Nitric oxide mediates hypoxia-induced cerebral vasodilation in humans

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Van Mil, Annette H. M., Aart Spilt, Mark A. van Buchem, Edward L. E. M. Bollen, Luc Teppema, Rudi G. J. Westendorp, and Gerard J. Blauw. Nitric oxide mediates hypoxia-induced cerebral vasodilation in humans. J Appl Physiol 92: 962–966, 2002. First published October 12, 2001; 10.1152/japplphysiol.00616.2001.—Nitric oxide (NO) plays a pivotal role in the regulation of peripheral vascular tone. Its role in the regulation of cerebral vascular tone in humans remains to be elucidated. This study investigates the role of NO in hypoxia-induced cerebral vasodilatation in young healthy volunteers. The effect of the NO synthase inhibitor Nω-monomethyl-L-arginine (L-NMMA) on the cerebral blood flow (CBF) was assessed during normoxia and during hypoxia (peripheral O2 saturation 97 and 80%, respectively). Subjects were positioned in a magnetic resonance scanner, breathing normal air (normoxia) or a N2-O2 mixture (hypoxia). The CBF was measured before and after administration of L-NMMA (3 mg/kg) by use of phase-contrast magnetic resonance imaging techniques. Administration of L-NMMA during normoxia did not affect CBF. Hypoxia increased CBF from 1,049 ± 113 to 1,209 ± 143 ml/min (P < 0.05). After L-NMMA administration, the augmented CBF returned to baseline (1,050 ± 161 ml/min; P < 0.05). Similarly, cerebral vascular resistance declined during hypoxia and returned to baseline after administration of L-NMMA (P < 0.05 for both). Use of phase-contrast magnetic resonance imaging shows that hypoxia-induced cerebral vasodilatation in humans is mediated by NO.

cerebrovasculature; vascular reactivity; Nω-monomethyl-L-arginine; magnetic resonance imagery

OVER A WIDE RANGE of systemic blood pressure, perfusion of brain tissue is kept constant by local regulation of vascular tone. This autoregulation of cerebral blood flow (CBF) is controlled by a combination of myogenic, neurogenic, and metabolic mechanisms (21). This complex autoregulatory mechanism is based on a tight coupling between O2 supply and metabolic demand. With a constant metabolic demand and O2 supply, changes in blood pressure are compensated by adjustments in vasomotor tone. On the other hand, a decrease in O2 supply or an increase in metabolic demand results in a decrease in vasomotor tone, causing an increase in CBF to match again with the O2 demand of the brain (19, 21). The mechanism underlying this coupling between O2 supply and cerebral vascular tone remains to be elucidated, although experimental data suggest that nitric oxide is involved (15). In various species it has been shown that nitric oxide synthase inhibitors attenuate hypoxia-induced cerebral vasodilatation (1, 2, 16). Recently, it has been shown that, in the human forearm, hypoxia-induced vasodilatation is mediated via the release of nitric oxide (4).

From experimental studies, there is increasing evidence that nitric oxide is involved in the regulation of cerebral vascular tone. Main sources of nitric oxide in the brain are neurons and endothelial cells (12, 15). Recently evidence has been provided that nitric oxide also plays a role in humans in the regulation of cerebral vasomotor tone (17, 28). On the basis of these data, together with the observations that nitric oxide might be involved in hypoxia-induced cerebral vasodilatation, it can be hypothesized that the coupling between O2 and cerebral vascular tone is mediated via the nitric oxide pathway.

It was the aim of the present study to investigate the role of nitric oxide in hypoxia-induced cerebral vasomotor relaxation in young healthy volunteers, by using the competitive nitric oxide synthase inhibitor Nω-monomethyl-L-arginine (L-NMMA) as a pharmacological tool. Phase-contrast magnetic resonance imaging (pcMRI) techniques were used to measure total CBF and changes in flow noninvasively (24).

METHODS

Subjects. Eight young male healthy volunteers (mean age 24 ± 3 yr) participated in the study. Physical and routine blood examinations, electrocardiogram (ECG), and conventional magnetic resonance imaging (MRI) of the brain (transverse relaxation time-weighted fast spin echo and fluid attenuated inversion recovery) revealed no abnormalities.

