Effect of nimodipine on intracellular brain pH, cortical blood flow, and EEG in experimental focal cerebral ischemia

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Intracellular brain pH, cortical blood flow, and electroencephalograms (EEG's) were recorded in severely and moderately ischemic regions in 10 control and 10 nimodipine-treated rabbits prior to and following major branch occlusion of the middle cerebral artery (MCA). Preocclusion cortical blood flow was 51 ml/100 gm/min and intracelluar brain pH was 7.01 in both the control and the treated animals. After MCA occlusion, the severely ischemic regions in the control group showed initial and 4-hour postocclusion flows of 12.7 and 5.2 ml/100 gm/min with a brain pH of 6.64 and 6.08, respectively. In animals given nimodipine after MCA occlusion, blood flow increased from 10.5 to 18.8 ml/100 gm/min, with an associated elevation in intracellular brain pH from 6.57 to 6.91. Comparable findings were observed in areas of moderate ischemia. Improvements in cortical blood flow, intracellular brain pH, and EEG attenuations produced by nimodipine were all statistically significant. Inspection of the cortex revealed reversal of cortical pallor and small-vessel spasm following treatment with nimodipine. It is hypothesized that nimodipine exerts its effects through reversal of ischemia-induced secondary vasoconstriction, and that this drug may be an important adjunctive treatment for patients with focal cerebral ischemia.

KEY WORDS
- calcium antagonist
- nimodipine
- brain pH
- cortical blood flow
- electroencephalography
- focal cerebral ischemia

Calcium antagonists may attenuate ischemic neuronal damage through two mechanisms: dilatation of cerebral vessels or prevention of excessive calcium influx into cytoplasmic and mitochondrial compartments. Calcium antagonists have been shown to cause vasodilatation in vitro to improve cerebral blood flow (CBF) in vivo in non-ischemic models, and to decrease the results of posts ischemic hypoperfusion in global ischemia models. The limited studies on the effects of calcium antagonists on CBF during focal cerebral ischemia are inconclusive. The demonstration of dihydropyridine binding sites in cerebral tissue and behavioral changes in animals given calcium antagonists suggests that these agents may act directly on cerebral tissue independent of their action on cerebral vasculature. The few experiments that assess the metabolic effects of calcium antagonists during ischemia yield contradictory results.

The rabbit has recently been shown as a model of focal cerebral ischemia in which branch occlusion of the middle cerebral artery (MCA) produced regions of both severe and moderate ischemia. These zones were assessed for 4 hours after MCA occlusion by measurement of intracellular brain pH, cortical blood flow, electroencephalography (EEG), and histology. By these parameters, severely ischemic regions were evolving infarcts, while the moderately ischemic regions were zones of ischemic penumbra.

The purpose of this current experiment was to evaluate the effects of the calcium antagonist nimodipine on CBF and neuronal metabolism in both severely and moderately ischemic focal regions by measurement of intracellular brain pH, cortical blood flow, and EEG. To approximate the clinical setting, nimodipine was given as an intravenous infusion after MCA occlusion.

Materials and Methods

Animal Preparation

Twenty-three New Zealand White rabbits, weighing between 3.5 and 4.5 kg each, were anesthetized with
ischemia, partially supplied by the division left intact?'

This border zone of moderate ischemia was located

Occlusion of one of these divisions will yield three

zones of flow: 1) a zone of severe ischemia; 2) a zone

in intracellular brain pH.

vation. Confirmation of suspected border zones was by

color changes of the cortex under microscopic obser-

approximately halfway between the occluded and pat-

ent branches and could be visually identified by subtle

infraorbital craniectomy similar to that used in the cat.52

The skin, subcutaneous tissue, and muscle were dissected from

the supraorbital ridge and parietal bone. Enucleation of the orbit facilitated removal of the supraorbital ridge with rongeurs. The craniectomy involved the parietal bone medial to the sagittal sinus, the temporal bone to the zygomatic arch, and the frontal bone down to the optic canal. The craniectomy was performed with a high-speed air drill and with the aid of an Olympus operating microscope so that no pressure was exerted against the underlying cortex. The dura was removed, and a thin sheet of plastic film (Saran Wrap) was placed on the cerebrum with irrigation to keep the brain moist and prevent surface oxygenation. The total blood loss for the operative procedure did not exceed 5 cc.

