Neuroprotective effects of nicardipine in a rat model of ischemia and reperfusion

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A reversible middle cerebral artery occlusion was performed in rats to determine whether nicardipine, a dihydropyridine voltage-sensitive Ca++ channel (VSCC) antagonist, exerts neuroprotective effects when administered 10 minutes following an ischemic insult, and if it does, whether this is due to its vasodilatory action and effect on cerebral blood flow (CBF) or to direct blockade of Ca++ entry into ischemic brain cells. An increase in the intracellular calcium, \([\text{Ca}^{++}]_i\), plays a major role in neuronal injury during cerebral ischemia. Although a large amount of Ca++ enters neurons through the VSCC during ischemia, inconsistent neuroprotective effects have been reported with the antagonists of the VSCC. An intraperitoneal injection of nicardipine (1.2 mg/kg) was administered to rats at 10 minutes after the onset of ischemia, and 8, 16, and 24 hours after occlusion. Cortical CBF was determined by laser-Doppler flowmetry. Neurological and neuropathological examinations were performed after 72 hours. Neuron-specific enolase, a specific marker for the incidence of neuronal injury, was measured in plasma. The CBF in the ischemic core and periphery, as well as brain temperature and physiological parameters, were not affected by nicardipine during occlusion or reperfusion. However, nicardipine treatment significantly improved motor neurological outcome by 32%, and the infarction and edema volume in the pallium as well as the edema volume in the striatum were significantly reduced by 28%, 37%, and 53%, respectively. Nicardipine also significantly reduced the neuron-specific enolase plasma levels by 50%, 42%, and 59% at 24, 48, and 72 hours after the occlusion, respectively. It is concluded that nicardipine may attenuate focal ischemic brain injury by exerting direct neuroprotective and antiedematous effects that do not depend on CBF.

Key Words * nicardipine * focal ischemia * cerebral blood flow * neuroprotection * neuron-specific enolase

Central nervous system trauma and ischemia produce a deregulation of cellular Ca++ homeostasis by numerous mechanisms, including energy depletion, cell membrane depolarization, excessive synaptic activity, and organelle dysfunction.[42] The pathological increases in intracellular Ca++ ions, \([\text{Ca}^{++}]_i\), which normally regulate numerous intracellular cascades, trigger further injury to neuronal and axonal elements. It is well known that during severe ischemia, when the plasma membrane is depolarized, a
large amount of Ca++ enters neurons through the voltage-sensitive Ca++ channels (VSCCs).[16] Calcium ions can also enter the cells through receptor-operated ion channels that open during activation of N-methyl-D-aspartate (NMDA) receptors.[24] Various classes of drugs that prevent [Ca++]i increases by blocking Ca++ influx (such as, VSCC blockers and NMDA antagonists) or acting directly on [Ca++]i (such as, cell-permeant Ca++-chelating agents) and/or attenuating the secondary processes triggered by [Ca++]i excess have been investigated as neuroprotective agents in cerebral ischemia.[42]

Dihidropyridine antagonists of the VSCC (that is, nimodipine, nicardipine, and nilvadipine) have been applied with variable success to reduce traumatic and/or ischemic central nervous system (CNS) injury. In some studies, the VSCC blockers produced modest or even negative results.[6,9,31,32,38,46] According to the "vascular" theory, these inconsistent favorable effects depend on the vasodilatory action and increase in cerebral blood flow (CBF), as for example in CNS injury due to aneurysmal subarachnoid hemorrhage (SAH).[42] In contrast, other studies indicated that VSCC blockers exert direct neuroprotective effects,[7,8,10,18,23,26,33,34] possibly by preventing increases in brain cytosolic-free Ca++.[14,43] In support of the "neuronal" theory it has been demonstrated that reduced Ca++ entry through VSCCs in different models of cerebral ischemia correlates well with decreases in histological damage and improved CNS functioning.[14,36,43,44]

