The Effects of a Calcium Antagonist (Nimodipine) on Basal Cerebral Blood Flow and Reactivity to Various Agonists

T.A. McCalden, Ph.D., R.G. Nath, M.S., and K. Thiele, B.S.

SUMMARY  In vitro studies suggest that cerebrovascular contraction is more dependent on the influx of calcium from the extracellular fluid than other general systemic blood vessels. Thus, it seems probable that, pharmacologic agents which prevent the movement of calcium into the smooth muscle would preferentially reduce cerebrovascular constriction leaving intact general systemic vascular tone. The drug nimodipine has proven effective in vivo in antagonizing cerebral vascular contractions to agonists such as 5-hydroxytryptamine, norepinephrine and high K+ solutions. The present study tested, in vivo, the effects of various doses of nimodipine on resting cerebral blood flow. The dose which showed the largest cerebral flow increase was then used to determine the action on the cerebrovascular constriction resulting from, 5-hydroxytryptamine infusion in the hypercapnic animal and hyperventilation to lower the PCO2.

Methods and Materials

Adult baboons (Papio 8-12 kg) were sedated with intramuscular phencyclidene (Sernylan, 10 mg/kg) or ketamine (5 mg/kg). They were then transported to the laboratory where full anesthesia was induced with sodium pentobarbital (Nembutal, 20 mg/kg i.v.). The animals were intubated and after succinyl choline (20 mg i.v.) were ventilated using a positive pressure respirator. The rate and depth of ventilation were adjusted so that the animals showed approximately 5% CO2 in end tidal expiratory air (CO2 measured continuously from the endotracheal tube) using a Goddard capnograph. The depth of anesthesia was monitored throughout using a single lead EEG and this was maintained showing a rhythm of 8–10 Hz using a ventilation gas mixture of 70% N2O in oxygen and supplemen-

mental bolus i.v. injection of sodium pentobarbital as required.

Catheters were placed in the femoral vein (for i.v. injections and infusion of nimodipine), and the femoral artery (for recording arterial blood pressure, and obtaining samples for blood PCO2 and PO2 analysis — Corning). An additional catheter was placed in a retrograde direction in the lingual artery and the tip of this catheter was placed at the junction of the external carotid and the carotid bifurcation. Then the external carotid, the lingual and all other branches of the external carotid were tied distally. Thus, 133Xenon injected as a bolus into the catheter travelled via the internal carotid artery to the brain. The arrival and subsequent clearance of the 133Xenon was monitored using a collimated sodium iodide crystal detector, mounted externally. The detector was placed over the parietal area of the brain and shielded and collimated to avoid counting xenon in non-cerebral tissues. The output from the crystal was amplified and subjected to pulse height analysis. The activity at 81 ± 5 KeV was displayed on a ratemeter and recorded on a 10-inch chart recorder. The graphic record of each clearance curve was then replotted as log10 xenon activity vs. time. Cerebral blood flow was determined from each graph using the ten minute height-over-area technique. A xenon partition coefficient of 1.07 was used throughout. The height of the curve was the xenon activity at time zero minus that at 10 minutes. The area of the curve above background was measured for each minute using the technique of Simpson and totalled over the whole 10 minutes of xenon clearance.

In 4 of these animals another catheter was pleced into the internal jugular vein and pushed intracranially. All other branches of the vein were ligated and this catheter was used to obtain samples of mixed cerebral venous blood. The hemoglobin (g/100 ml) and percentage saturation with oxygen were measured (Instrumentation Laboratories CO-Oximeter) in these venous samples and in simultaneously removed samples of arterial blood. Assuming that each gram of hemoglobin carries 1.36 ml of oxygen, the venous and arterial oxygen content was calculated. Hence the arterio-ve-
nous oxygen difference was determined and when this was multiplied by the cerebral blood flow, an index of cerebral oxygen consumption was obtained.

