

## The Effect of Nimodipine on Post-ischemic Cerebral Glucose Utilization and Blood Flow in the Rat

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The authors hypothesized that pretreatment with the calcium entry blocker nimodipine would preserve cerebral glucose utilization and maintain favorable brain blood flow after cerebral ischemia. Three groups of pentobarbital anesthetized rats were studied: control (group 1), ischemia (group 2), and ischemia plus nimodipine pretreatment, 1 mg·kg<sup>-1</sup> ip, 1 h prior to ischemia (group 3). Fore-brain ischemia was induced with bilateral carotid clamping, administration of trimethaphan, and blood withdrawal to obtain a mean arterial pressure of 50 mmHg. The carotid clamps were released and blood re-infusion was begun 9 min after the onset of an isoelectric EEG signal. Ten minutes later, determination of regional cerebral glucose utilization (rCGU) was begun by injecting <sup>3</sup>H-2-deoxyglucose in saline. After 60 min of reperfusion, regional cerebral blood flow (rCBF) was determined by the indicator fractionation method, using <sup>14</sup>C-iodoantipyrine. The brain was divided into hemisphere, diencephalon, cerebellum, and brainstem. Tissue radioactivities were determined by standard techniques. Compared to group 1, hemispheric rCGU (mean ± SEM, μmoles·100 g<sup>-1</sup>) was significantly (*P* < 0.05) reduced in groups 2 and 3 (40 ± 3 vs. 27 ± 2 and 22 ± 2). Hemispheric rCGU was not significantly different in groups 2 and 3. Group 2 exhibited significantly (*P* < 0.05) reduced rCBF (mean ± SEM, ml·100 g<sup>-1</sup>·min<sup>-1</sup>) in the hemispheres compared to control (85 ± 6 vs. 135 ± 17). However, nimodipine pretreatment prevented this post-ischemic hypoperfusion in group 3 (133 ± 18). Regional CBF to non-ischemic regions, *i.e.*, diencephalon and cerebellum, was not significantly reduced in group 2, but it was increased in group 3 compared to control. Nimodipine favorably influences reperfusion at 1 h after forebrain ischemia, but it does not affect post-ischemic regional hypometabolism in the early period of reperfusion and increases flow to non-ischemic brain. (Key words: Brain: blood flow, regional; glucose utilization, regional; ischemia. Calcium entry blockers: nimodipine.)

NIMODIPINE, A CALCIUM ENTRY BLOCKER with specific affinity for the cerebral vasculature,<sup>1</sup> has been shown to favorably influence post-ischemic reperfusion, histopathology, and neurological outcome.<sup>2-5</sup> There have been reports that link ionic calcium shifts during and after brain injury to production of deranged cellular metabolism by a variety of different mechanisms.<sup>6,7</sup> In

light of the drug's ability to affect intracellular ATP levels during ischemia and reperfusion in the rodent,<sup>8,9</sup> it seems reasonable to assume some influence on post-ischemic cerebral metabolism would be observed if the drug is given in a dose sufficient to confer cerebral protection. Furthermore, if the mechanism of action of nimodipine to protect the brain from ischemic damage is dependent on its ability to improve post-ischemic regional cerebral blood flow (rCBF), one would expect to observe an effect on post-ischemic regional cerebral glucose utilization (rCGU), if given in a dose sufficient to improve post-ischemic rCBF. The present study was undertaken to examine the hypothesis that a dose of nimodipine sufficient to improve post-ischemic rCBF would be sufficient to influence post-ischemic regional cerebral glucose utilization.

