SHORT THESIS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY (PHD)

Effects of vasoconstrictive factors on neurovascular coupling

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UNIVERSITY OF DEBRECEN
DOCTORAL SCHOOL OF NEUROSCIENCES

DEBRECEN, 2015

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The Examination takes place at Department of Neurology, Faculty of Medicine, University of Debrecen
15th of June 2015 at 11 a.m.

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The PhD Defense takes place at the Lecture Hall of Bldg. "A", Department of Internal Medicine, Faculty of Medicine, University of Debrecen
15th of June 2015 13:00 p.m.

1. INTRODUCTION

1.1. Cerebral circulation and mechanisms influencing the diameter of cerebral resistance vessels and blood flow

From one hand the cerebral blood flow is relatively constant within a wide range of perfusion pressure (autoregulation), from the other hand the regional cerebral blood flow quickly adapts to the continuously changing neuronal or metabolic activity (neurovascular coupling). Both autoregulation and neurovascular coupling (NVC) are based on vasodilatory and vasoconstrictive abilities of the resistance vessels (mainly arterioles), which mechanisms are controlled by complex regulation. Tone of cerebral resistance vessels and the regional cerebral blood flow are regulated by three basic mechanisms: 1) myogenic regulation which is based on the intrinsic properties of vascular smooth muscle cells , 2) metabolic/humoral regulation indicating the effect of metabolic products of neurons and astrocytes adjacent to the cerebral vessels, and 3) the neurogenic control of perivascular nerves. One of the characteristic features of the precapillary vascular segments with smooth muscle cells is that increase in intraluminal pressure leads to vasoconstriction, while decrease of intraluminal pressure causes vasodilation. This is the so-called Bayliss-effect, which has a particular importance in the mechanism of cerebral autoregulation.

Cerebral autoregulation refers to a mechanism that ensures relatively constant cerebral blood flow at a wide range of cerebral perfusion pressure (difference of mean arterial pressure and intracranial pressure). Besides myogenic control, humoral and metabolic regulations play also an important role in the regulation of cerebral circulation. It is well known that both increase of the partial pressure of blood CO2 (pCO2) and increase of pH lead to vasodilation, while decrease of pCO2 and increase of pH result in vasoconstriction. Additionally, a number of other metabolic and humoral factors influence the diameter of cerebral resistance vessels, such as the adenosine, lactate, NO, endothelin-1, prostacyclin, etc. Besides the myogenic and humoral regulations, neurogenic regulation of the cerebral resistance vessels was proved as well. Regarding the origin, the perivascular fibers might be sympathetic, parasympathetic, or trigeminal fibers, and the innervations is based on mainly the cholinergic and adrenergic systems.

1.2. The neurovascular coupling

Maintenance of cerebral homeostasis requires dynamic regulation of oxygen and glucose supply so as to match nutrient delivery to metabolic demand of active neurons. This is achieved by a tight spatial and temporal coupling between neuronal activity and blood flow, called neurovascular coupling (NVC). Although neurovascular research has made significant strides toward understanding how the brain neurovascular unit accomplishes rapid and spatial increases in blood flow following neuronal activation, the exact mechanisms remained unclear. In order to match regional cerebral blood flow with neuronal activity, the cerebral microcirculation was shown to be equipped with control mechanisms, regulated by different mediator systems and cell types such as neurons, endothelial cells as well as astrocytes. The regulation involves both vasodilating and vasoconstricting components, in which the pH may have a strong effect in both directions. An increase in pH (alkalosis) leads to vasoconstriction and thus decreased flow, whereas an acidosis induces vasodilation and increased cerebral blood flow (CBF). One of the best examples for the neurovascular coupling is the visual stimulation induced flow increase in the visual cortex. As a result of visual stimulation (e.g. reading) the neurons of the visual cortex are activated, leading to release of various neurotransmitters and vasoactive mediators. Due to increase of neuronal oxygen, glucose and ATP utilization, CO2 and adenosine are produced resulting in local vasodilation of arterioles. Vasodilation of resistance vessels in the activated visual cortex leads to increased regional cerebral blood flow, and consequently increased flow velocity in the supplying artery, namely in the posterior cerebral artery.

