Effect of Nimodipine on Canine Cerebrovascular Responses to 5-Hydroxytryptamine and Potassium Chloride After Exposure to Blood

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The stainless steel cannula inserting method was used to investigate the blocking effects of nimodipine on vascular responses to intraluminal administration of 5-hydroxytryptamine (5-HT) or potassium chloride (KCl) before and after application of abluminal blood containing thrombin in isolated and perfused canine basilar arteries. A transient elevation of perfusion pressure was observed initially, and during the course of the experiment the perfusion pressure gradually increased. Nimodipine significantly depressed both transient and prolonged changes of perfusion pressure. Dose-dependent vasoconstriction induced by 5-HT was significantly enhanced, while that evoked by KCl was significantly attenuated for up to 8 hours after the application of blood. Pretreatment with nimodipine inhibited vasoconstriction to 5-HT less effectively than to KCl both before and after application of blood. The proportion of the 5-HT-induced vasoconstriction, which was sensitive to nimodipine, was reduced after application of blood, while no such change was observed in the responses to KCl. It is suggested that the augmentation of cerebrovascular responses to 5-HT in the early stage of subarachnoid hemorrhage may be mediated mainly by changes in intracellular calcium utilization rather than by the increase of calcium influx through nimodipine-sensitive channels. (Stroke 1989;20:105-111)

Cerebral vasospasm, resulting in cerebral ischemia, is a major cause of morbidity and mortality in patients with subarachnoid hemorrhage (SAH) following rupture of an intracranial aneurysm. Although the pathogenesis of vasospasm is still not well understood, treatment with calcium antagonists has been suggested as an effective therapy for the prophylaxis and reversal of cerebral vasospasm.

It is well known that calcium antagonists are effective in inhibiting the action of a variety of vasoactive compounds on isolated smooth muscle by their inhibition of the influx of calcium, which is required to develop the contraction. This led Allen and Bahr to examine the calcium antagonist nifedipine in a canine model of delayed cerebrovascular spasm, and the results were sufficiently encouraging for human studies to be conducted. The present consensus seems to be that the calcium antagonists are useful in reducing the occurrence of the delayed ischemic neurologic deficit that is associated with late vasospasm, although whether this action arises from an effect on vasospasm per se or from some other mechanism is controversial. Certainly, in several animal models even large doses of nimodipine are not effective in reversing established vasospasm, and there is little angiographic evidence that nimodipine reverses spasm in humans. Nimodipine reverses the contractile action of a variety of compounds in vitro when isolated preparations of cerebral artery are examined, although it has recently been shown that this agent is less effective as an antagonist for some agents than others, and it has been suggested that putative mediators of spasm may be poorly antagonized by the dihydropyridines.

Cerebrovascular spasm may be considered as two separate phenomena: early vasospasm, which occurs within a few minutes of the hemorrhage and which has not been unequivocally demonstrated in humans, and late vasospasm, the onset of which occurs after several days, which is clinically important and which can lead to delayed ischemic neurologic deficit. It is not known whether
The cannulated artery was placed in a 200-ml bicarbonate solution at 37° C oxygenated with 95% O2 and 5% CO2 by means of a peristaltic pump (Model P-3, Pharmacia, Uppsala, Sweden) and a thermopump (Model E 52, Haake, Saddle Brook, New Jersey). The millimolar composition of the solution was as follows: Na+ 132, K+ 5.9, Ca2+ 2.5, Mg2+ 1.2, Cl− 122.7, HCO3− 25, SO42− 1.2, H3PO4− 1.2, and dextrose 11. The pH of the solution was maintained at 7.2–7.4 because reactivity had been shown to be sensitive to changes in pH in vascular preparations. The perfusion pressure was continuously measured with a pressure transducer (Model P23 ID, Gould, Oxnard, California) connected to a polygraph (Model 7D, Grass, Quincy, Massachusetts). Vasoconstriction and vasodilatation were recorded as an increase and a decrease in perfusion pressure, respectively. The preparation was allowed to equilibrate for 1 hour. Further details of this method have been published.

The cannula inserting method, which was originally developed by Hongo and Chiba for measuring vascular responsiveness of relatively large arteries of dogs, has been modified by Tsuji and Chiba to apply to various smaller vessels. Recently, it was found that this method could be applied to the examination of isolated basilar arteries of monkeys or dogs. Using the stainless steel cannula inserting method, we studied the blocking effects of nimodipine on 5-HT- and KCl-induced vasoconstrictions in isolated and perfused canine basilar arteries before and several hours after exposure of the artery to whole blood.

