Beneficial Effect of Nimodipine on Metabolic and Functional Disturbances in Rabbit Hippocampus Following Complete Cerebral Ischemia

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We investigated the effects of intravenous application of nimodipine on the neurophysiologic, biochemical, and morphologic consequences of 15 minutes of global cerebral ischemia in seven rabbits. In vivo dialysis of the hippocampus was used to determine changes in extracellular concentrations of extracellular calcium and amino acids and blood–brain barrier permeability. Ischemia without treatment produced a rapid disappearance of electroencephalographic activity, a decrease in the concentration of extracellular calcium, the release of neuroactive amino acids, and leakage of methionine to the tissue fluid, plus a significant increase of the blood–brain barrier permeability to fluorescein. Except for permeability and electroencephalographic activity, these parameters normalized during 45 minutes of recirculation; permeability and activity failed to normalize completely during 3 hours of recirculation. After 3 hours of recirculation, morphologic changes in the CA1 hippocampal area were observed. Treatment with nimodipine significantly enhanced electroencephalographic activity recovery and normalization during recirculation, reduced the decrease in extracellular calcium concentration, and prevented the increased permeability of the blood–brain barrier. Nimodipine protected the CA1 area from early morphologic changes and reduced leakage of methionine from brain cells. The beneficial cytoprotective effect of nimodipine, probably related to normalization of calcium homeostasis and blood–brain barrier permeability after ischemia, may reflect both vascular and cellular sites of action. (Stroke 1989;20:70–77)

I

The effects of ischemia-induced influx of calcium into nerve cells, which manifests itself by a decrease in extracellular calcium concentration (Ca\(^{2+}\)), plays a pivotal role in the pathogenesis of irreversible ischemic brain damage. Elevation of intracellular calcium concentration (Ca\(^{2+}\)) may induce important metabolic disturbances that involve calcium-dependent proteases and phospholipases, which results in the release of active and potentially toxic products such as lysocompounds, diacylglycerols, polyunsaturated fatty acids, and eicosanoids. Increase of Ca\(^{2+}\) may also be involved in the contraction of vascular smooth muscles, leading to a known postischemic hypoperfusion.

Calcium entry blockers including the dihydropyridine nimodipine [isopropyl(2-methoxyethyl)-1,4-dihydro-2,6-dimethyl-4(3-nitrophenyl)-3,5-pyrimidinedicarboxylate], known to inhibit some types of voltage-sensitive calcium channels (VSCC), have been used in the experimental therapy of ischemic brain damage. In spite of negative reports, a beneficial effect of nimodipine on cerebral ischemia (improved postischemic neurologic recovery and increased survival) was observed. This was paralleled by enhancement of cerebral blood flow (CBF); therefore, the protective effect of nimodipine was attributed almost exclusively to its known vasodilatatory action whereas the possible direct effects of nimodipine on ischemic calcium fluxes in neurons was studied less extensively.

In vivo brain microdialysis has great advantages for the complex dynamic monitoring of ischemia-evoked changes of pathophysiologic factors implicated in the pathogenesis of cerebral injury, factors such as Ca\(^{2+}\) extracellular concentrations of amino acids, and leakage of methionine to the tissue fluid, plus a significant increase of the blood–brain barrier permeability to fluorescein. Except for permeability and electroencephalographic activity, these parameters normalized during 45 minutes of recirculation; permeability and activity failed to normalize completely during 3 hours of recirculation. After 3 hours of recirculation, morphologic changes in the CA1 hippocampal area were observed. Treatment with nimodipine significantly enhanced electroencephalographic activity recovery and normalization during recirculation, reduced the decrease in extracellular calcium concentration, and prevented the increased permeability of the blood–brain barrier. Nimodipine protected the CA1 area from early morphologic changes and reduced leakage of methionine from brain cells. The beneficial cytoprotective effect of nimodipine, probably related to normalization of calcium homeostasis and blood–brain barrier permeability after ischemia, may reflect both vascular and cellular sites of action. (Stroke 1989;20:70–77)

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venous infusion of noradrenaline. The rabbits were during and after ischemia SABP was maintained by controlling bleeding into a pressure compensator. If necessary, additional doses of drugs were applied in the course of the experiments. The rabbits were anesthetized with pentobarbital and tracheosomtomized, relaxed with gallamine triethiodide, and artificially ventilated. If necessary, additional doses of drugs were applied in the course of the experiments. Fifteen minutes of complete, normothermic cerebral ischemia was induced by occlusion of the brachiocephalic trunk and the left subclavian and both internal thoracic arteries, with reduction of the systemic arterial blood pressure (SABP) to 60 mm Hg by pharmacologically maintained SABP, systemic arterial blood pressure. *Significantly different from baseline, p < 0.047 (Walsh test).

