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Adaptation of the hypothalamic blood flow to chronic nitric oxide deficiency is independent of vasodilator prostanoids

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Abstract

The aim of our study was to investigate the adaptation of the hypothalamic circulation to chronic nitric oxide (NO) deficiency in rats. Hypothalamic blood flow (HBF) remained unaltered during chronic oral administration of the NO synthase (NOS) inhibitor *N*^G-nitro-L-arginine methyl ester (L-NAME, 1 mg/ml drinking water) although acute NOS blockade by intravenous L-NAME injection (50 mg/kg) induced a dramatic HBF decrease. In chronically NOS blocked animals, however, acute L-NAME administration failed to influence the HBF. Reversal of chronic NOS blockade by intravenous L-arginine infusion evoked significant hypothalamic hyperemia suggesting the appearance of a compensatory vasodilator mechanism in the absence of NO. In order to clarify the potential involvement of vasodilator prostanoids in this adaptation, cyclooxygenase (COX) mRNA and protein levels were determined in the hypothalamus, but none of the known isoenzymes (COX-1, COX-2, COX-3) showed upregulation after chronic NOS blockade. Furthermore, levels of vasodilator prostanoid (PGI₂, PGE₂ and PGD₂) metabolites were also not elevated. Interestingly, however, hypothalamic levels of vasoconstrictor prostanoids (TXA₂ and PGF_{2α}) decreased after chronic NOS blockade. COX inhibition by indomethacin but not by diclofenac decreased the HBF in control animals. However, neither indomethacin nor diclofenac induced an altered HBF-response after chronic L-NAME treatment. Although urinary excretion of PGI₂ and PGE₂ metabolites markedly increased during chronic NOS blockade, indicating COX activation in the systemic circulation, we conclude that the adaptation of the hypothalamic circulation to the reduction of NO synthesis is independent of vasodilator prostanoids. Reduced release of vasoconstrictor prostanoids, however, may contribute to the normalization of HBF after chronic loss of NO.

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Keywords

Cerebral circulation; Hypothalamus; Chronic nitric oxide deficiency; Prostanoid

1. Introduction

Acute inhibition of nitric oxide (NO) synthesis causes sustained hypertension accompanied by a marked reduction of the cardiac output and regional blood flow, including the perfusion of the heart (Shah and MacCarthy, 2000) and brain (Faraci and Heistad, 1998). During chronic NO synthase (NOS) blockade, however, the coronary and the cerebral blood flows return to the normal level although the vasoconstriction in other organs remains unchanged (Benyó et al., 1995; Huang et al., 1995; Ito et al., 1995; Kelly et al., 2000; Puybasset et al., 1996; Tayama et al., 1998). In accordance, endothelial NOS (eNOS) knockout mice show normal blood flow in the heart (Gödecke et al., 1998) and brain (Atochin et al., 2003) indicating that the circulation of these organs is able to adapt to chronic NO deficiency. In case of the coronary circulation, it is well established that a vasodilator prostanoid, presumably prostacyclin, may function as a compensatory factor after the chronic loss of NO (Beverelli et al., 1997; Lacza et al., 2003; Marcelín-Jiménez and Escalante, 2001; Puybasset et al., 1996). The mechanism of the adaptation of the cerebral circulation to chronic NO deficiency is far less understood. Vasodilator prostanoids, released constitutively from brain microvessels (Gecse et al., 1982; Kis et al., 1999; Kövecs et al., 2001; Parfenova et al., 2002; Peri et al., 1995), are apparently involved in the maintenance of the resting cerebral blood flow in some species including humans and rats (Busija, 2002; Leffler, 1997) and enhancement of their influence may serve as a reserve regulatory mechanism in NO deficiency. We have observed a significant increase of the prostacyclin and prostaglandin E₂ levels in the cerebrospinal fluid of rats after acute NOS inhibition (Lacza et al., 2001b and unpublished observations), which may indicate the activation of a prostanoid mediated compensatory mechanism in NO deficiency. In a recent study, pial arteriolar relaxations in response to acetylcholine or hypercapnia were improved during chronic as compared to acute NOS blockade in female rats and the improvement of the vasoreactivity was reversible by indomethacin indicating the augmentation of the prostanoid contribution to these vasodilator responses in chronic NO deficiency (Xu et al., 2003).