The MCA was exposed through a retro-orbital cran- 

The MCA was identified at the anterior-inferior mar-

of the craniectomy between the frontal and tem-

ral lobes. As shown in Fig. 1, the MCA gives off an inferior temporal branch, and then ascends over the parietal cortex, separating into two main divisions. Occlusion of one of these divisions will yield three zones of flow: 1) a zone of severe ischemia; 2) a zone of normal flow supplied by the division left intact; and 3) between these two zones, a region of moderate ischemia, partially supplied by the division left intact.31

This border zone of moderate ischemia was located approximately halfway between the occluded and patient branches and could be visually identified by subtle color changes of the cortex under microscopic observation. Confirmation of suspected border zones was by measurement of a 60% reduction in cortical blood flow as compared to preocclusion flows, with only minimal changes in the intracellular brain pH. Severely ischemic regions had an 80% reduction in cortical blood flow as compared to preocclusion flows, with a dramatic drop in intracellular brain pH.

Data Recording

After the operative exposure, the animals were moved to an intravital microscope stage. Measurements of two

separate sites on the suprasylvian gyrus were taken at a 

PaCO₂ of 40, 20, 60, and 40 torr by altering the amount of inspired CO₂ and the respiratory rate. A normal

PaCO₂-cortical blood flow response curve insured that the brain had not been injured and was metabolically normal.29

Repeated measurement of a normal intracellular brain pH during this PaCO₂ response curve was further evidence of a physiologically intact cerebrum. After the PaCO₂ response curve, two measurements of a normal cortical blood flow and intracellular brain pH at a PaCO₂ of 40 torr were made prior to occlusion of the MCA with bipolar cautery.

Twenty rabbits were divided equally into two groups: a control group infused with the solvent polyethylene glycol, and a treatment group that received an infusion of nifedipine† (0.5 μg/kg/min) after MCA occlusion for the duration of the experiment. In each rabbit, regions of both severe and moderate ischemia were identified immediately after MCA occlusion. The x and y coordinates were recorded so these sites could be evaluated hourly. Intracellular brain pH and cortical blood flow were measured at all sites for 4 hours after MCA occlusion.

Three rabbits underwent a sham craniectomy without MCA occlusion. These animals served as time controls, and, after a normal PaCO₂ response curve was recorded, intracellular brain pH and cortical blood flow were assessed during a 4-hour testing period.

Electroencephalography was performed in all ani-

† Nimodipine supplied by Miles Laboratories, Elkhart, Indiana.
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the craniectomy. Arterial blood gases were measured hourly and blood pressure was recorded continuously. The animals were sacrificed with a lethal injection of potassium chloride.

Intracellular Brain pH-Cortical Blood Flow Measurements

The use of umbelliferone as a noninvasive in vivo technique for measuring intracellular brain pH and cortical blood flow has been described before. Umbelliferone is a lipid-soluble, pH-sensitive fluorescent indicator that freely diffuses across the blood-brain barrier. It is nontoxic and isolated to the intracellular compartment. Its ionic and molecular forms are fluorophors which have different fluorescent characteristics. The ratio of the ionic and molecular forms at 370-nm and 340-nm excitation, respectively, is pH-sensitive. It is possible to create a nomogram relating pH to the ratio of the 450-nm fluorescent curves of the indicator at 370-nm and 340-nm excitation.

Cortical blood flow was determined by the washout curve of the molecular form of umbelliferone over a 60-second time period, beginning after the arterial spike using the 1-minute initial slope index. Umbelliferone clearance is a measure of perfusion through the intracellular compartment and in effect demonstrates focal cortical blood flow. It is thought to be dependent on diffusion from capillary to glial cell to neuron. Since this technique measures flow only in the outer 1 mm of cortex, it eliminates the "look through" phenomenon obtained with xenon-133. Furthermore, it is noninvasive, unlike the hydrogen clearance technique, which requires the placement of microelectrodes.

pH Instrumentation

The microspectrofluorometer used in this experiment was equipped with the following; optics for bright field illumination which permitted low-intensity excitation energy; high-efficiency recording from an 80-μm avascular area of cortex; a high-speed filter wheel with four interference filters to allow synchronization of the emission recording system at 450 nm with the 370-nm and 340-nm excitation bands; and an emission recording system consisting of a high-sensitivity thermoelectrically cooled photomultiplier tube attached to a high-efficiency grating monochromator. Fluorescent emission signals were amplified by a cascaded electrometer amplifier and directed into a photo-demodulator synchronized with the filter wheel. The fluorescence washout curves were recorded on a dual strip-chart recorder. The instrumentation is fully described in previous reports.

