CHARLES UNIVERZITY IN PRAGUE
FACULTY OF PHYSICAL EDUCATION AND SPORT
DEPARTMENT OF PHYSIOTHERAPY

Diploma thesis

Effect of carbon dioxide on neurovascular coupling during cortical epileptic activity in rats

Supervizor:

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Prague, April 2010

Abstract

Title:

Effect of carbon dioxide on neurovascular coupling during cortical epileptic activity in rats

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Aim:

The aim of the study is to investigate the effect of CO_2 on neurovascular coupling in response to epileptiform activity after trancallosal electric stimulation in rats.

Methods:

Adult albino rats (250-350g, n=6) were anaesthetized with isoflurane and epidural silver EEG electrodes were implanted and fixed into the skull over sensorimotor cortices. To measure regional cerebral blood flow (rCBF) during epileptic activity a self-made metal holder for Laser Doppler Flowmeter (LDF) probe was fixed to thinned skull with dental acrylic. To measure arterial blood pressure (BP) and arterial blood gasses a plastic catheter was implanted into the common carotid artery. After postsurgical recovery animals were placed in a recording chamber. After 20 minutes of background recording effect of CO_2 on basal BP an rCBF was tested by inflating a mixture of 10%CO_2, 20%O_2 and 70%N_2 for 30seconds. To assess changes in neurovascular coupling a rat model for myoclonic seizures was used. Biphasic constant current suprathreshold stimulus (8Hz, 15s) was applied under normal or elevated CO_2 atmosphere.

Results:

Elevated CO_2 led to a significant increase of both regional cerebral blood flow and blood pressure. In addition, inhalation of 10%CO_2 during electrical stimulation of senzorimotor cortex (8Hz, 15s) caused significant increase in regional cerabral blood flow. Surprisingly, basal blood pressure did not increase after 10%CO_2 exposure during electrical stimulation of senzorimotor cortex. Transcallosal electrical stimulation produced cortical epileptic

afterdischarges which were paralleled by facial and fore limb clonic seizures. Elevated CO_2 reduced or completely blocked the stimulation-evoked epileptiform afterdischarges.

Conclusions:

Our data suggest that application of CO_2 may be effective in the acute treatment of epileptic seizures. The effect of CO_2 might be explained by both decreased excitability due to tissue acidosis and elevated rCBF levels.

The project was supported by grant no. P303-10-0999 of ASCR.

Key words:

regional cerebral blood flow, CO_2, LDF, neurovascular coupling, transcallosal electrical stimulation

Souhrn

Název práce:

Účinky oxidu uhličitého na neurovaskulární spojení během epileptické kortikální aktivity u potkanů

Vedoucí práce:

MUDr. Jakub Otáhal, PhD.
Katedra anatomie a biomechaniky FTVS UK
Oddělení vývojové epileptologie FGÚ AVČR

Cíl:

Cílem práce je zjistit, jaký vliv na neurovskulární spojení v mozku potkanů bude mít podání CO_2 v průběhu elektrické transcalosální stimulace.

Metody:

Experimenty byly provedeny na skupině 6 dospělých potkanů kmene Wistar (250 – 350 g). Potkanům byly pod aestezií implantovány stimulační a snímací elektrody k monitorování spontánního a evokovaného EEG a ručně vyrobený úchyt na sondu Laser Doppler Flowmeter k měření krevního průtoku v mozku, který byl přiložen kontralaterálně od stimulačních elektrod. Pro měř ení systémového tlaku krve (BP) a krevních plynů byl zaveden plastový katétr do krkavice. Po pooperační rekonvalescenci byli potkani umístěni do boxu, kde byli napojeni na měřící systém. Testovali jsme vliv CO_2 na krevní průtok v mozku (CBF) a BP v průběhu transcallosální stimulace. Měření mělo tři části: čistá stimulace, stimulace s inhalací CO_2, čistá stimulace. CO_2 bylo inhalováno ve směsi 10%CO_2, 20%O_2 and 70%N_2. Změny regionálního mozkového krevního průtoku byly monitorovány během transcalosální stimulace (8 Hz, 15 s) za normálních podmínek i za zvýšeného CO_2.

Výsledky:

Zvýšení CO_2 vedlo k významnému nárůstu jak rCBF tak i BP. Inhalací 10%CO_2 během transcalosální elektrické stimulace došlo ke zvýšení rCBF a překvapivě ke snížení BP. Epileptické AD, vyvolané transcallosální elektrickou stimulací, byly doprovázeny projevy klonického záchvatu ve faciální oblasti a na přední končetině. Zvýšení CO_2 redukovalo nebo kompletně blokovalo stimulací vyvolané následné výboje (AD).

Závěr:

Výsledná data poukazují na to, že aplikace CO_2 může být efektivní v akutní fázi léčby epileptického záchvatu. Účinnost CO_2 může být vysvětlena snížením excitability způsobené tkáňovou acidózou a zvýšením rCBF.

Tento projekt vznikl za podpory grantu č. P303-10-0999 ASCR.

Klíčová slova:

regionální mozkový krevní průtok, CO_2, LDF, neurovaskulární spojení, transcallosální stimulace

I declare that this diploma thesis has been based on my own individual work and all the additional information included have been taken from the list of literature that is integrated in this thesis.

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Martina Jindrová

Acknowledgements

I would like to thank MUDr. Jakub Otáhal, PhD. for his advice, instructions, guidance and for introducing me the principles of science research. I am also grateful to Carole Brožíčková for her relevant comments on my work.

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Content

1 Introduction

The human brain is a highly complex organ of fundamental importance in maintaining healthy bodily functions. The normal brain function relies on the robust coupling between neural activity and cerebral blood flow (neurovasculare coupling). When neurons in a specific brain region increase their synaptic activity, the cerebral metabolic needs to accrue and the regional cerebral blood flow demands an increase in temporally and spacially coordinated manner.

Epilepsy is one of the most common neurological diseases with a prevalence of approximately 1-1.5% in the general population. Epilepsy refers to conditions of chronic recurrent epileptic seizures (ES). ES represent the intermittent and self-limited clinical manifestations that result from abnormally excessive and synchronous activity of neurons. This interrupts the normal behaviour of the parts of the brain involved. In the part of the brain where the electrical hyperactivity occurs, the cerebral blood flow increase to satisfy neuronal metabolic demands. The recent investigations of cerebral blood flow shows that the changes of regional cerebral blood flow and perfusion occure during the epileptic activity.

The neurovasculare coupling is tightly controlled via numerous biological signaling pathways. The control may be via metabolic factors (CO_2, K^+,H^+, adenosine etc.) or via neurogenic factors (neurotransmitters, NO etc.). The CBF is physiologically dependent on Cerebral perfusion pressure (CPP) and Cerebral vascular resistance (CVR), which are under influences of neural, chemical, metabolic and physical factors in quite a different way from those of peripheral organs and tissues. In contrast to the dominant vasoconstrictor control in the periphery, the intracranial vascular tone is predominantly influenced by vasodilator mediators. Recent studies have indicated that nitroxidergic vasodilator nerve, endothelium-derived hyperpolarizing factor (EDHF), K^+ channel, nitric oxide (NO), endothelium-derived relaxing factor (EDRF), polypeptides, prostaniodes, etc. play important roles in the regulation of cerebral arterial and arteriolar tone.

Carbon dioxide (CO_2) is powerful modulator of cerebrovascular resistance and perhaps clinically most accesible one. Hypercapnia has been known to intensely increase CBF by dilating cerebral arteries and arterioles. Recent findings show that respiratory alkalosis triggers experimental febrile seizures in neonatal rats, which are blocked by an elevation of ambient CO_2 to 5%. Alterations in partial pressure of CO_2 (pCO_2) influence the cerebral circulation. Deviations from acid-based balance are known to have signifacant effects on neuronal excitability and it is of a great interest to find out more about mechanisms underlying CO_2-induced changes.

This study was designed to investigate the effect of CO_2 on neurovascular coupling in response to epileptiform activity after trancallosal electric stimulation in rats. A rat model for myoclonic seizures was used to assess changes in neurovascular coupling. Laser Doppler flowmeter was used for measuring regional rCBF during epileptic activity. To monitor spontaneous and evoked cortical EEG two recording electrodes were implanted and for stimulation two silver electrodes were implanted into the skull. To measure arterial BP and arterial blood gasses a plastic catheter was implanted into the common carotid artery. The aim of the theoretical part of this thesis is to introduce the physiology of cerebral blood flow regulation, the role of astrocytes, NO and CO_2; the basic functions of blood-brain barrier; epilepsy including classification, pathology, changes in CBF and neuroimaging in epilepsy.

2 Theoretical backround

2.1 Regulation of cerebral blood flow

The brain function is critically dependent on a continuous supply of blood. Therefore, the cerebral vasculature is endowed with neurovascular control mechanisms that assure that the blood supply of the brain is adequate to the energy needs of its cellular units. The regulation of cerebral blood flow (CBF) during brain activity involves the coordinated complex of cellular, metabolic, and vascular processes (Fig. 1). Various cellular processes of neurons, such as the restoration of ionic gradients and neurotransmitter recycling, require energy in the form of adenosine triphosphate (ATP). ATP is synthesized first by glycolysis, which is anaerobic and produces a small amount of ATP and then by oxidative glucose metabolism, which requires oxygen and produces a large amount of ATP. In the brain, about 90% of glucose is metabolized aerobically. Cerebral metabolism thus depends on a constant supply of both glucose and oxygen. A continuous supply of these two energy substrates is maintained by CBF, which delivers glucose and oxygen to neural tissue through the complex web of blood vessels in the brain's vascular system. Accordingly, during neural activity, increases in oxygen and glucose consumption are followed by an increase in CBF.

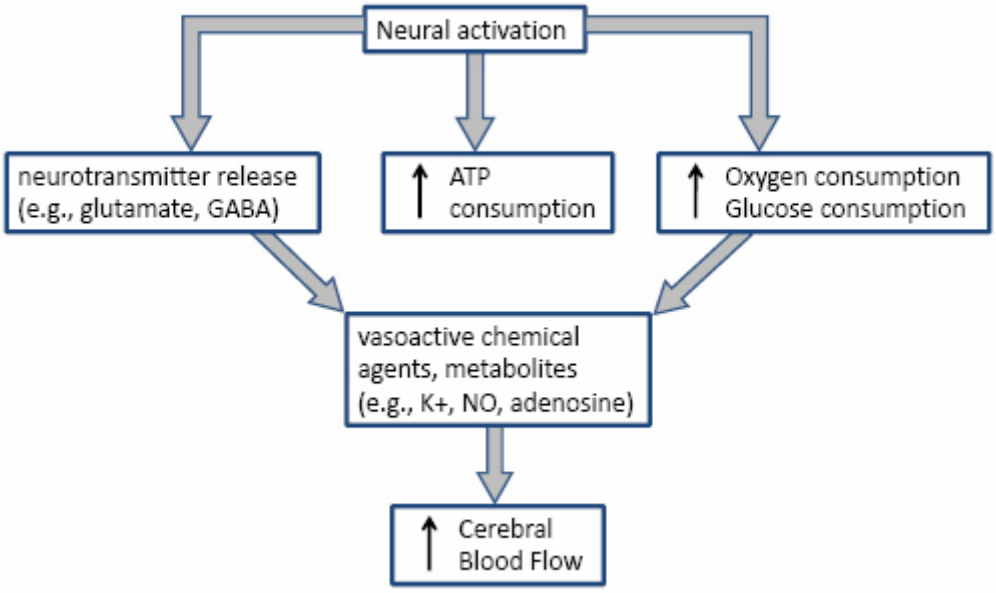


Fig.1 : Basic summary of physiological changes linking neural and vascular responses during brain aktivity (5).

A large body of evidence indicates that neural activity is closely related to CBF. The close spatial and temporal relationship between neural activity and CBF, termed neurovascular coupling, is at the basis of modern neuroimaging techniques that utilize the cerebrovascular changes induced by activation to map regional changes in function in behaviour of the human brain. However, in several brain pathologies, the interaction between neural activity and cerebral blood vessels is disrupted, and the resulting homeostatic unbalance may contribute to brain dysfunction (Iadecola, 2006).

While it is clear that alterations in neural activity and metabolism are correlated with changes in CBF, the mechanisms linking these processes are still under investigation. There are several hypotheses of cerebral blood regulation. One possibility is that cerebral blood flow is controlled directly by energy demand. This idea was originally proposed over a century ago by Roy and Sherrington (1890) In this view, regional CBF is controlled by feedback mechanisms that are sensitive to variations in the concentrations of ionic and molecular metabolic products. These products, such as K+, NO, adenosine, CO_2 and arachidonic acid metabolites, may directly or indirectly alter CBF by depolarizing (or hyperpolarizing) the vascular smooth muscle cells which trigger vasodilation (or vasoconstriction).

Another hypothesis is that local blood flow is controlled directly by a feedforward mechanism involving neuronal signaling via neurotransmitters (Lauritzen, 2005). Evidence of this mechanism suggests that astrocytes may play an important role in linking neurotransmitter activity to vascular response. Astrocytes are a critical component for glutamate recycling. A cascade of chemical events within the astrocyte may then link the rate of glutamate cycling to the production of vasoactive chemical agents. In this view, neurovascular coupling is mediated by neuronal signaling mechanisms via glial pathways, rather than by mechanisms that is sensitive to energy consumption. In addition, neurovascular coupling might be mediated by diffusion of products of neuronal activity without the involvement of glial cells (Iadecola, 2006).

In adition there is evidence that direct neuronal innervation of smooth muscle cells can also control CBF (Hamel, 2004).

2.1.1 Role of Astrocytes

Astrocytes are in an ideal anatomical position to enable the transfer of information from neural structures directly to vessels (Fig. 2). Processes of a single astrocyte can associate with numerous synapses, contact adjacent astrocytes to form a glial syncytium and form perivascular endfeet on arterioles and capillaries. Endfeet are enlarge astrocytic

compartments that appear to be specialized for direct interaction with vessels. Together with the colective arrangement of many other astrocyte processes, all cerebral arterioles in the brain are encased by endfeet. In addition to this anatomical propensity that suggests a prominent role in vasculature physiology astrocytes also wield signaling capability. This includes a long-range inter-astrocytic Ca^{2+} waves and an ability to sense transmitters such as glutamate, GABA, neuropinephrine and ATP, which may enable the transmission of relevant information about the extracellular environment to vessels. Furthermore, endfeet are themselves endowed with many elements taking part in cerebrovasculature control (Gordon, 2007).