### Methods and Materials

Twenty adult male Sprague-Dawley rats were allowed free access to food and tap water prior to experiments. Anesthesia was induced with pentobarbital 30 mg·kg<sup>-1</sup> ip. The trachea was exposed *via* a midline incision, cannulated, and ventilation controlled with a rodent ventilator. Arterial blood gases (ABG) were determined to maintain P<sub>CO<sub>2</sub></sub> between 35-40 torr. Supplemental oxygen was added to inspired air to maintain Pa<sub>O<sub>2</sub></sub> > 80 torr. Temperature was kept between 36-37° C by means of a heating lamp and monitored by rectal thermistor. Platinum needles were inserted into temporalis muscle bilaterally to obtain a bipolar frontal EEG signal. Cannulae were placed in both femoral arteries and one femoral vein *via* a femoral incision. Blood pressure and EEG were continuously recorded on a Grass model 7 polygraph (Grass, Inc., Quincy, MA). All operative sites were packed with fibrillar collagen. Heparin (Liquaemin, Organon, West Orange, NJ) 50 IU was given iv. Metocurine (Eli Lilly, Indianapolis, IN) 0.1 mg·kg<sup>-1</sup> was given iv for muscle relaxation.

Using the model described by Smith *et al.*,<sup>10,11</sup> cerebral ischemia was induced by trimethaphan (4 mg·kg<sup>-1</sup>) and withdrawal of venous blood to obtain a mean arterial pressure (MAP) of 50 torr and then bilateral carotid occlusion with vascular clamps. Zero time for ischemia was taken as the onset of an isoelectric EEG signal, occurring approximately 30 s after accomplishment of MAP reduction and carotid occlusion. At the end of 9 min, shed blood was re-infused and the carotid

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clamps released. The carotid arteries were inspected to verify patency. The amount of blood withdrawal required to maintain a MAP of 50 torr ranged from 3–6 ml. With the establishment of reperfusion, phenylephrine, 1–4  $\mu\text{g} \cdot \text{kg}^{-1}$ , was given to maintain MAP at or near pre-ischemic levels to prevent pressure-related perfusion deficits<sup>10</sup> and 0.5 ml of 8.4% Na HCO<sub>3</sub> given to counteract systemic acidosis.

Ten minutes after removal of the carotid clamps and restoration of blood pressure, determination of rCGU was begun. A bolus of 100  $\mu\text{Ci} \cdot \text{kg}^{-1}$  of <sup>3</sup>H-2-deoxyglucose in 0.3 ml normal saline was given iv and 200  $\mu\text{l}$  arterial samples were taken at 0.33, 0.66, 1, 3, 6, 10, 15, 25, 35, and 45 min. Donor blood was given after each sample to maintain euolemia. At 60 min of reperfusion, rCBF was determined by the indicator fractionation method.<sup>12–14</sup> A bolus of 20  $\mu\text{Ci} \cdot \text{kg}^{-1}$  of <sup>14</sup>C-iodoantipyrine (New England Nuclear, Boston, MA) in 0.3 ml normal saline was given iv, with simultaneous withdrawal of a reference flow sample from the femoral artery using a Harvard Pump (Harvard Instruments, South Natick, MA) at a rate of 0.786 ml  $\cdot$  min<sup>-1</sup>. After 10 s, the experiment was terminated by decapitation and the catheter was simultaneously withdrawn from the femoral artery. Additional donor blood (1–2 ml) was given iv immediately before rCBF determination in order to prevent changes in MAP during reference flow blood sample withdrawal. The brain was quickly removed and divided into hemispheres, diencephalon, cerebellum, and brainstem. The brain regions were placed in pre-weighed scintillation vials. Tissue was solubilized in Protosol (Dupont/NEN Research Products, Boston, MA). Blood obtained from the 10-s withdrawal from the femoral artery for reference flow activity was decolorized and treated with Protosol. The timed arterial blood samples were centrifuged to obtain plasma for glucose assay by the hexokinase method using a Gilford 240 spectrophotometer (Gilford Instruments, Oberlin, OH) and tracer activity determination. Blood, plasma and tissue radioactivity were assessed by liquid scintillation counting using a Packard Tricarb counter (Packard Instrument Co., Sterling, VA)

Separation of isotope activity in samples was accomplished by dual window counting, and disintegrations per minute for each isotope present in blood plasma and tissue samples were calculated from the appropriate formula.<sup>15</sup> Counting efficiencies were determined by an external standard, which was checked against internal standards of <sup>3</sup>H- and <sup>14</sup>C-toluene. Determination of rCGU was calculated as described by Savaki *et al.*<sup>16</sup> This modification of the original operational equation, as described by Sokoloff *et al.*,<sup>17</sup> takes into account changing arterial plasma glucose levels. A variation of this approach, using direct counting of tissue radioactivity