The aim of our present study was therefore to clarify the role of COX pathways in the adaptation of the cerebral circulation to NO deficiency. We have focused on the hypothalamic circulation, since in our earlier studies the hypothalamic blood flow (HBF) was shown to normalize within 1 week after the onset of the chronic NOS blockade (Benyó et al., 1995). Furthermore, in this brain region relatively high COX expression has been demonstrated (O'Banion, 1999; Kis et al., 2004; Simmons et al., 2004) and prostanoid release reportedly contributes to the maintenance of the resting HBF of rats under physiological conditions (Dahlgreen et al., 1981; Gerozissis et al., 1983; McCulloch et al., 1982).

2. Results

During the 1-week pretreatment period, the daily water intake was not different between control ($n=54$) and L-NAME-treated ($n=48$) animals (101 ± 7 vs. 96 ± 4 ml/kg/day, respectively). In animals subjected to NOS blockade, the average L-NAME consumption was 96 ± 4 mg/kg/day. After 1 week of L-NAME pretreatment, the animals showed marked hypertension (Table 1), but the HBF remained unchanged (0.86 ± 0.04 vs. 0.86 ± 0.04 ml/g/min in controls) in spite of the markedly reduced hypothalamic NOS activity (1.19 ± 0.16 vs. 4.85 ± 1.19 pmol citrulline/mg protein/min, $p=0.003$). Baseline arterial blood gas and acid-base parameters showed no significant differences between the control and the L-NAME pretreated experimental groups (Table 1).

Acute NO blockade achieved by intravenous injection of high dose (50 mg/kg) L-NAME induced marked hypertension (Fig. 1A) and reduction of the HBF (Fig. 1B) in control animals ($n=11$) but had no significant effect after chronic NOS blockade ($n=7$) indicating the appearance of an NO-independent mechanism in the maintenance of the HBF during chronic NO deficiency (Figs. 1A and B).

Intravenous L-arginine administration (30 mg/kg initial bolus followed by 10 mg/kg/min infusion) was without any significant effect on the mean arterial blood pressure (MABP), HBF, arterial blood gas tensions or pH in control animals ($n=11$, Figs. 2A and B). This observation is consistent with our previous report in cats (Kováč et al., 1992) and indicates that L-arginine availability is not a rate-limiting factor of the resting NO production. L-Arginine, however, was shown to reverse L-NAME-induced cerebrovascular NOS-inhibition in a previous study (Sándor et al., 1994), and therefore we analyzed its effect also in chronically L-NAME pretreated animals ($n=12$). L-Arginine infusion normalized the MABP (Fig. 2A) and at the same time evoked marked hypothalamic hyperemia (Fig. 2B), without changing arterial blood gas or acid–base parameters (data not shown). Since reversal of the NOS blockade by L-arginine elevated the HBF over its normal level (in spite of the reduction of the MABP to its physiological level), it was reasonable to hypothesize that a compensatory vasodilator mechanism has been activated in the hypothalamic vasculature during chronic NO deficiency. The potential involvement of COX isoenzymes and vasodilator prostanoids in this adaptation process has been addressed in the second part of the study.

During chronic NOS blockade, the urinary concentrations of prostacyclin and prostaglandin E₂ metabolites increased significantly, indicating the activation of COX pathways in the systemic circulation (Fig. 3). In the hypothalamus of control rats, RT-PCR revealed the constitutive expression of all three known COX isoenzymes. Chronic NOS blockade, however, did not induce upregulation of any of the mRNAs (Fig. 4A). Consistently, the COX-1 and COX-2 protein levels were not significantly different in L-NAME pretreated and control animals (Fig. 4B). Furthermore, levels of vasodilator prostanoids in the hypothalamus did not change after chronic NOS blockade (Table 2). Interestingly, however, the concentration of vasoconstrictor prostanoid metabolites decreased significantly in the hypothalamus of L-NAME pretreated animals (Table 2).