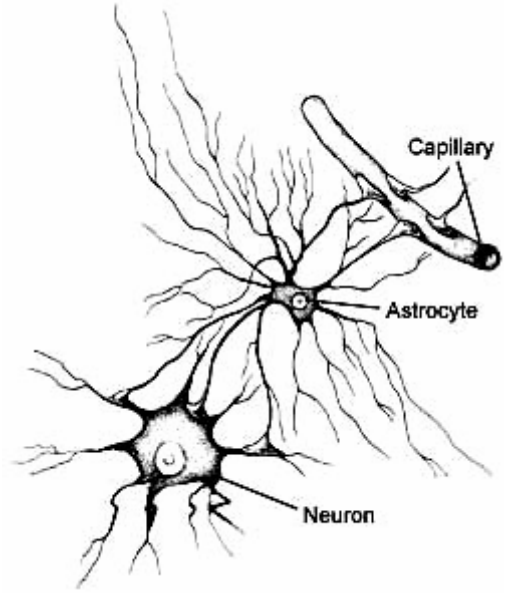


Fig. 2. Astrocyte (1)

2.1.1.1 Role of astrocytes - Ca^{2+} signals

Recent evidence (Iadecola 2004, Straub et al. 2006, Zonta et al. 2003) suggests that Ca^{2+}-dependent signaling events in astrocytes play a critical role in communicating neuronal activity to the cerebral microcirculation, resulting in profound changes in arteriolar diameter and local cerebral blood flow (Straub, 2007).

Astrocytes act as vital regulators of neuronal function, serving to modulate extracellular potassium concentration and extracellular volume, and remove neurotransmitters from synapses. These cells possess receptors for a range of neurotransmitters and neuropeptides. Glutamate-dependent generation of astrocytic Ca^{2+} signals is a common mechanism by neuronal information is transmitted to the cerebral vasculature. Specifically, activation of metabotropic glutamate receptors (mGluRs) located on astrocytic projections which surround synapses of glutamatergic neurons results in an increase in astrocytic cytosolic Ca^{2+} concentration that propagates through astrocytic

processes, ultimately resulting in a astrocytic Ca^{2+} concentration increase in the endfoot (Zonta, 2003).

These Ca^{2+} concentration increases through activation of the phospholipase C/inositol trisphosphate ($InsP_3$) cascade, and release of Ca^{2+} from $InsP_3$ sensitive channels located on the endoplasmic reticulum. Several studies using cultured astrocytes suggest that Ca^{2+} release from ryanodine receptors may also be involved in generating the astrocytic Ca^{2+} signal. In addition to glutamate, neurotransmitters such as GABA, somatostatin, and vasoactive intestinal peptide released from interneurons may likewise generate vasoactive effects through generation of astrocytic Ca^{2+} signals, in addition to direct innervation of vessels. These findings suggest that Ca^{2+} signaling within endfeet is highly dynamic in nature, and therefore likely to exert local control over Ca^{2+}-dependent vasoactive signaling pathways involved in neurovascular coupling (Straub, 2006).

Zonta et al. studied the role of astrocytic Ca^{2+} signaling in the dynamic regulation of the cerebral vasculature. They linked astrocytic Ca^{2+} changes to alterations in arteriolar diameter. In this study, astrocytic Ca^{2+} changes induced by neuronal activity or application of an mGluR agonist were measured in rat cortical brain slices and correlated with alterations in vessel diameter. Electrical stimulation of glutamatergic neurons induced consistent increases in astrocytic Ca^{2+} that were significantly attenuated (Zonta, 2003). Separate experiments showed that vasodilation occurred in a similar time frame to the increases in astrocytic Ca^{2+}, suggesting that astrocytic Ca^{2+} changes are coupled to changes in vessel diameter. These findings suggest that astrocyte-derived factors, generated in response to elevations in astrocytic Ca^{2+}, are capable of engaging cerebral arterioles, modulating arteriolar diameter, and dynamically regulating local cerebral blood flow (Straub, 2007).

Astrocytic Ca^{2+} increases have also been shown to induce vasoconstriction. Mulligan and McVicar (2004) found that elevation of Ca^{2+} within an individual astrocyte, induced through photolysis of the caged divalent ion chelator DMNP-EDTA, resulted in constriction of arterioles in brain slices. Cerebral arteriolar diameter was affected only when a large Ca^{2+} increase propagated into an endfoot. No change in the state of the arterioles was seen when a Ca^{2+} increase was selectively induced within an astrocytic cell body, or when a small Ca^{2+} increase was generated in an endfoot. Similar to the effects of photoreleasing Ca^{2+}, application of norepinephrine to slices induced an increase in astrocytic Ca^{2+} that was accompanied by vasoconstriction.

The chemical mediators of facilitating changes in cerebral arteriolar diameter by astrocytic Ca^{2+} have been studied. Zonta et al. (2003) provided evidence that the vasodilatory factor released from astrocytes following the astrocytic Ca^{2+} increase was

prostaglandin E_2 (PGE_2), a product of cyclooxygenase (COX) metabolism of arachidonic acid (AA) (Fig.3). This proposal was based primarily on a decrease in the number of vessels that dilated to neuronal stimulation in the presence of the COX inhibitor acetylsalicylic acid. Consistent with this finding, direct application of PGE_2 to brain slices resulted in vasodilation similar to that induced by the mGluR agonist t-ACPD. Additionally, this group has previously shown that PGE_2 is released in a pulsatile manner from cultured astrocytes (Zonta, 2003).

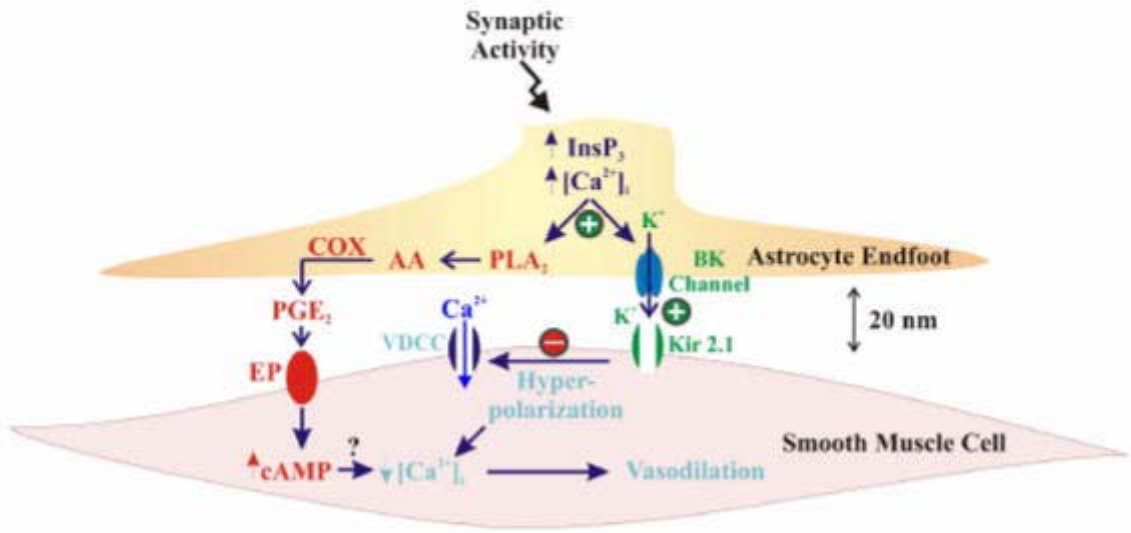


Fig. 3. Putative vasoactive signaling pathways involved in neurovascular coupling. Neuronal activity generates elevation of $[Ca^{2+}]_i$ in the astrocytic endfoot, through activation of $InsP_3R$, which activates astrocytic BK channels. BK channel activation leads to the local release of K^+ ions from endfeet surrounding cortical blood vessels, which activates Kir channels in arteriolar smooth muscle cells (SMCs). Local concentrations of K^+ ions between 3–15 mM increase SMC Kir channel conductance, causing SMC membrane potential hyperpolarization, closing voltage-dependent Ca^{2+} channels (VDCC), and leading to vasodilation. Higher local concentrations of K^+ (e.g. >20 mM) would cause membrane potential depolarization, and vasoconstriction. Through an alternate pathway, increased astrocytic $[Ca^{2+}]_i$ is postulated to activate phospholipase A_2 (PLA_2) to generate arachidonic acid (AA) and subsequently prostaglandin E_2 (PGE_2) through a cyclooxygenase (COX)-dependent mechanism. Activation of EP receptors by PGE_2 would lead to increased cAMP in SMCs, ultimately decreasing SMC $[Ca^{2+}]_i$ and generating vasodilation through an as yet unidentified mechanism (Straub, 2007).

AA can also be converted to epoxyeicosatrienoic acids (EETs) by the action of cytochrome P450 (CYP450) epoxygenases, to produce the vasoactive components 11,12 and 14,15 EET.It have been shown that EETs, which are potent vasodilators activate Ca^{2+}-sensitive, large conductance potassium channels (BK channels) in smooth muscle as well as astrocytes. In support of a potential role for EETs in neurovascular coupling, it has been recently implicated epoxygenase products in maintaining vasodilation of cortical arterioles following astrocytic AMPA receptor stimulation (Straub, 2007).

Mulligan and MacVicar (2004) postulated a role for 20-hydroxyeicosatetraenoic acid (20-HETE) in generating vasoconstriction of cerebral arterioles in response to neuronal activity. It was suggested that 20-HETE is synthesized by CYP450 hydroxylases in SMCs following diffusion of AA from astrocytic endfeet to SMCs. This proposal was based on the ability of PLA_2 and CYP450 hydroxylase inhibitors to prevent vasoconstriction induced by photolysis of caged Ca^{2+} in astrocytes. 20-HETE is suggested to inhibit BK channels in SMCs, leading to depolarization and constriction. However, the arterioles measured in this study were not pre-constricted, likely existing in a maximally dilated state. Under such conditions SMC BK channels are not engaged in the regulation of vessel diameter (Straub, 2007).

2.1.1.2 Role of astrocytes – K^+ channels

Potassium ions (K^+) are among the most potent vasodilatory signals in the cerebral vasculature. Elevation of extracellular potassium concentration activates strong inward rectifier potassium (Kir) channels and the Na^+/K^+-ATPase in SMCs of extracerebral arteries. Activation of SMC Kir channels results in SMC hyperpolarization which closes voltage-dependent Ca^{2+} channels in the SMCs and leads to decreased astrocytic Ca^{2+} and vasodilation. Astrocytes have been proposed to play a critical role in the spatial buffering of K^+ within the brain. It has been suggested that astrocytes function to move K^+ from regions of elevated extracellular potassium concentration around synapses to regions of lower extracellular potassium concentration around the vasculature (Straub, 2007).

Consistently with a potential role of K^+ in regulating cerebrovascular function, recent evidence suggests that K^+ released from astrocytic endfeet plays a critical role in generating rapid vasodilation of cerebral arterioles in response to neuronal activity (Filosa, 2006). Elevation of astrocytic Ca^{2+} leads to a vasodilation of pre-constricted arterioles in cortical brain slices that is significantly reduced (~70 % reduction) by treatment with the BK (potassium) channel blockers iberiotoxin, or by block of Kir channels with barium. This vasodilation is rapid, typically occurring within 2 seconds following onset of electrical stimulation to induce neuronal activity, consistent with the functional hyperemic response observed in vivo (Straub, 2007).

Approximately 40% of the astrocytic K^+ current in the absence of neuronal stimulation is carried through BK channels, which are preferentially expressed in endfeet (Price, 2002). In addition, neuronal stimulation dramatically increases single channel activity consistent with BK channels in “on-endfoot” patch clamp recordings from endfeet within cortical slices. SMCs isolated from parenchymal arterioles exhibit a high density of

Kir currents, suggesting that Kir channels in parenchymal arterioles may be a target of K^+ released from endfeet through BK channels. Furthermore, neurovascular coupling was diminished in cortical slices of rats whose BK channels were genetically ablated, but not in slices preincubated with the COX inhibitor indomethacin (10 μM). Interestingly, in the presence of indomethacin, the treatment with barium completely abolished vasodilation (Straub, 2007).

Gerrits et al. (2002) showed a decrease in whisker stimulation-evoked blood flow changes following block of BK channels with topical application of TEA or iberiotoxin or intravenous administration of TEA, suggesting a critical role for BK channel activity in the functional hyperemic response. Since neither TEA nor iberiotoxin affected neuronal activity, these findings are consistent with the assertion that BK channels in astrocytes and/or SMCs have a critical role in functional hyperemia.

These observations support a model of rapid neurovascular coupling in which neuronal activity is communicated to the vasculature through the Ca^{2+} dependent activation of endfoot BK channels and release of K^+ into the perivascular space, which induces hyperpolarization of SMCs through activation of SMC Kir channels, to promote vasodilation (Fig. 3). An interesting feature of this model is that extracellular K^+ is capable of accounting for both rapid vasodilation, as described above, and vasoconstriction, depending upon its concentration in the perivascular space and the SMC membrane potential (Straub, 2007).

2.1.2 Role of Nitric oxid (NO)

NO is a ubiquitous intercellular messenger involved in particular functions in the cardiovascular, immunological and nervous systems. In the cerebral cortex, nitric oxide is synthetized by endothelial cells and by a discrete population of neurons and glial cells expressing nitric oxide synthase (NOS). NO of endothelial and neuronal origin is involved in the regulation of cerebral blood flow. It contributes to vasodilatation, increased local blood flow, and decreased vascular resistance in cerebral circulation.

NO is formed by the constitutive isoforms of NO synthase, endothelial (eNOS), neuronal (nNOS) and inducible NOS (iNOS) and acts as a messenger between endothelial and smooth muscle cells, causing vascular relaxation, as a cytotoxic mediator in the immune system and as a neurotransmitter in certain neurons. NO is produced when NOS catalyzes L-arginine to L-citrulline in the presence of O2 and cofactors including flavin adenine dinucleotide, flavin mononucleotide, nicotinamide adenine dinucleotide phosphate (NADPH), tetrahydrobiopterin, and heme. Ca^{2+} is required for the activation of nNOS and endothelial eNOS. nNOS is constitutively expressed in the brain, peripheral

nerves, and kidneys, and eNOS is constitutively expressed mainly in endothelial cells (Todu, 2009).

Hoffmeyer et al. provide evidence that NO contributes to the basal cerebrovascular tone and to the CBF responses evoked by stimulation. They used 7-Nitroindazole, a relatively specific blocker of neuronal NOS, to attenuate activity-dependent CBF responses. They reported that the CBF response depends on changes in NOS activity (NOS inhibition reduced baseline CBF by 12% and CBF responses to all stimulation frequencies by 50%).

Endothelial NO causes vasodilatation, increases blood flow, lowers blood pressure and reduces smooth muscle proliferation. It also acts to prevent atherosclerosis and plays a crucial role as a neurotransmitter from the peripheral efferent nerves in cerebral arteries (Todu, 2003).

NO signaling appears to be essential for neural plasticity, that is, long-term potentiation in the hippocampus and long-term depression in the cerebellum. NO formed by *N*-methyl-D-aspartate (NMDA) receptor activation diffuses to adjacent nerve terminals to modulate neurotransmitter release (Todu, 2009).