Drug Preparation

Nimodipine is light-sensitive; therefore, all syringes and catheters used for mixing and infusion were covered in aluminum foil. Nimodipine was supplied at a concentration of 1 mg/5 ml of a solvent solution of polyethylene glycol. Aliquots of this solution were mixed in a corresponding volume of 0.9% NaCl to give a final volume of 20 cc at a pH of 5.55 to be infused over 4 hours at 0.5 μg/kg/min.

Statistical Analysis

For each hourly measurement, results at the severely ischemic sites in the 10 control animals were compared with the results at the severely ischemic sites in the 10 animals treated with nimodipine. Likewise, results at the moderately ischemic sites in the 10 control animals were compared to results at the moderately ischemic sites in the 10 nimodipine-treated animals. The statistical significance was calculated by a paired t-test for each hourly measurement. The deviation from mean value is expressed as standard error.

Results

Stability of the Preparation

In the three rabbits without MCA occlusion, there was no significant decline (paired t-test) in either intracellular brain pH or cortical blood flow for 4 hours after a normal PaCO2 response curve had been obtained. Intracellular brain pH was 6.98 ± 0.02 initially and 6.98 ± 0.03 at 4 hours. Cortical blood flow was 48.1 ± 3.7 ml/100 gm/min initially and 43.0 ± 4.0 ml/100 gm/min at 4 hours.

Intracellular Brain pH-Cortical Blood Flow

Severe Ischemia. Measurements of intracellular brain pH and cortical blood flow at severely ischemic sites are shown in Table 1 and Fig. 2. Pooling data from all 20 animals with MCA occlusion, preocclusion cortical blood flow was 51.8 ± 4.6 ml/100 gm/min and intracellular brain pH was 7.01 ± 0.04. In the 10 control animals, the cortical blood flow at the severely ischemic sites was 12.7 ± 2.3 ml/100 gm/min immediately after occlusion. Within 10 minutes, intracellular brain pH

<table>
<thead>
<tr>
<th>Time of Test (min)</th>
<th>Intracellular Brain pH</th>
<th>Cortical Blood Flow (ml/100 gm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>preocclusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-15</td>
<td>7.04 ± 0.3</td>
<td>6.98 ± 0.03</td>
</tr>
<tr>
<td>0</td>
<td>7.03 ± 0.05</td>
<td>7.00 ± 0.04</td>
</tr>
<tr>
<td>postocclusion</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>6.64 ± 0.06</td>
<td>6.57 ± 0.03</td>
</tr>
<tr>
<td>60</td>
<td>6.41 ± 0.08</td>
<td>6.69 ± 0.04</td>
</tr>
<tr>
<td>120</td>
<td>6.14 ± 0.14</td>
<td>6.73 ± 0.04</td>
</tr>
<tr>
<td>180</td>
<td>6.22 ± 0.10</td>
<td>6.85 ± 0.05</td>
</tr>
<tr>
<td>240</td>
<td>6.08 ± 0.15</td>
<td>6.91 ± 0.06</td>
</tr>
</tbody>
</table>

* Each group included 10 rabbits. Values are mean ± standard error.
was 6.64 ± 0.06. These sites continued to demonstrate reductions in both intracellular brain pH and cortical blood flow over the ensuing 4 hours. Four hours after occlusion, the intracellular brain pH was 6.08 ± 0.15 and blood flow was 5.2 ± 1.5 ml/100 gm/min. Compared to the preocclusion values, the reduction in both cortical blood flow and intracellular brain pH at each measured interval was statistically significant (p < 0.001).