In this study, a reversible 3-hour middle cerebral artery (MCA) occlusion performed in rats followed by 72 hours of reperfusion was used to determine whether nicardipine exerts neuroprotective effects after a focal ischemic insult, and if it does, whether these effects depend on CBF. The levels of neuron-specific enolase (NSE), a marker for the incidence of cerebral damage in acute and chronic ischemic brain infarctions,[3,17] were determined in systemic circulation and compared with neuropathological findings and neurological motor outcome. Preliminary results have been reported.[20]

**MATERIALS AND METHODS**

Male Sprague-Dawley rats weighing between 260 and 300 g (Charles River Breeders, Hollister, CA) were housed under diurnal light conditions with unlimited access to food and water and were allowed a minimum of 3 days for acclimation. All procedures were performed in accordance with the Animal Care Guidelines at the University of Southern California as approved by the National Institutes of Health.

**Nicardipine Administration**

Nicardipine hydrochloride (Wyeth-Ayerst Laboratories Inc., Philadelphia, PA), at a dose of 1.2 mg/kg, was administered intraperitoneally, 10 min after the onset of ischemia, and 8, 16, and 24 hours after a 3-hour reversible MCA occlusion and a 72-hour period of reperfusion.

**Focal Ischemia Study**

Rats were deprived of food for 12 hours before surgery. Reversible MCA occlusion was performed in eight nicardipine-treated and eight control rats using an intraluminal thread technique,[22] as recently modified in our laboratory.[21] The procedure involved initially anesthetizing the rats with metofane and maintaining the anesthesia by administration of intraperitoneal pentobarbital (50 mg/kg). Intraperitoneal atropine methyl nitrate (0.18 mg/kg) was given as premedication to prevent airway obstruction by mucus formation. The animals were allowed to breath spontaneously. Rectal temperature was maintained at 37±C by a thermostatically regulated heating pad. A PE-50 catheter was introduced into the right femoral...
artery for continuous monitoring of the mean arterial blood pressure, as well as for repeated sampling of blood for serial measurements of PaO₂, PaCO₂, pH (ABL 30 Acid-Base Analyzer; Radiometer, Copenhagen, Denmark), hematocrit, and blood glucose levels. With the aid of an operating microscope, the right common carotid artery (CCA) was exposed through a ventral midline incision and the external carotid artery (ECA) and the internal carotid artery (ICA) were isolated. The CCA was tied at approximately 8 mm from the bifurcation. The ECA was tied at approximately 5 mm from the bifurcation (permanent double knot), and a second loose knot was placed around the ECA origin. After placing a microvascular clip across the ICA adjacent to the origin of the ECA, a partial incision was made in the ECA midway between the permanent double knot and second loose knot. A No. 3-0 monofilament nylon suture with a rounded tip was introduced into the ECA lumen, the stump of the ECA was tightened around the intraluminal nylon suture to prevent bleeding, and the microvascular clip was removed. The nylon suture was then gently advanced from the ECA into the ICA lumen, and the tip was advanced 17.5 mm from the bifurcation to occlude the MCA at its origin at the circle of Willis. After 3 hours of occlusion, the thread was pulled out, the ECA was permanently tied at the level of bifurcation, and the CCA was opened to allow reperfusion.

Blood Flow and Head Temperature Measurements

Cortical CBF was monitored by laser-Doppler flowmetry in both the nicardipine-treated and control groups before and during the 3-hour occlusion and within 1 hour of reperfusion. A single 0.8-mm diameter laser-Doppler flow probe was positioned 0.1 mm above the dura over the cortical surface and connected to a tissue perfusion monitor (model BLF21; Transonic, Ithaca, NY). In the hemisphere ipsilateral to the occluded MCA the coordinates were: Point A, 1 mm posterior to the bregma and 5.4 mm lateral to the midline; Point B, 1 mm posterior to the bregma and 2.1 mm lateral to the midline; Point D, 1 mm anterior to the bregma and 3.4 mm lateral to the midline; and Point C in the contralateral hemisphere was 1 mm posterior to the bregma and 5.4 mm from the midline. At each point a small burr hole was drilled in the skull, and the bone carefully removed to prevent damage to the cortex. Steady-state baseline values were recorded before occlusion, and the CBF measured during occlusion and reperfusion was expressed as a percentage of the baseline values. Head temperature was monitored with a 36-gauge thermocouple temperature probe connected to a digital thermometer (Thermoregulator model 9000; Omega, Stanford, CT) in the temporalis muscle.