During neuronal activation action potentials develop, which process contributes to an increase of the extracellular K+ concentration. Increase of extracellular K+ concentration by 8-10 mmol/L is known to cause vasodilation of arterioles both in vitro and in vivo. Additionally, during long-term activation, decrease of ATP-level results in opening the ATP-sensitive K+ channels, which also leads to vasodilatation.

Increased energy demand during neuronal activation may cause relative glucose and oxygen deficiency. While the decreased oxygen level increases the cerebral blood flow only slightly and transiently, the adenosine, generated during the catabolism of ATP, is a potent vasodilator which plays a role in the development of neurovascular coupling. Lactate, generated during the brain activation, may also be an important mediator, causing vasodilation by increase of the H+ concentration.

Vasoactive neurotransmitters, released by either the local interneurons or the neurons of remote nuclei during neuronal activation, may also contribute to vasodilation, which process

is required for development of neurovascular coupling. It is well known that activation of glutamate receptors leads to vasodilatation and consequently increase in blood flow. Exogenous glutamate or N-methyl-D-aspartate (NMDA) in the neocortex and hippocampus may cause vasodilation of arterioles and pial cerebral microvessels, which process can be inhibited by NMDA receptor antagonists. The activated glutamate receptor results in increase of intracellular Ca2+ concentration and thus triggers the activation of Ca2+-dependent enzymes, including nNOS and phospolipase, which in turn leads to the production of vasodilator substances. One of these Ca2+-dependent enzyme is the neuronal nitric oxide synthase (nNOS), which is responsible for the synthesis of nitrogen monoxide (NO). It was demonstrated that the neuronal activity induced NO production was shown to contribute significantly to the neuronal activation induced flow increase that could be inhibited by nNOS inhibitors. Besides nNOS, phospholipase A2 is also activated by the glutamate-induced intracellular Ca2+ increase and leads to arachidonic acid production. Arachidonic acid is metabolized in the cyclo-oxygenase (COX) pathway, resulting in synthesis of vasoactive prostaglandins. The processes, mentioned in this section, contribute to vasodilation of resistance vessels, which serves for transfer of glucose and oxygen to the activated tissue and removal of the metabolic end-products.

1.3. Investigation of the neurovascular coupling in humans

The neurovascular coupling is a complex and precisely regulated process, which can be examined by various methods, including fMRI (functional magnetic resonance imaging), PET (positron emission tomography), SPECT (single photon emission computer tomography), near-infrared spectroscopy (NIRS) and transcranial Doppler (TCD). Functional MRI, as well as SPECT and PET are expensive and time consuming imaging tests, in addition, administration of radioisotopes in PET and SPECT studies is also required. Therefore, other, widely available, simple, non-invasive tests, like TCD and NIRS, with excellent temporal resolution are used in the every-day practice to investigate the neurovascular coupling. In our studies, functional TCD was applied to determine the visual stimulation induced vascular response. The TCD method is a cheap, non-invasive simple test with excellent temporal resolution and without major side-effect, which main advantages are that it can be repeated at any time and does not require administration of external agent. The main disadvantage of the method is that its spatial resolution is rather low, and in older females the insonation is not always possible due to thick cranial bones.

According to the functional anatomy of the brain, neurovascular coupling can be easily and reliably examined in humans by measurement of the visual stimulation-evoked flow velocity changes in the posterior cerebral arteries (PCAs). Visual stimulation activates the neurons in the visual cortex, which activation induces vasodilation of arterioles and consequently leads to increase in regional CBF. These regional changes result in increased flow and flow velocity in the supplying artery, in this case in the posterior cerebral artery.