TABLE 1. Selected Physiological Variables

	Group 1— Control (n = 5)	Group 2— Ischemia (n = 9)	Group 3— Ischemia + Nimodipine (n = 6)
Glucose (mg $\cdot$ dl <sup>-1</sup> )	181 $\pm$ 11	219 $\pm$ 16	274 $\pm$ 44
MAP (mmHg)	122 $\pm$ 6	111 $\pm$ 3	113 $\pm$ 6
Temperature ( $^{\circ}\text{C}$ )	36.3 $\pm$ 0.2	36.7 $\pm$ 0.2	36.8 $\pm$ 0.2
Hematocrit (%)	39 $\pm$ 1	37 $\pm$ 1	37 $\pm$ 2
pH	7.4 $\pm$ 0.02	7.38 $\pm$ 0.01	7.39 $\pm$ 0.01
P <sub>CO<sub>2</sub></sub> (mmHg)	39 $\pm$ 2	40 $\pm$ 1	37 $\pm$ 1
P <sub>O<sub>2</sub></sub> (mmHg)	85 $\pm$ 5	111 $\pm$ 22	102 $\pm$ 7

Physiological variables were obtained at the time of rCBF determination. All values are expressed as mean  $\pm$  SE.

rather than autoradiography for glucose utilization, has been previously reported by Mraovitch *et al.*<sup>18</sup> Regional CBF was calculated by the equations described by Van Uitert and Levy.<sup>13</sup>

Animals were divided into three groups: control (group 1), ischemia plus vehicle (group 2), or ischemia plus nimodipine (group 3). Nimodipine (Miles Pharmaceuticals, West Haven, CT) was prepared in a stock solution of 20 mg  $\cdot$  ml<sup>-1</sup> in polyethylene glycol, and further diluted to 0.5 mg  $\cdot$  ml<sup>-1</sup> with normal saline. Appropriate measures were taken to prevent exposure of drug solutions to light. Group 3 (n = 6) received 1 mg  $\cdot$  kg<sup>-1</sup> of nimodipine 1 h before induction of ischemia, immediately after induction of anesthesia. Group 2 (n = 9) received an equivalent amount of vehicle as group 3 1 h prior to induction of ischemia. Group 1 (n = 5) received an equivalent amount of vehicle as group 3 and underwent surgical preparation, but did not undergo carotid clamping and blood withdrawal.

Flow, metabolism, and other physiological data for all groups in the experiments were compared by ANOVA, and, if a significant difference was present, pairwise comparisons between groups were made by Student's *t* test with the Bonferroni correction.

## Results

There were no significant differences between groups with respect to weight, temperature, hematocrit, MAP, ABG, or plasma glucose levels. Furthermore, these physiological variables, measured both at the time of <sup>3</sup>H-2-deoxyglucose and <sup>14</sup>C-iodoantipyrine administration, were compared, and were not significantly different within each of the three groups. Physiological variables at the time of rCBF testing are shown in table 1. The time from induction of ischemia until EEG silence was similar in groups 2 and 3, occurring between 20 and 40 s after carotid clamping. Nimodipine did not affect the time to EEG silence after induction of ischemia. After reperfusion, the EEG remained

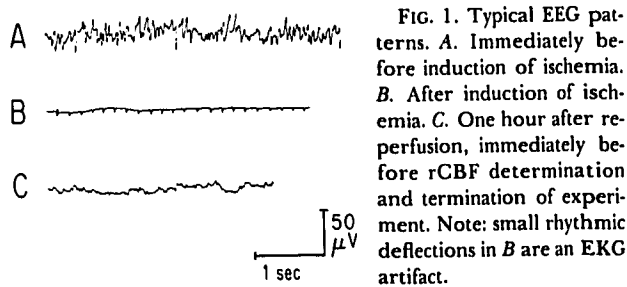


FIG. 1. Typical EEG patterns. A. Immediately before induction of ischemia. B. After induction of ischemia. C. One hour after reperfusion, immediately before rCBF determination and termination of experiment. Note: small rhythmic deflections in B are an EKG artifact.

isoelectric for approximately 20 min. After this time, very low amplitude 20–30 Hz activity was observed with occasional 1–3 Hz activity. There was no difference in EEG patterns between the nimodipine- and vehicle-treated animals. Typical EEG patterns are shown in figure 1.

