Changes in Cerebral Blood Flow and Metabolism Following Intraarterial or Local Administration of Nimodipine, Before and After Experimental Subarachnoid Hemorrhage in Baboons

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SUMMARY Experimental subarachnoid hemorrhage (SAH) was induced in baboons by repeated injections of autologous blood into cisterna chiasmatis and cisterna magna, a total of 14–33 ml being injected over 3–4 days. Cerebral blood flow (CBF; $^{133}$Xenon clearance) and cerebral metabolic rate of oxygen (CMRO$_2$) were measured before, and 7 days after, the first blood injection. The effect of the calcium channel blocker, nimodipine, used in a commercially available form for clinical application, was studied following continuous i.a. infusion ($0.1 \mu g \times kg^{-1} \times min^{-1}$) for an interval of 45 min, and also 20 and 60 min after intrathecal administration of $1 \mu g \times kg^{-1}$. During the infusion experiments, CBF was increased by 25–30% both before and after the cisternal blood injection. CMRO$_2$ was also enhanced, but much less. Nimodipine in doses given did not alter systemic blood pressure. Following intrathecal application, CBF and CMRO$_2$ slightly increased at 20 min only before experimental SAH.

DELAYED CEREBRAL ISCHEMIA following subarachnoid hemorrhage (SAH) — often due to rupture of an intracranial arterial aneurysm — is believed to have multifactorial pathophysiological mechanisms. 1,2 Associated clinical deterioration generally appears toward the end of the first week of bleeding, progressing to serious ischemic manifestations in the second week. 3,4 Accompanying changes in the cerebrovascular system are of vasoconstrictory nature, often appearing as angiographically visible vasospasm.

Irrespective of the type of stimulus inducing the contraction of smooth muscle cells, free calcium (activator calcium) is always required in the contraction mechanism, 5 and the degree of contraction is dependent on the amount of free calcium available at the ATPase on actin and myosin filaments. 6 Free calcium can principally originate from the release of intracellular pools, or from extracellular calcium entering across the muscle cell membrane. 7 8 Activator calcium for the cerebral vascular smooth muscle cells appear to be derived primarily from extracellular sources. 9–11 The peripheral vascular smooth muscle cells behave differently in that they contract mainly due to sequestration of calcium ions from intracellular organelles. For the contraction to be maintained, the smooth muscle cells need replenishment of the intracellular calcium pool via the influx of extracellular calcium. These data form the rational basis for the attempt to treat cerebral arteriolar vasoconstriction and spasm with inhibitors of calcium uptake.

Nifedipine and nimodipine 9–16 are the most potent members of a group of drugs, which also include verapamil, 16 D 600 12–17,18 and YC-93. 19,20 All inhibit the influx of extracellular calcium through the smooth muscle cell membrane.

Nimodipine (Bayer AG), which is used in the present study, has a preferential action on cerebral and coronary blood vessels, 21–24 probably because these vascular beds are more dependent on extracellular calcium for their contractile process. 9,25 It is a substituted pyridine with a molecular weight of 418.5. The drug is lipid soluble and is inactivated by light. The LD$_{50}$ for the drug given orally to dogs is greater than 1 g x kg$^{-1}$ body weight (Bayer AG, Toxicology Studies). The blocker is available for clinical trials for injection purposes as an ethanol solution, which may be used for systemic and topical application in conjunction with arterial aneurysm operations. The present study on baboons was undertaken to test the effect of the drug on cerebral blood flow (CBF) and metabolism, measured in terms of the oxygen consumption (CMRO$_2$) following local treatment with nimodipine via the intracisternal or intracarotid routes. Comparison of the effect on CBF and metabolism was made before and during the late phase of vasospasm induced by repeated injections of autologous blood into the basal cisterns. The effect of the alcohol component in the nimodipine solution was also taken into consideration.

