Nitric oxide: a modulator, but not a mediator, of neurovascular coupling in rat somatosensory cortex

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Lindauer, Ute, Dirk Megow, Hiroshi Matsuda, and Ulrich Dirnagl. Nitric oxide: a modulator, but not a mediator, of neurovascular coupling in rat somatosensory cortex. *Am. J. Physiol.* 277 (*Heart Circ. Physiol.* 46): H799–H811, 1999.—We investigated the role of nitric oxide (NO)/cGMP in the coupling of neuronal activation to regional cerebral blood flow (rCBF) in α-chloralose-anesthetized rats. Whisker deflection (60 s) increased rCBF by 18 ± 3%. NO synthase (NOS) inhibition by N^{ω}-nitro-L-arginine (L-NNA; topically) reduced the rCBF response to 9 ± 4% and resting rCBF to 80 ± 8%. NO donors [*S*-nitroso-*N*-acetylpenicillamine (SNAP; 50 μM), 3-morpholinosydnonimine (10 μM)] or 8-bromoguanosine 3′,5′-cyclic-monophosphate (8-BrcGMP; 100 μM)] restored resting rCBF and L-NNA-induced attenuation of the whisker response in the presence of L-NNA, whereas the NO-independent vasodilator papaverine (1 mM) had no effect on the whisker response. Basal cGMP levels were decreased to 35% by L-NNA and restored to 65% of control by subsequent SNAP superfusion. Inhibition of neuronal NOS by 7-nitroindazole (7-NI; 40 mg/kg ip) or soluble guanylyl cyclase by 1*H*-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ; 100 μM) significantly reduced resting rCBF to 86 ± 8 and 92 ± 10% and whisker rCBF response to 7 ± 4 and 12 ± 3%, respectively. ODQ reduced tissue cGMP to 54%. 8-BrcGMP restored the whisker response in the presence of 7-NI or ODQ. We conclude that NO, produced by neuronal NOS, is a modulator in the coupling of neuronal activation and rCBF in rat somatosensory cortex and that this effect is mainly mediated by cGMP. L-NNA-induced vasomotion was significantly reduced during increased neuronal activity and after restoration of basal NO levels, but not after restoration of cGMP.

whisker stimulation; laser Doppler flowmetry; cerebral circulation; nitric oxide synthase inhibition; guanylyl cyclase; vasomotion

THE INVOLVEMENT of the highly diffusible vasodilator bioradical nitric oxide (NO) in the regulation of regional cerebral blood flow (rCBF) is widely accepted. Conceptually, the role of NO in CBF regulation may be classified as either mediatory or modulatory. Thus a stimulus may induce activation of NOS, with the result of increased tissue NO levels and diffusion of NO to vascular smooth muscle cells, where vasodilatation occurs as a consequence of guanylate cyclase stimulation. In this scenario, NO acts as a mediator, and NO levels quantitatively determine the vascular response. Alternatively, a certain NO level, provided by a basal activity of NOS, may be required to provide vascular responsiveness to a certain stimulus. According to this model, NO acts as a "permissive" factor, which modulates the effect of other vasodilators produced by the stimulus. Recently, a number of cerebrovascular reactions to various stimuli in different brain regions have been investigated regarding a mediatory or modulatory (i.e., permissive) function of NO.

The cerebrovasodilation produced by hypercapnia involves NO, and a number of studies have demonstrated a permissive role for NO in all brain regions for this response. In the brain, vasodilation by CO_2 is substantially attenuated by the inhibition of NO synthase (NOS) (11, 18, 29), and this attenuation can be completely reversed by reestablishing basal levels of NO using NO donors, despite persisting NOS inhibition (22, 35). Other vasodilator reactions in the cerebral cortex in which NO acts permissively include the vascular responses to elevation in extracellular potassium (12) and α_2-adrenoceptor stimulation (7).

In contrast, in a cerebellar model of neurovascular coupling, NO has been convincingly shown to be a classic mediator of stimulation-induced vasodilation. In this model, electrical stimulation of cerebellar parallel fibers triggers monosynaptic excitatory postsynaptic potentials in target cells via non-*N*-methyl-D-aspartate receptors. This glutamate receptor-mediated local vasodilation can be attenuated by NOS inhibition but cannot be restored by NO donors or cGMP (1, 35).

NOS activity in the cerebral somatosensory cortex has been shown to be significantly lower than that in the cerebellum (9), whereas the neuroanatomy and neurochemistry of the polysynaptic somatosensory system is more complex. Although the involvement of NO in neurovascular coupling in the somatosensory cortex is well established (8, 9, 11), the exact role of NO in this system is currently unknown. We therefore investigated whether NO acts as a mediator or modulator in physiological functional activation of the rat whisker barrel system. We found that NO donors as well as cGMP completely restore the attenuation of the vasodilation to somatosensory stimulation due to NOS inhibition. The data support the hypothesis that NO acts as a permissive factor in the coupling of neuronal activation and rCBF in the somatosensory cortex of rats.

METHODS

General Preparation

Male Wistar rats (250–300 g) were anesthetized with isoflurane in 30% O_2-70% N_2O. After anesthesia was administered, the concentration of isoflurane was reduced from 3% to 0.8–1.5%. The animals were tracheotomized and artificially ventilated (small-rodent respirator, Effenberger, Pfaffing/Attel, Germany), and end-expiratory P_{CO_2} was monitored continuously (CO_2 Monitor Artema MM 200, Heyer, Bad Ems,

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Germany). Body temperature was measured and maintained at 38 ± 0.5°C using a heating pad. The left femoral artery and vein were cannulated, a continuous saline infusion (1 ml/h iv) was started, and systemic arterial pressure was recorded continuously (RFT Biomonitor, Zwönitz, Germany). Arterial P_{O_2}, P_{CO_2}, and pH were measured periodically (Compact 2, AVL, Bad Homburg, Germany). The animals were placed in a stereotactic frame, and a closed cranial window was implanted over the right whisker barrel cortex (center of the window 7 mm lateral and 3 mm caudal to the bregma; for details see Ref. 25). The dura mater was removed and the exposed brain continuously superfused (superfusion rate 1 ml/h) with artificial cerebrospinal fluid (aCSF) composed of (in mM) 3 K^+, 150 Na^+, 1.25 Ca^{2+}, 0.6 Mg^{2+}, 132 Cl^-, 24.5 HCO_3^-, 3.7 glucose, and 6 urea, which was equilibrated with a gas mixture containing 6.6% O_2-5.9% CO_2-87.5% N_2, leading to the following gas tensions and pH: P_{O_2}, 109 ± 9 mmHg; P_{CO_2}, 37.4 ± 3.5 mmHg; and pH, 7.36 ± 0.02. To measure somatosensory evoked potentials (SEPs) in animals of *study VIII* (see *Experimental Protocol* and Fig. 1) a Ag-AgCl ball-tip electrode was inserted under the closed cranial window in the center of the exposed cortex, not touching the pial surface (for details see Ref. 24). A reference electrode was placed at the contralateral side on the intact skull.

After surgery, anesthesia was switched to intravenous α-chloralose-urethan (50 mg/kg α-chloralose with 500 mg/kg urethan as a bolus, followed by a continuous infusion of 40 $mg \cdot kg^{-1} \cdot h^{-1}$ α-chloralose with 400 $mg \cdot kg^{-1} \cdot h^{-1}$ urethan). Because rats were not paralyzed, the adequacy of the level of anesthesia was assessed by testing motor responses to tail pinch.

Whisker Stimulation

Somatosensory stimulation was performed by continuous mechanical deflection of the mystacial vibrissae cut to a length of 1.5 cm on the left side of the rat's face with a frequency of 3–4 strokes/s by a custom-built stimulation device over a period of 60 s.

rCBF Monitoring

Regional cortical CBF was measured using a laser Doppler flowmeter (Blood Perfusion Monitor BPM^2, Vasamedic, St. Paul, MN) permitting continuous monitoring of rCBF with a spatial resolution of 1 mm^3 and a temporal resolution of 0.1 s. The flow probe (tip diameter 0.8 mm, fiber separation 250 μm) was mounted on a micromanipulator and positioned at the surface of the cranial window at the side of maximal response to whisker deflection. Relative changes of rCBF were calculated as a percentage of the baseline during the 60 s before stimulation, because laser Doppler flowmetry (LDF) does not measure flow quantitatively in absolute terms.

SEP Recording

For measurement of SEP in *study VIII*, 100 consecutive stimulations (whisker deflection at a frequency of 3 strokes/s) were performed, and the electroencephalogram was averaged using a commercial Nihon Kohden Neuropack Monitor. Maximum SEP amplitudes were measured between the first marked positive P wave (P_1) and the following negative wave (N_1).

cGMP Measurement

Brain tissue samples of three control animals (closed cranial window preparation, superfusion with physiological aCSF for 2 h) and three rats each after NOS inhibition, after NOS inhibition with subsequent *S*-nitroso-*N*-acetylpenicillamine (SNAP) application, or after guanylyl cyclase inhibition were harvested to measure cGMP levels in the tissue. At the end of the superfusion periods, the whole animals were snap-frozen in liquid N_2 in situ. In a −20°C environment, tissue samples were taken of the exposed cortex beneath the cranial window (0.5–0.75 mm thick) after the pial surface, including the pial vasculature, was carefully removed. The tissue samples were stored below −70°C in liquid N_2 until assayed for cGMP.

