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

DOCTORAL THESIS

by

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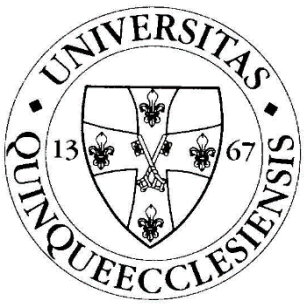

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List of Abbreviations

AA	Arachidonic acid
ABP	Arterial (mean) Blood Pressure
ACh	Acetylcholine
AMP	Pulse Amplitude of Intracranial Pressure (ICP)
BA	Basilar Artery
BHT	Breath Hold Test
CaBV	Cerebral arterial Blood Volume
cAMP	cyclic Adenosine Monophosphate
CBF	Cerebral Blood Flow
CBFV	Cerebral Blood Flow Velocity
CFF	Continuous Flow Forward
CoQ	Coenzyme Q
COX	Cyclooxygenase
CPP	Cerebral Perfusion Pressure
CPPopt	optimal Cerebral Perfusion Pressure
CVCi	Cerebrovascular Conductance Index
CVR	Cerebrovascular Resistance
DMT	Danish Myo Technology
DHE	Dihydroethidium
eNOS	endothelial NOS
FV	Flow Velocity
FVm	mean Flow Velocity
FVdia	diastolic Flow Velocity
FVsys	systolic Flow Velocity
GCS	Glasgow Coma Scale
ICP	Intracranial Pressure
ICU	Intensive Care Unit
MAP	Mitogen-Activated Protein
MCA	Middle Cerebral Artery

MP-RAGE	Magnetization Prepared Rapid Acquisition with Gradient Echo
MRI	Magnetic Resonance Imaging
mTBI	mild TBI
mtROS	mitochondrial ROS
NADPH	Nicotinamide Adenine Dinucleotide Phosphate
nCPPopt	non-invasive CPPopt
NMDA	N-methyl-D-aspartate
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
NOX	NADPH Oxides
nPAx	non-invasive PAx
nPRx	non-invasive PRx
NVC	Neurovascular Coupling
PCR	Polymerase Chain Reaction
PFF	Pulsatile Flow Forward
PG	Prostaglandin
PGD2	Prostaglandin D2
PGE2	Prostaglandin E2
PGF2	Prostaglandin F2
PGH2	Prostaglandin H2
PKA	Protein kinase A
PKC	Protein kinase C
PAx	Pulse Amplitude index
PRx	Pressure Reactivity index
PSS	Physiological Saline Solutions
ROS	Reactive Oxygen Species
RT-PCR	Real-Time PCR
SAH	Subarachnoid Hemorrhage
SHR	Spontaneously Hypertensive Rat

SS–31	Szeto-Schiller (SS) peptide
TBI	Traumatic Brain Injury
TCD	Transcranial Doppler
TMT	Trail Making Test
WKY	Wistar Kyoto rat

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 [14]. 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 [56]. 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 [59]. 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 [78]. 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. This so called "autoregulation of cerebral blood flow" is achieved by several mechanisms [4]. Based on Ohm's and Hagen-Poiseuille's laws autoregulation can be defined in terms of changes of vascular resistance or simply in terms of changes of vessel diameter as perfusion pressure varies [58]. The vascular resistance is adjusted to changes in perfusion pressure by the interaction of the pressure-induced intrinsic myogenic response of cerebral vessels, as well as metabolic effects of local compounds such as changes in H^+, K^+, O_2, adenosine concentration (so called „metabolic regulation") etc. [14]. It is important to note, that uniquely, in the cerebral circulation large arteries (i.e. middle cerebral artery) are important in autoregulatory function because they contribute to vascular resistance about 40 %. Also, various brain regions are involved differently in the regulation of CBF. Thus autoregulation of CBF shows both segmental and regional heterogeneity [24]. 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. Autoregulation of cerebral blood flow is profoundly affected by chemical and metabolic regulation. Accordingly, cerebrovascular tone is largely determined by the changes in partial pressure of carbon dioxide, and local metabolic by-products also modulate diameter and thus resistance of cerebral arteries and arterioles. 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

It is required to adequately adjust cerebral blood flow to neuronal activation in a tightly controlled temporal and spatial manner to maintain normal cellular function while the demand for oxygen and nutrients surges rapidly in the brain with increases in regional neuronal activity[69]. A feedforward homeostatic process termed 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 [64].

Neurovascular coupling's ability to increase cerebral blood flow in functionally active parts of the brain plays a central role in maintaining cognitive function. This is suggested by studies showing that impaired NVC due to hypertension, aging, obesity, traumatic brain injury etc. is associated with cognitive decline [68, 70] and that pharmacological impairment of neurovascular coupling leads to memory deficit and disturbances in gait function in mice [69]. To quantify NVC, the measurement of blood flow and activity of specific regions of the cerebral cortex is necessary. Several methods - transcranial Doppler (TCD), functional magnetic resonance imaging (fMRI), single photon emission computer tomography (SPECT), positron emission tomography (PET), electroencephalography (EEG) and near-infrared spectroscopy (NIRS) – are already proved to be efficient in the assessment of NVC [34, 38]. It is important to note, that in humans, by using the mentioned in vivo approaches it is not possible to understand the underlying molecular and cellular mechanisms of NVC. Previous studies suggested in various preclinical models and humans that astrocytic prostaglandin E2 (PGE_2) may play an important role in NVC (Figure 1) [5, 40, 52]. However, contradictory studies have shown that PGE_2 is rather a vasoconstrictor than a vasodilator in the brain [20], and it even may play a role in the development of vasospasm following subarachnoid bleeding [44].

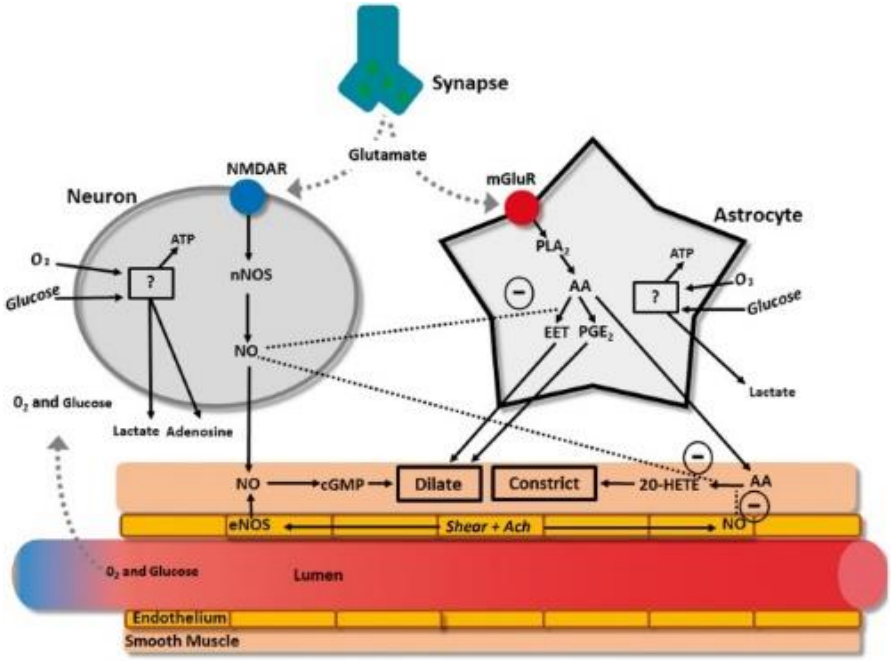

Figure 1. Main molecular mechanisms of neurovascular coupling and the role of PGE_2 (Phillips, Chan, Zheng, Krassioukov, & Ainslie, 2015). Through neuronal activation, the excitatory neurotransmitter, glutamate binds to NMDA receptor on the postsynaptic neuron, which increases nNOS activity and consequently NO concentration. Glutamate also binds to mGluR on astrocytes, which eventually leads to PGE2 production. Both NO and PGE2 dilate arterioles, therefore contribute to neurovascular coupling. (NMDAR: N-methyl-D-aspartate receptor; ATP: Adenosine triphosphate; nNOS: neuronal nitric oxide synthase; NO: nitric oxide; eNOS: endothelial nitric oxide synthase; cGMP: cyclic guanosine monophosphate; Ach: acetylcholine; mGluR: metabotropic glutamate receptor; PLA2: phospholipases A2; AA: arachidonic acid; PGE2: prostaglandin E2; 20-HETE: 20-Hydroxyeicosatetraenoic acid)

Prostaglandin E_2

Neurovascular hyperemia is predominantly mediated by cyclooxygenase (COX)-derived metabolites of arachidonic acid (AA) [50], of which Prostaglandin E_2 (PGE_2) has been proposed as a key mediator of both astrocyte- and neuron-mediated neurovascular coupling responses [5, 40]. Indirect evidence also supports a key role of COX-derived vasodilator metabolites in neurovascular coupling responses in humans [31]. This has also been confirmed by the administration of the COX inhibitor indomethacin: the consequent reduction of PGE_2 synthesis was shown to significantly attenuate neurovascular coupling responses in human subjects [10, 36, 41, 61]. 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 [53, 61].

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. Parenchymal arterioles are the major determinants of cerebrovascular resistance and this segment of the cerebrovascular tree is primarily involved in both maintenance of normal basal cerebral perfusion and moment-to-moment adjustment of CBF to metabolic needs of neurons via neurovascular coupling [26]. Theoretically, a test to support the potential role of PGE_2 in neurovascular coupling is the demonstration that PGE_2 can directly elicit vasodilation, at least in parenchymal resistance-sized arterioles. Contrary to expectations, a recent study reported that PGE_2 constricted rather than dilated parenchymal arterioles isolated from both *M. musculus* and *R. norvegicus* [20]. Furthermore, in preclinical models overproduction of PGE_2 has also been linked to pathological vasospasm associated with subarachnoid hemorrhage [44].

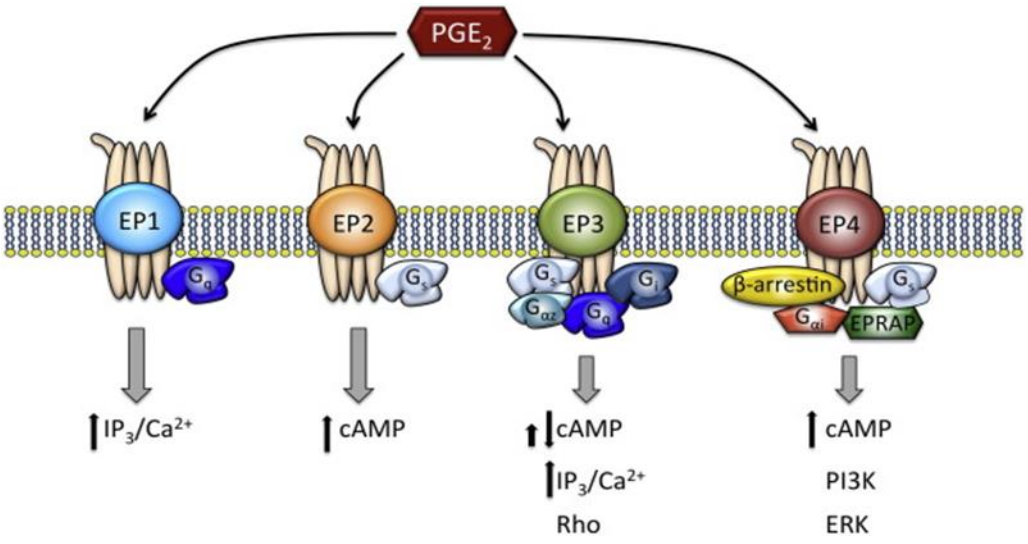

Figure 2. Receptors of PGE_2 and the different types of activated G-proteins and signaling pathways (Friedman, Ogletree, Haddad, & Boutaud, 2015). PGE_2: prostaglandin E2; EP: prostaglandin receptor; IP_3: inositol trisphosphate; Ca^{2+}: calcium ion; cAMP: cyclic adenosine monophosphate; Rho: GTPase Family; PI3K: phosphoinositide 3-kinase; ERK: extracellular signal-regulated kinase)

The contradictory findings, partially, could be explained by the different receptors of PGE_2, and the difference in the molecular mechanisms these receptors activate (Figure 2) [27].The vasomotor effect of PGE_2 - similarly to other prostaglandins an thromboxanes - is mediated by G protein coupled prostaglandin (EP) receptors, and in theory this could be the reason behind PGE_2's "two-faced" behavior. Accordingly, PGE_2 can evoke constriction or dilation, depending on the receptor subtype activated (Figure 2). 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). Previous studies lead to the hypothesis that expression of EP receptor subtypes in cerebral arterioles and therefore the vasoactive action of PGE_2 are species-dependent [79]. Importantly, to the best of our knowledge expression of EP receptors mediating dilator and constrictor vasomotor mechanisms and the vasoactive effects of PGE_2 in human cerebral arterioles have not yet been documented.

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 (n = 19, female: 7, male: 12, age: 53.7±2.9 years), which otherwise would have been discarded. The patients did not have known comorbidities. Preoperatively, contrast enhanced magnetization prepared rapid acquisition gradient echo (MP-RAGE) MRI sequences were carried out in order to visualize contrast enhancing areas with pathologically increased blood brain barrier permeability. Cerebral samples containing intraparenchymal arterioles and/or the arterioles themselves were removed carefully from non-enhancing normal cortical areas that had to be removed because of technical reasons in order to approach deep-seated tumors [67]. After removal, cerebral tissue from the fronto-temporo-parietal lobes was placed in 0°C - 4°C physiologic salt solution (PSS, 110.0 NaCl, 5.0 KCl, 2.5 CaCl2, 1.0 MgSO4, 1.0 KH2PO4, 5.5 glucose, and 24.0 mmol/L NaHCO3 equilibrated with a gas mixture of 95% air and 5% CO2, balanced with nitrogen at pH ~7.3). 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, Figure 3). 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 a subset of experiments (n = 5) endothelium was removed by inserting an air bubble through the lumen of the vessels. The lack of a functioning endothelium was verified by the absence of vasomotor response of the arteriolar rings to acetylcholine. Optimal passive tension (as determined from the vascular length-tension relationship) was applied for one hour (equilibration period).

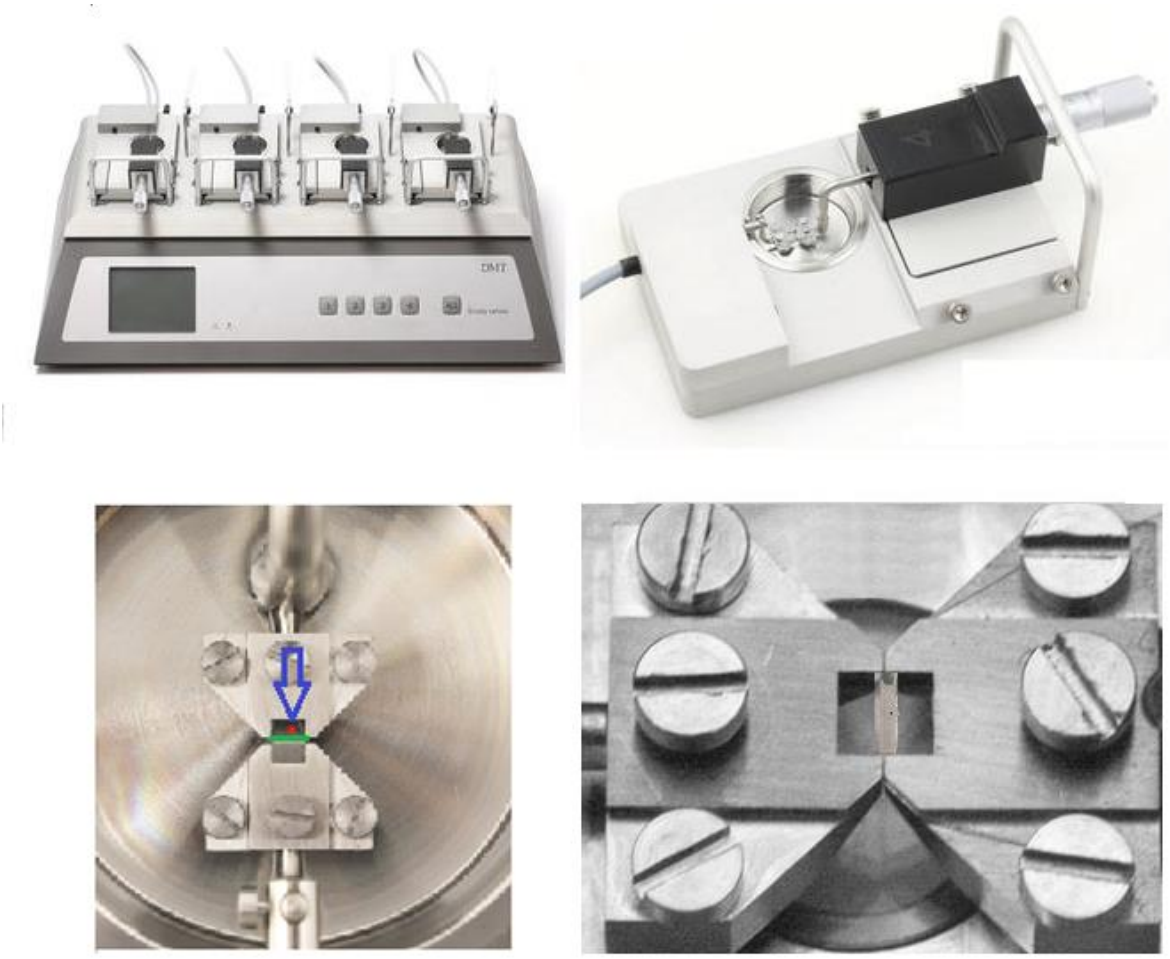

Figure 3. DMT multi-chamber 620M Wire Myograph system (dmt.dk). The DMT system (top left) contains 4 vessel-chambers (top right), and gives the opportunity to simultaneously execute experiments. Under operating microscope 1-1.5 mm long arterial segments are mounted (bottom left, green line and bottom right) and fixed with the help of 40 μm thick stainless steel micro wires and the screws. Then the chamber is filled with oxygenated physiologic salt solution. After an incubation period and normalization, the vasomotor effect of different pharmacological agents can be measured by detecting the tension on the wire caused by the contraction (or dilation) of the arterioles.

In an additional set of experiments Wistar Kyoto rats (WKY, 300 - 350 g, male, n = 10, purchased from Charles River Laboratories) were anesthetized (isoflurane), decapitated, the brains were removed and segments of basilar arteries were isolated and mounted in a wire myograph, as described previously [62]. 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.

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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) either in the presence or absence of a functional endothelium. Endothelial function was tested by investigating relaxation to acetylcholine (ACh; from 10^{-9} to 10^{-6} mol/L). Endothelium-independent relaxation was studied by administration of the NO donor sodium nitroprusside (from 10^{-9} to 10^{-6} 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 in the observed vasomotor effect of PGE2: the specific EP4 blocker BGC 20-1531 (10^{-6} mol/L for 5 min, Cayman Chemicals, Ann Arbor, MI), the EP2 receptor blocker PF-04418948 (10^{-6} mol/L for 5 min, Cayman Chemicals, Ann Arbor, MI) or the EP1 receptor blocker SC-51322 (10^{-6} mol/L for 5 min, Cayman Chemicals, Ann Arbor, MI). 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. Arteriolar rings were incubated in the presence of only one inhibitor in each experiment, and dose-responses were repeated, in a self-controlled manner. At the end of the experiments still intact functional reactivity was assessed again, then passive tension was obtained in Ca^{2+} free conditions, as published earlier [6].

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.

Quantitative real-time RT-PCR

A quantitative real-time RT-PCR method was used to analyze mRNA expression of EP1 and 4 receptors in endothelium-denuded human cerebral parenchymal arterioles (PTGER1 and PTGER4, n = 5) and rat arterial segments (Ptger1 and Ptger4, n = 5) according to previously described protocols [66]. In brief, RNA was isolated with the Pure LinkTM RNA Mini Kit (Life Sciences, Carlsbad CA, USA) according to the protocol suggested by the manufacturer and was reverse transcribed using the High Capacity cDNA kit (Applied Biosystems, Foster City CA, USA). The total amount of RNA was determined by NanoDrop (Thermo Scientific, Waltham MA, USA). qRT-PCR was performed using SensiFast SYBR Green reagent (BioLine, Luckenwalde, Germany). The following primer sequences were used: PTGER1, forward: GAGGGAGGGAGGAAGCGG, reverse CCGCAAGGGCTCATGTCAG, Ptger1, forward: ACAGGGGATGCTCCAAACAC, reverse: GGTGGGACGTGAATCCAGAA; PTGER4, forward: CTGCCGCTACAGACCCAG, reverse: CAAGGAGGCGGACGAATTGA, Ptger4, forward: CTCATCTGCTCCATTCCGCT, reverse: CCGGGTTTCTGCTGATGTCT. Amplification efficiencies were determined using a dilution series of a standard vascular sample. Quantification was performed using the efficiency-corrected ΔΔCq method as previously described [66].