Glutamate is the major excitatory neurotransmitter in brain and mediates its effects through activation of its receptors. NMDA (glutamate receptor) produces increases in neuronal activity and marked increases in cyclic GMP (cGMP) in brain tissue that are mediated by NO. Isolated neurons release NO in response to activation of receptors for NMDA and computer modeling suggests that neuronally derived NO may influence local vascular tone. Because NO is highly diffusible and is released extracellularly by neurons during activation, it has been hypothesized that neuronally derived NO may have an important influence on the local cerebral microcirculation (Faraci, 1993).

Faraci et al. examined whether NMDA produced dilatation of cerebral arterioles in vivo and whether the response to NMDA was dependent on activation of neurons and formation of NO.They confirm that activation of NMDA receptors produces dilatation of the cerebral microcirculation. Dilatation of cerebral arterioles in response to NMDA was inhibited by tetrodotoxin and L-NNA, suggesting that responses to NMDA were dependent on neuronal activation and production of NO. Further, they support the hypothesis that dilatation of cerebral arterioles in response to NMDA in vivo is mediated by NO produced by neurons in response to activation of receptors for NMDA.

It has been found by using different methods for CBF measurement that NOS inhibition substantially decreases the cerebrovascular dilation that follows hypercapnia in several species. The effect is observed when the administration of NOS inhibitors is either systemic or topical and it is reversed by L-arginine. CO_2 inhalation elevated brain cGMP,

while NOS inhibition reduced this response. Some authors have recently described that NO donor administration prevents the attenuation of hypercapnic vasodilatation by NOS inhibitors. Although the results are somewhat contradictory, depending on the NOS inhibitor used and the NO donor administration route, they suggest that NO may have a permissive rather than an effector role in the cerebrovascular response to hypercapnia (Estrada, 1998).

2.1.3 Role of CO_2

Cerebral blood flow (CBF) and its distribution are highly sensitive to changes in the partial pressure of arterial CO_2 (pCO_2). This physiological response is a vital homeostatic function that helps regulate and maintain central pH. The effect of pH on cerebral vascular tone is mediated by NO, prostanoids, cyclic nucleotides, potassium channels, and intracellular calcium. Several studies support an important role for each of these mediators in the response of the cerebral circulation to CO_2.

2.1.3.1 CO_2 mechanism

Increased pCO_2 relaxes cerebral arteries in vitro, which indicates that CO_2 can cause cerebral vascular relaxation independent of extravascular cells. In vivo, cerebral arteries respond to highly localized perivascular alteration of pCO_2 and pH, which indicates that mechanisms that affect cerebral vascular tone are localized to the area of the blood vessel wall. Cellular elements that could contribute to the cerebral vascular response to CO_2 include vascular cells (endothelium and smooth muscle) and extravascular cells (perivascular nerves, parenchymal neurons, and glia). When cerebral vascular tone is altered by a change in pCO_2, it is possible that CO_2 itself, CO_2 -mediated change in pH, or both are signals leading to a change in vascular tone. Applying acidotic or alkalotic solutions to the brain dilates or constricts cerebral arteries in vivo, which indicates that pH can affect cerebral vascular tone (Brian, 1998).

The relative change in CBF during variations of pCO_2 depends on several factors, including baseline CBF, cerebral perfusion pressure and anesthetic drugs. Reducing pCO_2 to 20 - 25 mmHg decreases the global CBF by 40 - 50%, and further reductions of pCO_2 do not reduce CBF any further. Increasing the pCO_2 to 80 mmHg or more produces a maximal increase in CBF of 100 - 200% in anesthetized animals. In conscious animals, however, increasing the pCO_2 to 80 mmHg increases CBF six times, but a half of the increase in CBF is a result of endogenous catecholamine release and activation of neuronal metabolism. This suggests that in conscious subjects, severe hypercapnia may

increase the flow by two mechanisms by a direct effect of CO_2 on cerebral blood vessels and an indirect effect by increasing brain metabolism and blood flow (Iadecola, 1994).

Brain blood flow is not homogeneous, and areas of the brain that receive more blood flow have a steeper flow response to changes in pCO_2 . The observation that baseline CBF influences the response of CBF to changes in pCO_2 also remains valid when CBF is elevated artificially by inhalational anesthetics (Brian, 1994).

2.1.3.2 Effect of hypercapnia on CBF

Blood oxygenation changes during typical brain activity are predominantly the result of the washout of deoxyhemoglobin by an increase in the supply of highly oxygenated (arterial) blood in conjunction with increases in blood volume. Hypercapnia induces an increase in blood flow and a decrease in the oxygen extraction fraction, and thus alters the vascular physiology of the brain that dictates the behavior of functionally evoked changes in oxy- and deoxyhemoglobin.

Huppert et al. hypothesized that hypercapnia leads to an increased baseline blood flow, which changes the model parameters by defining increased baseline oxygen saturation, increased baseline blood volume, and decreased vascular transit time. They investigated that baseline flow was changed by hypercapnia. The relationship between the neural and blood oxygenation signals (oxy - and deoxyhemoglobin) also changed, whereas the neurovascular and neurometabolic coupling relationships were the same. Thus, while under both normal and hypercapnia conditions, a linear relationship between neural activity and overall blood oxygenation was observed. Furthermore, they found that the hypercapnia-induced differences that were observed in the blood oxygenation responses are consistent with the mechanical effects expected from the changes in the model parameters based on measured differences in blood flow, volume, and oxygen saturation between normal and hypercapnic states (Huppert, 2009).

2.1.3.3 Effect of hypocapnia on CBF

Hypocapnia leads to cerebral hypoxia and causes respiratory alkalosis. A study suggests that hypocapnia may decrease cerebral blood flow velocity (cBFV) through the constriction of arterioles. Debreczeni et al. exemined the changes in cBFV, which were caused by voluntary hyperventilation-induced hypocapnia. There was a significant decrease of cBFV (about 30% less than the reference value) after 2 min voluntary hyperventilation.

Hyperventilation is considered an integral component of the anesthetic management of patients undergoing intracranial surgery. Intraoperative hyperventilation appears to be a clinically useful intervention to control intracranial pressure (ICP), to offset the effect of inhaled anesthetics, and to enhance operative exposure (Brian, 1998). Gelb et al. investigated the effect of hyperventilation on ICP in patients undergoing craniotomy. They found that hyperventilation decreased the risk of increased brain bulk by 45% and ICP during hyperventilation was lower than that during normoventilation.

2.2 Blood-brain barrier

The blood-brain barrier (BBB) is a highly specialized brain endothelial structure of the fully differentiated neurovascular system. In concern with pericytes, astrocytes and microglia, the BBB separates components of the circulating blood from neurons. Moreover, the BBB maintains the chemical composition of the neuronal milieu which is required for proper functioning of neuronal circuits, synaptic transmission, synaptic remodeling, angiogenesis and neurogenesis in the brain.

The BBB breakdown altered transposrt of molecules between blood and brain and brain and blood, aberrant angiogenesis, vessel regression, brain hypoperfusion, and inflamantory responses may initiate or contribute to a vicious circle of the disease process resulting in progressive synyptic and neuronal dysfunction and loss in disorders such as Alzheimer´s disease, Parkinson´s disease, Multiple sclerosis, Epilepsy and others (Zlokovic, 2008).

2.2.1 Physiology

The barrier results from the selectivity of the junctional complexes between the cerebral endothelial cells. These complexes are comprised of tight and adherens junctions. Such restrictive angioarchitecture at the BBB reduces paracellular diffusion, while minimal vesicle transport aktivity in brain endothelial cells limits transcellular transport. Under normal conditions, this largely prevents the extravasation of large and small solutes and prevents migration of any type of blood-borne cell (Stamatovic, 2008).

The cytoplasm of brain endothelial cell (EC) is of uniform thickness, with very few pinocytotic vesicles and a lack of fenenstrations. The wall thickness of brain capillaries is approximately 40 % than in other types of EC. It speculated that this decrease in wall thickness is an adaption to the restrictive permeability of the BBB, alowing nutrients a

shortened transport time to cross through the membrane and cytoplasm and enter the brain parenchyma.

In general, brain endothelial cells have a very low number of vesicles under normal conditions compared to other types of ECs. However, during some disease states the number of vesicles can increase. Fusion between vesicles may eventually lead to the formation of transendothelial channels and/or vesicle/vacuolar organells (VVO). Brain ECs also posses a well developed tubular system formed by membrane-bound tubules that intrude deeply in to the ECs from both the luminal and abluminal poles (Lossinky, 2004).

The BBB is an enzymatic barrier capable of metabolizing drugs and nutrients. These enzymes are principally directed at metabolizing neuroactive blood-borne solutes. Enzymes such as y-GTP, alkaline phosphatase and aromatic acid decarboxylase are found at elevated concentrations in cerebral microvessels, yet are often in low concentration or absent in non-neuronal capillaries. These enzymes are often polarized between the luminal and abluminal membrane surafce of brain ECs (Matter, 2003).

The BBB possesses a wide array of tranporters. The BBB has very high levels of the glucose transporter GLUT-1 and the large neutral amino acid transporter LAT1, that faciliate movement of nutriens from blood to brain. There are also efflux transporters such as P-glycoprotein and organic anion tranporters that move compounds from brain to blood. Other transporters are involved in ion homeostasis and the transport of signaling molecules. Besides transporters, Brain ECs posses several ion channels , which control important endothelial functions (Lossinky, 2004).

2.2.1.1 Tight Junctions (TJs) and Adherens Junctions (AJs)

The most important factors responsible for BBB impermeability are the junctional comlexes existing between the ECs of brain microvessels. It is generally accepted that TJ seal the interendothelial cleft forming of a continuous blood vesel, while the AJ are important for initiating and maintaining endothelial cell-cell contact. The TJs between the ECs lead to high endothelial electrical resistence and low paracellular permeability. The electrical resistence is in the range of 1500-2000 $\Omega.cm^2$ (pial vessels) compared to 3-33 $\Omega.cm^2$ in other tissues (Gonzales, 2003).

TJ and AJ are composed of transmembrane proteins and cytoplasmic plaque proteins. The former proteins physically associate with their counterparts on the plasma membrane of adjacent cells, whereas the latter provide a link between transmembrane TJ/AJ proteins and the actin cytoskeleton but also participate in intracellular signaling. Transmembrane proteins of the TJ include occludin, claudins and Junctional adhesion molecule (JAM-A, JAM-B, JAM-C). The TJ cytoplasmic plaque proteins are subdivided

depending on whether they contain a PDZ motif. PDZ containing proteins include members of the family membrane – associated guanylate-kinase (MAGUK) homologues (ZO-1, -2, -3), partitioning –defective proteins (Par3, Par6) afidin, etc. PDZ lacking plaque proteins include as cingulin, 7H6, Rab 13, ZONAB, heterotrimetric G protein, etc. The major transmembrane protein of the AJ is vascular endothelium cadherin. The AJ cytoplasmatic plaque includes proteins of the catenin family (Tab. 1) (Gonzales, 2003; Bazzoni, 2006).

TJs exist between the ECs and endcircle the cells like a continuous belt. Morphologically, they are represented by closely connected fragments of adjacent ECs known as the zonula occludens. At these locations , membranes are completely fused and form a five-layer construction. The number of fusion points between TJs differs and due to these differences, the level of tightness in different regions is also diverse (Bernacki, 2008).

Junctional complex	**Proteins**	**Localization**	**Function**
Tight junction (TJ cytoplasmatic plaque proteins)	Occluding	Membrane , multipass membrane protein, cell-cell contact, tight junction	Formation and regulation of TJ paracellular barrier permeability, interacts with ZO-1, -2, -3, F-actin
	Claudin 3, 5, 12	Membrane , multipass membrane protein, cell-cell contact, tight junction	Tight junction-specific obliteration of the intercellular space, directly interacts with ZO-1, -2, -3, F-actin
	JAM-A, -B, -C	Mebrane, single-pass type I membrane protein, cell contact, tight junction	JAM-A tight junction formation, neutrophile and monocytes transmigration, interaction with Par3 JAM-B lymphocytes transmigration, interaction with JAM-C JAM-C cell-cell adhesion, leukocytes transmigration, interaction with JAM-B
	ZO-1, -2, -3	Cytoplasm, the cytoplasmic face of tight and adherens junction	ZO-1 stabilization of junctions, signal transduction, interact with occludin, claudins, cingulin, ZO-3 ZO-2 stabilization of TJ and AJ, interacts with occludin ZO-3 interacts with occludin, claudins, ZO-1
	Par 3, Par 6	Cytoplasm, intracytoplasmic membrane, the cytoplasmic face of TJ	TJ assembly, the Par6, Par3 complex links GTP bound Rho small GTPases to atypical proteinkinase C proteins
	Afidin	Cytoplasm, the cytoplasmic face of TJ	Assembly of TJ and AJ, interaction JAMs, catherin, catenin, F-actin, nectin
	Cingulin	Cytoplasm, the cytoplasmic face of TJ	Formation and regulation of the TJ permeability, anchoring the TJ proteins to actin based cytoskeletons, interaction with ZO-1, JAMs

	7H6	Cytoplasm, colocalizes with actin stress fibers	F-actin cross-linking protein, anchor actin to other TJ an AJ proteins, inetract with ZO-1, catenin
	Rab3b, Rab 13	Lipid anchor, the cytoplasmic face of TJ, cytoplasmic vesicle membrane	Participation in polarized transport, assembly and/or activity of TJ
	PKC	Cytoplasm, membrane, endosome	Cell polarization processes, biogenesis of TJ, interaction with Par3, Par6, Rac, Cdc42, actin cytoskeleton
	G protein	Membrane , multipass membrane protein, cell-cell contact, tight junction	TJ biogenesis, stabilization of TJ, regulation of permeability
Adherence Junction	Ve-catherin	Membrane, single-pass type I membrane protein, cell-cell and cell-matrix boundaries	Control the cohesion and organization of the intercellular junctions
	Catenin	Multiprotein cell-cell adhesion complex, cytoplasm, the cytoplasmic side of AJ, cytoskeleton	With Ve-cadherin produces a complex which linked to the actin filament network, and it is imporatant for Ve-catherin cell-adhesion properties

Tab. 1 Brain endothelial junctional complex proteins, their localization and function (Gonzales, 2003)

2.2.1.2 Role of Astrocytes in BBB

Astrocytes are critical in the development and maintenance of BBB charakteristics. Two main types of astrocyte cells can be distinguished in the brain. Protoplasmic cells exist in the grey matter and fibrillary cells are present in the white matter. Due to their localization astrocytic end-feet have a few specific characteristics. For example , high concentrations of the water channel aquaporin 4 (AQP4) and the K+ channels Kir4.1 are palced within the orthogonal arrays of particles (OAPs). This accumulation is connected with the expresion and action of agrin-heparin sulphate proteoglycan. This protein is produced in the axtracellular matrix sheet by the basal lamina. Agrin is important for BBB integrity and accumulates when the barrier tightens. Furthemore, its splice variant Y0Z0 is a specific element of the capilary basal lamina formed by brain ECs. (Abott, 2006; Bernacki, 2008)

Astrocytes play a major role in neuronal metabolism, nutrition and discharge of used substrates (Abbot, 2006).