In the 10 nimodipine-treated animals, cortical blood flow was 10.5 ± 1.3 ml/100 gm/min and intracellular brain pH was 6.57 ± 0.03 at the severe ischemia sites immediately after occlusion. The difference in these values as compared to the control results was not significant. One hour after the addition of nimodipine, cortical blood flow was 24.2 ± 3.3 ml/100 gm/min as compared to 8.0 ± 1.6 ml/100 gm/min in the control animals, and at 4 hours it was 18.8 ± 3.0 ml/100 gm/min. This 90% to 130% increase in tissue perfusion was statistically significant at each hourly measurement as compared to the controls (p < 0.001). There was also a reversal of the decline in pH observed in control animals, with eventual normalization of intracellular pH at the 4th hour. Four hours after occlusion, intracellular brain pH was 6.91 ± 0.06 in the treated animals as compared to 6.08 ± 0.15 in control rabbits. This improvement in intracellular brain pH was statistically significant at each hourly interval (p < 0.001).

There were no significant differences in systemic parameters between the control and treated groups (Table 2). The dosage of nimodipine used in this experiment did not cause a decline in systolic blood pressure and did not alter arterial pH.
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TABLE 2

Measurements of systemic parameters in control and nimodipine-treated groups*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Animal Group</th>
<th>Preocclusion Values</th>
<th>Postocclusion Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>15 Min</td>
<td>60 Min</td>
</tr>
<tr>
<td>MABP (mm Hg)</td>
<td>control</td>
<td>122.8 + 1.0</td>
<td>121.5 + 6.0</td>
</tr>
<tr>
<td></td>
<td>treated</td>
<td>110.1 + 5.4</td>
<td>112.5 + 2.8</td>
</tr>
<tr>
<td>PaCO2 (torr)</td>
<td>control</td>
<td>40.5 + 1.0</td>
<td>39.0 + 1.1</td>
</tr>
<tr>
<td></td>
<td>treated</td>
<td>37.8 + 0.6</td>
<td>39.3 + 0.7</td>
</tr>
<tr>
<td>PaO2 (torr)</td>
<td>control</td>
<td>172.5 + 8.1</td>
<td>168.5 + 9.6</td>
</tr>
<tr>
<td></td>
<td>treated</td>
<td>157.7 + 7.2</td>
<td>151.4 + 8.4</td>
</tr>
<tr>
<td>pH</td>
<td>control</td>
<td>7.298 + 0.015</td>
<td>7.284 + 0.022</td>
</tr>
<tr>
<td></td>
<td>treated</td>
<td>7.292 + 0.026</td>
<td>7.274 + 0.015</td>
</tr>
</tbody>
</table>

* Values are mean ± standard error. MABP = mean arterial blood pressure.

TABLE 3

Measurements at moderately ischemic sites in animals with and without treatment*

<table>
<thead>
<tr>
<th>Time of Test (min)</th>
<th>Intracellular Brain pH</th>
<th>Cortical Blood Flow (ml/100 gm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>preocclusion</td>
<td>7.04 ± 0.03</td>
<td>6.98 ± 0.03</td>
</tr>
<tr>
<td>0</td>
<td>7.03 ± 0.03</td>
<td>7.00 ± 0.04</td>
</tr>
<tr>
<td>postocclusion</td>
<td>6.92 ± 0.06</td>
<td>6.84 ± 0.06</td>
</tr>
<tr>
<td>15</td>
<td>6.85 ± 0.06</td>
<td>6.88 ± 0.06</td>
</tr>
<tr>
<td>60</td>
<td>6.81 ± 0.06</td>
<td>6.93 ± 0.04</td>
</tr>
<tr>
<td>120</td>
<td>6.86 ± 0.06</td>
<td>6.95 ± 0.04</td>
</tr>
<tr>
<td>180</td>
<td>6.74 ± 0.09</td>
<td>7.07 ± 0.05</td>
</tr>
<tr>
<td>240</td>
<td>6.74 ± 0.09</td>
<td>7.07 ± 0.05</td>
</tr>
</tbody>
</table>

* Each group included 10 rabbits. Values are mean ± standard error.