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Exclusion criteria were current smoking, use of drugs or
more than three alcoholic drinks a day, a body mass index
greater than 26 kg/m², hypertension, claustrophobia, dyslip-
idemia, diabetes mellitus, signs and symptoms of cardiovas-
cular disease, or any other significant abnormalities in phys-
ical examination, blood analysis, ECG, or standard MRI
scans. Before the start of the experiments, subjects abstained
from nonsteroidal anti-inflammatory drugs for at least 10
days and from alcoholic and caffeine-containing beverages for
at least 12 h. The protocol was approved by the Medical
Ethical Committee of the Leiden University Medical Center
and conformed with the principles outlined in the Declara-
tion of Helsinki; all subjects gave written, informed consent.

Procedures. During the experiments, the subjects were in
the supine position with their heads comfortably stabilized.
A well-fitted face mask was applied for administration of gas
mixtures (normal pressurized air mixture and N₂-O₂ mix-
ture), and a deep antecubital vein was cannulated for infu-
sion of l-NMMA. Then the subjects were positioned in a
magnetic resonance system operating at a field strength of 1.5
T (ACS-NT, Philips Medical Systems, Best, The Nether-
lands) under continuous audio and video surveillance. Heart rate was derived from a one-lead ECG and was con-
tinuously monitored. Blood pressure was measured semicon-
tinuously with intervals of 3 min by use of an automatic
device. Inspired O₂ (MiniOx 3000, Come Care Medical, The
Netherlands), peripheral O₂ saturation (SpO₂), breath rate,
and end-tidal CO₂ (Millennia, In vivo Research, Orlando, FL)
were monitored continuously.

CBF was measured noninvasively in the basilar artery and
both internal carotid arteries by use of a gradient echo pMRI
technique as described previously with the following param-
eters: time to repeat/echo time 16/9 ms; flip angle 7.5°; 5 mm
slice thickness; field of view 250 mm and one number of
signal averages (11). Triggering was retrospective using a
peripheral pulse unit. The flow measurements were analyzed
on a Sun UltraSparc 10 workstation with internally devel-
oped software package FLOW (26). Total CBF was defined as
the summed flow measured in the basilar artery and both
internal carotid arteries (expressed as ml/min). The mean
values of two consecutive CBF measurements, measured
with an interval of 5 min, were used for further analysis.

Study protocol. The study was performed in a single-
blinded fashion and consisted of 2 study days with an inter-
val of 1 wk. On one day the effect of the competitive nitric
oxide synthase inhibitor l-NMMA on CBF was assessed
during normoxia (SpO₂ 97%), and on the other day this was
done during hypoxia (SpO₂ 80%). The procedures were done in
random order.

After they were positioned in the scanner, the subjects
started breathing ambient air through the face mask. After
an equilibrium period of 20 min, basal cerebral blood flow
was measured twice. Subsequently, the subjects either con-
tinued breathing ambient air or started breathing a vari-
ad N₂-O₂ mixture. The objective of the N₂-O₂ mixture was
to obtain a SpO₂ of 80%. Therefore, the N₂-O₂ mixture was
continuously adjusted for each subject during the experi-
ments. After 20 min of a stable SpO₂ at either 97% or 80%,
cerebral blood flow was measured twice. Then l-NMMA (Cli-
nalpaha, Laufelfingen, Germany) was administered intrave-
nously in a dose of 3 mg/kg in 5 min, by use of a constant-rate
infusion pump (Spectris MR injector, Medrad Europe, Beel,
The Netherlands). Five minutes after this infusion, CBF was
measured twice.

Analysis. Results are given as means ± SD. Measurements
of total CBF are expressed in absolute values. Changes in
cerebral vascular resistance are expressed as percent
changes from baseline. Cerebral vascular resistance is calcu-
lated by mean arterial pressure (mmHg) divided by total
cerebral blood flow (ml/min). The Wilcoxon signed-rank test
for paired observations were used to evaluate the statistical
significance of the data. The data were analyzed blinded to
the gas phase, i.e., normoxia and hypoxia. P values <0.05
were regarded as significant.

RESULTS

No adverse effects of hypoxia or the administration of
l-NMMA were observed. The subjects’ rating of
whether they had been exposed to hypoxia or normoxia
was not better than by chance, illustrating the success
of subject blinding. In one subject, the experiments
were stopped during the first 20-min stabilization pe-
riod because of claustrophobia. Therefore the data
were analyzed on seven subjects. Hypoxia was easily
achieved and maintained at a SpO₂ level of 79.1 ± 0.3%.
Basal CBF was comparable on the 2 study days, 862 ±
139 ml/min and 907 ± 253 ml/min, respectively (not signif-
ant).

