Effect of Nimodipine on Cerebral Blood Flow and Metabolism in Rats During Hyperventilation

William L. Young, MD, and Shu Chien, MD, PhD

Nimodipine shows promise in the prevention and treatment of brain ischemia. We examined the interaction of nimodipine pretreatment in a dose sufficient to prevent postischemic hypoperfusion and hyperventilation. We studied four groups of rats: normocarbia plus vehicle (Group 1, n=5), hypocarbia plus vehicle (Group 2, n=4), normocarbia plus nimodipine (Group 3, n=7), and hypocarbia plus nimodipine (Group 4, n=6). Groups 3 and 4 received 1 mg/kg i.p. nimodipine, and Groups 1 and 2 received an equivalent amount of vehicle. Ventilation was left unaltered in Groups 1 and 3 or increased to lower PaCO₂ to 21–24 mm Hg in Groups 2 and 4. Determination of regional cerebral glucose utilization (rCGU) was carried out using the [³H]2-deoxyglucose method, and regional cerebral blood flow (rCBF) was determined by the indicator fractionation method using [¹⁴C]iodoantipyrine. The brain regions studied were the cerebral hemispheres, the diencephalon, the cerebellum, and the brainstem. Hyperventilation in Groups 2 and 4 from approximately 38 to 22 mm Hg reduced rCBF to 60% of normocarbic levels (p<0.05). The slope and intercept of this response were similar in vehicle- and nimodipine-pretreated rats. Nimodipine modestly decreased mean arterial blood pressure by 20% and increased plasma glucose concentration by 60% (p<0.05). Although nimodipine tended to increase rCBF and decrease regional cerebrovascular resistance (rCVR), this was significant only for hemispheric rCVR (p<0.05). There was a borderline effect for nimodipine to increase rCGU, especially during hypocarbia. Although nimodipine pretreatment significantly increased plasma glucose levels, it did not interfere with the ability to use hyperventilation for rCBF manipulation. (Stroke 1989;20:275–280)
mental oxygen was added as necessary. Cannulas were placed via femoral incisions in both femoral arteries and one femoral vein. Blood pressure and EEG were continuously recorded on a Grass Model 7 polygraph (Grass Instrument Co., Quincy, Massachusetts). Temperature was maintained at 36–37°C by means of a heating lamp and monitored by a rectal thermistor. After all operative sites were packed with fibrillar collagen, 50 IU heparin (Liquaemin, Organon Inc., West Orange, New Jersey) was given intravenously.

Nimodipine (Miles Pharmaceuticals, West Haven, Connecticut) was prepared in a stock solution of 20 mg/ml in polyethylene glycol and further diluted to 0.5 mg/ml with normal saline. Appropriate measures were taken to prevent exposure of the drug solutions to light. Experiments were performed on four groups of rats: normocarbia plus vehicle (Group 1, n=5), hypocarbia plus vehicle (Group 2, n=4), normocarbia plus nimodipine (Group 3, n=7), and hypocarbia plus nimodipine (Group 4, n=6). Groups 3 and 4 received 1 mg/kg i.p. nimodipine immediately after induction of anesthesia; Groups 1 and 2 received an equivalent amount of vehicle.

One hour after the induction of anesthesia and the administration of drug or vehicle, ventilation was left unaltered (normocarbia) in Groups 1 and 3 but was increased in Groups 2 and 4 by keeping the tidal volume constant and raising the ventilator rate to lower PacO₂ to 21–24 mm Hg (hypocarbia).

Ten minutes after the induction of hypocarbia in Groups 2 and 4 or after an equivalent normocarbic period in Groups 1 and 3, determination of rCGU was begun. An intravenous bolus of 100 μCi/kg [3H]2-deoxyglucose in 0.3 ml normal saline was given, and 200-μl arterial samples were taken at 0.33, 0.66, 1, 3, 6, 10, 15, 25, 35, and 45 minutes. Donor blood was given intravenously after each sample to maintain euvolemia.

Sixty minutes after the [3H]2-deoxyglucose injection, rCBF was determined by the indicator fractionation method. An intravenous bolus of 20 μCi/kg [14C]iodoantipyrine (New England Nuclear, Boston, Mass.) in 0.3 ml normal saline was given, with simultaneous withdrawal of a reference sample from a femoral artery catheter using a Harvard pump at a rate of 0.786 ml/min. After 10 seconds, the experiment was terminated by decapitation of the rat and the femoral artery catheter was simultaneously withdrawn from the femoral artery. Additional donor blood (1–2 ml) was given immediately before rCBF determination to prevent changes in mean arterial blood pressure (MABP) during reference sample withdrawal.