Neurological Examination

All 16 rats were killed following neurological examination, which was performed 72 hours after reperfusion. Neurological outcome was scored on a 6-point scale:[21,47] a score of 0 indicated no deficit (normal); a score of 1 (failure to extend left forepaw fully), a mild focal deficit; a score of 2 (circling to the left), a moderate focal deficit; a score of 3 (falling to the left), a severe focal deficit; rats with a score of 4 did not walk spontaneously and had a depressed level of consciousness; and a score of 5 was stroke-related death (not observed in the present study).

Measurement of Volume of Injury

Each brain was placed in ice-cold phosphate-buffered saline, chilled for 20 minutes, and sliced into 2-mm coronal sections. The area of injury in each of the 16 rat brains was delineated by incubation of unfixed 2-mm coronal brain slices in 2% triphenyltetrazolium chloride in 0.173 M sucrose and 50 mM K⁺ phosphate buffer (pH 7.4) for 20 minutes at 37°C and then stored in 10% formalin as reported. Serial
coronal sections were displayed on a digitizing video screen using a commercially available imaging system (Jandel Scientific, San Rafael, CA), and the areas of nonstaining tissue were determined in each section. The volume of injury was calculated by summing the affected areas from each coronal section and multiplying that number by the thickness of the section. The volumes of the control and lesioned hemispheres were calculated, and the amount of injury was expressed as a percentage of total cerebral volume (% injury volume) and in absolute terms in cubic millimeters. The injury volume of gray matter structures (the pallium and striatum) was corrected for brain edema in the lesioned hemisphere by subtracting the volume of the normal tissue in the lesioned hemisphere from the volume of the control (normal) hemisphere, based on a previously described method.[35] The edema volume was calculated by subtracting the volume of the normal gray matter in the control hemisphere from the volume of gray matter in the lesioned hemisphere.[21,35,47] Measurements of the volume of injury were made separately for the pallium and striatum. A coronal section from each brain was obtained at the level of the optic chiasm. Representations of the injury areas of individual sections from each animal in the control and nicardipine-treated groups were superimposed to gain an impression of the topography and incidence of infarction. The boundary of infarction was redrawn for each rat on the corresponding coronal section and the following areas were delineated: the infarct area in which all rats were affected 100%, the infarct area in which 50% or more of the rats were affected, and the infarct area in which less than 50% of the rats were affected.

**Determination of NSE Plasma Levels**

The level of NSE was determined in plasma in the control and nicardipine-treated groups at 24, 48, and 72 hours after MCA occlusion. For measurement of plasma NSE concentrations, a NSE-selective radioimmunoassay (RIA) was used (Pharmacia Inc., Uppsala, Sweden). Human NSE was used as a standard in this RIA. Iodine-125 NSE antibody (antiserum raised in rabbits) was incubated for 3 hours at 23°C with standard concentrations of NSE or plasma samples (50 µl). Bound NSE was separated from free NSE using a sepharose anti-rabbit immunoglobulin G antibody. The Iodine-125 was counted and the concentration of NSE was determined from a standard curve. The sensitivity of the assay was 150 pg per tube and the EC50 was 1200 pg per tube. Data were normalized to nanograms per milliliter of plasma NSE.