Western blotting

Endothelium-denuded human cerebral parenchymal arterioles were crushed with Potter's homogenizer in ice-cold lysis buffer (50 mM Tris-base, pH 7.4, 150 mM NaCl, 10% glycerol, 1 mM EGTA, 1 mM Na-orthovanadate, 5 µM ZnCl2, 100 mM NaF, 10 µg/ml aprotinin, 1 µg/ml leupeptin, 1 mM PMSF, 1% Triton X-100) and were centrifuged at 35.000×g at 4 °C for 20 min. The protein concentration of the supernatants was measured by spectrophotometry on 595 nm (Lowry's method, Detergent Compatible Protein Assay Kit, Bio-Rad, Hercules, CA, USA). Samples containing 50 µg of protein were mixed with 4× Laemmli buffer (25 ml 1 M Tris–HCl, pH 6.8, 40 ml glycerol, 8 g SDS, 10 ml 100 mM EDTA, 10 ml 100 mM EGTA and 1 ml 1% bromophenol blue brought up to 100 ml with distilled water) and boiled for 5 minutes. The proteins were separated in 10% SDS-polyacrylamide gel based on their size. The gels were electro-blotted onto PVDF membranes (Hybond-P, GE Healthcare, UK). The membranes were blocked in 5% nonfat dry milk dissolved in TBS-Tween (10 mM Tris-base, 150 mM NaCl, 0.2% Tween-20, pH 8.0). PGER1 (Cayman Chemical Company, 1:200) or PGER4 (Novus Biologicals, 1:200) primary antibody was added diluted in 3% BSA-TBS-Tween solution and incubated overnight. Not bound antibodies were washed five times by TBS-Tween and membranes were incubated with secondary anti-rabbit antibodies conjugated with horseradish-peroxidase (HRP) (Pierce, Thermo Fischer Scientific, Rockford, IL, USA) diluted 1:50.000 in 5% nonfat dry milk blocking solution. After five washes in TBS-Tween the chemiluminescent signal was detected (Immobilon Western, Millipore Corporation, Billerica, MA, USA). The blots were stripped and reprobed with ß-actin (Cell Signaling Technology, 1:2.000) antibody to check the equal loading of proteins [65].

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

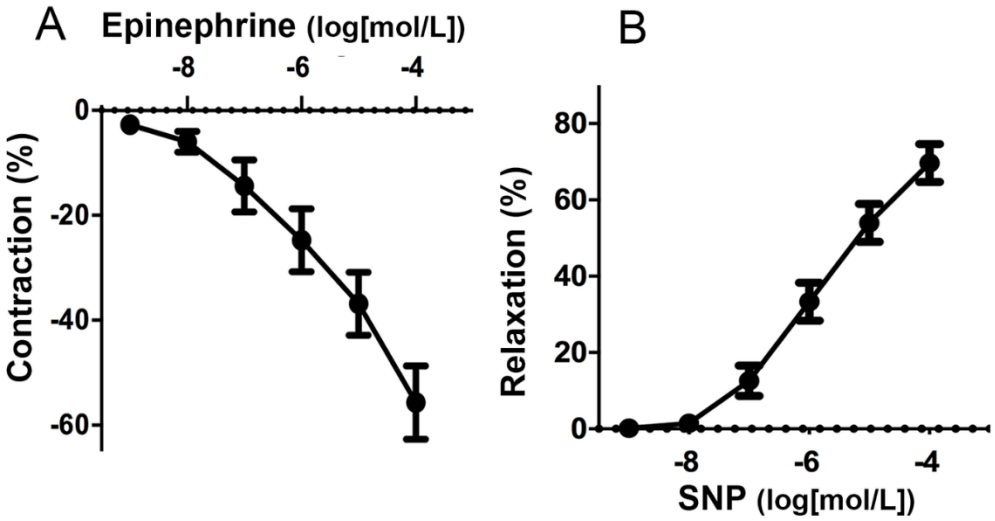

Figure 4. ***Dose-dependent vasomotor effects elicited by vasoactive agents in human cerebral parenchymal arterioles.*** ***A****: cumulative administration of the beta adrenergic vasoconstrictor agent epinephrine and* ***B****: the NO donor vasodilator sodium nitroprusside (SNP) in human isolated cerebral parenchymal arterioles. Note that pharmacologically elicited vasodilator and vasoconstrictor responses were intact in the vascular preparations used, demonstrating that the vessels were functionally intact. Data are mean ± S.E.M. n=12 to 15 for each data point.*

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

The smooth muscle layer of the studied human cerebral parenchymal arterioles was functionally intact as demonstrated by the dose-dependent constriction evoked by epinephrine (Figure 4A) and the dose-dependent dilation of the vessels to sodium-nitroprusside (Figure 4B).

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 5A. Summary data are shown in Figure 5B. The presence of a functional endothelium was verified by demonstration of vasorelaxation to acetylcholine (10^{-5} mol/L) (Figure 5C). Denudation of the endothelium did not affect the vasomotor response to PGE_2 (Figure 5B). We found that rat basilar arteries (BA) exhibit dose-dependent contraction in response to administration of PGE_2 (Figure 6A-B) and that in rat BAs mRNA expression of the constrictor EP1 (Ptger1) receptors was significantly higher than expression of the dilator EP4 (Ptger4) (Figure 6C), extending previous findings of Dabertrand et al.

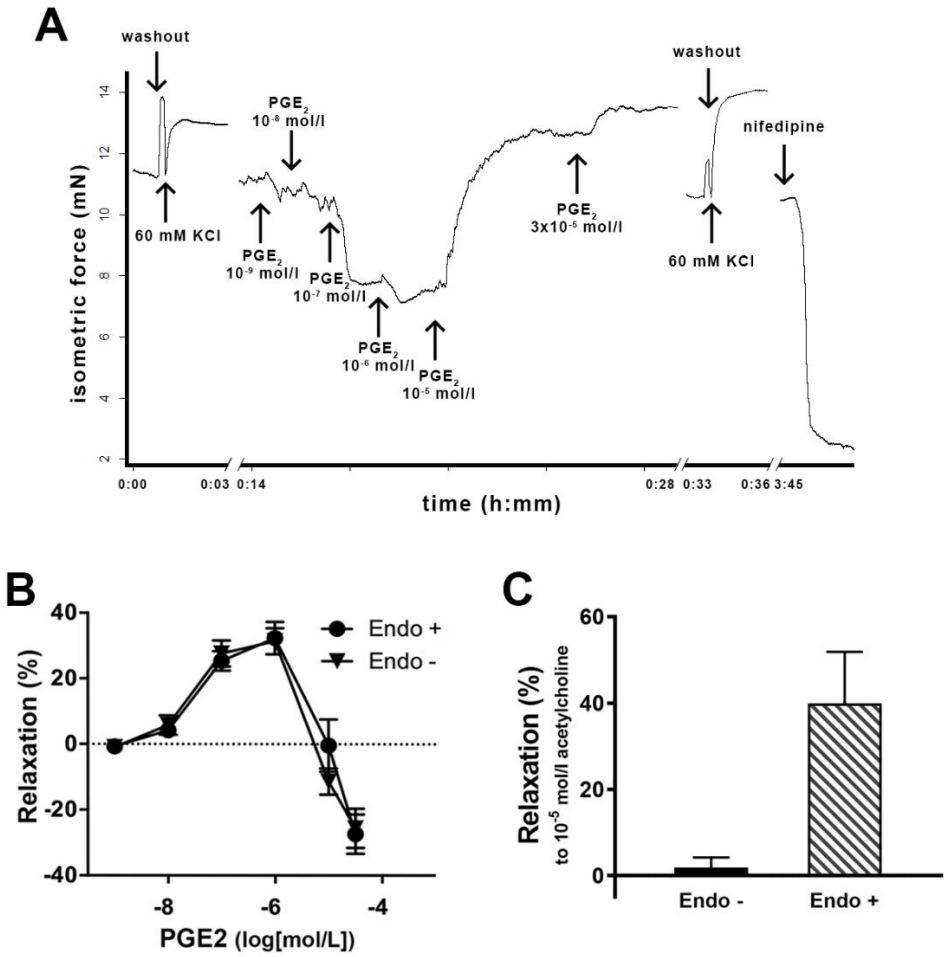

Figure 5. ***Prostaglandin E2 (PGE_2) elicits biphasic vasomotor responses in human cerebral parenchymal arterioles.*** ***A****: Original recording showing the effect of PGE_2 on the tone of an isolated segment of a human cerebral parenchymal arteriole. Note that at lower concentrations PGE_2 elicits substantial vasorelaxation, whereas at higher concentrations a significant vasoconstriction becomes manifest.* ***B****: 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.* ***C****: acetylcholine-induced, endothelium dependent relaxation of the vessels. Responses are expressed as percentage changes in vasomotor tone. Data are mean ± S.E.M. n=5 to 7 for each data point.*

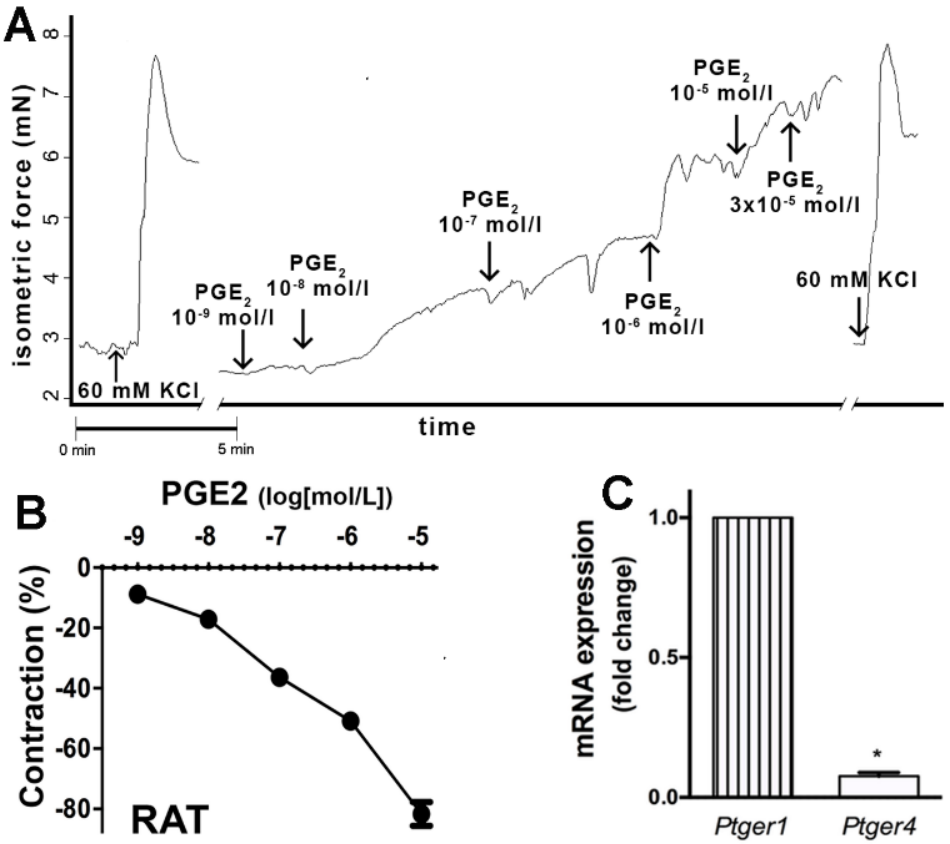

Figure 6. ***Prostaglandin E2 (PGE_2) elicits contractions in cerebral arteries of rats.*** *Original recording (**A**) and summary data (**B**) of PGE_2-induced vasomotor responses of isolated basilar arteries of Whistar-Kyoto rats (n = 6). Responses are expressed as the percentage change in vasoconstriction (increase in vasomotor tone) from baseline. Note that PGE_2 induces dose-dependent constriction of rat cerebral arteries, supporting previous findings of Dabertrand et. al. Data are mean ± S.E.M.* ***C**: mRNA expression of the PGE_2 EP1 (Ptger1) and EP4 (Ptger4) receptors in isolated rat basilar arteries. Data are mean ± S.E.M. (n = 5 for each group) *P<0.05.*

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 7A-B).

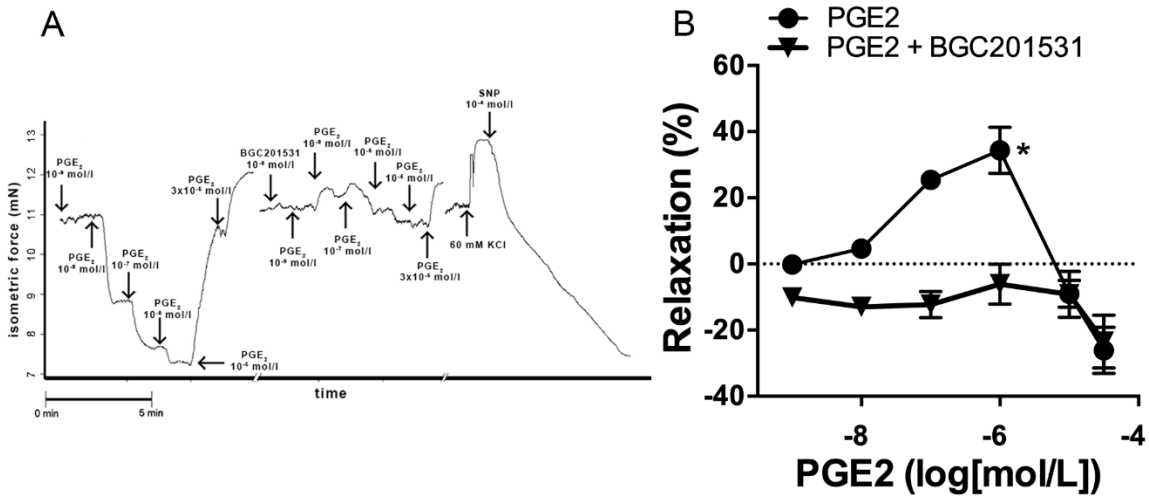

Figure 7. ***Role of PGE_2 receptor subtype EP4 in mediation of PGE_2-induced vasodilation in human cerebral parenchymal arterioles.*** *Representative original recording of an experiment (A) and summary data (B) of PGE_2-induced changes in vasomotor tone of human isolated cerebral parenchymal arterioles in the absence and presence of BGC201531 (10^{-6} mol/L), a specific inhibitor of the PGE_2 receptor subtype 4 (EP4). 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 8A-B). 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.

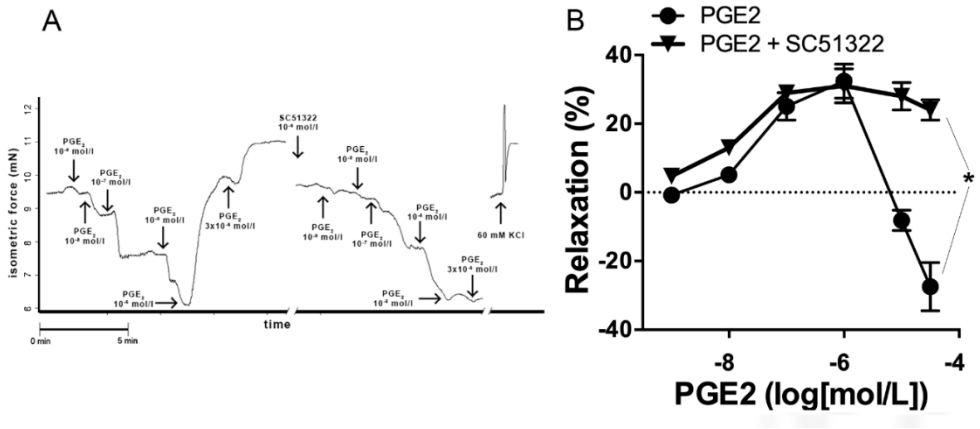

Figure 8. ***Role of PGE_2 receptor subtype EP1 in mediation of PGE_2-induced vasoconstriction in human cerebral parenchymal arterioles.*** *Representative original recording of an experiment (A) and summary data (B) of changes in vasomotor tone of human isolated cerebral parenchymal arterioles induced 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. PGE_2-induced responses without SC51322.*

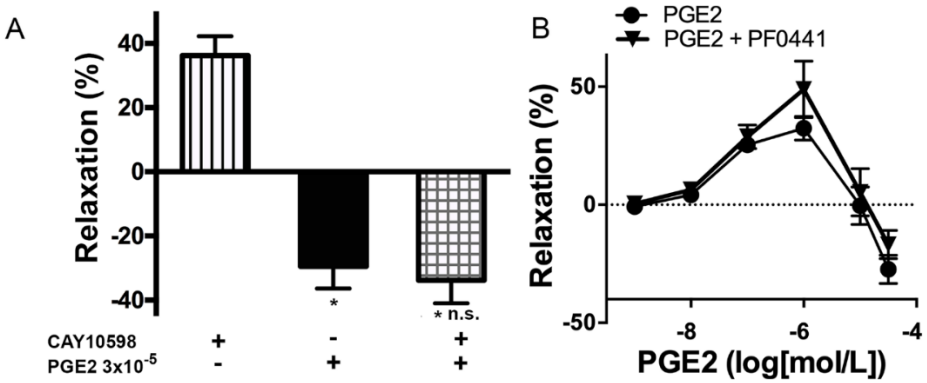

Figure 9. ***Role of PGE_2 receptor subtype EP2 and EP4 in mediation of PGE_2-induced vasomotor responses in human cerebral parenchymal arterioles. A:*** *The EP4 agonist CAY10598 evokes relaxation of isolated human cerebral parenchymal arterioles, which is blocked by the EP1-dependent constrictor response induced by high concentration of PGE_2. Data are mean ± S.E.M. (n=6) *P<0.05 vs. CAY10598-induced response, n.s.(non-significant) vs. PGE_2 induced contraction.* ***B:*** *PGE_2-induced changes in vasomotor tone of human isolated cerebral parenchymal arterioles in the absence and presence of PF0441 (10-6 mol/L), a specific inhibitor of the PGE_2 receptor subtype 2 (EP2). Data are mean ± S.E.M. (n=6)*

This concept is further supported by the findings that 10^{-6} mol/L CAY10598, a specific EP4 receptor agonist, induced relaxations in human cerebral parenchymal arterioles (Figure 9A) to a similar extent as did 10^{-6} mol/L PGE_2 (Figure 4B). However, it did not have any effects when the vessels were pre-constricted with the $3x10^{-5}$ mol/L PGE_2. Administration of the EP2 blocker PF-04418948 was without effect on the PGE_2-induced vasomotor responses of human cerebral parenchymal arterioles (Figure 9B).

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 (Figure 10A-B).

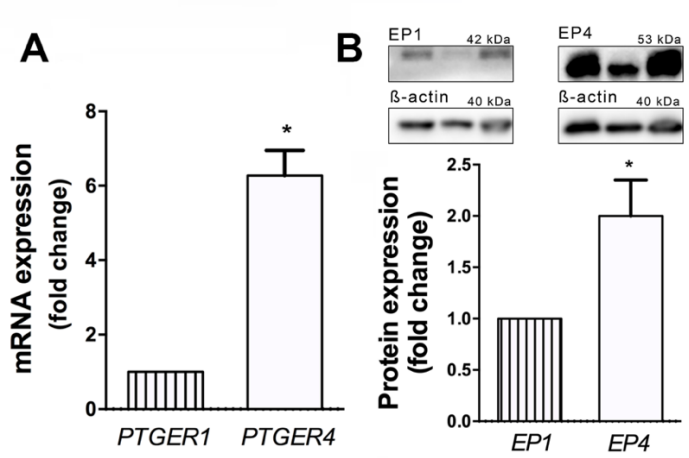

Figure 10. ***Expression of EP1 and EP4 receptors in human cerebral parenchymal arterioles.*** *mRNA (**A**) and protein (**B**) expression of EP1 and EP4 receptors (gene PTGER1 and PTGER4, respectively) in isolated human cerebral parenchymal arterioles. Data are mean ± S.E.M. (n=9 for QRT-PCR, and n=6 for western blotting for each group) *P<0.05.*

Summary of results of Part I.