Normal Effect of Nimodipine
At least two control cerebral blood flow determinations were made in each animal with measurements of arterial PCO$_2$ maintained between 35 to 40 mm Hg and PO$_2$ at approximately 100 mm Hg. The EEG was examined before each flow determination and maintained with a dominant rhythm of 8–10 Hz. Blood pressure was monitored to ensure that it was within the limits of cerebral autoregulation. Then the xenon was injected and recording of the 10 minute clearance curve begun. During this time, samples of arterial and venous blood were removed for oxygen content determinations. After these controls, nimodipine was infused intravenously at doses ranging from 0.1 µg/kg/minute to 1000 µg/kg/minute. A maximum of two doses were tested in each animal. The blood flow, and all ancillary measurements were repeated between 10 and 20 minutes after the nimodipine infusion was begun. The nimodipine infusion solution was prepared daily from a previously prepared concentrated stock solution. This stock solution (50 mg/ml) was prepared in the dark by dissolution of the drug in polyethylene glycol. The stock was stored in amber glass bottles in the freezer. Final preparation of the infused solution was done under subdued lighting and the infusion syringes and catheters were protected from laboratory light with a covering of aluminum foil.

Nimodipine and Cerebral Vasoconstrictor Agonists
The dose of nimodipine having a maximal effect on cerebral blood flow was identified and used in this part of the study. Continuous i.v. infusion of the nimodipine was used over a 60-minute period. During this time measurements of cerebral blood flow were made with hypercapnia (PCO$_2$ approximately 50 mm Hg), hypercapnia plus intra-arterial infusion of 5-hydroxytryptamine (5HT) at 10 µg/kg/min, and hyperventilation to reduce PCO$_2$ (approximately 25 mm Hg).

Statistics
The data was subject to one way analysis of variance and t tests. A p value of less than 0.05 was considered significant.

Results
Dose-Response to Intravenous Nimodipine
The 16 animals studied showed a mean baseline cerebral blood flow (CBF H/A) of 48 ± 2 ml/min per 100 grams of brain. This value was obtained with a PaCO$_2$ of 38.5 ± 0.7 mm Hg; a PaO$_2$ of 105.0 ± 3.1 mm Hg; and an arterial blood pressure of 113 ± 3 mm Hg. Figure 1 shows, at the left side, this base cerebral blood flow and the alteration away from that basal value with nimodipine (NIM) infusion. The lowest dose (0.1 µg/kg/min) produced no significant alteration in flow, or any other variable (table 1). NIM infusion at 1 µg/kg/min produced a modest 18% increase in CBF H/A, again with no significant alteration in BP or blood gases (table 1). Higher levels of NIM infusion caused a significant decrease in BP and this effect seemed to remove any beneficial effect of the low dose CBF dilation (table 1). There were no significant alterations in blood gases. Table 1 also shows the mean changes of cerebrovascular resistance (CVR) with each dose of NIM. At 1.0 µg/kg/min the CVR was significantly decreased from the control values indicating that a relaxation of the cerebral resistance vessels had occurred. The higher values for dose of NIM were also associated with a significantly decreased value of CVR from control. This maintained relaxation of the cerebral resistance vessels was accompanied by a significant reduction in arterial blood pressure. Despite the fact that these alterations in BP were within the limits of cerebrovascular autoregulation, the increase in CBF was not maintained.

The biexponential $^{133}$xenon clearance curves were resolved into one fast and one slowly clearing compart-

![Graph showing the effect of nimodipine on cerebral blood flow](http://stroke.ahajournals.org/)

**Figure 1.** The basal cerebral blood flow (H/A, gray matter and white matter) are shown as the first histogram bar in each of the three sections of the figure. The alterations away from that basal flow with infusion of nimodipine are shown as the columns 1 to 5 at the right side of each base measuremen. The vertical bars represent plus and minus one standard error of the mean.

