Role of Nitric Oxide in the Regulation of Cerebral Blood Flow in Humans

Chemoregulation Versus Mechanoregulation

Shahar Lavi, MD; Rania Egbarya, MD; Ronit Lavi, MD; Giris Jacob, MD, DSc

Background—From animal studies it emerged that nitric oxide is important for the modulation of CO2-mediated cerebral blood flow (CBF chemoregulation) but not for the pressor-dependent mechanism (mechanoregulation). This hypothesis was tested in 18 healthy subjects.

Methods and Results—Peak velocity (PV), diastolic velocity (DV), and mean velocity (MV) were measured by transcranial Doppler of the middle cerebral artery. Chemoregulation was assessed during normocapnia, hypocapnia, and after inhaled mixture of 95% O2 +5% CO2. Mechanoregulation was evaluated by incremental doses of phenylephrine. Measurements were repeated during infusion of sodium nitroprusside (SNP). Regional cerebrovascular resistance (CVR) was calculated as mean blood pressure (BP)/MV. SNP infusion decreased mean BP by 7 mm Hg and CVR decreased from 1.38±0.08 to 1.29±0.09 mm Hg/cm·s⁻¹; P=0.01, resulting in unaffected CBF. Phenylephrine (25 to 250 µg) caused a similar increase in BP in a dose-response fashion before and during SNP infusion. Despite the increments in BP and CVR, CBF remained unaffected. During hyperventilation (end-tidal CO2 ≈24 mm Hg), CVR increased by 75±3% and PV and DV decreased by 27±2% and 43±2%, respectively (P<0.001 for all). SNP infusion blunted the vasoconstrictive effect of hypocapnia; CVR increased only by 57±5%, and PV and DV decreased by 23±2% and 35±3%, respectively, (P<0.05 for all). Similarly, SNP augmented the vasodilatory effect of hypercapnia.

Conclusions—Exogenous nitric oxide donor affects the basal cerebral vascular tone without affecting the CBF mechanoregulation. However, it selectively affects only the chemoregulatory mechanism (CO₂-dependent). Thus, the CO₂–NO axis is a cardinal pathway for CBF regulation in humans. (Circulation. 2003;107:1901-1905.)

Key Words: nitric oxide receptors, adrenergic, alpha I cerebral vascular disorders I cerebral vascular circulation

Cerebral blood flow (CBF) regulation is an intrinsic property of small blood vessels to maintain a constant blood flow despite the oscillations in systemic blood pressure (BP).¹ This mechanoregulation (pressure-dependent) is interrupted if BP values outrange 60 to 160 mm Hg and during alterations in CO2 concentration.² ³ The latter chemoregulatory mechanism (CO2 reactivity) has an important role in the determination of CBF during metabolic perturbations, independent of BP fluctuations. For instance, in healthy subjects, CBF increases by 50% during hypercapnia and decreases ≈35% during hyperventilation, without major changes in BP.⁴

Both autoregulatory mechanisms are independently operating. The cellular mechanism behind mechanoregulation is unknown. Even though it has emerged from animal studies that nitric oxide (NO) is necessary to maintain CO2-mediated CBF, it does not appear to be involved in cerebral mechanoregulation.⁵ ⁷

Numerous human investigations were conducted during the past decade to explore the role of NO in CBF regulation.

NO synthase inhibitors, for example, L-NMMA (N⁶-monomethyl-L-arginine), decrease CBF and attenuate the vasodilatory response to hypercapnia,⁸ ⁹ whereas NO donors, for example, sodium nitroprusside (SNP) conferred inconsistent results.¹⁰ ¹³ The conflicting results are due to the use of a different study design, different cerebral flow measurement methodology, unhealthy subjects, or the effect of anesthetic medication. Also, to our knowledge, there is no single study that considered both mechanisms, chemoregulation and mechanoregulation of CBF, in the same healthy individual under the effect of NO donors.

Therefore, the aim of the present study was to separately elucidate the aforementioned mechanisms. We hypothesized that NO is involved in the chemoregulation but not in the mechanoregulation of CBF. Thus, we monitored hemodynamics, end-tidal CO2 (EtCO2), and CBF velocities by using transcranial Doppler (TCD) before and during normocapnia, hypocapnia, and hypercapnia (CO2 vasomotor reactivity) and during manipulation of BP, before and after the continued infusion of an NO donor in healthy volunteers.