**Statistical Analysis**

Physiological variables, infarction and edema volumes, and NSE levels were compared between groups using Student's t-test. Nonparametric data (neurological outcome scores) were subjected to the Kruskal-Wallis test. A probability value of less than 0.05 was considered statistically significant.

Preocclusion was defined as 10 minutes before MCA occlusion. Data obtained for MCA occlusion at 20, 40, 60, 80, 100, 120, and 160 minutes (except for T) were pooled because there was no significant difference at different time points. Data for reperfusion at 10 and 60 minutes (except for T) were pooled because there was no significant difference. Data for the temperature taken at the temporalis muscle were obtained 10 minutes before and 60 minutes after MCA occlusion and 60 minutes after reperfusion.

**RESULTS**

**Physiological Variables**

No differences in physiological variables (including the temporalis muscle temperature) were noted.
between the nicardipine-treated and control groups before MCA occlusion, during occlusion, or during reperfusion (Table 1), except that mean arterial blood pressure during reperfusion was significantly lower in the nicardipine-treated group when compared to the control groups' preocclusion values.

![Table 1](image)

**Figure 1** illustrates changes in CBF during MCA occlusion and reperfusion. No significant differences in baseline tissue perfusion units were observed between nicardipine-treated and control rats, indicating similar preocclusion CBF values. Reductions in CBF during the 3-hour occlusion period were comparable between the two groups: 15 to 17% (p < 0.001) and 15 to 18% (p < 0.001) of baseline in the ischemic core of control and nicardipine-treated rats, respectively, and 33 to 37% (p < 0.001) of baseline in the periphery of the ischemic core in both groups. The trend toward CBF restoration during reperfusion both in the ischemic core and periphery of the ischemic core was again comparable between the two groups, and there was no significant difference as a result of nicardipine treatment. During 1 hour of reperfusion in the periphery of the ischemic core, CBF rose to between 70% and 73% of baseline (p < 0.01) in control group, and to between 70% and 76% (p < 0.01) in nicardipine-treated group. In the ischemic core of control and nicardipine-treated rats, CBF was restored to approximately 32 to 35% (p < 0.001) and 34 to 37% (p < 0.001) of preocclusion values, respectively.
Neurological Outcome

There was a significant difference in the outcome between control and nicardipine-treated rats (Table 2). In the control group two of eight rats had severe focal neurological deficit (score 4), whereas in the nicardipine-treated group no rat exhibited a score of 4. A relatively mild neurological deficit (score 1 and 2) was found in six (75%) of eight nicardipine-treated rats, and in only three (38%) of eight control rats. Nicardipine treatment resulted in an average reduction of 29% in the score.

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>NEUROLOGICAL SCORES FOR EIGHT CONTROL RATS AND EIGHT NICARDIPINE-TREATED RATS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Score</td>
</tr>
<tr>
<td>control (8 rats)</td>
<td>0 2 1 3 2</td>
</tr>
<tr>
<td>nicardipine (8 rats)</td>
<td>0 3 3 2 0</td>
</tr>
</tbody>
</table>