The values for rCBF and rCGU are shown in table 2, and relative changes are depicted in figure 2. During the first hour of reperfusion after ischemia, the hemispheric rCGU (mean  $\pm$  SEM,  $\mu\text{moles} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$ ) in the target area of the ischemic insult, the hemispheres, was  $27 \pm 2$  in group 2 and  $22 \pm 2$  in group 3. Both of these values are significantly different from the control hemisphere values of  $40 \pm 3$  ( $P < 0.05$ ). There was no difference in rCGU between the animals pretreated with vehicle and those pretreated with nimodipine. There was no difference in rCGU patterns in the three experimental groups in areas distant from ischemia, *i.e.*, in diencephalon, cerebellum, or brainstem.

The rCBF values (mean  $\pm$  SEM,  $\text{ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$ ) indicate that group 2 animals experienced a significant reduction in rCBF ( $85 \pm 6$ ) after 1 h of reperfusion compared to the control group ( $135 \pm 17$ ). Nimodipine

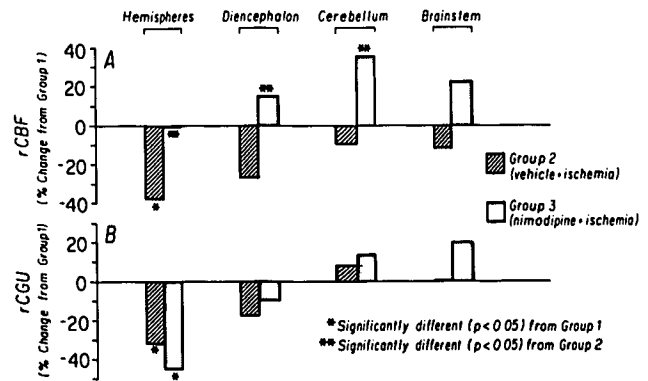


FIG. 2. Relative changes in rCGU during first hour of reperfusion (A) and in rCBF at 60 min of reperfusion (B) after forebrain (hemispheric) ischemia. Although nimodipine pre-treatment prevents post-ischemic hypoperfusion in hemispheres, rCGU is unaffected.

pretreatment prevented this rCBF reduction in group 3, where hemispheric rCBF ( $133 \pm 18$ ) was essentially the same as group 1, although significantly different from hemispheric flow in group 2 ( $P < 0.05$ ). Compared to group 1, group 2 rCBF values in diencephalon, cerebellum, and brainstem were not significantly different. Nimodipine pretreatment resulted in rCBF values in group 3 that were significantly increased compared to group 2 in diencephalon ( $171 \pm 17$  versus  $110 \pm 10$ ) and cerebellum ( $187 \pm 25$  versus  $125 \pm 12$ ). Although rCBF in group 3 brainstem tended to be greater than group 2, this did not achieve statistical significance ( $173 \pm 20$  versus  $125 \pm 12$ ).

## Discussion

The methodology used in this study is a combination of widely used techniques for determination of rCGU and rCBF that are usually employed for quantitative autoradiography.<sup>10-12,17,19,20</sup> By using <sup>14</sup>C-iodoantipyrine and <sup>3</sup>H-2-deoxyglucose in the same animal, it is possible to take advantage of their different energy spectra to separate tracer activities by use of a dual window scintillation counting technique.<sup>15</sup> One disadvantage of using this method is the lack of spatial resolution that is possible with autoradiography, with respect to localization of flow and metabolism. There may be local changes in flow and metabolism that occur during ischemia and reperfusion that would not be detected by this technique.<sup>19,21</sup> The technique is useful, however, because it is relatively easy to perform, and simultaneous testing for flow and metabolism may be performed in the same animal. It is probably most useful in testing physiological conditions that are not rapidly changing and in a relatively steady state. A drawback to the application of this technique to the present study is the tim-