- PGE2 induces biphasic vasomotor responses in human cerebral parenchymal arterioles.
- PGE2 at low concentrations elicits vasodilation by activating EP4 receptors and likely plays a critical role in neurovascular coupling.
- Human cerebral parenchymal arterioles predominantly express EP4 receptors.
- PGE2 at higher concentrations elicits vasoconstriction by activating EP1 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 [1, 8]. Since then, the technology and the assessing method went through profound development, and nowadays it is used in various clinical settings for various purposes: detection of vasospasm after subarachnoid hemorrhage; diagnosis of cerebral circulatory arrest; intraoperative and postoperative detection of hypoperfusion and emboli related to carotid endarterectomy; residual AVM diagnosis; monitoring in TBI etc. [25, 57]. The basics of this ultrasonography technique are not different from those sonographies used outside the skull: based on the Doppler phenomenon the velocity of red blood cells are calculated from the shift in the wavelength between the emitted and reflected sound waves. When trying to look inside of cerebral arteries we have to insonate through the skull bone, which attenuates the strength of the ultrasound signal [8]. To achieve better signal-to-noise ratio lower insonating frequencies (2 MHz) can be used [39, 76]. Most of the time, the trans-temporal insonation is used to measure velocities in the middle cerebral arteries (MCA). From the raw data of instantaneous flow velocities, 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) [29, 37, 49].

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 [45]. 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) [11, 81].

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) [17]. It is calculated in 5-minute time windows by correlating mean values of ABP and ICP every 10 seconds, ie. 30-30 data points, 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 [18]. 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 [72].

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 [3, 12].

The so-called slow waves (B-waves) are intrinsic factors of ICP, they reflect to vasogenic activity of cerebral autoregulation and have a frequency range of 0.005 - 0.05 Hz [16, 42, 60]. This means that a minimum of 5 minutes are required to collect enough data to characterize the relationship between ABP and ICP. Using the 5-minute time window and the averages calculated every 10 seconds is essential to calculate moving correlation in a reliable way. On the other hand, the correlation coefficient calculated over a wider time range is sensitive to other conditions (e.g. metabolic changes, nursing activities, drug interactions), which would distort the parameter characteristic of autoregulation [16].

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. Cerebrovascular dysregulation plays a critical role in secondary damage of brain tissue induced by traumatic brain injury (TBI) [30, 51]. In severe TBI patients both the impairment of autoregulation of CBF and neurovascular hyperemia can be observed. It has been shown that disturbed autoregulatory function determines the outcome of severe TBI patients [7]. 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 [16, 18]. 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 [16]. 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. At present a clinical trial analyzing the feasibility of treatment based on optimal perfusion pressure is ongoing under the name COGiTATE (http://cppopt.org/).

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 [82]. This prevents the applicability of PRx in patients in whom intracranial pressure cannot be measured (moderate or mild TBI patients etc.) 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. A feasibility study from 2017 provides evidence that in healthy individuals TCD is capable to detect increased cerebral blood flow caused by different cognitive tasks, implying the activation of NVC mechanisms [77]. It also has been shown that with similar methods, a significant deterioration of NVC functions is detectable in patients with acute ischemic stroke [54]. These results make TCD a cost-efficient, non-invasive tool to investigate fundamental physiological and pathological mechanisms, but they also add another element to TCD's already wide scope of use, that 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 [55, 77].

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: data was collected from 14 traumatic brain injury (TBI) patients, whose recordings contained plateau waves (transient intracranial hypertension) which resulted in a significant difference (mean±SD: 25.3±5.9 mmHg) 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 [13, 19, 71]. 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 asses 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. Furthermore, since healthy controls are necessary to undertake various tasks, it is important that only non-invasive approaches are feasible. 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). Both device have an analogue signal output. BP and FV signals were sent to a data acquisition (DAQ) A/D converter device (DT9816, Measurement Computing Corporation, Norton MA, USA) in order to acquire synchronized data (Figure 11). Then synchronized digital data were transferred to ICM+ software (Cambridge Enterprise, Cambridge, United Kingdom; http://www.neurosurg.cam.ac.uk/icmplus). Our protocol was based on the work of Ronney B. Panereai et al. [54, 55, 77]. 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. The tasks were as follows:

1. Count backwards from 100 by 7
2. Name words in one minute that begin with a specific letter
3. Trail making test (TMT), originally used as part of a neuropsychological toolset to quickly asses cognitive functions, where the subject is required to connect numbers (and letters) in ascending (or alphabetical) order [9]
4. Clock-drawing test [2]
5. Elbow flexion (active, passive)

6. Breath holding test

Changes in BP and FV from baseline to plateau during the different activities were analyzed via ICM+ and Excel software.

Figure 11. ***Hardware used to detect physiological changes in blood pressure and intracranial blood flow velocity indicating functioning neurovascular coupling.*** *Top left: Blood flow velocity (FV) was detected by a transcranial Doppler ultrasound device (Multi-Dop T, Compumedics DWL, Singen, Germany). Top right: A head frame was used to steady the TCD's transducers at correct positions in order to continuously measure blood flow in booth middle cerebral arteries. Bottom left: A non-invasive finger cuff monitor (CNAP® Monitor 500 HD, CNSystems, Graz, Austria), to record beat-to-beat blood pressure continuously based on blood volume changes in the finger (plethysmography). Bottom right: Analogous BP and FV signals are converted to digital signals and forwarded to a computer by a data acquisition (DAQ) A/D converter device (DT9816, Measurement Computing Corporation, Norton, MA, USA)*

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 [73].

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).

With TCD monitoring, the cross-sectional area of the middle cerebral artery is unknown, and the CBF cannot be precisely calculated. In these equations, CBF can be substituted with CBFV, and so the relative changes in CaBV can be estimated (Figure 12).

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.

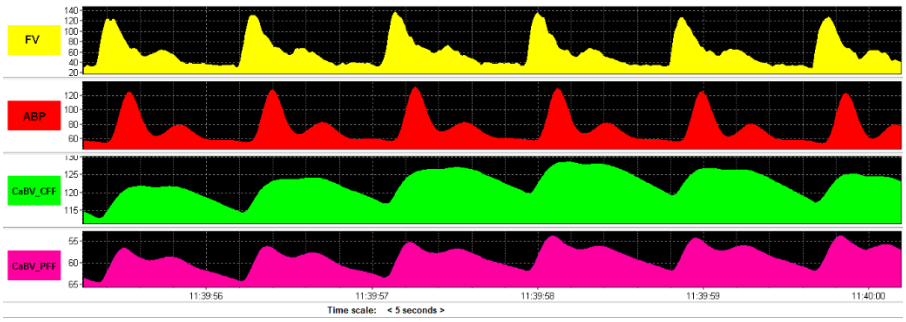

Figure 12. ***Waveforms of flow velocity, arterial blood pressure, and changes in cerebral arterial blood volume (CaBV)*** *– calculated both with the continuous flow forward (CFF) and the pulsatile flow forward (PFF) models. The pulsatile nature of CaBV with both methods is visible, but more prominent peaks appear with the PFF model.*

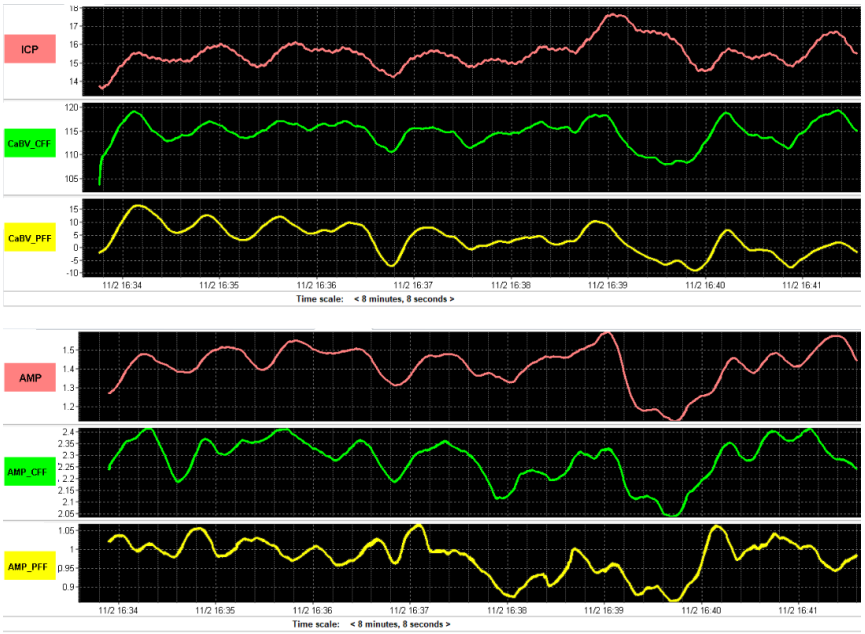

*Figure 13. A representative example of good coherence between slow waves in intracranial pressure (ICP) and cerebral arterial blood volume (CaBV) (**upper panel**), and between slow waves of pulse amplitude of ICP (AMP) and the pulse amplitude of CaBV (**lower panel**). Both the continuous flow forward (CFF) and pulsatile flow forward (PFF) models were used to for the calculations.*

Different calculation methods of FVm can cause an error in measurements

During measurements in patients with transient intracranial hypertension (Figure 15), 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) ($p<0.05$) (Figure 16A). 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 16B). In many cases, a strong correlation appeared between ICP and the errors (Figure 17).

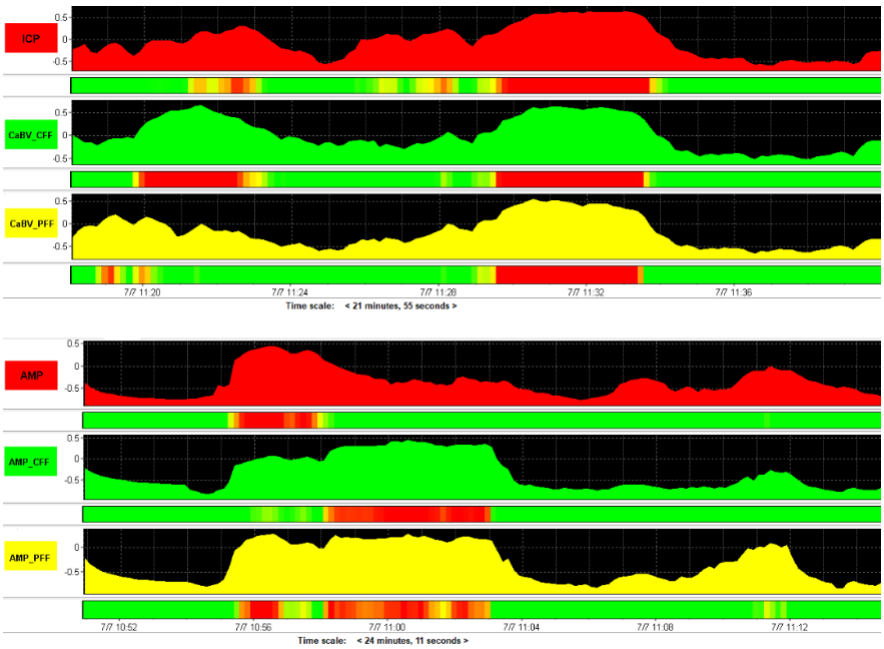

*Figure 14. Signals of pressure reactivity index (PRx), non-invasive PRx (nPRx) (**upper panel**), pulse amplitude index (PAx), and non-invasive PAx (nPAx) (**lower panel**). Both the continuous flow forward (CFF) and pulsatile flow forward (PFF) models were used to calculate non-invasive autoregulation indices.*

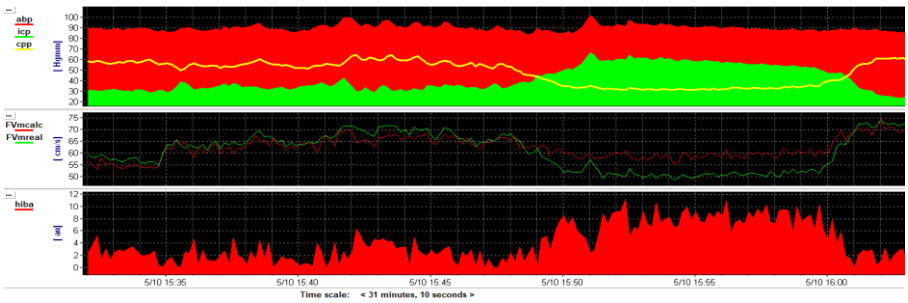

*Figure 15. **Transcranial Doppler (TCD), arterial blood pressure (ABP) and intracranial pressure (ICP) monitoring using ICM+ software.** On the upper panel a drastic, 15 min long rise in ICP (plateau wave) is visible together with a constant ABP and the consequent drop in cerebral perfusion pressure (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.*

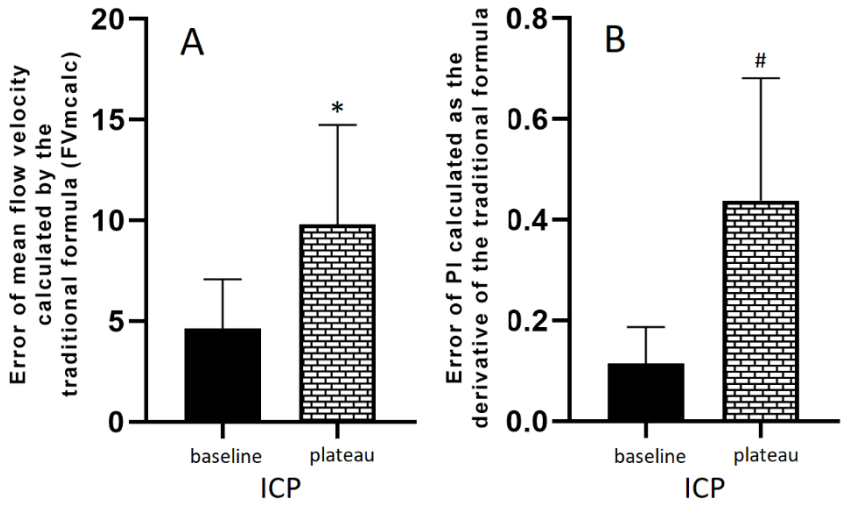

*Figure 16. Both the error of the traditional formula FVmcalc and the error of its derivative PI significantly increase from baseline to plateau. Data are mean ± SD (n=12 for each group) *P<0.05 vs. baseline; #P<0.005 vs. baseline (paired Student's t-test)*

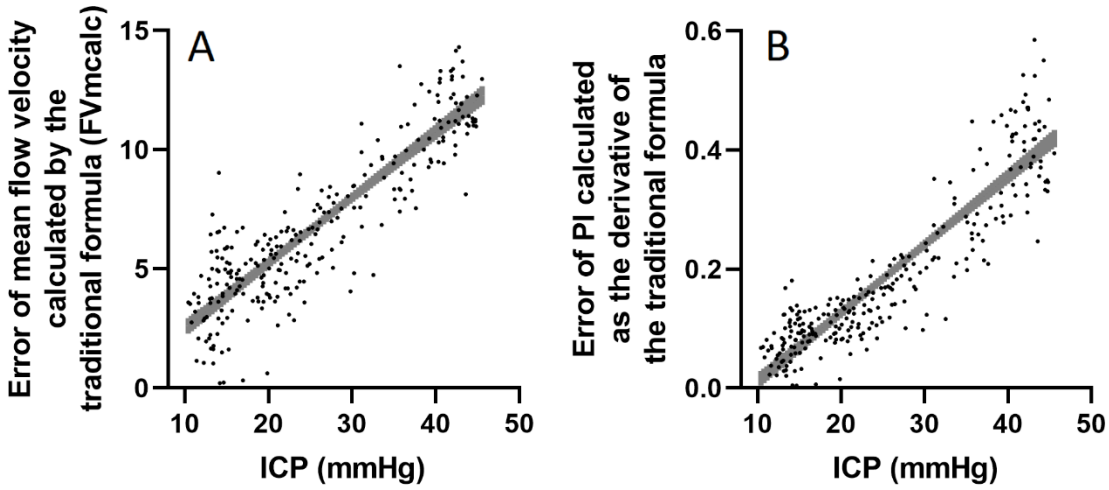

Figure 17. ***Representative plots of errors of estimated mean flow velocity and pulsatility index against intracranial pressure (ICP).*** *Panel A shows the strong correlation between error of the estimated mean flow velocity (FVmcalc) and ICP during the monitoring of a patient with plateau wave. On Panel B the error of pulsatility index (PI) when calculated from FVmcalc instead from FVmreal is visible.*

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, although FV during other tests was also higher than FV on baseline. 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.

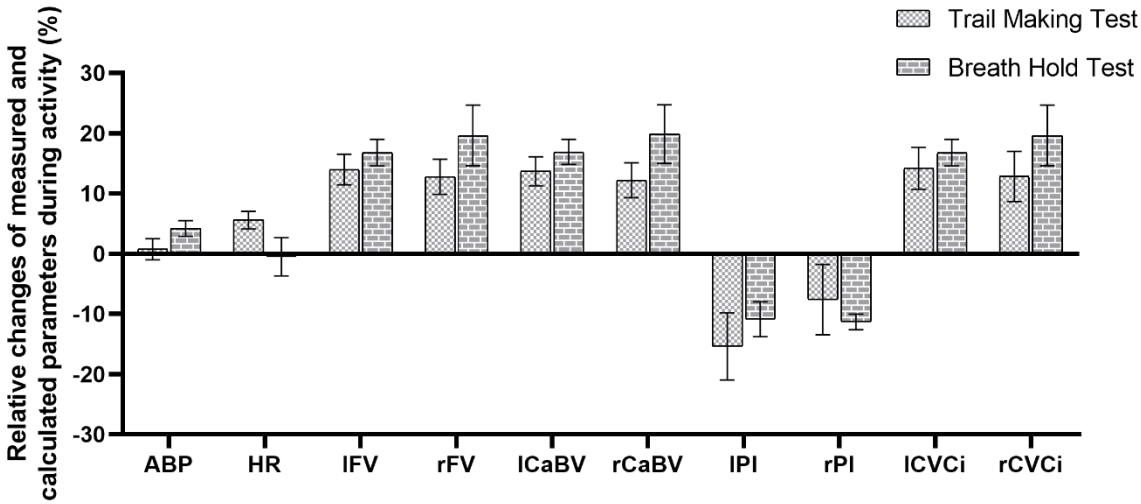

Figure 18. ***Relative changes of measured and calculated parameters during Trail Making Test (TMT) and Breath Hold Test (BHT)*** *(mean +/- SEM). ABP – Arterial Blood Pressure, HR – Hearth Rate, l – left, r – right, FV – Flow Velocity, CaBV – Cerebral Arterial Blood Volume, PI – Pulsatility Index, CVCi – Cerebrovascular Conductance Index*

During both the Trail Making Test and Breath Hold Test mean values of blood flow velocity, and consequently the estimated cerebral arterial blood volume increased relatively to baseline, and this effect was observable in both hemispheres (TMT: lFV – 14%, rFV – 12.8%, lCaBV – 13.7%, rCaBV – 12.2%; BHT: lFV – 16.8%, rFV – 19.6%, lCaBV – 16.9%, rCaBV – 19.9%). Blood pressure and hearth rate changes differed during these activities, as BHT did not alter HR (-0.5%) but increased ABP (4.1%) whereas in case of TMT the effect was the opposite (ABP – 0.7%, HR – 5.6%). Not surprisingly, the

increased blood flow velocities measured in the MCAs caused by vasodilation in the distal arterioles, coincide with decreased cerebrovascular resistance (TMT: lPI – -15.4%, rPI – -7.6%; BHT: lPI – -10.9%, lPI – -11.3%). Finally, as a result of the mild or not existent changes of ABP compared to FV, the cerebrovascular conductance indices were also increasing (TMT: lCVCi – 14.2%, rCVCi – 12.8%; BHT: lCVCi – 16.8%, rCVCi – 19.6%) (Figure 18).

