

**Effect of painful afferent stimulation on regional
cerebral blood flow and global cerebral blood volume
in anesthetized rats**

PhD thesis



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Introduction

Neuronal activation and functional hyperemia are not completely clarified physiological functions in the central nervous system. Increase in blood flow following neuronal activation is called activation-flow coupling or neurovascular coupling in the literature. The first theory of neurovascular coupling is originated from Roy and Sherrington in 1890 and called metabolic theory. This more than 100 years old theory is not able to explain totally the dynamism of the circulatory parameters in activation-flow coupling. However the exact mechanism of activation-flow coupling is not completely clarified, functional neuroradiology is based on this phenomenon.

There are several opportunity to model functional activation, the effect of non-noxiuos stimulation on cerebral circulation are widely examined. Investigations of pain-induced circulatory changes are more complex, because of the activation of sympathetic system and elevated mean arterial pressure, so there are quite a few data in the literature about pain-induced regional cerebral blood flow and blood volume. Our workgroup examined the effect of somatic afferent, painful stimulation on regional cerebral blood flow and global cerebral blood volume in anaesthetized rats.

The aims of our investigation were fourfold:

1. to measure the effect of noxious, painful C-fiber stimulation (sciatic nerve) in three different regions of the anesthetized rat brain (hypothalamus, thalamus and cortex)
2. to determine the possible involvement of different vasoactive substances like: potassium ion, free radical NO, prostaglandins or endogenous opioid peptides in pain-related blood flow changes
3. to determine the role of the activated sympathetic nervous system in the pain-induced regional blood flow and gCBV changes
4. to estimate gCBV changes during painful stimuli and to clarify the role of L-arginine-NO system in pain-induced gCBV changes

Methods

Operation

Experiments were performed in anesthetized (1.3 g/kg intraperitoneally injected urethane), relaxed (2.0 mg/kg intravenously injected pancuronium bromide) male Wistar rats, weighing 280-400 g. The depth of the anesthesia was continuously monitored by checking the cornea and cutan reflexes and supplementary doses of urethane were administered intravenously when necessary. The trachea was cannulated, and the animals were ventilated with a positive pressure with room air and supplemental oxygen. The right femoral artery was cannulated to measure systemic arterial blood pressure and heart rate, and to obtain arterial blood samples, and the right femoral vein was cannulated for drug administration. In some experiments the left femoral artery was also cannulated for intraarterial transfusion of homologue blood. Core temperature was measured with a rectal probe, the thermometer was connected to an electronically regulated heating pad and the temperature was maintained between 37.0-37.5 °C.

Regional cerebral blood flow (rCBF) was measured with laser-Doppler (LD) flowmetry in the contralateral hemispherium: in the hind limb area of the sensory cortex and in the medial part of the thalamus. The LD flux signals were measured with a two-channel laser-Doppler flow monitor. In another setup Auckland's H₂ gas clearance method was used to measure regional blood flow in contralateral thalamus and in hypothalamus.

Global cerebral blood volume (gCBV) was measured by Tomita's photoelectric method with Sándor's modification. In short, a miniaturized light source (1 mm wide, 1,5 mm long tungsten lamp) was positioned between the two hemispheres of the rat through a 1,3 mm in diameter hole of the skull and was fixed with dental cement. Photodiodes were attached to both sides of the skull to the outer surface of the lamina interna of the parietal bone and were secured with light-absorbent dental cement. Assuming that the light intensity, the distance between the lamp and the photodiode, and the light extinction caused by the brain tissue, remain unchanged during the experiment, the light intensity changes are depending on the changing blood content of

the transilluminated brain tissue, and can be quantified for cerebral blood volume changes and the gCBV values can be expressed in volume %.

The blood flow and blood volume signals were continuously recorded together with the arterial pressure and heart rate changes in a polygraph at the same time, both flow and volume and pressure signals were digitized and stored on an external hard drive (sampling rate 20 Hz).

The head of the animal was secured in a stereotaxic frame. After exposing the skull from the occiput to the forehead, a 1.5mm in diameter burr hole was opened under operation microscope, probes or Pt electrodes were placed into the three different regions (sensory cortex, thalamus, hypothalamus) The positions of both probes were determined by using Paxinos's and Watson's stereotaxic coordinates. After completing the experiments, the brains were removed, fixed in formaldehyde solution, and dissected for checking the correct position of the probes. CBV measurement was carried out simultaneously with H₂ polarography without icv. drug administration.

