

Despite In Vitro Increase in Cyclic Guanosine Monophosphate Concentrations, Intracarotid Nitroprusside Fails to Augment Cerebral Blood Flow of Healthy Baboons

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Background: During cerebral angiography, intracarotid infusion of sodium nitroprusside (SNP), an endothelium-independent nitric oxide donor, fails to increase cerebral blood flow (CBF) of human subjects. A confounding effect of intracranial pathology or that of radiocontrast could not be ruled out in these experiments. The authors hypothesized that, if nitric oxide was a significant regulator of CBF of primates, then intracarotid SNP will augment CBF of baboons.

Methods: In *in vivo* studies, CBF (intraarterial ^{133}Xe technique) was measured in healthy baboons during isoflurane anesthesia at (1) baseline and during (2) induced hypertension with intravenous phenylephrine, (3) concurrent infusions of intravenous phenylephrine and intracarotid SNP, and (4) intracarotid verapamil (positive control drug). In *in vitro* studies, the authors measured tissue cyclic guanosine monophosphate (cGMP) by radioimmunoassay after incubating vascular rings obtained from freshly killed baboons (1) with increasing concentrations of SNP and (2) after SNP exposure following preincubation with the radiocontrast agent, iohexol.

Results: In the *in vivo* studies, coinfusion of intravenous phenylephrine and intracarotid SNP did not increase CBF. However, intracarotid verapamil significantly increased CBF (from 26 ± 7 to $43 \pm 11 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$; $P < 0.0001$) without a change in mean arterial pressure. In the *in vitro* studies, incubation of intracranial arterial rings in SNP resulted in dose-dependent increases in cGMP concentrations. A similar increase in cGMP content was evident despite iohexol preincubation.

Conclusions: Collectively, these results suggest that, in healthy baboons, intracarotid SNP does not decrease arteriolar resistance, although SNP could affect proximal arterial tone, as demonstrated by the *in vitro* increase in cGMP content of these vessels.

THE recent resurgence of interest in the cerebrovascular effects of sodium nitroprusside (SNP), an endothelium-

independent nitric oxide (NO) donor, can be attributed to the observation that, in patients with cerebral vasospasm, intrathecal SNP augments cerebral blood flow (CBF).^{1,2} Furthermore, in primates, intraarterial NO donors reverse the decrease in CBF resulting from experimental cerebral vasospasm.^{3,4} Yet in the absence of vasospasm, NO donors such as SNP do not augment CBF when given by the intravenous route to human and nonhuman primates.⁵⁻⁹ In rodents and goats, unlike in human subjects, intraarterial infusion of SNP increases CBF.¹⁰⁻¹⁴ The reasons for these differing results between rodents and primates are unclear. Intracranial pathology or use of a radiocontrast agent during cerebral angiography could have inhibited the response to SNP.

Because of striking similarities between the cerebral circulation of human and nonhuman primates, we conducted parallel *in vivo* and *in vitro* studies on healthy baboons.¹⁵ Intraarterial SNP infusion has been extensively used to test NO-mediated vasodilation in the peripheral arterial beds.¹⁶⁻¹⁹ Our hypothesis is that if NO is a major determinant of CBF, then intracarotid infusion of a NO donor, such as SNP, should increase CBF in healthy baboons.

We first replicated the drug infusion protocol used in human subjects on anesthetized baboons.¹³ Having failed to find an increase in CBF after intracarotid SNP infusions, we then undertook additional *in vitro* studies of arteries obtained from freshly killed baboons. We measured changes in tissue cyclic guanosine monophosphate (cGMP) content of intracranial and extracranial segments of internal carotid artery (ICA) after incubation with increasing concentrations of SNP. Furthermore, to rule out a confounding effect of radio-contrast agent on the outcome of *in vivo* studies, we preincubated primate arterial rings in iohexol and measured changes in tissue cGMP concentration in iohexol-exposed arterial rings after SNP incubation.

Materials and Methods

In Vivo Studies

The experiments were conducted on a colony of six adult baboons after we obtained approval from the Institutional Animal Care and Use Committee, in accordance with the guidelines set by the National Institute of Laboratory Animal Resources. For the *in vivo* studies,

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six animals were sedated with intramuscular ketamine (20 mg/kg), intubated, and mechanically ventilated. Anesthesia was maintained with 1–1.5% isoflurane. The puncture site in the groin was infiltrated with 0.5% bupivacaine. A 4.5-French introducer sheath (Check-Flo; Cook Co. Inc., Bloomington, IN) was placed in the femoral artery, through which a 4-French coaxial catheter (Cook Co. Inc.) was placed in the common carotid artery. A microcatheter (12-French, Fastrack; Target Therapeutics, Boston Scientific International, Cedex, France) was then placed *via* the coaxial sheath in the ICA. Satisfactory positioning of catheter was verified by a biplane angiogram. To rule out any catheter-induced vasospasm, the placement of the microcatheter was considered to be satisfactory if the following conditions were fulfilled: (1) there was free flow of angiographic contrast; (2) a pressure wave could be recorded through the microcatheter; and (3) mean pressure recorded through the microcatheter in the ICA was not less than 90% of mean arterial pressure (MAP) recorded in the femoral artery.