COX inhibition by diclofenac (10 mg/kg iv) did not affect HBF or other physiological variables in control ($n=8$) or L-NAME-treated animals ($n=10$, Fig. 5A). In contrast, indomethacin (5 mg/kg iv) significantly reduced the HBF in control rats ($n=17$, Fig. 5B) without changing the MABP or arterial blood gas and acid–base parameters (data not shown). However, the HBF-decreasing effect of indomethacin was not enhanced in L-NAME pretreated animals ($n=13$, Fig. 5B). The vehicle of indomethacin (2 ml/kg saline iv) did not influence either the HBF or any other measured variables (data not shown). These results altogether indicate that the adaptation of the hypothalamic circulation to the reduction of NO synthesis is independent of vasodilator prostanoids. Reduction of vasoconstrictor prostanoid release, however, may significantly contribute to the normalization of the HBF after the loss of NO.

3. Discussion

Chronic NOS blockade in normotensive rats is a well established model of specific vascular dysfunction and hence of early vascular disease, since several pathophysiological states including essential hypertension, atherosclerosis and vasospasm are associated with some degree of deficiency of NO production or action (Faraci and Heistad, 1998). In case of endothelial damage or other NO-depleting situations, alternative vasodilator mechanisms may be upregulated in order to compensate for the loss of NO. Characterization of these

compensatory mechanisms may help to improve therapeutic strategies in cardiovascular diseases.

Previous studies have indicated the ability of the cerebral circulation to adapt to chronic NO deficiency. First, it has been demonstrated that the resting cerebral blood flow, which is significantly reduced after acute NOS inhibition, returns to its physiological level during chronically diminished NO synthesis (Atochin et al., 2003; Benyó et al., 1995; Huang et al., 1995; Kelly et al., 2000). Second, chronic NOS blockade influences less severely the cerebral vasodilator responses evoked by hypercapnia, acetylcholine or NMDA than the acute blockade (Pelligrino et al., 1996; Wang et al., 1994; Xu et al., 2003). In the present study, although we were unable to identify the compensatory mechanism(s) responsible for the normalization of the hypothalamic blood flow, we have clarified three important aspects of the adaptation during chronic NO deficiency.

First, our observation that acute NOS blockade by L-NAME infusion induced a dramatic HBF decrease in control but not in chronically L-NAME-treated animals clearly indicates that during chronic NO deficiency an NO-independent vasodilator mechanism is involved in the maintenance of the HBF. This conclusion is not obvious since the constitutive NOS activity of the hypothalamus is only reduced by approximately 90% during chronic oral L-NAME treatment (Benyó et al., 1995). Taken into account that in case of diminished NO production the cerebrovascular reactivity to NO increases (Kovách et al., 1992; Moncada et al., 1991), the remnant 10% NOS activity could contribute significantly to the maintenance of the cerebral blood flow. Indeed, acute L-NAME treatment was shown to reduce the blood flow in the neocortex, hippocampus and striatum of rats subjected to chronic NOS blockade (Kelly et al., 1995), indicating that the remnant NO production contributes to the maintenance of the blood flow in these brain regions. Our results, however, indicate the importance of an NO-independent mechanism in the normalization of the hypothalamic blood flow during chronic NOS blockade.

Second, the observation that reversal of the chronic NOS blockade by L-arginine infusion elevates the HBF over its normal level (in spite of the reduction of the MABP to its physiological level) suggests the appearance of a vasodilator mechanism, which is activated to compensate the loss of NO. This has to be a very effective mechanism, since the high regional blood flow increase during simultaneous arterial pressure decrease indicates a remarkable reduction of the cerebrovascular resistance in the hypothalamus. Numerous observations in different experimental systems indicated that prostacyclin may act as a backup vasodilator in chronic NO deficiency. For instance, elevated plasma levels of prostacyclin and increased urinary excretion of the prostacyclin metabolite 6-keto-prostaglandin $F_{1\alpha}$ were reported after chronic inhibition of NOS in rats (Cao et al., 1999; Danielson and Conrad, 1996; Henrion et al., 1997; Tomida et al., 2003). Furthermore, the endothelium-dependent relaxation by bradykinin was sensitive to indomethacin in coronary arteries of dogs subjected to chronic NOS blockade but not in controls (Puybasset et al., 1996). A bradykinin-induced increase in prostacyclin production was greater in coronary arteries taken from nitro-L-arginine-treated dogs, which difference was attributable to the upregulation of the endothelial COX-1 isoform during chronic inhibition of NOS (Beverelli et al., 1997; Puybasset et al., 1996).