Samples were sonicated at 0°C in 5% trichloroacetic acid (TCA), and the precipitate was separated by centrifugation (15,000 *g*, 15 min, 4°C). TCA was removed from the supernatant by precipitation with K_2CO_3, and samples were diluted in the assay buffer delivered with the immunoassay. cGMP enzyme immunoassay (Amersham Life Science) was performed according to the recommendations for acetylation assay. Confidence of the sample preparation was tested with control samples containing defined quantities of cGMP. Protein concentration was determined with bicinchoninic acid (BCA Assay, Pierce), and cGMP content was expressed as femtomoles per milligram of protein.

Experimental Protocol

After anesthesia was switched to α-chloralose, baseline blood gases were measured, and the baseline increases in rCBF elicited by 60-s whisker deflection or hypercapnia (*study IX*) were performed. Hypercapnia was induced by the addition of 5% CO_2 (in 20% O_2-75% N_2) to the inspiratory air for 3 min (time for rCBF increase to reach a steady state), and blood gases were measured again to verify the level of hypercapnia. Figure 1 shows the overall experimental protocol.

Studies I–IV: Topical application of 3-morpholinosydnonimine, SNAP, papaverine, or 8-bromoguanosine 3′,5′-cyclic monophosphate after NOS inhibition with N^ω-nitro-L-arginine. After the baseline rCBF increase to 60-s whisker deflection was recorded, N^ω-nitro-L-arginine (L-NNA; 1 mM in aCSF; Sigma Chemicals) was superfused over the cranial window for 2 h. The response to 60-s whisker deflection was tested again after 1 and 2 h of L-NNA application. Using citrulline-conversion HPLC assay, we have previously shown (24) that superfusion of L-NNA at this concentration for 2 h inhibits NOS activity in our preparation in the somatosensory cortex under the cranial window by ~ 85%. In *study I* (n = 6) and *study II* (n = 4), either of the NO donors 3-morpholinosydnonimine (SIN-1; 10 μM in aCSF containing 1 mM L-NNA; Research Biochemicals International) or SNAP (50 μM in aCSF containing 1 mM L-NNA; Research Biochemicals International), respectively, was then added to the superfusion solution for 30 min to restore resting rCBF to pre-L-NNA levels. The response to whisker deflection was measured again. In *study III* (n = 5) and *study IV* (n = 5), the NO-independent vasodilator papaverine (1 mM in aCSF containing 1 mM L-NNA; Sigma Chemicals) or the cell-permeable cGMP analog 8-bromoguanosine 3′,5′-cyclic monophosphate (8-BrcGMP; 100 μM in aCSF containing 1 mM L-NNA; Sigma Chemicals), respectively, was used instead of the NO donors SIN-1 or SNAP to reestablish the pre-L-NNA resting rCBF level.

Study V: Determination of cortical cGMP levels under the closed cranial window. To test the effect of NOS inhibition, the effect of NO donor application subsequent to NOS inhibition, or the efficacy of guanylyl cyclase inhibition on the basal cGMP level in the cerebral cortex, tissue samples under the cranial window were harvested after 2 h of normal aCSF

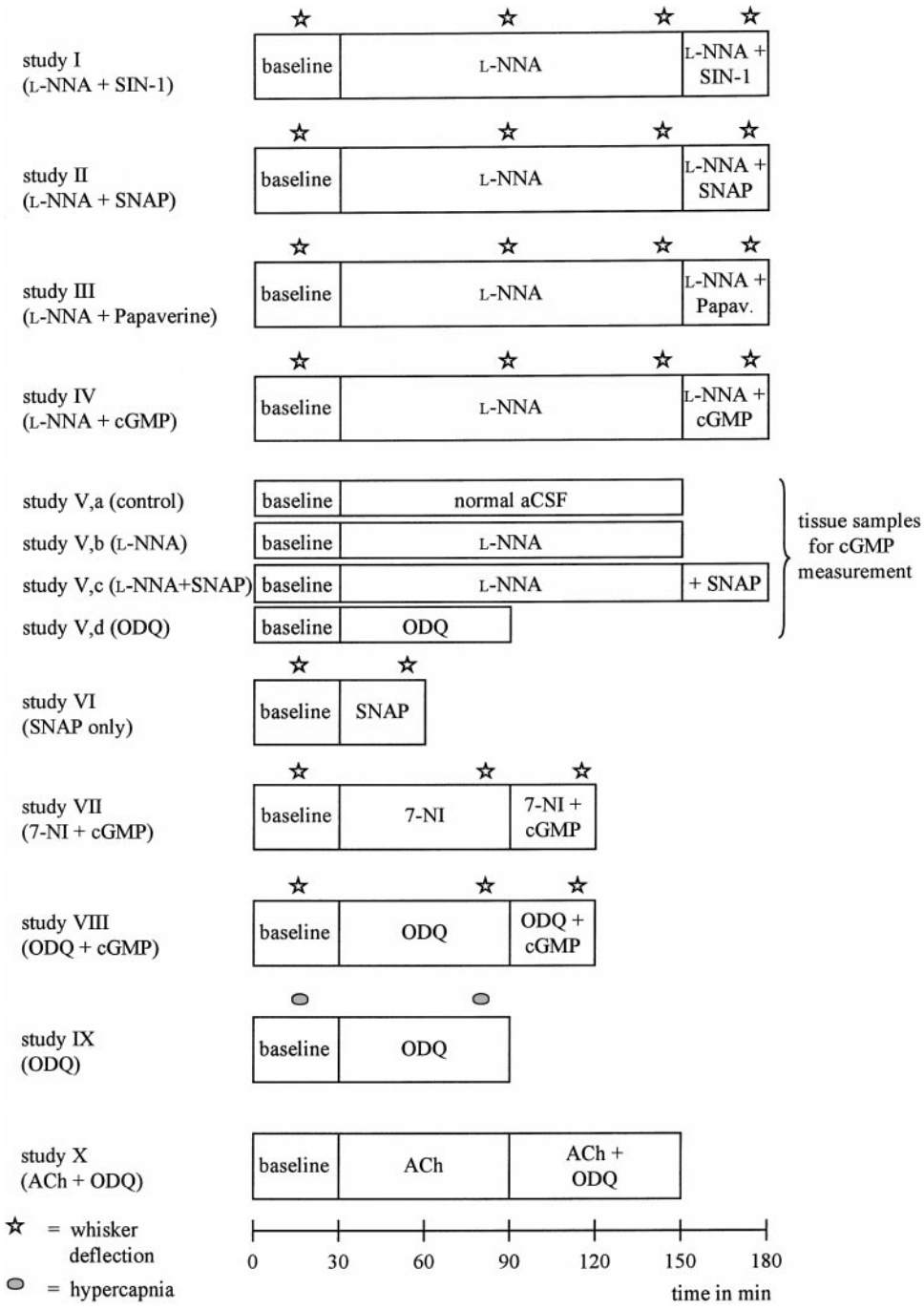

Fig. 1. Experimental paradigm for *studies I–X*. L-NNA, N^{ω}-nitro-L-arginine; SIN-1, 3-morpholinosydnonimine, SNAP, *S*-nitroso-*N*-acetylpenicillamine; Papav, papaverine; aCSF, artificial cerebrospinal fluid; 7-NI, 7-nitroindazole; ODQ, 1*H*-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one.

superfusion (*study V,a*, $n = 3$), after 2 h of topical L-NNA application without (*study V,b*, $n = 3$) or with subsequent SNAP application (*study V,c*, $n = 3$), or after 1 h of topical 1*H*-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) application (*study V,d*, $n = 3$).

Study VI: Topical application of SNAP without previous NOS inhibition. The effect of increased resting rCBF level by SNAP alone without previous NOS inhibition on the magnitude of the rCBF increase to whisker deflection was tested in *study VI* ($n = 5$). The aim of this study was to investigate the effect of further elevated basal NO levels on the rCBF response to whisker deflection, because SNAP application after NOS inhibition in *study II* elevated the resting rCBF beyond pre-L-NNA levels.

Study VII: Topical application of 8-BrcGMP after inhibition of the neuronal NOS with 7-nitroindazole. After the baseline rCBF increase to 60-s whisker deflection was recorded, 7-nitroindazole (7-NI; 40 mg/kg ip in 1.5 ml of peanut oil; Sigma Chemicals) was administered. Later (60 min), the response to 60-s whisker deflection was tested again. 8-BrcGMP (100 μM in normal aCSF; Sigma Chemicals) was then added to the superfusion solution for 30 min to restore resting rCBF to pre-7-NI levels. The response to whisker deflection was measured again ($n = 5$).

Study VIII: Topical application of 8-BrcGMP after inhibition of the NO-sensitive guanylyl cyclase with ODQ. After the baseline rCBF increase to 60-s whisker deflection was recorded, ODQ (100 μM in aCSF; Research Biochemicals Inter-

national) (16) was superfused over the cranial window for 1 h and the response to 60-s whisker deflection was tested again. 8-BrcGMP (100 μM in aCSF containing 100 μM ODQ; Sigma Chemicals) was then added to the superfusion solution for 30 min to restore resting rCBF to pre-ODQ levels. The response to whisker deflection was measured again (*study VIII*, $n = 4$). In additional rats of *study VIII* ($n = 2$), the effect of guanylyl cyclase inhibition on increased neuronal activity during whisker deflection was tested by recording whisker deflection-induced SEPs at baseline conditions, after 1 h of ODQ superfusion, and after an additional 30 min of 8-BrcGMP application.