Hemispheric CBF was determined by intraarterial ^{133}Xe injection technique.^{20,21} The technique involved a compact injection of approximately 0.8 mCi of Xe isotope flushed with a bolus of normal saline (3 ml/3–5 s). Such an injection results in an instantaneous input function. The injected ^{133}Xe is rapidly lost through the lungs. Thus, there is no need for deconvolution analysis of the washout curve. A cadmium-telluride scintillation detector was placed over the middle cerebral artery distribution to record the radiation washout. The placement over the region of interest was confirmed by angiography. The washout of the tracer was recorded for 90 s after ^{133}Xe injection. Blood flow was determined by analyzing the slope of the ^{133}Xe washout curve between 20 and 80 s after tracer injection. Assuming a ^{133}Xe blood:brain partition coefficient of 1, this method yields a CBF value (milliliters per 100 grams per minute) that is biased toward gray matter. Intracarotid drug infusions were continued after bolus injection of ^{133}Xe for 90 s. Physiologic variables were recorded at the end of tracer washout and included end-tidal isoflurane concentrations, end-tidal carbon dioxide concentrations, heart rate, MAP, and ICA pressure. A sample of arterial blood was obtained with each CBF measurement for determining hematocrit and arterial carbon dioxide partial pressure. Cerebrovascular resistance (CVR; millimeters of mercury per milliliter per 100 grams per minute) was calculated by ICA pressure divided by CBF.

In vivo drug infusion was designed with the following objectives: (1) to test autoregulatory response to induced systemic hypertension; (2) to avoid systemic hypotension during intracarotid SNP infusion; and (3) to test pharmacologic response to intracarotid verapamil. The protocol consisted of the following four repeat CBF measurements: (1) Baseline measurements were made

during intracarotid infusion of normal saline. (2) Second CBF measurement was undertaken during induced hypertension with infusion of intravenous phenylephrine: 20- μg bolus followed by 0.2- $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ infusion. This dose of phenylephrine increases MAP by 10–15%. We selected phenylephrine to induce hypertension because during physiologic circumstances, phenylephrine lacks direct cerebrovascular effect and is therefore useful in augmenting cerebral perfusion pressure. Intracarotid saline was infused during systemic hypertension. (3) Third CBF measurement was undertaken during concurrent infusions of intravenous phenylephrine and intracarotid SNP. Based on previous experience, intracarotid SNP (0.5 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) is sufficient to decrease MAP by 10–15% and reverse phenylephrine-induced systemic hypertension. (4) The final CBF measurement was undertaken during intracarotid infusion of verapamil (0.2 mg/min).¹⁴ Verapamil, a calcium channel-blocking drug, increases CBF when given by the intracarotid route. The dose of verapamil was based on our previous human studies.^{14,22} It was used to test *in vivo* pharmacologic reactivity of the preparation. We did not randomize the four stages of the experiments. The sequence of drug infusions was aimed to ensure smooth transition from one stage of the experiment to the next so as to minimize the time required for the study and to avoid any time-dependent increase in CBF during isoflurane anesthesia.²³

In Vitro Studies

The *in vitro* studies were conducted on tissues obtained from freshly killed healthy baboons that were a part of a separate study. These animals served as controls and had received intraarterial saline infusion and undergone a magnetic resonance image scan before being killed by intravenous injection of pentobarbital chloride.

Intracranial Arteries Versus Extracranial Internal Carotid Artery Segments. To investigate whether cGMP content of primate cerebral arteries increases after exposure to SNP, we measured changes in tissue cGMP concentrations of segments of intracranial arteries and extracranial portions of the ICA. The extracranial ICA, approximately 1 cm in length, was used as a control for intracranial arteries because the behavior of the arteries changes dramatically after traversing the skull base.²⁴ Immediately after animal sacrifice, the brains were removed from the skull. We obtained segments of the intracranial arteries from the Circle of Willis and the middle cerebral artery that were approximately 6–10 cm in length. All harvested vessels were incubated in physiologic HEPES solution. After obtaining intracranial arteries, the extracranial portion of ICA was harvested separately. Tissue harvesting was completed within 45 min of animal sacrifice. The fascia on the surface of the vessel was dissected, and the cleaned vessels were divided into approximately five equal sections, approximately 2 mm

