Calcium Channel Blockers Correct Acidosis in Ischemic Rat Brain Without Altering Cerebral Blood Flow

Leo Berger, MD, and Antoine M. Hakim, MD, PhD

We compared the effects of intravenous infusions of 40 μg/kg/min verapamil (n = 5), 0.5 μg/kg/min nimodipine (n = 5), and 5 ng/kg/min prostacyclin (n = 6) and no treatment (n = 6) on local cerebral pH and local cerebral blood flow in middle cerebral artery-occluded rats 90 minutes after the ischemic insult. Local cerebral pH and local cerebral blood flow were determined simultaneously by a double-label autoradiographic technique. The infusions were started 15 minutes after completion of the occlusion and ended at decapitation 90 minutes after completion of the occlusion. Cortical pH for four regions in the ischemic middle cerebral artery territory of rats receiving verapamil or nimodipine was normalized (mean±SEM 6.90 ±0.02 and 7.01 ±0.01, respectively, for the parietal, sensorimotor, frontal, and auditory cortexes), while mean±SEM pH in rats receiving prostacyclin was 6.79 ±0.01; in untreated rats, mean±SEM pH in the same brain regions was 6.72 ±0.01. Local cerebral pH in the verapamil- or nimodipine-treated rats was thus significantly different from that in untreated rats (p<0.05). Local cerebral blood flow in treated rats was not different from that in untreated ones. Our findings suggest that calcium channel blockers correct ischemic cerebral acidosis by metabolic mechanisms rather than by changes in blood flow. (Stroke 1988;19:1257–1261)

Published reports have shown that calcium channel blockers attenuate postischemic acidosis, but different mechanisms have been invoked to explain this effect. Some studies have stressed the vasodilating properties of calcium channel blockers on the cerebral vasculature when reporting increased local cerebral blood flow (LCBF) and increased local cerebral pH (LCpH)1 or improved neurologic outcome.1–3 We have reported that 4 hours after middle cerebral artery (MCA) occlusion, rats receiving calcium channel blockers show no significant changes in LCBF despite improved LCpH. This suggested to us that the calcium channel blocker was acting through a metabolic effect,4 but the possibility that LCBF increased substantially during the 4 hours between occlusion and our determinations, resulting in a washout of acids, led us to determine LCBF and LCpH in rats 90 minutes after MCA occlusion.

Materials and Methods

Male Sprague-Dawley rats weighing 250 g were fed a regular laboratory diet (Ralston Purina Inc., Richmond, Virginia) but were fasted overnight prior to the experiment. On the day of the experiment, each rat was anesthetized with 1.5% halothane and polyethylene catheters were placed in one femoral artery and both femoral veins. The left MCA was then occluded using the method of Tamura et al5; the entire procedure usually required 75 minutes. Completeness of the occlusion was verified intraoperatively by lack of passage of 0.5 ml Evans blue injected intravenously. Starting 15 minutes after completion of the occlusion and until decapitation 75 minutes later, one of the following agents was infused through the femoral vein:

Verapamil. From Knoll Pharmaceuticals, Markham, Canada; 2.5 mg/ml in isotonic aqueous solution, infused to deliver 40 μg/kg/min in five rats.

Nimodipine. From Miles Laboratories, Rexdale, Canada; 67 μg/ml of carrier (200 g 96% ethanol, 170 g polyethylene glycol 400, 2 g sodium citrate, and 0.5 g citric acid), infused to deliver 0.5 μg/kg/min in five rats. The volumetric rate of infusion was approximately 2 μl/min. The syringe and catheter
were covered completely with aluminum foil to counteract sensitivity of nimodipine to light. No group was treated with only nimodipine carrier as previous experiments had shown that LCpH and LCBF in carrier-treated rats were statistically indistinguishable from those in untreated controls.4

Prostacyclin. From The Upjohn Co., Kalamazoo, Michigan; 0.5 mg in 50 ml glycine buffer. A 1:6 dilution of this prostacyclin-glycine solution in saline was prepared and infused within 12 hours at 5 ng/kg/min in six rats. The volume infused was 0.875 μl/min.