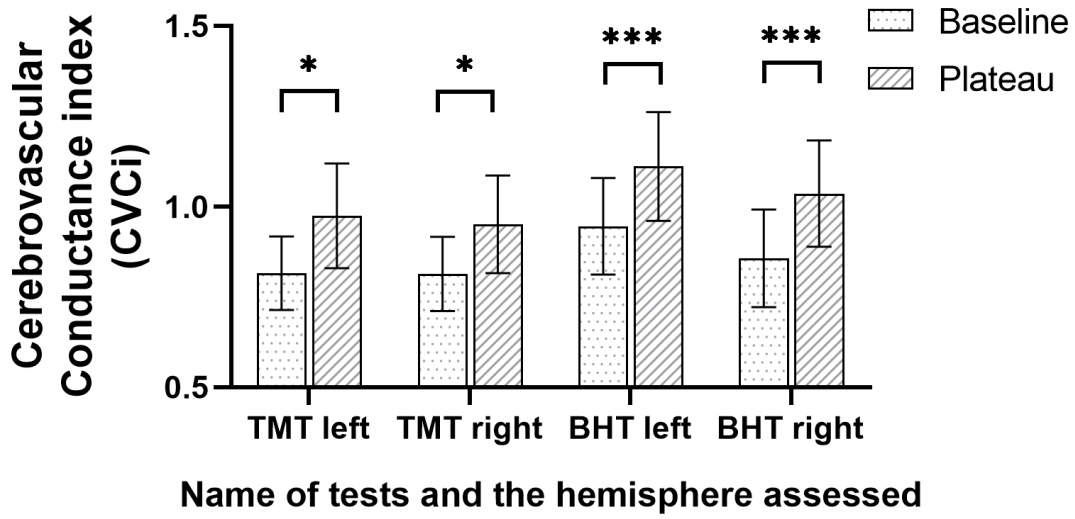

Figure 19. ***Cerebrovascular Conductance index (CVCi) before and during TMT and BHT tests*** *(mean +/- SEM) in the left and right hemispheres. TMT – Trail Making Test, BHT – Breath Hold Test. (*P<0.05; ***P<0.0005)*

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.)

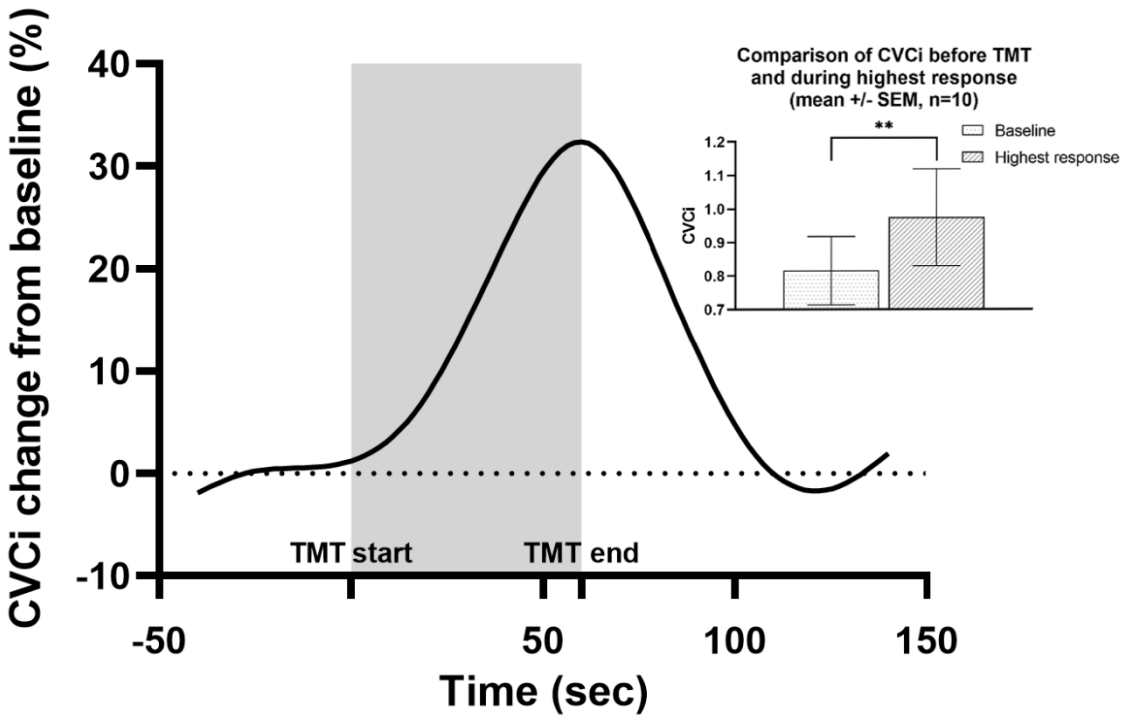

Figure 20. ***Representative figure of the change of cerebrovascular conductance index (CVCi) during Trail Making (TMT) test.*** *The gray area represents the interval during which TMT was carried out. Summary data in inlet shows, that there was a significant increase in CVCi after subjects were started to perform the cognitive activity (**P<0.01, paired t-test)*

As shown on Figure 20 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.

Summary of results of Part II.

- nPRx and nPAx – the non-invasive counterparts of PRx and PAx – calculated by the estimation of changes in cerebral arterial blood volume could be used to describe cerebral autoregulation.
- When calculating mean flow velocities of TCD recording, the integration of the pulse wave is recommended, especially when substantial changes in ICP are expected.
- Cerebrovascular responses to neurovascular coupling are detectable with non-invasive methods.
- Multiple forms of cerebral blood flow regulation are becoming accurately measurable by easy-to-use, cheap and non-invasive methods, leading to new possibilities to monitor pathophysiological aspects of neurosurgical conditions.

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 (Figure 4). 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 [53, 61] and initiation of neurovascular coupling responses during increased neuronal activity in humans [10, 31, 41, 61]. 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 [46]. Our results extend the findings of earlier studies demonstrating that PGE_2 at low concentrations dilates segments of the human middle cerebral artery [21]. Vasodilation in response to low concentrations of PGE_2 has also been observed in human pial arteries [74] and middle cerebral arteries of non-human primates [32]. 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 [21]. In line with these findings we found also constriction to PGE2 in human cerebral parenchymal arterioles when higher concentrations of PGE_2 were applied (Figure 4). 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 (Figure 7). Previous studies [33, 48] support this concept of the cross-talk between EP1 and EP4 receptors in the vascular smooth muscle cells.

Accordingly, the findings that arterioles preconstricted by PGE_2 cannot be dilated by the EP4 agonist CAY10598 (Figure 9) may be consistent with inhibition of EP4 receptor-mediated cAMP production by overactivation of EP1 receptors. EP4 receptors have been desensitized promptly in response to PGE_2 at the concentration of 10^{-6} mol/L [23], which may provide an additional mechanism to explain our results. 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[28]), EP1 receptor mediated constriction of cerebral parenchymal arterioles becomes manifest leading to brain ischemia.

Interestingly, the vasomotor action of PGE_2 exhibits strong species-dependency. In the vasculature of murine species the vasoconstrictor EP1 receptors are overexpressed, but our study demonstrates that human cerebral parenchymal arterioles predominantly express vasodilator EP4 receptors (Figure 10). These findings extend previous observations that contractile EP1 receptors do not have a widespread expression in tissues of higher species and are more expressed in murine species [15]. Species-dependent differences in the expression profile of PGE_2 receptors in the cerebral vessels likely underlie the species-specific vasomotor and hemodynamic effects of PGE_2. Accordingly, consistent with the higher expression level of EP1 receptors (Figure 6), arteries and arterioles from R. norvegicus, M. musculus, and C. porcellus predominantly exhibit constriction in response to administration of PGE_2 [20, 35, 75]. It has to be noted here, that we examined responses of cerebral arteries of rats, which are not directly comparable to the human vessels studied here, and they are not involved in neurovascular hyperemia, either. However, as mentioned, in a very well-controlled study by Dabertrand et al. arterioles of the rat and mice responded with contraction to PGE_2 as well, supporting our hypothesis above. Also, these previous findings question the direct role of PGE_2 in

neurovascular coupling in the mice and rat and govern the attention to other likely mediators of the hyperemic response in these preclinical models [20]. The significance of the species-specific role of PGE_2 in regulation of cerebral blood flow is presently not well understood. While functional hyperemia is likely equally important in each species, it is likely that each species utilizes predominantly a different combination of synergistic neurovascular coupling pathways (e.g. involving the release of epoxy-eicosatrienoic acids, purinergic mediators, NO and/or K+ in addition to prostaglandins). Functional studies showing that administration of inhibitors of COX significantly attenuates neurovascular coupling responses [10, 41, 61] is consistent with the concept that PGE_2 plays an important role in dilation of resistance arterioles and mediation of functional hyperemia in the human brain. The differences between species in PGE2-evoked vasomotor responses and neurovascular coupling have to be taken into account by future studies when translating PGE2-related cerebrovascular findings in rats (and mice) to human conditions. 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 [22, 64].

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. These local vascular mechanisms provide the basis of in vivo vascular processes, which then determine neuronal function. For example, attenuated neurovascular coupling was shown to impair cognitive function in rats [63]. Therefore, 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. These indices can be calculated similarly to PRx and PAx if ICP is changed to CaBV. Figure 13 demonstrates that a change in CaBV is reflected in a corresponding change in ICP – which is the rationale of the usability of PRx [18] – but this could also explain the similarities between the invasive and non-invasive indices shown in Figure 14. 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 [80].

The usability of either nPRx or nPAx is limited, because these indices depend on continuous TCD monitoring technology. However, these techniques develop quickly, therefore further studies aiming for the investigation of nPRx and nPAx would be useful, so that clinicians can utilize the abovementioned advantages immediately after the necessary improvements are made.

During plateau waves, with increasing ICP, the error of the estimated FVm and its derivatives are also increasing (Figure 15). This observation most likely occurs because the pulse waveform changes, therefore altering the relationship between FVs-FVd-FVm

[43, 45]. An example of this altering relationship is when heart rate changes, and the generalization that the heart spends twice as much time in diastole then in systole becomes invalid, therefore the error in the formula will increase [47]. However in this study, we focused on the elevation of ICP, while the heart rate was relatively constant. This leads to the conclusion, that other physiological or pathophysiological changes must happen in the brain that alter the storage of blood in the intracranial space, the compliance of the brain, the vasoreactivity of the arteries or some other mechanism that leads to a new waveform and consequently a new link between FVs-FVd-FVm. More research is needed to identify the drive behind this transformation, but until then if a change in mean ICP is expected, then the calculation of FVmreal is recommended.

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 (Figure 18). 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). It should be noted however, that all the activities we studied induced changes in both hemispheres (Figure 19). The applied tasks the subjects needed to perform are most likely not specific for strictly localized centers and functions in the brain. For example, the most promising test - in terms of the most prominent effect – the Trail Making Test requires the activation of multiple brain structures (centers of drawing, calculation, movement, etc.). Therefore, more studies are needed to identify the best circumstances in which NVC can be examined more accurately.

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.

5. Szarka, N., Toth, L., Czigler, A., Kellermayer, Z., Ungvari, Z., Amrein, K., … Toth, P. (2019). Single mild traumatic brain injury induces persistent disruption of the blood-brain barrier, neuroinflammation and cognitive decline in hypertensive rats. *International Journal of Molecular Sciences*, *20*(13), 1–10.

6. Toth, L., Czigler, A., Horvath, P., Kornyei, B., Szarka, N., Schwarcz, A., … Toth, P. (2020). Traumatic brain injury-induced cerebral microbleeds in the elderly. GeroScience, 43(1), 125–136. https://doi.org/10.1007/s11357-020-00280-3

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.

13. Beqiri, E., Ercole, A., Aries, M. J., Cabeleira, M., Czigler, A., Liberti, A., … Smielewski, P. (2021). Optimal Cerebral Perfusion Pressure Assessed with a Multi-Window Weighted Approach Adapted for Prospective Use: A Validation Study. Acta Neurochirurgica. Supplement, 131, 181–185. https://doi.org/10.1007/978-3-030-59436-7_36

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

References

1. Aaslid R, Markwalder TM, Nornes H (1982) Noninvasive transcranial Doppler ultrasound recording of flow velocity in basal cerebral arteries. J Neurosurg 57(6):769–774 doi: 10.3171/jns.1982.57.6.0769

2. Agrell B, Dehlin O (1998) The clock-drawing test. Age Ageing 27(3):399–403 doi: 10.1093/ageing/27.3.399

3. Aries MJH, Czosnyka M, Budohoski KP, Kolias AG, Radolovich DK, Lavinio A, Pickard JD, Smielewski P (2012) Continuous monitoring of cerebrovascular reactivity using pulse waveform of intracranial pressure. Neurocrit Care 17(1):67–76 doi: 10.1007/s12028-012-9687-z

4. Armstead WM (2016) Cerebral Blood Flow Autoregulation and Dysautoregulation. Anesthesiol Clin 34(3):465–477 doi: 10.1016/j.anclin.2016.04.002

5. Attwell D, Buchan AM, Charpak S, Lauritzen M, MacVicar BA, Newman EA (2010) Glial and neuronal control of brain blood flow. Nature 468(7321):232–243 doi: 10.1038/nature09613

6. Bailey-Downs LC, Sosnowska D, Toth P, et al (2012) Growth Hormone and IGF-1 Deficiency Exacerbate High-Fat Diet-Induced Endothelial Impairment in Obese Lewis Dwarf Rats: Implications for Vascular Aging. Journals Gerontol Ser A Biol Sci Med Sci 67A(6):553–564 doi: 10.1093/gerona/glr197

7. Balestreri M, Czosnyka M, Hutchinson P, Steiner LA, Hiler M, Smielewski P, Pickard JD (2006) Impact of intracranial pressure and cerebral perfusion pressure on severe disability and mortality after head injury. Neurocrit Care 4(1):8–13 doi: 10.1385/NCC:4:1:008

8. Bathala L, Mehndiratta M, Sharma V (2013) Transcranial doppler: Technique and common findings (Part 1). Ann Indian Acad Neurol 16(2):174 doi: 10.4103/0972-2327.112460

9. Bowie CR, Harvey PD (2006) Administration and interpretation of the Trail Making Test. Nat Protoc 1(5):2277–2281 doi: 10.1038/nprot.2006.390

10. Bruhn H, Fransson P, Frahm J (2001) Modulation of cerebral blood oxygenation by indomethacin: MRI at rest and functional brain activation. J Magn Reson Imaging 13(3):325–334 doi: 10.1002/jmri.1047

11. Bryant R, Sanni O, Moore E, Bundy M, Johnson A (2014) An uncertainty analysis of mean flow velocity measurements used to quantify emissions from stationary sources. J Air Waste Manage Assoc 64(6):679–689 doi: 10.1080/10962247.2014.881437

12. Carrera E, Kim D-J, Castellani G, Zweifel C, Czosnyka Z, Kasparowicz M, Smielewski P, Pickard JD, Czosnyka M (2010) What shapes pulse amplitude of intracranial pressure? J Neurotrauma 27(2):317–324 doi: 10.1089/neu.2009.0951

13. Castellani G, Zweifel C, Kim DJ, Carrera E, Radolovich DK, Smielewski P, Hutchinson PJ, Pickard JD, Czosnyka M (2009) Plateau waves in head injured patients requiring neurocritical care. Neurocrit Care 11(2):143–150 doi: 10.1007/s12028-009-9235-7

14. Cipolla MJ (2016) The Cerebral Circulation, Second Edition. Colloq Ser Integr Syst Physiol From Mol to Funct 8(1):1–80 doi: 10.4199/C00141ED2V01Y201607ISP066

15. Coleman RA, Smith WL, Narumiya S (1994) International Union of Pharmacology classification of prostanoid receptors: properties, distribution, and structure of the receptors and their subtypes. Pharmacol Rev 46(2):205–29

16. Czosnyka M, Czosnyka Z, Smielewski P (2017) Pressure reactivity index: journey through the past 20 years. Acta Neurochir (Wien) 159(11):2063–2065 doi: 10.1007/s00701-017-3310-1

17. Czosnyka M, Miller C (2014) Monitoring of Cerebral Autoregulation. Neurocrit Care 21(S2):95–102 doi: 10.1007/s12028-014-0046-0

18. Czosnyka M, Smielewski P, Kirkpatrick P, Laing RJ, Menon D, Pickard JD (1997) Continuous Assessment of the Cerebral Vasomotor Reactivity in Head Injury. Neurosurgery 41(1):11–19 doi: 10.1097/00006123-199707000-00005

19. Czosnyka M, Smielewski P, Piechnik S, Schmidt EA, Al-Rawi PG, Kirkpatrick PJ, Pickard JD (1999) Hemodynamic characterization of intracranial pressure plateau waves in head-injured patients. J Neurosurg 91(1):11–19 doi: 10.3171/jns.1999.91.1.0011

20. Dabertrand F, Hannah RM, Pearson JM, Hill-Eubanks DC, Brayden JE, Nelson MT (2013) Prostaglandin E 2 , a Postulated Astrocyte-Derived Neurovascular Coupling Agent, Constricts Rather than Dilates Parenchymal Arterioles. J Cereb Blood Flow Metab 33(4):479–482 doi: 10.1038/jcbfm.2013.9

21. Davis RJ, Murdoch CE, Ali M, Purbrick S, Ravid R, Baxter GS, Tilford N, Sheldrick RLG, Clark KL, Coleman RA (2004) EP 4 prostanoid receptor-mediated vasodilatation of human middle cerebral arteries. Br J Pharmacol 141(4):580–585 doi: 10.1038/sj.bjp.0705645

22. Demuth HU, Dijkhuizen RM, Farr TD, et al (2017) Recent progress in translational research on neurovascular and neurodegenerative disorders. Restor Neurol Neurosci 35(1):87–103 doi: 10.3233/RNN-160690

23. Desai S, April H, Nwaneshiudu C, Ashby B (2000) Comparison of Agonist-Induced Internalization of the Human EP2 and EP4 Prostaglandin Receptors: Role of the Carboxyl Terminus in EP4 Receptor Sequestration. Mol Pharmacol 58(6):1279–1286 doi: 10.1124/mol.58.6.1279

24. Duffin J, Sobczyk O, McKetton L, Crawley A, Poublanc J, Venkatraghavan L, Sam K, Mutch WA, Mikulis D, Fisher JA (2018) Cerebrovascular Resistance: The Basis of Cerebrovascular Reactivity. Front Neurosci

25. Fatima N, Shuaib A, Chughtai T, Ayyad A, Saqqur M (2019) The role of transcranial doppler in traumatic brain injury: A systemic review and meta-analysis. Asian J Neurosurg 14(3):626 doi: 10.4103/ajns.AJNS_42_19

26. Fernandez-Klett F, Offenhauser N, Dirnagl U, Priller J, Lindauer U (2010) Pericytes in capillaries are contractile in vivo, but arterioles mediate functional hyperemia in the mouse brain. Proc Natl Acad Sci 107(51):22290–22295 doi: 10.1073/pnas.1011321108

27. Friedman EA, Ogletree ML, Haddad E V., Boutaud O (2015) Understanding the role of prostaglandin E2 in regulating human platelet activity in health and disease. Thromb Res 136(3):493–503 doi: 10.1016/j.thromres.2015.05.027

28. Gaetani P, Marzatico F, Baena RR y (1992) Ex vivo release of eicosanoids after aneurysmal subarachnoid hemorrhage: a preliminary experience in humans1. Acta Neurol Scand 86(2):184–189 doi: 10.1111/j.1600-0404.1992.tb05063.x

29. Gosling RG, King DH (1974) Arterial assessment by Doppler-shift ultrasound. Proc R Soc Med 67(6 Pt 1):447–9

30. Greve MW, Zink BJ (2009) Pathophysiology of traumatic brain injury. Mt Sinai J Med A J Transl Pers Med 76(2):97–104 doi: 10.1002/msj.20104

31. Hahn T, Heinzel S, Plichta MM, Reif A, Lesch K-P, Fallgatter AJ (2011) Neurovascular Coupling in the Human Visual Cortex Is Modulated by Cyclooxygenase-1 (COX-1) Gene Variant. Cereb Cortex 21(7):1659–1666 doi: 10.1093/cercor/bhq236

32. Hayashi S, Park MK, Kuehl TJ (1985) Relaxant and contractile responses to prostaglandins in premature, newborn and adult baboon cerebral arteries. J. Pharmacol. Exp. Ther.