We designed an in vivo dialysis method to evaluate the effect of an intravenously applied VSCC blocker (nimodipine) on ischemia-evoked changes of Ca\(^{2+}\) and extracellular concentrations of some neuroactive amino acids and on BBB permeability during and for up to 3 hours after 15 minutes of complete cerebral ischemia. In parallel, the effect of nimodipine on the functional recovery of neurons was estimated by monitoring the bioelectric activity of the cortex and hippocampus, and we compared these data with the morphologic picture.

Materials and Methods

We used seven albino rabbits weighing 2.5–3.5 kg. We treated the rabbits with intravenous nimodipine, and we compared the results with those from 15 untreated (control) rabbits, which we had published earlier. Control experiments disclosed the lack of any effect of infusion of 5 \(\mu\)g/kg/min of the vehicle (30% ethanol).

One day before the experiment, the rabbits were implanted with a transhippocampal dialysis fiber as described previously. On the day of the experiment, the rabbits were anesthetized with pentobarbital and noramidopyrine methanesulfonate sodium, tracheostomtomized, relaxed with gallamine triethiodide, and artificially ventilated. If necessary, additional doses of drugs were applied in the course of the experiments. Fifteen minutes of complete, normothermic cerebral ischemia was induced by occlusion of the brachiocephalic trunk and the left subclavian and both internal thoracic arteries, with reduction of the systemic arterial blood pressure (SABP) to 60 mm Hg by controlling bleeding into a pressure compensator. During and after ischemia SABP was maintained by intra-arterial transfusion of autologous blood and intravenous infusion of noradrenaline. The rabbits were observed during 3 hours of recirculation after ischemia, and then the brains were perfused and prepared for light microscopy.

The application of nimodipine consisted of a single 10 \(\mu\)g/kg i.v. bolus injection 15 minutes before ischemia, which was followed by a continuous infusion of 1 \(\mu\)g/kg/min i.v. starting after 10 minutes of ischemia and continued for the 2 hours of recirculation.

The basic physiologic parameters were controlled, and the bioelectric activity of the cortex and hippocampus was recorded. For labeling with calcium-45, the rabbits were perfused via the dialysis fiber with 2.5 \(\mu\)l/min Krebs-Ringer bicarbonate medium starting 3.5 hours before ischemia and continuing for 2 hours after ischemia. Dialysate samples were analyzed for calcium-45 radioactivity to monitor changes in Ca\(^{2+}\) in the hippocampus, using high-performance liquid chromatography to monitor changes in extracellular concentrations of amino acids, or spectrofluorometrically to estimate BBB permeability to fluorescein. For this purpose, 10 mg/kg i.v. sodium fluorescein was injected as a 1% solution 30 minutes before ischemia.

Results

An analysis of the changes in physiologic indexes characterizing the cardiovascular and respiratory status of the control rabbits has been published. Data in Table 1 present physiologic parameters in seven rabbits treated with nimodipine. Administered as an intravenous bolus 15 minutes before ischemia, nimodipine induced a transient, 1–5-minute 40–60 mm Hg decrease of SABP after 35–60 seconds; baseline SABP was 100–125 mm Hg. Thirty to sixty seconds after nimodipine administration, heart rate varied from 240 to 252/min, whereas baseline heart rate varied from 228 to 288/min. The remaining parameters did not change with nimodipine administration before ischemia. During ischemia, SABP was pharmacologically maintained within the range 70–50 mm Hg and the heart rate was 192–312/min. During recirculation, reduced SABP developed, but it was less accentuated in the nimodipine-treated than in the control rabbits.
Figure 1. Effect of nimodipine (NIMOD) on evolution of electroencephalographic changes in rabbits during 15 minutes of complete cerebral ischemia and during recirculation (recovery). Top: Untreated. Bottom: Nimodipine applied intravenously. Hipp., hippocampus. Time constant 0.03 second.
TABLE 2. Effect of Intravenous Nimodipine Treatment on Changes in Bioelectric Activity in Rabbit Cortex and Hippocampus During and After 15 Minutes of Complete Cerebral Ischemia