2.2.1.3 Role of Pericytes in BBB

Pericytes are small vessel wall-associated cells that originate developmentally fromthe mesoderm and differ from mesenchymal cells. They are separeted from ECs by the basal lamina but they are gab junctions provide the contact spots.

In the brain, pericytes are responsible for the rugulation of EC activity, mediation of inflammation and control of capillary-like structures (CLS) (Bernacki, 2008).

In the rat brain, pericytes covar approximately 25% of capillary outer surfaces. Pericytes are placed at capillary straight parts or locatedat capillary connections. Co-culture stadies have revealed that capillaries change their phenotype from multilateral to spindle-like when they connect to CLS. However this type of association accurs only in the presence of astrocytes (Ramsauer, 2002).

2.2.1.4 Role of neurons in BBB

Given the dynamic nature of neuronal activity and the considerable metabolic needs of nervous tissue, the microcirculation of the brain must be highly responsive to the tissue it supplies. Indeed, metabolic coupling of regional brain activity to blood flow is the basic of the functional neuroimaging (Buxton, 1997).

There is evidence that communication between neurons and the vasculature may not simply regulate blood flow, but BBB permeability as well (Brain, 2005).

In previous studies anatomical evidence has been found for direct innervation of the microvascular endothelium and associated astrocytic processes by noradrenergic, seratonergic, cholinergic and GABA-ergic neurons. Chemical lesion of the locus coeruleus from which the noradrenergic projections to the vasculature originate, increases the vulnerability of the BBB to acute hypertension. Significant loss of cholinergic innervation of cortical microvessels has been observed in Alzheimer´s disease (Brain, 2005).

2.2.1.5 Role of the Extracellular Matrix (EM)

The EM of the basal lamina also interacts with the cerebarl microvascular endothelium. Disruption of the extracellular matrix is strongly associated with increased BBB permeability in pathological states. Matrix proteins can influence the expression of endothelial TJ proteins (Rascher, 2002).

2.2.2 Pathophysiology

Disruption of the BBB is generally believed to be harmfull in most circumstances as it can cause the influx of leukocytes, potentially neuroactive compounds and water from blood. Many CNS diseases, including a diverse range of inflammatory diseases, diabetes, cancer and microbial infection, cause such a disruption. The most progressive BBB breakdown is associated with a diverse range of CNS inflammantory conditions (Stamatovic, 2008).

Inflammatory reactions occur in the brain in various CNS disease, including autoimmune, neurodegenerative and epileptic disorderes. Proinflammatory and antiinflammatory cytokines and related molecules have been discribed in CNS and plasma, in experimental models of seizures and in clinical cases of epilepsy. Inflammation involves both the innate and the adaptive immune systems and shares molecules and pathways also activated by systemic infection. Experimental studies in rodens show that inflammatory reactions in the brain can enhance neuronal excitability, impair cell survival, and increase the permeability of the blood-brain barrier to blood-borne molecules and cells (Vezzani, 2005).

One of the mechanisms by which cytokines contribute to the inflammatory response at the level of the BBB and blood – CSF barrier is by increasing the expression of selectins and adhesion molecules , chemokines, and their receptors on endothelial and epithelial cells. These molecules, by interacting with integrin molecules on leukocyte membrane surface are responsible for leukocyte recruitment from the bloodstrea, promoting their adhesion and eventual entry into the perivascular space, CSF and CNS parenchyma (Ransohoff, 2003).

2.2.3 The BBB and Epilepsy

Transient changes have been demonstrated in the physiology and structures of the BBB in various CNS injuries such as status epilepticus, infections, and traumatic and ischemic events. An impaired BBB and inflammatory state are common features of neurological diseases associated with the late onset of epilepsy. Proinflammatory cytokines are elevated in experimental animal brains after ischemia and in the CSF of stroke and epilepsy patients. Cytokine release causes subsequent up-regulation of endothelial and neutrophil adhesion molecules in human cerebrovascular endothelial cells during hypoxic injury, leading to transmigration of leukocytes across the endothelium and the BBB. Leukocyte recruitment may trigger signal transduction cascades, resulting in tight junction disorganization and BBB breakdown. Although the mechanism of delayed onset of epilepsy remains unclear, available data suggest that inflammation and breakdown of the BBB are necessary components of epileptogenesis following brain injury (Choi, 2008).

2.3 Epilepsy

2.3.1 Definition

Epilepsies are one of the most common neurological conditions with a prevalence of approximately 1-1.5% in the general population and, therefore, are considered a public health problem (Lopes, 2008). It has been estimated that about 7%–8% of the population experience at least one epileptic seizure during their lifetimes. The basic mechanism of epileptic seizures has not been fully elucidated (Karis, 2008).

Epilepsy refers to conditions of chronic recurrent epileptic seizures (ES). ES represent the intermittent and self-limited clinical manifestations (signs and symptoms) that result from abnormally excessive and synchronous activity of neurons. Seizures are categorized as partial (with the initial activation of a limited number of neurons in a part of one hemisphere) or generalized (with the initial activation of neurons throughout both hemispheres).

Epilepsy, however, is more than just recurrent seizures. It includes a specific etiology, associated neurologic abnormalities, a genetic background, environmental factors, responses to various therapies, and other factors. The disability and the effect on quality of life may be different for each individual (David, 2002).

2.3.2 Pathogenesis

Normal neuronal activity occurs in a nonsynchronized manner, with groups of neurons inhibited and excited sequentially during the transfer of information between different brain areas. Seizures occur when neurons are activated synchronously.

Interictal spike discharge are often observed on EEG recordings from epileptic patients. These are due too synchronous depolarization of a group of neurons in an abnormally axcitable area of brain. This is known as the paroxymal depolarizing shift and is followed by a hyperpolarozing afterpotential that is the celular correlate of the slow wave that follows spike discharges on the EEG. The shift is produced by depolarizing currents generrated at excitatory synapses and by subsequent influx of sodium or calcium through voltage-gated channels.

Normally, discharging excitatory neurons activate nearby inhibitory interneurons that suppress the activity of the discharging cell and its neighbors. Most inhibitory synapses utilize the neurotransmitter GABA. Voltage-gated and calcium – depend potassium currents are also activated in the discharging neuron to suppress excitability. Adenosine generated from adenosine triphosphate (ATP) released during exitation futher

suppresses neuronal extitation by binding to adenosine receptors present on nearby neurons. Disruption of these inhibitory mechanisms by alternations in ion channels, or by injury to inhibitory neurons by synapses, may allow for the development of a seizure focus. Groups of neurons may become synchronized if local excitatory circuits are enhauced by reorganization of neural networksafter brain injury.

Spread of the local discharge occurs by a combination of mechanisms. During paroxysmal depolarizing shift, extra cellular potassium accumulates, depolarizing nearby neurons. This involves increased calcium influx through voltage-gated channels and through the NMDA subtype of glutamate receptor-gated ion channels. NMDA receptor-gated channels pass calcium ion but are relatively quiescent during normal synaptic transsmision because they are blocked by magnesium ions. Magnesium block is relieved by depolarization. In contrast, the effect of inhibitory synaptic neurotransmission appears to decrease with high-frequency stimulation. This may be partly due to rapid desensitization of GABA receptors at high concentrations of released GABA. The net effect of these changes is to recruit neighboring neurons into a synchronous discharge and cause a seizure (McPhee, 2006).

2.3.3 Classification

The classification of epileptic seizures by the International League Against Epilepsy was last revised in 1989 (Tab. 2). The classification is important because etiologic diagnosis, appropriate treatment, and accurate prognostication all depend on the correct identification of seizures and epilepsy. There are 2 main seizure types: partial seizures and primary generalized seizures. Partial (formerly referred to as focal) seizures show either clinical or EEG evidence of onset from a localized area within the cerebral hemisphere. The nature of the signs and symptoms in most cases indicate the region of the brain involved by the epileptic process. Partial seizures are designated as simple or complex. Complex partial seizures are associated with loss of consciousness. In simple seizures, the epileptic process is usually confined to neocortical structures, and the limbic system and brain stem are spared. Most simple seizures are less disabling than those associated with loss of consciousness. Partial seizures can spread and develop into secondarily generalized seizures. Primary generalized seizures originate simultaneously from both cerebral hemispheres, and clinical manifestations involve both sides of the body. Primary generalized seizures first occur at an earlier age, and are more likely to be associated with a family history of seizure disorders, but are less likely to be associated with focal cerebral lesions. Some seizures remain unclassified because the underlying mechanism of their origin or propagation is unknown (Karis, 2008).

Partial Seizures
(seizures with focal onset)

I. Simple partial seizures (consciousness not impaired)
- **1.with motor signs**
- **2.with somatosensory or special-sensory symptoms**
- **3.with autonomic symptoms**
- **4.with psychic symptoms**

II. Complex partial seizures (consciousness impaired)

1. starting as simple partial seizures
- **without automatisms**
- **with automatisms**
- **with impairment of consciousness at onset with out automatisms**

III. Partial seizures evolving into secondarily generalized seizures

Generalized Seizures

I. Absence seizures and atypical absence seizures
(may have the following components: mild clonic, atonic, tonic, autonomic activities or automatic behavior)

II. Myoclonic seizures

III. Clonic seizures

IV. Tonic seizures

V. Tonic-clonic seizures

VI. Atonic seizures

Unclassified Epileptic Seizures

Tab. 2 International classification of epileptic seizures (Commision on classification and terminology of the ILAE)

2.3.4 Syndroms

Classifying into syndromes takes a number of characteristics into account, including the type of seizure; typical EEG recordings; clinical features such as behavior during the seizure; the expected course of the disorder; precipitating features; expected response to treatment, and genetic factors (2).

Epileptic syndromes can be either idiopathic or symptomatic of underlying brain damage or disease. In general, idiopathic forms have a better prognosis in terms of both seizure control and eventual remission than symptomatic forms. Epileptic syndromes include: seizure syndromes in newborns, febrile convulsions, West syndrome (infantile spasms), Lennox-Gastaut syndrome, childhood absence epilepsy, juvenile myoclonic epilepsy, Rolandic epilepsy, Landau-Kleffner syndrome, Rasmussen's encephalitis (syndrome), progressive myoclonic epilepsy, temporal lobe epilepsy, and frontal lobe epilepsy. Types of epilepsy that appear in infancy range from benign to severe, although persistent epilepsy that begins during the first year of life tends to remain severe, sometimes throughout life. Some authorities would not include febrile (fever-generated)

seizures in this list. Many consider them to be non-epileptic seizures because they cease to be a problem as the child grows older (2).

2.3.4.1 Temporal lobe epilepsies (TLE)

TLE can be devided into those arising from from the mesiobasal structures (amygdala and hippocampus, within the medial and inferior aspect of the temporal lobe) and those arising from the temporal neocortex.

Mesiotemporal lobe epilepsy is prototype of partial epilepsy syndromes. A family history of epilepsy is common and the patient often has a history of febrile convulsions. Unprovoked seizures frequently begin in childhood. Memory deficits are common but are most profound when the memory-dominant temporal lobe is involved. Seizures become more frequent and severe after several years and are commonly intractable. Temporal lobectomy achieves seizure freedom in 80% ti 90% of those with refractory seizures of mesiobasal origin (David, 2002).

Amygdalohippocampal sclerosis and atrophy are the most common pathological findings and are readily identified with appropriate magnetic resonance imaging techniques. The scalp-recorded ictal EEG patterns may consist of rhytmic slow waves or sharp waves and can be unilateral or bilateral, regardless of the distribution of the disease. Intra cranial recording with mesiobasal depth electrodes may reveal low-amplitude fast activity, rhytmic spikes or rhytmic slow waves at seizure onset. These findings help to differentiate a mesiobasal from a neocortical seizure onset, altought seizures arising from the parahippocampal gyrus may have similar EEG findings. Interictal positron emission tomography may show a temporal lobe hypometabolism. Ictal single-photon emission computed tomographic studies reveal temporal lobe hyperperfusion, whereas the interictal image may reveal temporal lobe hypoperfusion (Newton, 1996).

Seizures of mesiobasal origin are more common than neocortical. Simple partial seizures often begin with an indescribable strange sensation, rising epigastric discomfort or nausea. Other prominent initial signs and symptoms include fear, anxiety, sensory phenomena autonomic signs and psychic symptoms (David, 2002).

2.3.4.2 Frontal lobe epilepsies (FLE)

Seizures arising from the frontal lobe are characterized as simple partial seizures, complex partial seizures, secondarily generalized seizures or combination of these. Frequent daily events are more characteristic of frontal lobe than temporal lobe epilepsies. The seizures are generally shorter in duration compared with temporal lobe seizures and

are more commonly associated with a rapid secondary generalization. The symptoms of simple partial seizures of frontal origin may be difficult for the patient to discribe. With complex partial seizures of frontal lobe origin there may be minimal to no postictal confusion (David, 2002).

In frontal lobe epilepsies, the interictal EEG may show no abnormalities, but background asymmetries and frontal spikes or sharp waves with or without associated slow waves are characteristic. Secondary bilateral synchronization of the interictal epileptiform activity (and slow activities) is a feature of frontal lobe epilepsies originating from the interhemispheric and inferior cortical regions. The ictal EEG recordings of frontal lobe epilepsies may reveal various patterns that accompany the initial ictal symptoms, such as unilateral attenuation of the background activities, lowamplitude rhythmic fast activity, rhythmic spike or sharp waves, or slow waves that may be unilateral or bilateral. Intracranial depth or subdural electrode evaluations are generally necessary for localizing the epileptogenic zone. Magnetic resonance imaging may reveal structural lesions or developmental anomalies in about a half of frontal lobe cases (Lorenzo, 1995).

2.3.4.3 Status epilepticus (SE)

SE was defined by its duration, that is, as continuous seizures that last for more than 30 minutes or two or more sequential seizures without full recovery of consciousness between seizures. More recently, authors suggest that SE should be defined as any seizure lasting longer than 5 minutes based on natural history data that show typical generalized convulsive seizures that resolve spontaneously after 3-5 minutes.

Although any type of seizure can be sustained or recurrent, the most frequent and potentially dangerous type of SE is generalized convulsive SE. The overall mortality rate is about 20%. Death is most often is related to an underlying cause of brain injury.javascript:showcontent('active','references'); The mortality rate is highest in elderly patients with hypoxic or ischemic central nervous system (CNS) insults (3). In the study by Kang et al., neuroligical outcomes and recurrence of SE were found to be strongly associated with etiology and the seizure type.

2.3.5 Epilepsy and Cerebral blood flow

It is generally accepted that during normal cortical processing increases in neuronal activity simultaneously increase the cerebral metabolic rate of oxygen and glucose, leading to an increase in CBF and cerebral blood volume (CBV), as the brain

attempts to perfuse active neurons with oxygenated hemoglobin. Studies demonstrated a rapid decrease in tissue oxygenation and an increase in deoxygenated hemoglobin that precedes the increase in CBF.