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From the J. Recanati Autonomic Dysfunction Center (S.L., R.E., G.J.), Departments of Cardiology (S.L.) and Anesthesiology (R.L.), Rambam Medical Center, and Technion-Israel Institute of Technology, Haifa, Israel.
Correspondence to Giris Jacob, MD, DSc, Jacob Recanati Autonomic Dysfunction Center, Medicine A, Rambam Medical Center, PO Box 9602, Haifa 31096, Israel. E-mail G_Jacob@rambam.health.gov.il
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Baseline → hyperventilation → hypercapnia → phenylephrine DRC

SNP infusion

Phenylephrine DRC → hyperventilation → hypercapnia

Figure 1. Protocol timing design. DRC indicates dose-response curve; SNP, sodium nitroprusside.

Methods

Subjects
Eighteen healthy volunteers were recruited through an advertisement at the university near Rambam Medical Center. Enrolled subjects had a high-quality TCD signal and no history of drug abuse, smoking, or use of any medications. Subjects refrained from alcohol or products containing caffeine 24 hours before study sessions. Studies were approved by the institutional review board of Rambam Medical Center and by the Ministry of Health, and informed consent was obtained.

Protocol
Studies were conducted after overnight fasting in a quiet, partially darkened room with an ambient temperature of ~24°C. Two venous 21-gauge catheters were inserted for SNP infusion and phenylephrine administration. After instrumentation and a 30-minute supine rest, subjects underwent hemodynamic and TCD measurements at baseline, during hyperventilation, and during CO₂ inhalation. The protocol was repeated during SNP infusion. The protocol procedures are illustrated graphically in Figure 1.

Measurements
Continuous 3-lead ECG and beat-to-beat radial arterial BP measured with a Tonometric Monitor (CBM 7000, Colin) were monitored and displayed on a computer screen and on a thermal array recorder (TA-6000, Gould). Cuff blood pressure and calibration of the hemodynamics and CBF are depicted in the Table. Studies were conducted after overnight fasting in a quiet, partially darkened room with an ambient temperature of ~24°C. Two venous 21-gauge catheters were inserted for SNP infusion and phenylephrine administration. After instrumentation and a 30-minute supine rest, subjects underwent hemodynamic and TCD measurements at baseline, during hyperventilation, and during CO₂ inhalation. The protocol was repeated during SNP infusion. The protocol procedures are illustrated graphically in Figure 1.

Results are expressed as mean±SEM. A paired 2-sided t test was used for comparison between measurements of the various procedures. Linear regression analysis was used for the correlation between the various parameters and for the determination of the chemoregulatory index. Two-way ANOVA for repeated measurements was used to assess the effect of SNP infusion on the various parameters. Data were analyzed with Quattro Pro (version 7; Corel Corp, Ltd) and GraphPad Prism (version 3.0; GraphPad Software, Inc). The level selected for statistical significance was set at probability value <0.05.

Hemodynamic Response to Sodium Nitroprusside

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>+SNP</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP, mm Hg</td>
<td>122±2</td>
<td>113±2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>67±1</td>
<td>60±2</td>
<td>0.002</td>
</tr>
<tr>
<td>MBP, mm Hg</td>
<td>85±1</td>
<td>78±1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>62±2</td>
<td>69±3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PV, cm/s</td>
<td>99±5</td>
<td>97±5</td>
<td>0.4</td>
</tr>
<tr>
<td>DV, cm/s</td>
<td>49±3</td>
<td>50±3</td>
<td>0.7</td>
</tr>
<tr>
<td>MV, cm/s</td>
<td>65±4</td>
<td>64±4</td>
<td>0.3</td>
</tr>
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</table>

All values are expressed as mean±SEM.

Results
Seventeen participants (12 male, 5 female) completed the protocol. The subjects’ mean age, height, weight, and body mass index were 27±0.7 years, 177±2 cm, 75±3 kg, and 23.9±0.7 kg/m², respectively. Baseline and SNP effects on hemodynamics and CBF are depicted in the Table.

Systolic and mean BP and heart rate (HR) were 122±2, 85±1 mm Hg and 62±2 bpm, respectively. The mean dose of SNP required to reach the target BP was 0.4±0.04 µg/kg per minute. MAP decreased by 7±1 mm Hg and HR increased by 9±2 bpm. Concurrently there was a significant decrease in basal CVR (1.38±0.09 versus 1.29±0.09 mm Hg/cm·s⁻¹, P=0.01), resulting in unchanged cerebral velocity indexes, as depicted in the Table. It is noteworthy to mention that SNP did not cause any relevant side effects in any of the participants.