33. von Hayn K, Werthmann RC, Nikolaev VO, Hommers LG, Lohse MJ, Bünemann M (2010) G q -mediated Ca 2+ signals inhibit adenylyl cyclases 5/6 in vascular smooth muscle cells. Am J Physiol Physiol 298(2):C324–C332 doi: 10.1152/ajpcell.00197.2009

34. Hendrikx D, Smits A, Lavanga M, De Wel O, Thewissen L, Jansen K, Caicedo A, Van Huffel S, Naulaers G (2019) Measurement of Neurovascular Coupling in Neonates. Front Physiol 10(FEB):1–13 doi: 10.3389/fphys.2019.00065

35. Högestätt ED, Uski TK (1987) Actions of some prostaglandins and leukotrienes on rat cerebral and mesenteric arteries. Gen Pharmacol Vasc Syst 18(2):111–117 doi: 10.1016/0306-3623(87)90235-7

36. Hortobágyi L, Kis B, Hrabák A, Horváth B, Huszty G, Schweer H, Benyó B, Sándor P, Busija DW, Benyó Z (2007) Adaptation of the hypothalamic blood flow to chronic nitric oxide deficiency is independent of vasodilator prostanoids. Brain Res 1131:129–137 doi: 10.1016/j.brainres.2006.11.009

37. Hoskins PR, Johnstone FD, Chambers SE, Haddad NG, White G, McDicken WN (1989) Heartrate variation of umbilical artery Doppler waveforms. Ultrasound Med Biol 15(2):101–105 doi: https://doi.org/10.1016/0301-5629(89)90158-0

38. Huneau C, Benali H, Chabriat H (2015) Investigating human neurovascular coupling using functional neuroimaging: A critical review of dynamic models. Front Neurosci 9(DEC):1–12 doi: 10.3389/fnins.2015.00467

39. Kwon J-H, Kim JS, Kang D-W, Bae K-S, Kwon SU (2006) The Thickness and Texture of Temporal Bone in Brain CT Predict Acoustic Window Failure of Transcranial Doppler. J Neuroimaging 16(4):347–352 doi: 10.1111/j.1552-6569.2006.00064.x

40. Lacroix A, Toussay X, Anenberg E, et al (2015) COX-2-Derived Prostaglandin E2 Produced by Pyramidal Neurons Contributes to Neurovascular Coupling in the Rodent Cerebral Cortex. J Neurosci 35(34):11791–11810 doi: 10.1523/JNEUROSCI.0651-15.2015

41. St. Lawrence KS, Ye FQ, Lewis BK, Frank JA, McLaughlin AC (2003) Measuring the effects of indomethacin on changes in cerebral oxidative metabolism and cerebral blood flow during sensorimotor activation. Magn Reson Med 50(1):99–106 doi: 10.1002/mrm.10502

42. Lazaridis C, Robertson CS (2016) The Role of Multimodal Invasive Monitoring in Acute Traumatic Brain Injury. Neurosurg Clin N Am 27(4):509–517 doi: 10.1016/j.nec.2016.05.010

43. Leliefeld PH, Gooskens RHJM, Peters RJM, Tulleken CAF, Kappelle LJ, Han K Sen, Regli L, Hanlo PW (2009) New Transcranial Doppler Index in Infants with Hydrocephalus: Transsystolic Time in Clinical Practice. Ultrasound Med Biol 35(10):1601–1606 doi: 10.1016/j.ultrasmedbio.2009.04.024

44. Maeda Y, Tani E, Miyamoto T (1981) Prostaglandin metabolism in experimental cerebral vasospasm. J Neurosurg 55(5):779–785 doi: 10.3171/jns.1981.55.5.0779

45. Mascia L, Fedorko L, TerBrugge K, Filippini C, Pizzio M, Ranieri VM, Wallace MC (2003) The accuracy of transcranial Doppler to detect vasospasm in patients with aneurysmal subarachnoid hemorrhage. Intensive Care Med 29(7):1088–1094 doi: 10.1007/s00134-003-1780-5

46. Maubach KA, Davis RJ, Clark DE, Fenton G, Lockey PM, Clark KL, Oxford AW, Hagan RM, Routledge C, Coleman RA (2009) BGC20-1531, a novel, potent and selective prostanoid EP4 receptor antagonist: A putative new treatment for migraine headache. Br J Pharmacol 156(2):316–327 doi: 10.1111/j.1476-5381.2009.00027.x

47. Moran D, Epstein Y, Keren G, Laor A, Sherez J, Shapiro Y (1995) Calculation of Mean Arterial Pressure During Exercise as a Function of Heart Rate. Appl Hum Sci 14(6):293–295 doi: 10.2114/ahs.14.293

48. Neuschäfer-Rube F, Oppermann M, Möller U, Böer U, Püschel GP (1999) Agonist-Induced Phosphorylation by G Protein-Coupled Receptor Kinases of the EP4 Receptor Carboxyl-Terminal Domain in an EP3/EP4 Prostaglandin E 2 Receptor Hybrid. Mol Pharmacol 56(2):419–428 doi: 10.1124/mol.56.2.419

49. Newell DW, Aaslid R, Stooss R, Reulen HJ (1992) The relationship of blood flow velocity fluctuations to intracranial pressure B waves. J Neurosurg 76(3):415–421 doi: 10.3171/jns.1992.76.3.0415

50. Newman EA (2013) Functional Hyperemia and Mechanisms of Neurovascular Coupling in the Retinal Vasculature. J Cereb Blood Flow Metab 33(11):1685–1695 doi: 10.1038/jcbfm.2013.145

51. Overgaard J, Tweed WA (1974) Cerebral circulation after head injury. Part 1: Cerebral blood flow and its regulation after closed head injury with emphasis on clinical correlations. J Neurosurg 41(5):531–541 doi: 10.3171/jns.1974.41.5.0531

52. Phillips AA, Chan FH, Zheng MMZ, Krassioukov A V., Ainslie PN (2016) Neurovascular coupling in humans: Physiology, methodological advances and clinical implications. J Cereb Blood Flow Metab 36(4):647–664 doi: 10.1177/0271678X15617954

53. Rosenegger DG, Tran CHT, Wamsteeker Cusulin JI, Gordon GR (2015) Tonic Local Brain Blood Flow Control by Astrocytes Independent of Phasic Neurovascular Coupling. J Neurosci 35(39):13463–13474 doi: 10.1523/JNEUROSCI.1780-15.2015

54. Salinet ASM, Panerai RB, Robinson TG (2014) The Longitudinal Evolution of Cerebral Blood Flow Regulation after Acute Ischaemic Stroke. Cerebrovasc Dis Extra 4(2):186–197 doi: 10.1159/000366017

55. Salinet ASM, Robinson TG, Panerai RB (2012) Reproducibility of cerebral and peripheral haemodynamic responses to active, passive and motor imagery paradigms in older healthy volunteers: A fTCD study. J Neurosci Methods 206(2):143–150 doi: 10.1016/j.jneumeth.2012.02.011

56. Schaller B, Graf R (2005) Different Compartments of Intracranial Pressure and Its Relationship to Cerebral Blood Flow. J Trauma Inj Infect Crit Care 59(6):1521–1531 doi: 10.1097/01.ta.0000197402.20180.6b

57. Sharma D, Prabhakar H (2018) Transcranial Doppler Ultrasonography. In: Prabhakar HBT-NT (ed) Neuromonitoring Tech. Elsevier, pp 113–145

58. Silverman A, Petersen NH (2021) Physiology, Cerebral Autoregulation.

59. Sonig A, Jumah F, Raju B, Patel N V., Gupta G, Nanda A (2020) The Historical Evolution of Intracranial Pressure Monitoring. World Neurosurg 138:491–497 doi: 10.1016/j.wneu.2020.03.028

60. Spiegelberg A, Preuß M, Kurtcuoglu V (2016) B-waves revisited. Interdiscip Neurosurg Adv Tech Case Manag 6:13–17 doi: 10.1016/j.inat.2016.03.004

61. Szabo K, Rosengarten B, Juhasz T, Lako E, Csiba L, Olah L (2014) Effect of non-steroid anti-inflammatory drugs on neurovascular coupling in humans. J Neurol Sci 336(1–2):227–231 doi: 10.1016/j.jns.2013.10.048

62. Szarka N, Pabbidi MR, Amrein K, et al (2018) Traumatic brain injury impairs myogenic constriction of cerebral arteries: Role of mitochondria-derived H 2 O 2 and TRPV4-Dependent Activation of BK ca Channels. J Neurotrauma 35(7):930–939 doi: 10.1089/neu.2017.5056

63. Tarantini S, Hertelendy P, Tucsek Z, et al (2015) Pharmacologically-induced neurovascular uncoupling is associated with cognitive impairment in mice. J Cereb Blood Flow Metab 35(11):1871–1881 doi: 10.1038/jcbfm.2015.162

64. Tarantini S, Tran CHT, Gordon GR, Ungvari Z, Csiszar A (2017) Impaired neurovascular coupling in aging and Alzheimer's disease: Contribution of astrocyte dysfunction and endothelial impairment to cognitive decline. Exp Gerontol 94:52–58 doi: 10.1016/j.exger.2016.11.004

65. Tarjányi O, Berta G, Harci A, Bacsa EB, Stark B, Pap M, Szeberényi J, Sétáló G (2013) The role of Src protein in the process formation of PC12 cells induced by the proteasome inhibitor MG-132. Neurochem Int 63(5):413–422 doi: 10.1016/j.neuint.2013.07.008

66. Toth P, Csiszar A, Tucsek Z, Sosnowska D, Gautam T, Koller A, Schwartzman ML, Sonntag WE, Ungvari Z (2013) Role of 20-HETE, TRPC channels, and BK Ca in dysregulation of pressure-induced Ca 2+ signaling and myogenic constriction of cerebral arteries in aged hypertensive mice. Am J Physiol Circ Physiol 305(12):H1698–H1708 doi: 10.1152/ajpheart.00377.2013

67. Toth P, Rozsa B, Springo Z, Doczi T, Koller A (2011) Isolated Human and Rat Cerebral Arteries Constrict to Increases in Flow: Role of 20-HETE and TP Receptors. J Cereb Blood Flow Metab 31(10):2096–2105 doi: 10.1038/jcbfm.2011.74

68. Toth P, Szarka N, Farkas E, Ezer E, Czeiter E, Amrein K, Ungvari Z, Hartings JA, Buki A, Koller A (2016) Traumatic brain injury-induced autoregulatory dysfunction and spreading depression-related neurovascular uncoupling: Pathomechanisms, perspectives, and therapeutic implications. Am J Physiol - Hear Circ Physiol 311(5):H1118–H1131 doi: 10.1152/ajpheart.00267.2016

69. Toth P, Tarantini S, Csiszar A, Ungvari Z (2017) Functional vascular contributions to cognitive impairment and dementia: Mechanisms and consequences of cerebral autoregulatory dysfunction, endothelial impairment, and neurovascular uncoupling in aging. Am J Physiol - Hear Circ Physiol 312(1):H1–H20 doi: 10.1152/ajpheart.00581.2016

70. Toth P, Tarantini S, Tucsek Z, et al (2014) Resveratrol treatment rescues neurovascular coupling in aged mice: role of improved cerebromicrovascular endothelial function and downregulation of NADPH oxidase. Am J Physiol Circ Physiol 306(3):H299–H308 doi: 10.1152/ajpheart.00744.2013

71. Tymko MM, Donnelly J, Smielewski P, Zeiler FA, Sykora M, Haubrich C, Nasr N, Czosnyka M (2019) Changes in cardiac autonomic activity during intracranial pressure plateau waves in patients with traumatic brain injury. Clin Auton Res 29(1):123–126 doi: 10.1007/s10286-018-0579-y

72. Uchino H, Ushijima K, Ikeda Y (2015) Neuroanesthesia and Cerebrospinal Protection. Neuroanesthesia Cerebrospinal Prot

73. Uryga A, Kasprowicz M, Calviello L, Diehl RR, Kaczmarska K, Czosnyka M (2019) Assessment of cerebral hemodynamic parameters using pulsatile versus non-pulsatile cerebral blood outflow models. J Clin Monit Comput 33(1):85–94 doi: 10.1007/s10877-018-0136-1

74. Uski TK, Andersson KE, Brandt L, Ljunggren B (1984) Characterization of the prostanoid receptors and of the contractile effects of prostaglandin F2 alpha in human pial arteries. Acta Physiol Scand 121(4):369–378

75. Uski TK, Högestätt ED (1992) Effects of various cyclooxygenase and lipoxygenase metabolites on guinea-pig cerebral arteries. Gen Pharmacol Vasc Syst 23(1):109–113 doi: 10.1016/0306-3623(92)90056-P

76. Wijnhoud AD, Franckena M, van der Lugt A, Koudstaal PJ, Dippel en DWJ (2008) Inadequate Acoustical Temporal Bone Window in Patients with a Transient Ischemic Attack or Minor Stroke: Role of Skull Thickness and Bone Density. Ultrasound Med Biol 34(6):923–929 doi: 10.1016/j.ultrasmedbio.2007.11.022

77. Williams CAL, Panerai RB, Robinson TG, Haunton VJ (2017) Transcranial Doppler ultrasonography in the assessment of neurovascular coupling responses to cognitive examination in healthy controls: A feasibility study. J Neurosci Methods 284:57–62 doi: 10.1016/j.jneumeth.2017.04.013

78. Wilson MH (2016) Monro-Kellie 2.0: The dynamic vascular and venous pathophysiological components of intracranial pressure. J Cereb Blood Flow Metab 36(8):1338–1350 doi: 10.1177/0271678X16648711

79. Yokoyama U, Iwatsubo K, Umemura M, Fujita T, Ishikawa Y (2013) The Prostanoid EP4 Receptor and Its Signaling Pathway. Pharmacol Rev 65(3):1010–1052 doi: 10.1124/pr.112.007195

80. Zeiler FA, Czosnyka M, Smielewski P (2018) Optimal cerebral perfusion pressure via transcranial Doppler in TBI: application of robotic technology. Acta Neurochir (Wien) 160(11):2149–2157 doi: 10.1007/s00701-018-3687-5

81. Zeiler FA, Donnelly J, Calviello L, Lee JK, Smielewski P, Brady K, Kim D-J, Czosnyka M (2018) Validation of Pressure Reactivity and Pulse Amplitude Indices against the Lower Limit of Autoregulation, Part I: Experimental Intracranial Hypertension. J Neurotrauma 35(23):2803–2811 doi: 10.1089/neu.2017.5603

82. Ziegler D, Cravens G, Poche G, Gandhi R, Tellez M (2017) Use of Transcranial Doppler in Patients with Severe Traumatic Brain Injuries. J Neurotrauma 34(1):121–127 doi: 10.1089/neu.2015.3967

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Prostaglandin E_2, a postulated mediator of neurovascular coupling, at low concentrations dilates whereas at higher concentrations constricts human cerebral parenchymal arterioles

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ABSTRACT

There is considerable controversy regarding the vasoactive action of prostaglandin E_2 (PGE_2). On the one hand, indirect evidence implicates that astrocytic release of PGE_2 contributes to neurovascular coupling responses mediating functional hyperemia in the brain. On the other hand, overproduction of PGE_2 was also reported to contribute to cerebral vasospasm associated with subarachnoid hemorrhage. The present study was conducted to resolve this controversy by determining the direct vasoactive effects of PGE_2 in resistance-sized human cerebral parenchymal arterioles.

To achieve this goal PGE_2-induced isotonic vasomotor responses were assessed in parenchymal arterioles isolated from fronto-temporo-parietal cortical tissues surgically removed from patients and expression of PGE_2 receptors were examined.

In functionally intact parenchymal arterioles lower concentrations of PGE_2 (from 10^{-8} to 10^{-6} mol/l) caused significant, endothelium-independent vasorelaxation, which was inhibited by the EP4 receptor blocker BGC201531. In contrast, higher concentrations of PGE_2 evoked significant EP1-dependent vasoconstriction, which could not be reversed by the EP4 receptor agonist CAY10598. We also confirmed previous observations that PGE_2 primarily evokes constriction in intracerebral arterioles isolated from *R. norvegicus*. Importantly, vascular mRNA and protein expression of vasodilator EP4 receptors was significantly higher than that of vasoconstrictor EP1 receptors in human cerebral arterioles.

PGE_2 at low concentrations dilates whereas at higher concentrations constricts human cerebral parenchymal arterioles. This bimodal vasomotor response is consistent with both the proposed vasodilator role of PGE_2 during functional hyperemia and its putative role in cerebral vasospasm associated with subarachnoid hemorrhage in human patients.

1. Introduction

Demand for oxygen and nutrients in the brain surges rapidly with increases in regional neuronal activity, which require prompt adjustment of cerebral blood flow (CBF) to maintain normal cellular function [24]. Matching cerebral blood flow to neuronal activity is accomplished through a feed-forward homeostatic process termed neurovascular coupling (or "functional hyperemia"), which is orchestrated by activated neurons and astrocytes releasing vasodilator mediators [20].

Prostaglandin E_2 (PGE_2), a cyclooxygenase (COX)-derived

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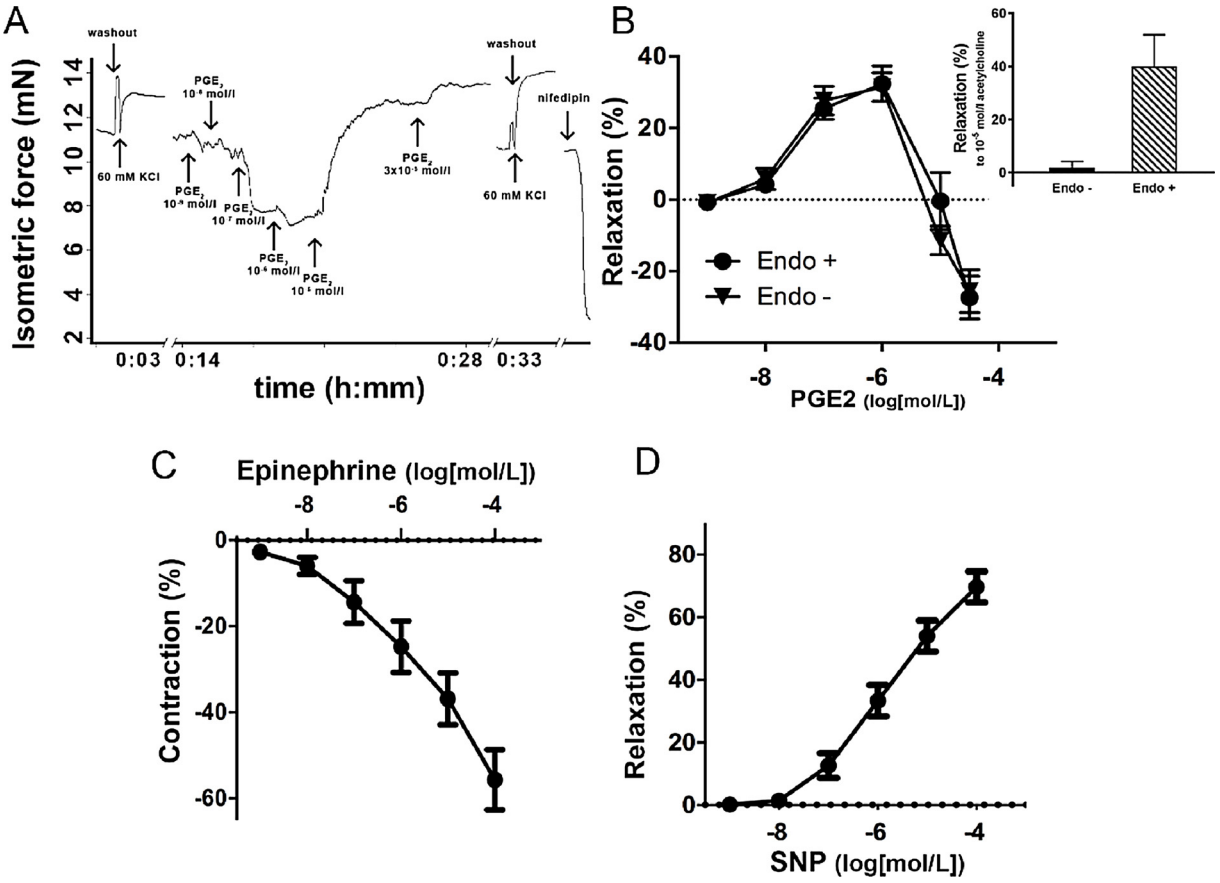

Fig. 1. Prostaglandin E2 (PGE2) elicits biphasic vasomotor responses in human cerebral parenchymal arterioles. A: Original recording showing the effect of PGE2 on the tone of an isolated segment of a human cerebral parenchymal arteriole. Note that at lower concentrations PGE2 elicits substantial vasorelaxation, whereas at higher concentrations a significant vasocontraction becomes manifest. B: Summary data showing PGE2-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. Inlet shows Acetylcholine-induced, endothelium dependent relaxation of the vessels. Responses are expressed as percentage changes in vasomotor tone. Data are mean ± S.E.M. n = 5–7 for each data point. Panels C and D depict dose-dependent vasomotor effects elicited by cumulative administration of the beta adrenergic vasoconstrictor agent epinephrine (Panel C) and the NO donor vasodilator sodium nitroprusside (SNP; Panel D) in human isolated cerebral parenchymal arterioles. Note that pharmacologically elicited vasodilator and vasoconstrictor responses were intact in the vascular preparations used. Data are mean ± S.E.M. n = 12–15 for each data point.

metabolite of arachidonic acid (AA) has been proposed as a key mediator of both astrocyte- and neuron-mediated neurovascular coupling responses [1,12]. Indirect evidence also supports a key role of COX-derived vasodilator metabolites in neurovascular coupling responses in humans [9]. Accordingly, administration of indomethacin, which inhibits COX and reduces PGE_2 synthesis, was shown to significantly attenuate neurovascular coupling responses in human subjects [3,17,18]. In addition, there is also evidence suggesting that COX-derived PGE_2 exerts a tonic vasodilatory influence in the cerebral circulation contributing to the maintenance of normal CBF [16,18].