The third conclusion of our present study is, however, that prostacyclin or other vasodilator prostanoids are not involved in the adaptation of the hypothalamic circulation to chronic NO deficiency. Although we have observed increased urinary excretion of vasodilator prostanoid metabolites during chronic NOS blockade, the hypothalamic levels of these prostanoids remained unchanged. Furthermore, none of the COX isoforms showed an increased expression in the hypothalamus of rats subjected to chronic L-NAME treatment. In accordance, the influence of indomethacin or diclofenac on the HBF was not changed after chronic NOS

blockade. All these data clearly indicate that neither prostacyclin nor any other vasodilator prostanoids play a significant role in the adaptation of the hypothalamic circulation to chronic NO deficiency.

We have observed a significant reduction in the hypothalamic levels of vasoconstrictor prostanoids after chronic NOS blockade. This finding is particularly interesting in the light of our previous observations showing that the cerebral vasoconstriction induced by inhibition of the resting NO synthesis is partly due to the increased reactivity of the cerebral vessels to TXA₂ (Benyó et al., 1998; Lacza et al., 2001a). During chronic NOS blockade, however, the reduction of vasoconstrictor prostanoid release may compensate this vascular hyperreactivity and contribute to the normalization of the cerebral vascular resistance and blood flow.

A further interesting finding of the present study is the difference between the effects of indomethacin and diclofenac on the resting HBF. Similar differences between the effectiveness of these two COX inhibitors were reported previously: indomethacin but not diclofenac inhibited the cerebral hyperemic response to hypercapnia (Quintana et al., 1988) and to acetazolamide-induced extracellular acidosis (Wang et al., 1993) in rats. Both compounds, in doses used in our present and the above cited studies, were shown to inhibit prostanoid production in the rat brain (Abdel-Halim et al., 1978). However, indomethacin was also reported to inhibit cerebrovascular prostacyclin receptors, which effect could explain its stronger influence on the resting HBF (Parfenova et al., 1995). The difference of the relative potencies of these inhibitors towards the different COX isoenzymes, i.e., the higher affinity of indomethacin to COX-1 and diclofenac to COX-2 (Chandrasekharan et al., 2002; Mitchell et al., 1994), is also a possible explanation for the different HBF-effects. Recent observations that COX-1-deficient mice show diminished blood flow in some brain regions including the hypothalamus (Niwa et al., 2001) also suggest that this isoenzyme mediates the release of vasodilator prostanoid(s) influencing the resting cerebrovascular tone.

In conclusion, the present study demonstrates that during chronic NO deficiency the hypothalamic blood flow remains unaltered and activation of one or more vasodilator mechanisms appear to be responsible for this adaptation. Constitutive prostanoid release, presumably by COX-1, contributes to the maintenance of the hypothalamic blood flow under physiological conditions, but the influence of this mechanism is not enhanced during chronic NO deficiency. Although the enhanced urinary excretion of PGI₂ and PGE₂ metabolites indicates COX activation in the systemic circulation during chronic NOS blockade, we conclude that the adaptation of the hypothalamic circulation to the reduction of NO synthesis is independent of vasodilator prostanoids. Reduced release of vasoconstrictor prostanoids, however, may significantly contribute to the normalization of HBF after chronic loss of NO.

4. Experimental procedures

All investigations were performed in adult male Wistar rats (b.w. 300–400 g) under the guidelines of the Hungarian Law of Animal Protection (243/1988) and approved by the Semmelweis University Committee on the Ethical Use of Experimental Animals (590/99 Rh). During the pretreatment period, the animals were housed individually and drinking either normal tap water or a 1 mg/ml L-NAME solution for 1 week.