1.4. Role of TCD in examination of neurovascular coupling

Development of transcranial Doppler in 1982 allowed to examine the flow velocity in the cerebral arteries, as well as to assess the vascular resistance by measurement of the pulsatility index (PI). Due to a fast development in the technology, the transcranial Doppler has been widely used in the neurology practice today. While 2 decades ago the TCD was only used to monitor the vasospasm after subarachnoid hemorrhage and to diagnose arterial obstructions intracranially, now it plays a role in the investigation of neurovascular coupling, cerebrovascular reactivity, cerebral autoregulation, intracranial pressure, as well as in the detection of microemboli. Moreover, the method can be applied in the diagnosis of brain death, but it is also suitable to investigate the cerebral parenchyma and cerebral perfusion. Monitoring of changes of flow velocities induced by different stimuli is called functional TCD. Functional TCD (fTCD) can be used to measure the flow velocity changes after acetazolamide or CO2 administration, after apnoe or hyperventilation, but also during visual or cognitive stimulation. According to previous observations, flow velocity changes in one artery are directly proportional to the flow changes in the territory of that artery, supposing that the diameter of the artery is constant. Based on data of previous studies, vasoactive stimuli usually influence the diameter of the small, resistance vessels and not or much less the size of the large intracranial arteries. Therefore, flow changes in the territory of an artery can be concluded from the flow velocity changes in the artery.

2. AIMS

Our previous studies proved that significant vasodilation, caused by either hypercapnia or acetazolamide administration, did not affect the neurovascular coupling since the visually evoked relative flow velocity in the posterior cerebral arteries increased by the same measure under the effect of vasodilating agents as under the control condition. These data suggest that despite a significant cerebral vasodilation, caused by hypercapnia or acetazolamide, cortical

neuronal activation evokes further local vasodilation, maintaining the adaptation of CBF according to neuronal activity.

In the present study, our aim was to examine whether a hypocapnia (alkalosis-) or non-steroidal anti-inflammatory drug (NSAID) induced cerebral vasoconstriction inhibits the neuronal activation evoked vascular response. In order to determine the effect of the hypocapnia and NSAID induced cerebral vasoconstriction on the neurovascular coupling, visually evoked flow velocity changes were measured in the posterior cerebral arteries (PCAs) of young healthy subjects during control phases as well as hyperventilation (HV), and NSAID phases. To obtain a measure of neuronal activity, visual-evoked-potentials (VEP) were also examined.

The two main aims of our study were the followings:

I. Do widely used, non-selective, non-steroid anti-inflammatory drugs (NSAIDs), indomethacin and naproxen, given orally in usual therapeutic doses, inhibit neurovascular coupling in healthy humans?

II. Does vasoconstriction, induced by hypocapnia (hyperventilation), affect the neurovascular coupling in human subjects?

3. SUBJECTS AND METHODS

3.1. Subjects

The study was approved by the local ethics committee, and each volunteer gave written, informed consent. Cerebrovascular risk factors such as smoking habit, arterial hypertension, obesity, diabetes mellitus, and hyperlipidemia, as well as history of migraine, coronary or peripheral artery diseases were screened, and subjects with risk factors were excluded. The included subjects did not take any medicine regularly. The study protocol included a complete neurological examination and routine clinical laboratory tests (serum ions, blood urea nitrogen, creatinine, fasting glucose, hepatic enzymes, creatinin-kinase, hemostasis screening test, serum lipids, and inflammatory markers). Blood was drawn after an overnight fast between 8 and 10 a.m.

The functional TCD tests were performed in the morning in a quiet room at about 23°C while the subjects were sitting comfortably. All volunteers had abstained from caffeine overnight before the study.

3.2. Functional Transcranial Doppler (fTCD)

Two 2-MHz probes were mounted by an individually fitted headband. In all cases, the P2 segment of the PCA was insonated on both sides at a depth of 58 mm. Peak systolic and end diastolic blood flow velocities were recorded with a Multidop T2 Doppler device (DWL, Überlingen, Germany). The reason for the separated evaluation of peak systolic and end diastolic blood flow velocitieswas that these indices are known to show different time courses in dynamic blood flow regulation. Being less influenced by Doppler artefacts, the peak systolic velocity index was used. The other reason for using peak systolic flow velocities in the present study was that this flow parameter reflected the dynamic flow regulation most appropriately. As a stimulation paradigm, we used a new magazine with emotionally neutral text that the volunteers could read freely. This "reading" test had been previously validated against a checkerboard stimulation paradigm. The stimulation protocol consisted of 10 cycles with a resting phase of 20 s and a stimulation phase of 40 s for each cycle.