TABLE 2. rCBF and rCGU Values Determined after 9 Min of Acute Forebrain Ischemia

Region	Group 1—Control (n = 5)	Group 2—Ischemia (n = 9)	Group 3—Ischemia + Nimodipine (n = 6)
Hemisphere			
rCBF	135 $\pm$ 17	85 $\pm$ 6*	133 $\pm$ 18†
rCGU	40 $\pm$ 3	27 $\pm$ 2*	22 $\pm$ 2*
Diencephalon			
rCBF	149 $\pm$ 12	110 $\pm$ 10	171 $\pm$ 17†
rCGU	39 $\pm$ 1	32 $\pm$ 3	35 $\pm$ 3
Cerebellum			
rCBF	138 $\pm$ 15	125 $\pm$ 12	187 $\pm$ 25†
rCGU	34 $\pm$ 2	37 $\pm$ 4	39 $\pm$ 2
Brainstem			
rCBF	142 $\pm$ 11	125 $\pm$ 12	173 $\pm$ 20
rCGU	35 $\pm$ 2	35 $\pm$ 4	42 $\pm$ 4

Regional CBF and rCGU values (mean  $\pm$  SE) are expressed in  $\text{ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$  and  $\mu\text{moles} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$ , respectively.

\*  $P < 0.05$  different than group 1.

†  $P < 0.05$  different than group 2.

ing of the tracer injection and the relative periods that the metabolism and flow determinations reflect, since flow and metabolism conditions are not in a steady state during reperfusion after ischemia. The rCBF measurement is essentially instantaneous, whereas the rCGU calculation is heavily weighted for the first 15–20 min after  $^3\text{H}$ -2-deoxyglucose injection.<sup>17</sup> Since the rCGU tracer was given after 10 min of reperfusion, the metabolic state of brain tissue from 10–30 min of reperfusion is primarily reflected in our rCGU values, while the rCBF is measured at 60 min of reperfusion. Although it is not possible to draw conclusions about the coupling between flow and metabolism at 60 min of reperfusion, the object in this study was to see the early, *i.e.*, 10–30 min after reperfusion, metabolic consequences of nimodipine pretreatment associated with improvement in post-ischemic rCBF at 60 min. The fact that the EEG activity returned in both ischemic groups (groups 2 and 3) at approximately 20 min, and remained essentially the same until 60 min of reperfusion, suggests that metabolism was probably not changing, or changing relatively slowly, during the latter half of the period reflected in the rCGU values.

Application of the operational equation for rCGU determination, as proposed by Sokoloff *et al.*,<sup>17</sup> uses a different set of rate constants for gray and white matter. Since we did not measure the distribution of gray and white matter in our sampled brain regions, we calculated rCGU for each brain region assuming it consisted of 50% gray and 50% white matter by weight. This assumption will lead to a value for rCGU which is not strictly quantitative, as the proportion of gray to white matter varies somewhat throughout different regions of the brain, and drugs or ischemia may affect gray and white matter differently.<sup>17</sup> However, the ratio of gray to white matter is probably not different between experimental groups, and, therefore, experimental results would reflect relative changes.

There is evidence that treatment with calcium entry blockers may improve the neurological outcome of a cerebral ischemic event.<sup>6,22</sup> Agents that mitigate against adverse neurological outcome would find a potentially wide application in anesthetic practice for use during procedures that carry with them an attendant risk of cerebral ischemia, such as neurovascular and cardiac surgery.