The *in vivo* experiments were carried out in 102 rats anesthetized with Urethane (1.3 g/kg *ip*; Sigma, St. Louis, MO, USA) and spontaneously breathing via a trachea cannula. Catheters were inserted into both femoral arteries (to measure blood pressure and for blood sampling) and into the left femoral vein for drug administration. The skull was fixed in a stereotaxic head-holder. Body temperature was kept constant between 36 and 38 °C with a controlled heating lamp. Systemic arterial pressure was continuously recorded on a polygraph (Model 7E, Grass,

Quincy, MA, USA). Hypothalamic blood flow (HBF) was determined by using Aukland's H₂-gas clearance method (Aukland et al., 1964), as described (Horváth et al., 2003). Briefly, a 100- μ m in diameter Teflon-coated Pt electrode with a 1-mm bare tip was introduced stereotactically into the ventromedial hypothalamic area. H₂ washout curves were produced by H₂-gas inhalation and were recorded on the polygraph. Bi-exponential analysis of the washout curves by a computer program based on the Marquardt algorithm (Marquardt, 1963) was used to calculate the HBF values.

Blood gas parameters (*PCO*₂, *PO*₂, O₂ saturation) and pH in femoral arterial samples were measured by a Radiometer Blood Gas Analyzer (ABL-300, Copenhagen, Denmark) at the times of HBF determinations. Mean arterial blood pressure (MABP) was determined at the same time. After the last measurements, the anesthetized animals were rapidly exsanguinated and hypothalamic tissue samples were excised and frozen rapidly. Total hypothalamic NOS activity was measured on the basis of the formation of labeled citrulline from labeled L-arginine (Nagy et al., 2000, Sándor et al., 1994). Tissue samples were homogenized in brain homogenizing solution containing 50 mM Tris-HCl (pH 7.4), 0.3 M sucrose, 0.1 mM EDTA, 1 mM dithioerythritol and 1 mM phenyl-methyl-sulfonylfluorid (PMSF, protease inhibitor). Homogenates were added to the samples containing 5 mM HEPES, 1 mM NADPH, 10 μ M tetrahydrobiopterin, 30 μ M calmodulin, 2.5 mM CaCl₂ at pH 7.4 and reaction was initiated by adding 20 μ M ³H-arginine (all final concentrations). After 30 min incubation, the reaction was stopped, samples were put onto 2-cm Dowex-50 $\times$ 8 resin columns and eluates were mixed with 5-ml dioxane-based scintillation fluid and measured in a Beckman TriCarb liquid scintillation spectrometer. Protein contents were determined by the biuret reaction, after precipitating the samples with HClO₄ and redissolved in NaOH. NOS-specific activities were calculated in picomole citrulline formed per minute per milligram protein units.

For the determination of hypothalamic tissue prostanoid levels (prostaglandin D₂, E₂ and F_{2 α} , as well as the stable prostacyclin and thromboxane A₂ metabolites 6-keto-prostaglandin F_{1 α} and thromboxane B₂, respectively), two groups of animals, pretreated with the same protocol as described above, were rapidly exsanguinated in deep ether anesthesia. Hypothalamic tissue samples were frozen rapidly and kept at -75 °C until further analysis. In order to measure prostanoid concentrations, the hypothalamus was homogenized in 4 volumes of water (weight/volume) with an UltraTurrax and afterwards with a glass potter. Deuterated internal standards were added and prostanoids were extracted with 1 ml ethyl acetate/hexane (7:3, v/v) as described (Schweer et al., 1994). The sample was evaporated and reconstituted in 1 ml water. Further derivatization and gas chromatography/triple quadrupole mass spectrometry (GC/MS/MS) conditions were described previously (Schweer et al., 1994).

For the determination of COX mRNA expression by RT-PCR, total RNA was isolated from hypothalamic tissue samples by SV Total RNA Isolation System (Promega, Madison, WI, USA). We used the same primer sets to detect COX-1, COX-2 and β -actin mRNA as previously described (Kis et al., 2003). For COX-3 detection, the sense primer (5'-CAGAGTCATGAGTCGTGAG; 1376773–1376791 bases of GenBank NW_047653.1) was designed to bind to intron 1 and the antisense (5'-AGAGGGCAGAATGCGAGTAT; 501–520 bases of GenBank S67721) to bind to exon 5 of the rat COX-1 gene, as previously described (Kis et al., 2004). The expected length of the RT-PCR product was 573 base pairs. Our COX-3 primer set distinguishes between COX-1 and COX-3 and also distinguishes between COX-3 and "partial" COXs (PCOX-1a, PCOX-1b) because our antisense primer binds to exon 5 which is lacking in PCOXs (Chandrasekharan et al., 2002). The conditions of the PCR reactions were set according to the instructions of the Qiagen OneStep RT-PCR Kit. Briefly, the RT reaction was performed at 50 °C for 30 min, followed by the initial PCR activation step at 95 °C for 15 min. Thirty-seven PCR cycles were performed with the following parameters: denaturation 30 s at 94 °C, annealing 45 s at 58 °C and extension at 72 °C for 1 min. The last step of the process

was a final extension at 72 °C for 10 min. The COX PCR products were identified by the size and the sequence. Automated DNA sequencing of the PCR products was performed on an ABI Prism 377 DNA sequencer (Applied Biosystems, Foster City, CA, USA).