Despite these advances, there is considerable controversy regarding the vasoactive action of PGE_2. Parenchymal arterioles are the major determinants of cerebrovascular resistance and this segment of the cerebrovascular tree is primarily involved in both maintenance of normal basal cerebral perfusion and moment-to-moment adjustment of CBF to metabolic needs of neurons via neurovascular coupling [8]. Therefore, a critical test to support a potential role of PGE_2 in neurovascular coupling and maintenance of basal CBF is the demonstration that PGE_2 can directly elicit vasodilation in parenchymal resistance-sized arterioles. Contrary to expectation, a recent study reported that PGE_2 constricted rather than dilated parenchymal arterioles isolated from both *M. musculus* and *R. norvegicus* [5]. Furthermore, in preclinical models overproduction of PGE_2 has also been linked to pathological vasospasm associated with subarachnoid hemorrhage [13].

The vasomotor effect of PGE_2 is mediated by G protein coupled prostaglandin (EP) receptors, and in theory PGE_2 evokes 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). Previous studies lead to the hypothesis that expression of EP receptor subtypes in cerebral arterioles and therefore the vasoactive action of PGE_2 are species-dependent. Importantly, expression of EP receptors mediating dilator and constrictor vasomotor mechanisms and the vasoactive effects of PGE_2 in human cerebral arterioles have not yet been documented.

The present study was conducted to resolve the existing controversy regarding the role of PGE_2 in regulation of CBF by determining the direct vasoactive effects of PGE_2 as well as expression of EP receptor subtypes in resistance-sized human cerebral parenchymal arterioles.

2. Materials and methods

2.1. Isolation of human parenchymal arterioles and vasomotor studies

Following written informed consent, we obtained cortical (gray matter) samples from patients undergoing neurosurgical removal of cerebral tumors (n = 19, female:7, male:12, age: 53.7 ± 2.9 years), which otherwise would have been discarded. The patients did not have

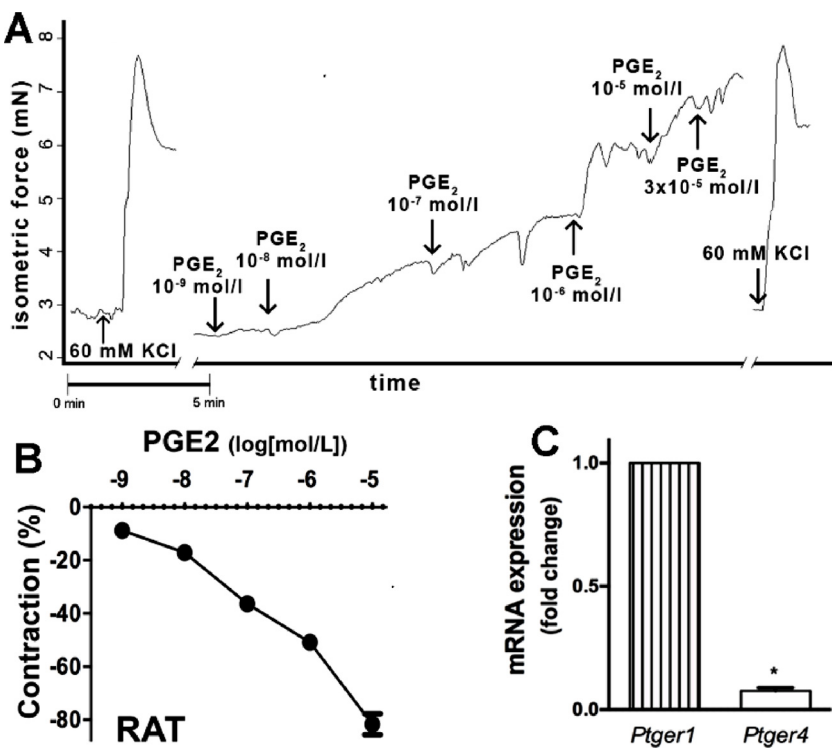

Fig. 2. Prostaglandin E2 (PGE_2) elicits contractions in cerebral arteries of rats. Original recording (A) and summary data (B) of PGE_2-induced vasomotor responses of isolated basilar arteries of Whistar-Kyoto rats (n = 6). Responses are expressed as the percentage change in vasoconstriction (increase in vasomotor tone) from baseline. Note that PGE_2 induces dose-dependent constriction of rat cerebral arteries, supporting previous findings of Dabertrand et al. [5]. Data are mean ± S.E.M. B: mRNA expression of the PGE_2 EP1 (Ptger1) and EP4 (Ptger4) receptors in isolated rat basilar arteries. Data are mean ± S.E.M. (n = 5 for each group) *P < 0.05.

known comorbidities. Preoperatively, contrast enhanced magnetization prepared rapid acquisition gradient echo (MP-RAGE) MRI sequence were carried out in order to visualize contrast enhancing areas with pathologically increased blood brain barrier permeability. Cerebral samples containing intraparenchymal arterioles and/or the arterioles themselves were removed carefully from non-enhancing normal cortical areas that had to be removed because of technical reasons in order to approach deep-seated tumors [23]. After removal, cerebral tissue from the fronto-temporo-parietal lobes was placed in 0 °C – 4 °C physiologic salt solution (PSS, 110.0 NaCl, 5.0 KCl, 2.5 $CaCl_2$, 1.0 $MgSO_4$, 1.0 KH_2PO_4, 5.5 glucose, and 24.0 mmol/L $NaHCO_3$ equilibrated with a gas mixture of 95% air and 5% CO_2, balanced with nitrogen at pH ~7.3). 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 a subset of experiments (n = 5) endothelium was removed by inserting an air bubble through the lumen of the vessels. The lack of a functioning endothelium was verified by the absence of vasomotor response of the arteriolar rings to acetylcholine. Optimal passive tension (as determined from the vascular length-tension relationship) was applied for one hour (equilibration period).

In additional set of experiments Wistar Kyoto rats (WKY, 300–350 g, male, n = 10, purchased from Charles River Laboratories) were anesthetized (isoflurane), decapitated, the brains were removed and segments of basilar arteries were isolated and mounted in a wire myograph as described [19].

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. 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 the EU Directive 2010/63/EU, and are reported in compliance with the ARRIVE guidelines.

2.2. Pharmacological studies

Vasomotor responses of preconstricted (phenylephrine 10^{-5} mol/L) human cerebral parenchymal arteriolar preparations were assessed in response to cumulative addition of increasing concentrations of PGE_2 (from 10^{-9} to 3×10^{-5} mol/L) either in the presence or absence of a functional endothelium. Endothelial function was tested by investigating relaxation to acetylcholine (ACh; from 10^{-9} to 10^{-6} mol/L). Endothelium-independent relaxation was studied by administration of the NO donor sodium nitroprusside (from 10^{-9} to 10^{-6} 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 in the observed vasomotor effect of PGE_2: the specific EP4 blocker BGC 20–1531 (10^{-6} mol/L for 5 min, Cayman Chemicals, Ann Arbor, MI), the EP2 receptor blocker PF-04418948 (10^{-6} mol/L for 5 min, Cayman Chemicals, Ann Arbor, MI) or the EP1 receptor blocker SC-51322 (10^{-6} mol/L for 5 min, Cayman Chemicals, Ann Arbor, MI). 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 3×10^{-5} mol/L PGE_2. Arteriolar rings were incubated in the presence of only one inhibitor in each experiment, and dose-responses were repeated, in a self-controlled manner. At the end of the experiments still intact functional reactivity was assessed again, then passive tension was obtained in Ca2+ free conditions, as published earlier [2].

In additional control experiments vasomotor responses were assessed in rat basilar artery preparations in response to cumulative addition of increasing concentrations of PGE_2 (from 10^{-9} to 3×10^{-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.

Quantitative real-time RT-PCR

A quantitative real-time RT-PCR method was used to analyze mRNA expression of EP1 and 4 receptors in endothelium-denuded human

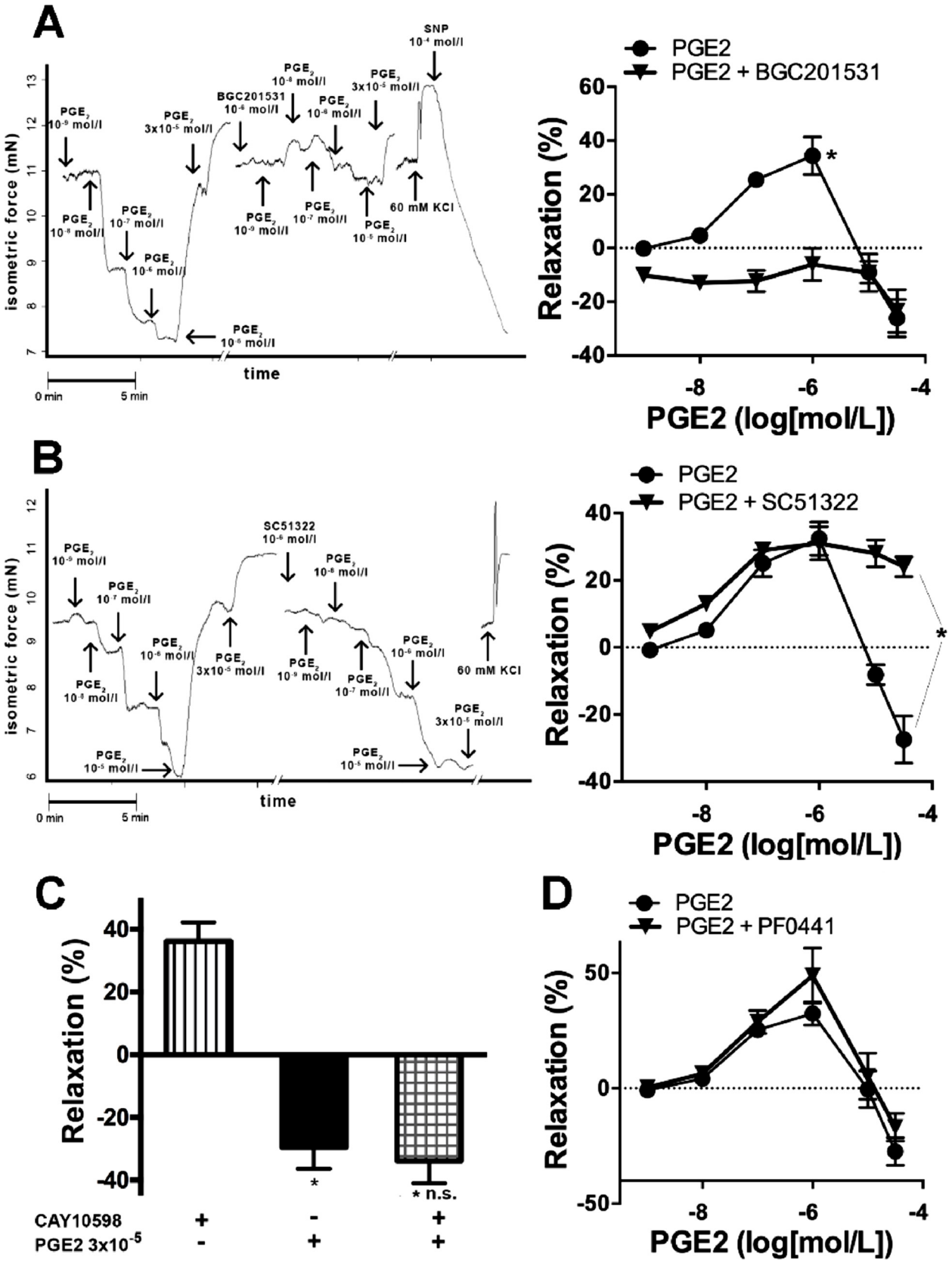

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Fig. 3. Role of PGE2 receptor subtypes in mediation of PGE2-induced vasomotor responses in human cerebral parenchymal arterioles. A: PGE2-induced changes in vasomotor tone of human isolated cerebral parenchymal arterioles in the absence and presence of BGC201531 (10-6 mol/L), a specific inhibitor of the PGE2 receptor subtype 4 (EP4). Original recording of an experiment and summary data are shown. Data are mean ± S.E.M. (n = 6) *P < 0.05 vs. vehicle control. B: Changes in vasomotor tone of human isolated cerebral parenchymal arterioles induced by PGE2 in the presence of the PGE2 receptor subtype 1 (EP1) blocker SC51322 (10-6 mol/L). Note that after SC51322 administration high concentrations of PGE2 (10-5 and 3 × 10-5 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. PGE2-induced responses without SC51322. C: The EP4 agonist CAY10598 evokes relaxation of isolated human cerebral parenchymal arterioles, which is blocked by the EP1-dependent constrictor response induced by high concentration of PGE2. Data are mean ± S.E.M. (n = 6) *P < 0.05 vs. CAY10598-induced response, n.s.(non-significant) vs. PGE2-induced contraction. D: PGE2-induced changes in vasomotor tone of human isolated cerebral parenchymal arterioles in the absence and presence of PF0441 (10-6 mol/L), a specific inhibitor of the PGE2 receptor subtype 2 (EP2). Data are mean ± S.E.M. (n = 6).

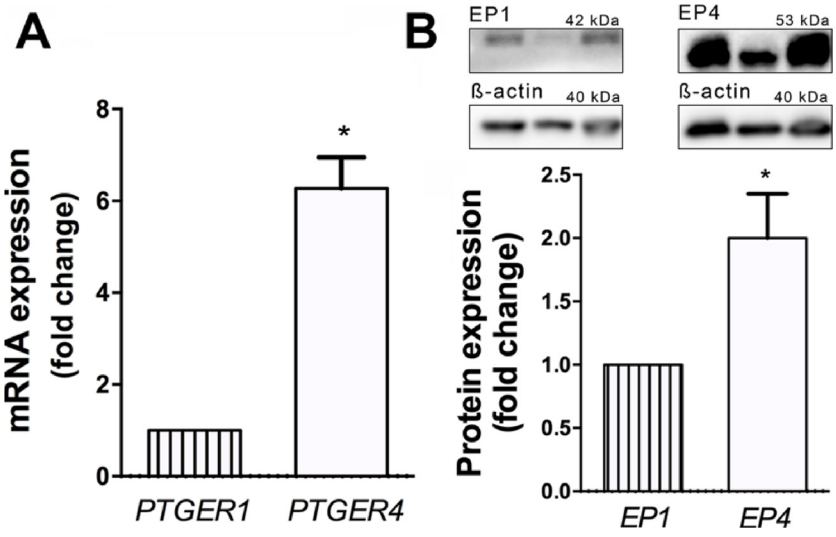

Fig. 4. Expression of EP1 and EP4 receptors in human cerebral parenchymal arterioles. mRNA (A) and protein (B) expression of EP1 and EP4 receptors (gene PTGER1 and PTGER4, respectively) in isolated human cerebral parenchymal arterioles. Data are mean ± S.E.M. (n = 9 for QRT-PCR, and n = 6 for western blotting for each group) *P < 0.05.

cerebral parenchymal arterioles (*PTGER1* and *PTGER4*, n = 5) and rat arterial segments (*Ptger1* and *Ptger4*, n = 5) according to previously described protocols [22]. In brief, RNA was isolated with the Pure Link™ RNA Mini Kit (Life Sciences, Carlsbad CA, USA) according to the protocol suggested by the manufacturer and was reverse transcribed using the High Capacity cDNA kit (Applied Biosystems, Foster City CA, USA). The total amount of RNA was determined by NanoDrop (Thermo Scientific, Waltham MA, USA). qRT-PCR was performed using SensiFast SYBR Green reagent (BioLine, Luckenwalde, Germany). The following primer sequences were used: *PTGER1*, forward: GAGGGAGGGAGGAA GCGG, reverse CCGCAAGGGCTCATGTCAG, *Ptger1*, forward: ACAGGG GATGCTCCAAACAC, reverse: GGTGGGACGTGAATCCAGAA; *PTGER4*, forward: CTGCCGCTACAGACCCAG, reverse: CAAGGAGGCGGACGAA TTGA, *Ptger4,* forward: CTCATCTGCTCCATTCCGCT, reverse: CCGGG TTTCTGCTGATGTCT. Amplification efficiencies were determined using a dilution series of a standard vascular sample. Quantification was performed using the efficiency-corrected ΔΔCq method as previously described [22].

2.3. Western blotting

Endothelium-denuded human cerebral parenchymal arterioles were crushed with Potter's homogenizer in ice-cold lysis buffer (50 mM Tris-base, pH 7.4, 150 mM NaCl, 10% glycerol, 1 mM EGTA, 1 mM Na-orthovanadate, 5 µM ZnCl2, 100 mM NaF, 10 µg/ml aprotinin, 1 µg/ml leupeptin, 1 mM PMSF, 1% Triton X-100) and were centrifuged at 35.000 × g at 4 °C for 20 min. The protein concentration of the supernatants was measured by spectrophotometry on 595 nm (Lowry's method, Detergent Compatible Protein Assay Kit, Bio-Rad, Hercules, CA, USA). Samples containing 50 µg of protein were mixed with 4 × Laemmli buffer (25 ml 1 M Tris–HCl, pH 6.8, 40 ml glycerol, 8 g SDS, 10 ml 100 mM EDTA, 10 ml 100 mM EGTA and 1 ml 1% bromophenol blue brought up to 100 ml with distilled water) and boiled for 5 min. The proteins were separated in 10% SDS-polyacrylamide gel based on their size. The gels were electro-blotted onto PVDF membranes (Hybond-P, GE Healthcare, UK). The membranes were blocked in 5% nonfat dry milk dissolved in TBS-Tween (10 mM Tris-base, 150 mM NaCl, 0.2% Tween-20, pH 8.0). PGER1 (Cayman Chemical Company, 1:200) or PGER4 (Novus Biologicals, 1:200) primary antibody was added diluted in 3% BSA-TBS-Tween solution and incubated overnight. Not bound antibodies were washed five times by TBS-Tween and membranes were incubated with secondary anti-rabbit antibodies conjugated with horseradish-peroxidase (HRP) (Pierce, Thermo Fischer Scientific, Rockford, IL, USA) diluted 1:50.000 in 5% nonfat dry milk blocking solution. After five washes in TBS-Tween the chemiluminescent signal was detected (Immobilon Western, Millipore Corporation, Billerica, MA, USA). The blots were stripped and reprobed with ß-actin (Cell Signaling Technology, 1:2.000) antibody to check the equal loading of proteins [21].

2.4. 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.

3. Results

3.1. PGE_2 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 Fig. 1A. Summary data are shown in Fig. 1B. The presence of a functional endothelium was verified by demonstration of vasorelaxation to acetylcholine (10^{-5} mol/L) (Fig. 1B inlet). Denudation of the endothelium did not affect the vasomotor response to PGE_2 (Fig. 1B). The smooth muscle layer of the studied human cerebral parenchymal arterioles was functionally intact as demonstrated by the dose-dependent constriction evoked by epinephrine (Fig. 1C) and the dose-dependent dilation of the vessels to sodium-nitroprusside (Fig. 1D). We found that rat basilar arteries (BA) exhibit dose-dependent contraction in response to administration of PGE_2 (Fig. 2A-B) and that in rat BAs mRNA expression of the constrictor EP1 (*Ptger1*) receptors was significantly higher than expression of the dilator EP4 (*Ptger4*) (Fig. 2C), extending previous findings of Dabertrand et al. [5].

