Basal cerebral blood flow is dependent on the nitric oxide pathway in elderly but not in young healthy men
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Objective
Brain perfusion is tightly regulated over a wide range of blood pressures by local regulation of cerebral blood flow (CBF). Ageing is associated with impaired CBF and impaired nitric oxide mediated vasodilator responses. The role of nitric oxide in the regulation of basal CBF in young and older subjects was investigated, using the nitric oxide synthase inhibitor L-NMMA as pharmacological tool.

Methods
We used a gradient echo phase-contrast magnetic resonance imaging technique to investigate the role of nitric oxide in the regulation of cerebral blood flow in young (25±7.1 years; n=8) and old (78±6.6 years; n=7) volunteers. The study was performed in a double-blinded fashion and consisted of two study days. On one day the effects of the intravenously infused L-NMMA on CBF and blood pressure was measured and on the other day the effects of a matching placebo.

Results
Basal CBF was significantly lower in old compared to young subjects (590±20 vs 704±20 ml/min), while the cerebral vascular resistance (CVR) levels were significantly higher (0.15±0.01 (arbitrary units) vs 0.12±0.01, respectively). Infusion of L-NMMA significantly increased mean arterial pressure in both groups (2.8±1.2 mmHg; P=0.02 in the young and in the old subjects 5.6±1.1 mmHg; P<0.001). Infusion of L-NMMA significantly decreased CBF (49±12 ml/min; P<0.001) and increased CVR (0.02±0.004; P<0.001) in the old subjects but did not significantly influence cerebral circulation in the young subjects.

Conclusion
We conclude that compared to young subjects, in old people CBF is impaired, and dependent on the intactness of the nitric oxide pathway.
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Introduction

Brain perfusion is tightly regulated over a wide range of blood pressures by local regulation of cerebral blood flow (CBF)\textsuperscript{18}. Nevertheless, ageing is associated with impaired CBF\textsuperscript{26}.

Possible explanations for this age dependent impairment of CBF, is a reduction in cerebral metabolism or loss of cerebral tissue at old age (atrophy)\textsuperscript{89,90}. Alternatively, this reduced cerebral perfusion at old age could be a pathological condition, caused by vascular damage, e.g. atherosclerosis and lipohyalinosis. In contrast to the first two possibilities, the latter pathological state would cause a potential imbalance between oxygen supply and demand of the brain, triggering auto regulatory mechanisms to induce cerebral vasodilation. An important mechanism to regulate CBF at a local level is the nitric oxide pathway (for review see \textsuperscript{91}).

Nitric oxide is produced by the endothelium from L-arginine upon various triggers, amongst others hypoxia and hypercapnia, both important stimuli to increase cerebral perfusion \textsuperscript{91}. Both ageing and atherosclerosis are associated with impaired endothelial function and consequently impaired nitric oxide mediated vasodilation\textsuperscript{89,91,92}. Since cerebral vascular disease is a disorder of the elderly it is possible that endothelial dysfunction might be the cause of a relative hypoperfusion of the brain at old age, causing ischaemic damage. This relative hypoperfusion would cause an imbalance between oxygen supply and demand, triggering the endothelium to enhance the production and release of nitric oxide.

In the present study we examined the role of nitric oxide in the regulation of basal cerebral vascular tone in older and young subjects. We used the nitric oxide synthase inhibitor L-NMMA as pharmacological tool.

Methods

Subjects

Eight young (mean age 25±7.1 years) and 7 old (mean age 78±6.6 years) non-smoking healthy male volunteers, participated in this study after giving informed consent. Physical and routine blood examinations, ECG and conventional MRI scans of the brain (T\textsubscript{2} weighted fast spin echo and fluid attenuated inversion recovery (FLAIR)) revealed no major abnormalities. Exclusion criteria were current smoking, use of drugs and more than three alcoholic drinks a day, body mass index greater than 26 kg/m\textsuperscript{2}, hypertension, claustrophobia, dyslipidemia, diabetes mellitus, signs and symptoms of cardiovascular disease or any other significant abnormalities in physical examination, blood analysis,
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ECG, or standard MRI-scans. Before the start of the experiments, subjects abstained from non-steroidal anti-inflammatory drugs for at least 10 days, from alcoholic and caffeine containing beverages for at least 12 hours. The protocol corroborated the principles outlined in the Declaration of Helsinki and was approved by the ethical committee of the Leiden University Medical Center.

Procedures

During the experiments the subjects were in the supine position with their heads comfortably stabilised. Lying in the MR-scanner (ACS-NT15; Philips Medical Systems, Best, The Netherlands), heart rate was continuously monitored and blood pressure was measured automatically with intervals of 5 min. CBF was measured non-invasively in the basilar artery and both internal carotid arteries using a gradient echo phase-contrast MRI technique as described previously with the following parameters: TR/TE 16/9 ms; flip angle 7.5°; 5 mm slice thickness; FOV 250 mm and one NSA. Triggering was retrospective using of a peripheral pulse unit. The flow measurements were analysed on a Sun UltraSparc 10 workstation with the internally developed software package FLOW®. Total CBF was defined as the summed flow measured in the basilar artery and both internal carotid arteries and expressed as ml/min using a gradient echo phase-contrast MRI technique.

Study protocol

The study was performed on two occasions with an interval of 1 week in a double-blinded placebo controlled fashion, randomising infusion of the nitric oxide synthase inhibitor L-NMMA and saline as the matching placebo. L-NMMA (Cinalpha, Läufelfingen, Germany) was administered intravenously with an initial bolus of 3 mg/kg in 5 min, followed by a constant infusion of 30 g/kg/min for 50 min, using a constant rate infusion pump (Spectris MR injector, Medrad Europe BV, Beel, The Netherlands). CBF was measured at baseline prior to the infusions and then semi-continuously for 45 min with intervals of 5 min.