Noxious and non-noxious somatosensory stimulation

For acute somatosensory stimulation, the sciatic nerve was prepared, placed on a bipolar silver electrode and was transected distal from the stimulation. The nerve was protected from drying with a warm paraffin oil pool. The stimulation was carried out with an S44 Stimulator, with either noxious or non-noxious parameters. For *noxious stimulation* a square wave, pulse-train stimulation was used with 30 V, 5Hz, and 0.5ms for 2 min, and for *non-noxious stimulation* a 0.2V, 5 Hz, 0.5 ms pulse train stimulation was applied for 2 min.

For noxious stimulation we used the same parameters as Evans. By using these parameters the somatic afferent C-fibers, which are responsible for carrying nociceptive impulses to the central nervous system, could be stimulated. For non-noxious

stimulation using these parameters somatosensory C-fibers remain silent and there is no significant increase in MAP and HR.

The duration of the noxious stimulation was 2 min. This duration was important because it caused no nerve damage (stimulation was repeatable), but was long enough to obtain at least a one-minute-long plateau period to calculate circulatory parameters after the initial peak of the changes. The non-noxious stimulation was carried out with the same duration as the noxious one to allow a comparison.

Drug administration

Drugs were administered either peripherally or centrally. For peripheral (intravenous, i.v.) drug administration the right femoral vein was used. For central (intracerebroventricular, i.c.v.) drug administration, a 1.5 mm in diameter burr hole was opened on the left side of the skull, 0.6 mm behind the bregma, 2.5 mm lateral to the midline. A 15 mm long, 1.0 mm in diameter polyethylene tube was inserted into the left lateral ventricle (with its tip 4.5 mm below the skull surface, in an 80° angle to the vertical), and was secured with dental cement. When drugs were injected, the obturator was removed and a microinjection syringe was introduced into the i.c.v. cannula. The i.c.v. injections were given in a volume of 3.5-10.0 µL, in 1 min.

The possible role of the different vasodilator mechanisms in the stimulation-induced rCBF increase was examined with the aid of selective, specific blocking agents. The effect of NOS blocking agent N^o-nitro-L arginine methyl ester (L-NAME, 30 mg/kg i.v., in 0.1 mL/100g fluid volume), cyclooxygenase inhibitor indomethacine (IMA, 300 µg/kg i.c.v., in 1.0 µL/100g fluid volume.), the K⁺ATP-channel blocker glibenclamide (GLI, 10 µg/rat i.c.v., in 7 µL/rat fluid volume), the general opiate-receptor inhibitor naloxone (NLX, 100 µg/kg i.c.v., in 1.0 µL/100g fluid volume), the sympathetic α-receptor blocker phenoxybenzamine (PBA, 50 µg/rat i.c.v., in 3.5 µL/rat fluid volume) and the sympathetic β-receptor blocking agent propranolol (PRO, 10 µg/rat i.c.v., in 3.5 µL/rat fluid volume) were investigated on regional cerebral blood flow. The effect of the blocking agents or their vehicles on the stimulation-induced flow increase was studied 15-20 min after the drug administration.

Experimental protocol

All measurements started after a 20 minute-long resting period following surgery and stabilization of the systemic arterial pressure, blood flow and volume signals, normal blood gas values and core temperature. The first 2 min control stimulation was followed by a 15-20 min recovery period and then either vehicle, or one of the specific blocking agents was injected i.v., or i.c.v. Then, 20 min after the drug injection the stimulation was repeated with the same parameters. Only one of the blocking agents was administered, and only once in the same animal. Cortical and thalamic blood flow as well as systemic arterial pressure, heart rate and body temperature was continuously recorded and arterial blood gas samples were taken to all stimulation.

Noxious somatic afferent stimulation resulted not only in acute rCBF changes but in simultaneous, acute arterial pressure changes as well. Therefore, two tests were carried out to investigate, whether the observed regional cerebral flow changes are simply the consequences of the systemic arterial pressure increase, or not. First, in a group of animals rapid intraarterial transfusion of homologue blood (obtained from other rats of the same strain) was used to elevate the arterial pressure in the same extent as it was observed following noxious sciatic nerve stimulation. The effect of the rapid blood transfusion on the regional cortical and thalamic blood flow was compared to that observed following acute sciatic nerve stimulation. Second the effect of non-noxious sciatic nerve stimulation on the local cortical and thalamic blood flow as well as on the systemic arterial pressure was measured.