Studies IX and X: Functional confirmation of the efficacy of guanylyl cyclase inhibition by topical ODQ application. The efficacy of guanylyl cyclase inhibition by topical application of ODQ was proven functionally by testing its effect on the hypercapnia-induced cerebral vasodilation ($n = 3$; baseline conditions vs. 1 h of ODQ application) and the reversal of the ACh (1 mM in aCSF; Sigma Chemicals)-induced vasodilation by subsequent ODQ (100 μM in aCSF containing 1 mM ACh) application ($n = 3$).

Data Analysis

All data are presented as means ± SD. Mean rCBF response to vibrissae stimulation was calculated from the average of all data points sampled during the 60 s immediately before stimulation and the average of the 60-s stimulation period. Mean rCBF increase is expressed as a percentage of the mean baseline value. To avoid calculation artifacts due to high vasomotion amplitudes after NOS or guanylyl cyclase inhibition, every calculated mean rCBF value was carefully corrected using values achieved by manual control on the diagram printouts. Mean rCBF response to hypercapnia was calculated from the average of all data points sampled during the 60-s immediately before CO_2 inhalation and the average of all data points sampled during the last 60 s of the 3-min period of CO_2 inhalation. Mean rCBF increase during hypercapnia is expressed as a percentage of the mean baseline value per millimeter of Hg of arterial P_{CO_2} increase. Mean rCBF response to topically applied ACh was calculated from the average of all data points sampled during the 60 s immediately before ACh superfusion and the average of all data points sampled during the 60 s at the end of the 1-h application period of ACh. Whisker deflection-induced rCBF responses in the observation period at different time points were compared using repeated-measures ANOVA; hypercapnia- or ACh-induced hyperemic values at baseline and after 1 h of ODQ application were compared using the paired-samples Student's *t*-test. cGMP tissue levels at control and after NOS inhibition without or with SNAP application were compared using one-way ANOVA (all pairwise multiple comparison procedures with Bonferroni correction); cGMP tissue levels with and without guanylyl cyclase inhibition with ODQ were compared using the unpaired-samples Student's *t*-test (SigmaStat, Jandel). *P* values <0.05 were considered statistically significant.

Spectral Analysis

To quantify oscillatory activity in the rCBF data as measured with LDF, selected 1-min LDF recording intervals were subjected to fast Fourier transform (FFT) spectral analysis using MATLAB software (MathWorks, Natick, MA). Data were expressed as percent changes from baseline. LDF raw data were filtered with a Hamming window and subjected to FFT and power spectral density calculation. Spectral power at respiration frequency was excluded from the analysis, and heart rate was not included in the raw signal because the data were collected by a moving 1-s average. The maximum spectral power density in each 1-min interval was used for further comparisons. Power spectral density is expressed as the square of the percent LDF change from baseline, per minute. Frequencies are expressed as cycles per minute.

RESULTS

The physiological variables were within normal ranges throughout the experiments (Table 1).

NOS Inhibition-Induced Reduction of Vascular Response to Whisker Deflection: Influence of SIN-1, SNAP, papaverine, or 8-BrcGMP

Sixty seconds of whisker deflection increased LDF rCBF in the whisker barrel cortex by 18.1 ± 3.3% (*studies I–IV*, $n = 20$). Two hours of NOS inhibition by topical application of L-NNA led to a significant reduction of resting rCBF to 80.3 ± 7.8% and a significant

Table 1. *Physiological variables*

Study	SAP, mmHg	Pa_{CO_2}, mmHg	Pa_{O_2}, mmHg	pH
I ($n = 6$)				
Baseline	131 ± 15	33 ± 3	145 ± 21	7.43 ± 0.02
3 h	136 ± 10	32 ± 2	137 ± 20	7.42 ± 0.02
II ($n = 4$)				
Baseline	132 ± 22	34 ± 1	90 ± 18	7.40 ± 0.03
3 h	141 ± 26	32 ± 2	111 ± 12	7.40 ± 0.02
III ($n = 5$)				
Baseline	129 ± 14	34 ± 5	118 ± 17	7.42 ± 0.07
3 h	138 ± 16	33 ± 3	110 ± 13	7.41 ± 0.05
IV ($n = 5$)				
Baseline	139 ± 23	31 ± 2	133 ± 14	7.43 ± 0.01
3 h	151 ± 15	29 ± 2	133 ± 20	7.42 ± 0.03
V,a ($n = 3$)				
Baseline	101 ± 4	34 ± 2	162 ± 20	7.42 ± 0.02
2.5 h	109 ± 8	36 ± 1	121 ± 13	7.41 ± 0.02
V,b ($n = 3$)				
Baseline	106 ± 29	35 ± 5	167 ± 42	7.42 ± 0.04
2.5 h	123 ± 13	34 ± 2	124 ± 26	7.42 ± 0.01
V,c ($n = 3$)				
Baseline	106 ± 5	39 ± 1	191 ± 6	7.43 ± 0.02
3 h	128 ± 9	42 ± 3	177 ± 21	7.40 ± 0.03
V,d ($n = 3$)				
Baseline	109 ± 11	38 ± 3	188 ± 11	7.42 ± 0.01
1.5 h	113 ± 12	40 ± 2	163 ± 17	7.41 ± 0.01
VI ($n = 5$)				
Baseline	135 ± 7	36 ± 6	130 ± 28	7.42 ± 0.03
1 h	139 ± 9	35 ± 4	153 ± 38	7.40 ± 0.02
VII ($n = 5$)				
Baseline	121 ± 14	34 ± 3	144 ± 32	7.41 ± 0.03
2 h	113 ± 12	31 ± 3	141 ± 33	7.40 ± 0.05
VIII ($n = 6$)				
Baseline	115 ± 15	33 ± 1	136 ± 16	7.44 ± 0.03
2 h	121 ± 3	34 ± 1	120 ± 14	7.41 ± 0.03
IX ($n = 3$)				
Baseline	106 ± 18	33 ± 3	164 ± 12	7.44 ± 0.04
1.5 h	106 ± 8	35 ± 4	148 ± 13	7.42 ± 0.02
X ($n = 3$)				
Baseline	107 ± 27	35 ± 0	164 ± 15	7.43 ± 0.06
2.5 h	119 ± 20	37 ± 1	172 ± 47	7.41 ± 0.04

Values are means ± SD for n = no. of animals. SAP, systemic arterial pressure; Pa_{CO_2}, arterial P_{CO_2}; Pa_{O_2}, arterial P_{O_2}. See Fig. 1 and *Experimental Protocols* for descriptions of study groups.

attenuation of the stimulation-induced rCBF response by ~50% (9.5 ± 4.2%; for detailed data in each group see Figs. 2–5). Superfusion of the NO donor SIN-1 (10 μM) during continued NOS inhibition reestablished resting CBF to pre-L-NNA levels and completely restored the attenuated blood flow response to whisker deflection (Fig. 2). To exclude the possibility that the effect of SIN-1 was caused by superoxide, simultaneously produced by this NO donor (17), we used the NO donor SNAP in another set of experiments. Qualitatively comparable results were obtained (*study II*): superfusion of the NO donor SNAP in a concentration of 50 μM during further NOS inhibition not only

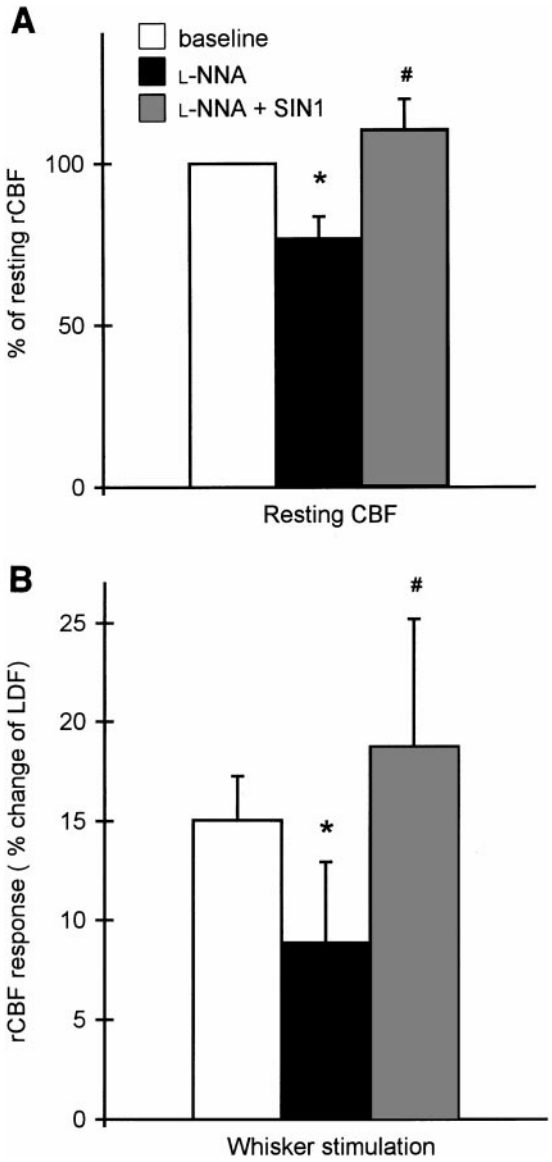

Fig. 2. Effect of topical application of NO donor SIN-1 (10 μM) on L-NNA (1 mM)-induced attenuation of resting regional cerebral blood flow (CBF; *A*) and mean rCBF response during 60-s whisker-stimulation period (*B*) (*study I*). NOS inhibition by L-NNA significantly reduces resting (*A*) as well as whisker deflection-stimulated (*B*) rCBF. SIN-1 superfusion during NOS inhibition reestablishes resting rCBF (*A*) and completely restores rCBF response to whisker stimulation (*B*). $^{*}P < 0.05$ compared with pre-L-NNA data (baseline). $^{\#}P < 0.05$ compared with L-NNA data.