There is a theoretical rationale for employing calcium entry blockers in the treatment of brain ischemia. Calcium ions are normally maintained with a 10,000-fold gradient from the extra- to intracellular space.<sup>6</sup> With loss of ATP production during severe ischemia, there is a shift of extracellular calcium into the cytosol,<sup>4,23</sup> some of which appears to concentrate in mitochondria.<sup>6,7</sup> This is due to failure of ATP-dependent

ionic pumps that maintain the normal transmembrane calcium gradient.<sup>7</sup> Uncoupling of mitochondrial oxidative phosphorylation, liberation of free fatty acids through the action of phospholipases, and derangements in neurotransmitter metabolism may result as a consequence of these events.<sup>4</sup> A potential mechanism of action for calcium entry blockers may be that they directly prevent this neuronal calcium overload.<sup>6</sup> Calcium-induced spasm of arterial smooth muscle during reperfusion after ischemia may provoke cerebral vasospasm and account for the well-described syndrome of post-ischemic hypoperfusion.<sup>6</sup> Another potential cerebral protective mechanism for calcium entry blockers is the preferential vasodilation of the cerebral vasculature and favorable effects on red cell rheology with subsequent amelioration of post-ischemic hypoperfusion.<sup>22</sup>

In this study, we have investigated the effect of nimodipine pretreatment on rCBF and rCGU after incomplete forebrain ischemia, employing the model described by Smith *et al.*<sup>10,11</sup> The model has been shown to yield a titratable level of damage, with increasing recruitment of brain regions involved in permanent injury by increasing the ischemia time from 2–10 min. For this investigation, we chose a time (9 min) that would yield damage to the hemispheres, including cortex, hippocampus, and caudateputamen. In their model, Smith *et al.*<sup>10,11</sup> induced ischemia during nitrous oxide anesthesia. In our study, we employed pentobarbital anesthesia, which may confer some degree of protection from ischemic damage. The preserved EEG activity observed at the dosage and timing employed in this study, reflecting a light level of barbiturate anesthesia, suggests that any protective effect would be minimal.<sup>24</sup> We have assumed that our animals experienced a similar ischemic insult to that sustained in the reports by Smith *et al.*<sup>10,11</sup> Although we did not verify the extent of histopathological damage, the EEG phenomena we observed were similar to theirs.

Our values for post-ischemic hypometabolism roughly agree with the observations of Pulsinelli *et al.*,<sup>12</sup> who observed a reduction in forebrain rCGU to 35% of control values after 30 min of forebrain ischemia using the four-vessel occlusion model. This rCGU depression persisted for up to 48 h after reperfusion. Their study also correlated the relationship of cerebral metabolic rate for oxygen ( $\text{CMR}_{\text{O}_2}$ ) with rCGU under the conditions of their experiment. The  $\text{CMR}_{\text{O}_2}$  was reduced from  $9.0 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$  to  $3.2 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$ , which was essentially the same as the fall in rCGU for the ischemic regions. Although there is some question as to the applicability of the rate constants for 2-deoxyglucose under conditions of ischemia,<sup>25</sup> the correlation between oxygen and glucose metabolism during ischemia in the rat appear to correlate. At any rate, relative

changes between vehicle and nimodipine pre-treatment would be valid.

Our results indicate that, after 1 h of reperfusion, the hemispheres experienced post-ischemic hypoperfusion, as evidenced by the 37% reduction in group 2 hemispheric rCBF as compared to group 1. Nimodipine pre-treatment prevented this flow reduction, and maintained flows at levels similar to those observed in control animals. Other groups have reported various degrees of flow improvement after transient ischemia.<sup>2,3,5,19</sup> With nimodipine pre-treatment, improved neurological outcome is associated with improved reperfusion blood flow in the canine global cerebral ischemia model<sup>2</sup> and some improvement of histopathological outcome in the rodent model of focal ischemia.<sup>5</sup> Nimodipine also caused a relative hyperemia in presumably nonischemic areas of brain, *i.e.*, diencephalon and cerebellum. That nimodipine increases flow in normal brain has been described by other authors.<sup>24</sup> Whether or not this propensity to increase rCBF in normal brain would have an adverse effect on intracranial pressure in the treatment of regional brain ischemia has not been studied, and was not addressed in this investigation.