Analysis

The results are presented as means±SEM. Cerebral vascular resistance (CVR) was calculated from the simultaneously measured total CBF and mean arterial pressures (MAP), and expressed in arbitrary units. Results between the two study groups at baseline were compared using the non-parametric Mann–Whitney U test, whereas the effect of L-NMMA was analysed with a linear mixed model for repeated
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Results

The clinical characteristics of the volunteers at the day of screening are listed in Table 1. Baseline MAP was significantly higher in the old subjects (91±13 mmHg) compared to the young individuals (74±27 mmHg; P<0.05). Baseline CBF in the old subjects (590±53 ml/min) was significantly lower compared to the young individuals (704±57 ml/min; P<0.05; Table 2). Consequently, baseline CVR was significantly higher in the old subjects compared to the young individuals (Table 2). In both groups L-NMMA induced a significant increase in MAP during the 55 min observation (Figure 1). In the young subjects the MAP increased 2.8±1.2 mmHg (P=0.02) and in the old subjects 5.6±1.1 mmHg (P<0.001). Compared to saline, L-NMMA induced a significant decrease in CBF (49.9±11.5 ml/min) and an increase in CVR (0.02±0.004) in the old subjects (P<0.001), but did not affect CBF (4.0±14.5 ml/min) or CVR (0.005±0.003) in the young individuals (Figure 1).

Discussion

The main finding of our study is that in elderly individuals basal total CBF is dependent on the nitric oxide pathway, whereas in younger individuals this is not the case, suggesting that in old age the range of cerebral auto regulation is impaired.

Table 1 Clinical characteristics of volunteers

<table>
<thead>
<tr>
<th></th>
<th>Young</th>
<th>Old</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>25±2.5</td>
<td>78±2.5*</td>
</tr>
<tr>
<td>Body Mass Index (kg/m²)</td>
<td>21±0.31</td>
<td>24±0.77*</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>133±6</td>
<td>143±5*</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>72±5</td>
<td>80±4*</td>
</tr>
<tr>
<td>Heart rate (min⁻¹)</td>
<td>72±5</td>
<td>75±3</td>
</tr>
</tbody>
</table>

All values are mean and corresponding standard error. *P<0.05 compared to mean of young subjects.

Table 2 Baseline values of cerebral blood flow, mean arterial pressure, and cerebral vascular resistance for study subjects at two occasions, the day of NaCl administration and the day of L-NMMA administration

<table>
<thead>
<tr>
<th></th>
<th>NaCl Day</th>
<th>L-NMMA Day</th>
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<tbody>
<tr>
<td></td>
<td>Young</td>
<td>Old</td>
</tr>
<tr>
<td>CBF (ml/min)</td>
<td>713±30</td>
<td>567±21*</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>73±3</td>
<td>91±5*</td>
</tr>
<tr>
<td>CVR</td>
<td>0.11±0.01</td>
<td>0.16±0.01*</td>
</tr>
</tbody>
</table>

NaCl, Natrium chloride (=saline); L-NMMA, NG-monomethyl-arginine; CBF, cerebral blood flow; MAP, mean arterial pressure; CVR, cerebral vascular resistance. All values are mean and corresponding standard error. *P<0.05 compared to mean of young subjects.
Total CBF is kept constant over a wide range of systemic blood pressure by local regulation of vascular tone. This auto regulation of CBF is controlled by a combination of myogenic, neurogenic, and metabolic mechanisms. This complex auto regulatory mechanism is based on a tight coupling between oxygen supply and demand. Recently, it has been shown that in humans nitric oxide is involved in this cerebral vascular auto regulation, using L-NMMA as a pharmacological tool. In the present experiments, total CBF was determined as the summed flows in the internal and carotid arteries and basilar artery. Since the flow in these arteries is nutrient brain flow, variations in CBF reflect changes at the arteriolar level in the brain.

Aging is associated with a decrease in basal CBF. The present findings corroborate these data, with an estimated decrease of 2 ml/min per year. In a recent study we found strong evidence that this age related decrease in CBF is independent of brain volume (unpublished data).
Systemic administration of L-NMMA did not influence basal CBF in the young subjects, although systemic blood pressure was significantly increased. The absence of a cerebral vasoconstrictor response does not inevitably preclude the role of nitric oxide in maintaining basal CBF in young individuals, but merely indicate the that cerebral auto regulation is not depending on the intactness of the nitric oxide pathway in the young. It can be argued that the absent of effect of L-NMMA on CBF in the young subjects is caused by a lack of effectiveness of L-NMMA at young age. However, this seems less likely as ample experiments in various vascular beds has shown that L-NMMA effectively blocks the NO-production in young individuals, also in the cerebral circulation.\textsuperscript{74,93}

The finding that, in the older individuals infusion of L-NMMA did cause an increase in systemic blood pressure and a decrease in basal CBF, and consequently an increase in CVR shows two things. First, it confirms that in humans nitric oxide is involved in the regulation of cerebral vascular tone\textsuperscript{83,93}. Second, in contrast to young subjects, in old age basal CBF is depending on the intactness of the nitric oxide pathway, suggesting that cerebral auto regulation is impaired in elderly. The cause of this phenomenon is unknown, but it can be speculated that atherosclerosis and endothelial dysfunction may play a significant role, since the endothelium plays a cardinal role in the local regulation of organ perfusion. Age is the main risk factor for atherosclerosis causing impaired endothelial mediated vasodilatation, via amongst other vasodilators, a reduced response to nitric oxide\textsuperscript{89,90}.

In conclusion the present findings show that in contrast to young individuals, in old subjects basal CBF is impaired and dependent on the intactness of the nitric oxide pathway, suggesting an impaired cerebral auto regulation in old age.