Table 1. Effect of Intracarotid Infusion of Nitroprusside on Cerebral Blood Flow of Primates (N = 10)

| Parameter (n = 10) | Base | IV Phenylephrine | IV Phenylephrine + Intracarotid SNP | Intracarotid Verapamil |
|--|-----------|---------------------|--|---------------------------|
| Hematocrit, % | 34 ± 3 | 34 ± 3 | 34 ± 3 | 34 ± 4 |
| Paco ₂ , mmHg | 33 ± 4 | 33 ± 5 | 33 ± 4 | 33 ± 4 |
| Heart rate, beats/min | 86 ± 13 | 77 ± 18* | 81 ± 17 | 86 ± 15 |
| MAP, mmHg | 69 ± 12 | 87 ± 15*‡§ | 75 ± 13† | 66 ± 12 |
| P _{ica} , mmHg | 68 ± 10 | 84 ± 14*‡§ | 73 ± 13† | 63 ± 10 |
| CBF, ml · 100 g ⁻¹ · min ⁻¹ | 26 ± 7 | 24 ± 5 | 27 ± 7 | 43 ± 11*†‡ |
| CVR, mmHg · ml ⁻¹ · 100 g ⁻¹ · min ⁻¹ | 2.8 ± 0.8 | 3.6 ± 1.1* | 2.8 ± 1.0† | 1.6 ± 0.6*†‡ |

Data presented as mean ± SD. Statistical analysis was performed using factorial analysis of variance and *post hoc* Bonferroni–Dunn test.

Significantly different from *base, †IV phenylephrine, ‡IV phenylephrine plus intracarotid sodium nitroprusside (SNP), and §intracarotid verapamil.

CBF = cerebral blood flow; CVR = cerebrovascular resistance; MAP = mean arterial pressure; P_{ica} = pressure in internal carotid artery.

wide. Vessels were washed to remove any blood or clots in the vascular lumen. They were incubated for 10 min in freshly prepared, light-protected solutions of SNP dissolved in physiologic saline. The concentrations of SNP used were 0, 10⁻⁹, 10⁻⁷, 10⁻⁵, and 10⁻³ M. After 10 min of incubation with SNP, the reaction was arrested by 5% trichloroacetic acid. The vessels were then frozen at -80°C for subsequent cGMP determination.

To determine cGMP content, vascular rings were thawed and weighed. The cGMP concentrations were determined by a radioimmunoassay technique using a commercial kit (Biomedical Technologies, Inc., Stoughton, MA). To assure quality control, the test plot of standard solutions was obtained for each run. Two identical tubes for each standard and sample were prepared to assess test-retest intraexperimental reliability. Only cGMP values that were within the linear portion of the standard curve were included in the data set. The results also were rejected if the count obtained from the paired samples differed by ≥ 10%. The cGMP content is expressed in picomoles per milligram of the tissue.

Effect of Iohexol on Cyclic Guanosine Monophosphate Response to Sodium Nitroprusside. To determine whether preincubation with iohexol altered

cGMP response to SNP, the distal 3 cm of the common carotid artery was harvested from the animals described above. The harvested arteries were cleaned and then divided into eight approximately equal rings, approximately 4 mm in length, that were randomly assigned to one of two groups: SNP and control. To test the response of these rings at normoosmotic concentrations, a pair of rings from each animal was incubated in HEPES to provide control data. One ring from each of the six pairs was then exposed to 10⁻⁵ M SNP (nitroprusside group) and the other to normal saline (control group) for 10 min. The concentration of SNP corresponded to the estimated cerebral arterial blood concentration of SNP during our *in vivo* studies. After 10 min of SNP incubation, the reaction was arrested with trichloroacetic acid. The six remaining rings from each animal were used for testing the effect of iohexol preincubation. To simulate clinical use of radiocontrast, three pairs of rings were preincubated for 10 min in one of the three concentrations of iohexol (5, 20, or 100%). The incubation period of 10 min represented the cumulative time of exposure to contrast agent during angiography for *in vivo* experiments. One of each pair of rings was subsequently