<table>
<thead>
<tr>
<th>Dose of nimodipine (µg/kg/min)</th>
<th>CBF H/A</th>
<th>BP (mm Hg)</th>
<th>CVR (mm Hg/ml)</th>
<th>PaCO$_2$ (mm Hg)</th>
<th>PaO$_2$ (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1</td>
<td>48 ± 2</td>
<td>113 ± 3</td>
<td>0.3 ± 0.18</td>
<td>-0.9 ± 0.7</td>
<td>-1.0 ± 0.4</td>
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<tr>
<td>1.0</td>
<td>48 ± 2</td>
<td>113 ± 3</td>
<td>0.3 ± 0.18</td>
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The mean alteration (± 1ST) from baseline values in cerebral blood flow (CBF H/A), arterial blood pressure (BP), cerebrovascular resistance (CVR = BP/CBF) and arterial blood gases (PaCO$_2$ and PaO$_2$) are shown (* = significantly different from baseline, p < 0.05).
ments. Infusion of NIM at 1 μg/kg/min significantly increased (p < 0.01) gray matter CBF by 17 ± 5 ml/m
per 100 g from the mean baseline value of 55 ± 4 ml/min per 100 g. No significant alteration in gray
matter flow occurred at other NIM doses or at any NIM dose in cerebral white matter (baseline value = 6 ±
0.5 ml/min per 100 g). These results are shown in figure 1.

Effects of Vasodilator NIM on Cerebral Oxygen Consumption
In four of the previous 16 animals arterial and cerebral venous concentrations of oxygen were measured. These values were determined under basal conditions and while NIM (at 1 and 10 μg/kg/min) were infused. Using the simultaneously obtained measurement of CBF H/A, cerebral consumption of oxygen (CMRO₂) was determined. The basal CMRO₂ of 2.55 ± 0.53 mls O₂/min per 100 g of brain was not significantly altered by either NIM at 1 or 10 μg/kg/min (2.87 ± 0.28 and 3.29 ± 0.73 mls O₂/min per 100 g respectively).

Nimodipine and the Response to CO₂ and 5HT Infusion
The normal baboon with basal blood gas levels does not exhibit significant CBF vasconstrictor response to intra-carotid infusion of 5HT. The vasoconstrictor sensitivity may be unmasked by elevation of the PaCO₂ above 50 mm Hg. This elevation of PaCO₂ from a mean of 39.5 ± 0.4 mm Hg to 54.4 ± 0.8 mm Hg (without alteration in PaO₂) was accompanied by an increase in CBF H/A from 56 ± 3 ml/min per 100 g to 110 ± 15 ml/min per 100 g (figure 2, left side). This alteration in flow was significant (p < 0.05, n = 5) and represents a CO₂ reactivity of 3.73 ± 0.75 ml/min per mm Hg rise in PaCO₂. The hypercapnia was maintained at a PaCO₂ of 54.8 ± 0.7 mm Hg and a simultaneous infusion of 5HT (10 μg/kg/min) was begun into the internal carotid artery. The 5HT significantly decreased the CBF from its hypercapnic value by 36 ± 4 ml/min per 100 g.

In a second group of 5 animals the CO₂ reactivity and the 5HT response were tested during i.v. infusion of nimodipine (NIM) at 1 μg/kg/min. The right side of figure 2 shows that during NIM infusion an increase in CBF H/A occurred and that subsequent induction of hypercapnia (PaCO₂ = 56.0 ± 2.9 mm Hg) caused a further increase in flow. However, when the hypercapnic increase in flow is calculated the reactivity to CO₂ is significantly (p < 0.05) lower (1.01 ± 0.16 ml/min per mm Hg) than that for the normal group of animals (3.73 ± 0.75). Subsequent, infusion of 5HT (10 μg/kg/min) into the NIM treated, hypercapnic animals caused a decrease in CBF H/A. However this decrease of 20 ± 8 ml/min per 100 g with 5HT was significantly (p < 0.05) smaller than the response found in the normal animals.