Normoxia. During normoxia, there was no change in
CBF (816 ± 124 and 825 ± 137 ml/min) or any of the
other parameters (Fig. 1). Administration of l-NMMA
during normoxia did not significantly affect CBF
(825 ± 137 and 815 ± 198 ml/min, before and after
l-NMMA, respectively; not significant) heart rate,
mean arterial pressure, or end-tidal CO₂ (Fig. 1). Con-
sequently, the vascular resistance did not change dur-
ing normoxia.

Hypoxia. Hypoxia (SpO₂ 80%) was induced by lower-
ing the inspired O₂ from 21.0 ± 0.5% to 13.1 ± 0.8%.
During hypoxia, CBF increased from 1,049 ± 113 to
1,209 ± 143 ml/min, respectively (P < 0.05; Fig. 2).
Hypoxia had no significant effect on the steady-state
end-tidal CO₂ (38.7 ± 6.9 vs. 38.4 ± 5.8 mmHg) or mean
arterial pressure (82 ± 8 vs. 83 ± 8 mmHg). Heart rate increased from 70 ± 8 to 78 ± 5 beats/min
(P < 0.05). The calculated cerebral vascular resistance
decreased by 17 ± 10% during hypoxia (P < 0.05; Fig. 2).

During hypoxia, administration of l-NMMA de-
creased CBF significantly from 1,209 ± 143 to 1,050 ±
161 ml/min (P < 0.05; Fig. 2). Mean arterial pressure
increased from 83 ± 8 to 85 ± 5 mmHg after adminis-
tration of l-NMMA (P < 0.05). Heart rate decreased
not significantly from 78 ± 13 to 70 ± 8 beats/min.

DISCUSSION

The main finding of the study is that acute hypoxia
induced an increase in total CBF that could be blunted
by the competitive nitric oxide synthase inhibitor
l-NMMA, providing evidence that hypoxia-induced ce-
rebral vasodilatation is mediated by the release of
nitric oxide. Because l-NMMA is a nonselective nitric
oxide synthase inhibitor, acting on both endothelial
and neuronal nitric oxide synthase, the present study
does not provide evidence for the source of nitric oxide
mediating cerebral vasodilatation during hypoxia.

This is the first study using pMRI to investigate
hypoxia-induced cerebral vascular reactivity in hu-
mans. To date, Doppler ultrasonography is commonly
used to quantify blood flow in the common carotid artery, internal carotid artery, and middle cerebral artery. A clear advantage of MRI over Doppler ultrasound is the considerable reduction of random error that can be attributed to inaccuracy in measuring the cross-sectional area and varying angles of approach with ultrasonography (13). Furthermore, the position of the basilar artery makes it very difficult to perform measurements in this artery with the use of ultrasound. Therefore, total cerebral blood flow cannot be determined by Doppler ultrasound. Finally, because Doppler ultrasound measures only the highest flow velocities in the center of the vessel, the real total blood flow in the vessels is overestimated. With the present MRI technique, the flow velocities of the total area of the vessels measured are averaged, i.e., in the basilar artery and both internal carotid arteries, reflecting total CBF (5, 11, 18, 24, 25).

The normal hemodynamic response to acute hypoxia is an increase in heart rate and a slight decrease in mean arterial pressure due to a redistribution of blood flow to organs with a greater O2 dependency, e.g., skeletal muscles, kidneys, intestines, and the brain (27). The mechanism underlying this hypoxia-induced vasodilatation in selective vascular beds is not fully understood. In animal experiments, it has been demonstrated that nitric oxide synthase inhibitors attenuate hypoxia-induced cerebral vasodilatation (1, 2, 14, 16). The present data are the first to show in humans that hypoxia-induced cerebral vasodilatation is mediated by nitric oxide, corroborating studies in the human forearm (4). Because the cerebral vascular resis-

![Fig. 1. Percent change in cerebral vascular resistance, cerebral blood flow, mean arterial pressure, and heart rate during normoxia. L-NMMA, N\textsuperscript{G}-monomethyl-L-arginine.](image1)