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Disappearance of activity (sec)</th>
<th>First signs of recovery (min)</th>
<th>Continuous activity (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cortex</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>15</td>
<td>15.5±1.0</td>
<td>37.7±2.2*</td>
<td>72.6±3.6</td>
</tr>
<tr>
<td>Nimodipine</td>
<td>7</td>
<td>17.0±2.0</td>
<td>10.7±2.8*</td>
<td>35.1±1.6*</td>
</tr>
<tr>
<td><strong>Hippocampus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>33.5±5.2</td>
<td>36.2±2.9</td>
<td>78.8±7.2</td>
</tr>
<tr>
<td>Nimodipine</td>
<td>7</td>
<td>22.4±2.9*</td>
<td>13.8±2.6*</td>
<td>35.1±1.6*</td>
</tr>
</tbody>
</table>

Data are mean±SEM. Control data from Pluta et al.2
*Significantly different from control, p<0.05 (Mann-Whitney U test).

In control rabbits subjected to 15 minutes of cerebral ischemia,2 electroencephalograms (EEGs) recorded from the cortex and hippocampus (Figure 1, top) demonstrated a typical evolution of changes. After the arrest of the blood supply to the brain we observed a rapid disappearance of EEG activity, and during recirculation we observed a slow reappearance of both the first signs of EEG activity and continuous activity (Table 2). Normalization of the EEG recordings was not observed during 3 hours of recirculation (Figure 1, top). Activity of the vasomotor centers, evaluated as to the ability of the rabbit to maintain its SABP, disappeared 3.9±0.3 minutes (mean±SEM) after the onset of ischemia and recovered after 15±1 minutes of recirculation.

In treated rabbits, bolus injection of nimodipine 15 minutes before ischemia produced an increase in the number and amplitude of slow waves. Five to ten minutes after injection, the EEG recordings normalized completely (Figure 1, bottom). Arrest of the blood supply to the brain led to changes in cortical and hippocampal bioelectric activity; 13-28 seconds (in the cortex) and 13-33 seconds (in the hippocampus) after the onset of ischemia, EEG activity disappeared. During ischemia, EEGs recorded from the cortex and hippocampus were isoelectric. The time to disappearance of activity of the vasomotor centers varied from 3.5 to 4.5 minutes, with a mean±SEM of 4.1±0.2 minutes. The recovery of cerebral activity during recirculation was evaluated in the treated rabbits as reappearance of cortical and hippocampal EEGs and the components of the vasomotor centers. The first signs of recovery of spontaneous EEG activity of the cortex (after 3-20 minutes) and hippocampus (after 7-26 minutes) (Table 2) were spindles of alpha and theta waves, with increasing amplitudes and changes in the records of 10 µV amplitude; they were initially separated by numerous isoelectric periods of varying duration. However, at that time the EEG recordings from the hippocampus were definitely less developed and contained more slow waves with low amplitudes and slight deflections with an amplitude of 10 µV. Later during recirculation, the duration and frequency of spindles increased and showed a tendency to join in continuous activity. This provided a background for the appearance of low-voltage waves of middle frequency with rapidly increasing amplitude. In the meantime, isoelectric periods completely disappeared and continuous EEG activity was observed. At that time the recordings from the hippocampus were definitely poorer than those from the cortex (Figure 1, bottom). Continuous EEG activity was recorded from the cortex and hippocampus after 28-40 minutes of recirculation in the treated rabbits (Table 2). The recovery of EEG activity from the cortex and hippocampus was very rapid, reaching a wave frequency and amplitude close to baseline after 2 hours of recirculation. Restitution of the
activity of the vasomotor centers was observed after 8–14 minutes of recirculation (mean±SEM 10.8±0.8 minutes). After 2 hours of recirculation, the infusion of nimodipine was stopped, which did not attenuate the accelerated recovery of EEG activity from the cortex and hippocampus (Figure 1, bottom).