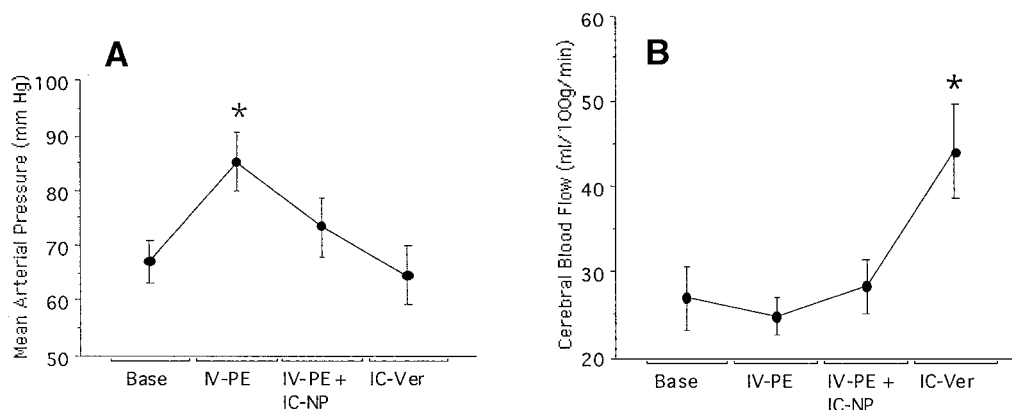


Fig. 1. Changes in (A) mean arterial pressure (MAP, millimeters of mercury) and (B) cerebral blood flow (CBF, milliliters per 100 g per minute) in baboon experiments (n = 10). Intravenous phenylephrine (IV-PE) resulted in a significant increase in MAP. Concurrent infusion of IV-PE and intracarotid nitroprusside (IC-SNP) decreased MAP to near baseline levels (Base). Intracarotid verapamil (IC-Ver) had no effect on MAP. CBF was unchanged at baseline, during IV-PE infusion, and concurrent infusion of IV-PE and intracarotid nitroprusside (IC-SNP). IC-Ver significantly increased CBF. Error bars represent SE; asterisks represents significant differences from the other three stages of the experiment.

Table 2. Increase in Tissue cGMP Concentrations After Incubation of Intracranial and Extracranial Arterial Segments with Sodium Nitroprusside

| SNP Concentration, m | cGMP Content per Milligram of Tissue, pm/mg | |
|-------------------------|---|-------------------|
| | Intracranial, n | Extracranial, n |
| 0 (base) | 0.12 ± 0.08 (6)* | 0.02 ± 0.01 (5) |
| 10 ⁻⁹ (base) | 0.14 ± 0.08 (4)* | 0.02 ± 0.01 (4) |
| 10 ⁻⁷ (base) | 0.14 ± 0.08 (4)* | 0.02 ± 0.10 (5) |
| 10 ⁻⁵ (base) | 0.51 ± 0.10 (6)*† | 0.20 ± 0.15 (5)†‡ |
| 10 ⁻³ (base) | 0.73 ± 0.19 (6)*† | 0.38 ± 0.19 (5)†‡ |

Data presented as mean ± SD. Statistical analysis was performed using factorial analysis of variance and *post hoc* Bonferroni-Dunn test.

*Significant difference between intracranial and extracranial vessels.

†Significantly different from baseline and 10⁻⁹ m sodium nitroprusside (SNP). ‡Significantly different from baseline and 10⁻⁷ m SNP.

cGMP = cyclic guanosine monophosphate.

incubated in saline or nitroprusside. The cGMP content was determined as described above.

The data are presented as mean ± SD. The data were analyzed by repeated-measures analysis of variance for *in vivo* studies and by factorial analysis of variance for *in vitro* studies and *post hoc* Bonferroni-Dunn test.

Results

In Vivo Studies

Experiments were conducted on a colony of six adult baboons (weighing 14 ± 4 kg). All animals fulfilled the criteria for optimum placement of the microcatheter. End-tidal isoflurane, hematocrit, and arterial carbon dioxide partial pressure did not change during the experiments (table 1). MAP increased during infusion of intravenous phenylephrine (69 ± 12 to 87 ± 15 mmHg;

$P < 0.001$). However, MAP was comparable to baseline values during concurrent infusion of intravenous phenylephrine plus intracarotid SNP (69 ± 12 and 75 ± 13 mmHg; nonsignificant) and during intracarotid verapamil infusion (69 ± 12 and 66 ± 12 mmHg; nonsignificant; fig. 1A). A decrease in heart rate was noted during intravenous phenylephrine infusion (86 ± 13 and 77 ± 18 beats/min; $P < 0.002$). Intravenous phenylephrine did not affect CBF (26 ± 7 to 24 ± 5 ml · 100 g⁻¹ · min⁻¹; nonsignificant). However, concurrent infusion of intravenous phenylephrine and intracarotid SNP had no effect on CBF (24 ± 5 to 27 ± 7 ml · 100 g⁻¹ · min⁻¹; nonsignificant). In contrast, infusion of verapamil caused a significant increase in CBF (27 ± 7 to 43 ± 11 ml · 100 g⁻¹ · min⁻¹; $P < 0.0001$; fig. 1B). Compared with baseline, intravenous phenylephrine increased CVR (2.8 ± 0.8 to 3.6 ± 1.1 mmHg · ml⁻¹ · 100 g⁻¹ · min⁻¹; $P < 0.0001$). Concurrent infusion of intravenous phenylephrine and intracarotid SNP decreased CVR to levels comparable to baseline (2.8 ± 1.0 vs. 2.8 ± 0.8 mmHg · ml⁻¹ · 100 g⁻¹ · min⁻¹, respectively; nonsignificant). However, only intracarotid verapamil decreased CVR to less than baseline values (1.6 ± 0.6 vs. 2.8 ± 0.8 mmHg · ml⁻¹ · 100 g⁻¹ · min⁻¹; $P < 0.0001$).