* p < 0.05 by Kruskal-Wallis test.
Neuropathological Examination

The total volume of injury corrected for the edema volume was reduced by 19% in the nicardipine-treated group relative to the control group (Table 3). The total edema volume was also reduced by 42% in the nicardipine-treated group. These decreases were caused by a significant decrease in the injury volume (27%; \( p < 0.05 \)) and edema volume in the pallium (37%; \( p = 0.058 \)), indicating that nicardipine reduces the expansion of injury into cortical regions. The injury volume in the striatum was not affected by nicardipine, whereas there was a significant 52% decrease in the edema volume. The total area of brain injury determined at the level of the optic chiasm (anterior coronal block), and expressed as a percentage of the coronal section was decreased by 20% in nicardipine-treated rats, comparable to a 24% decrease in brain injury expressed as a percentage of the total hemispheric volume.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Nicardipine</th>
<th>p value</th>
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<tbody>
<tr>
<td>infarct volume corrected for edema</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>striatum (mm(^3))</td>
<td>44.1 ± 6.7</td>
<td>44.3 ± 10.7</td>
<td>0.972</td>
</tr>
<tr>
<td>pallium (mm(^3))</td>
<td>106.1 ± 16.1</td>
<td>77.5 ± 15.9</td>
<td>0.003</td>
</tr>
<tr>
<td>total (mm(^3))</td>
<td>150.2 ± 18.4</td>
<td>121.8 ± 11.5</td>
<td>0.002</td>
</tr>
<tr>
<td>edema volume</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>striatum (mm(^3))</td>
<td>10.6 ± 5.6</td>
<td>5 ± 4.7</td>
<td>0.048</td>
</tr>
<tr>
<td>pallium (mm(^3))</td>
<td>18.1 ± 8.5</td>
<td>11.4 ± 3.2</td>
<td>0.058</td>
</tr>
<tr>
<td>total (mm(^3))</td>
<td>28.7 ± 13.4</td>
<td>16.4 ± 6.6</td>
<td>0.035</td>
</tr>
<tr>
<td>area of brain injury</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% coronal section at the optic chiasm</td>
<td>29.4 ± 5.1</td>
<td>23.4 ± 2.2</td>
<td>0.008</td>
</tr>
<tr>
<td>% total hemisphere volume</td>
<td>63.6 ± 12.2</td>
<td>48.4 ± 4.7</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*Values are given as the mean ± SD for eight control and eight nicardipine-treated animals. The p value was obtained using Student's t-test.

Figure 2 illustrates that 100% of the control rats were injured in the striatum and mediolateral cortex; 50% or more exhibited changes in dorsolateral and basolateral cortex, and less than 50% showed changes in ventrolateral cortex. No nicardipine-treated rat exhibited changes in dorsolateral cortex; there was also significant improvement in mediolateral cortex, that is, less than 50% of the nicardipine-treated rats exhibited changes in this area, in contrast to 100% of control rats.
Fig. 2. Graphic illustration showing the incidence and topography of infarction at the level of the optic chiasm during reversible MCA occlusion in control (left) and nicardipine-treated (right) rats.

**Plasma Levels of NSE**

The changes in NSE plasma levels are illustrated in Fig. 3. Following a 3-hour reversible MCA occlusion there was a significant increase in serum NSE levels in control versus the nicardipine-treated animals by 2.9-fold, 1.3-fold, and 1.5-fold at 24, 48, and 72 hours of reperfusion, respectively. Nicardipine treatment resulted in significant decreases in NSE values compared with the control group; the reductions in NSE levels were by 50%, 42%, and 59% at 24, 48, and 72 hours of reperfusion, respectively.

Fig. 3. Levels of NSE in the plasma of control rats (open bars), in MCA model rats (black bars), and in MCA model rats after nicardipine treatment (hatched bars) 1, 2, and 3 days after MCA occlusion.
DISCUSSION

The present study demonstrates that nicardipine treatment attenuates focal ischemic brain injury in a rat model of reversible MCA occlusion by exerting neuroprotective and antiedematous effects that do not depend on the CBF. The CBF in the ischemic core and the periphery of ischemic core, as well as brain temperature and other physiological parameters, were not affected by nicardipine treatment either during occlusion or reperfusion. However, nicardipine significantly improved motor neurological score by 32% 72 hours after the initiation of the 3-hour reversible MCA occlusion. Neuropathological analysis of brain tissue indicated that the infarction and edema volume in the pallium and the edema volume in the striatum were significantly reduced by nicardipine at 72 hours by 28%, 37%, and 53%, respectively. The effect of nicardipine in alleviating the neuronal injury in this model has been confirmed by significant reductions of the NSE levels in the serum by 50%, 42%, and 59% at 24, 48, and 72 hours after occlusion, respectively.