The other goal of our experiment was to detect of changes in gCBV during stimulation. In this experimental setup beside the measurement of contra or ipsilateral gCBV, regional thalamic or hypothalamic blood flow was also estimated by Aukland's H₂ gas clearance technique.

Arterial blood gas values were determined immediately before and after all sciatic nerve stimulation in 250 μ L arterial blood samples and they were maintained within normal limits throughout the experiments.

In order to verify the stimulation-induced activation of the central neurons in the investigated cortical and thalamic regions, the C-fos antigen immunoreactivity test was used. C-fos is a proto-oncogene that is expressed within the neurons following depolarization and the protein product, C-fos protein, can be identified by immunohistochemical techniques. C-fos expression is a highly sensitive and reliable marker for neuronal activity throughout the neuraxis following peripheral stimulation. Frozen sections of the brains were prepared and the C-fos antigen immunoreactivity test was performed, using the routine immunoperoxidase technique with ABC kit.

Data analysis

All data are presented as mean \pm SD. Local cerebral blood flow changes were calculated from the LD curves in a way that the initial peak response (immediately after the onset of the stimulus) was neglected, since its shape and magnitude showed large variations. Instead, the average of all data points sampled during the plateau phase of the 2-min stimulation was calculated and was compared to the averaged values of the same parameters in the 2-min pre-stimulation period. Evaluations of the mean arterial blood pressure (MABP, mm Hg) and heart rate (HR, beat/min) responses were performed in a similar way. The stimulation-induced flow changes were expressed in percentage of the pre-stimulation control values. Statistical analysis of the effect of somatosensory stimulation, drug administration or homologous blood transfusion on the measured circulatory and blood gas parameters was carried out by using Student's paired t-test. Variations in the values of the measured parameters in the different experimental groups during the control periods (i.e. before drug administrations) were analyzed by using the analysis of variance (ANOVA) for repeated measures and the Newmann-Keuls test were used to estimate individual differences.

Results

There was a marked expression of C-fos immunoreactivity in all investigated animals in the midline thalamic nuclei, and also in the first somatosensory area of the cortex projecting to the hindlimb of the rats subjected to noxious stimuli. In contrast, few neuronal cells contained Fos-positive cell nuclei in the unstimulated control rats and in the animals subjected to non-noxious stimulation.

The effect of sciatic nerve stimulation on systemic circulatory parameters and regional cerebral blood flow

C-fiber stimulation with noxious parameters resulted in an immediate rise of both cBF and tBF. At first, the flow increase reached a peak within 5 sec, then (after 15-20sec) a plateau was developed, and finally (at the end of the 120-sec stimulation) the blood flow returned to the resting level in one-to three minutes. During noxious stimulation cBF increased by $46.7\pm 23.9\%$ and tBF increased by $44.3\pm 18.2\%$ compared to the pre-stimulation control value (n=44, p=0,001, Fig. 1).

Sciatic nerve stimulation with noxious parameters resulted in significant MABP and HR increases (Fig. 1). MABP increased from 97 ± 11 mm Hg to 131 ± 14 mm Hg, and HR changed from $511\pm 34/\text{min}$ to $538\pm 32/\text{min}$. (n=44, p=0,001). The dynamics of the MABP and HR changes were similar to those of the regional cerebral flow changes.

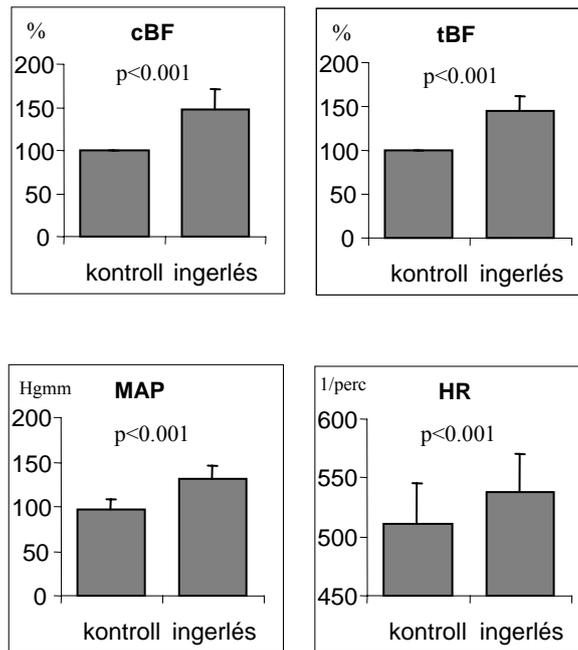


Figure 1. Synchronous changes in regional cerebral blood flow in thalamus (*tBF*), cortex (*cBF*) and in mean arterial pressure and heart rate ($n=44$, $p<0,01$)