Western blotting method was described in details elsewhere (Szabó et al., 2004). Briefly, the hypothalamic tissue samples were prepared as described above and protein content of the homogenate was determined by biuret reagent and then the samples were heated in a buffer containing 10% glycerol, 2.5% 2-mercaptoethanol, 5% SDS and 0.1% bromophenol blue marker dye. After heat treatment, equal protein amounts (70 µg in 10 µl) were applied onto the gel. After the electrophoretic separation, samples were transferred to nitrocellulose membranes. Samples were then blocked with 3% BSA for 24 h at 4 °C and tested for COX-1 and COX-2 isoenzymes using primary antibodies (Cayman, Ann Arbor, MI, USA, 120 min, room temperature, 1:500 dilution). Anti-rabbit horseradish peroxidase-conjugated secondary antibodies (Sigma, 1:2000 dilution) were then added for 30 min and finally membranes were treated with enhanced chemiluminescence solution (Amersham, Buckinghamshire, UK) to develop chemiluminescent bands that were visualized on an X-ray film (Medifort, Forte, Vác, Hungary). Bands were converted to computer-edited files. Positive controls were used for both isoenzymes and molecular mass standard was also run.

In order to measure the urinary excretion of prostacyclin and prostaglandin E₂ metabolites, the animals were housed individually in metabolic cages (Techniplast, Buguggiate, Italy). Urine samples were collected daily from control and L-NAME-treated animals. The concentration of urinary prostacyclin metabolites (2,3-dinor-6-keto-prostaglandin F_{1α} and 6-keto-prostaglandin F_{1α}) was determined by a commercial enzyme immunoassay kit (Assay Designs Inc., Ann Arbor, MI, USA). The concentration of the urinary PGE₂ metabolite 11α-hydroxy-9,15-dioxo-2,3,4,5,20-pentanor-19-carboxyprostanic acid was measured by GC/MS/MS as described previously (Schweer et al., 1994). Urinary prostanoid concentrations were normalized with the concentration of creatinine, which was measured by the Metra creatinine assay kit (Quidel, San Diego, CA, USA).

L-NAME (Sigma), indomethacin (Merck) and diclofenac (Sigma) were given in intravenous doses of 50, 5 and 10 mg/kg, respectively, dissolved in 2 ml/kg saline. Intravenous L-arginine (Sigma, dissolved in saline) administration consisted of a 30 mg/kg initial bolus followed by 10 mg/kg/min infusion with a rate of 0.1 ml/min.

All values are presented as mean±SEM; *n* represents the number of animals. Statistical analysis was performed using ANOVA for repeated measurements or one-way ANOVA followed by Tukey's post hoc test. For comparison of two groups, Student's unpaired *t*-test was used. A *p* value of less than 0.05 was considered to be statistically significant.

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Fig. 1.

Acute L-NAME administration induces marked hypertension and reduction of the hypothalamic blood flow in control but not in chronically NOS blocked animals. Mean arterial blood pressure (A) and hypothalamic blood flow (B) before (0 min) as well as 10, 20 and 40 min after intravenous injection of 50 mg/kg L-NAME in control (open circles, $n=11$) and chronically L-NAME pretreated rats (filled circles, $n=7$). *** $p < 0.001$ vs. "0 min".

Fig. 2.

L-Arginine administration normalizes the blood pressure and elevates the hypothalamic blood flow over its physiological level in chronically NOS blocked animals. Mean arterial blood pressure (A) and hypothalamic blood flow (B) before (0 min) as well as 10, 20 and 40 min after the onset of intravenous L-arginine infusion in control (open circles, $n=12$) and chronically L-NAME pretreated rats (filled circles, $n=12$). *** $p<0.001$ vs. "0 min".