Material and Methods

Animals

The experiments were performed on 11 baboons (Papio cynocephalus) of either sex, weighing 9.2–21.8 kg. The animals were housed in individual cages 4–8 weeks before (and during) the experiments. They were fed with standard monkey pellets, supplemented with fruit and vegetables, and tap water ad lib.
Anesthesia

Anesthesia was initiated with phencyclidine hydrochloride (Sernylan, BioCeutic; 1 mg × kg\(^{-1}\) i.m.) followed by thiopental sodium (Abbott; 7.5 mg × kg\(^{-1}\) i.v.). The animals were intubated endotracheally and respiration was controlled in a semi-open circuit. The anesthesia was maintained with a continuous infusion of phencyclidine (0.2–0.5 mg × kg\(^{-1}\) × hr\(^{-1}\)), supplemented with 70% nitrous oxide and 30% oxygen. EEG was monitored continuously in order to allow for adjustment of the phencyclidine dose. Muscular relaxation was achieved initially with suxamethonium chloride (Celocurin-klorid, Vitrum; 25 mg i.v.) and then continued with 50 mg suxamethonium chloride i.m.

Blood Flow Measurements

CBF was determined by an intra-arterial injection and cerebral clearance method.\(^\text{26, 27}\) The tracer, \(^{133}\text{Xe}\), was dissolved in 0.9% saline to a final volume of 0.5 ml, which was injected manually into the right internal carotid artery via a catheter placed in the linguo-facial artery. The injection time was close to 1.5 sec. Ipsilateral extracerebral contamination of \(^{133}\text{Xe}\) was minimized by clamping the external carotid artery and by extirpation of the temporal muscle. The clearance rate for \(^{133}\text{Xe}\) was measured from the temporoparietal area of the brain during a 10-min period with a single, collimated sodium iodide scintillation crystal of 25 mm diameter and 15 mm in collimator depth. The stochastic (height-over-area) method was used for calculating CBF.\(^\text{27}\)

Physiological Parameters

The \(P_{CO_2}\) was measured during the second and seventh min, respectively, of the 10 min \(^{133}\text{Xe}\) clearance period. The average value was maintained near 40 mm Hg and not allowed to exceed the range of 38–42 mm Hg. The \(P_{O_2}\) was kept between 100 and 160 mm Hg. The CMRO\(_2\) was obtained by multiplying the arteriovenous difference of oxygen content with CBF. Venous blood was drawn from a catheter inserted immediately above the confluens sinuum. Superior sagittal sinus pressure (SSP) was monitored continuously during the experiment. EEG was recorded via permanently inserted extradural electrodes.

Intracisternal Blood Injections

The blood injections were started 3–5 days after the baseline CBF measurements had been obtained. The animals were sedated with phencyclidine (1 mg × kg\(^{-1}\) i.m.) and the anesthesia was maintained with fluothane using a face mask.

Blood injections were usually carried out three times with two-day intervals. Fresh autologous blood was obtained from a femoral artery puncture. The first and second injections were given into the cisterna magna and the third through the orbit into the cisterna chiasmatis. A short-beveled needle (1.5 mm o.d.) with a styllet was passed percutaneously into the cisterna magna, and 5–10 ml cerebrospinal fluid (CSF) were withdrawn and replaced with blood until the respiratory rhythm became depressed. This usually occurred when 1–4 ml more than the amount of CSF aspirated had been administered. For the intrachiasmatic injections, the same type of needle was passed through the conjunctiva and the optic foramen. Three to 6 ml blood was injected; it was seldom possible to aspirate more than 1–2 ml CSF prior to the injection. The animals thus received a total amount of 14–33 ml blood intracisternally.

Further technical details have been described elsewhere.\(^\text{28}\)

Nimodipine Solution

The commercially available nimodipine preparation for clinical application (Bayer AG) was used in the present experiments. The concentration of the calcium uptake blocker is 0.1 mg/ml in a solution of distilled water containing 150 mg × ml\(^{-1}\) each of ethanol and polyethylene glycol. Nimodipine was omitted from the solution given in the control experiments. Both solutions were diluted with 0.9% saline to a suitable volume (approximately 0.5 ml × min\(^{-1}\)) for the infusion experiments carried out at room temperature (20°C), and to a 2-ml volume with fresh (37°C) CSF immediately before cisternal administration. Syringes and catheters were wrapped in aluminum foil in order to avoid photodecomposition of nimodipine.

Experimental Design

Animals received the calcium uptake blocker, nimodipine, either intrathecally (i.th.) or intra-arterially (i.a.) before and after experimental SAH. The same amount of solvent was given in control experiments. In the i.th. experiments, cisterna magna was punctured percutaneously, and 1.8 ml CSF was aspirated, giving a total of 2 ml, which was injected into the cistern rapidly to secure an efficient distribution in the subarachnoid space. The i.a. infusion (0.1 \(\mu\)g × kg\(^{-1}\) × min\(^{-1}\)) was performed via a catheter placed in the linguo-facial artery, the infusion being discontinued only for the bolus injection of tracer in the CBF measurements.