The two different type of rCBF measurement (LD-flowmetry and H₂ gas clearance) provide absolute data about rCBF changes in thalamus and in hypothalamus. Sciatic nerve stimulation is also resulted in a significant rCBF increase in thalamic blood flow and hypothalamic flow. (Figure 2.) Thalamic blood flow increased by 97%, (from 0.67 ± 0.1 ml/g/min to 1.32 ± 0.3 ml/g/min, $p=0.0002$, $n=8$) and hypothalamic blood flow by 47%, (from 0.70 ± 0.3 ml/g/min to 1.03 ± 0.3 ml/g/min, $p=0.0007$, $n=6$). After NOS blockade, both thalamic and hypothalamic blood flow decreased significantly (from 0.65 ± 0.2 ml/g/min to 0.42 ± 0.2 ml/g/min in the thalamus, and from 0.61 ± 0.2 ml/g/min to 0.47 ± 0.2 ml/g/min in the hypothalamus). The NOS blockade, however, did not abolish the flow-effect of sciatic stimulation: rCBF increased from 0.42 ± 0.2 ml/g/min to 0.61 ± 0.2 ml/g/min in the thalamus, $p=0.021$, $n=8$, and from 0.48 ± 0.2 ml/g/min to 0.52 ± 0.3 ml/g/min in the hypothalamus, $p=0.028$, $n=6$).

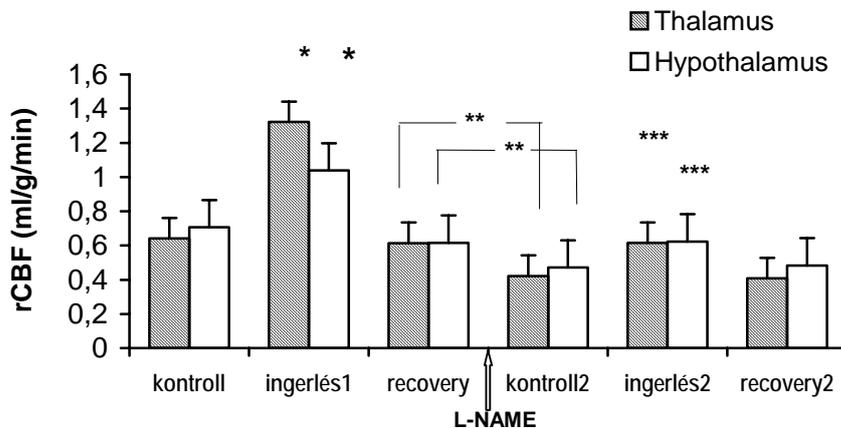


Figure 2. Regional cerebral blood flow (rCBF) in the ventral nucl. of the thalamus (n=8) and in the ventromedial nucl. of the hypothalamus (n=6). Bars represent means \pm SEM. * $p < 0.05$ compared to the corresponding control value; ** $p < 0.05$ compared to the corresponding recovery flow value before L-NAME administration; *** $p < 0.05$ compared to the corresponding control value after L-NAME administration

PaO₂, PaCO₂, and pH values remained unchanged during the two-minute noxious stimulation compared to the pre-stimulation control values

Effect of painful stimulation on cerebral blood volume

Hemispheric blood volumes remained unaffected by the noxious stimuli, either ipsilaterally, or contralaterally to the stimulated sciatic nerve (Figure 3.). Ipsilateral gCBV was 5.68 ± 1.42 vol% in the steady state control period, 6.07 ± 1.42 vol% during stimulation, and 5.7 ± 1.3 vol% in the post-stimulatory, recovery period. Contralateral gCBV values were 5.02 ± 0.6 vol% in the control phase, 5.21 ± 0.58 vol% during stimulation, and 5.05 ± 0.6 vol% in the recovery phase. NOS-blockade by L-NAME resulted in a significant reduction of gCBV both ipsilaterally (from 5.7 ± 1.3 vol% to 4.58 ± 1.6 vol%, $p = 0.017$, $n = 6$) and contralaterally (from 5.05 ± 0.6 vol% to 4.24 ± 0.9 vol%, $p = 0.002$, $n = 12$). L-NAME administration, however, did not alter the effect (or, rather the lack of an effect) of sciatic nerve stimulation on gCBV. After NOS blockade