The brain was quickly removed and divided into the cerebral hemispheres, diencephalon, cerebellum, and brainstem. The brain regions were placed in preweighed scintillation vials. Tissue was solubilized in Protosol (Du Pont/New England Nuclear Research Products, Boston, Massachusetts). Blood from the 10-second reference withdrawal was decol-lorized and treated with Protosol. The timed arterial blood samples were centrifuged to obtain plasma for determining tracer activity and for glucose assay by the hexokinase method using a Gilford 240 spectrophotometer (Gilford Instruments, Oberlin, Ohio). Blood, plasma, and tissue radioactivities were assessed by liquid scintillation counting using a Packard Tricarb counter (Packard Instrument Co., Inc., Sterling, Virginia).

Separation of isotope activities in samples was accomplished by dual window counting, and radioactivities (disintegrations per minute) for each isotope present in blood, plasma, and tissue samples were calculated from the appropriate formula. Counting efficiencies were determined by an external standard, which was checked against internal standards of [3H]toluene and [14C]toluene. rCGU was calculated using the operational equation described by Savaki et al. This modification of the original operational equation, as described by Sokoloff et al. takes into account changing arterial plasma glucose levels. A variation of this approach, using direct counting of tissue radioactivity rather than autoradiography to determine rCGU, has been reported. rCBF was calculated by the equations described by Van Uitert and Levy. Regional cerebrovascular resistance (rCVR) was derived from the ratio of systemic MABP to rCBF.

We compared flow, metabolism, and other physiologic data for all groups using two-way analysis of variance. Using a linear regression model, we regressed hemispheric rCBF on PacO₂ for combined Groups 1 and 2 and for combined Groups 3 and 4. Using Student's two-tailed paired t test, we compared physiologic variables obtained at the time of rCBF determination with values obtained at the beginning of rCGU determination within each group. A probability value of <0.05 was taken as the threshold of significance for all statistical testing.

**Results**

Physiologic variables, shown in Table 1, remained unchanged within each group during the course of the experiments from the time of [3H]2-deoxyglucose injection until [14C]iodoantipyrine injection. There was no difference in the EEG patterns among the four groups. Nimodipine pretreatment significantly decreased MABP (by 20%) and increased plasma glucose levels (by 60%).

rCGU, rCBF, and rCVR for all brain regions are shown in Table 2. Hyperventilation from approximately 38 to 22 mm Hg reduced rCBF to 60% of normocarbic levels in Groups 2 and 4 (p<0.05). There was no interaction between hyperventilation and nimodipine pretreatment, which implies that the differences in outcome variables between normocarbic and hypocarbic rats were of similar magnitude and direction in the rats pretreated with either vehicle or nimodipine. Similarly, the differences in outcome variables between rats pretreated with vehicle or nimodipine were of similar magni-
TABLE 1. Selected Physiologic Variables in Rats During Determination of Effects of Nimodipine on Cerebral Blood Flow and Metabolism During Hyperventilation

<table>
<thead>
<tr>
<th>Group 1: vehicle+ normocarbia (n=5)</th>
<th>Group 2: vehicle+ hypocarbia (n=4)</th>
<th>Group 3: nimodipine+ normocarbia (n=7)</th>
<th>Group 4: nimodipine+ hypocarbia (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose concentration (mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature (° C)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PaCO₂ (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pch (mm Hg)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SEM. MABP, mean arterial blood pressure.

November and Chien  Nimodipine and Hyperventilation  277

physiologic variables were obtained at time of determination of regional cerebral blood flow, immediately before injection of [14C]iodoantipyrine. Values are mean±SEM. MABP, mean arterial blood pressure.

*Significant nimodipine effect.
†Significant hyperventilation effect.

Nimodipine pretreatment did not significantly affect rCBF in any brain region. Nimodipine decreased rCVR, but only in the cerebral hemispheres. Nimodipine pretreatment had a borderline effect to increase hemispheric rCGU (p=0.097), brainstem rCGU (p=0.06), and hemispheric rCBF (p=0.073) and to decrease cerebellar rCVR (p=0.091).

Linear regression analysis of hemispheric rCBF vs. PaCO₂ for the combined Groups 1 and 2 had a slope of 3.04 (n=9, r=0.74) and for the combined Groups 3 and 4 a slope of 3.47 (n=13, r=0.61). Neither the slopes nor the intercepts of the two regression lines were significantly different, and both lines correspond to a CO₂ reactivity of approximately 2%/mm Hg. Similarly, there was a good correlation between hemispheric rCVR and PaCO₂ for the combined Groups 1 and 2 and for the combined Groups 3 and 4. The slopes of the regression lines were nearly identical (−0.027 vs. −0.028), differing only in their intercepts (2.07 vs. 1.76).