The effects of the VSCC blockers have been examined in a number of experimental models of ischemia and the results are somewhat conflicting. Significant neuroprotective effects have been demonstrated,[7,8,10,14,18,23,26,33,34,36,43,44] although negative results have been reported as well.[2,9,11,31,32,45,46] Possible factors contributing to the variable results include differences in species, type of anesthesia used, dosages and timing of VSCC blocker treatment, type of ischemia (global vs. focal, complete vs. incomplete), presence or absence of reperfusion, and timing of evaluation. This study confirms the neuroprotective effects of VSCC blockers in focal ischemic brain injury and suggests that the effects produced by nicardipine may be due to its ability to act as an antagonist of Ca++ influx into neurons, rather than on its vasoactive properties and increase in CBF. It has been previously reported that nicardipine may reduce Ca++ accumulation in regional cerebral ischemia in rats,[14] as well as increase cytosolic-free Ca ++ in focal cerebral ischemia and reperfusion in cats.[43]

The results obtained in our study, including reduction of brain injury in the periphery of the ischemic core, significant improvement in mediolateral cortex, and complete prevention of damage to dorsolateral cortex after nicardipine administration are well supported by similar histological improvements found with nimodipine or nilvadipine treatment in different models of experimental ischemia.[14,26,34,36,43] It seems that the timing of the treatment is most crucial to maximize the beneficial effects of the VSCC antagonists, which tend to be effective when administered before or immediately after the ischemic insult. We administered nicardipine as early as 10 minutes after the onset of ischemia and maintained the dosage for 24 hours. It has been shown that even after 5 minutes of ischemia the VSCCs are open, Ca++ moves intracellularly, and specific binding of the VSCC antagonists (nimodipine) increases in severely affected ischemic regions earlier than in those with moderate reductions in perfusion.[15] It has been suggested that when binding of the VSCC blockers declines in an ischemic region in which it was previously increased, infarction is likely. Although significant transport of highly lipophilic VSCC antagonists across the blood-brain barrier (BBB) has been shown,[51] only binding to ischemic tissue was found to be specific and saturable, whereas binding in nonischemic tissue was much lower and nonspecific.[19]

Three subtypes of VSCCs (L, T, and N) in neurons with different dihydropyridine sensitivities and
electrophysiological properties have been identified.[28] It has been suggested that in vivo binding of dihydropyridine Ca++ antagonists in ischemic tissue is to the high-affinity binding state of the L-type VSCC,[19] which occurs in depolarized cell membranes.[39] However, the physiological importance of the high-affinity binding to ischemic brain tissue is not clear,[15,19] and it is not known whether this process is directly related to the improvement in histological outcome observed following treatment with dihydropyridine Ca++ antagonists.[7,8,10,14,18,23,26,33,34,36,43,44] Alternatively, low, nonspecific binding to brain tissue before it becomes affected by severe ischemia may be important for attenuating neuronal damage.

Small increases in [Ca++]i observed in nimodipine- and nicardipine-treated animals during ischemia may be explained by a synaptic Ca++ entry through other subtypes of VSCCs and/or by a receptor-operated Ca++ entry during activation of NMDA receptors.[24,42] It has been demonstrated that brain injury after either focal or global ischemia is mediated, at least in part, by excitatory amino acids (EAAs).[1,4,5,13,29,30] The release of EAAs during ischemia is thought to result in overactivation of NMDA receptors, resulting in an excessive Ca++ neuronal influx. It has been suggested that this process can lead to neuronal cell death, but it could be specifically inhibited by drugs that antagonize NMDA-stimulated processes. The effectiveness of selective antagonists of the NMDA receptors has been shown in experimental cerebral ischemia and in some clinical trials.[12,48,49]