ipsilateral gCBV was 4.58 ± 1.6 vol% in the control period, 4.6 ± 1.3 vol% during stimulation, and 4.8 ± 1.3 vol% in the post-stimulatory, recovery period. Contralateral gCBV values after L-NAME were 4.24 ± 0.9 vol% in the control phase, 4.4 ± 0.85 vol% during stimulation, and 4.36 ± 0.96 vol% in the recovery phase.

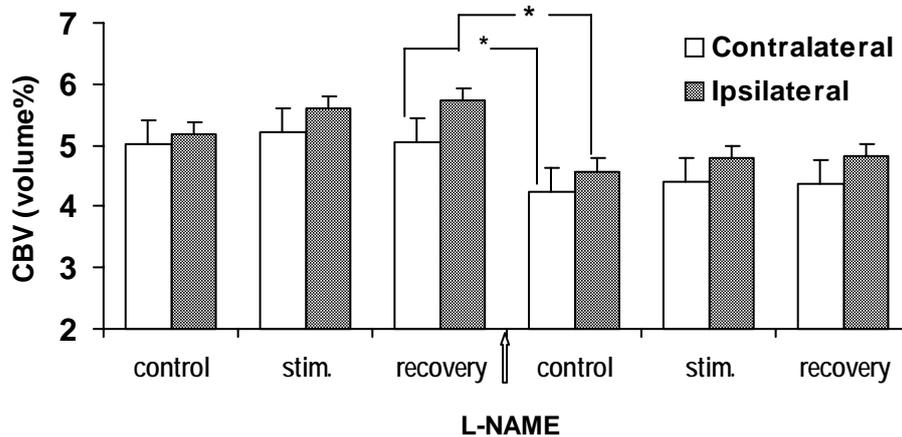


Figure 3. Total cerebral blood volume (gCBV) measured in the ipsilateral (n=5) and contralateral hemisphere (n=12), compared to the stimulated sciatic nerve. Bars represent means \pm SEM; * $p < 0.05$ compared to the corresponding gCBV values of the recovery phase before L-NAME administration

The effect of non-noxious stimulation and MAP elevation

Sciatic nerve stimulation with non-noxious parameters, caused no significant flow changes either in the sensory cortex or in the thalamus (cBF change was $-0.3 \pm 5.1\%$ / $p=0,18$, $n=6$ /, tBF change was $+0.2 \pm 3.0\%$ / $p=0,47$, $n=6$ / compared to the pre-stimulation flow values).

Stimulation with non-noxious parameters caused no significant changes either in the systemic mean arterial pressure values (from 96 ± 11 mm Hg to 93 ± 9 mm Hg, $n=6$) or in the heart rate (from 495 ± 51 /min to 495 ± 50 /min, $n=6$).

In a series of experiments rapid intraarterial blood transfusion was induced in order to produce acute, sustained MABP increase in the rats with similar magnitude to that

observed previously in the same animal during electric stimulation. The transfusion-induced MABP elevation resulted in a significant increase of the cortical blood flow, although the $18.4 \pm 15.6\%$ increase of cBF was less than half of the flow increase observed following noxious sciatic nerve stimulation. Thalamic blood flow, however, remained practically unchanged at the same time (increased only by $2.6 \pm 8.4\%$, $n=8$, $p=0.43$). Acute elevation of the systemic arterial pressure by blood transfusion, failed to affect thalamic blood flow and increased only moderately the cortical blood flow.

Mediators of pain-induced regional cerebral blood flow and global blood volume changes

Inhibition of the L-arginine - nitric oxide system by *L-NAME* resulted in different changes in the three regions. A significant reduction of the stimulation-induced flow increase in the thalamus (from $36.1 \pm 20.5\%$ to $21.3 \pm 14.1\%$, $n=6$, $p=0.001$ with LD flowmetry, and from 0.67 ± 0.1 ml/g/min to 0.21 ± 0.11 ml/g/min with H_2 gas clearance) but caused no statistically significant reduction in the flow increase in the sensory cortex (from $36.4 \pm 30.6\%$ to $25.4 \pm 16.9\%$, $n=6$, $p=0.10$ LD-flowmetry). [Table 1.]