Discussion

Our study demonstrates that nimodipine in a dose sufficient to prevent postischemic hypoperfusion does not prevent manipulation of rCBF by hyperventilation. We have previously demonstrated that the dosage employed in this study is capable of...
improving rCBF in rats after 10 minutes of forebrain ischemia, with a clinically acceptable effect on systemic arterial blood pressure.\(^1\)\(^6\) Significant hypotension produced by nimodipine in the setting of impaired cerebrovascular autoregulation would be counterproductive to any protective properties that the drug might confer. In our present study, nimodipine modestly reduced MABP by approximately 20% without concomitantly affecting rCBF. Although not statistically significant, there was a distinct trend for nimodipine to increase rCBF and decrease rCVR by approximately 20% and 30%, respectively, in all brain regions. The relatively high variance and small sample size contributed to our inability to demonstrate significant differences. In the cerebral hemispheres at least, it appears that rCBF was maintained by a reduction in rCVR. The calculation of rCVR deserves mention in that it was estimated by the ratio of MABP to CBF. Although cerebrovascular resistance is properly calculated by taking the ratio of cerebral perfusion pressure (MABP minus central venous pressure) to CBF, the ratio of MABP to CBF is a reasonable approximation in a group of animals without myocardial dysfunction and with similar intraoperative fluid management. Since nimodipine is a vasodilator and may decrease central venous pressure, rCVR may be slightly underestimated in Groups 2 and 4.

In general, there appears to be a considerable variation in the reported CBF response of normal brain to nimodipine.\(^2\)\(^6\)\(^9\)\(^18\)\(^19\) This may be attributable in part to varying anesthetic techniques, varying routes of administration, methodologic differences, and interspecies variation. Steen et al\(^2\) reported improved neurologic outcome after global ischemia in dogs using an intravenous infusion of 1 \(\mu g/\)kg/min. This dosage had little effect on baseline CBF measured globally before ischemia and improved postischemic hypoperfusion compared with controls. In unanesthetized rabbits, Haws et al\(^8\) used radiolabeled microspheres to study rCBF with several different intravenous doses of nimodipine. Both 0.1 and 1 \(\mu g/\)kg/min raised rCBF from control in all regions, including white matter, by approximately 50–100%. However, nimodipine given in a dose that did not change baseline rCBF significantly impaired the response of the pial circulation to changes in blood pressure and \(P_{CO_2}\) in anesthetized cats and impaired the rCBF response to changes in blood pressure in anesthetized monkeys.\(^19\) Harris et al\(^18\) studied the effect of nimodipine on rCBF in baboons under \(\alpha\)-chloralose anesthesia using the hydrogen clearance technique. In their closed-skull preparation, lingual artery infusion of 0.6 \(\mu g/\)kg/min nimodipine did not result in significant increases in rCBF although the autoregulatory response to hypotension was significantly impaired. Using quantitative autoradiography to measure local cerebral blood flow (ICBF), Mohamed et al\(^9\) reported that gray matter structures in the cerebral hemispheres and diencephalon gave the proportionately greatest increases in ICBF with an intravenous dose of 1 \(\mu g/\)kg/min nimodipine compared with doses of up to 4 \(\mu g/\)kg/min. They suggest that this lower dose may provide maximal cerebrovascular relaxation or that the effect of increases in drug concentration above 1 \(\mu g/\)kg/min are counteracted by concomitant systemic hypotension. They observed no increase in white matter ICBF at any dosage of nimodipine.

Between \(P_{CO_2}\) values of 20 and 80 mm Hg, there is an approximately 2% change in CBF per mm Hg change in \(P_{CO_2}\).\(^2\)\(^6\)\(^9\)\(^18\)\(^19\) In our study, both nimodipine- and vehicle-pretreated rats retained \(CO_2\) responsiveness to moderate hypocarbia of a magnitude employed clinically. The \(CO_2\) vs. rCBF regression lines had similar slopes in rats pretreated with vehicle or nimodipine, in contradistinction to other reports.\(^17\)\(^18\) In the baboon study of Harris et al\(^18\) an open-skull preparation was also evaluated in which the same dosage and route of administration of nimodipine significantly increased baseline rCBF by 24–26% after 15 and 45 minutes of drug infusion. In these baboons, the \(CO_2\) reactivity was severely impaired, being reduced to approximately 25% of control values. McCalden et al\(^7\) also reported nimodipine-induced changes in \(CO_2\) responsiveness. Hyperventilation from a \(P_{CO_2}\) of 38 to 28 mm Hg did not result in a significant change in CBF. In both of these studies, however, the degrees of hypocarbia were more modest than in our present study.