During the resting periods, volunteers were instructed to close their eyes; during the stimulation phases, they read silently. Changes between phases were signaled acoustically with a tone. After a short time delay at the beginning of the visual stimulation, cerebral blood flow velocity increased rapidly, overshot and then stabilized at a constant but lower level. After closure of the eyes, the flow velocity decreased and reached the baseline value. Within each person, flow velocity data of ten cycles were averaged. To ensure independence from the insonation angle and to allow comparisons between volunteers, absolute data were transformed into relative changes of cerebral blood flow velocity in relation to baseline. Baseline was calculated from the blood flow velocity averaged for a time span of 5 s at the end of the resting phase, before the beginning of the stimulation phase. Relative flow velocities were expressed in % of baseline value. To analyze the maximum increase of relative flow velocity changes, the highest of the values obtained during the stimulation phase was taken from each subject.

Pulsatility index (PI) was calculated during the whole experiment using the following equation: PI = (peak systolic flow velocity – end diastolic flow velocity) / mean flow velocity. PI at the resting phase was used for statistical analysis, and calculated from the PI averaged for a time span of 5 seconds at the end of the resting phase, before the beginning of the stimulation phase.

3.3. Visual evoked potential test

Besides visually evoked flow velocities, visual-evoked-potentials (VEP) were also investigated over the occipital cortex and amplitudes and latencies of P100 waves were calculated to estimate the neuronal activity of the visual cortex (Neuro unit Pack, Nihon Kohden Corp.).

3.4. Experimental protocols

I. In the study, examined the effect of NSAIDs on cerebral circulation, at first the visually evoked flow response and visual-evoked-potentials (VEP) were examined without medication (control phase, n=15). After the control examination, 3 × 25 mg indomethacin was given orally to the same volunteers (n = 15) for 2 days, after which the visually evoked flow test and examination of visual-evoked-potentials were repeated. Four weeks later, the same protocol was performed in the same subjects (n=15) after oral administration of 2 × 550 mg naproxen for 2 consecutive days.

II. In the hyperventilation study, at first the visually evoked flow response and visual-evoked-potentials (VEP) were examined during normocapnia (control phase). After this phase, the same volunteers were taught to hyperventilate at a rate of 35–40 breaths per minute and the visually evoked flow test was repeated under hyperventilation (HV) without changing the positions of the TCD probes. As a side effect of hyperventilation, the subjects felt dizziness, therefore they were asked to maintain hyperventilation for 5 minutes. Since the measurement was started 1 minute after the beginning of hyperventilation, only 4 cycles were performed and averaged during the HV phase. Relative flow velocities in the control and hyperventilation phases were calculated in each individual and expressed as percent of the control and hyperventilation baseline flow velocity values, respectively.

To control the effectiveness of hyperventilation, end-tidal CO2 was recorded (Capnograd, Novametrix Medical Systems Corp., Wallingford, USA) during the whole examination period, and capillary blood gasses were checked before and at the end of the hyperventilation phase. Blood pressure was measured noninvasively in the sitting position before and at the end of both normo- and hyperventilation. Besides visually evoked flow velocities, visual-evoked-potentials (VEP) were also investigated over the occipital cortex under normo- and hyperventilation.

3.5. Statistical analysis

Data were expressed as means± standard deviation (SD). Tests for normal distribution were performed, and the homogeneity of the variances was checked by an F test. Results of bilateral measurements were averaged within one subject. Repeated measures analysis of variance (ANOVA) was applied to compare absolute and relative changes of visually evoked cerebral blood flow velocities between the various phases. Paired t-test was used to compare the pulse rate, breathing frequency, end tidal CO2, resting flow velocity and resting pulsatility index (PI), maximum relative flow velocity change, amplitude and latency of the visual-evoked-potential (P100 wave), and blood gasses before and during hyperventilation. A difference of $p<0.05$ was considered statistically significant.

4. RESULTS

4.1. Non-steroidal anti-inflammatory drugs (indomethacin and naproxen) effect's on neurovascular coupling in humans

Fifteen volunteers (8 men and 7 women) were included with a mean age of 25 ± 4 years. Blood pressure, pulse rate, and parameters of the visual-evoked-potentials (amplitude and latency of P100 wave) were similar in the control, indomethacin and naproxen phases.

The resting peak systolic flow velocity was significantly lower after administration of either naproxen or indomethacin than in the control phase. Both NSAIDs caused increase of increase of pulsatility index compared to the control period ($p<0.01$).