Epilepsy is an abnormal physiologic state which, unlike normal somatosensory processing, places supra-normal demands on the brain's autoregulatory mechanisms as a result of an enormous increase in the metabolic rate of oxygen following both interictal and ictal events. Hence, neurovascular-coupling mechanisms that apply in the normal situation may not be relevant to the epileptic brain. Whether or not CBF is adequate to meet the increased metabolic demands of epilepsy has been a long-standing debate. The initial hypoxia–hypoperfusion hypothesis, derived from histologic similarities between ischemic and epileptic brain damage, proposed that the cell damage following status epilepticus was caused by cerebral anoxia. Later studies refuted this theory based on findings that the relative increase in CBF was greater than the relative increase in cerebral metabolism. The cellular damage associated with status epilepticus was not identical to hypoxic injury (Schwartz, 2007).

Yoo et al. investigate the regional cerebral blood flow (rCBF) changes in patients with idiopathic generalized epilepsy (IGE). They compared brain single photon emission computed tomography (SPECT) images of drug naive IGE patients with those of age/sex matched healthy volunteers. The analysis showed that the rCBF of the IGE patients was significantly reduced in the anterior and posterior cingulate gyri, bilateral anterior nuclei and right dorsolateral nucleus of the thalamus, right superior colliculus of the midbrain, and the cerebellum at the level of uncorrected $p < 0.005$. In the small volume correction analysis for the thalamus and brainstem, the rCBF was also significantly decreased in the same brain regions at the level of FDR corrected $p < 0.05$. No brain regions of the IGE patients had increased rCBF.

Çarçak et al. studied regional cerebral blood flow (rCBF) changes in brain regions involved in seizures in both epileptic (genetic absence epilepsy rats from Strasbourg) and nonepileptic rats to map the differences that may be related to the resistance to kindling. The results show that rates of rCBF increased in stimulated epileptic and nonepileptic groups compared to nonstimulated controls. The rCBF increase in stimulated epileptic rats was larger and more widespread than that observed in stimulated nonepileptic rats (Fig.4). The rCBF increase in the somatosensory cortex, ventrobasal and anterior thalamic nuclei, hypothalamus, subthalamic nucleus, piriform, entorhinal and perirhinal cortex, amygdala, CA2 region of hippocampus, and substantia nigra was statistically significantly larger in stimulated epileptic rats compared to stimulated nonepileptic rats. This widespread activation in GAERS involves the somatosensory cortex and thalamus,

which are both known to be involved in the expression of absence seizures as well as numerous limbic regions thought not to play a role in the expression of absence seizures, suggesting an interaction between corticothalamocortical and limbic circuitries (Çarçak, 2009).

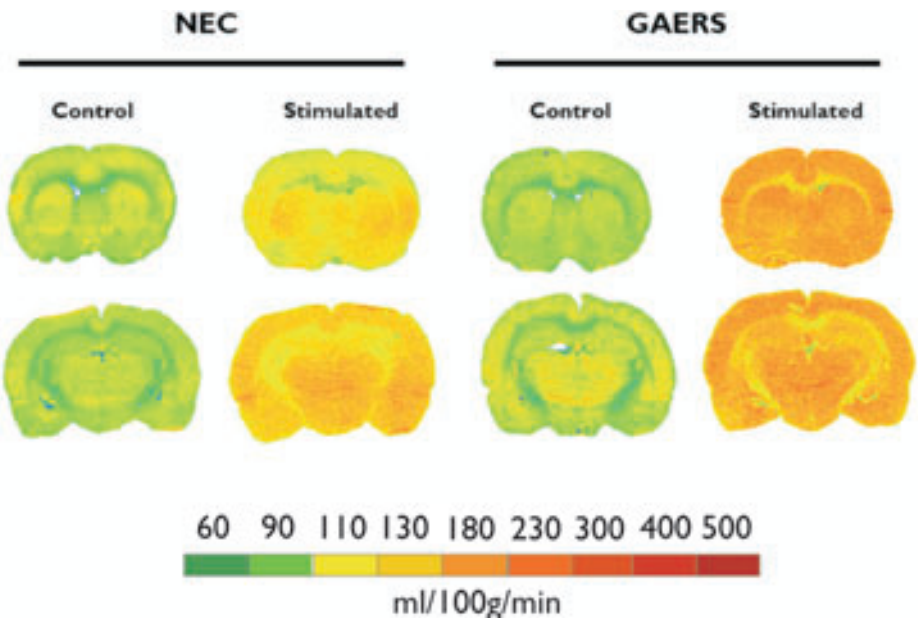


Fig. 4. Color-coded images from iodoantipyrine-labeled brain sections showing the distribution of cerebral blood flow rates during a stage 2 kindled seizure in NECs (nonepileptic rats) and GAERS (epileptic rats). The seizure-induced hyperperfusion is larger and more widespread in GAERS than in NECs (Çarçak, 2009).

2.3.6 Neuroimaging in epilepsy

Structural and functional neuroimaging continue to play an increasing role in the presurgical evaluation of patients with epilepsy. Assessing brain structure or function with imaging studies is essential in the diagnosis and management of epileptic seizure disorders.

2.3.6.1 Structural imaging

Volumetric MRI (VMRI)

Volumetric MRI has been used for quantifying structural pathology in patients with neurological disease and has been especially useful for predicting preoperative neuropsychological functioning and post-operative decline in patients with epilepsy. Volumetric MRI is important in validating the role of the hippocampus and other medial

temporal lobe structures in learning and memory, and in determining which patients are at greatest risk for memory decline following temporal lobectomy (McDonald, 2008).

A recent study found that left hippocampal volume and perirhinal volume asymmetry independently predicted verbal fluency and confrontational naming in patients with TLE (temporal lobe epilepsy). In TLE, the most commonly reported findings are associations between hippocampal or amygdalar volumes and depression. One study found that a reduction in left hippocampal volume was associated with depression in TLE, but only for patients with a right-sided seizure focus (Alessio, 2006).

Voxel-based morphometry (VBM)

VBM is an automated, quantitative imaging method that involves voxel-wise comparisons of the distribution of grey and white matter between groups of subjects. VBM studies have demonstrated reductions in temporal and extratemporal tissue concentrations in TLE, and there is some data linking regional reductions to cognitive impairment. For example, Bonilha et al. performed VBM on 36 TLE patients with hippocampal atrophy and found that reductions in gray matter concentration of the hippocampus, entorhinal cortex, and perirhinal cortex were associated with general and verbal memory impairment. In addition, gray matter reductions in limbic and frontal structures, including the orbitofrontal and cingulate cortex, predicted poorer memory performances in TLE (McDonald, 2008).

Diffusion tensor imaging (DTI)

DTI tractography is a relatively new MRI technique for visualizing and quantifying the integrity of white matter tracts in the brain by measuring a degree of water diffusion and its directionality. That is, the diffusivity of water is stronger along white matter fibers and is decreased orthogonal to those fibers. By applying diffusion-weighted gradients in multiple directions, the degree of anisotropy of overall motion in a voxel can be measured and expressed as an index of the directionality of diffusion or the relative volume of water movement (Wakana, 2007).

^{1}H MRS

^{1}H MRS is an imaging technique that allows for measurements of specific brain metabolites. The most commonly evaluated metabolite is N-acetylaspartate (NAA), which is located primarily within healthy neurons and precursor cells. A reduction in NAA signal, or in its ratio to other metabolites such as creatine (Cr), is thought to reflect neuronal loss or dysfunction (McDonald, 2008).

2.3.6.2 Functional imaging

Positron emission tomography (PET)

PET images regional or focal cerebral activity according to the degree of uptake of radioactive agents. One advantage of PET is the availability of a wide variety of radioactive ligands for investigating pathophysiological mechanisms underlying the epileptic process. The rate of glucose uptake can be estimated with 2-[18F]fluoro-2-deoxy-D-glucose ([18F]FDG), and blood flow can be determined with 15O-labeled water (H215O). Several other agents are available for assessing cellular receptors of compounds such as benzodiazepine, dopamine, and opiates (Elson, 2002).

In TLE, studies of cognitive functioning have demonstrated associations between left perirhinal and inferior temporal hypometabolism and poor verbal associative memory , lateral temporal metabolic asymmetry and impaired verbal IQ and memory , left lateral temporal and thalamic metabolic asymmetry and impaired verbal memory and fluency, and left temporal pole hypometabolism and recognition of famous faces (McDonald, 2008).

Functional MRI (FMRI)

FMRI measures regional changes in cerebral blood flow, thus providing an indirect measure of neuronal activity. Increases in neural activity cause changes in the MR signal via a mechanism called the blood oxygen level-dependent effect. Increased neural activity causes an increased demand for oxygen and the vascular system actually overcompensates for this, increasing the amount of oxygenated haemoglobin relative to deoxygenated hemoglobin.

The concentration of deoxy-haemoglobin depends on the cerebral metabolic rate of oxygen ($CMRO_2$), cerebral blood flow (CBF) and cerebral blood volume (CBV). The common model assumes that neuronal activity increases oxygen consumption, increasing deoxy-haemoglobin concentration. After about 2 s, this initial change is followed by increased CBF, overcompensating for oxygen consumption and increasing CBV due to the elastic properties of blood vessels. The overall measured response, called the haemodynamic response function (HRF), reflects the residual effect of changes in $CMRO_2$, CBF and CBV (Schleim, 2009).

Magnetoencephalography (MEG)

MEG is an imaging technique used to measure the magnetic fields produced by electrical activity in the brain via extremely sensitive devices such as superconducting quantum interference devices. The clinical uses of MEG are in detecting and localizing

epileptiform spiking activity in patients with epilepsy, and in localizing eloquent cortex for surgical planning in patients with brain tumors or intractable epilepsy. MEG localizations of the central sulcus obtained from somatosensory evoked magnetic fields show strong agreement with these invasive recordings. MEG studies assist in clarification of the functional organization of primary somatosensory cortex and to delineate the spatial extent of hand somatosensory cortex by stimulation of the individual digits. This agreement between invasive measures of localization of cortical tissue and MEG recordings implies the effectiveness of MEG analysis (McDonald, 2008).

3 Reaserch questions and hypothesis

Aim

The aim of the study is to investigate the effect of CO_2 on neurovasculare coupling in response to epileptiform activity after trancallosal electric stimulation in rats.

Reaserch questions

1. What is the effect of CO_2 inhalation on cerebral blood flow (CBF)?

2. What is the effect of CO_2 inhalation on basal blood pressure (BP)?

3. What are the changes in excitability of brain tissue and in epileptic activity during CO_2 inhalation?

Hypothesis

1. CO_2 inhalation during the trancallosal electric stimulation causes vasodilatation of cerebral arteries and elevate CBF.

2. CO_2 inhalation during the trancallosal electric stimulation cause increases in basal BP.

3. Excitability of brain tissue decreases immediately after the CO_2 inhalation during the trancallosal electric stimulation.

4. CO_2 causes decreased pH.

4 Methods

4.1 Characteristics of animal groups

A rat model for myoclonic seizures was used in this investigation in which electrical stimulation of the somatosensory cortex elicits bilateral epileptiform afterdischarges (ADs) in the EEG, paralleled by clonic seizures. The rats used in this reaserch were male Wistar albino rats of 250-350g body weight. Rats were housed in separate cages in controlled environment with stable temperature 22 ± 1ºC and 12-h light/dark cycle with free access to food and water. All experiments were approved by Animal care and Use Committee of the Institute of Physiology and in agreement with the Animal Protection Law of the Czech Republic (compatible with European Community Council Directives 86/609/EEC).

4.2 Surgical preparation

Rats (n=6) were anaesthetized with 5% isoflurane and anesthesia was maintained with 2-3% isoflurane during the surgical procedure. The epidural silver EEG electrodes were implanted and fixed into the skull over sensorimotor cortices. Two stimulation silver electrodes were implanted epidurally over the right sensorimotor cortex 2mm apart. Two recording electrodes were implanted epidurally over both hemispheres to monitor spontaneous and evoked cortical EEG and reference and ground electrodes were put over the cerebellum (Fig. 5).

To measure regional cerebral blood flow (rCBF) during epileptic activity a self-made metal holder for Laser Doppler flowmeter (LDF) probe (Perimed,, Sweden) was fixed to skull with dental acrylic. The skull was thinned under the holder to enable laser rays transmit to the tissue. Laser Doppler probe was placed directly contralaterally to the stimulating electrodes (i.e. area with highest activation and the ADs rise) (Fig. 5). To measure arterial blood pressure (BP) and arterial blood gasses a plastic catheter PE50 was implanted into the common carotid artery. To prepare carotid artery the midline cut on the neck was used. To prevent thrombosis a plastic catheter PE50 was filled up with 1% heparin solution. To pull a plastic catheter PE50 from the midline incision to occipital area a subcutaneous tunnel was created. To prevent damage to extracorponal area an elastic jacket was used.

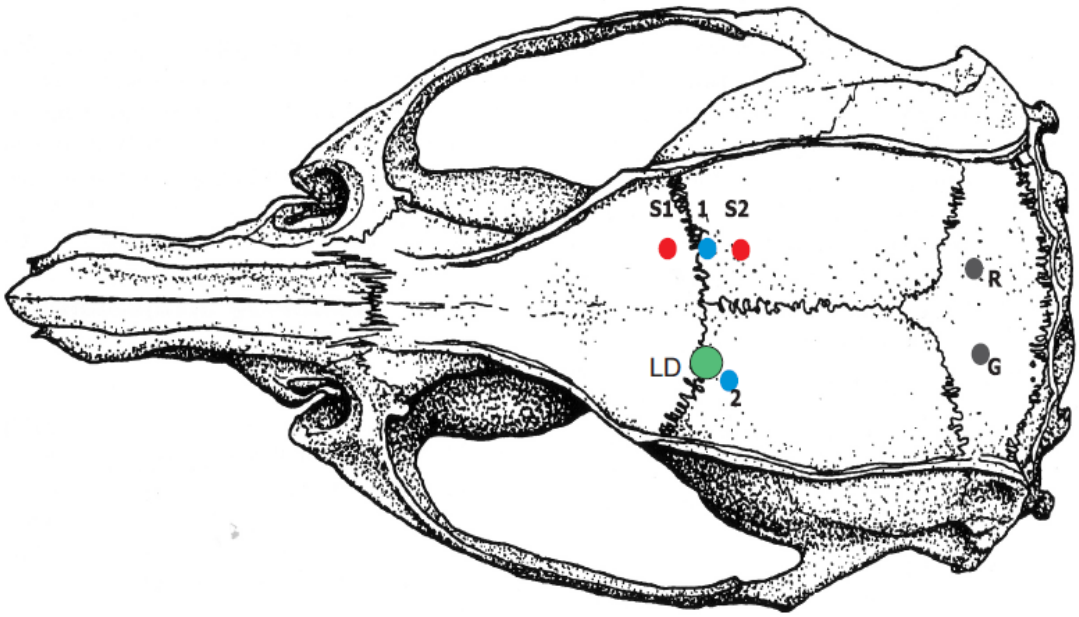


Fig.5 Localization of the electrodes. Silver electrodes were implanted epidurally over the senzoriomoror cortical area (AP = 1, L = 2) for stimulation (marked red), Laser Doppler probe was placed over the left sensorimotor cortical area (AP = 0, L =2) (marked green), registration electrodes were placed over the left and right sensorimotor cortical areas (marked blue). Reference and ground electrodes were put over the cerebellum.

