Comparison of Intraluminally Versus Extraluminally Administered Nimodipine on Serotonin-Induced Cerebral Vascular Responses In Vitro and In Situ

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The purpose of our study was to compare the ability of intraluminally and extraluminally administered nimodipine to inhibit serotonin-induced cerebral vascular responses in vitro and in situ. No difference was noted in the ability of nimodipine, whether administered intraluminally or extraluminally, to reduce the contractile response of extraluminally administered serotonin in a closed, pressurized, in vitro bovine middle cerebral artery preparation; histologic studies indicated that the tight endothelial junctions normally found in cerebral arteries remained intact in this preparation. In cats, pretreatment with nimodipine did not significantly reduce the ability of intracisternally injected serotonin to decrease cerebral blood flow; however, nimodipine did reduce the changes in cerebral artery diameter normally noted angiographically after serotonin injection. Although minor differences were noted between the intraluminal and extraluminal routes of administration of nimodipine in situ, in general the effects were comparable. (Stroke 1989;20:1065-1070)

Calcium entry blocking agents are currently in vogue as both prophylactic and postinsult treatment measures for intracerebral hemorrhage, focal ischemia, global ischemia and vasospasm following subarachnoid hemorrhage. However, the effectiveness of these drugs in preventing or reversing cerebral vascular constriction varies depending upon species, route and time of administration. In vitro, the effectiveness of most calcium blocking agents in antagonizing a serotonin (5-HT)-induced contraction in cerebral arteries is well documented. However, recent clinical and laboratory evidence suggests that systemic therapy with nimodipine (NM) may not significantly affect the degree of angiographically visualized spasm, nor does NM when given intravenously to baboons significantly reduce the vasoconstrictor response to 5-HT.

In a chronic vasospasm study using dogs, prolonged oral administration of NM did not significantly reduce the severity of arterial narrowing. However, in the same animal model, angiographic evaluation 20-30 minutes after the subarachnoid administration of NM revealed partial resolution of chronic spasm in four of the six dogs. Similar results with topically or intrathecally administered calcium antagonists have been reported by others.

We wondered if the above differences in the effectiveness of calcium blocking agents were related to internal or external perfusion routes. Thus, we compared the ability of NM to inhibit an extraluminally induced 5-HT contraction in vitro (bovine middle cerebral artery) and in situ (cats) when given intraluminally and extraluminally.

Materials and Methods

The in vitro model consisted of 1-cm sections of bovine middle cerebral arteries (MCAs) cannulated between glass tubes, placed in a photodiameter gauge and bathed intraluminally and extraluminally with an artificial Krebs-type solution. Intraluminal pressure was maintained at 75 mm Hg. A more complete description of this model has been described. We obtained 5-HT creatinine sulfate from Sigma Chemical Co. (St. Louis, Missouri) and dissolved it in deionized water before adding it to
the extraluminal surface of the artery in a cumulative dose manner. NM was a gift from Miles Pharmaceuticals (West Haven, Connecticut). NM was dissolved in ethanol and kept in the dark until used. Initially, two control 5-HT dose–response curves were obtained, then NM was added intraluminally or extraluminally 10 minutes before repeating the 5-HT dose–response curves. The MCA sections were washed after exposure to NM until the control 5-HT response returned to its original value. Each section was exposed to both intraluminally and extraluminally placed NM in random order. Drug amounts are reported as final bath concentrations.

To determine if the blood–cerebrospinal fluid (CSF) barrier was still intact in this in vitro preparation, six MCA sections were prepared as for experimentation, then perfused with Evans blue/albunin (EBA) complex (200 mg Evans blue, 1 g albunin in 10 ml normal Krebs) for 10 minutes at a flow rate of 4.1 ml/min and 75 mm Hg pressure. After fixation in 10% buffered formalin, these sections were sectioned and examined for EBA leakage under a fluorescence microscope. Evans blue forms a complex with albumin and does not cross the blood-CSF barrier. Two coronary arteries from the same animal were similarly prepared and examined for EBA leakage. The EBA complex fluoresces red under the microscope (Hg lamp; HBO 200, primary filter; Schott BG12, secondary filter; Schott OG 4 [Yonkers, New York]).