CBF, metabolism, and other parameters were measured 20 and 60 min after the i.th. administration, after 45 min of continuous i.a. infusion. In the SAH animals, the nimodipine treatment (or administration of solvent) was performed on the seventh day after the first intracisternal blood injection (i.e. 3 days following the last injection).

Results

Intracisternal blood injection resulted in a statistically significant, 16.3% reduction in CBF and a 12.6% fall in CMRO\(_2\), as shown in table 1. It can also be seen from table 1 that experimental SAH did not cause any significant changes in \(P_{CO_2}\), MABP, or blood hemo-
Table 1: Effect of Experimental Subarachnoid Hemorrhage (SAH) on Cerebral Blood Flow (CBF) and Cerebral Metabolic Rate of Oxygen (CMRO₂). Mean Arterial Blood Pressure (MABP), Pulse Rate, Sagittal Sinus Pressure (SSP), and Hemoglobin Concentration in 11 Anesthetized Baboons Before and 1 Week After Cisternal Blood Injections

<table>
<thead>
<tr>
<th>Physiological parameter</th>
<th>Before SAH</th>
<th>After SAH</th>
<th>Paired t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBF (ml x 100 g⁻¹ x min⁻¹)</td>
<td>58.2 ± 10.7</td>
<td>48.7 ± 1.9</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>CMRO₂ (ml x 100 g⁻¹ x min⁻¹)</td>
<td>3.26 ± 0.14</td>
<td>2.85 ± 0.13</td>
<td>p &lt; 0.01</td>
</tr>
<tr>
<td>P_CO₂ (mm Hg)</td>
<td>39.1 ± 0.3</td>
<td>39.8 ± 0.3</td>
<td>n.s.</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>113 ± 5.2</td>
<td>110 ± 3.3</td>
<td>n.s.</td>
</tr>
<tr>
<td>Pulse rate (per min)</td>
<td>90 ± 4.6</td>
<td>113 ± 7.0</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>SSP (mm Hg)</td>
<td>3.2 ± 1.2*</td>
<td>10.4 ± 3.7†</td>
<td>n.s.</td>
</tr>
<tr>
<td>Hemoglobin (g%)</td>
<td>114.9 ± 4.0*</td>
<td>101.1 ± 5.9*</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Mean values ± SEM, n.s. = non-significant.

*For technical reasons only 10 animals included.
†For technical reasons only 8 animals included.

Continuous i.a. infusion of the calcium uptake blocker at a rate of 0.1 μg x kg⁻¹ x min⁻¹ before experimental SAH significantly elevated both CBF and CMRO₂, in comparison with baseline values, as measured 45 min after starting the treatment (fig. 2). This effect was emphasized when comparing with the controls receiving the ethanol-containing solvent, which tended to lower both flow and metabolism. Following SAH, nimodipine increased CBF to the same extent as before the blood injections, though the enhancement in CMRO₂ was no longer statistically significant. The mean level of MABP remained unchanged in the experiments, and there was no statistically significant effect of nimodipine on SSP.

Discussion

Attempts were made in baboons to simulate the late phase of cerebral vasospasm following SAH in patients by repeated cisternal injections of autologous blood according to a model described in detail elsewhere.28 The blood injections give rise to angiographically visible constriction of the major brain vessels, associated with a decrease in CBF. Also the oxygen consumption of the brain is reduced to a corresponding degree.28

The use of agents blocking the uptake of extracellular calcium into the vascular smooth musculature has been suggested as a means to overcome the circulatory disturbance following SAH.9-10-12 Thus, blockade of the calcium channels counteracts the experimentally induced constriction of pial vessels from cat,14 dog,9 and man.12 Nimodipine prevents depression of CBF following brief total ischemia within the brain of