4.3 Recordings and processing

After postsurgical recovery (1 day) animals ware placed into a plexiglas box (18x28x35 cm; supplied with a gas inlet and outlet), in order to prevent rotation of animal and they were connected to the recording system. To measure BP plastic catheter was connected to pressure sensor nad continuously recorded with Spike2 software. To evalute fast rCBF oscilations a small probe was inserted into the skull holder. Low pass filter was sat to time constant 0,03s. Analog output of LDF was connected to CDE digitalization unit and recorded with Spike2 software. EEG-data were acquired at 2kHz and filtered at 2-500Hz (Tucker-Davis Technologies, FL, USA). After 20 minutes of background recording we tested the effect of CO_2 on basal BP and rCBF by inflating a mixture of 10%CO_2, 20%O_2 and 70%N_2 for 30 seconds. ADs (afterdischarges) were elicited by means of a 15 sec stimulation train of biphasic rectangular pulses (1 ms duration) at a frequency of 8 Hz.

Biphasic constant current suprathreshold stimulus was applied under normal or elevated CO_2 atmosphere to elicit ADs. Absolute values of for AD threshold intensities were designated for individual animals. Stimulation sessions consisted of three (air exposure, CO_2 exposure, air exposure), with an interval of 15 min between the end of an AD and the next stimulation. Arterial blood gasses were analysed by ABL5 Blood gas system (BGA), which was designed for use on human blood in laboratory areas. The plastic catheter was disconnected and 150µl of fresh blood was sampled in to the glass capilary and immediately transported for measurment with BGA. At each blood taking the plastic catheter was fill up with 0,1% heparin solution. After all experiments rats were over-anaesthetized.

The data were calculated in Microsoft Excell list and data were analyzed offline using Matlab software (Mathworks, Inc., Natick, MA). Values are represented as mean ± SEM.

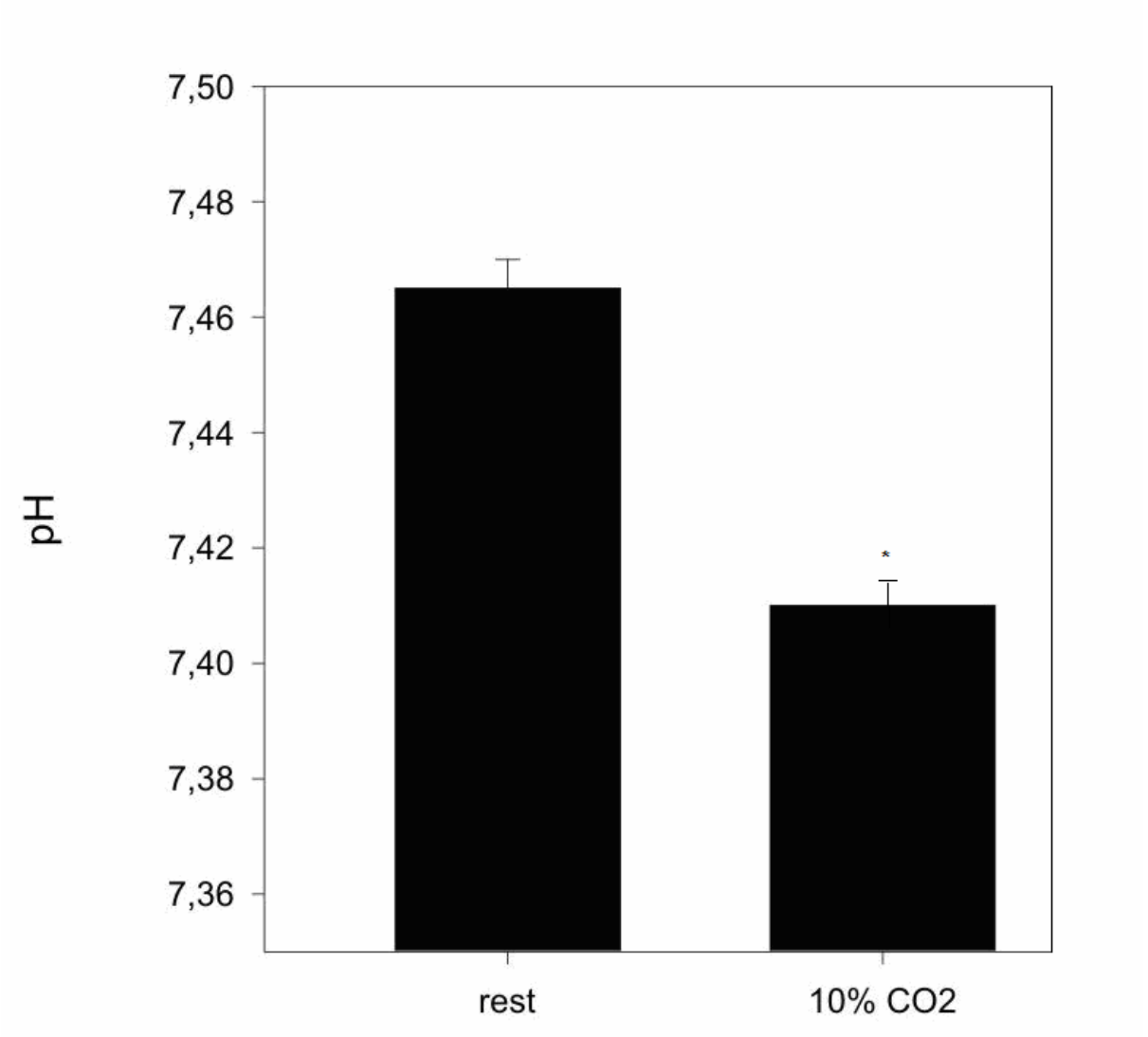
4.4 Statistical analysis

Statistical significance was tested using paired *t*-test, *t*-test and Wilcoxon Signed Rank Test. Analysis of variance was tested with Friedman Repeated Measures Analysis of Variance on Ranks and One Way Repeated Measures Analysis of Variance.

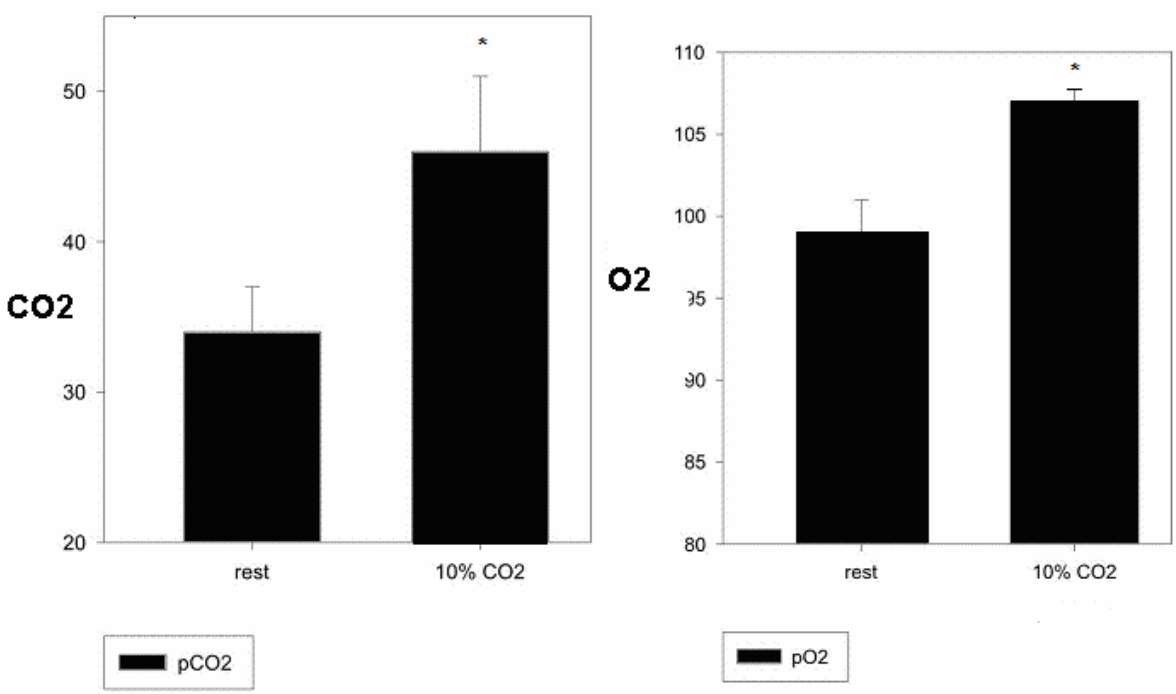
5 Results

5.1 CO_2 affects pCO_2, pO_2 a pH

To investigate the effect of CO_2 on blood pH the blood gases (p CO_2, p O_2) were analysed before and after aplication of 10% CO_2. After the exposure of 10% CO_2 blood pH decreased from 7.465 ± 0.005 to 7.41 ± 0. (Graph 1.). Decrease of pH was caused by pCO_2 increase in arterial blood from 34 ± 3torr to 46 ± 5torr (Graph 2.). These changes were accompanied by increase of pO_2 from 99 ± 2torr to 107 ± 1torr. (Graph 2.)



Graph 1. Blood gas analysis of pH. Blood pH decreased from 7.465 ± 0.005 to 7.41 ± 0 after the 10% CO_2 exposure. (* = P < 0,05)

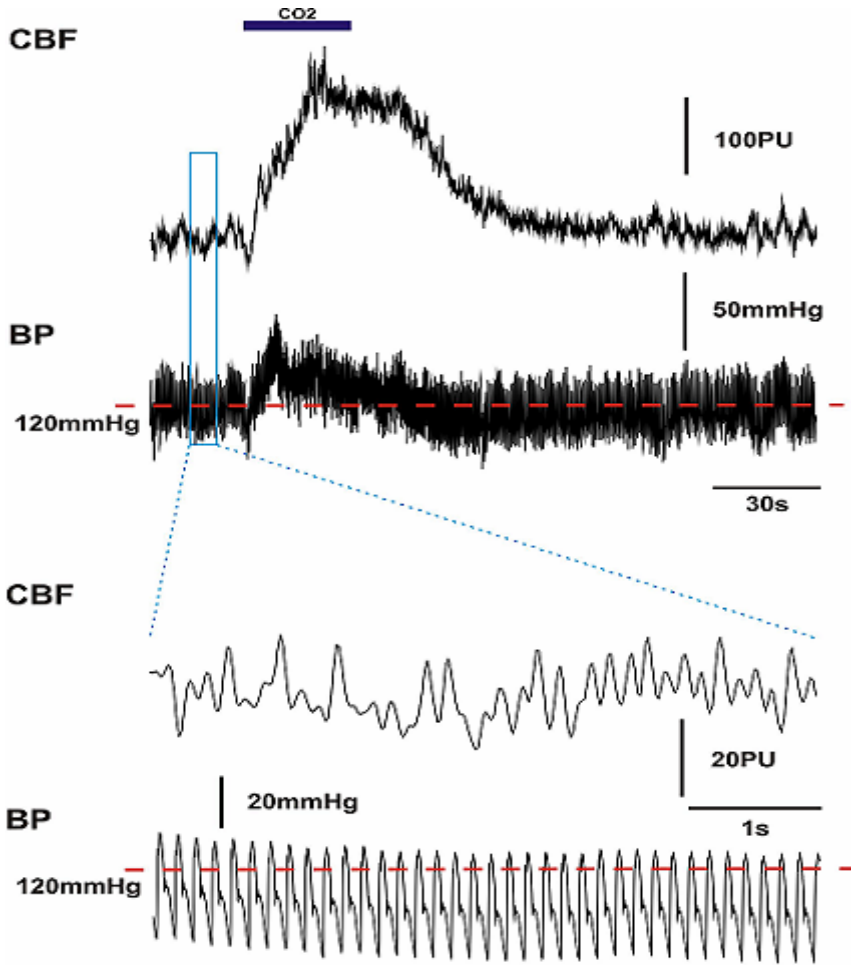


Graph 2. Blood gas analysis of pCO_2 (left part) and pO_2 (right part). pCO_2 increase in arterial blood from 34 ± 3torr to 46 ± 5torr (* = P < 0,05). pO_2 increase in arterial blood from 99 ± 2torr to 107 ± 1torr (* = P < 0,05).

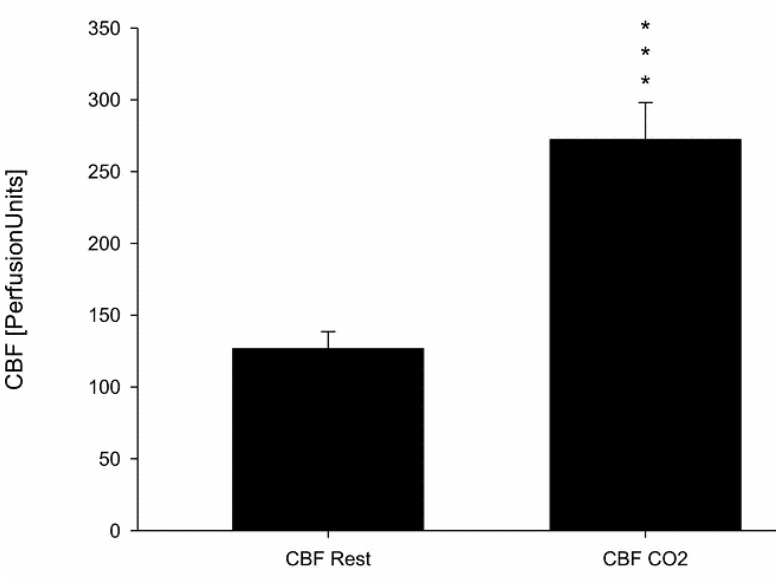
5.2 Effect of CO_2 on rCBF and BP

To measure regional cerebral blood flow (rCBF) Laser Doppler flowmeter was used. A self-made metal holder for Laser Doppler flowmeter probe was fixed to skull with dental acrylic. To evalute fast rCBF oscilations a small probe was inserted into the skull holder. Low pass filter was sat to time constant 0,03s. Analog output of LDF was connected to CDE digitalization unit and recorded with Spike2 software. After the inflating a mixture of 10%CO_2, 20%O_2 and 70%N_2 for 30 seconds rCBF significantly increased from 126.6 ± 11.9PU to 272.2 ± 25.9PU (Graph 4.). Latency of the rCBF maxima was 25.4 ± 2.3s (P = 0.044) (Graph 6.).