Neither pre- or post-treatment with nimodipine, in a dose sufficient to improve post-ischemic global CBF after 10 min of aortic occlusion in the dog, affected levels of various cerebral metabolites after 120 min of reperfusion.<sup>2,3</sup> In those studies, global CMR<sub>O<sub>2</sub></sub> in both pre- and post-treated animals fell to approximately 50% of control level after 5–10 min of reperfusion. After return of EEG activity at 17–19 min of reperfusion, global CMR<sub>O<sub>2</sub></sub> gradually increased to approximately 75% by 30–40 min and remained at this level until the termination of the experiment at 120 min. The reductions in hemispheric rCGU after 10–30 min of reperfusion after forebrain ischemia that we observed are of a similar magnitude to the CMR<sub>O<sub>2</sub></sub> reductions seen in the canine studies. In the gerbil, nimodipine retards the fall in cortical and striatal ATP during ischemia from common carotid artery occlusion.<sup>8</sup> In the rat, nimodipine enhances recovery of ATP after 2 h of reperfusion from incomplete forebrain ischemia.<sup>9</sup> Since nimodipine appears to have a sparing effect on neuronal ATP levels after ischemia in rodent models of ischemia, it was hypothesized that nimodipine would prevent calcium interference in post-ischemic mitochondrial oxidative phosphorylation, and lead to a preserved or improved rate of glucose utilization, which would be accompanied by higher rCBF to post-ischemic brain.

Nimodipine, when administered in levels sufficient to prevent post-ischemic hypoperfusion at 1 h of reperfusion, does not have any effect on rCGU over the first 10–30 min of reperfusion. This suggests that the improvement in rCBF after ischemic reperfusion may not be related to a direct effect on neuronal metabolism. Apparently, cerebral metabolism in this early period of recirculation is not modified by pretreatment of nimodipine, and attainment of near normal flows in ischemic brain regions are not associated with early recovery of metabolic function, although further studies must be done to conclusively state that nimodipine does not affect post-ischemic rCGU during the later course of reperfusion. On the basis of our results, we would suggest that attainment of normal flows in post-ischemic brain by pre-treatment with nimodipine does not prevent calcium overload of neurons and subsequent metabolic depression. It is also possible that the metabolic depression observed in the post-ischemic period is not directly related to calcium overload and cannot be prevented by calcium entry blockade. Further studies must be done to determine the dose thresholds for improvement of rCBF, preservation of ATP and associated glucose metabolism, and their relationship to neurological outcome.

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## References

1. Towart R, Wehninger E, Meyer H, Kazda S: The effects on nimodipine, its optical isomers and metabolites on isolated vascular smooth muscle. *Arzneimittelforschung* 32:338–346, 1982
2. Steen PA, Newberg LA, Milde JH, Michenfelder JD: Nimodipine improves cerebral blood flow and neurologic recovery after complete global ischemia in the dog. *J Cereb Blood Flow Metab* 3:38–43, 1983
3. Steen PA, Newberg LA, Milde JH, Michenfelder JD: Cerebral blood flow and neurologic outcome when nimodipine is given after complete cerebral ischemia in the dog. *J Cereb Blood Flow Metab* 4:82–87, 1984
4. Steen PA, Gisvold SE, Milde JH, Newberg LA, Scheithauer BW, Lanier WL, Michenfelder JD: Nimodipine improves outcome when given after complete cerebral ischemia in primates. *ANESTHESIOLOGY* 62:406–414, 1985
5. Mohamed AA, Gotoh O, Graham DI, Osborne KA, McCulloch J, Mendelow AD, Teasdale GM, Harper AM: Effect of pretreatment with the calcium antagonist nimodipine on local cerebral blood flow and histopathology after middle cerebral artery occlusion. *Ann Neurol* 18:705–711, 1985
6. White BC, Winegar CD, Wilson RF, Hoehner PJ, Trombley JH: Possible role of calcium blockers in cerebral resuscitation: A review of the literature and synthesis for future studies. *Crit Care Med* 11:202–207, 1983
7. Siesjo BK: Cell damage in the brain: A speculative synthesis. *J Cereb Blood Flow Metab* 1:155–185, 1981