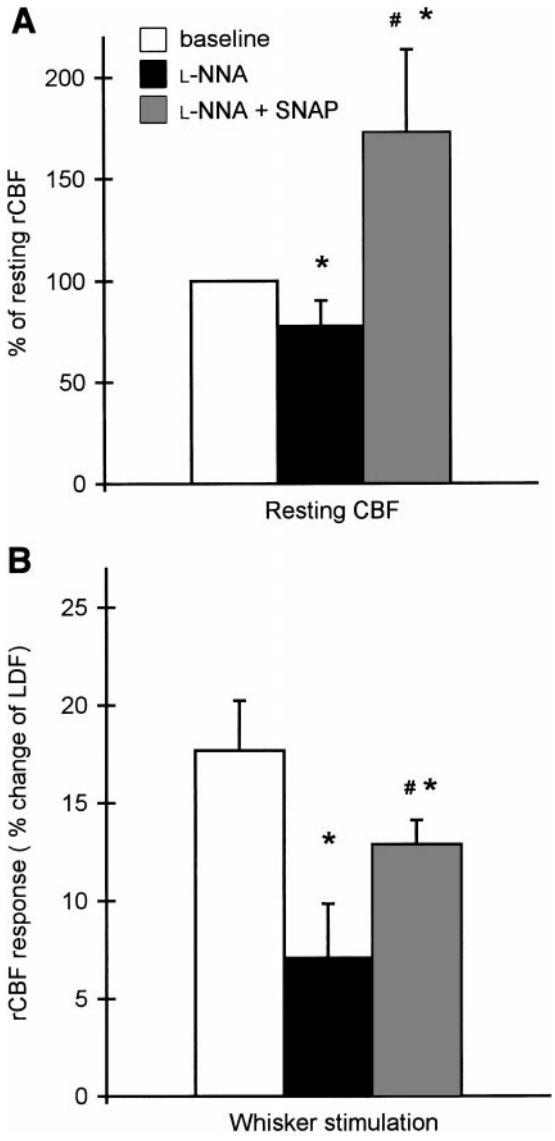

Fig. 3. Effect of topical application of NO donor SNAP (50 μM) on L-NNA (1 mM)-induced attenuation of resting rCBF (*A*) and mean rCBF response during 60-s whisker-stimulation period (*B*) (*study II*). NOS inhibition by L-NNA significantly reduces resting (*A*) as well as whisker deflection-stimulated (*B*) rCBF. During SNAP superfusion and continued NOS inhibition, resting rCBF significantly exceeds pre-L-NNA level (*A*) and only partially restores rCBF response to whisker stimulation (*B*). $^{*}P < 0.05$ compared with pre-L-NNA data. $^{\#}P < 0.05$ compared with L-NNA data.

reestablished resting rCBF after NOS inhibition but also far exceeded the pre-L-NNA level (Fig. 3*A*). The rCBF response to whisker deflection was only partially restored after NO application (Fig. 3*B*). To test whether the elevated resting rCBF per se influenced the whisker stimulation-induced rCBF increase, we applied SNAP in the same concentration of 50 μM without previous NOS inhibition (*study VI*). Resting rCBF was elevated to the same level (172.8 ± 40.9% in *study II* and 181.6 ± 45.0% in *study VI*), and the rCBF response to whisker deflection was equally reduced compared with baseline vasodilation (12.9 ± 1.2% in *study II* and 11.1 ± 4.1% in *study VI*).

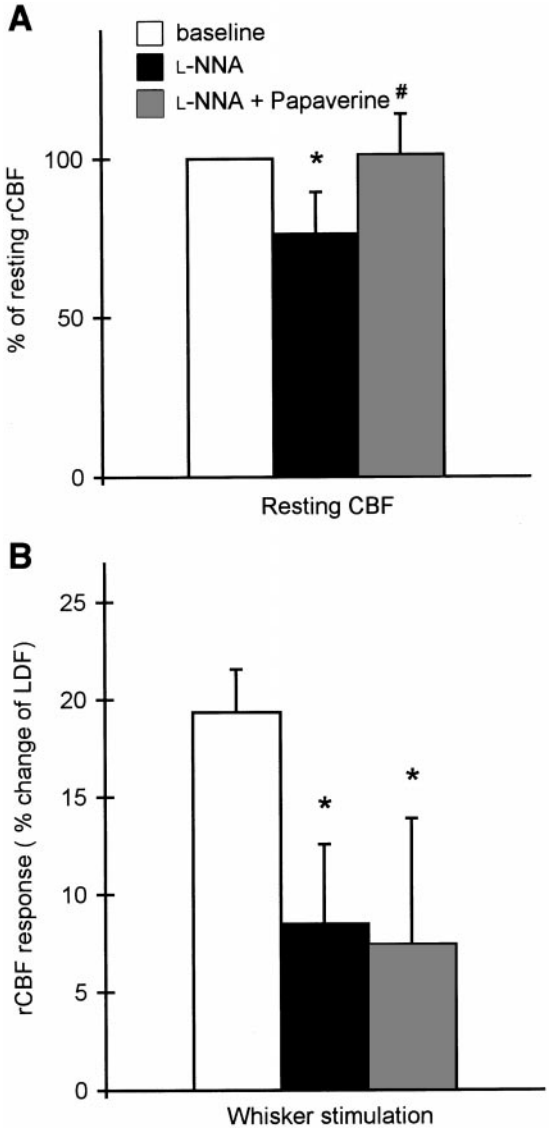

Fig. 4. Effect of topical application of NO-independent vasodilator papaverine (1 mM) on L-NNA (1 mM)-induced attenuation of resting rCBF (*A*) and mean rCBF response during 60-s whisker-stimulation period (*B*) (*study III*). NOS inhibition by L-NNA significantly reduces resting (*A*) as well as whisker deflection-stimulated (*B*) rCBF. Papaverine superfusion during NOS inhibition reestablishes resting rCBF (*A*) but has no effect on NOS inhibition-induced attenuation of rCBF response to whisker stimulation (*B*). $^{*}P < 0.05$ compared with pre-L-NNA data. $^{\#}P < 0.05$ compared with L-NNA data.

In contrast to SIN-1 and SNAP, papaverine, an NO-independent vasodilator, failed to restore the reduced rCBF response to whisker stimulation while reestablishing resting rCBF (Fig. 4).

To further investigate the mechanism of action of NO in restoring the stimulation response while NOS is inhibited, the cell-permeable cGMP analog 8-BrcGMP was tested in five rats. Reestablishing basal cGMP levels in the tissue after NOS inhibition led to a complete restoration of the vascular response to whisker deflection (Fig. 5).

In additional animals we tested the responsiveness of the vasculature beneath the cranial window to the vasodilators used to restore resting CBF after NOS inhibition under control conditions without L-NNA application. SNAP (50 μM in aCSF), papaverine (1 mM in aCSF), and 8-BrcGMP (100 μM in aCSF) increased LDF rCBF in the estimated LDF sample volume of 1 mm^3 after 30 min of topical application in control animals by 81.6 ± 45, 41.1 ± 17.6, and 31.0 ± 11.7% and after 2 h of NOS inhibition by 72.8 ± 40.9, 37.0 ± 13.7, and 32.1 ± 5.6%, respectively. There was no significant difference in the responsiveness of the vasculature between the control conditions and after L-NNA application.