NOS-blockade by L-NAME resulted in a significant reduction of gCBV both ipsilaterally (from 5.7 ± 1.3 vol% to 4.58 ± 1.6 vol% , $p=0.017$, $n=6$) and contralaterally (from 5.05 ± 0.6 vol% to 4.24 ± 0.9 vol%, $p=0.002$, $n=12$). L-NAME administration, however, did not alter the effect (or, rather the lack of an effect) of sciatic nerve stimulation on gCBV. After NOS blockade ipsilateral gCBV was 4.58 ± 1.6 vol% in the control period , 4.6 ± 1.3 vol% during stimulation, and 4.8 ± 1.3 vol% in the post-stimulatory, recovery period. Contralateral gCBV values after L-NAME were 4.24 ± 0.9 vol% in the control phase, 4.4 ± 0.85 vol% during stimulation, and 4.36 ± 0.96 vol% in the recovery phase. [Figure 3.]

Indomethacine had no significant effect on the stimulation-induced flow increase in either the cortical or the thalamic region (cBF increase changed from $31.8 \pm 4.3\%$ to $38.5 \pm 20.7\%$, $n=6$, $p=0.48$; tBF increase changed from $47.3 \pm 17.6\%$ to $37.7 \pm 8.3\%$, $n=6$, $p=0.25$).

The flow increase following noxious stimulation was not influenced at all after general opiate receptor blockade by *naloxone*, either in the cortex or in the thalamus (cBF increase changed from 57.6±13.8% to 64.6±33.2%, n=7, p=0.24; and tBF increase changed from 48.0±14.5% to 47.0±18.8%, n=7, p=0.78). [Table 1.]

Sympathetic β -receptor blockade by *propranolol* caused a significant decrease in the pain- induced regional cerebral flow increase in both the cortical and the thalamic regions (cBF increase was reduced from 41.6±26.8% to 27.3±15.7%, n=8, p=0.01; and tBF increase was reduced from 42.7±24.7% to 30.6±18.6%, n=8, p=0.04).

The i.c.v. administered sympathetic α -receptor inhibitor *phenoxybenzamine* had no effect on the stimulation induced flow changes in either of the investigated brain regions (cBF increase changed from 52.8±28.5% to 53.5±28.2%, n=7, p=0.92 and tBF increase changed from 50.7±22.2% to 50.0±23.7% , n=7, p=0.91).

Glibenclamide, the specific K^+ ATP-channel inhibitor reduced considerably the stimulation-induced regional flow increase, both in the thalamus and in the sensory cortex. (cBF increase was reduced from 55.3±21.9% to 33.1±18.8% , n=10, p=0.03; and tBF increase was reduced from 41.7±8.3% to 22.9±19.6%, n=10, p=0.01). [Table 1.]

Inhibitor	ΔcBF	ΔtBF	n
Propranolol	-14,3±4,6 *	-12,2±5,1% #	8
Glibencamide	-22,2±6,8% *	-18,8±5,4 *	10
L-NAME	-10,9±6,2% NS	-14,8±2,7% *	6
Indometacine	6,7±7,3% NS	-9,6±6,5% NS	6
Naloxone	7,0±9,5% NS	-1,0±4,4% NS	7
Phenoxybenzamine	0,7±8,3% NS	-0,8±8,2% NS	7

Table 1. Regional differences of inhibitors on cerebral blood flow changes during sciatic nerve stimulation, * $p < 0,01$; # $p < 0,05$

Noxious stimulation resulted in an increased MABP and HR, as discussed above. These changes, however, were not altered significantly by any of the administered blocking agents. [Table 2].