In Vitro Studies

Intracranial Versus Extracranial Arteries. We obtained sets of extracranial (n = 5) and intracranial (n = 6) ICA segments from six freshly killed baboons. Sufficient length of ICA could not be obtained from one animal. Three samples were lost during processing, and two values were outside the calibration range of the cGMP assay. Results of 50 of the 55 specimens were available for analysis (table 2). The cGMP content per

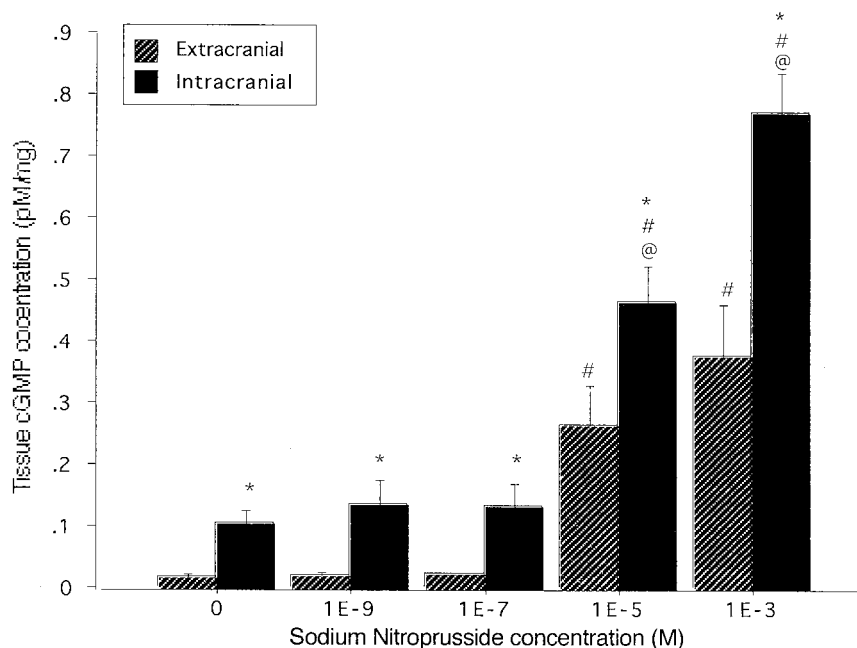


Fig. 2. Increase in tissue cyclic guanosine monophosphate (cGMP) concentrations (picomole per milligram of tissue) in arterial segments of the Circle of Willis and extracranial portion of internal carotid artery after 10-min incubation in increasing concentrations of sodium nitroprusside (SNP). Error bars represent SE. *Significant differences between intracranial and extracranial arteries. #Significant difference from 0, and 10⁻⁹ M SNP concentrations. @Significant difference from 10⁻⁷ M SNP concentration.

Table 3. Effect of Preincubation of Vascular Rings with Iohexol on Tissue cGMP Concentrations After Incubation with Sodium Nitroprusside (10^{-5} M)

| | Saline, n | 10^{-5} M Nitroprusside, n | Significance |
|--------------------------------|----------------------|------------------------------|--------------|
| Normoosmotic challenge (total) | 0.03 ± 0.04 (6) | 0.07 ± 0.05 (6) | NS |
| Hyperosmotic challenge (total) | 0.03 ± 0.02 (17) | 0.1 ± 0.08 (18) | $P = 0.003$ |
| Iohexol (100%) | 0.02 ± 0.02 (6) | 0.11 ± 0.09 (6) | $P = 0.04$ |
| Iohexol (20%) | 0.02 ± 0.02 (6) | 0.02 ± 0.01 (6) | $P = 0.03$ |
| Iohexol (5%) | 0.03 ± 0.02 (5) | 0.1 ± 0.08 (6) | NS |

Data presented as mean \pm SD. Statistical analysis was performed using factorial analysis of variance and *post hoc* Bonferroni-Dunn test.