Figure 1. Per cent changes in cerebral blood flow (CBF), cerebral metabolic rate of oxygen (CMRO₂), and mean arterial blood pressure (MABP) at 20 and 60 min following intrathecal (i.th.) administration of 1 μg x kg⁻¹ nimodipine, before and after experimental subarachnoid hemorrhage (SAH). Values are mean ± SEM, number of animals within parenthesis. Differences between mean values from experiments with nimodipine (hatched bars) and the ethanol-containing solvent (open bars) according to Student’s t-test are indicated, together with differences between absolute baseline values and experimental values according to the paired t-test (*0.01 < p < 0.05); n.s. = non-significant.
EFFECTS OF NIMODIPINE FOLLOWING SAH/Sahlin et al

cats and it also tends to increase CBF in rats following 15 min of incomplete (forebrain) ischemia. In baboons, continuous i.v. infusion of 2 μg x kg⁻¹ x min⁻¹ of nimodipine induces an approximately 30% increase in CBF without significantly affecting CMRO₂. Under these conditions, systemic blood

![Figure 2](http://stroke.ahajournals.org/)

**Figure 2.** Per cent changes in cerebral blood flow (CBF), cerebral metabolic rate of oxygen (CMRO₂), and mean arterial blood pressure (MABP) induced by continuous i.a. infusion of 0.1 μg x kg⁻¹ x min⁻¹ nimodipine during 45 min, before and after experimental subarachnoid hemorrhage (SAH). Values are mean ± SEM, number of animals within parenthesis. Differences between mean values from experiments with nimodipine (hatched bars) and the ethanol-containing solvent (filled bars) according to Student's t-test are indicated, together with differences between absolute baseline values and experimental values according to the paired t-test (*0.01 < p < 0.05, **0.001 < p < 0.01); n.s. = non-significant.

pressure was reported to fall by a mean of about 15%. A more variable effect of i.a. infusion on CBF of baboons has been reported at a dose of 0.6 μg x kg⁻¹ x min using the hydrogen clearance technique to measure blood flow.

In the present experiments, flow and metabolism of the brain were measured after administration of a commercially available nimodipine solution containing ethanol and designed for clinical application. It has been shown studies on humans and laboratory animals that large quantities of ethanol induce cerebral vasodilation, whereas smaller doses have no cerebrovascular effects, or cause a reduction in CBF. This agrees with the slight reduction in CBF seen in the baboons (before experimental SAH) which received the ethanol-containing solvent in an amount corresponding to 9 mg x kg⁻¹ x hr⁻¹ of ethanol. The effect is probably due to a depression of brain metabolism.

Infusion of nimodipine (0.1 μg x kg⁻¹ x min⁻¹) i.a. increased CBF by 25% above baseline values. In view of the above-mentioned action of the ethanol-containing solvent, the figure probably is a slight underestimation. The cerebrovascular effect agrees with that reported by Harper et al. There is reason to believe that the response is a combination of a direct effect of nimodipine on the brain vasculature and an effect associated with an increased CMRO₂.

Calcium uptake antagonists have been advocated in the treatment of the cerebrovascular disturbances following SAH. Following experimental SAH in baboons, previously reported in a preliminary form, we found that i.a. infusion of nimodipine in the dose mentioned above increases CBF by 25% one week following the cisternal blood injection, without any statistically significant effect on brain metabolism. The dose used did not affect MABP. Following peroral administration (1 mg x kg⁻¹ every 8 hours) the effect of nimodipine in baboons one or two weeks after experimental SAH was not clearcut, possibly because the drug was given at too long intervals. In a randomized and placebo-controlled double-blind study on 125 neurologically normal patients given nimodipine perorally within 96 hrs following SAH, Allen et al reported a statistically significant reduction in the incidence of ischemic neurologic deficits during a 21-day treatment period. However, this has not been confirmed in subsequent clinical studies.

Vasodilating agents, recently also including nimodipine, are clinically used for irrigation or local application following clipping of intracranial aneurysms in order to minimize vasospasm. Sometimes, this is followed by continued local application through a catheter during the immediate postoperative days. In view of this, it was considered important to evaluate the effect of locally administered nimodipine on CBF and CMRO₂, following experimental SAH in the baboons. The effect of nimodipine applied i.th. was less pronounced than that following i.a. infusion. Flow and metabolism were significantly, and slightly, increased 20 min after administration, whereas no statistically significant effect was seen at 60 min. No changes
could be recorded after experimental SAH, possibly due to inefficient access of the antagonist following local blockade of the subarachnoid space as a consequence of the blood injections.

References
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