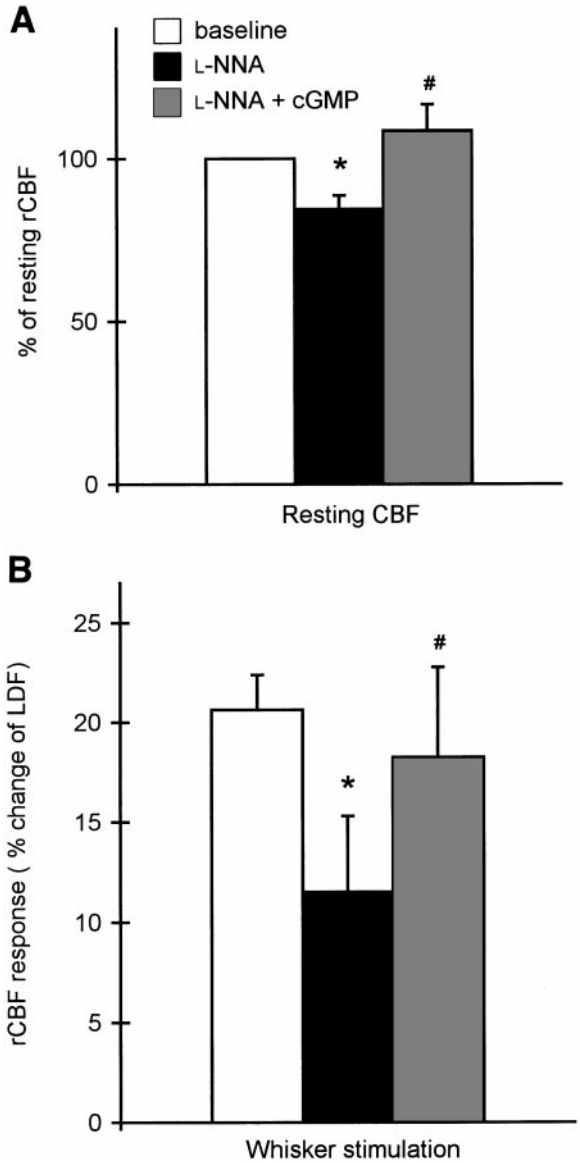

Fig. 5. Effect of topical application of cell-permeable cGMP analog 8-bromoguanosine 3′,5′-cyclic monophosphate (8-BrcGMP; 100 μM) on L-NNA (1 mM)-induced attenuation of resting rCBF (*A*) and mean rCBF response during 60-s whisker-stimulation period (*B*) (*study IV*). NOS inhibition by L-NNA significantly reduces resting (*A*) as well as whisker deflection-stimulated (*B*) rCBF. 8-BrcGMP superfusion during NOS inhibition reestablishes resting rCBF (*A*) and completely restores rCBF response to whisker stimulation (*B*). $^{*}P < 0.05$ compared with pre-L-NNA data. $^{\#}P < 0.05$ compared with L-NNA data.

Influence of NOS Inhibition, NO Donor Application, or Guanylyl Cyclase Inhibition on Basal Cortical cGMP Levels

To test the effect of NOS inhibition on the basal cGMP level in the cerebral cortex, tissue samples under the cranial window were taken after 2 h of normal aCSF superfusion (*study V,a*) and compared with those taken after 2 h of topical L-NNA application (*study V,b*), after 2 h of topical L-NNA application followed by SNAP superfusion (*study V,c*), or after 1 h of ODQ application (*study V,d*). cGMP content in cortical gray matter ≤500–1,000 μm under the brain surface beneath the cranial window in control rats was 219 ± 56 fmol/mg protein. NOS inhibition after 2 h of topical L-NNA application reduced basal cGMP levels by ~65% to 76 ± 18 fmol/mg protein ($P < 0.05$), and application of the NO donor SNAP partially restored brain cGMP content to 141 ± 12 fmol/mg protein (*study V,c*; $P > 0.05$ compared with control and L-NNA application).

Inhibition of the NO-dependent soluble guanylyl cyclase by topical ODQ application for 1 h significantly reduced basal cGMP levels by ~46% to 118 ± 23 fmol/mg protein (*study V,d*; $P < 0.05$).

Effect of Inhibition of Neuronal NOS on Resting rCBF and Whisker Stimulation-Induced Vasodilation and Influence of cGMP Substitution After Enzyme Blockade

In *study VII*, the effect of specific inhibition of neuronal NOS with 7-NI on resting rCBF and on the whisker deflection-induced rCBF response was tested in four animals. Systemic application of 7-NI led to a significant reduction of resting rCBF to 85.7 ± 6.5% (Fig. 6*A*), which is comparable to nonspecific NOS inhibition with L-NNA. In addition, 1 h after 7-NI application the whisker deflection-induced rCBF response was attenuated by >50% (6.6 ± 3.6%, $P < 0.05$). 7-NI in the same concentration as used in our study has been shown to reduce forebrain NOS activity by 50% within 30 min, and this reduction is constant over a time period of 2 h (37). Topical application of 8-BrcGMP for 30 min (started 1 h after 7-NI injection) restored both the reduced resting rCBF and the attenuated whisker deflection-induced vasodilation (Fig. 6). Without cGMP superfusion, resting rCBF and whisker deflection-induced rCBF responses remained reduced >1.5 h after 7-NI application (7.2 ± 2.0% whisker response, 7-NI control experiments).

Effect of Inhibition of the Soluble Guanylyl Cyclase on Resting rCBF and Whisker Stimulation-Induced Vasodilation and Influence of cGMP Substitution After Enzyme Blockade

In *study VIII*, the effect of inhibition of the soluble guanylyl cyclase with ODQ on resting rCBF and the whisker deflection-induced rCBF response was tested in four animals. ODQ superfusion led to a slight but statistical significant reduction of resting rCBF (91.6 ± 9.6%, $n = 4$) (Fig. 7*A*). Guanylyl cyclase inhibition attenuated the whisker deflection-induced rCBF response by ~30% to 12.4 ± 3.1% ($P < 0.05$). During ODQ

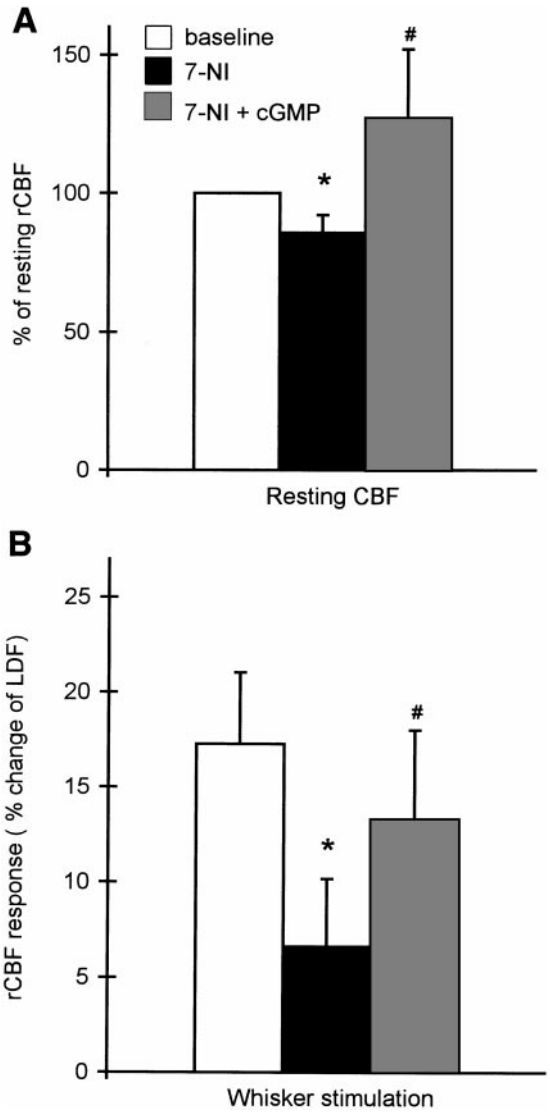

Fig. 6. Effect of topical application of 8-BrcGMP (100 μM) on 7-NI (40 mg/kg ip)-induced attenuation of resting rCBF (*A*) and mean rCBF response during 60-s whisker-stimulation period (*B*) (*study VII*). Neuronal NOS inhibition by 7-NI significantly reduces resting (*A*) as well as whisker deflection-stimulated (*B*) rCBF. 8-BrcGMP superfusion during neuronal NOS inhibition reestablishes resting rCBF (*A*) and restores rCBF response to whisker stimulation (*B*). $^{*}P < 0.05$ compared with pre-7-NI data. $^{\#}P < 0.05$ compared with 7-NI data.

superfusion, topical application of 8-BrcGMP completely restored both the reduced resting rCBF and the attenuated whisker deflection-induced vasodilation (Fig. 7). To rule out the possibility that the attenuation of the whisker deflection-induced rCBF response during guanylyl cyclase inhibition was a consequence of a reduction in stimulation-induced neuronal activity, we measured whisker stimulation-induced SEPs in additional animals ($n = 2$) at baseline conditions, during ODQ application, and after cGMP-level restoration. No change in SEP amplitudes occurred as a result of guanylyl cyclase inhibition or cGMP application (SEP amplitudes: 69.9 ± 3.1 μV at baseline, 72.8 ± 8.1 μV during ODQ application, and 70.8 ± 6.7 μV during ODQ and cGMP application), indicating a partial uncou-