The in vivo experimental protocol was approved by the Institutional Animal Care and Use Committee of Pennsylvania Hospital. Thirty-three cats weighing 3–5 kg were premedicated with 35 mg/kg i.p. ketamine hydrochloride and 0.05 mg/kg i.p. atropine sulfate. Standard surgical technique was used to catheterize the left femoral artery and vein. The arterial catheter was used to monitor systemic arterial blood pressure and to routinely sample arterial blood gases (Paco2 31.4±3.8 mm Hg, Paco2 105.8±10.7 mm Hg, pH 7.34±0.06; mean±SD). The venous catheter was used to administer various drugs. Body temperature was measured with a rectal probe and maintained with a warming blanket and/or heat lamp. The cats were then intubated, paralyzed with 0.05 mg/kg i.v. pancuronium bromide, and placed on a respirator breathing a 3:1 mixture of nitrous oxide: oxygen; 10 mg/kg i.m. ketamine hydrochloride and 0.05 mg/kg i.v. pancuronium bromide were administered every hour. Each cat was placed in a stereotactic frame and the scalp was incised from the nasion to the occipital condyles, exposing the skull. Electroencephalograms were recorded from both hemispheres using platinum electrodes. Cerebral blood flow (CBF) was measured by the hydrogen clearance technique. Two holes 1/16 in. in diameter were drilled at midparietal bone, approximately 1 cm lateral to the sagittal suture on each side, for the placement of the platinum electrodes. These electrodes were driven 3–5 mm into the previously drilled parietal holes, and a silver/silver chloride reference electrode was placed in the temporalis muscle. After baseline values (100%) for CBF, arterial blood gases, and systemic blood pressure were recorded, a 19-gauge angiographic catheter (Cordis Corporation, Miami, Florida) was placed into the cisterna magna just below the occipital bone. Backflow of clear CSF was used as an indication of an atraumatic insertion.

After baseline values were measured to ascertain the effects of the intrathecal catheter, the cats were divided into three groups. Eight cats served as controls and received no NM. Seven cats were pretreated with 200 µg NM dissolved in ethanol and injected into the carotid artery via the lingual artery (intraluminal group). Eight cats were pretreated with the same dose of NM injected intracisternally (extraluminal group). Preinjection CBF and blood pressure were recorded for all groups. Forty-five minutes later, the cats were injected with 5×105 M 5-HT hydrochloride dissolved in 0.5 ml saline warmed to room temperature into the cisterna magna. CBF and blood pressure were recorded within 10 minutes and thereafter every 30 minutes for 180 minutes, at which time the cats were killed by euthanasia. All experiments were performed between March 3 and May 17, 1988.

Angiograms were taken in 10 cats (four control, three intraluminal, and three extraluminal). A catheter was placed in the right common carotid artery to facilitate the injection of 3–4 ml contrast material (Renografin 76, Squibb, New Brunswick, New Jersey) into the cerebral circulation. The angiograms were taken on a 2-second delay, and the cats were placed in a flat posterior–anterior orientation with respect to the skull and film. Angiograms were taken before (baseline) and after NM injection. After the intracisternal 5-HT injection, films were obtained 30, 90, 150, and 210 minutes later. The developed angiograms showed the circle of Willis and its accessory vessels very clearly. The arteries examined included the MCA, the basilar, posterior communicating, and cavernous portion of the internal carotid arteries. The diameters of these vessels as percent of baseline were calculated using a Zeiss operating microscope equipped with a measuring reticle (Thornwood, New York), and the sum of the changes were considered.

The in vitro data were expressed as mean±SEM change in outside diameter and were analyzed using Student’s t test for unpaired data. For the in situ data, CBF and vessel diameter results were expressed as mean±SEM percent of baseline. Preliminary t tests comparing left and right CBF indicated no significant differences between groups. Changes in CBF and vessel diameter were compared among groups using a two-way analysis of variance (ANOVA) for repeated measures. Subsequently, the vessel diameter data were subjected to a one-way ANOVA and the Newman-Keuls method.
FIGURE 1. Graphs of effects of three concentrations of nimodipine (NM) on mean±SEM serotonin (5-HT)–induced contractions of bovine middle cerebral artery in vitro. NM was administered intraluminally (●, n=6) or extraluminally (○, n=6) 10 minutes before application of 5-HT. Though NM significantly (*p<0.05) reduced 5-HT–induced contractions compared with control (△, n=8), there was no difference between intraluminal and extraluminal routes.

for all pairwise comparisons. A probability level of p<0.05 was accepted as significant.