If \(P_{CO_2}\) is kept in the range 23–25 mm Hg, it is generally accepted that hyperventilation is a safe and effective means of inducing a global reduction in CBF and cerebral blood volume in patients with increased intracranial pressure, decreased intracranial compliance, and/or during neurosurgical procedures requiring brain relaxation for operative exposure. At lower \(P_{CO_2}\) levels, cerebral vasoconstriction and systemic alkalosis may result in cerebral ischemia and derangement of oxidative metabolism.\(^21\) As reviewed by Harp and Wollman,\(^2\) cerebral glucose uptake does not increase until \(P_{CO_2}\) is lowered to approximately 10 mm Hg with a concomitant fall in \(O_2\) consumption by 10%, indicating anaerobic glycolysis. Vigorous hyperventilation may induce a relative ischemia with a subsequent reduction in amounts of cellular ATP. This leads to stimulation of phosphofructokinase, the rate-controlling enzyme of anaerobic glycolysis, and results in enhanced glucose uptake and lactate production. In rats, nimodipine does not appear to affect cerebral glucose utilization in normal brain under normocapnic conditions.\(^6\) However, nimodipine affects cerebral metabolism in that it is capable of producing EEG changes and possesses moderate psychotropic properties in humans.\(^22\) Perhaps nimodipine lowers the sensitivity of the brain to the hypocarbia-induced increases in anaerobic metabolism. In this case, one would expect a simultaneous fall in oxygen consumption, which we did not measure. Harris et al\(^18\) demonstrated an increased rCBF threshold for extra-
cellular $K^+$ and $Ca^{2+}$ homeostasis during cerebral ischemia in nimodipine-treated animals; that is, animals treated with nimodipine did not tolerate reductions in CBF as well as control animals. These authors suggest that nimodipine may increase the susceptibility of cellular energy metabolism to ischemic damage. Our observed trend toward increases in rCGU in nimodipine-treated rats, especially those rendered hypocarbic, may reflect a similar lowering of the threshold for derangements in cellular energy metabolism during induced hypocarbia and should be investigated using different doses under the varying conditions employed clinically.

Another issue to be addressed is the significant increases in plasma glucose levels brought about by nimodipine in this study. This same effect has been described by others. Increased plasma glucose levels are associated with a worsened outcome after cerebral ischemia in several animal models, and circumstantial evidence for the same mechanism exists in humans. Furthermore, this mechanism may be operative even at relatively modest elevations of plasma glucose concentration, as we encountered in this study. A relative hyperglycemia may partially offset any cerebral protective effects induced by nimodipine.

Although controversial, nimodipine has been suggested as a means to prevent ischemic complications of vasospasm in patients with aneurysmal subarachnoid hemorrhage. Nimodipine pretreatment may be used in patients undergoing neurovascular procedures such as aneurysm clipping where the possibility exists for ischemia as a result of surgical interruption of cerebral end-arteries. Since nimodipine is a vasodilator, patients who might otherwise benefit from its cerebral protective properties might be placed at risk from deleterious increases in cerebral blood volume that may cause an increase in intracranial pressure and/or prevent adequate brain relaxation intraoperatively. Although nimodipine has been reported to interfere with the responsiveness of the cerebral circulation to hypocarbia, our study demonstrates the effectiveness of hyperventilation in reducing CBF after pretreatment with a dose that improves postischemic hypoperfusion. Although nimodipine shows promise as a pharmacologic means of protecting or resuscitating brain from ischemic insults, further work is needed to define the interaction of the drug with autoregulatory mechanisms and the pathophysiology of cerebral ischemic syndromes in commonly encountered clinical settings.

Acknowledgments

The authors wish to thank Juan Rodriguez, Daniel Battista, and Kenneth Josowitz, BA, for technical assistance; Linda Rolnitzy, BS, MPH, for statistical consultation; Octavio Morales, BS, and George Scheussler, PhD, for computer support; Joyce Ouchi for assistance in preparation of the manuscript; Isak Prohovnik, PhD, Amiram Barkai, PhD, A. Donald Finck, MD, and Edward D. Miller, MD, for helpful suggestions; and Miles Pharmaceuticals for supplying nimodipine.

References


KEY WORDS: calcium channel blockers • cerebral blood flow • autoregulation • rats
Effect of nimodipine on cerebral blood flow and metabolism in rats during hyperventilation.
W L Young and S Chien

Stroke. 1989;20:275-280
doi: 10.1161/01.STR.20.2.275

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/20/2/275

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/