Nimodipine and the Response to Hyperventilation
In 5 animals treated with nimodipine at 1 μg/kg/min hyperventilation reduced the arterial PCO₂ from 37.8 ± 1.1 mm Hg to 27.7 ± 0.8 mm Hg. Associated with this change the CBF H/A was not significantly altered showing a small increase from 50 ± 7 ml/min per 100 g to 52 ± 6 ml/min per 100 g. This indicates a response to hypocapnia of increased CBF by 0.08 ± 0.24 ml/min per 100 g of tissue for each mm Hg decrease in PaCO₂. This is a response which is abnormal since a similar sized control group of animals showed a decreased CBF by 0.91 ± 0.38 ml/min/100 g for each mm Hg decrease in PaCO₂ from 39.5 ± 0.4 to 27.6 ± 1.2 mm Hg. A decreased flow of approximately 1.0 ml/min/100 g for each mm Hg is expected.5

Conclusions
The present study shows that i.v. infusion of nimodipine at 1 μg/kg/min causes a modest increase in basal cerebral blood flow without significant alterations in cerebral metabolism or systemic blood pressure. The percentage increase in flow was approximately 20% using the CBF height over area method of analysis. This compares well with previously published literature using nimodipine infusion at 2 μg/kg/min.9 We have further demonstrated that this improvement of cerebral perfusion is largely confined to the rapidly clearing component of the xenon clearance curve (cerebral gray matter). This result with a drug putatively blocking calcium influx into vascular smooth muscle is not unexpected. Previous studies have shown that the cerebral vessels are more dependent on the presence of extra-cellular calcium for sustaining contraction, than other general systemic arter-
ies. In addition, in vitro studies have shown that calcium channel blockers, such as nimodipine, will antagonise cerebrovascular contraction more easily.

A vasodilator action of a drug such as nimodipine probably has a mechanism involving the antagonism of a steady "tonic" influx of calcium across the smooth muscle cell membrane. It might be further suggested that this influx of calcium must be due to the opening of these channels in response to a cerebrovascular agonist. The nimodipine may therefore have mediated its dilator effects through blocking the effects of one or more constrictor agonists. In general, blood vessels are responsive to three kinds of agonists. These are endogenous vasomotor substances (either neural or hormonal), and chemical, or autoregulatory mechanisms. The present study indicates that the nimodipine interfered with the response to alterations in arterial CO2 tension. This change cannot be explained by the combination of a nimodipine dilatation and a CO2 dilatation producing a supra maximal dilator stimulus. Therefore the nimodipine appears to block the mechanism utilized by alterations in CO2. This interaction is further underlined by the decreased cerebral vasoconstrictor responses to a decreased arterial CO2. It may be that CO2 and NIM have similar mechanisms of action.

At first sight the results with 5HT infusion in the hypercapnic animal suggest that the normal reduction of 36 ml/min per 100 g with 5HT infusion was significantly reduced to 20 ml/min per 100 g in the NIM treated animals. This may not be the case. The 5HT does not have much effect on CBF in the normocapnic animal, and to unmask the 5HT effect it is necessary to vasodilate with hypercapnia. The relative lack of vasoconstrictor response to 5HT in the NIM treated animal may be due to the failure of hypercapnia to produce adequate vasodilation.

Table 1 provides some indirect evidence concerning the state of autoregulatory adjustments of CBF to decreased BP. The fall in BP with NIM infusion at the three highest doses negated the increase in CBF found at the 1.0 µg/kg/min dose. This was despite the fact that the cerebral resistance vessels remained dilated (as shown by the continued significant decrease in CVR). Thus, the cerebrovascular dilatation which should have occurred to "autoregulate" CBF during the NIM induced fall in BP seems not to have occurred. This observation is in contrast to previous reports of intact vasodilator autoregulation in the baboon, and the matter therefore remains unresolved. Further study of NIM in the baboon seems necessary.

Acknowledgments
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