Visual stimulation resulted in an increase of peak systolic flow velocity in the control phase, as well as after administration of indomethacin or naproxen. For absolute values of flow velocity, repeated measures analysis of variance (ANOVA) detected significant group main effect ($p < 0.01$), indicating significant difference in the flow velocity time courses during the visual stimulation between the control, indomethacin and naproxen phases. Besides the group main effect, the time-of-measurement main effect and the group with time-of-measurement interaction were also significant ($p<0.001$ and $p<0.01$, respectively). This means that during the visual stimulation the peak systolic velocities of blood flow in the posterior cerebral arteries were significantly different at the different time points of measurement and that the pattern of velocity changes induced by visual stimulation was different in the control, indomethacin, and naproxen phases. Using 2-group comparisons, when data of control phase were compared with either the indomethacin or naproxen phase, the group main effect

remained significant ($p<0.01$ and $p<0.05$, respectively), however, when data of indomethacin and naproxen phases were compared, no significant group main effect could be detected ($p = 0.44$).

In order to compare the different phases, absolute flow velocity values were normalized to the baseline data (last 5 seconds of the resting phase) and expressed in % of the baseline flow velocity. Repeated measures analysis of variance revealed significant group main effect ($p<0.01$) and significant time-of-measurement main-effect ($p<0.001$) also when relative changes were analyzed during the visual stimulation. It means that not only the absolute, but also the relative changes in flow velocity were different in the control, indomethacin, and naproxen phases, as well as at different time points. The significant group with time-of-measurement interaction ($p < 0.01$) indicated that the pattern of relative changes during visual stimulation was different in the three phases. When 2-group comparisons were performed, repeated measures ANOVA revealed significant group main effect when data of control phase were compared to indomethacin or naproxen phase ($p < 0.01$, and $p < 0.05$, respectively). However, no significant group main effect was found ($p = 0.24$) when indomethacin and naproxen phases were compared. To analyze the maximum increase of relative flow velocity changes, the highest of the values obtained during the 40-second visual stimulation was taken from each subject in the control, indomethacin, and naproxen phases. The maximum increase of the visually evoked relative flow velocity was significantly higher during the control phase, than in the indomethacin and naproxen phases.

4. 2. Effects of hyperventilation (hypocapnia) induced vasocontriction on neurovascular coupling in humans

We examined 14 healthy volunteers (7 women and 7 men) with a mean age of 25 ± 4 years.

The breathing frequency increased significantly ($p<0.001$) during hyperventilation and resulted in a significant decrease ($p<0.001$) of the end-tidal CO2, capillary blood pCO2, and a significant increase of blood pH and capillary blood pO2. According to the hypocapnia induced vasoconstriction, the resting flow velocity decreased, while the pulsatility index increased significantly as a result of HV ($p<0.01$). Blood pressure did not change during hyperventilation, however, pulse rate increased significantly ($p<0.001$). Parameters of the visual-evoked-potentials (amplitude and latency of P100 wave) were similar under normo- and hyperventilation.

Visual stimulation resulted in an increase of peak systolic flow velocity in both the normoventilation and hyperventilation phases. Absolute changes in velocity of flow as well as

relative changes were analyzed. For absolute values of flow velocity, repeated measures analysis of variance detected significant group (i.e. normo- versus hyperventilation) main effect ($p < 0.001$) and significant time-of-measurement main effect ($p<0.001$). This means that during the visual stimulation the peak systolic velocity of blood flow in the posterior cerebral arteries was significantly different between the normo- and hyperventilation phases, and that velocity of flow was significantly different at the different time points of measurement. The group with time-of-measurement interaction was also significant (pb0.01), indicating that the pattern of the visually evoked flow velocity time course was different during normo- and hyperventilation.

