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Changes in total cerebral blood flow and morphology in aging

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Chapter 5

Nitric oxide mediates hypoxia-induced cerebral vasodilation in humans

Nitric oxide mediates hypoxia-induced cerebral vasodilation in humans

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Purpose

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.

Materials and methods

The effect of the NO synthase inhibitor NG-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.

Results

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).

Conclusions

Use of phase-contrast magnetic resonance imaging shows that hypoxia-induced cerebral vasodilatation in humans is mediated by NO.

Introduction

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 mechanisms¹⁸. 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^{18,32}. The mechanism underlying this coupling between O₂ supply and cerebral vascular tone remains to be elucidated, although experimental data suggest that nitric oxide is involved⁶⁷. In various species it has been shown that nitric oxide synthase inhibitors attenuate hypoxia-induced cerebral vasodilatation⁶⁸⁻⁷⁰. Recently, it has been shown that, in the human forearm, hypoxia-induced vasodilatation is mediated via the release of nitric oxide⁷¹.

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^{67,72}. Recently evidence has been provided that nitric oxide also plays a role in humans in the regulation of cerebral vasomotor tone^{73,74}. 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 vascular relaxation in young healthy volunteers, by using the competitive nitric oxide synthase inhibitor NG-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 noninvasively⁴⁹.

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. 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, dyslipidemia, diabetes mellitus, signs and symptoms of cardiovascular disease, or any other significant abnormalities in physical 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 hours. The protocol was approved by the Medical Ethical Committee of the Leiden University Medical Center and conformed with the principles outlined in the Declaration 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 mixture), and a deep antecubital vein was cannulated for infusion of L-NMMA. Then the subjects were positioned in a magnetic resonance system operating at a field strength of 1.5 T (ACS-NT15; Philips Medical Systems, Best, The Netherlands) under continuous audio and video surveillance. Heart rate was derived from a one-lead ECG and was continuously monitored. Blood pressure was measured semicontinuously 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 pcMRI technique as described previously with the following parameters: 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 averages⁷⁵. Triggering was retrospective using a peripheral pulse unit. The flow measurements were analyzed on a Sun UltraSparc 10 workstation with internally developed software package FLOW^{®29}. 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 interval of 1 week. On one day the effect of the competitive nitric oxide synthase inhibitor L-NMMA on CBF was assessed during normoxia (SpO_2 97%), and on the other day this was done during hypoxia (SpO_2 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 continued breathing ambient air or started breathing a variable N_2 - O_2 mixture. The objective of the N_2 - O_2 mixture was to obtain a SpO_2 of 80%. Therefore, the N_2 - O_2 mixture was continuously adjusted for each subject during the experiments. After 20 min of a stable SpO_2 at either 97% or 80%, cerebral blood flow was measured twice. Then L-NMMA (Clinalpha, Läfelfingen, Germany) was administered intravenously 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 \pm 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 calculated 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 period because of claustrophobia. Therefore the data were