Results

In the in vitro experiments, only the extraluminal surface of a MCA section was exposed to 5-HT. NM effectively inhibited 5-HT–induced contractions, and pretreatment with 10^{-7} M NM reduced maximum contraction by >50%. There were no significant differences in the ability of intraluminally and extraluminally administered NM to inhibit a 5-HT–induced response to 10^{-8}, 10^{-7}, or 10^{-6} M NM (Figure 1).

In the six MCA sections perfused with EBA solution, no fluorescence was detected in the smooth muscle or adventitial layers; fluorescence was contained to the endothelial layer. In the two bovine coronary arteries, red fluorescence was detected evenly diffused through all layers of the cross-sectioned artery, indicating that, unlike in the MCA, a highly permeable endothelial layer existed in the coronary artery.

In the in vivo experiments, 5-HT injected intracisternally into the control cats reduced CBF by 40% in both hemispheres (Figure 2). CBF 10 minutes later (at 3 hours) was significantly depressed compared with baseline. Extraluminally and intraluminally administered NM increased CBF, but not significantly. In cats pretreated with NM, CBF significantly decreased (approximately 25%) immediately after the 5-HT injection. However, CBF returned to near-baseline levels by the end of the experiment. There was no significant difference between the intraluminal and extraluminal routes, nor between treated and control cats as to the effects of 5-HT on CBF.

NM reduced blood pressure by 25% when administered intraluminally and by 33% when administered extraluminally (Figure 3); a partial recovery occurred within 30 minutes. Serotonin decreased blood pressure by 35% in all groups, indicating that NM offered little protection against the systemic pressure effects of 5-HT.

In control cats, 5-HT significantly (p<0.01) reduced angiographic vessel diameter immediately, and this reduction persisted for approximately 120 minutes (Figure 4); by 180 minutes after 5-HT injection (at 6 hours), cerebral vessel diameters had returned to within 8% of baseline. Extraluminal and intraluminal NM produced immediate, but not significant, 22% and 3% increases in vessel diameter, respectively. In NM-treated cats, both routes of administration offered significantly more protection against the immediate 5-HT–induced contractions. By 120 minutes after 5-HT injection, the cerebral vessel diameters of the extraluminally treated group

FIGURE 2. Graph of effects of nimodipine (NM) on mean±SEM serotonin (5-HT)–induced changes in cerebral blood flow in cats in situ. NM (200 μg) was administered intraluminally (●, n=7) or extraluminally (○, n=8) 45 minutes before intracisternal injection of 5×10^{-4} M 5-HT. Control cats (△, n=8) received no NM. Blood flow at 3 hours was significantly (*p<0.05) less than baseline (100%) for all three groups.
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Figure 3. Graph of effects of nimodipine (NM) on mean±SEM serotonin (5-HT)-induced changes in systemic arterial blood pressure in cats in situ. NM (200 μg) was administered intraluminally (■, n=7) or extraluminally (□, n=8) 45 minutes before intracisternal injection of 5×10⁻⁴ M 5-HT. Control cats (△, n=8) received no NM. Pressure at 2 hours was significantly (*p<0.05) less than baseline (100%) for both NM-treated groups, and pressure at 3 hours was significantly less than baseline in all three groups. Effects in the extraluminal groups were significantly greater than those in the control or intraluminally treated groups. In intraluminally treated cats, cerebral vessel diameter remained reduced by 12%, similar to control, by 180 minutes.

Discussion

We attempted to clarify the controversy that surrounds the use of calcium antagonists to prevent cerebral ischemia associated with vasoconstriction. Our results indicate that there is little difference in the effectiveness of NM whether given intraluminally or extraluminally and that there is not always a direct relation between changing CBF and angiographically documented changes in cerebral vessel diameter. Our experiments focused on the prophylactic use of NM since many in vitro studies have pointed out the specificity of calcium antagonists as inhibitors of cerebral vascular contractions as opposed to relaxing agents.22 In monkey cerebral vessels, Bevan et al23 found that diltiazem administered before experimental hemorrhage protects against arterial narrowing. Also, several in vivo studies have shown beneficial effects of pretreatment with NM.2,4