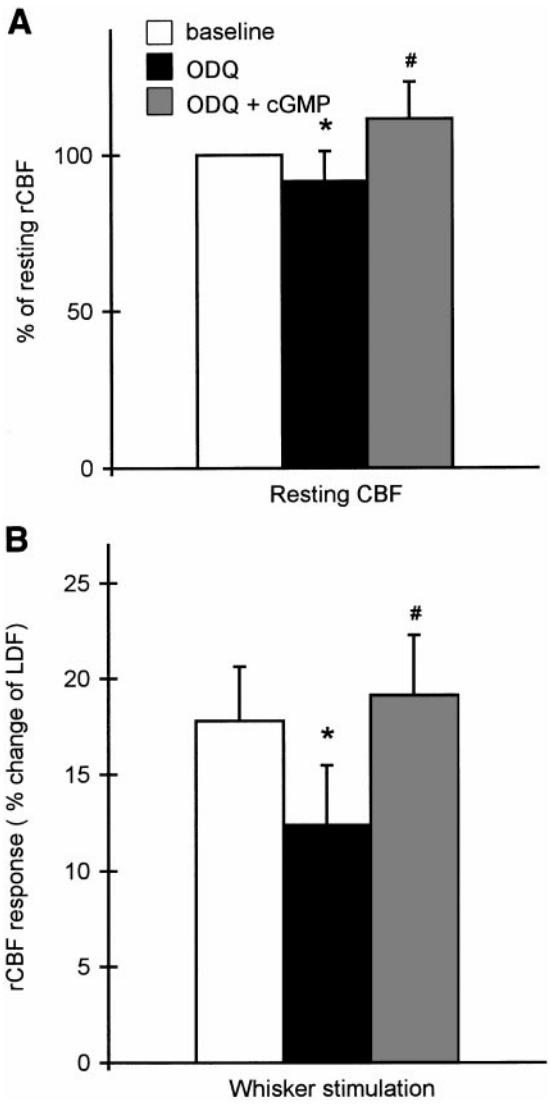

Fig. 7. Effect of topical application of 8-BrcGMP (100 μM) on ODQ (100 μM)-induced attenuation of resting rCBF (*A*) and mean rCBF response during 60-s whisker-stimulation period (*B*) (*study VIII*). Guanylyl cyclase inhibition by ODQ both significantly reduces resting rCBF (*A*) and attenuates whisker deflection-stimulated rCBF (*B*). 8-BrcGMP superfusion during guanylyl cyclase inhibition reestablishes resting rCBF (*A*) and completely restores rCBF response to whisker stimulation (*B*). $^{*}P < 0.05$ compared with pre-ODQ data. $^{\#}P < 0.05$ compared with ODQ data.

pling of neuronal activity and rCBF during ODQ application.

In addition to cGMP measurements in *study V,d*, where we have shown that inhibition of the NO-dependent soluble guanylyl cyclase during topical application of ODQ significantly reduces basal cGMP levels by ~46%, the efficacy of guanylyl cyclase inhibition was also tested functionally by two different, cGMP-dependent stimuli. Hypercapnia-induced vasodilation has been shown to nearly completely depend on smooth muscle cGMP levels in a permissive way (22, 35), whereas the ACh-induced vasodilation is endothelium dependent and mediated by an increase in smooth muscle cGMP levels (34). Therefore, in additional rats (*studies IX* and *X*, $n = 3$ each) we measured hypercapnic vasodilation (P_{CO_2} 50–55 mmHg) or ACh-induced rCBF increase at baseline conditions and after 1 h of ODQ application. The hypercapnia-induced rCBF increase as well as the ACh-induced rCBF increase was practically abolished by guanylyl cyclase inhibition (*study IX*: 2.4 ± 0.3%/mmHg at baseline and 0.5 ± 0.4%/mmHg after ODQ application, $P < 0.05$; *study X*: 23.2 ± 6.9% at baseline and 3.3 ± 2.9% after ODQ application, $P < 0.05$).

Effect of NOS Inhibition and Guanylyl Cyclase Blockade and Subsequent NO Donor 8-BrcGMP or Papaverine Application on rCBF Oscillation (Vasomotion)

In all experiments, NOS inhibition by L-NNA induced rhythmic oscillations (6–11 cycles/min) of rCBF with a mean frequency of 9.5 ± 1 cycles/min with high amplitudes (vasomotion), as has already been shown in a previous paper (10). During whisker deflection, the amplitude of the NOS inhibition-induced rhythmic vasomotion is strongly reduced (Fig. 8; typical example in Fig. 10). L-NNA-induced vasomotion of resting rCBF is significantly attenuated during subsequent application of both NO donors (SIN-1, SNAP) and tended to decrease during papaverine application. During 8-BrcGMP superfusion, no change in NOS inhibition-induced rhythmic vasomotion was observed (Fig. 9), whereas the whisker deflection-induced rCBF response was completely restored (Fig. 5, typical example in Fig. 10). Guanylyl cyclase blockade by ODQ also induced rhythmic oscillations of rCBF, with the amplitudes

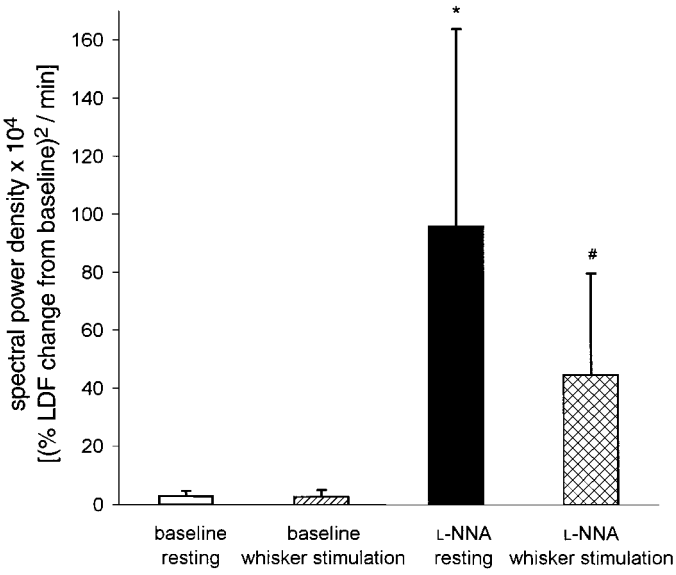

Fig. 8. Quantification of oscillatory activity in laser Doppler flowmetry (LDF) rCBF data by fast Fourier transform (FFT) spectral analysis at rest and during whisker deflection. NOS inhibition by L-NNA induces rhythmic oscillations (mean frequency 9.5 ± 1 cycles/min) of resting rCBF with high amplitudes. During whisker deflection, amplitude of NOS inhibition-induced rhythmic vasomotion is strongly reduced. $^{*}P < 0.05$ compared with baseline situation. $^{\#}P < 0.05$ compared with NOS inhibition (L-NNA resting).

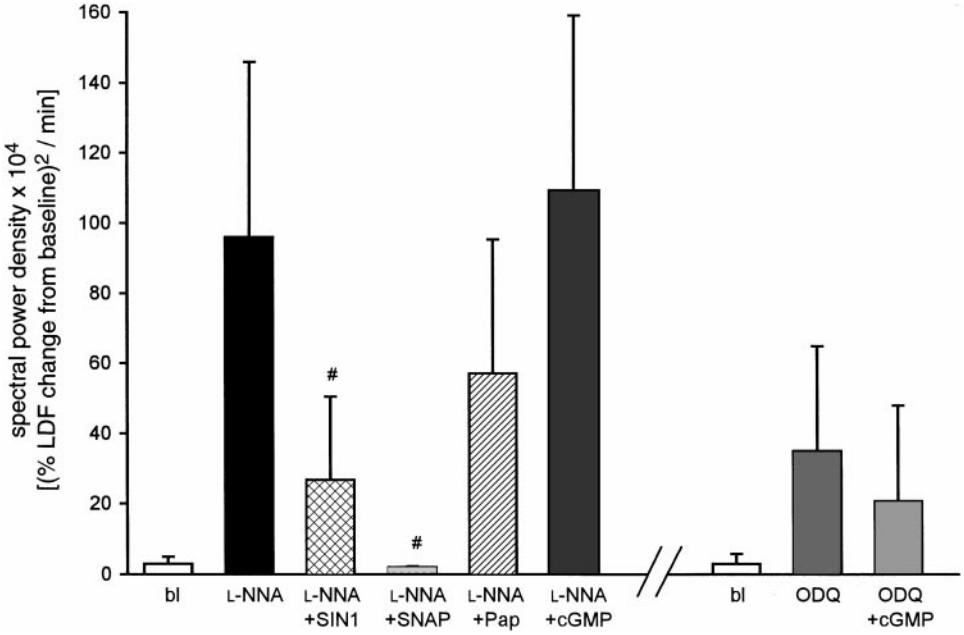

Fig. 9. Quantification of oscillatory activity in resting LDF rCBF data by FFT spectral analysis: NOS inhibition by L-NNA induces rhythmic oscillations of resting rCBF with high amplitudes. L-NNA-induced vasomotion of resting rCBF is significantly attenuated during subsequent application of both NO donors (L-NNA + SIN-1, L-NNA + SNAP) and tends to decrease during papaverine application (L-NNA + Pap). During 8-BrcGMP superfusion (L-NNA + cGMP), no change in NOS inhibition-induced rhythmic vasomotion occurs. Guanylyl cyclase blockade by ODQ also induces rhythmic oscillations of rCBF, with amplitudes being much smaller than after NOS inhibition with L-NNA, and cGMP application after ODQ (ODQ + cGMP) does not significantly change guanylyl cyclase inhibition-induced rhythmic vasomotion. bl, Baseline. $^{\#}P < 0.05$ compared with NOS inhibition (L-NNA) alone.

being much smaller than after NOS inhibition with L-NNA. cGMP application after ODQ did not significantly change guanylyl cyclase inhibition-induced rhythmic vasomotion (Fig. 9).