Fig. 3.

Chronic L-NAME administration increases the production of vasodilator prostanoids. Urinary concentrations of prostacyclin (PGI₂, panel A) and prostaglandin E₂ (PGE₂, panel B) metabolites in control (open circles, *n*=5) and in L-NAME-treated (filled circles, *n*=5) rats before ("Day 0") and for 1 week after the onset of the treatment. Values are normalized with the urinary creatinine content. **p*<0.05, ***p*<0.01 vs. "Day 0".

Fig. 4.

Chronic NOS blockade does not influence the mRNA and protein levels of COX isoenzymes in the hypothalamus. (A) Semiquantitative RT-PCR indicating equal expression of COX-1, COX-2 and COX-3 mRNA in the hypothalamus of control (open bars, $n=4$) and chronically L-NAME pretreated (filled bars, $n=4$) rats. Densitometric analysis of the RT-PCR results normalized by β -actin. (B) Western blot analysis indicating equal expression of COX-1 and COX-2 proteins in the hypothalamus of control (open bars, $n=6$) and chronically L-NAME pretreated (filled bars, $n=6$) rats. Densitometric analysis of the Western blot results normalized by β -actin.

Fig. 5.

The effect of COX-inhibition by diclofenac or indomethacin on the hypothalamic blood flow is not impaired in animals subjected to chronic NOS blockade. Hypothalamic blood flow before (0 min) as well as 10, 20 and 40 min after intravenous injection of 10 mg/kg diclofenac (A) or 5 mg/kg indomethacin (B) in control (open circles, $n=8$ and 17 on panels A and B, respectively) and chronically L-NAME pretreated rats (filled circles, $n=10$ and 13 on panels A and B, respectively). *** $p<0.001$ vs. "0 min".

Table 1

Baseline physiological parameters in the different experimental groups

Experimental group (acute treatment)	I. L-NAME		II. L-Arginine		III. Diclofenac		IV. Indomethacin		V. Vehicle	
Subgroup (chronic pretreatment)	Control (n=11)	L-NAME (n=7)	Control (n=12)	L-NAME (n=12)	Control (n=8)	L-NAME (n=10)	Control (n=17)	L-NAME (n=13)	Control (n=6)	L-NAME (n=6)
MABP (mmHg)	91±3	133 ±6***	98±4	140 ±7***	93±6	130 ±3***	94±4	125 ±3***	91±5	120±5*
PO ₂ (mmHg)	93±2	98±4	87±2	84±2	101±2	100±3	95±2	99±3	102±4	97±2
O ₂ sat. (%)	96.6±0.3	97.4±0.3	95.7±0.2	95.7±0.2	97.4±0.2	97.3±0.3	96.5±0.3	96.6±0.3	96.9±0.4	96.7±0.3
PCO ₂ (mmHg)	39.5±1.5	44.2±1.4	41.9±0.6	37.9±1.1	42.3±1.0	42.3±1.0	44.8±0.7	41.2±1.1	43.7±3.2	42.9±1.3
pH	7.37±0.02	7.40 ±0.01	7.35±0.01	7.38 ±0.01	7.38±0.01	7.38 ±0.01	7.33±0.01	7.31 ±0.01	7.32±0.03	7.33 ±0.02

Mean arterial blood pressure (MABP) as well as O₂ and CO₂ tensions (PO₂ and PCO₂), O₂ saturation and pH of arterial blood samples were determined before acute treatment with the respective drug or vehicle. * and *** indicate significant differences vs. the corresponding "control" values ($p < 0.05$ and 0.001 , respectively).

Table 2
Prostanoid concentrations (pg/mg tissue) in the hypothalamus of control and L-NAME pretreated rats

	Control (n=9)	L-NAME (n=9)
6-Keto-Prostaglandin F _{1α}	9.8±1.9	9.3±2.3
Prostaglandin E ₂	13.6±1.8	11.6±2.2
Prostaglandin D ₂	11.6±1.3	12.2±2.9
Thromboxane B ₂	40.2±4.2	26.1±4.1*
Prostaglandin F _{2α}	37.7±3.9	18.6±3.9**

* $p<0.05$.

** $p<0.01$ vs. the corresponding “control” values.