**Moderate Ischemia.** Measurements of intracellular brain pH and cortical blood flow at moderately ischemic sites are shown in Table 3 and Fig. 3. In the 10 control animals, initial postocclusion cortical blood flow was 20.0 ± 2.0 ml/100 gm/min or approximately 40% of preocclusion flows. This was associated with an intracellular brain pH of 6.92 ± 0.06. While the reduction in flow was significant (p < 0.001), the mild drop in pH was not. For the first 3 hours, blood flow was stable, but then fell significantly at the 4th hour to 14.5 ± 3.6 ml/100 gm/min (p < 0.05). Intracellular brain pH slowly worsened at each hourly measurement and at 4 hours was 6.74 ± 0.09 (p < 0.01).

At the moderately ischemic sites in the 10 nimodipine-treated animals, initial postocclusion blood flow was 24.8 ± 1.0 ml/100 gm/min, approximately a 40% reduction of preocclusion flows. This was associated with an intracellular brain pH of 6.84 ± 0.06, slightly lower than the control value of 6.92 ± 0.06. These two parameters in nimodipine-treated animals compared well with the results from the control group. In the nimodipine-infusion group, blood flow increased to 38.0 ± 3.3 ml/100 gm/min at the 1st hour (versus 20.4 ± 2.0 ml/100 gm/min in the control animals), and was 29.7 ± 3.2 ml/100 gm/min at 4 hours. This 20% to 53% improvement in cortical blood flow was maintained for the duration of the experiment and was statistically significant compared to the controls (p < 0.001). The nimodipine-treated animals demonstrated maintenance of a normal intracellular brain pH compared to the downward trend observed in the control group. Four hours postocclusion, intracellular brain pH was 7.07 ± 0.05 in the treated animals versus 6.74 ± 0.09 in the control animals (p < 0.001).

**Electroencephalographic Correlates**

The baseline EEG of the rabbit cortex consists of a 6- to 10-Hz rhythm (Fig. 4). Since the EEG leads were located at the margins of the craniectomy, they summed the electrical activity across both normal and ischemic cortex. In the 10 control animals, eight demonstrated loss of amplitude or a slow 1- to 2-Hz rhythm immediately after MCA occlusion (Fig. 4 upper). In these eight rabbits, the EEG continued to demonstrate progressive loss of amplitude and frequency over the ensuing 4 hours. None of these animals had improvement of electrical activity.

In the 10 nimodipine-treated animals, immediately after occlusion seven demonstrated initial EEG changes consisting of either a loss of amplitude or a slow 1- to 2-Hz delta waves. With the addition of nimodipine, five of these seven animals had improvement in cortical electrical activity (p < 0.05). Two patterns of improvement were observed. Three had normalization to the 6- to 10-Hz baseline rhythm (Fig. 4 center) and two improved from a 1- to 2-Hz to a 4- to 6-Hz rhythm (Fig. 4 lower).

**Discussion**

Previous evaluation of this model has shown that severely ischemic sites represent developing infarcts while moderately ischemic sites are zones of penumbra. In this current experiment, nimodipine at 0.5 μg/kg/min, given as a continuous intravenous infusion after MCA occlusion, improved both the electrical and metabolic function of ischemic neurons. This improvement in metabolic function as assessed by intracellular
brain pH occurred in both severely and moderately ischemic regions. Concomitant with this normalization in brain pH was an increase in cortical blood flow. Within the limits of this experiment, it is not possible to determine if improved intracellular brain pH was the result of inhibition of calcium flux into the neuron or was secondary to the increase in blood flow alone. These two potential mechanisms will be addressed separately in terms of this current experiment.