Neither the NO donors SIN-1 or SNAP nor 8-BrcGMP or papaverine application had an effect on oscillation frequency.

DISCUSSION

The key findings of this study were as follows. *1*) The NOS inhibition-induced attenuation of the rCBF response to increased neuronal activity during whisker deflection in rats can be fully restored by reestablishing basal NO levels. *2*) This basal NO level in cortical tissue in rats is mainly produced by the neuronal isoform of the constitutive NOS. *3*) NOS inhibition leads to a significant decrease of basal cGMP levels in the cerebral cortex. The permissive role of NO during whisker stimulation-induced vasodilation is significantly mediated by an NO-dependent basal cGMP concentration in the tissue. *4*) NOS inhibition-induced rhythmic rCBF oscillations (6–11 cycles/min) are significantly reduced during increased neuronal activity (whisker stimulation) and are significantly attenuated after restoration of basal NO levels but not after restoration of basal cGMP levels.

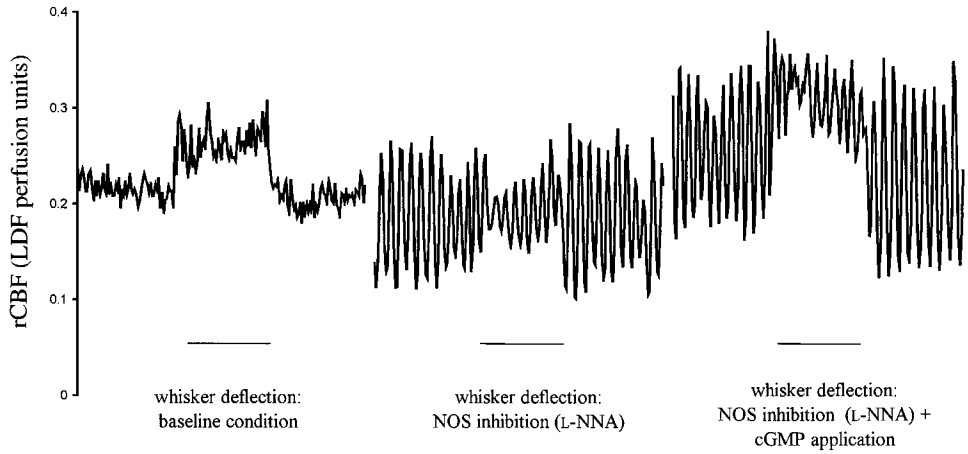

Fig. 10. Typical example of modulatory role of cGMP on whisker stimulation-induced rCBF response (*study IV*). Under baseline conditions, whisker deflection (horizontal bars indicate 60-s stimulation period) leads to a rapid and stable increase of rCBF (22.1% rCBF increase in this example). NOS inhibition (topical application of L-NNA) induces rhythmic oscillations of resting rCBF with high amplitudes (vasomotion) and significantly reduces resting rCBF as well as whisker deflection-induced rCBF response (5.3% rCBF increase in this example). Note that vasomotion amplitude is strongly reduced during increased neuronal activity (whisker stimulation period). During cGMP substitution (NOS still inhibited), resting rCBF is restored and rCBF response to whisker deflection is reestablished (20.0% rCBF increase in this example). Note that cGMP application has no effect on NOS inhibition-induced rhythmic oscillations of resting rCBF, which are again significantly reduced during whisker stimulation.

Several methodological aspects have to be considered. The sample volume of the LDF rCBF measurement and the tissue volume reached by drug superfusion or SEP measurement may not be exactly the same. Nevertheless, it can be assumed that the complete whisker barrel cortex is included by topical drug application in our study. Because the LDF probe was positioned exactly over the center of the whisker barrel cortex, avoiding larger pial vessels, the whole LDF sample volume is reached by the substances applied topically. To avoid tissue volume change-induced differences in the SEP recordings throughout the experiments, the recording electrode, positioned adjacent to the LDF probe, is not in direct contact with the brain surface, measuring evoked potentials generated by the whole whisker cortex area.

To show that during NOS inhibition the responsiveness of the vasculature beneath the cranial window is unchanged in response to NO production-independent vasodilators, all vasodilators used to restore resting CBF after NOS inhibition have been tested under control conditions without L-NNA application. The reaction to topical papaverine application was significantly lower as described by others (21). Because of the comparably high sample volume of the LDF probe, papaverine has to penetrate ≤1 mm into the cortex to influence the microcirculation within the whole sample volume. There is evidence that, during topical application, the concentration substances with even lower molecular weight than papaverine may strongly be reduced several hundred micrometers below the brain surface (19). The superficial rCBF increase in response to 10^{-4} M papaverine shown by Iadecola and Xu (21) is comparable to the LDF rCBF increase in our study. In addition, a nonspecific, papaverine-induced cGMP increase due to nonselective phosphodiesterase inhibition (3) can be ruled out because, in contrast to external cGMP application, papaverine did not reestablish the whisker-induced rCBF increase during NOS inhibition.

It is not clear whether reestablishing resting rCBF by external NO or cGMP application to brain tissue also reestablishes the physiological NO/cGMP level equally in all cells of the brain tissue. Because cGMP and NO are acting as neurotransmitters and second messengers, topical application after NOS inhibition may induce an artificial NO/cGMP concentration in the tissue compared with the physiological situation. In addition, a concentration gradient from the surface into the tissue may occur as a result of topical drug application. The restoration of resting CBF by superfusing vasodilators in the present study may therefore result from a greater dilation of pial arterioles than from intraparenchymal vessels, which may be different from the physiological situation under normal conditions. The neurovascular coupling cascade, normally including the NO/cGMP system, may be altered after the NO/cGMP levels are artificially reestablished during drug superfusion following NOS inhibition, as has been shown for ATP-dependent potassium-channel activation in response to other vasodilators, e.g., adenosine or CO_2/H^+ (33).

In the present study, we address the question of whether NO acts as a mediator in neurovascular coupling or whether it is a permissive factor, as has already been shown for hypercapnic vasodilation (22, 35). During somatosensory stimulation by whisker deflection, the rCBF response is significantly reduced during NOS inhibition by the nonspecific blocker L-NNA (current study and Ref. 11) as well as by the specific blocker of the neuronal NOS (type I), 7-NI (current study and Refs. 8 and 9). Compared with control experiments, during NOS inhibition whisker stimulation-induced SEPs (24) as well as cerebral glucose utilization (9) are unchanged, suggesting a partial uncoupling of neuronal activity and rCBF response during NOS blockade.

We have shown here that topical application of the NO donor SIN-1 restores the reduction in resting rCBF and reestablishes the whisker stimulation-induced hyperemia during NOS inhibition. In addition, the synthetic cGMP analog 8-BrcGMP was also found to restore the NOS inhibition-induced reduction of resting as well as stimulated rCBF. A nonspecific effect of the vasodilation produced by SIN-1 or 8-BrcGMP on the whisker stimulation-induced rCBF response can be ruled out, because the NO-independent vasodilator papaverine, while restoring resting rCBF, did not reestablish the attenuated response during NOS inhibition. A time-dependent decrease of the inhibitory effect of NOS inhibition by L-NNA or 7-NI seems unlikely to account for our results. The topically applied NOS inhibitor L-NNA is superfused over the brain cortex throughout the whole experimental period and has been shown by us previously (24) to reduce NOS activity after 2 h of L-NNA superfusion to 16 ± 9% in the brain tissue beneath the cranial window in the whisker barrel cortex. Yoshida et al. (37) have shown that 7-NI application (in the concentration given in our study) reduces NOS activity by ~50% after 30 min and that this reduction remains stable for ≤120 min. In our study the attenuation of the whisker stimulation-induced rCBF response after 7-NI application is stable over the experimental period.

The NO donor SIN-1 has been shown to produce superoxide anions simultaneously with NO liberation (17). Because reactive oxygen species are potent vasodilators, the restoration of the whisker stimulation-induced rCBF response may be caused by superoxide production rather than a specific NO-dependent effect. Therefore, we also tested the NO donor SNAP, which does not generate reactive oxygen species while liberating NO.

SNAP application in the concentration used in our study not only restored resting rCBF to pre-L-NNA levels after NOS inhibition but also led to significantly higher resting blood flow values. While counteracting the NOS inhibitory effect of L-NNA with SNAP, the whisker stimulation-induced rCBF response was only partially reestablished. This may be explained by the rCBF baseline increase by SNAP, because SNAP application without NOS inhibition, while increasing resting rCBF, reduces the rCBF response to whisker deflection to the same level as with SNAP application during

NOS inhibition. In a recent study an attenuation of the vascular response to hypercapnia has been shown during elevated resting rCBF with SIN-1 as well as with papaverine (22). Further experiments have to be conducted to elucidate whether the lack of complete restoration of the whisker stimulation-induced rCBF response after SNAP application in *study II* is mediated by the very high basal NO levels in the tissue or whether it is caused by the decrease in resting vasotonus, regardless of the vasodilator used. Compared with cGMP tissue concentrations during NOS inhibition and with cGMP levels in control animals beneath the cranial window, the tissue cGMP levels are only partially reestablished during SNAP application after NOS inhibition, despite increased resting rCBF values. These still-reduced cGMP levels may also account for the incomplete restoration of the whisker stimulation-induced rCBF response. cGMP-independent effects of NO may be responsible for the elevated resting rCBF during SNAP application after NOS inhibition.