Mechanoregulation
Incremental boluses of phenylephrine caused an increase in BP and CVR in a dose-response fashion. The graded increment in BP did not solicit any changes in the CBF parameters. Also, SNP infusion did not affect these flow indexes. The corresponding changes in SBP, PV, and CVR against the related dose of phenylephrine before and during the infusion of SNP are illustrated in Figure 2, A and B, respectively. The 2-way ANOVA for repeated measurements did not reveal any significant differences between the presented parameters before and during SNP infusion.

Chemoregulation (CO₂ Vasoreactivity)
Hyperventilation caused a similar decrease in EtCO₂ from 43±0.7 to 25±0.6 mm Hg before SNP infusion and from 41±0.9 to 24.5±0.5 mm Hg during SNP infusion. Although BP was not significantly affected after 4 minutes of hyperventilation (systolic and mean BP increased by 5±2 and 3±2 mm Hg, respectively), HR increased by 14±2 bpm. These hemodynamic increments remained unchanged during
SNP infusion. PV and MV decreased from 99 ± 5 to 72 ± 4 cm/s and 65 ± 4 to 40 ± 3 cm/s, respectively. CVR increased by 75% from 1.36 ± 0.09 to 2.3 ± 0.14 mm Hg/cm·s⁻¹ (P < 0.001). SNP infusion blunted the magnitude of cerebral vasoconstriction induced by the same hypocapnic maneuver as expressed by a lesser increase in CVR (~15%, P = 0.05). Also, there was a smaller decrease in PV and DV, as shown in Figure 3A.

Hypercapnia induced by inhaled mixture of CO₂ 5% and O₂ 95% for 6 minutes resulted in a similar increase in EtCO₂ (47 ± 0.5 and 48 ± 0.3 mm Hg before and during SNP infusion, respectively). Systolic and mean BP increased during hypercapnia compared with baseline (120 ± 3 versus 130 ± 3, P = 0.002 and 81 ± 3 versus 87 ± 3 mm Hg, P = 0.02, respectively), without significant changes in HR. Hypercapnia caused an increase in PV (96.5 versus 115.7 cm/s; P < 0.001), DV (49.2 versus 61.4; P < 0.001), and MV (64.7 versus 80.2 cm/s; P < 0.001) and a significant decrease in CVR (1.3 ± 0.08 versus 1.15 ± 0.08 mm Hg/cm·s⁻¹; P < 0.001). During SNP infusion, a further increment in PV and DV and a drop in CVR were observed, as illustrated in Figure 3B.

The vasoreactivity index, extrapolated from the individual linear correlation between EtCO₂ values against the corresponding MVs, dropped significantly by 10% during SNP infusion, as shown in Figure 4B. Also, SNP caused a decrease in the corresponding CVRs, as illustrated in Figure 4A (1 subject).

**Discussion**

The present study demonstrates that an exogenous NO donor reduces basal CVR without affecting CBF indexes, blunts the cerebral vasoconstrictor effect of hypocapnia, and augments the vasodilatory effect of hypercapnia, that is, causes left shifting of the vasomotor reactivity slope (CO₂ versus CBF). Also, α₁-adrenoreceptor agonist infusion causes an increase in the local cerebral resistance without affecting CBF indexes. This phenomenon remains intact during SNP infusion.

Normally, cerebral perfusion pressure, governed by systemic arterial BP, quantifies the CBF in primates. Although the mechanoregulation is the major regulatory mechanism of CBF, other mechanisms such as metabolic (eg, chemoregu-
lulation) and neurogenic mechanisms are implicated in this process.17

Because of the paucity of data on the mechanoregulation and lack of precise investigational means, our knowledge regarding this autoregulatory mechanism in humans is unsatisfactory. Autonomic nervous system activation scarcely affects the CBF despite the presence of adrenoreceptors in the cerebral vasculature.18 Human isolated pial arteries demonstrate poor innervation and weak responsiveness to adrenoceptor agonists.19 Furthermore, patients with severe autonomic failure have preserved mechanical autoregulatory mechanism despite destruction of vascular autonomic fibers.20,21 Also, ganglionic blockade and α2-adrenoreceptor and α1-adrenoreceptor activation did not significantly affect the mechanoregulation in healthy volunteers.18,22 The results of this study support our aforementioned presumptions. In our subjects, phenylephrine increased the regional cerebral vascular resistance in a dose-response fashion, although not enough to decrease CBF. Therefore, the mechanoregulatory mechanism eventually remains intact during α1-adrenoreceptor activation. Even though SNP at the given dose causes a significant reduction in the resting CVR, the phenylephrine dose response, BP, and CBF remain unaffected.