In order to compare the visually evoked peak systolic flow velocity changes in different individuals and different (i.e., normo- and hyperventilation) phases, relative flow velocities were calculated in the control and hyperventilation phases in relation to the corresponding baseline values. Repeated measures analysis of variance revealed significant group main effect ($p<0.001$) and significant time-of measurement main-effect ($p<0.001$) also when relative changes were analyzed during the visual stimulation. It means that not only the absolute, but also the relative changes in flow velocity differed between the normo- and hyperventilation phases and were different at the different time points. The time-of-measurement interaction was significant as well ($p<0.001$), indicating that the pattern of relative changes during visual stimulation was different in the normo- and hyperventilation phases, i.e., the temporal changes of the relative flow velocity during normo- and hyperventilation were not parallel but show different dynamics. To analyze the maximum increase of relative flow velcity changes, the highest of the values obtained during the 40-second visual stimulation was taken from each subject in both normo- and hyperventilation phases. The maximum increases of the visually evoked relative flow velocities were 26±7% and 12±5% during normoventilation and hyperventilation, respectively ($p < 0.001$).

5. DISCUSSION

Our results proved that widely used non-selective NSAIDs (indomethacin and naproxen), as well as hypocapnia resulted in a decrease of the resting flow velocity and an increase of the pulsatility index in the posterior cerebral arteries, suggesting vasoconstriction of cerebral resistance vessels. Furthermore, administration of either indomethacin or naproxen and hypocapnia decreased the visually evoked flow velocity response in the posterior cerebral arteries compared to the control phase, without affecting the visual-evoked potential parameters. These findings indicate that indomethacin or naproxen (administered orally in

usual therapeutic dose) and hyperventilation attenuate the cortical activation induced flow increase, i.e. neurovascular coupling. Since no significant difference could be detected between the effects of the two different NSAIDs, our data showed that not only indomethacin but other NSAIDs, such as naproxen, may also affect the neuronal activation–flow coupling. To our best knowledge, these were the first TCD studies showing inhibited neurovascular coupling caused by hypocapnia and NSAIDs.

5.1.1. Hemodynamic effects of NSAIDS (indomethacin and naproxen) on neurovascular coupling

Although a number of NSAIDs are available, most of the studies that aimed to investigate the effect of NSAIDs on cerebral hemodynamics, used indomethacin. This was probably because indomethacin was shown to be a potent vasoconstrictor, and thus it was widely used to facilitate the closing of patent ductus arteriosus in infants or to decrease intracranial pressure. In addition, the vasoactive effects of other non-steroid anti-inflammatory drugs were not so consistent: ibuprofen did not decrease CBF in animal experiments, and contrary to indomethacin, neither diclofenac, nor ibuprofen inhibited CO2 reactivity. Although high-dose aspirin, administered intravenously, was shown to reduce CBF in a rabbit model, others observed neither decrease of CBF, nor inhibition of CO2 reactivity or neurovascular coupling when aspirin was given in doses of 500–1200 mg to human subjects. Therefore, vasoconstriction was reported as a unique characteristic of indomethacin among NSAIDs, and a COX independent mechanism was supposed as well.

In the present study, decrease of baseline flow velocity and increase of pulsatility index were observed after administration of indomethacin or naproxen suggesting vasoconstriction of cerebral resistance vessels. Demonstrating a decrease of basal flow velocity, our results are in agreement with other studies that detected significant decrease of CBF after administration of indomethacin. However, contrary to other observations, our data proved that not only the indomethacin, but also the naproxen led to vasoconstriction of cerebral resistance vessels.

Besides decreasing basal CBF, indomethacin was shown to inhibit vasodilatory processes, including hypercarbia and acetazolamide induced flow increase. It is also known from animal experiments that the inhibition of NO-synthase or COX significantly decreases the neuronal activation induced flow response, indicating that both NO and prostacyclin play an important role in neurovascular coupling. In addition to animal experiments, a human study also proved that indomethacin attenuated the vasodilatory response during functional

brain activation. In that study, functional MRI investigations were performed in healthy volunteers and the BOLD (blood oxygenation level-dependent) MRI contrast was examined in the occipital cortex during visual stimulation, before and after intravenous administration of indomethacin or acetylsalicylate. While indomethacin significantly inhibited the neuronal activation–flow coupling, no similar effect of acetylsalicylate could be shown. In line with this observation, we also proved that indomethacin impaired neurovascular coupling. Our findings, however, suggest that besides indomethacin, naproxen also has a vasoconstrictive effect, leading not only to decreased cerebral blood flow velocity but also to disturbed cortical activation induced flow response. In our study, this effect of indomethacin and naproxen was shown to be present not only in experimental conditions, but also in every-day clinical practice, when NSAIDs are administered in usual therapeutic doses.