Effects of Nimodipine on Neuronal Metabolism

One of the precipitating pathways of irreversible neuronal damage is thought to be an increase in intracellular calcium.9,40-42 A failure of energy-dependent Na+\(^{+}\)-K+ transport leading to increased extracellular K+ will cause depolarization of the neuronal membrane. This results in opening of voltage-sensitive calcium channels with a rise in intracellular free calcium.20,59 This influx of calcium is enhanced by failure of the adenosine triphosphate (ATP)-dependent Na+\(^{+}\)-Ca+ antiport system; failure of the ATP-dependent sequestration of Ca+ by the endoplasmic reticulum; and the electrophoretic accumulation of calcium by the mitochondria, which will uncouple oxidative phosphorylation.12,33,35 This uncountered rise in intracellular calcium is thought to activate phospholipase A and C, which will attack membrane phospholipids resulting in the production of free fatty acids.41,42 This loss of membrane phospholipids will increase the permeability of neuronal and mitochondrial membranes, which will further alter calcium homeostasis with additional detrimental effects on oxidative phosphorylation. The accumulation of free fatty acids will cause deleterious effects on neuronal membranes, both directly and from their metabolites. In incomplete ischemia (as in this experiment), fatty acids, especially arachidonic acid, may be oxidized along the cyclo-oxygenase and lipoxygenase pathways. The end result would be the accumulation of prostaglandins, thromboxanes, leukotrienes, and possibly free radicals.8,42,60 Thromboxane A2 is a potent vasoconstrictor,25 leukotrienes alter membrane permeability and cause vasoconstriction,58 and free radicals if present would attack the membrane.8 Furthermore, intracellular acidosis could cause neuronal death by the denaturing of proteins with loss of normal metabolic function and by enhancing glial edema with further compromise of nutrient delivery.36 If calcium antagonists inhibit voltage-sensitive channels, prevention of calcium flux into the cell would attenuate the above cascade of events.

Studies on the direct effects of calcium antagonists and neuronal metabolism during ischemia are limited and contradictory.5,14,15,19,23,33,45 In the study reported here, repeated in vivo measurement of intracellular brain pH during ischemia assessed both acidosis and energy production. The acidosis observed in the first 5 to 10 minutes was primarily lactic acid production from anaerobic metabolism,50 whereas the acidosis in the ensuing 4 hours was probably largely due to the accumulation of free fatty acids, a reflection of energy failure with activation of the calcium-mediated mechanisms discussed above.28,50,59 In regions of both severe and moderate ischemia, nimodipine significantly improved intracellular brain pH. The reversal of EEG's in five of seven animals with postocclusion attenuation was further evidence of improved metabolic function of neurons subjected to ischemia. Both the restoration of pH
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FIG. 5. Experimental changes in the cortical vasculature of a nimodipine-treated rabbit. A: Conducting vessels (arrows) prior to middle cerebral artery occlusion. B: Fifteen minutes after occlusion, secondary vasospasm is present evidenced by cortical pallor and by vasoconstriction of both conducting vessels (large arrows) and penetrating arterioles (small arrows). The draining veins are also constricted. C: Fifteen minutes after nimodipine infusion there is partial reversal of the secondary vasospasm. Cortical pallor is improved, and the conducting vessels (arrows) are dilated. D: Thirty minutes after the nimodipine infusion was begun, the penetrating arterioles (small arrows), the conducting vessels (large arrows), and the draining veins are all dilated.

and improvement in EEG may have been secondary to inhibition of calcium entry into the cytoplasm or mitochondria. If true, then the mechanism of phospholipase A and C activation with the production of free fatty acids would not have occurred. Intracellular pH would improve over the ensuing 4 hours, since this would reflect predominantly free fatty acid concentrations. Furthermore, prevention of calcium flux into the mitochondria would have diminished the uncoupling of oxidative phosphorylation and improved the overall energy level. Alternatively, the improvement in intracellular brain pH may have been due solely to the increase in cortical blood flow above the threshold of ionic failure.

Effects of Nimodipine on Cerebral Blood Flow

Compared to the control group, nimodipine caused a significant increase in cortical blood flow in severely (90% to 130%) and moderately ischemic regions (20% to 53%). The dose of nimodipine chosen was based on work by Haws, et al.,17 who demonstrated in the rabbit that 0.5 µg/kg/min of nimodipine gave a maximum increase in CBF without lowering arterial blood pressure. This improvement in CBF is consistent with both in vitro and in vivo experiments.

Calcium antagonists have been shown in vitro to cause vasodilatation on vessels preconstricted with a variety of agents including K+, prostaglandin F2α, soro-
ton, noradrenaline, phenylephrine, plasma, and blood. This response has been observed in arteries obtained from man, dogs, cats, and rabbits.\(^{1,2,17,18,22,26,30,55,56}\) Most in vivo nonischemic models demonstrate an increase in CBF with calcium antagonists\(^{14,15,25,26,32,39,53}\) and support in vitro studies.