NS = no significant difference from saline incubation.

milligram of tissue was higher in intracranial than in extracranial arteries. Incubation with low concentrations of SNP (10^{-9} and 10^{-7} M) did not increase; however, incubation with 10^{-5} and 10^{-3} M SNP significantly increased cGMP concentrations of both intracranial and extracranial arteries (fig. 2). Compared with baseline values, there was a 19-fold maximal increase in cGMP content in extracranial vessels compared with a sixfold maximal increase in intracranial vessels.

Effect of Iohexol on Cyclic Guanosine Monophosphate Generation after Sodium Nitroprusside. Data from 47 of the 48 rings from six sets of primate rings that were incubated during this experiment were available for analysis. One sample was lost during processing. Twelve arterial rings were challenged at normoosmotic concentration, six each with saline and six with 10^{-5} M SNP. Arterial segments in the hyperosmotic group that were incubated in saline had a mean cGMP concentration of 0.03 ± 0.02 pmol/mg ($n = 17$). The cGMP content in rings incubated in SNP despite exposure to the three concentrations of iohexol, collectively, was significantly higher (0.1 ± 0.08 pmol/mg; $n = 18$; $P = 0.003$). There was no difference between rings incubated in different concentrations of iohexol (5, 20, and 100%; table 3 and fig. 3).

Discussion

The result of this study suggest that, in healthy anesthetized baboons, as in humans subjects undergoing angiography, intracarotid SNP ($0.5 \mu\text{g} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) fails to augment CBF despite evidence of normal autoregulatory vasoconstriction and pharmacologic response to the Ca^{2+} channel blocker verapamil. Yet intracranial arteries of baboons demonstrate a dose-dependent increase in cGMP content after SNP incubation. Furthermore, the lack of response to SNP during angiography cannot be attributed to the use of radiocontrast agent because preincubation of carotid arterial rings in iohexol did not influence the cGMP increase after SNP exposure. Collectively, these findings suggest that, in healthy baboons, SNP is relatively ineffective in relaxing the resistance arterioles to augment CBF, although SNP could relax proximal cerebral arteries.

Despite the general impression that SNP is a potent cerebral vasodilator, there are conflicting reports regarding its ability to augment CBF of higher primates. Three previous human studies on patients undergoing angiographic interventions have failed to demonstrate an increase in CBF after intracarotid infusion of SNP.¹²⁻¹⁴ All of these studies were undertaken during angiography on patients with suspected cerebrovascular pathologies. Arguably, it is possible that the response to SNP during these studies was inhibited by the underlying disease or by the radiocontrast agent required for angiography. It is not feasible to undertake intracarotid drug challenge studies in healthy human subjects because of the remote possibility of neurologic complications associated with cerebral angiography. Hence, to verify our human observations, we had to consider a nonhuman primate such as a baboon. In the presence of vasospasm, intracarotid NO donors have been shown to augment CBF of monkeys and baboons.^{3,4} We therefore set up specific criteria for satisfactory placement of the endovascular catheters that ruled out vasospasm.

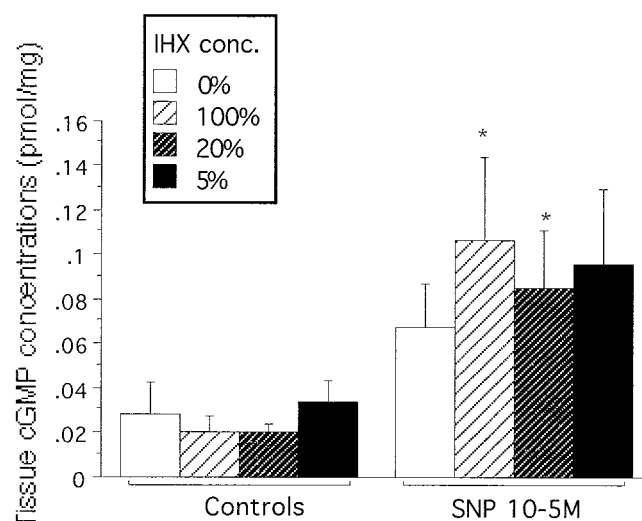


Fig. 3. Increase in tissue cyclic guanosine monophosphate (cGMP) concentration after exposure to 10^{-5} M sodium nitroprusside (SNP). The arterial rings were subjected to normoosmotic or hyperosmotic challenges with three concentrations of iohexol (5, 20, 100%). Rings were incubated in saline or 10^{-5} M of SNP. Error bars represent SE. *Significant differences in control and SNP groups.