The development of gene technology and production of selective inhibitors of COX isoenzymes allowed to detect the COX isotype responsible for neurovascular coupling. Lack of attenuation of the vibrissal stimulation induced flow increase in the somatosensory cortex of COX1 deficient mice, but the decrease in flow response after selective inhibition of COX2 isoenzyme provided evidence for a role of COX2 in the mechanism of neuronal activity–flow coupling. In line with these results, intravenous administration of meloxicam, a preferential COX2 inhibitor, decreased the stimulation induced CBF and BOLD responses in rats. On the other hand, COX-1 genotype (L237M, rs5789)-dependent decrease in enzymatic function in heterozygous L/M carriers was associated with a 42% reduction of visual stimulation evoked hemodynamic response in a functional near-infrared spectroscopy (NIRS) study, indicating that in addition to COX2, the COX1 isoenzyme also modulates neurovascular coupling in humans.

5.1.2. Conclusion

Our observations indicate that vasoconstrictor effects should be considered when non-selective NSAIDs are given, which, besides reducing CBF, may impair cerebral vasodilation as well as neurovascular coupling.

These vascular effects were shown in the present study in young, healthy human subjects after oral administration of the usual therapeutic doses of indomethacin or naproxen. Although scientific data provide evidence for a functional metabolic buffer, potential vascular effects of NSAIDs should not be ignored in patients with advanced steno-occlusive lesions of the brain supplying arteries, because decreased cerebral blood flow, impaired vasodilation of resistance vessels, and attenuated cortical activity induced flow response, caused by indomethacin or

naproxen, may increase the risk of cerebral ischemia in hemodynamically compromised patient.

5.2. Effect of hypocapnia induced vasoconstriction on the neurovascular coupling

Our results proved that despite the unchanged visual-evoked potential, the visually evoked relative flow velocity changes were significantly lower during hyperventilation than normoventilation. These findings indicate inhibition of the cortical activation induced flow increase during hypocapnia, in other words, the neuronal activation evoked local vasodilation could not overcome the hypocapnia induced vasoconstriction. To our best knowledge this is the first human study which proves that the hypocapnia induced vasoconstriction significantly inhibits the neuronal activation evoked flow response. Since the flow velocities and the cortical neuronal activity–flow coupling are influenced by hypocapnia, our observations called the attention that standard conditions are necessary including constant breath rate and pCO2 during dynamic flow investigations.

The decrease of baseline flow velocity and increase of pulsatility index in the posterior cerebral arteries undoubtedly proved significant vasoconstriction of cerebral microvessels during hyperventilation. Therefore, not surprisingly, the absolute flow velocity changes were much less during hyperventilation than normoventilation. In order to compare the two phases (normo- and hyperventilation phases), absolute data were transformed into relative changes of flow velocity in relation to the corresponding baseline values. Although the visual stimulation resulted in increase of relative flow velocity in both phases, it was significantly lower during hyperventilation (hypocapnia) than normoventilation (normocapnia), showing that the hypocapnia induced vasoconstriction significantly inhibited the cortical neuronal activity evoked flow changes. While the visually evoked flow velocity response during hyperventilation (12±5%) was less than 50% of the increase of flow velocity in the control phase the parameters of the visual-evoked-potentials did not change significantly, indicating preserved visual cortex activity and disturbance of cortical neuronal activity–flow coupling. Our data suggest that about half of the physiological blood flow increase may be sufficient to maintain the same neuronal function during visual stimulation, i.e. such a huge physiological flow increase under normocapnia is not necessary to maintain the normal visual-evoked-potential. In line with previous findings which showed that cerebral blood flow increases exceeded the cerebral metabolic rate of oxygen by a factor of 2–10, our results also indicate that the neurovascular coupling operates with a considerable safety factor under physiological

conditions. Nevertheless, stronger reduction of pCO2 pressure and therefore forced alkalosis may have deleterious effect on neuronal function. Although not in physiological conditions, hyperventilation was shown to induce ischemic areas in patients with closed head injury due to a perfusion lowering effect. Other pathological conditions (e.g. disturbances of oxigenisation or cases of hypoperfusion) may also contribute to the potentially deleterious effect of the hypocapnia induced vasoconstriction.