Experiments on the effects of calcium antagonists on CBF and ischemia can be divided into models of global and focal ischemia. Experiments on total brain ischemia convincingly show that calcium antagonists attenuate postischemia hypoperfusion.\(^{21,25,44,45}\) The limited studies on calcium antagonists in focal ischemia are contradictory. Harris, et al.\(^{15}\) demonstrated an increase in CBF after MCA occlusion in the baboon. However, the thresholds for edema and disturbances in ion homeostasis were increased. Roy, et al.,\(^{37}\) found a decrease in blood flow after MCA occlusion with increased brain water content along with evidence of a steal phenomenon in cats given verapamil. However, they also demonstrated that diltiazem improved CBF in regions of moderate ischemia without enhancing cerebral edema. Smith, et al.,\(^{43}\) found evidence of a steal phenomenon with regions of both hyperemia and hypoperfusion in rats pretreated with nimodipine prior to forebrain ischemia. Recovery of EEG recordings or somatosensory evoked potentials was not enhanced. The only study on calcium antagonists in man for treatment of acute focal ischemia was by Gelmers.\(^{11}\) Five patients received 15 \(\mu g/kg\) and five received 30 \(\mu g/kg\) of nimodipine given as an intracarotid infusion over 10 minutes. The CBF (as measured by inhalation of xenon-133) demonstrated a dose-dependent increase in blood flow. In three patients, an inverse steal phenomenon was suggested by an increase in flow to the region of focal ischemia as compared to the ipsilateral hemisphere.

In this current experiment, nimodipine given after MCA occlusion improved cortical blood flow in both severely and moderately ischemic focal regions. Although the number of sites measured in each animal was limited, there was no evidence of a steal phenomenon between adjacent zones of severe and moderate ischemia. The mechanism of vasodilatation may have been inhibition of secondary vasospasm, the immediate focal constriction of conducting vessels known to occur in core regions of ischemia distinct from vasospasm due to subarachnoid hemorrhage.\(^{49,54,57}\) This focal constriction evolves over a temporal profile that can be related to ischemic changes of the underlying cortex. The mechanism of secondary vasospasm is unclear, but has been postulated to be a result of an increase in extracellular \(K^+\), which would cause muscle contraction or of an influx of \(Ca^+\) into vascular smooth muscle. An increase in membrane permeability to \(Ca^+\) could be due to opening of \(K^+\)-depolarized calcium channels, interaction of surface receptors with an extracellular messenger like serotonin or norepinephrine,\(^{49}\) or by activation of \(3',5'\)-guanosine monophosphate (cyclic GMP).\(^{27}\) Brandt, et al.,\(^{7}\) showed that the topical application of nifedipine (20 to 40 \(\mu g/ml\)) caused a dose-dependent dilatation of arterioles and venules during focal ischemia, consistent with the hypothesis that secondary vasospasm is \(Ca^+\)-dependent. Those results support the current proposal that nimodipine antagonized secondary vasospasm resulting in an increase in cortical blood flow. As shown in Fig. 5, this mechanism was documented visually.

This increase in cortical blood flow may have been the cause of improvement in both intracellular brain pH and EEG. Two thresholds of cerebral ischemia have been documented as CBF is reduced: the threshold of electrical failure at 18 to 15 ml/100 gm/min\(^{5,51}\) and the threshold of ionic failure occurring at approximately 10 ml/100 gm/min.\(^{5,16}\) Ionic failure probably closely approximates irreversible neuronal death. Based on these current experiments, a threshold of pH failure can be postulated to occur at approximately 12 ml/100 gm/min as measured by umbiliforone clearance under light halothane anesthesia.\(^{33}\) Not surprisingly, this threshold of pH failure is essentially that of ionic failure. Nimodipine elevated cortical blood flows above the levels of electrical failure and of ionic or pH failure. This improvement in flow would explain the restoration of both intracellular brain pH and EEG independent of any possible effects of nimodipine on calcium flux into the neuron.

Acknowledgments

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