3.2. Role of EP1 and EP4 receptors in PGE2-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 (Fig. 3A).

Constrictions of arterioles evoked by high concentrations of PGE_2 (10^{-5} and 3×10^{-5} mol/L) were inhibited by SC51322 (10^{-6} mol/L), a specific antagonist of the PGE_2 receptor subtype 1 (EP1; Fig. 3B). Upon inhibition of EP1-mediated constrictor response (SC51322) to high concentrations of PGE_2, a PGE2-induced vasodilatory effect became manifest (Fig. 3B), suggesting that activation of the constrictor EP1 receptor by high PGE_2 concentrations masks the effects of activation of vasodilatory EP4 receptors. This concept is further supported by the findings that 10^{-6} mol/L CAY10598, a specific EP4 receptor agonist, induced relaxations in human cerebral parenchymal arterioles (Fig. 3C) to a similar extent as did 10^{-6} mol/L PGE_2 (Fig. 1B). However, it did not have any effects when the vessels were pre-constricted with the 3×10^{-5} mol/L PGE_2 (Fig. 3C). Administration of the EP2 blocker PF-04418948 was without effect on the PGE_2-induced vasomotor responses of human cerebral parenchymal arterioles (Fig. 3D).

3.3. 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 (Fig. 4A-B).

4. Discussion

This is the first study to demonstrate that PGE_2 at physiological concentrations elicits significant dilation in human cerebral parenchymal arterioles (Fig. 1). This finding provides direct support for the hypothesis that PGE_2 regulates the tone of cerebral resistance arterioles thereby controlling cerebral blood perfusion in human subjects. It is likely that PGE_2-mediated dilation of cerebral parenchymal arterioles contributes both to maintenance of basal cerebral blood perfusion [16,18] and initiation of neurovascular coupling responses during increased neuronal activity in humans [3,9,17,18]. PGE_2-induced dilation of resistance arteries likely also plays a critical role in the pathogenesis of migraine [14]. Our results extend the findings of earlier studies demonstrating that PGE_2 at low concentrations also dilates segments of the human middle cerebral artery [6]. Vasodilation in response to low concentrations of PGE_2 has also been observed in human pial arteries [26] and middle cerebral arteries of non-human primates [10]. These results suggest that most likely segmental differences cannot be observed in PGE_2-evoked responses of human cerebral vessels along the cerebrovascular tree.

Importantly, we found that PGE_2 at high concentrations elicits significant constriction of human cerebral parenchymal arterioles (Fig. 1). Similar findings were previously observed in segments of the human middle cerebral artery, as well [6]. Our pharmacological studies demonstrate that PGE_2 elicits vasodilation by activating EP4 receptors, which is masked at the higher PGE_2 concentration range by a strong EP1 receptor mediated vasoconstrictor effect (Fig. 3). These results provide an explanation for the virtually conflicting roles of PGE_2 in regulation of cerebral blood flow. 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. Our findings taken together with the results of earlier studies [29,15] also raise the possibility that there is a cross-talk between EP1 and EP4 receptors in the vascular smooth muscle cells. Accordingly, the findings that arterioles preconstricted by PGE_2 cannot be dilated by the EP4 agonist CAY10598 (Fig. 3) may be consistent with inhibition of EP4 receptor-mediated cAMP production by overactivation of EP1 receptors. EP4 receptors have been desensitized promptly in response to PGE_2 at the concentration of 10^{-6} mol/L [7], which may provide an additional mechanism to explain our results. These possibilities should be tested in future studies.

Interestingly, the vasomotor action of PGE_2 exhibits strong species-dependency. While our study demonstrates that human cerebral parenchymal arterioles predominantly express vasodilator EP4 receptors (Fig. 4), in the vasculature of murine species the vasoconstrictor EP1 receptors are overexpressed. These findings extend previous observations that contractile EP1 receptors do not have a widespread expression in tissues of higher species and are more expressed in murine species [4]. Species-dependent differences in the expression profile of PGE_2 receptors in the cerebral vessels likely underlie the species-specific vasomotor and hemodynamic effects of PGE_2. Accordingly, consistent with the higher expression level of EP1 receptors (Fig. 2), arteries and arterioles from *R. norvegicus, M. musculus*, and *C. porcellus* predominantly exhibit constriction in response to administration of PGE_2 [5,11,28]. It has to be noted here, that we examined responses of cerebral arteries of rats, which are not directly comparable to the human vessels studied here, and they are not involved in neurovascular hyperemia, either. However, as mentioned, in a very well-controlled study by Dabertrand et al. arterioles of the rat and mice responded with contraction to PGE_2 as well, supporting our hypothesis above. Also, these previous findings question the direct role of PGE_2 in neurovascular coupling in the mice and rat and govern the attention to other likely mediators of the hyperemic response in these preclinical models [5]. The significance of the species-specific role of PGE_2 in regulation of cerebral blood flow is presently not well understood. While functional hyperemia is likely equally important in each species, it is likely that each species utilizes predominantly a different combination of synergistic neurovascular coupling pathways (e.g. involving the release of epoxy-eicosatrienoic acids, purinergic mediators, NO and/or K^+ in addition to prostaglandins). Functional studies showing that administration of inhibitors of COX significantly attenuates neurovascular coupling responses [3,17,18] is consistent with the concept that PGE_2 plays an important role in dilation of resistance arterioles and mediation of functional hyperemia in the human brain.

4.1. Limitations and perspectives of the study

PGE_2, especially through endothelial PGE_2 receptors is a pivotal player in different inflammatory processes in the central nervous

system, therefore PGE_2-dependent vasomotor responses can be profoundly changed in various pathological conditions associated with enhanced inflammatory reaction, for example brain tumors. This should be studied in the future. Mediation of neurovascular coupling responses varies between brain regions, therefore it is logical to hypothesize that differences in PGE_2 receptor expression and PGE_2-dependent vasomotor function might be different between brain areas and structures (i.e grey matter vs. white matter), as well. This hypothesis could be tested in the future. A serious limitation of ours studies is that the vessels studied here are the largest in the subpial arteriolar system, larger than the precapillary arterioles. Detailed examination of segmental vasomotor responses to PGE_2 and expression of EP receptors along the vascular tree should be carried out in the future. We studied expression of EP receptors in arterioles, in which we showed lack of a functional endothelial layer pharmacologically. Distribution and morphological relation between the studied receptors, and their changes in pathological conditions should be established in whole vessels in the future. The cross talk between EP1 and EP4 receptors in the PGE_2-evoked vasomotor function of cerebral arterioles should be further studied in an appropriately chosen preclinical model with similar vasomotor responses to PGE_2 that can be observed in human cerebral arterioles.

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Declaration of Competing Interest

None.

Contribution

The experiments were conducted in the Department of Neurosurgery and Institute for Translational Medicine, University of Pecs Medical School, AC, LT, NSZ, ZK and PT designed the experiments; AC, LT, KSZ, NSZ, AH, MV, ZK performed and analyzed the experiments; AC, AK, ZU, ASZ, AB and PT interpreted the data, AC and PT wrote the manuscript and ASZ, ZU, AK, AB revised the manuscript. All authors approved the final version of the manuscript.

References

[1] D. Attwell, A.M. Buchan, S. Charpak, M. Lauritzen, B.A. Macvicar, E.A. Newman, Glial and neuronal control of brain blood flow, Nature 468 (2010) 232–243.
[2] L.C. Bailey-Downs, D. Sosnowska, P. Toth, M. Mitschelen, T. Gautam, J.C. Henthorn, P. Ballabh, A. Koller, J.A. Farley, W.E. Sonntag, A. Csiszar, Z. Ungvari, Growth hormone and IGF-1 deficiency exacerbate high-fat diet-induced endothelial impairment in obese lewis dwarf rats: implications for vascular aging, J. Gerontol. A Biol. Sci. Med. Sci. 67 (A) (2012) 553–564.
[3] H. Bruhn, P. Fransson, J. Frahm, Modulation of cerebral blood oxygenation by indomethacin: MRI at rest and functional brain activation, J. Magn. Reson. Imaging 13 (2001) 325–334.
[4] R.A. Coleman, W.L. Smith, S. Narumiya, International Union of Pharmacology classification of prostanoid receptors: properties, distribution, and structure of the receptors and their subtypes, Pharmacol. Rev. 46 (1994) 205–229.
[5] F. Dabertrand, R.M. Hannah, J.M. Pearson, D.C. Hill-Eubanks, J.E. Brayden, M.T. Nelson, Prostaglandin E2, a postulated astrocyte-derived neurovascular coupling agent, constricts rather than dilates parenchymal arterioles, J. Cereb. Blood Flow Metab. 33 (2013) 479–482.
[6] R.J. Davis, C.E. Murdoch, M. Ali, S. Purbrick, R. Ravid, G.S. Baxter, N. Tilford, R.L. Sheldrick, K.L. Clark, R.A. Coleman, EP4 prostanoid receptor-mediated vasodilatation of human middle cerebral arteries, Br. J. Pharmacol. 141 (2004) 580–585.
[7] S. Desai, H. April, C. Nwaneshiudu, B. Ashby, Comparison of agonist-induced internalization of the human EP2 and EP4 prostaglandin receptors: role of the carboxyl terminus in EP4 receptor sequestration, Mol. Pharmacol. 58 (2000) 1279–1286.
[8] F. Fernandez-Klett, N. Offenhauser, U. Dirnagl, J. Priller, U. Lindauer, Pericytes in capillaries are contractile in vivo, but arterioles mediate functional hyperemia in the mouse brain, Proc Natl Acad Sci U S A 107 (2010) 22290–22295.
[9] T. Hahn, S. Heinzel, M.M. Plichta, A. Reif, K.P. Lesch, A.J. Fallgatter, Neurovascular coupling in the human visual cortex is modulated by cyclooxygenase-1 (COX-1) gene variant, Cereb. Cortex 21 (2011) 1659–1666.
[10] S. Hayashi, M.K. Park, T.J. Kuehl, Relaxant and contractile responses to prostaglandins in premature, newborn and adult baboon cerebral arteries, J. Pharmacol. Exp. Ther. 233 (1985) 628–635.
[11] E.D. Hogestatt, T.K. Uski, Actions of some prostaglandins and leukotrienes on rat cerebral and mesenteric arteries, Gen. Pharmacol. 18 (1987) 111–117.
[12] A. Lacroix, X. Toussay, E. Anenberg, C. Lecrux, N. Ferreiros, A. Karagiannis, F. Plaisier, P. Chausson, F. Jarlier, S.A. Burgess, E.M. Hillman, I. Tegeder, T.H. Murphy, E. Hamel, B. Cauli, COX-2-derived prostaglandin E2 produced by pyramidal neurons contributes to neurovascular coupling in the rodent cerebral cortex, J. Neurosci. 35 (2015) 11791–11810.
[13] Y. Maeda, E. Tani, T. Miyamoto, Prostaglandin metabolism in experimental cerebral vasospasm, J. Neurosurg. 55 (1981) 779–785.
[14] K.A. Maubach, R.J. Davis, D.E. Clark, G. Fenton, P.M. Lockey, K.L. Clark, A.W. Oxford, R.M. Hagan, C. Routledge, R.A. Coleman, BGC20-1531, a novel, potent and selective prostanoid EP receptor antagonist: a putative new treatment for migraine headache, Br. J. Pharmacol. 156 (2009) 316–327.
[15] F. Neuschafer-Rube, M. Oppermann, U. Moller, U. Boer, G.P. Puschel, Agonist-induced phosphorylation by G protein-coupled receptor kinases of the EP4 receptor carboxyl-terminal domain in an EP3/EP4 prostaglandin E(2) receptor hybrid, Mol. Pharmacol. 56 (1999) 419–428.
[16] D.G. Rosenegger, C.H. Tran, J.I. Wamsteeker Cusulin, G.R. Gordon, Tonic local brain blood flow control by astrocytes independent of phasic neurovascular coupling, J. Neurosci. 35 (2015) 13463–13474.
[17] K.S. St Lawrence, F.Q. Ye, B.K. Lewis, J.A. Frank, A.C. McLaughlin, Measuring the effects of indomethacin on changes in cerebral oxidative metabolism and cerebral blood flow during sensorimotor activation, Magnetic Reson. Med. 50 (2003) 99–106.
[18] K. Szabo, B. Rosengarten, T. Juhasz, E. Lako, L. Csiba, L. Olah, Effect of non-steroid anti-inflammatory drugs on neurovascular coupling in humans, J. Neurol. Sci. 336 (2014) 227–231.
[19] N. Szarka, M.R. Pabbidi, K. Amrein, E. Czeiter, G. Berta, K. Pohoczky, Z. Helyes, Z. Ungvari, A. Koller, A. Buki, P. Toth, Traumatic brain injury impairs myogenic constriction of cerebral arteries: role of mitochondria-derived H2O2 and TRPV4-Dependent activation of BKca channels, J. Neurotrauma 35 (2018) 930–939.
[20] S. Tarantini, C.H.T. Tran, G.R. Gordon, Z. Ungvari, A. Csiszar, Impaired neurovascular coupling in aging and Alzheimer's disease: contribution of astrocyte dysfunction and endothelial impairment to cognitive decline, Exp. Gerontol. 94 (2017) 52–58.
[21] O. Tarjanyi, G. Berta, A. Harci, E.B. Bacsa, B. Stark, M. Pap, J. Szeberenyi, G. Setalo Jr, The role of Src protein in the process formation of PC12 cells induced by the proteasome inhibitor MG-132, Neurochem. Int. 63 (2013) 413–422.
[22] P. Toth, A. Csiszar, Z. Tucsek, D. Sosnowska, T. Gautam, A. Koller, M.L. Schwartzman, W.E. Sonntag, Z. Ungvari, Role of 20-HETE, TRPC channels, and BKCa in dysregulation of pressure-induced Ca2+ signaling and myogenic constriction of cerebral arteries in aged hypertensive mice, Am. J. Physiol. Heart Circ. Physiol. 305 (2013) H1698–1708.
[23] P. Toth, B. Rozsa, Z. Springo, T. Doczi, A. Koller, Isolated human and rat cerebral arteries constrict to increases in flow: role of 20-HETE and TP receptors, J. Cereb. Blood Flow Metab. 31 (2011) 2096–2105.
[24] P. Toth, S. Tarantini, A. Csiszar, Z. Ungvari, Functional vascular contributions to cognitive impairment and dementia: mechanisms and consequences of cerebral autoregulatory dysfunction, endothelial impairment, and neurovascular uncoupling in aging, Am. J. Physiol. Heart Circ. Physiol. 312 (2017) H1–H20.
[26] T.K. Uski, K.E. Andersson, L. Brandt, B. Ljunggren, Characterization of the prostanoid receptors and of the contractile effects of prostaglandin F2 alpha in human pial arteries, Acta Physiol. Scand. 121 (1984) 369–378.
[28] T.K. Uski, E.D. Hogestatt, Effects of various cyclooxygenase and lipoxygenase metabolites on guinea-pig cerebral arteries, Gen. Pharmacol. 23 (1992) 109–113.
[29] K. von Hayn, R.C. Werthmann, V.O. Nikolaev, L.G. Hommers, M.J. Lohse, M. Bunemann, Gq-mediated Ca2+ signals inhibit adenylyl cyclases 5/6 in vascular smooth muscle cells, Am. J. Physiol., Cell Physiol. 298 (2010) C324–332.

Errors and Consequences of Inaccurate Estimation of Mean Blood Flow Velocity in Cerebral Arteries

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Introduction

The mean flow velocity (FVm) in the cerebral arteries is a key parameter in transcranial Doppler (TCD) ultrasonography. Many TCD devices calculate FVm using the systolic flow velocity (FVs) and diastolic flow velocity (FVd) with the traditional formula $FVm_{calc} = (FVs + 2 \times FVd)/3$ [1]. This assumes a specific linear relationship between all components. FVm can be calculated more accurately as the time integral of the current flow velocities divided by the integration period (FVm_{real}) [2, 3].

Materials and Methods

We retrospectively reviewed flow velocity (FV) and intracranial pressure (ICP) signals collected with TCD ultrasonography and intraparenchymal ICP monitors. The data were gathered from 14 patients with a traumatic brain injury (TBI) over the duration of their admission to the Neurosciences Critical Care Unit (NCCU) at Addenbrooke's Hospital (Cambridge, UK). We performed all analyses using ICM+ (Cambridge Enterprise, Cambridge, UK; http://www.neurosurg.cam.ac.uk/icmplus) and R software. All recordings contained plateau waves (transient intracranial hypertension), which resulted in a significant difference in ICP (mean ± standard deviation (SD) 25.3 ± 5.9 mmHg) between the baseline and plateau phases. Differences in the FVm_{calc} and FVm_{real} indices and the derivative pulsatility index (PI) were also assessed.

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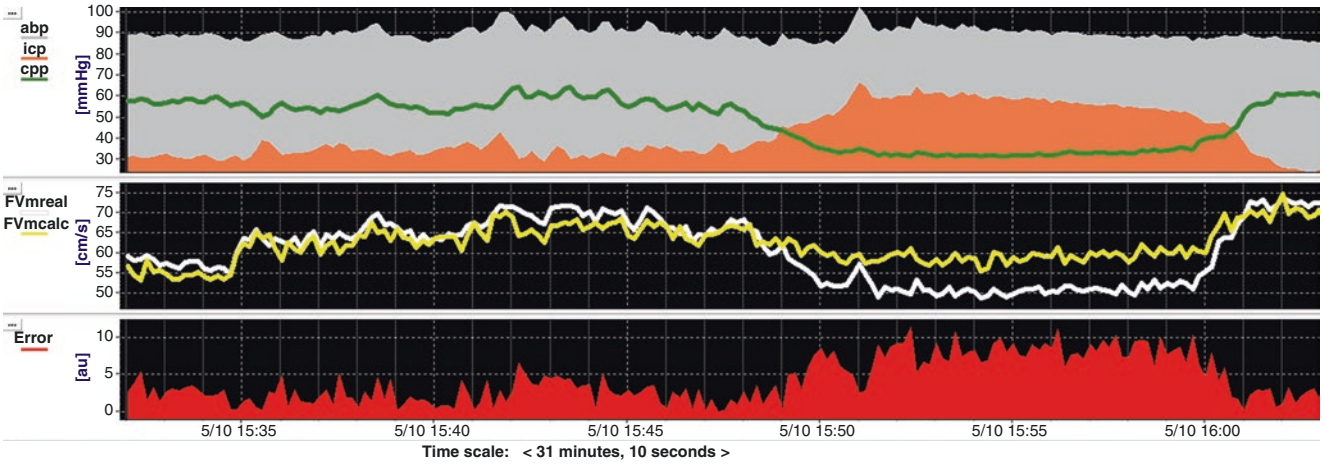

Fig. 1 Transcranial Doppler (TCD) arterial blood pressure (ABP) and intracranial pressure (ICP) monitoring using ICM+ software. *Top:* A drastic 15-min-long rise in ICP (plateau wave) is visible together with constant ABP and a consequent drop in cerebral perfusion pressure (CPP). *Middle:* The mean flow velocity in the middle cerebral arteries is calculated using both the traditional formula (FVmcalc) and the more accurate formula calculating it as the time integral of the current flow velocities divided by the integration period (FVmreal). *Bottom:* The difference between the two parameters is visible. An increase in the error coincides with the plateau wave

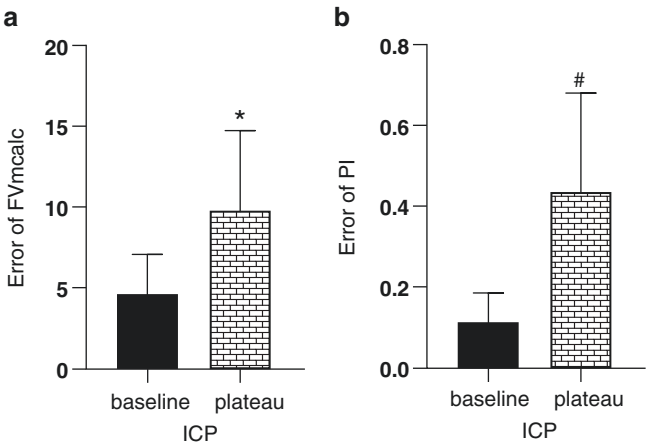

Fig. 2 Errors in both the traditional formula for calculation of the (**a**) mean flow velocity (FVmcalc) and its derivative (**b**) pulsatility index (PI) significantly increase from the baseline to the plateau. The data are expressed as mean ± standard deviation ($n = 12$ in each group). $^{*}P < 0.05$ vs. baseline, $^{\#}P < 0.005$ vs. baseline (paired Student's t test)

Results

During measurements, the averages of FVm_{calc} and FVm_{real} differed significantly ($P < 0.05$), and the mean ± SD of the absolute value of this difference was 6.1 ± 2.7 cm/s (Fig. 1). During plateau waves, when ICP rose, the error significantly increased from the baseline to the plateau (from 4.6 ± 2.4 to 9.8 ± 4.9 cm/s, $P < 0.05$) (Fig. 2a). Similarly, the error in PI calculated with FVm_{calc} also increased during plateau waves (from 0.11 ± 0.07 to 0.44 ± 0.24, $P < 0.005$) (Fig. 2b). In many cases, there appeared to be a strong correlation between ICP and the errors (Fig. 3).