No drug. Six untreated rats underwent the same procedure but were not infused with any drugs.

Sixty minutes after completion of the occlusion, the procedure for the simultaneous autoradiographic measurement of LCpH and LCBF6 was started. Briefly, once occlusion was completed and the wound was closed, exposure to the anesthetic was stopped and the rat was immobilized below the waist by a plaster cast. Three hours after completion of the occlusion, 85 μCi of [14C]dimethylxaloxidinedione ([14C]DMO) (specific activity 55 mCi/mmol; Amersham Corp., Oakville, Canada) dissolved in saline was injected intravenously at 100 μCi/ml. Blood samples for arterial blood gas measurement were taken 30, 45, and 55 minutes after [14C]DMO injection, and blood samples for [14C]DMO concentration measurement were drawn at 30, 55, 56, and 60 minutes. Other physiologic measurements, including blood glucose concentration, hematocrit, blood pressure, and temperature, were repeated twice during the study. Sixty minutes after [14C]DMO injection, 30 μCi of [14C]idoantipyrine ([14C]IAP) (specific activity 1.47 mCi/mmol; Amersham) in 1.8 ml of saline was injected intravenously. The injection protocol and the collection of samples conform to the method of Sakurada et al.7

The rat was decapitated 1 minute later; the brain was removed and immediately frozen in Freon 12 chilled to −55°C with liquid nitrogen, mounted, coated with embedding matrix, and cut into consecutive 20-μm sections using a cryostat at −22°C (American Optical Co., Buffalo, New York). Approximately 80 sections distributed across the brain were collected on glass coverslips, dried at room temperature, and subsequently exposed to Kodak SB-5 x-ray film (Rochester, New York) for 3 days. For each section taken for autoradiography, an adjacent section was submitted for histologic examination (see below). Calibrated [14C]methylmethacrylate standards (New England Nuclear, Boston, Massachusetts) were included in every autoradiography.

Following this exposure, which yielded the first film containing both [14C]DMO and [14C]IAP, the brain sections were exposed in a fume hood (temperature 28°C, air flow 83 ml/min) for 10 days, resulting in total sublimation of [14C]DMO. There was no loss of [14C]DMO while the brain sections were being exposed to the first film.8 The sections were then exposed as above for an additional 3 days, resulting in a second film containing only [14C]IAP. Autoradiographic concentration of [14C]DMO was then calculated by subtraction.

 Autoradiographic concentration was measured using a densitometer (model 52, Photovolt Co., New York, New York) equipped with a 0.1-mm aperture. Each brain region was identified by comparing its location with that in the atlas of König and Klippel.8 Once identified, the autoradiographic concentration for a brain region was read at least once on at least five different sections. The same sections were used to read any one brain region in both films. Control sections from rats receiving similar quantities of only [14C]DMO or [14C]IAP were included with every experiment to monitor total loss of [14C]DMO and absence of any effect on concentration of [14C]IAP.

LCpH was calculated from the measured activity of [14C]DMO in plasma ([DMO]), the plasma pH (pH), and the autoradiographic concentration of [14C]DMO in the tissue ([DMO]). [DMO] and pH were the means of the last three readings obtained. Using the equation [DMO]/[DMO] = (10^pH−6.13 +1)/(10^pH−6.13 +1), regional tissue pH (pH), a weighted average of intracellular and extracellular pH, was then calculated.9,10

Because [DMO] is very stable, declining at approximately 6.8%/hr, plasma [14C]IAP concentrations were calculated by subtracting the last [DMO] value from the total 14C activity in plasma. Plasma [14C]IAP concentrations were then used with the regional [14C]IAP concentration to calculate LCBF using the method of Sakurada et al.7

For histologic studies, the sections were collected on glass coverslips and soaked for at least 1 hour in a fresh solution of 25 ml of 0.4 M sodium cacodylate, 4 ml of 50% glutaraldehyde, and 70 ml of distilled water. The sections were then transferred into cacodylate buffer until staining with cresyl violet. The stained sections showed areas of decreased dye uptake, which on histologic examination demonstrated morphologic changes consistent with ischemic cell damage (hyperchromatic cytoplasmic clumping with irregular nuclear and cytoplasmic membranes). For each experiment, the section with the largest area of decreased staining was chosen visually for quantification. A photographic print was then produced and digitized (Digitplan; Zeiss Inc., Thornwood, New York). The area of infarction was computed as percentage of the cross-sectional area of the entire section.