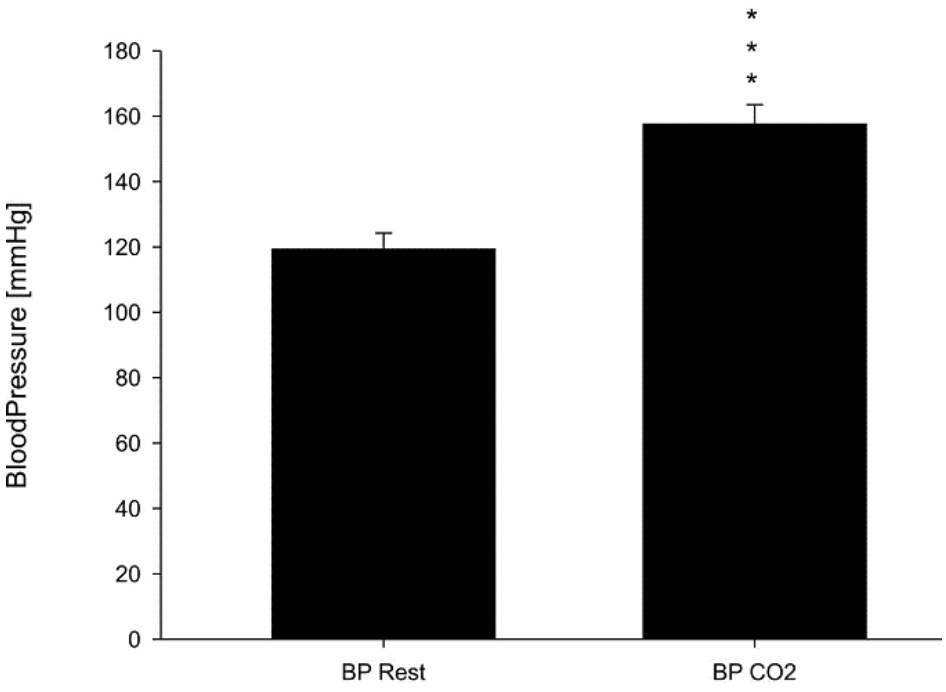
To measure arterial blood pressure (BP) and arterial blood gasses a plastic catheter PE50 was implanted into the common carotid artery. A plastic catheter was connected to pressure sensor nad continuously recorded with Spike2 software. After the inflating a mixture of 10%CO_2, 20%O_2 and 70%N_2 for 30 seconds BP significantly increased from 119.3 ± 5.0mmHg to 157.5 ± 6.0mmHg (P = <0.001) (Graph 5). Latency of the BP maxima was 16.9 ± 3.5s (P= 0.044) (Graph 6.).



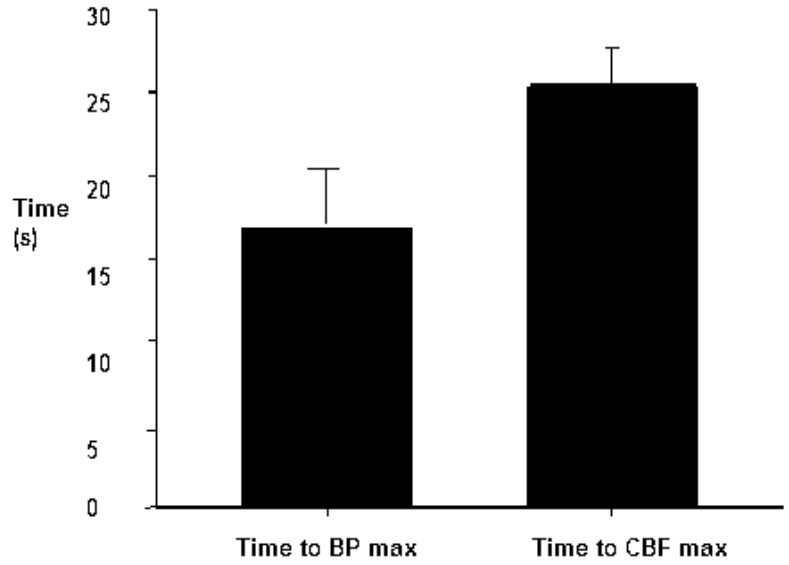
Graph 3. Effect of CO_2 on CBF and BP. The enlarged view of CBF recording shows individual heart oscillation.The enlarged view of BP recording shows individaul pressure oscilation. After the aplication of CO_2 there is a significant increase of both rCBF to 272.2 ± 25.9PU and BP to 157.5 ± 6.0mmHg.



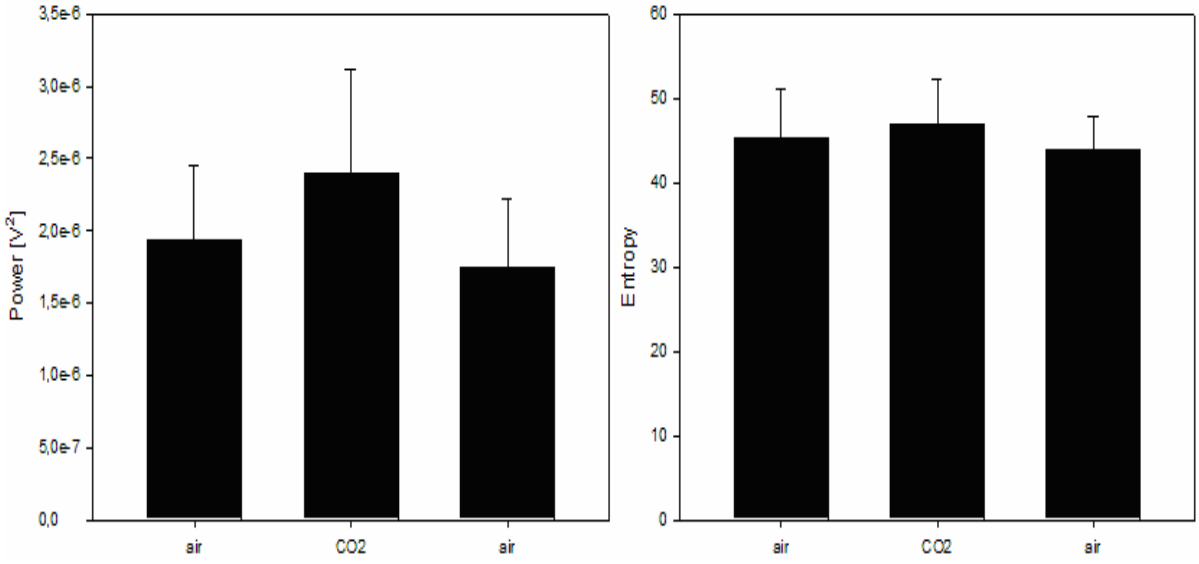
Graph 4. Effect of 10% CO_2 on rCBF. rCBF significantly increased from 126.6 ± 11.9PU to 272.2 ± 25.9PU (*** = P < 0.001).



Graph 5. Effect of 10% CO_2 on Blood pressure. BP significantly increased from 119.23± 5.0 mmHg to 157.5 ± 6.0mmHg (*** = P <0.001).



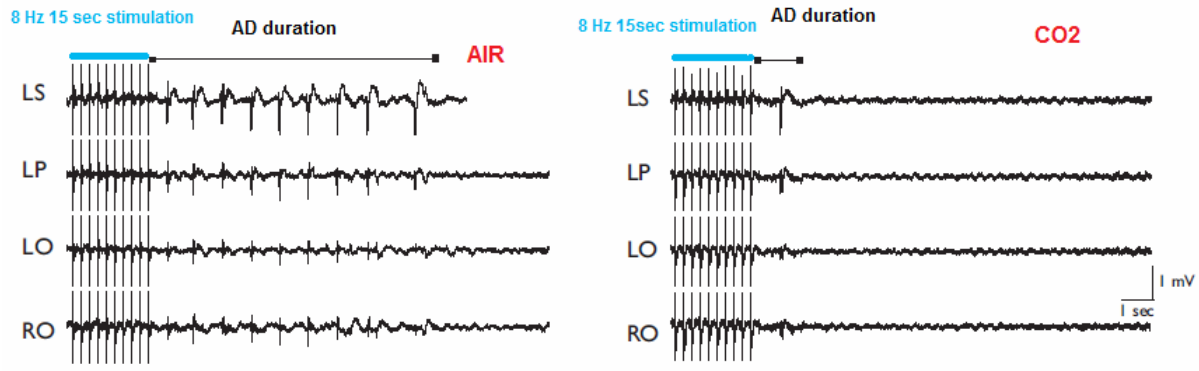
Graph 6. Latency of rCBF and BP maxima after 10% CO_2. Right column- latency of rCBF max, left column- latency of BP max. Latency of the rCBF maxima was 25.4 ± 2.3s (P = 0.044). Latency of the BP maxima was 16.9 ± 3.5s (P = 0,044).



Entropy and Power of the EEG signal. There is not a statistically significant difference in Entropy of EEG signal (P = 0.949). There is not a statistically significant difference in Power of the EEG signal. (P = 0.810).

5.3 CO_2 strongly reduces cortical AD duration in rats

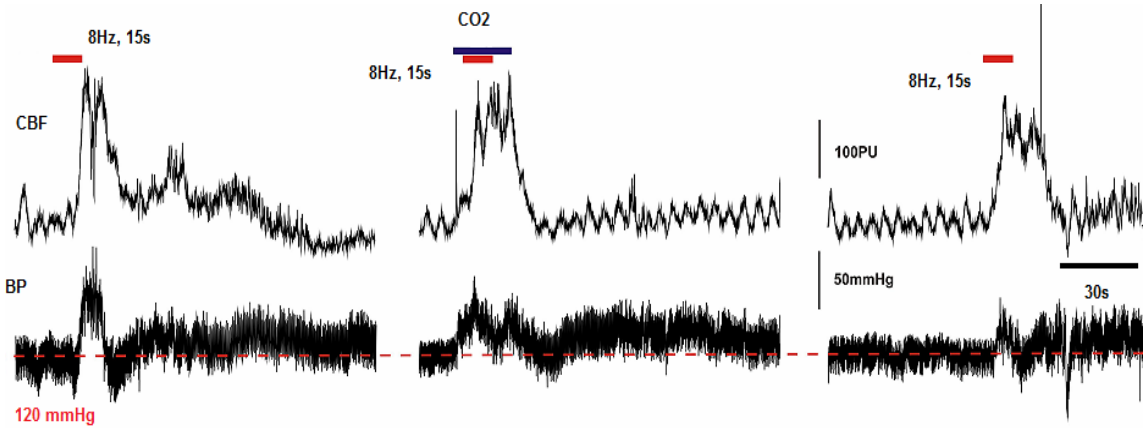
A rat model was used for myoclonic seizures where epileptic afterdischarges (ADs) are elicited in freely moving rats by stimulation of the sensorimotor cortex. ADs consisted of a spike-and-wave pattern seen bilaterally in the EEG that was accompanied behaviourally by clonic seizures. EEG monitoring revealed that the administration of CO_2 in normal air reduced or completely blocked stimulation-induced cortical ADs in unanesthetized adult rats (Graph 1). Transcallosal electrical stimulation produced cortical epileptic ADs which were paralleled by facial and fore limb clonic seizures.



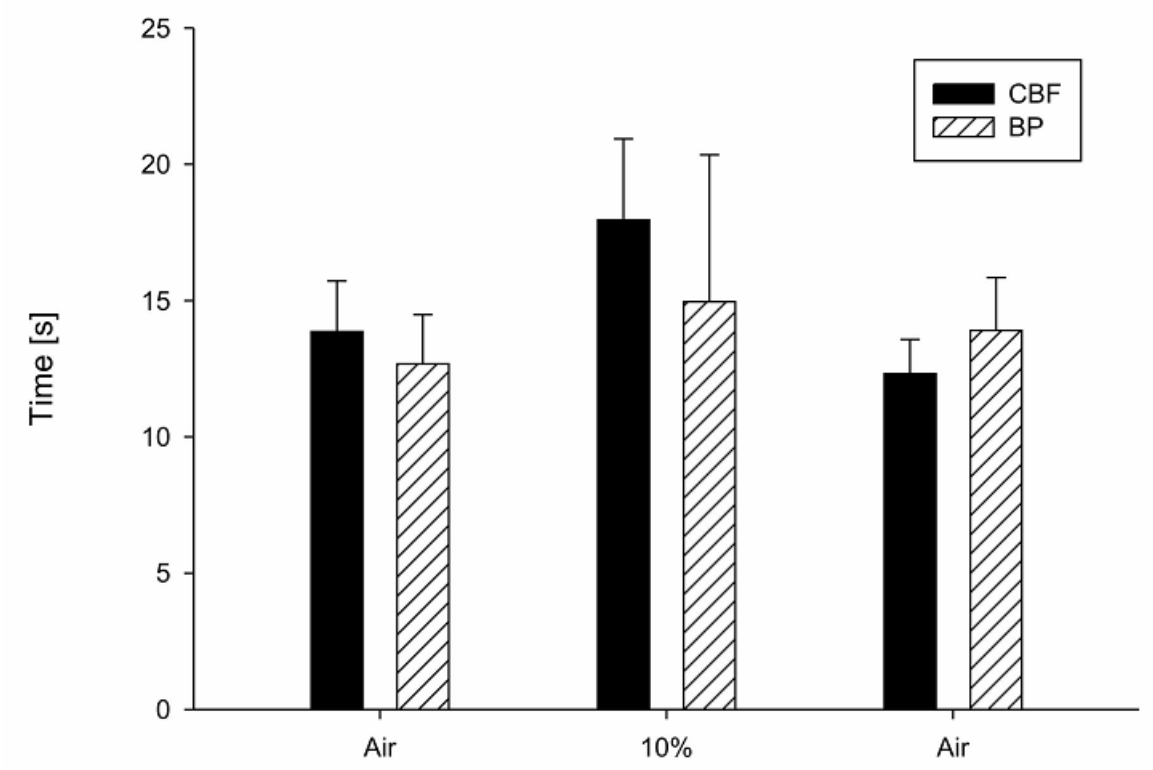
Graph 1. Cortical epileptiform afterdischarges in an adult rat in normal air and in the presence of 10% CO_2 evoked by electrical stimulation of the right senzorimotor cortex. Cortical ADs were seen bilaterally and were paralleled by clonic seizures of forelimb muscles. There was a near-complete block of ADs in the presence of CO_2 and CO_2 strongly reduced the behavioral seizures accompanying the ADs. LS: left somatosensory, LP: left parietal, LO: left occipital, RO: right occipital (Brožíčková, 2009).