It has recently been suggested that a key feature of Ca++ neurotoxicity is that [Ca++]i must rise in specific subcellular sites to trigger secondary cascades, which cause neuronal degeneration.[42] The theory has emerged that NMDA-mediated Ca++ influx is responsible for increases of so-called "toxic" [Ca++]i, whereas Ca++ influx mediated by VSCCs may not always trigger Ca++-dependent toxic cascades.[40,41] The apparent link between the neuroprotective effects of VSCC antagonists and decreases in [Ca++]i in ischemic brain[14,43] mitigate against this theory. It seems that the intracellular [Ca++]i dynamic is complex and may involve several regulatory sites that could not be regarded as being controlled in isolation from one another.[50]

It is well known that the ischemic/traumatic process significantly increases [Ca++]i levels by affecting Ca++ efflux caused by failure of the adenosine triphosphate­requiring pump (energy depletion), as well as by significant depolarization-induced Ca++ influx via L-type VSCCs.[16,42] Thus, Ca++ channel blockers may be very beneficial in this situation if given before or immediately after an ischemic insult, because they would tend to lower the total pool of [Ca++]i.[14,43] It is conceivable that by lowering [Ca++]i, these agents may alter the diffusion of free Ca++ ions within the cell, possibly favoring the diffusion of excess Ca++ away from the NMDA receptors, which combined can modify the coupling between Ca++ influx and Ca++-activated intracellular processes.

A significant reduction in the NSE level in plasma and improvement in neurological motor score confirm that nicardipine preserves the functional neuronal pool after MCA occlusion. It has been shown that cortical infarction and neurological dysfunction after MCA occlusion are associated with neuronal depletion and vascular redistribution of levels of brain NSE, resulting in a measurable increase in plasma NSE.[3] as confirmed in this study. Such diffusion of NSE into the cerebral vasculature and systemic circulation from ischemic tissue has been shown to serve as a sensitive marker for the incidence of cerebral damage.[3,17] Beneficial effects of the VSCC antagonists on CNS functions, such as the
recovery of electroencephalographic amplitude[18,23,43] and an increase in the amplitude of somatosensory evoked potentials[36] have been previously shown. It has been demonstrated that these recoveries during reperfusion correlate well with the reduced level of [Ca++]i, but not with changes in CBF.[43,44] This has been confirmed in the present model, because the CBF in the nicardipine-treated group, both in the ischemic core and the periphery, returned during reperfusion to values that were not different from the vehicle-treated group.

In addition to observed effects, it has been shown in experimental models of subarachnoid hemorrhage that nicardipine has a significant vasodilating effect, reducing angiographic vasospasm.[25,37] These effects were associated with recovery of the Na+ pump and reduction of lipid peroxidation.[25] Reduction of Ca++ influx into cerebrovascular smooth muscles could explain vasodilation.[42] The fact that Ca++ activates phospholipase A2 and C enzymes[42] plays an important role in generating arachidonic acid and other substrates for peroxidative and hydrolytic processes that amplify the production of reactive oxygen species leading to membrane disruption and cell death. The significant antiedematous effect of nicardipine observed in this study could be related to major stability of cellular membranes, and in particular of the BBB, disruption of which in ischemia is responsible for the formation of vasogenic edema. Cerebral microvascular endothelium, the site of the BBB, in contrast to endothelium of larger cerebral vessels expresses VSCC binding sites for dihydropyridine antagonists[27] and is capable of sequestering a significant amount of Ca++ channel blockers from the circulation.[51] These events could be directly responsible for nicardipine-mediated stabilization of the BBB membranes.

CONCLUSIONS

This study sheds light on the somewhat underscored cerebroprotective potential of VSCC blockers in focal cerebral ischemia. We suggest that with appropriate early timing following an ischemic insult these agents could antagonize a number of pathogenic processes that would ultimately lead to ischemic cell death. In particular, they may be useful if combined with antagonists of receptor-mediated Ca++ influx (NMDA receptor antagonists) that would synergistically lower [Ca++]i and interrupt intracellular cascades that trigger injury to neuronal and axonal elements.

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References


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