Our results indicate that NM offers significant protection against 5-HT-induced reductions in outer vessel diameter in vitro and against angiographic changes in vessel diameter in situ. The effects of 5-HT in these two models were similar. The maximum contraction produced by 5-HT in vitro was a 33% reduction in outer vessel diameter (data not shown), and the maximum reduction in vessel diameter as measured angiographically approached 30%. The inhibiting effect of NM in vitro was greater than that in situ. We believe this is due to the greater accessibility to the site of action in vitro compared with in situ. In all cases, 5-HT was given extraluminally. Review of the literature indicates that for a given species, cerebrovascular responses elicited by the intrathecal administration of vasoactive agents are similar to those obtained in corresponding isolated arteries.24-26 We used 5-HT in our experiments because of its potent cerebral vasoactivity27-28 and to avoid the possible vagaries of the blood-induced spasm model.29-30 It was also not possible to use whole blood in our in vitro model because blood clouded the bath, making it impossible to monitor changes in vessel diameter. Still, our results using 5-HT in vivo parallel the CBF, arterial systemic blood pressure,31 and angiographic32 changes seen in the blood-induced spasm and intracerebral hemorrhage models when examined over similar time frames.

Unlike other studies that seemed to favor the intrathecal route of administration,6-9,14 our results did not offer any clear advantages to either route. CBF in cats treated with intraluminal NM was not significantly different from that in extraluminally treated cats, whereas there was only one significant difference (at 5 hours) between the two routes in angiographic vessel diameter (Figure 4). Several other studies have shown varying and contradictory results with the use of calcium antagonists depending on the time or method of administration.5,7,9,14,33 One difference that should be pointed out is that in our in situ studies, NM was administered via the lingual artery to avoid the first-pass metabolism of the intravenous route. Total systemic clearance of the dihydropyridine calcium channel blockers is

Figure 4. Graph of effects of nimodipine (NM) on mean±SEM serotonin (5-HT)-induced contractions of cat cerebral arteries as seen angiographically in situ. NM (200 μg) was administered intraluminally (■, n=3) or extraluminally (□, n=3) 45 minutes before intracisternal injection of 5×10⁻⁴ M 5-HT. Control cats (△, n=4) received no NM. Diameter in both NM-treated groups was significantly (*p<0.05) greater than control at 3 and 4 hours; diameter in extraluminal group was significantly (>p<0.05) greater than control and intraluminal at 5 hours.
high and approaches liver blood flow when given intravenously or orally.\textsuperscript{34,35} The route of administration can influence the slope of the concentration-effect curve due to the formation of active metabolites or to stereoselective first-pass metabolism.\textsuperscript{36} This could explain the negative findings seen when NM is given systemically by the venous or oral route of administration.\textsuperscript{14,33}

Our angiographic and CBF data appear somewhat different, supporting other studies that have pointed out that there is not always a direct relation between changing CBF and angiographically documented changes in cerebral arterial diameter, at least not until the vessel constricts to \(\leq 50\%\) of control caliber.\textsuperscript{37-39} However, a number of studies indicate a good correlation between CBF and \(>50\%\) constriction on angiography,\textsuperscript{40-42} and this only adds to the confusion surrounding the use of calcium antagonists when attempts are made to document their effectiveness either angiographically or by changes in CBF.

Since in our in vitro experiments there was no significant difference in the ability of intraluminal or extraluminal NM to block 5-HT\textsuperscript{-}induced contraction, it is clear that NM easily passes through the endothelial layer to reach the smooth muscle cells. Histologic observations indicated an intact endothelial layer that was impermeable to the EBA lial layer.\textsuperscript{43} Also, the fact that in vivo, NM produced relatively equal drops in systemic arterial blood pressure within the same time frame regardless of the route of administration suggests that intracisternally administered NM quickly crosses the blood-brain barrier.

In conclusion, though we noted minor differences depending on how changes were documented, in general the intraluminal and extraluminal routes of administration of NM produced comparable results.

Acknowledgments

The authors wish to thank Chris Deitch for technical assistance and for help in preparation of the manuscript. Also, the statistical expertise of Dr. H. Menduke in the analysis of this data was greatly appreciated.

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**KEY WORDS** • nimodipine • serotonin • cats
Comparison of intraluminally versus extraluminally administered nimodipine on serotonin-induced cerebral vascular responses in vitro and in situ.

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*Stroke*. 1989;20:1065-1070
doi: 10.1161/01.STR.20.8.1065

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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