![Fig. 2. Percent change in cerebral vascular resistance, cerebral blood flow, mean arterial pressure, and heart rate during hypoxia. \({}^{*}P < 0.05\) compared with baseline \((t = 0)\); \(^{#}P < 0.05\) compared with values after 30 min of hypoxia \((\text{before administration of L-NMMA}) (t = 30)\).](image2)
tance declined during hypoxia and returned to baseline after L-NMMA administration, the changes in CBF cannot be attributed to blood pressure elevation. Furthermore, myogenic autoregulatory mechanisms would cause an opposite response by increasing vasomotor tone on blood pressure elevation to maintain CBF constant. The hypoxia-induced vasodilatation is probably the result of metabolic autoregulation, causing an increase in CBF and thus an increase in O₂ supply to match the metabolic demand. The present finding suggests that in healthy subjects nitric oxide plays an important role in enhancing the cerebral blood flow response to hypoxia.

The fact that the end-tidal CO₂ was not influenced by hypoxia virtually excludes the distorting influence of changes in PCO₂ on hypoxia-induced cerebral vasodilatation in the present study (28). Normally, the hypoxic ventilatory response consists of an acute carotid body-mediated increase in ventilation followed by a secondary (subacute) decrease, the so-called hypoxic ventilatory depression, to a new steady-state level above control. Part of the hypoxic ventilatory depression is thought to be due to the hypoxia-induced rise in cerebral blood flow resulting in an increased washout of CO₂ from brain tissue (7, 9, 10). In our setup, the end-tidal PCO₂ was allowed to change with changing levels of ventilation. The fact that in our experiments the steady-state normoxic and hypoxic end-tidal PCO₂ did not change indicates that the opposite effects of the initial increase and secondary decrease in ventilation were cancelled out.

In contrast to normoxia, L-NMMA induced a small increase in mean arterial pressure of 6% during hypoxia. This is probably caused by inhibition of hypoxia-induced nitric oxide-mediated systemic vasodilatation by L-NMMA. The fact that L-NMMA did not influence CBF during normoxia might indicate that nitric oxide plays a limited role in the maintenance of basal cerebral vascular tone under nonpathological basal conditions. Others described a significant fall in basal CBF after L-NMMA administration (29). Both findings are in agreement with the observation that L-NMMA reduced basal cerebral blood flow in healthy volunteers only at higher doses (17). Possibly we did not achieve complete nitric oxide blockade. In the present study, the absence of an effect of L-NMMA on the resting cerebral blood flow and on the systemic circulation helped to interpret the present results, because it allowed similar starting conditions during the normoxia and hypoxia occasions. The fact that L-NMMA blunted the hypoxia-induced cerebral vasodilatation demonstrated that it effectively blocked the nitric oxide pathway during hypoxia in the doses used.

An unexpected finding of this study is that basal cerebral blood flow is significantly different between the normoxic and hypoxic conditions. Because the study is performed in a randomized single-blind fashion, this finding must be accidental. This is confirmed by the fact that when baseline flows are analyzed by study day, no significant differences are observed. Nevertheless, the present results show that the intraindividual variation of cerebral blood flow on various occasions is large. However, the results during normoxia clearly show that on a specific occasion basal cerebral blood flow is remarkably stable, even after infusion of L-NMMA. Therefore, it can be assumed that the vascular responses observed during hypoxia are caused by hypoxia and the subsequent administration of L-NMMA and not based on accidental variations in cerebral blood flow.

The present finding that nitric oxide plays a role in hypoxia-induced cerebral vasodilatation in humans may have relevant clinical implications. To date, data on the role of nitric oxide in ischemic brain disease are scarce. It has been suggested that after cerebral ischemia, nitric oxide initially has beneficial vascular actions of in promoting CBF (22). However, from studies in peripheral vascular beds there is accumulating evidence that atherosclerosis impairs nitric oxide-mediated vasodilatation, probably via endothelial damage (6, 8, 20, 23). Recently, it has been shown that, in patients with signs and symptoms of atherosclerotic disease, cerebrovascular responsiveness is also impaired (3). It can be speculated that this is caused by an impaired production and release of nitric oxide, by either endothelial dysfunction or neuronal damage. Further studies on the role of nitric oxide in the maintenance of brain perfusion are important to unravel the pathogenesis of cerebrovascular disease.

In conclusion, by using pMRI techniques measuring cerebral blood flow, it is shown that hypoxia-induced cerebral vasodilatation in humans is mediated by nitric oxide.

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