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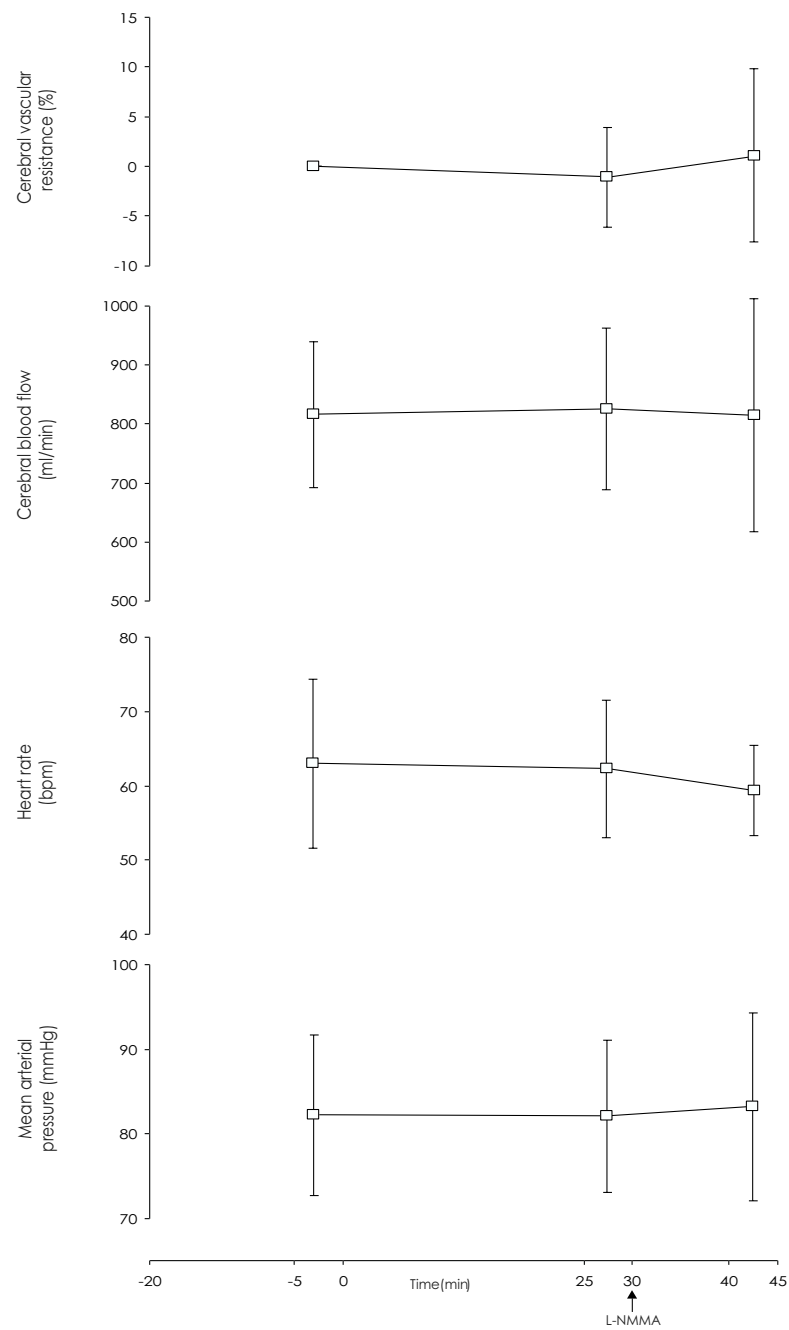


Figure 1 Percent change in cerebral vascular resistance, cerebral blood flow, mean arterial pressure, and heart rate during normoxia. L-NMMA, NG-monomethyl-L-arginine.