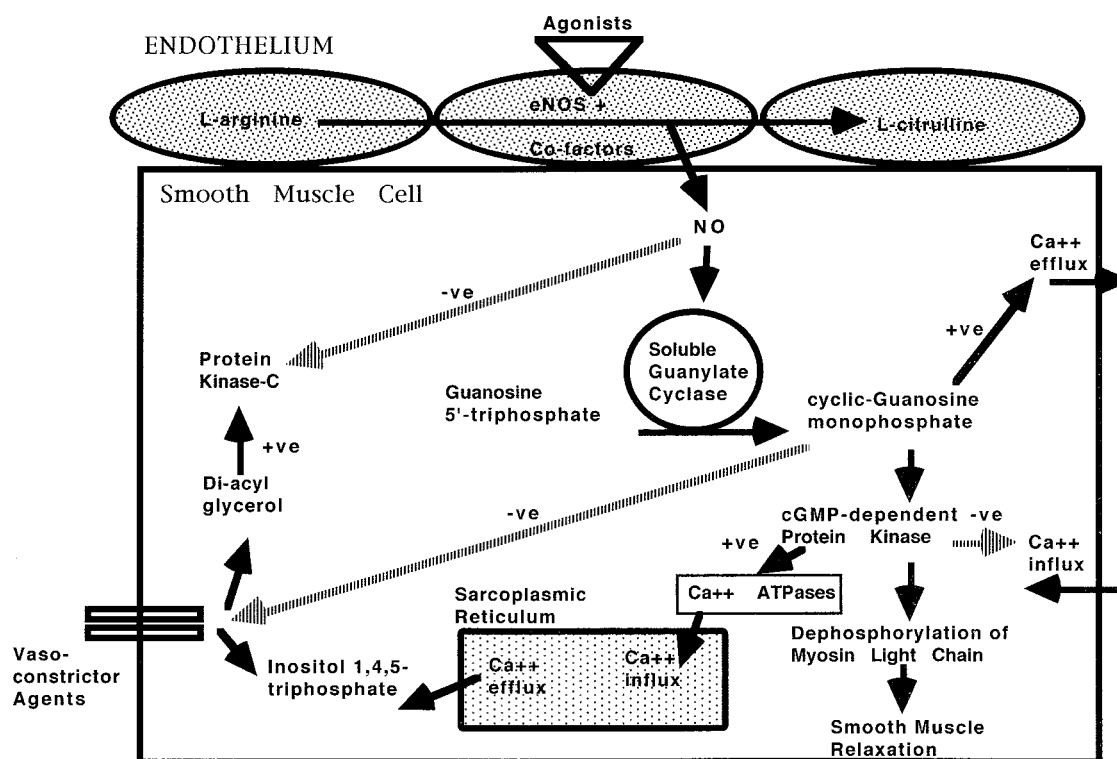


Fig. 4. Major actions of nitric oxide (NO) and cyclic guanosine monophosphate (cGMP) in the regulation of vascular tone. The generation of NO from L-arginine requires cofactors that include oxygen, nicotinamide dinucleotide phosphate (NADPH), and tetrahydrobiopterin (BH₄). NO diffuses into the vascular smooth muscle cell to activate soluble guanylate cyclase and generates cGMP to activate cGMP-dependent protein kinase (PKG). Activation of PKG leads to the lowering of intracellular Ca⁺⁺ concentration and the dephosphorylation of myosin light chain to relax the smooth muscle. However, NO may also antagonize the effects of constrictor agents by inhibiting protein kinase C. (Adapted with permission from Lang *et al.*³⁵)

As shown in figures 1A and 1B, the results of our *in vivo* studies on baboons are similar to those obtained from sedated humans using an identical drug infusion protocol.¹³ Despite the background general anesthesia in baboons, an intact autoregulatory response to induced hypertension could be demonstrated, as was evidenced by an increase in CVR during phenylephrine infusion. Intracarotid SNP significantly decreased MAP and restored CVR to baseline values, although it did not increase CBF. The decrease in CVR during concurrent infusion of SNP and phenylephrine could well be caused by the reversal of autoregulatory vasoconstriction secondary to a decrease in MAP. However, only intracarotid verapamil decreased CVR below baseline and increased CBF by approximately 68%. The increase in CBF after intracarotid verapamil is similar to that reported in the literature. In the current study we have calculated the CVR while assuming that the intracranial pressure does not change during intraarterial drug infusion. It can be argued that the changes in intracranial pressure might have affected the net cerebral perfusion pressure. Because of infections and the risk of neurologic complications, it is difficult to undertake intracranial pressure measurements during primate survival experiments. However, the conventional concepts of cerebral perfusion pressure and CVR have been challenged in recent

years.^{25,26} Therefore, we place more emphasis on changes in CBF rather than those in CVR.