Inhibitor	Δ MAP (mmHg)		Δ HR (1/min)		n
	before	after	before	after	
L-NAME	26,9 \pm 7,2	17,1 \pm 10,0	15,2 \pm 12,8	8,3 \pm 8,6	20
indometacine	28,5 \pm 6,4	28,2 \pm 8,8	27,7 \pm 20,3	21,2 \pm 9,1	6
naloxone	36,6 \pm 8,9	34,3 \pm 12,2	18,6 \pm 20,8	18,3 \pm 7,5	7
propanolole	34,5 \pm 10,9	31,2 \pm 12,4	26,9 \pm 21,7	25,2 \pm 20,4	8
phenoxybenzamin	37,4 \pm 8,5	39,5 \pm 13,9	29,5 \pm 21,3	33,5 \pm 23,2	7
glibencamide	35,2 \pm 11,8	28,4 \pm 13,9	29,4 \pm 21,5	32,6 \pm 33,9	10

2. táblázat. Effect of inhibitors on pain-induced changes of mean arterial pressure (MAP) and heart rate (HR). Data represent mean \pm SD.

Discussion

In the current study sciatic nerve of anesthetized rats was stimulated electrically with either noxious or non-noxious parameters and the effect of the stimulation on the local tissue blood flow and local neuronal activity of the sensory cortex, of the thalamus and of the hypothalamus was studied with LD flowmetry, H₂ gas clearance method and with the C-fos immunoreactivity test. Somatic afferent C-fiber stimulation with noxious parameters resulted in a marked, statistically significant increase of the local blood flow and local cellular activity all in the sensory cortex, in the thalamus and in the hypothalamus, but non-noxious stimulation caused no such changes. During stimulation global hemispherical blood volume remained unchanged in both side. The pain-induced elevation of the cortical and thalamic blood flow was not simply a consequence of the increased perfusion pressure. Rapid homologous blood transfusion was used in order to increase the systemic MABP to the same level as observed after noxious stimulation. The transfusion-induced MABP increase resulted in a regionally different flow-response in both investigated brain regions: it failed to affect thalamic blood flow, and increased only moderately the blood flow in the cortex. One may hypothesize that this second observation is a consequence of the regional differences in the autoregulatory capacity of the two investigated brain areas.

On the other hand, pain-induced regional blood flow increase are not due to the elevated

systemic arterial pressure, it caused by specific and regional different vasodilatatory mechanisms.

The present study shows for the first time that the *total* cerebral blood volume (gCBV) remains unaltered during acute, long-lasting noxious somatosensory stimulation of the anesthetized rat. One possible way to explain the steadiness of gCBV in the present study is that the 47-97 % blood flow increase in two small regions of the brain is probably much too small to influence total cerebral blood volume. It is more than likely, however, that pain induced enhancement of rCBF is not restricted to the two investigated region, as it was demonstrated by several studies [6, 7, 11, 17]. One may hypothesize, that the balance in gCBV is kept by redistributing the existing blood pool among various brain regions: those neuronal populations requiring more blood supply receive more blood from those regions not engaged by the stimulation-induced neuronal processes [15, 18]. To test this hypothesis, however, one has to use different methodology (e.g. SPECT-CT scanning) to develop a detailed map about rCBF changes parallel to the stimulation-induced gCBV alterations.

Role of vasoactive substances on regional cerebral blood flow

Activation of sympathetic nervous system

The Roy-Sherrington hypothesis fails to fully explain the exact mechanism of activation flow coupling, because (1) increase in rCBF is found to be greater (2) and more quickly as it could be explained by increased concentration of metabolic substances in the neighborhood. Neural regulation is resulted in the first, quick phase (30ms) of neurovascular coupling. However basic neuroanatomical and neurophysiological evidences exist about direct neural innervation of cerebral blood vessels since more than 20 years, experimental data obtained most of the investigators are quite doubtful and controversial. The main conclusion of these data on the neural regulation of cerebral blood flow can be summarized as follows:

- resting sympathetic tone in steady-state situation have minor or no influence at all in

the cerebrovascular bed. The role of sympathetic axon terminals in the cerebral vessel wall become significant under stress situation.

- sympathetic nerves protect cerebral blood flow and blood brain barrier (BBB) functions by limiting cerebrovascular dilatation especially in hypertension
- this protective effect is observed during hypoxia and hypercapnia as well
- peripheral painful stimulation may result in different rCBF response in different species

In our study sympathetic beta-activation is resulted in pain-induced blood flow increase since propranolol attenuated significantly the pain-induced hyperemia both in thalamus and cortex. At the same time, alpha-receptor inhibitor phenoxybenzamin failed to influence pain-induced blood flow changes in these regions.