5.3. Clinical importances of our results

Oral administration of indomethacin or naproxen in usual, therapeutic doses, as well as hyperventilation significantly decreased the resting cerebral blood flow velocity, increased the pulsatility index in posterior cerebral arteries (PCAs) and impaired the visually evoked flow velocity responses without affecting the visual-evoked-potential, indicating impairment of neurovascular coupling in humans.

These changes may be associated with an increased risk of adverse clinical outcome in patients with decreased cerebral blood supply (e.g. significant carotid stenosis, ischemic penumbra, vasospasm due to subarachnoid hemorrhage), decreased oxygen supply (e.g. pulmonary disorders, severe anemia), or increased oxygen and blood demand (e.g. epileptic seizure).

6. SUMMARY- NEW RESULTS

- Oral administration of non-steroid anti-inflammatory drugs, such as indomethacin and naproxen, in usual, therapeutic doses significantly decreased the resting cerebral flow velocity and increased the pulsatility index in posterior cerebral arteries (PCAs), indicating that besides indomethacin, other NSAIDs may also cause vasoconstriction of cerebral arterioles.
- Indomethacin and naproxen significantly impaired the visually evoked flow velocity responses without affecting the visual-evoked-potential, that is both NSAIDs inhibited the neurovascular coupling.
- No significant difference could be detected between the effects of naproxen and indomethacine on the cerebral resistance vessels, indicating that naproxen has similar vasoconstrictor effect to indomethacine.

- Hiperventilation significantly impaired the visually evoked flow velocity responses without affecting the visual-evoked-potential, indicating impairment of neurovascular coupling in humans.
- While the visually evoked flow velocity responses decreased by more than 50% during hyperventilation, the parameters of the visual-evoked-potentials did not change significantly. These data indicate inhibition of neurovascular coupling, and suggest that about half of the physiological cerebral blood flow increase may be sufficient during visual stimulation to maintain normal neuronal function. Therefore, it may be concluded that neurovascular coupling operates with a considerable safety factor in physiological conditions.
- Oral administration of indomethacin or naproxen in usual, therapeutic doses, as well as hyperventilation caused cerebral vasoconstriction and inhibited neurovascular coupling. These changes may be associated with an increased risk of adverse clinical outcome in patients with decreased blood/oxygen supply or increased blood/oxygen demand.

7. IN EXTENSO PUBLICATIONS OF THE AUTHOR

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Subject: Ph.D. List of Publications

Candidate: Katalin Judit Szabó
Neptun ID: IRYH3T
Doctoral School: Doctoral School of Neurosciences
MTMT ID: 10038815

List of publications related to the dissertation

1. **Szabó, K.**, Rosengarten, B., Juhász, T., Lakó, É., Csiba, L., Oláh, L.: Effect of non-steroid anti-inflammatory drugs on neurovascular coupling in humans.
J. Neurol. Sci. 336 (1-2), 227-231, 2014.
DOI: http://dx.doi.org/10.1016/j.jns.2013.10.048
IF:2.262 (2013)

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Total IF of journals (all publications): 8,151
Total IF of journals (publications related to the dissertation): 4,615

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of Web of Science, Scopus and Journal Citation Report (Impact Factor) databases.

10 April, 2015

DEBRECENI EGYETEM KENÉZY ÉLETTUDOMÁNYI KÖNYVTÁRA EGYETEMI ÉS NEMZETI KÖNYVTÁR

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8. AKNOWLEDGEMENTS

I would like to express my sincere thanks for **László Oláh,** M.D., my supervisor, for the opportunity of joining his research team, for providing selfless support and continuous help.

I am grateful to Professor **László Csiba**, for his continuous professional help and advices.

I am thankful to **Bernhard Rosengarten** for his professional advice.

I am really thankful to **Edina Pallagi,** M.D, **Paluskáné Peterman Tünde** for their valuable contributions and technical assistance.

I am grateful to **László Kardos** for the statistical analysis.

I am especially thankful to my dear **Family**, my Father and Mother and my Husband for their support during my whole life.

Last, but not least, I am also grateful to my **Dear Friends, Colleagues**. Without their participation in our investigations, we would not complete this work.