One of the significant criticisms of the above study is the use of only a fixed dose of intracarotid nitroprusside. We selected this dose of intracarotid SNP because we had observed minimal systemic hypotension with this dose. We estimated that this dose of SNP would generate a cerebral arterial blood concentration of approximately 10⁻⁵ M. This concentration was determined by dividing the dose of SNP by the estimated ICA blood flow volume. In cranial windows, micromolar concentrations of topical SNP concentrations are sufficient to increase in arterial diameters.²⁷ There are no data yet regarding the kinetics of intracarotid SNP; therefore, precise intracarotid doses and infusion rates are difficult to estimate. Rate of drug injection, baseline blood flow, protein binding, lipid solubility, and extraction fraction between brain:blood are some of the factors that influence tissue uptake by the brain.²⁸⁻³⁰

In the case of SNP, the release of NO is a one-stage nonenzymatic reaction.^{31,32} NO has greater affinity for oxyhemoglobin than for reduced hemoglobin.³³ Despite scavenging by arterial blood, intraarterial NO is a reliable tool to test for endothelium-independent vasodilation.³⁴ In addition, NO is highly lipid soluble and thus should easily penetrate through the endothelial cells into the

vascular smooth muscle.³⁵ Once within the muscle cell, NO activates guanylate cyclase to increase the cGMP concentration that eventually leads to muscle relaxation. However, NO might relax vascular smooth muscle cells by cGMP-independent mechanisms (fig. 4).³⁵ These cGMP-independent mechanisms might be particularly important in disease states such as cerebral vasospasm.³⁶

At first glance, our *in vivo* results with ¹³³Xe measurements seem to be in conflict of our *in vitro* results. While *in vivo* we observed a relative failure of SNP to augment CBF, *in vitro*, intracranial arteries of baboons demonstrated a robust dose-dependent increase in cGMP after SNP incubation. In the current study we observed a sixfold increase in cGMP in intracranial arteries after SNP incubation. In isolated arterial segments, a twofold to eightfold increase in cGMP content occurs after SNP incubation, while smooth muscle cells cultured from cerebral arteries can demonstrate a much more robust (40–80-fold) increase.^{37,38} The significant increase in cGMP content of intracranial arteries after SNP incubation suggests that the lack of *in vivo* response to intracarotid SNP is not caused by a lack of guanylate cyclase in the large cerebral arteries of primates.

The next question we addressed was whether the radiocontrast agent, iohexhol, used during cerebral angiography interfered with SNP-mediated vasodilation. The effect of iohexhol on cerebrovascular reactivity has not been reported. NO-mediated vasodilation has been observed during coronary angiography despite the use of radiocontrast agents.³⁴ To simulate clinical use, we exposed the cerebral arterial rings for 10 min to iohexhol. This represents the average time of contrast exposure during angiography. Further, during the *in vivo* experiments, the radiocontrast was diluted before injection. The dilution of contrast decreases viscosity and facilitates injection through the narrow lumen of the microcatheter. After injection, the contrast is further diluted by arterial blood. Therefore, we incubated the arterial rings in a range of iohexhol concentrations. However, collectively, SNP exposure of these arterial rings resulted in an increase in cGMP despite iohexhol preincubation, and iohexhol does not seem to impair cGMP response to SNP (table 3).

A possible explanation for the apparently contradictory results of our *in vivo* and *in vitro* studies could be that SNP primarily affects the tone of large cerebral arteries. In the absence of cerebral vasospasm, direct intraarterial pressure measurements reveal only a minimal decrease in pressure in the large cerebral arteries of human subjects.^{39–41} Thus, during physiologic conditions, the tone of large cerebral arteries in higher primates does not determine the CBF.⁴² Dahl *et al.*⁴³ used simultaneous measurement of CBF by single photon emission computed tomography and blood flow velocity in the middle cerebral artery by transcranial Doppler

during intravenous nitroglycerin infusion.⁴³ Nitroglycerin, a NO donor, did not increase CBF but did decrease middle cerebral artery blood flow velocity. Therefore, the investigators concluded that nitroglycerin dilates the middle cerebral artery but does not dilate the resistance arterioles.

In conclusion, this study demonstrates that *in vivo*, in the absence of cerebral vasospasm, intracarotid SNP infusion does not augment CBF of healthy anesthetized baboons. Yet *in vitro*, proximal intracranial arteries of these animals demonstrate a robust dose-dependent increase in cGMP concentrations after SNP incubation. We therefore infer that SNP can relax proximal cerebral arteries. However, when delivered by the intraarterial route, SNP is relatively ineffective in relaxing the resistance arterioles to augment CBF.

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