5.4 CO_2 affects CBF and BP during transcallosal electric stimulation

Epileptic afterdischarges were elicited in freely moving rats by stimulation of the sensorimotor cortex. Stimulation sessions consisted of three exposure (air exposure, CO_2 exposure, air exposure), with an interval of 15 min between the end of an afterdischarge and the next stimulation. Elevated CO_2, however, reduced or completely blocked the stimulation-evoked epileptiform afterdischarges. During stimulation and epileptic afterdischarges rCBF significantly increased during CO_2 exposure. rCBF rapidly increased to 190.8 ± 29.2PU with latency of the rCBF maxima 13.9 ± 1.9s during first air exposure. During CO_2 exposure r CBF increased to 232.2 ± 46.2PU with latency of the rCBF maxima 18.0 ± 3.0s and increased to 187.6 ± 48.4PU with latency of the rCBF maxima 12.3 ± 1.3s after second air exposure. BP increased to 38.3 ± 4.7mmHg with latency of the PB maxima 12.7 ± 1.8s during first air exposure. During CO_2 exposure BP decreased to 30.0 ± 6.1mmHg with latency of the BP maxima 15.0 ± 5.4s and decreased to 20.9 ± 4.9mmHg with latency of the PB maxima 13.9 ± 1.9s during first air exposure.



Graph 6. Effect of CO_2 on CBF and BP during transcallosal electrical stimulation. During stimulation with exposure of CO_2 rCBF and BP significantly increased. Red mark- 15 sec stimulation train of biphasic rectangular pulses (1 ms duration) at a frequency of 8 Hz, blue mark- exposure of CO_2.



Graph 7. Latency of the rCBF and BP maxima after stimulation session (air exposure, CO_2 exposure, air exposure). Latency of both rCBF and BP significantly increased after exposure of CO_2. Black column- Latency of CBF, striped column- Latency of BP.

6 Discussion

6.1 Discusion of results

The goal of this research project was to identify the effect of 10%CO_2 on neurovascular coupling in response to epileptiform activity after trancallosal electric stimulation in rats and on physiological fuctions of brain tissue and blood vessels. The fundamental hypothesis was that CO_2 exposure during the trancallosal electric stimulation causes vasodilatation of cerebral arteries, increases regional cerebral blood flow and in basal blood pressure. Further hypothesis suggested that excitability of brain tissue decreases immediately after CO_2 exposure during trancallosal electric stimulation. Our last hypothesis was that CO_2 causes a decrease of pH in arterial blood. We tried to prove our hypotheses by blood gases analysis, meassurement of regional cerebral blood flow (rCBF), survey of basal blood pressure (BP) and by brain activity monitoring.

The first hypothesis concerning an increase in rCBF and in BP during CO_2 exposure in the trancallosal electric stimulation was partially confirmed. Inhalation of 10%CO_2 during electrical stimulation of senzorimotor cortex (8Hz, 15s) caused significant increase in regional cerabral blood flow. Surprisingly, basal blood pressure did not increase. BP decreased during stimulation with CO_2 exposure as well as during following stimulation without CO_2. Therefor it can be assumed that inhalation of CO_2 has no effect on basal blood pressure during the trancallosal electric stimulation. Latency of the maximal amplitude of rCBF and latency of BP significantly increased after stimulation with 10%CO_2 exposure. Comparison of these two latency periods however shows that they were not completely simultaneous. The same results were reached also in meassurement of latency after aplication of CO_2 without stimulation. This implies that regional cerebral blood flow is not completely dependable on basal blood pressure.

The second hypohesis concerning the effect of CO_2 on excitability of brain tissue was fully confirmed. We found that tracallosal electric stimulation produces cortical epileptical afterdischarges. These afterdischarges were accompanied by clonic seizures in facial area and in fore limb. After CO_2 exposure these symptoms were reduced and also stimulation evoked afterdischarges were reduced or completely blocked. Same results were confirmed in work of Tolner et al. (2008), in which they studied the effect of 5%CO_2 on cortical apileptic activity. For somatosenzoric cortex stimulation in animal models they used a bipolar electrode (8Hz, 15s, 1,5-7,5mA). The electrodes for afterdischarges monitoring were placed contralaterally. To asses tha effect of CO_2 on afterdischarges duration they used a reference and experimental group of eight rats. The experimental

group was stimulated in four phases by inhalation of 5%CO_2 in artificial air (gas mixture of 18%O_2 in N_2). The reference group was stimulated in normal air. Results show significantly shorter afterdicharges in experimental group. These data correspond to our data – inhalation of CO_2 during tracallocal stimulation decreases duration of cortical afterdischarges and reduces symptoms of clonic epileptic seizure. Tolner et al. further studied the effect of 5%CO_2 on cortical excitability during single evoked potencials meassurement. In the presence of 5%CO_2, the peak-to-peak amplitude (N1P2) of cortical potentials was reduced to about 84%. Notably, the effect was visible within 30 seconds after the start of gas administration (Fig. 6). These data demonstrate a rapid suppression of cortical excitability by CO_2 in unanesthetized subjects.

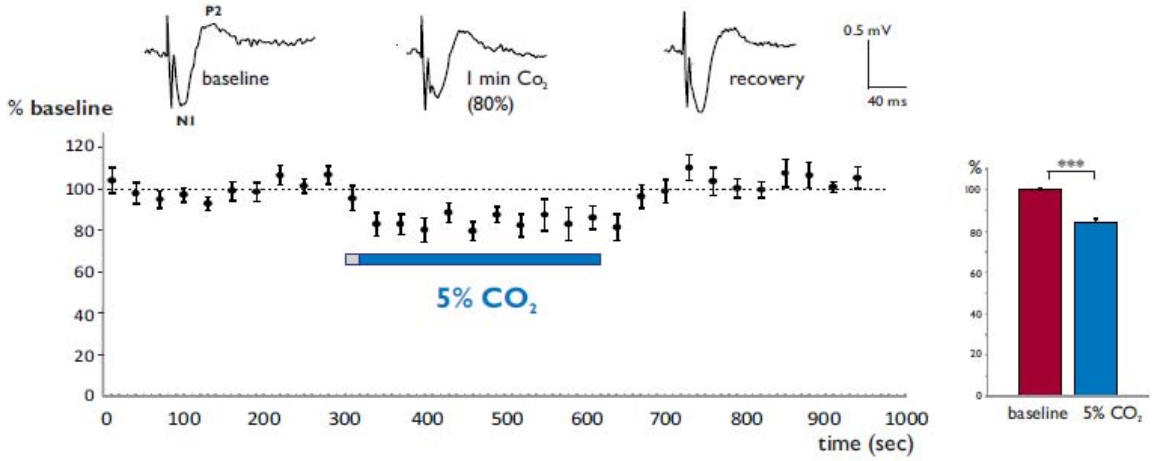


Fig.6 5% CO_2 rapidly reduces the somatosensory cortical response to single pulse stimulation in freely behaving rats. Figure shows example responses during baseline, 5%CO_2 and recovery and depict the measurement of the N1P2 amplitude (Tolner, 2008).

Hypothesis on the effect of CO_2 on blood pH was also confirmed. In blood gases analysis was determined the difference in pH before and after aplication of 10%CO_2. After aplication of CO_2 pH level decreased remarkably. The decrease in pH was caused by considerable increase of CO_2 in arterial blood accompanied by an increase in pO_2. This physiological effect of CO_2 causes malfunction of acidobasic balance and can lead to CNS depression. Hypocapnia increases neuronal excitability and is used diagnostically to induce seizures and therapeutically to prolong seizures during electroconvulsive therapy. Conversely, hypercapnia decreases neuronal excitability, causes sedation, and has a long history as an anesthetic. CO_2 levels influence tissue pH, which has also been linked to neuronal excitability. Dulla et al. (2005) hypothesized that CO_2-induced changes in pH

may alter neuronal excitability by altering extracellular adenosine and ATP concentrations. They used a combination of electrophysiology, an enzymatic adenosine biosensor and two-photon pH imaging to investigate the relationship between changes in brain CO_2 levels and altered neuronal excitability. Their results show that CO_2 influences cortical excitability via an interaction between extracellular pH, ATPmetabolizing enzymes and receptors for ATP and adenosine. These changes are responsible for the profound neuronal effects of CO_2 and suggest mechanisms to explain the global neurological effects of altered CO_2, such as seizures and sedation.

Experimental meassurements of physiological effects of CO_2 on brain tissue conducted in our study show a remarkable increase both in regional cerebral blood flow and basal blood pressure after CO_2 exposure. The increase of blood pressure during higher pCO_2 is probably caused by vasodilatation of cerebral arteries, which develops as a reaction on stimulation of so called ischemic recetors in brain stem. Hypercapnia directly stimulates vasomotorical area in truncus cerebri and possibly effects cells in C1 area. These neurons are stimulated when the blood pressure in cerebral arteries decreases under 60mmHg and that leads to their ischemia. The increase in intracranial pressure disturbs blood supply if vasomotorical area and hypercapnia increases neuronal excitability. As a result the arterial pressure increases.

We did not find a statisticaly significant difference in comparison of power of EEG signal in CO_2 exposure and of normal air exposure. However, there was a perceptible tendency to energy increase in CO_2 inhalation. CO_2 was to rats applied by direct allowance of artificial air (10%CO_2, 18%O_2, N_2) into the meassuring box. During the massurement of power of EEg signal in normal air, there was no air intake in the box. Rats may have reacted on the air drift, which could have led to increased brain activity of meassured areas and increased EEG signal power. Meassurements of entropy of EEG signal showed the same results.

6.2 Discusion of methodology

Methodology of chosen meassuring instruments carried a few aspects that might have had an influence on validity of acquired data. Laser Doppler Flowmeter (LDF) is relatively new method for tissue perfusion meassurement. The advantage of LDF is the possibility to coduct continuous meassurements for several hours without the must to kill the experimental animal (unlike many other methods for brain blood flow meassurements). On the other hand LDF enables determination of relative changes in perfusion but does not determinate absolute values. Another limiting factor might be a higher risk of influence of blood flow monitoring caused by extracranial aposition of LDF probe. It suggests that epidural placement might be more efficient. Large brain arteries are in the area of blood flow meassurement. Imperfect placement of LDF probe might be a source of inaccuracies in cerebral blood flow monitoring. Meassurements were performed in special boxes constructed to limit rats movements, however, animals managed to move or turn around. That might influence results or damage the probe. Brännström et al. (1999) tried to avoid these aspects in their study. They introduced a permanently anchored flow probe adapter centered round the burr hole. In this way, the flow probe could be repeatedly positioned, after removal and reattachment, at exactly the same anatomical location of the cortex. The flow probe rotates smoothly in the connector thus preventing the fiber from being twisted when the rat is moving around. However, as revealed in the baseline blood flow recording experiments CBF data were neither influenced by probe rotation nor tilting of the head to different positions. Repeated cortical CBF recording showed a stable CBF baseline.

Another factor that could have affected meassurement results was the aplication of artificial and normal air during investigation of CO_2 effects on brain tissue. In normal conditions we did not allow any air in the box. During the application of artificial air into the box, the air drift was flowing in directly on rats heads, which might have distracted them. Constant air flow from the tube to the box would be preferable.

7 Conclusions

The principal aim of the study was to investigate the effect of 10%CO_2 on neurovascular coupling in response to epileptiform activity after trancallosal electric stimulation in rats. In our experiments we studied the effect of 10%CO2 on regional cerebral blood flow, blood pressure, pH and duration of epileptic afterdischarges. Our results show that elevated CO_2 led to a significant increase of both regional cerebral blood flow and blood pressure, which returned to original values shortly after inflation of normal atmosphere. In addition, inhalation of 10%CO_2 during electrical stimulation of senzorimotor cortex (8Hz, 15s) caused significant increase in regional cerabral blood flow. This points to our hypothesis that CO_2 inhalation during the trancallosal electric stimulation causes vasodilatation of cerebral arteries and elevates regional cerebral blood flow. Surprisingly, basal blood pressure did not increase after 10%CO_2 exposure during electrical stimulation of senzorimotor cortex. Transcallosal electrical stimulation produced cortical epileptic afterdischarges which were paralleled by facial and fore limb clonic seizures. According to our hypothesis elevated CO_2 reduced or completely blocked the stimulation-evoked epileptiform afterdischarges and during stimulation and epileptic afterdischarges regional cerebral blood flow rapidly increased. Similar results occured in blood gas analysis. After the exposure of 10%CO_2 blood pH significantly decreased. Decrease of pH was caused by pCO_2 increase in arterial blood and these changes were accompanied by increase of pO_2. This denotes that our hypotheses were confirmed.

In total, our findings suggest that application of CO_2 may be effective in the acute treatment of epileptic seizures. The effect of CO_2 might be explained by both decreased excitability due to tissue acidosis and elevated regional cerebral blood flow levels.

The experiment was a component of a long-term research program at Institute of Physiology, Academy of Sciences of the Czech Republic, Department of Developmental Epileptology. The results were presented at 7. Conference of Czech Neuroscience Society and 1. Conference of Slovak Neuroscience Society (Prag, 2009).

I would very gratefully end up my study by saying that I apreciate to have the opportunity to take part in this study project. It gave me the possibility to look behind the courtains of the real science and into the work of scientists.

8 List of abbrevations

- **AA**- arachidonic acid
- **AJ**- Adherens Junctions
- **AD**- Afterdischarge
- **ATP**- adenosine triphosphate
- **BBB**- blood-brain barrier
- **BK channels**- potassium channels
- **CBV**- cerebral blood volume
- **CBF**- cerebral blood flow
- **cGMP**- cyclic GMP
- **CLS**- capillary-like structures
- **$CMRO_2$**- cerebral metabolic rate of oxygen
- **COX**- cyclooxygenase
- **CO_2**- carbon dioxide
- **Cr**- creatine
- **DTI**- Diffusion tensor imaging
- **EC**- endothelial cell
- **EETs** -epoxyeicosatrienoic acids
- **ES**- epileptic seizures
- **FLE**- Frontal lobe epilepsies
- **FMRI**- Functional MRI
- **HRF**- haemodynamic response function
- **IGE**- idiopathic generalized epilepsy
- **LCBF**- local cerebral blood flow
- **LDF**- Laser Doppler flowmeter
- **MEG**- Magnetoencephalography
- **mGluRs**- metabotropic glutamate receptors
- **NAA**- N-acetylaspartate
- **NADPH**- nicotinamide adenine dinucleotide phosphate
- **NMDA**- *N*-methyl-D-aspartate receptor
- **NO**- nitric oxide
- **NOS**- NO synthase, iNOS- inducible, eNOS- endothelial, nNOS- neuronal
- **pCO_2** -partial pressure of arterial CO_2
- **PET**- positron emission tomography
- **PGE2**- prostaglandin E2P
- **pO_2** -partial pressure of arterial O_2
- **rCBF**- regional cerebral blood flow
- **SE**- Status epilepticus
- **SMC**- smooth muscle cell
- **SPECT**- single photon emission computed tomography
- **VBM**- Voxel-based morphometry (VBM)
- **TJ**- Tight Junctions
- **TLE**- Temporal lobe epilepsies
- **VMRI**- Volumetric MRI
- **VVO**- vesicle/vacuolar organells
- **20-HETE-20**-hydroxyeicosatetraenoic acid

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