ATP-sensitive K⁺-ion channels

K⁺_{ATP}-channels play a significant role in mediation of pain-induced blood flow changes in the thalamus as well as in the sensory cortex. In our study, glibenclamide, the specific blocking agent of this ion-channel was the most effective inhibitor of the noxious stimulation-induced flow increase in both investigated brain areas. One may hypothesize, therefore, that in the current study increased release of norepinephrine from perivascular nerve endings could be responsible for the activation of the K⁺_{ATP}-channels in the smooth muscle cells of the cortical and thalamic vessels, and for the consequent local vasodilation and flow increase in these regions.

L-arginine-NO system

Steady-state regional cerebral blood flow is influenced by L-arginine-NO system To the maintenance of resting cerebral blood flow about 2/3 of the vasodilation is associated with NO from the vasodilator nerve that continuously receives efferent impulses from the brain and liberates neurotransmitter, and the remaining 1 /3 is due to NO from extraneuronal tissues, possibly the endothelium.

The current study shows for the first time that during *noxious stimulation* NO plays a significant role in activity-flow coupling in the thalamus and hypothalamus, but it may

not have the same role in the sensory cortex. Pain-induced elevation of the cortical blood flow after NOS blockade showed a decreasing tendency but the effect did not reach the level of significance. Regionally different reactivity in the presence of L-NAME can originate from regionally different nitroxigerc neuronal pattern.

Endogenous opioid peptides

Endogenous opioid peptides may influence neuro-vascular coupling via μ -receptor. The main role of opioid substances in nervous system is inhibition of neurotransmission. Direct vasomotor effect of opioid peptides is weak or does not exist. However β -endorphin plays a key role in hypothalamic autoregulation. *Regional cerebral blood flow increase following noxious sciatic nerve stimulation was not significantly influenced by the specific opiate receptor blocking agent naloxone.*

Prostaglandins

Products of cyclooxygenase enzyme may have an impact of cerebrovascular regulation; their role in activation-flow coupling following non-noxious stimulation is possible. *Regional cerebral blood flow increase following noxious sciatic nerve stimulation was not significantly influenced by COX-inhibitor indomethacine.*

Effect of painful stimuli on hemispheric blood volume

In the present study we provided experimental evidence for the first time, that (1) during acute, long-lasting noxious somatosensory stimulation – 2 min long electrical stimulation of the sciatic nerve-, which was able to induce highly significant increases in the regional cerebral blood flow, the *total* cerebral blood volume remained unchanged; (2) NO-synthase contributes to the maintenance of the steady state value of the total cerebral blood volume, in the presence of L-NAME gCBV decreases significantly, (3) but L-arginine-NO synthase blockade does not alter the effect of noxious stimulation on gCBV. To understand the regulatory mechanisms, participating in the maintenance of the remarkable steadiness of gCBV regulation (gCBV autoregulation?) one needs further investigations.

Summary of our results

1. Somatic afferent C-fiber stimulation of the sciatic nerve with noxious parameters resulted in a marked, statistically significant increase of the regional blood flow and local cellular activity in the sensory cortex, in the thalamus and in the hypothalamus
2. K^+ _{ATP}-channels is crucial elements in the mediation of pain-induced local flow increase at the sensory-cortical and thalamic levels of the pain transmission. The role of the L-arginine - nitric oxide pathway in this process is regionally different: participation of NO in the mediation of pain-induced flow increase is significant in the thalamus and hypothalamus, but its role is not so evident in the sensory cortex. Arachidonic-acid derivatives or endogenous opiates are not involved in the pain-induced regional cerebral blood flow changes
3. The pain-induced elevation of regional blood flow was not simply a consequence of the increased perfusion pressure, since rapid homologous blood transfusion is failed to demonstrate such regional blood flow changes as observed after noxious stimulation. β -receptor blockade with i.c.v. injected propranolol resulted in a statistically significant reduction of the pain-induced cortical and thalamic flow increase, while α -receptor blockade by phenoxybenzamine did not influence the pain-induced blood flow increase. One may hypothesize that pain-induced activation in sympathetic nervous system – by activation of sympathetic perivascular nerves-, resulted in regional blood flow increase by direct neural mechanism.
4. During acute, long-lasting noxious somatosensory stimulation, which was able to induce highly significant increases in the regional cerebral blood flow, the total cerebral blood volume remained unchanged. NO-synthase contributes to the maintenance of the steady state value of the total cerebral blood volume, but its blockade does not alter the effect of noxious stimulation on gCBV.

Articles and abstracts related to the topics of the thesis

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