† Kazda S, Hoffmeister F, Garhoff B, Towart R: Prevention of the post-ischaemic impaired reperfusion of the brain by nimodipine (abstract). *Acta Neurol Scand* 60(suppl 72):302, 1979

8. Heffez DS, Passonneau JV: The effect of nimodipine on cerebral metabolism during ischemia and recirculation in the Mongolian gerbil. *J Cereb Blood Flow Metab* 5:523-528, 1985
9. Mabe H, Nagai H, Takagi T, Umemura S, Ohno M: The effect of nimodipine on cerebral functional and metabolic recovery following ischemia in the rat brain. *Stroke* 17:501-505, 1986
10. Smith M-L, Auer RN, Siesjo BK: The density and distribution of ischemic brain injury in the rat following 2-10 min of forebrain ischemia. *Acta Neuropathol (Berl)* 64:319-332, 1984
11. Smith M-L, Bendek G, Dahlgren N, Rosin I, Wieloch T, Siesjo B: Models for studying long-term recovery following forebrain ischemia in the rat. A 2-vessel occlusion model. *Acta Neurol Scand* 69:385-401, 1984
12. Pulsinelli WA, Levy DE, Duffy TE: Regional cerebral blood flow and glucose metabolism following transient forebrain ischemia. *Ann Neurol* 11:499-509, 1982
13. Van Uitert RL, Levy DE: Regional brain blood flow in conscious gerbils. *Stroke* 9:67-72, 1978
14. Patlack CS, Blasberg RG, Fenstermacher JD: An evaluation of errors in the determination of blood flow by the indicator fractionation and tissue equilibration (Kety) methods. *J Cereb Blood Flow Metab* 4:47-60, 1984
15. Klein PD, Eisler WJ: An improved description of separation and performance capabilities of liquid scintillation counters in dual isotope studies. *Anal Chem* 38:1453-1457, 1966
16. Savaki HE, Davidson L, Smith C, Sokoloff L: Measurement of free glucose turnover in brain. *J Neurochem* 35:495-502, 1980
17. Sokoloff L, Reivich M, Kennedy C, Des Rosiers MH, Patlack CS, Pettigrew KD, Sakurada O, Shinohara M: The <sup>14</sup>C deoxyglucose method for the measurement of local cerebral glucose utilization: Theory, procedure, and normal values in the conscious and anesthetized albino rat. *J Neurochem* 28:897-916, 1977
18. Mraovitch S, Costantino I, Ruggiero D, Reis D: Widespread reductions in cerebral blood flow and metabolism elicited by electrical stimulation of the parabrachial nucleus in rat. *Brain Res* 341:283-296, 1985
19. Smith M-L, Kagstrom E, Rosen I, Siesjo BK: Effect of the calcium antagonist nimodipine on the delayed hypoperfusion following incomplete ischemia in the rat. *J Cereb Blood Flow Metab* 3:543-546, 1983
20. Mohamed AA, McCulloch J, Mendelow AD, Teasdale GM, Harper AM: Effect of the calcium antagonist nimodipine on local cerebral blood flow: Relationship to arterial pressure. *J Cereb Blood Flow Metab* 4:206-211, 1984
21. Welsh FA: Regional evaluation of ischemic metabolic alterations. *J Cereb Blood Flow Metab* 4:309-316, 1984
22. Gisvold SE, Steen PA: Drug therapy in brain ischaemia. *Br J Anaesth* 57:96-109, 1985
23. Harris RJ, Symon L, Branston NM, Bayhan M: Changes in extracellular calcium activity in cerebral ischemia. *J Cereb Blood Flow Metab* 1:203-209, 1981
24. Michenfelder JD, Theye RA: Cerebral protection by thiopental during hypoxia. *ANESTHESIOLOGY* 39:510-517, 1973
25. Wise RJS, Rhodes CG, Gibbs JM, Hatazawn J, Palmer T, Frackowiak RSF, Jones T: Cerebral oxygen metabolism and blood flow in human cerebral ischemic infarction. *J Cereb Blood Flow Metab* 2:231-335, 1983