Each brain region in both the ipsilateral and contralateral hemispheres was submitted to one-way analysis of variance for LCpH and LCBF in the verapamil, prostacyclin, and control groups.

To determine the differences among group means, a modified t test11 was applied; p<0.05 was declared significant.

Results

The mean ± SEM venous glucose concentrations, arterial blood gases, arterial blood pressure, hemat-
TABLE 1. Physiological Parameters in Rats Subjected to Middle Cerebral Artery Occlusion

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Treatment group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Verapamil (n = 5)</td>
</tr>
<tr>
<td>pH</td>
<td>7.35 ± 0.01</td>
</tr>
<tr>
<td>PacO₂ (mm Hg)</td>
<td>43.4 ± 1.3</td>
</tr>
<tr>
<td>PaO₂ (mm Hg)</td>
<td>116.6 ± 1.7</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>10.6 ± 0.8</td>
</tr>
<tr>
<td>Temperature (° C)</td>
<td>36.0 ± 0.3</td>
</tr>
<tr>
<td>Mean arterial blood pressure (mm Hg)</td>
<td>95.8 ± 2.5</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.52 ± 0.01</td>
</tr>
</tbody>
</table>

Values are mean±SEM. *Significantly different from untreated control at p<0.05.

crit, and body temperature in each group of rats are shown in Table 1. The only significant difference was the lower PaO₂ in the nimodipine group compared with untreated control rats.

Figure 1 shows LCpH in several ipsilateral brain regions in the four groups of rats 90 minutes after MCA occlusion. Those regions indicated by an asterisk had a LCpH significantly higher than that in the untreated control group. Verapamil and nimodipine corrected the LCpH in the visual cortex and in all four ischemic cortical regions supplied by the MCA (parietal, sensorimotor, frontal, and auditory cortex) while prostacyclin improved LCpH significantly in only one of the five ischemic regions (auditory cortex).

Figure 2 shows LCBF 90 minutes after MCA occlusion in the same regions and groups as in Figure 1. All ipsilateral cortical regions in the MCA vascular territory had lower LCBF than those in the contralateral hemisphere, and the groups were indistinguishable.

The mean cross-sectional areas of infarction for the verapamil group was 9.0 ± 2.1%, for the nimodipine group 5.3 ± 0.9%, for the prostacyclin group 8.6 ± 1.5%, and for the untreated control group 7.7 ± 1.2%. Only the nimodipine group had a significantly different (smaller) infarct area than control.

Discussion

There is considerable controversy in the literature about the effects of calcium channel blockers in ischemic cerebral tissue. Most studies have reported increased LCBF in ischemic regions of the brain in animals pretreated with calcium channel blockers such as nimodipine.1,2 In animals treated only after occlusion, however, some reports1,2 have stated that LCBF increased while others12,13 found LCBF unchanged. Steen et al,2,14 while noting increased CBF in animals treated with nimodipine before or after ischemia found improved neurologic outcome in pretreated but no change in posttreated animals.

We have reported that rats treated immediately after MCA occlusion with calcium channel blockers show no significant change in LCBF 4 hours later despite improved LCpH.4 This suggested to us that the calcium channel blocker improved pH through a metabolic effect, but the possibility that CBF increased substantially in the 4 hours between occlusion and our measurements, resulting in a washout of acids, led us to measure LCBF and LCpH earlier after occlusion.

We used a double-label autoradiographic technique that allows simultaneous measurement of...
Our results show that 90 minutes after the onset of focal cerebral ischemia, verapamil and nimodipine, both calcium channel blocking agents, corrected LCBF in all five ischemic cortical regions studied without significantly changing LCBF. Prostacyclin, a vasodilator, had a minimal effect on cerebral pH compared with the calcium channel blockers and did not change LCBF in ischemic regions.