Our results, in which NO donors reestablish the whisker stimulation-induced rCBF response while NOS activity is inhibited, clearly indicate a modulatory rather than a mediatory function for NO in neurovascular coupling in the somatosensory cortex.

Further evidence for a permissive role of NO in neurovascular coupling and the critical role of the cGMP system in these responses is our finding that inhibition of the NO-sensitive guanylyl cyclase with ODQ significantly reduced the blood flow response to whisker stimulation, which was restored by 8-BrcGMP. ODQ application for 1 h reduced cortical cGMP concentrations by ~46%, which is in good agreement with the situation in the hippocampus as shown in other studies (13, 32). In addition, the efficacy of guanylyl cyclase inhibition was verified by near complete inhibition of hypercapnia- as well as acetylcholine-induced hyperemia. An nonspecific effect of ODQ leading to complete vasoparalysis seems unlikely because the whisker stimulation-induced rCBF response is only partially reduced by ~50% of the control response. In addition, it has been shown (36) that vasoreactivity to the guanylyl cyclase-independent vasodilators adenosine and 8-BrcGMP are unchanged after ODQ application. In an earlier study (11), we showed that topical L-NNA application also leads to a near complete reduction of the hypercapnic vasodilation (3.11 ± 0.5%/mmHg at control conditions and 0.2 ± 0.3%/mmHg after NOS inhibition), comparable to the results with ODQ in the present study. This argues for the hypothesis that hypercapnic hyperemia is critically dependent on the basal cGMP concentration in the tissue, produced by a basal NO level. In addition, the hypothesis of a basal NO production in the cerebral cortex as a requirement for a basal cGMP level in the tissue is strongly supported by our results. Two hours of topical L-NNA application not only decreases brain NOS activity by ~85% (24) but also reduces basal tissue cGMP by ~65%. In studies in which microdialysis was used to measure cGMP levels in the cerebellum and hippocampus of freely moving rats (13, 31, 32), it was reported that the basal extracellular level of cGMP was equally reduced to 20 and 50%, respectively, by the administration of L-NNA or ODQ, supporting our hypothesis of a basal NO tonus as a requirement for a basal cGMP level in the tissue. The cGMP concentration in the somatosensory cortex beneath the cranial window under normal conditions as measured in our study is smaller than that described by Irikura et al. (23) in the forebrain of rats. This discrepancy may be caused by region-specific differences of cGMP content in the brain, being highest at the pial surface, which we purposely removed in our tissue samples taken for cGMP measurement.

Similarly to NOS inhibition (24), guanylyl cyclase inhibition does not affect neuronal activation, as shown by unaltered whisker stimulation-induced SEPs, measured by a noncontact electrode in the cranial window. A possible lack of sensitivity of the recording methodology used in the present study has to be taken into consideration as discussed by Ngai et al. (27). They reported that noncontact and pial surface contact electrodes yield different results, which they attributed to the inferior spatial resolution in the noncontact electrode. In their setup (pial surface contact electrode), NOS inhibition significantly reduces stimulation-induced neuronal activity. In contrast, we and others (1, 9, 24, 28) have shown that NOS inhibition does not affect stimulus evoked neuronal activity or the increase in cerebral glucose utilization. The reasons for the discrepancy remain unclear. The lack of definitive neuronal activity measurements, however, may not detract from the present conclusion of a permissive role of NO, because theoretically a basal level of NO may be required for neuronal activity as well.

Our present results complement a recent study on the role of NO in neurovascular coupling using the well-characterized model of electrical parallel fiber stimulation in the cerebellum (1, 35). Parallel fiber stimulation triggers monosynaptic excitatory postsynaptic potentials via activation of glutamate receptors. This excitatory synaptic activity-dependent local vasodilation is attenuated by NOS inhibition (1, 20). However, neither NO donors nor cGMP is effective in restoring the response (1, 35), indicating a mediatory role of NO during neuronal activation in this system. The production of NO and cGMP on glutamate stimulation has been demonstrated in cerebellar slices (6), whereas currently there is no evidence for glutamatergic NO production in the somatosensory cortex. NOS activity in the rat cerebellum is ~10-fold higher than in the somatosensory cortex (9). Thus, besides profound neurochemical, neuroanatomic, and functional differences between the cerebellar parallel fiber system and the primary somatosensory cortex, the discrepant findings concerning the role of NO in neurovascular coupling may be best explained by differences in NOS levels in both systems. Taken together, the studies by Akgören et al. (1) and Yang and Iadecola (35) and our results suggest that there are region-specific, profound differences in basic vascular functions of the brain.

Where does the basal, permissive NO level in the somatosensory cortex originate? Because brain vascu-

lar endothelial cells are rich in NOS, and because type III (endothelial) NOS is therefore located close to vascular smooth muscle, a contribution of the endothelium to the basal NO level required for neurovascular coupling in the somatosensory cortex may seem likely. However, it is interesting to note that *1*) pharmacological inhibition of type I (neuronal) NOS using 7-NI reduces basal vascular tone almost to the same degree as combined inhibition of types I and III (endothelial) NOS with L-NNA (current study and Refs. 9 and 35); *2*) type III NOS knockout mice exhibit normal rCBF responses to whisker stimulation (2); and *3*) together with evidence derived from experiments using type I NOS knockout mice (26) or the type I NOS-specific inhibitor 7-NI in rats (current study and Ref. 8), these findings point to the critical involvement of neuronal NOS in this response. On the basis of these results, we postulate that the basal NO tone relevant for coupling is produced mainly by type I, not type III, NOS.

The phenomenon of periodic rCBF oscillations (vasomotion) and its mediators or modulators has been investigated for decades but still remains unclear. Vasoconstrictors and vasodilators have been reported to enhance and reduce, respectively, vasomotion of cerebral vessels in vivo (15). In the present study we have shown that amplitudes of NOS inhibition-induced periodic rCBF oscillations (vasomotion) (10) are significantly reduced during increased neuronal activity. It is interesting to note that spontaneous rhythmic fluctuations in this frequency range of deoxygenated hemoglobin as detected by blood oxygenation level-dependent (BOLD) magnetic resonance imaging in humans have been shown to focally decrease in functionally active cortical areas (4). In addition to functional activation, application of NO donors significantly depressed NOS inhibition-induced oscillatory activity, but vasomotion was only completely abolished when NO application far exceeded pre-NOS inhibition levels (significant higher resting rCBF during SNAP application after NOS inhibition in *study II* with only partially restored tissue cGMP content). Restoration of the baseline vasotonus after NOS inhibition with the NO-independent vasodilator papaverine also slightly, but not significantly, reduced vasomotion amplitudes, whereas cGMP substitution had no effect on NOS inhibition-induced oscillatory activity. Blockade of the NO-dependent guanylyl cyclase with ODQ also induced vasomotion with significantly smaller amplitudes than during NOS inhibition, whereas cGMP substitution had no significant effect on ODQ-induced oscillatory activity. Taken together, our findings suggest that NO itself, which is an important modulator of the rCBF response to somatosensory stimulation, also plays a modulatory role in oscillatory activity of vascular smooth muscle. In contrast to neuronal activity-dependent vasoreactivity, this modulation seems not to be mediated by cGMP. Because papaverine, acting at intracellular Ca^{2+} stores, also slightly reduced NOS inhibition-induced vasomotion, and because NO has been shown to directly activate calcium-dependent potassium channels in vascular smooth muscle cells without cGMP production (5), we speculate that rhythmic oscillations may at least in part be mediated by oscillations in intracellular potassium concentration, regulated via calcium-dependent potassium channels. Besides this cGMP-independent component of NOS inhibition-induced vasomotion, there also seems to exist a small cGMP-dependent component regulating oscillatory activity in vascular smooth muscle cells, because inhibition of guanylyl cyclase by ODQ, while reducing tissue cGMP concentration by ~46%, also induced small-amplitude vasomotion, which was unaffected by external cGMP substitution. In conclusion, NO-dependent as well as NO-independent mechanisms seem to play a role in the oscillatory activity of vascular smooth muscle cells (14). Under physiological conditions, cerebrovascular vasomotion seems to be suppressed by the basal NO level.

In conclusion, we have demonstrated that NOS inhibition as well as inhibition of the NO-sensitive guanylyl cyclase attenuates the vasodilation produced by somatosensory stimulation during whisker deflection in rats. The attenuation of the functional activation-induced vasodilation is reversed by NO donors or cGMP. The data suggest that the rCBF response to somatosensory stimulation requires a basal level of NO/cGMP but not NOS activation. During whisker stimulation, NO in the somatosensory cortex acts as a modulator rather than a mediator of vascular relaxation, permitting vasodilation mediated by other agents. Further experiments have to elucidate the nature of these mediators, which may include parenchymal factors such as potassium, protons, and adenosine or a hematogenic mechanism such as the oxygenation-sensitive, allosteric release of NO from cysteineβ93 *S*-nitroso-hemoglobin (30).

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