Discussion

During plateau waves, with increasing ICP, errors in the estimated FVm and its derivatives also increase. This observation most likely occurs because the pulse waveform changes, thereby altering the FVs–FVd–FVm relationship [4, 5]. An example of such as changing relationship is when the heart rate changes and the generalization that the heart spends twice as much time in diastole as it does in systole becomes invalid; therefore, the error in the formula will increase [6]. However, in this study, we focused on elevation of ICP while the heart rate was relatively constant. This leads to the conclusion that other physiological or pathophysiological changes must happen in the brain that alter the storage of blood in the intracranial space, the compliance of the brain, the vasoreactivity of the arteries, or some other mechanism that leads to a new waveform and consequently a new link between FVs, FVd, and FVm. More research is needed to identify the driver behind this transformation; until then, if a change in the mean ICP is expected, then use of the FVm_{real} formula is recommended.

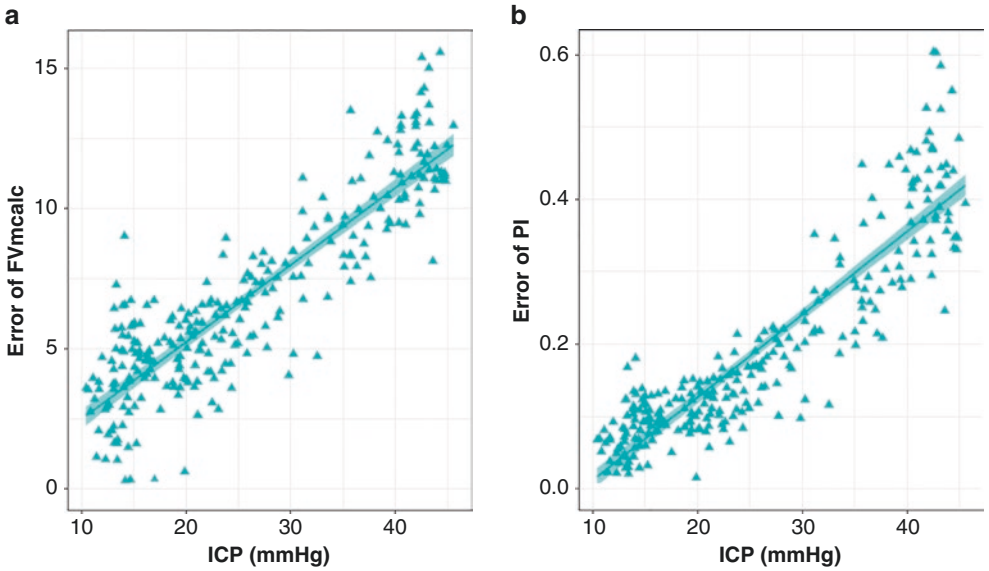

Fig. 3 Representative plots of errors against intracranial pressure (ICP). (**a**) There is a strong correlation between the error in the estimated mean flow velocity (FVm) and ICP during monitoring of a patient exhibiting a plateau wave. (**b**) With increasing ICP, there is an increasing error in the pulsatility index calculated using the traditional formula for FVm calculation (FVmcalc) instead of the more accurate formula calculating it as the time integral of the current flow velocities divided by the integration period (FVmreal)

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References

1. Bathala L, Mehndiratta MM, Sharma VK (2013) Transcranial Doppler: technique and common findings (part 1). Ann Indian Acad Neurol 16(2):174–179
2. Bryant R, Sanni O, Moore E, Bundy M, Johnson A (2014) An uncertainty analysis of mean flow velocity measurements used to quantify emissions from stationary sources. J Air Waste Manage Assoc 64(6):679–689
3. Zeiler FA, Donnelly J, Calviello L, Lee JK, Smielewski P, Brady K, Kim D-J, Czosnyka M (2018) Validation of pressure reactivity and pulse amplitude indices against the lower limit of autoregulation, part I: experimental intracranial hypertension. J Neurotrauma 35(23):2803–2811
4. Carrera E, Kim D-J, Castellani G, Zweifel C, Czosnyka Z, Kasparowicz M, Smielewski P, Pickard JD, Czosnyka M (2010) What shapes pulse amplitude of intracranial pressure? J Neurotrauma 27(2):317–324
5. Leliefeld PH, Gooskens RHJM, Peters RJM, Tulleken CAF, Kappelle LJ, Sen Han K, Regli L, Hanlo PW (2009) New transcranial Doppler index in infants with hydrocephalus: transsystolic time in clinical practice. Ultrasound Med Biol 35(10):1601–1606
6. Moran D, Epstein Y, Keren G, Laor A, Sherez J, Shapiro Y (1995) Calculation of mean arterial pressure during exercise as a function of heart rate. Appl Hum Sci 14(6):293–295

Usability of Noninvasive Counterparts of Traditional Autoregulation Indices in Traumatic Brain Injury

Andras Czigler, Leanne A. Calviello, Frederick A. Zeiler, Peter Toth, Peter Smielewski, and Marek Czosnyka

Introduction

The pressure reactivity index (PRx) is one of the commonly used parameters to describe autoregulation in traumatic brain injury (TBI). It quantifies the changes in vascular smooth muscle tone that occur as a result of variations in transmural pressure. It is calculated as the moving linear correlation coefficient between mean arterial blood pressure (ABP) and intracranial pressure (ICP) [1].

In certain cases (i.e. after craniectomy), PRx might falsely indicate good autoregulation due to the increased compliance of the intracranial space and the altered status of ICP. In these situations, the correlation of ABP and the pulse amplitude of ICP (AMP) could be a better descriptor of cerebrovascular reactivity. This index is called the pressure-amplitude index (PAx) [2].

Since ICP is needed to calculate both PRx and PAx, both indices are considered to be invasively quantified markers of cerebral autoregulation. PRx and PAx are applicable for this purpose because a change in cerebral arterial blood volume (CaBV) results in a corresponding change in ICP. Therefore, PRx and PAx are indirect descriptors of the relationship between the mean arterial blood pressure (ABP) and the instantaneous blood volume inside the cranial space. However, with the help of the transcranial Doppler ultrasound (TCD) technique, it is possible to approximate CaBV noninvasively solely from cerebral blood flow velocities. The disadvantage of this method is that because of the unknown cross-sectional area of the insonated blood vessels, the direct calculation of blood volume is not possible. In this brief study, we aimed to investigate whether noninvasive estimation of relative CaBV with different models could be used to describe the cerebrovascular reactivity of TBI patients.

Materials and Methods

TBI patients received both continuous invasive (ABP and ICP) monitoring and daily noninvasive monitoring with TCD 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+ soft-

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ware (Cambridge Enterprise, Cambridge, United Kingdom; http://www.neurosurg.cam.ac.uk/icmplus). The database was fully anonymized, no data on patient identifiers were available, and therefore no additional ethical approval or formal patient or proxy consent was needed. PRx and PAx were calculated as the correlation coefficients between 30 samples of 10-s averages of ABP and ICP (or the amplitude of ICP in the case of PAx).

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 the assumption made about the nature of outflow, two different methods can be used to model changes in CaBV [3]:

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

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, CBF_a—cerebral blood flow, ABP—arterial blood pressure, and CVR—cerebrovascular resistance (CVR = meanABP/meanCBFa).

In a 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, so it can be calculated by averaging the inflow over several cardiac cycles (in this study, we used 5-min 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.

With TCD monitoring, the cross-sectional area of the middle cerebral artery is unknown, and the CBF cannot be precisely calculated. In these equations, CBF can be replaced with CBFV, so the relative changes in CaBV can be estimated (Fig. 1).

The noninvasive counterparts of PRx (nPRx) and PAx (nPAx) 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.

Results

Discussion

With TCD it is possible to derive noninvasive indices – nPRx and nPAx – of cerebrovascular reactivity by estimating the relative changes in CaBV. These indices can be calculated similarly to PRx and PAx if ICP is changed to CaBV. Figure 2 demonstrates that a change in CaBV is reflected in a corresponding change in ICP – which is the rationale of the usability of PRx [1]—but this could also explain the similarities between the invasive and noninvasive indices shown in Fig. 3. This analogous behavior opens up possibilities for the use of these noninvasive cerebrovascular reactivity indices: they may become clinically useful in the subacute phase of neuro-intensive care because they can provide further infor-

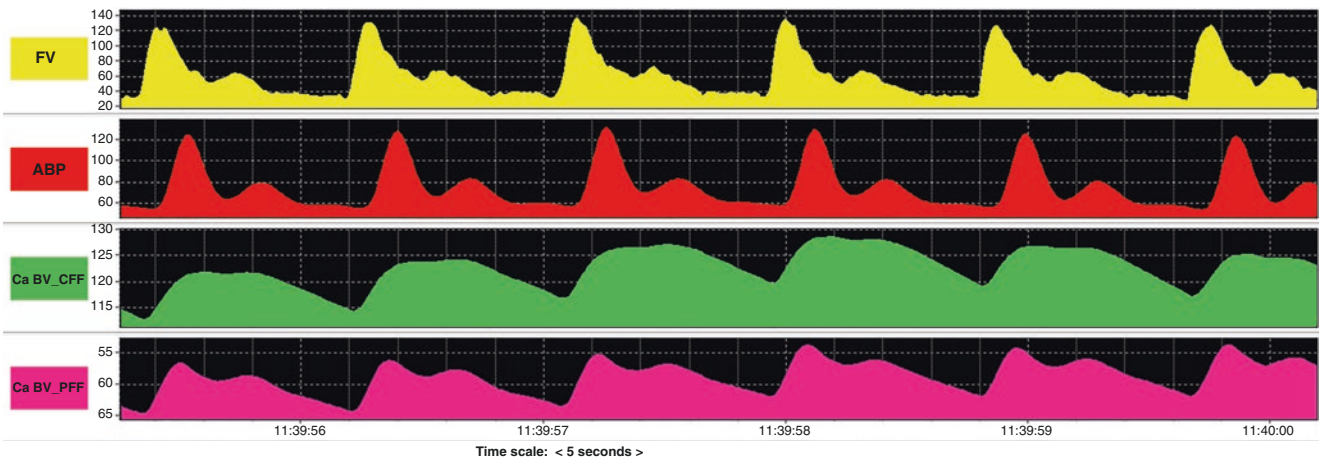

Fig. 1 Waveforms of flow velocity, arterial blood pressure and changes in CaBV, calculated both with the continuous flow forward and the pulsatile flow forward models. The pulsatile nature of CaBV with both methods is visible, but more prominent peaks appear with the PFF model

mation about autoregulation even after the removal of invasive ICP monitors. With other noninvasive 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, noninvasive optimal cerebral perfusion pressure (nCPPopt) instead of traditionally invasive optimal cerebral perfusion pressure (CPPopt) could be determined and used to guide treatment.

The usability of either nPRx or nPAx is limited because these indices depend on continuous TCD monitoring technology. However, these techniques develop quickly, so further studies aimed at the investigation of nPRx and nPAx would be useful, which would enable clinicians to utilize the previously mentioned advantages immediately after the necessary improvements are made.

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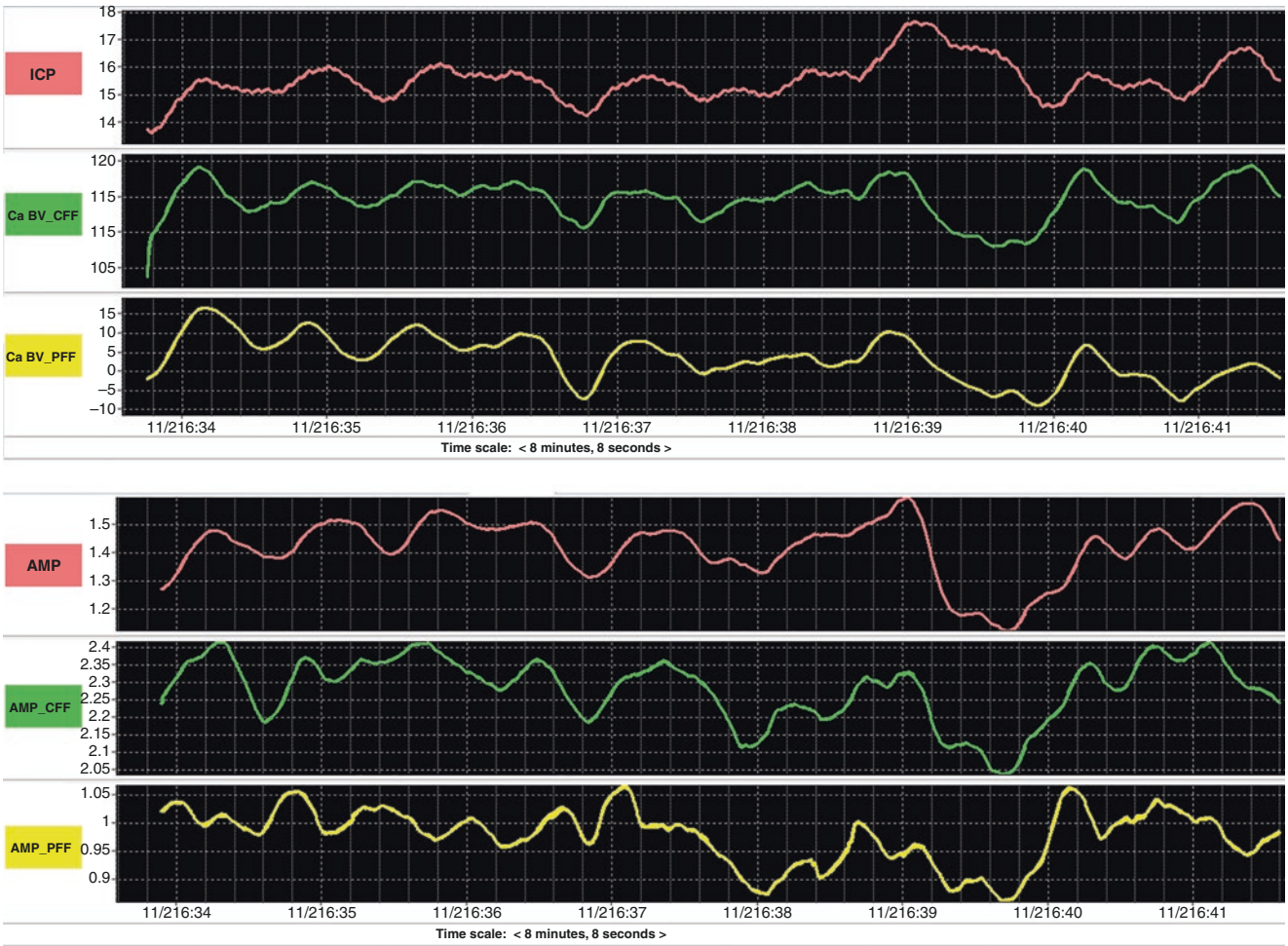

Fig. 2 A representative example of good coherence between slow waves in ICP and CaBV (upper panel) and between slow waves of AMP and pulse amplitude of CaBV (lower panel). Both the CFF and PFF models were used for the calculations

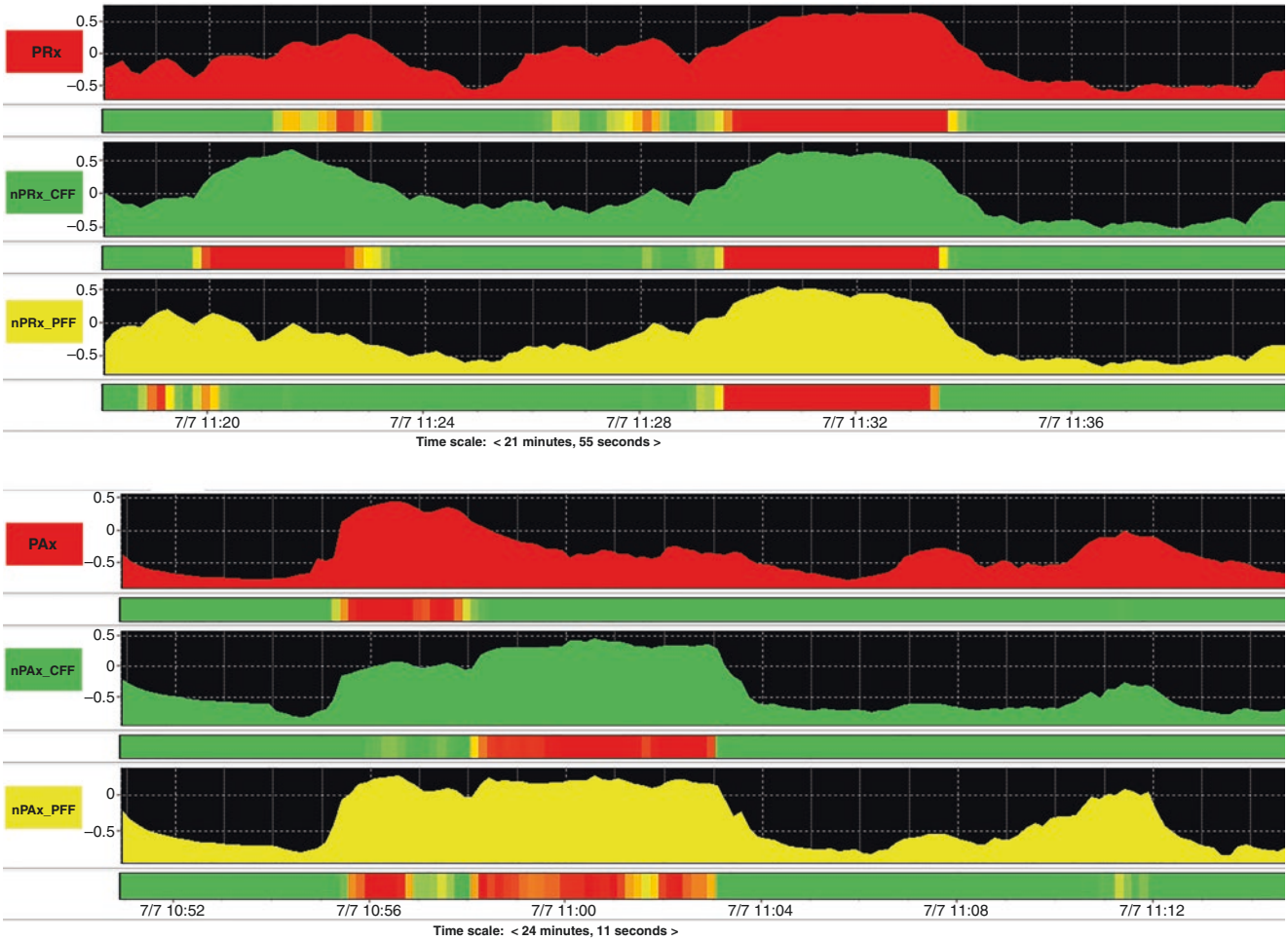

Fig. 3 Signals of PRx, nPRx (upper panel), PAx and nPAx (lower panel). Both the CFF and PFF models were used to calculate noninvasive autoregulation indices

References

1. Czosnyka M, Smielewski P, Kirkpatrick P, Laing RJ, Menon D, Pickard JD (1997) Continuous assessment of the cerebral vasomotor reactivity in head injury. Neurosurgery 41(1):11–19
2. Aries MJH, Czosnyka M, Budohoski KP, Kolias AG, Radolovich DK, Lavinio A, Pickard JD, Smielewski P (2012) Continuous monitoring of cerebrovascular reactivity using pulse waveform of intracranial pressure. Neurocrit Care 17(1):67–76
3. Uryga A, Kasprowicz M, Calviello L, Diehl RR, Kaczmarska K, Czosnyka M (2019) Assessment of cerebral hemodynamic parameters using pulsatile versus non-pulsatile cerebral blood outflow models. J Clin Monit Comput 33(1):85–94