Comparing our results 90 minutes after MCA occlusion with previous data obtained 4 hours after MCA occlusion, we conclude that calcium channel blockers do not raise LCBF in ischemic cortical regions at either time while still achieving normal pH. This implies that the correction of pH is unlikely to be caused by an increase in CBF. Nimodipine treatment was associated with a smaller infarct than verapamil, prostacyclin, or no treatment 90 minutes after occlusion. However, previous work in our laboratory did not show improvement of infarct size with nimodipine 4 hours after occlusion using similar methods.\(^4\) We interpret these conflicting data as evidence of the difficulty in assessing drug efficacy on the basis of histologic effects after relatively short periods of ischemia. The literature on this subject is also controversial. There are published studies of rats receiving nimodipine after the onset of ischemia that show no change in histologic ischemic damage.\(^{12,15}\) However, other studies in which nimodipine was given before ischemia\(^3\) or when treatment and observation of the animals continued for longer periods before sacrifice\(^6\) have shown improvement in infarct size with nimodipine treatment.

The occurrence of brain tissue acidosis during ischemia has been known for many years\(^{17-19}\) and has been postulated to play an important role in influencing recovery after an ischemic insult.\(^{20,21}\) The acidosis can best be explained by enhanced lactate production\(^{22,23}\) as glycolysis is stimulated to support ATP production. Experiments have shown that animals infused with glucose before the onset of ischemia had higher lactic acid concentrations and poorer outcome. In addition, the highest lactic acid concentrations and the worst outcome were noted in glucose-infused animals exposed to incomplete ischemia, presumably because incomplete ischemia allowed appreciable amounts of glucose to enter the ischemic cells and contributed to further anaerobic glycolysis.\(^{24}\) Thus, while acidosis is probably not the major determinant of cell death, it appears to constitute one of the suboptimal conditions that adversely affect cell survival.\(^{21}\)

Our data indicate that the correction of LCpH achieved with verapamil or nimodipine is not associated with improved LCBF. Rather, the LCpH correction may be related to decreased production of lactic acid or improved export of acid. Previous work has shown that normalization of LCpH after ischemia depends mainly upon cellular metabolism of lactate, as capillary permeability to ions is very low.\(^{24-26}\) Evidence has been accumulating that intracellular Ca\(^{2+}\) accumulation is important in the evolution of ischemia leading to neuronal death.\(^{22,23,27,28}\) Deshpande et al\(^{29}\) have recently demonstrated that calcium accumulates before cell necrosis occurs, implying that loss of calcium homeostasis is an early event and not an epiphenomenon of cell injury.

Verapamil can inhibit early Ca\(^{2+}\) uptake of ischemic hippocampal cells.\(^{30}\) By blocking intracellular Ca\(^{2+}\) accumulation and preserving mitochondrial function, calcium channel antagonists may allow oxidative metabolism to continue in cells not totally deprived of oxygen and may contribute to lowering lactic acid levels in ischemic tissue by attenuating production and enhancing oxidation of lactic acid. Diltiazem, another calcium channel antagonist, has been shown to reduce lactate production in ischemic heart muscle.\(^{31}\) Compromised but still-viable

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

**Figure 2.** Mean±SEM local cerebral blood flow ipsilateral to middle cerebral artery occlusion in rats in untreated control and treatment groups. When SEM was small, bar is not indicated. All ipsilateral cortical brain regions had values significantly lower than contralateral regions (not shown), but ipsilateral regions in control and treatment groups were indistinguishable. Overall mean±SEM for ipsilateral cortical regions: control, 61±5; prostacyclin, 49±2; verapamil, 51±3; and nimodipine, 49±11; contralateral cortical regions: control, 228±12; prostacyclin, 49±2; verapamil, 51±3; and nimodipine, 154±21 ml/100 g/min.
ischemic cerebral tissue, as in the so-called ischemic penumbra, may benefit most from therapeutic intervention with calcium channel blocking agents because of this protective action.

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References


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