Intact endothelial function and NO are cardinal for most vascular bed tone regulation.23 This potent vasodilator probably is involved in the regulation of cerebral circulation.24 However, from animal studies, it appears that NO is involved in the chemoregulation (vide infra) without significantly affecting the pressor-dependent regulation.6,25 Also, in humans, under anesthetic effect, the direct infusion of SNP into the internal carotid artery with NO synthase inhibitor. Furthermore, the effect of L-NMMA on regional CBF is linearly correlated with PacO2 and is reversed by L-arginine, suggesting a graded relation of NO production and PacO2.5 In one investigation in humans, hypercapnia-induced vasodilation was significantly blunted by administration of L-NMMA.9 Our study shows that NO is involved not only in the CBF response to hypercapnia but also in the CBF response to normocapnia and hypocapnia. These results confirm that in humans, the chemoregulation is significantly affected by NO.

The molecular relation between CO2 and NO pathway is quite challenging. Few animal studies have investigated this interaction. These investigations concluded, indirectly, that several mechanisms might be involved. For instance, the fast changes in pH that occur during hypocapnia and hypercapnia are important modulators of NO synthase.32 Also, pharmacological manipulations of opiate receptors,33 prostaglandins production,34 and ATP-dependent K+ channel activation35 were found to be involved in the modulation of CO2–NO cerebral vasomotor reactivity. The detailed discussion of this issue is beyond the scope of this article, and the final mechanism of this pathway remains to be explored.

Although the sympathetic nervous system appears to have a marginal role in the CO2 modulation of CBF, NO has a pivotal task in this process. To our knowledge, the current investigation is the first to show that in the same individuals, both hypocapnia and hypercapnia are significantly affected by this endothelial relaxing factor. Even low doses of an NO donor, without causing major systemic hemodynamic perturbations, blunt hyperventilation-mediated cerebral vascular constriction and enhances the vasodilatory effect of hypercapnia. Consequently, the vasomotor CO2-reactivity slope is shifted to the left. These findings support the importance of NO in the autochemoregulation of CBF in humans.

Basal resting CBF is partially determined by NO. Inhibition of NO synthase by systemic L-NMMA causes a 20% decrease in CBF as measured by PET and regional angiography. This effect is reversed by L-arginine.8,30 In our subjects, the mild decrease in BP and CVR (~7%), at the given dose of SNP, results in unaffected CBF.

Animal studies demonstrate that NO participates in the cerebral CO2-dependent vasomotor reactivity. In rats, the NO synthase inhibitor L-NAME (N^6-nitro-L-arginine methyl ester) increases CVR and attenuates the response to hypercapnia.7 On the other hand, in anesthetized dogs, hypocapnia does not cause a significant change in CVR or CBF during infusion of SNP.31 In primates, the increment in CBF in response to hypercapnia is completely blocked by infusion of the internal carotid artery with NO synthase inhibitor. Furthermore, the effect of L-NMMA on regional CBF is linearly correlated with PacO2 and is reversed by L-arginine, suggesting a graded relation of NO production and PacO2.5 In one investigation in humans, hypercapnia-induced vasodilation was significantly blunted by administration of L-NMMA.9 Our study shows that NO is involved not only in the CBF response to hypercapnia but also in the CBF response to normocapnia and hypocapnia. These results confirm that in humans, the chemoregulation is significantly affected by NO.

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**Study Limitations**

The increase in BP obtained during hypercapnia (steady state) might involve mechanoregulation. Given the lack of effect of BP on CBF parameters before and during SNP infusion, the effects of hypercapnia on CBF can be attributed only to chemoregulation. The given low dose of SNP was limited to avoid significant side effects resulting in major hemodynamic perturbations or reactive psychological responses that may affect the stability of the CBF measurements. Also, for ethical reasons, we restricted the dose response of phenylephrine to
a limited component of the wide automechanoregulatory range. We should mention that we studied only one extreme of the pressor autoregulation without exploring the hypotensive arm of such a mechanism. Regrettably, there is no accepted method to cause a transient hypotension without affecting the NO pathway. Higher doses of SNP and higher concentrations of CO2 may be required in future studies to further confirm our findings. The validity of the TCD method has been mentioned above.

Conclusions
According to the present investigation, NO is involved in the determination of the basal cerebrovascular tone without being implicated in the pressor-mediated cerebral flow autoregulation (mechanoregulation). The CO2–NO axis appears to be a cardinal pathway in the chemoregulation of CBF. Whether this mechanism is neurally or endothelially mediated (or both) remains to be explored in humans.

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References
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