Fifteen minutes of cerebral ischemia in control rabbits induced biphasic changes in Ca\(^{2+}\); an initial rise of 10% was followed by a decrease of 26.4% after 10 minutes of recirculation, whereas baseline Ca\(^{2+}\) was restored 35 minutes later (Figure 2). Bolus injection of nimodipine before ischemia produced a brief, 19% elevation of calcium-45 efflux to the dialysate. An additional peak (17%) was observed early during ischemia and was followed by a negligible decrease (7.5%) of calcium-45 activity. In the treated rabbits, baseline Ca\(^{2+}\) was restored after approximately 22 minutes of recirculation (Figure 2).

Changes in extracellular concentrations of six amino acids are presented in Figure 3. Baseline concentrations in the control and treated groups were for glutamate (Glu) 2.69 and 3.06 µM, for aspartate (Asp) 0.49 and 0.55 µM, for glutamine (Gln) 39.2 and 19.4 µM, for methionine (Met) 0.82 and 1.27 µM, for taurine (Tau) 1.67 and 1.32 µM, and for phosphoethanolamine (PEa) 1.17 and 0.74 µM. Fifteen minutes of cerebral ischemia in the control group produced significant but transient elevation of extracellular concentrations relative to baseline of Glu (by 320%), Asp (by 205%), and Tau (by 650%). A delayed increase of PEa concentration (by 820%) was also observed, whereas changes of Gln and Met concentrations were much less pronounced. The extracellular concentration of Met progressively increased during recirculation. Intravenous administration of nimodipine did not significantly influence the baseline concentrations of Glu, Asp, Met, and Tau in the dialysate; concentrations of Gln and PEa decreased. Nimodipine infusion did not change Glu, Gln, and Tau concentrations during and immediately after ischemia relative to control but did cause significant elevation in Asp and decrease in Met concentrations (Figure 3). The difference between the groups in the ischemia-evoked relative increase of PEa concentration actually results from different baseline concentrations; this difference is negligible when PEa concentration is calculated in absolute units.

Fluorescein was applied intravenously as a tracer of BBB permeability. In five control rabbits, 15 minutes of cerebral ischemia produced a 40% drop...
FIGURE 4. Effect of intravenous nimodipine (dashed line) on permeability of blood–brain barrier in rabbits to fluorescein before, during, and after 15 minutes of cerebral ischemia. Mean of five untreated control (solid line; data from Pluta et al) and four nimodipine-treated rabbits; SEM did not exceed 20%. *significantly different from control (p<0.05, Mann-Whitney U test).

of fluorescence in the dialysate, followed by a rapid increase to 160% of baseline, which remained stable for the remaining 100 minutes of recirculation. Intravenous bolus injection of nimodipine before ischemia produced a transient 40% elevation of fluorescence in the dialysate, with a decrease during ischemia; during recirculation, fluorescein concentration in the dialysate remained constant at 90% of baseline (Figure 4).

In the control rabbits the hippocampal CA1 sector contained a mixed population of degenerated and unchanged neurons (Figure 5C). Shrinkage of the neuronal cytoplasm dominated, with corkscrew-like torsion of the apical dendrites. Ischemic neuronal changes occurred very frequently in control rabbits, but complete disappearance of the pyramidal neurons was not observed. Edema and dispersion of the cells were prominent features in the pyramidal layer and fascia dentata. Edema was characterized by rarefaction of the tissue, particularly marked around the vessels. In rabbits treated with nimodipine, the CA1 sector of the hippocampus appeared as that in rabbits not subjected to cerebral ischemia (Figure 5, A and B).

Discussion

There are contradictory data in the literature concerning improvement of the neurologic state and survival of ischemic animals treated with nimodipine.9-12,21-22 In our study we limited the recirculation period after ischemia to 3 hours. Within that period an evident improvement with nimodipine treatment was found in the morphologic appearance of CA1 neurons in the hippocampus; EEG activity after ischemia returned significantly sooner and the appearance of final recordings after 2 hours of recirculation was much better than in control rabbits. Since changes of the EEG power and amplitude reflect the efficiency of CBF and oxygen delivery to brain tissues,23 our results conform with findings that nimodipine improves postischemic CBF,5,10,21 which does not exclude the direct influence of nimodipine on neuronal cells.