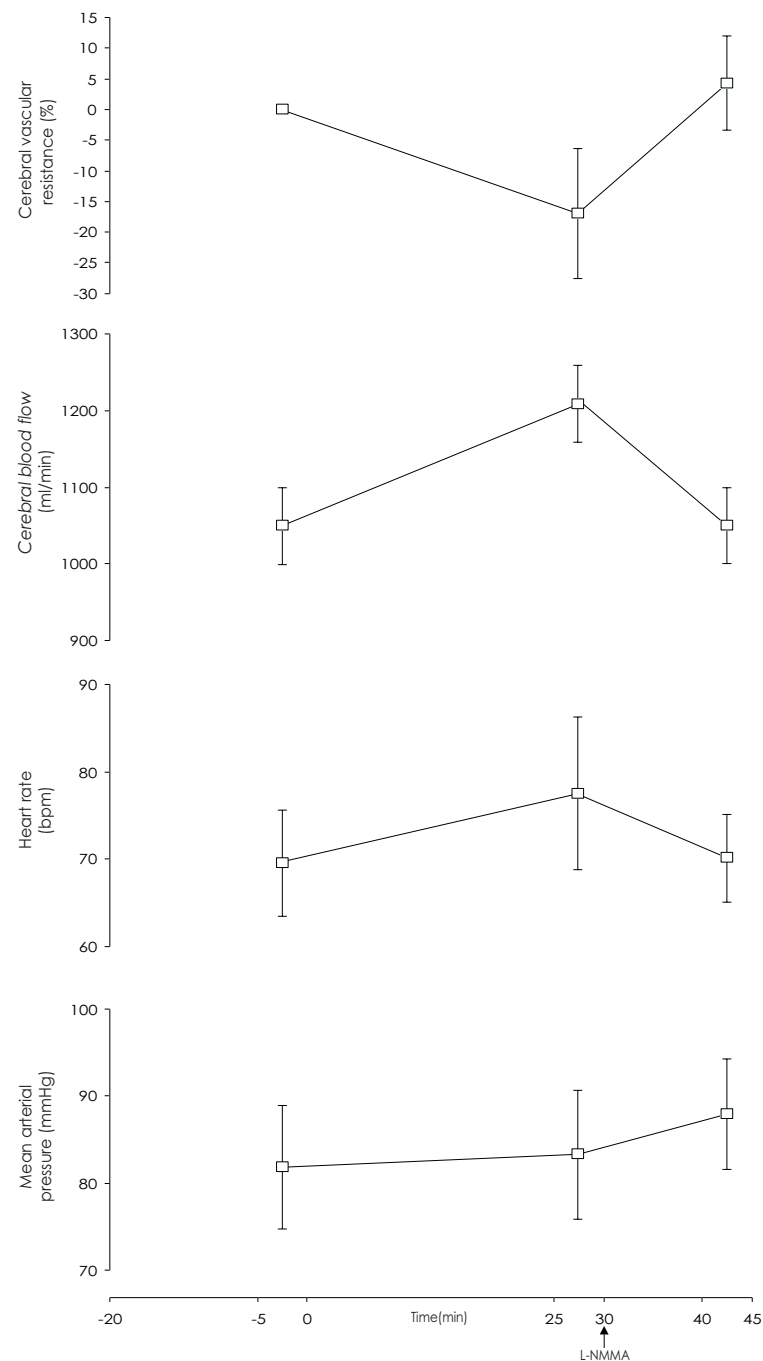


Figure 2 Percent change in cerebral vascular resistance, cerebral blood flow, mean arterial pressure, and heart rate during hypoxia.

analyzed on seven subjects. Hypoxia was easily achieved and maintained at a SpO_2 level of $79.1 \pm 0.3\%$. Basal CBF was comparable on the 2 study days, 862 ± 139 ml/min and 907 ± 253 ml/min, respectively (not significant).

Normoxia

During normoxia, there was no change in CBF (816 ± 124 and 825 ± 137 ml/min) or any of the other parameters (Figure 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 CO_2 (Figure 1). Consequently, the vascular resistance did not change during normoxia.

Hypoxia

Hypoxia (SpO_2 80%) was induced by lowering the inspired O_2 from $21.0 \pm 0.5\%$ to $13.1 \pm 0.8\%$. During hypoxia, CBF increased from $1,049 \pm 113$ to $1,209 \pm 143$ ml/min, respectively ($P < 0.05$; Figure 2). Hypoxia had no significant effect on the steady-state end-tidal CO_2 (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 declined by $17 \pm 10\%$ during hypoxia ($P < 0.05$; Figure 2).

During hypoxia, administration of L-NMMA decreased CBF significantly from $1,209 \pm 143$ to $1,050 \pm 161$ ml/min ($P < 0.05$; Figure 2). Mean arterial pressure increased from 83 ± 8 to 88 ± 5 mmHg after administration 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 cerebral 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 pcMRI to investigate hypoxia-induced cerebral vascular reactivity in humans. 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 ultrasonography¹⁹. 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^{22,49,75-77}.

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 O_2 dependency, e.g., skeletal muscles, kidneys, intestines, and the brain⁷⁸. 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^{68-70,79}. 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 forearm⁷¹. Because the cerebral vascular resistance 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 O_2 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 CO_2 was not influenced by hypoxia virtually excludes the distorting influence of changes in PCO_2 on hypoxia-induced cerebral vasodilation in the present study⁷⁴. 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 CO_2 from brain tissue⁸⁰⁻⁸². In our setup, the end-tidal PCO_2 was allowed to change with changing levels of ventilation. The fact that in our experiments the steady-state normoxic and hypoxic end-tidal PCO_2 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 vasodilation 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 administration⁸³. Both findings are in agreement with the observation that L-NMMA reduced basal cerebral blood flow in healthy volunteers only at higher doses⁷³. 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 CBF⁸⁴. However, from studies in peripheral vascular beds there is accumulating evidence that atherosclerosis impairs nitric oxide-mediated vasodilatation, probably via endothelial damage⁸⁵⁻⁸⁸. Recently, it has been shown that, in patients with signs and symptoms of atherosclerotic disease, cerebrovascular responsiveness is also impaired⁶⁴. 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 pcMRI techniques measuring cerebral blood flow, it is shown that hypoxia-induced cerebral vasodilation in humans is mediated by nitric oxide.