To evaluate the metabolic effects of nimodipine treatment, we measured the complex ischemia-evoked changes in $Ca^{2+}_{E}$, extracellular concentrations of amino acids, and penetration of fluorescein through the BBB. Biphasic changes of $Ca^{2+}_{E}$ in ischemic brain, with a slight elevation of $Ca^{2+}_{E}$ reflecting shrinkage of the extracellular compartment24 followed by a long-lasting drop as a result of calcium influx to the intracellular compartment, does not differ from the results of studies with a calcium-sensitive electrode.25 The participation of different calcium channels in the latter phenomenon has been postulated.13 Delayed normalization of $Ca^{2+}_{E}$ after ischemia probably reflects a retardatory effect of impaired reperfusion on the metabolic recovery of the brain.
Nimodipine infused intravenously reduced the maximal ischemic drop of Ca\(^{2+}\) by 70\% and accelerated the normalization of Ca\(^{2+}\) after ischemia by 20 minutes, which probably resulted from a combination of inhibitory action on some classes of calcium channels and improvement of CBF. It is difficult to evaluate the extent to which each effect participates in the complex phenomenon. A class of VSCC (N channels) is known to be dihydropyridine-insensitive, as are receptor-operated channels (ROC) linked to N-methyl-D-aspartate and kainate glutamatergic receptors. Therefore, one cannot expect that nimodipine could completely prevent any ischemia-evoked calcium fluxes in the brain. The few and contradictory data available describing effects of calcium entry blockers on the decrease of Ca\(^{2+}\), whereas Tau may attenuate this effect. The inhibition of calcium influx to neurons by dihydropyridine calcium channel blockers does not necessarily interfere with the ischemia-evoked release of neuroactive amino acids since presynaptic VSCC of the \(N\) type, which are important for neurotransmitter release, are nimodipine-insensitive. In fact, apart from the peculiar modifications of Asp release, we observed no other changes in concentrations of neuroactive amino acids. Nimodipine applied intravenously significantly reduced the efflux of methionine from brain cells, which we had observed in control rabbits after ischemia. This may serve as additional evidence for the cytoprotective effects of nimodipine.

The use of a dialysis technique offered the possibility of measuring the permeability of the BBB to fluorescein. The injection of nimodipine before ischemia produced a transient elevation of fluorescence in the dialysate. The transport of fluorescein to the brain tissues is conditioned by the CBF; however, after ischemia the penetration of fluorescein from the blood vessels increased significantly, undoubtedly due to the leakage of the BBB, in spite of posts ischemic hypoperfusion developing very quickly after 10–15 minutes of reactive hyperemia. This finding is in accordance with suggestions that the BBB may be opened to micromolecules shortly after recirculation. This increased BBB permeability appeared to be completely prevented by intravenous application of nimodipine. On the other hand, phenothiazine antagonists of calmodulin were reported to reduce efficiently the leakage of the BBB. The inhibition of calmodulin is one of the known side effects of dihydropyridines, which suggests a possible mechanism of the observed effect. It seems that nimodipine may be more effective than other calcium channel blockers in BBB protection as no effect of verapamil on increased BBB permeability in ischemic pathology was reported.

Some of our findings (namely, the acceleration of EEG recovery and diminution of the unspecific leakage of methionine) clearly point at beneficial effects of nimodipine treatment on posts ischemic brain damage. Our other data, which include such effects of nimodipine as the diminution of calcium influx to the hippocampal cells during and after ischemia and protection of the BBB early after recirculation, concern the possible mechanisms of ischemic injury and pharmacologic mechanisms of nimodipine action. It seems that there is a direct causal relation between the effects of nimodipine on calcium homeostasis and BBB permeability and the final effect of this treatment: the protection and/or facilitation of posts ischemic recovery. The effectiveness of a calcium channel blocker (nimodipine) in the therapy of brain ischemic injury gives further evidence of the universal and multiple role of calcium in the pathomechanism of stroke.

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References


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