Materials and Methods

We anesthetized 27 mongrel dogs of either sex weighing 15–28 kg with 30 mg/kg i.v. sodium pentobarbital and killed them by rapid exsanguination. The basilar artery with a 5-mm thickness of the brainstem attached was carefully removed, and a stainless steel cannula with three small holes 3–7 mm from the distal sealed end (21 or 23 gauge; 0.83 or 0.68 mm o.d., respectively, and 3 or 4 cm in length) was inserted into the lumen of the basilar artery under magnification. The distal part of the artery was tied to the cannula. Two long steel clips were placed close to and parallel with the basilar artery and attached to a plastic plate to avoid leakage of the solution from small branches. It was thus possible to supply the perfusion stream from the holes of the cannula as a unidirectional flow through the intraluminal surface of the isolated artery. The cannula was slightly thinner than the artery, and the flow rate (2.0–3.0 ml/min) was determined at the beginning of the experiment to obtain a baseline perfusion pressure of approximately 50 mm Hg (50.2±3.1 mm Hg, mean±SEM) in the resting state. The time from isolation to perfusion of the artery was approximately 1 hour. The cannulated artery was placed in a 200-ml cup-shaped glass bath and perfused with Krebs' bicarbonate solution at 37° C oxygenated with 95%
Nimodipine itself produced little change in perfusion pressure at doses from 0.1 to 10 µg. Nimodipine administration sometimes caused a small vasoconstriction of <10 mm Hg, which proved to be due to the vehicle, ethanol, while an equally small but prolonged vasodilation appeared to arise from the effects of nimodipine itself. A higher dose of nimodipine (10 µg) significantly inhibited 5-HT-induced vasoconstriction apparently in a noncompetitive fashion, whereas lower doses of nimodipine (0.1, 1 µg) had no significant effect (Figure 1). The vasoconstriction evoked by 1 µg 5-HT was reduced to 60% after 10 µg nimodipine. On the other hand, increasing doses of nimodipine (0.1-10 µg) blocked the vasoconstriction induced by KCl to a much greater extent than that induced by 5-HT (Figure 2). Vasoconstriction caused by 3 mg KCl was almost completely attenuated after 10 µg nimodipine.

When Krebs' solution was replaced with whole blood and thrombin, a transient elevation of perfusion pressure was immediately observed. Intraluminal perfusion of the basilar artery with Krebs' solution was maintained continuously during this procedure. To ensure that this effect did not arise from temperature change on administration of the clot, control experiments were performed using Krebs' solution kept at room temperature. The effects of this treatment were very small and transient. The baseline perfusion pressure gradually increased for several hours after blood application. This change, which presumably arose from increased reactivity of the vessel arising from agents associated with or released from the clot, was not caused by mechanical obstruction of the perfusion system since the luminal surface was not exposed to the clot. After pretreatment with 10 µg nimodipine the maximum increase in perfusion pressure and the duration of the initial response were depressed significantly as was the prolonged elevation of baseline perfusion pressure. These results are shown in Table 1.

After blood application, vasoconstriction induced by 0.01, 0.1, and 1 µg 5-HT was significantly augmented, while that induced by 1 and 3 mg KCl was greatly attenuated. The vasoconstriction induced by 1 µg 5-HT was substantially larger 4 and 8 hours after exposure to blood (Figure 3), while the maximum response to 3 mg KCl was reduced over the first hour to approximately 60% of the control value, after which it remained almost stable (Figure 4).

The 5-HT-induced vasoconstriction was enhanced and KCl-induced vasoconstriction was reduced 4 hours after exposure to blood even in preparations treated with a high dose (10 µg) of nimodipine immediately before testing with the agonist. Figure 5 shows the dose–response curves to 5-HT and KCl.
Table 1. Effects of Nimodipine on Responses of Dog Basilar Artery to Clot of Autologous Blood Containing Thrombin

<table>
<thead>
<tr>
<th>Transient response</th>
<th>Control</th>
<th>10 μg nimodipine</th>
</tr>
</thead>
<tbody>
<tr>
<td>mm Hg</td>
<td>22.0±3.9</td>
<td>5.5±0.6*</td>
</tr>
<tr>
<td>n</td>
<td>14</td>
<td>13</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Elevation of baseline perfusion pressure</th>
<th>1 hr after blood treatment</th>
<th>4 hr after blood treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>mm Hg</td>
<td>7.8±1.8</td>
<td>38.5±6.6</td>
</tr>
<tr>
<td>n</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>mm Hg</td>
<td>2.4±0.7*</td>
<td>15.8±2.4*</td>
</tr>
<tr>
<td>n</td>
<td>8</td>
<td>13</td>
</tr>
</tbody>
</table>

*p<0.005, 0.01, respectively, different from control.

Discussion

Whether vascular reactivity of isolated cerebral arteries to vasoactive substances in the late stage of vasospasm after SAH is increased or decreased is still controversial. 5-HT has been reported to produce a decreased response in cerebral arteries after SAH in dogs19 and monkeys,11 whereas other reports suggested an increase in dogs,28 cats,17 and rabbits.18,20 The response to KCI decreases in monkeys11 and dogs19,28 but increases in rabbits.29 There have been few examinations of the changes in vascular responsiveness that occur shortly after SAH, despite the fact that these changes may be critical to the development of late vasospasm. Toda et al19 observed that contractile responses to 5-HT in canine middle cerebral arteries 2 hours after experimental SAH were slightly enhanced vis-à-vis their contralateral control, but this increase was not significant; no changes in response to KCI were observed. Young et al20 reported that rabbit basilar artery exhibited an initial reduction in response to 5-HT up to 6 hours after SAH and then hypersensitivity, which was maximal 36 hours after SAH; the response to KCI was increased at 1 hour, decreased at 12 hours, and increased again at 36 hours although the significance of these changes remains unknown.

We used a different methodology from that usually employed for the examination of vascular reactivity in vitro. Our method has a number of advantages over the use of rings or helical strips. First, it is easy to apply blood or putative spasmogens from blood to only the abluminal surface, while drugs can be applied to the interior of the vessel. This pro-

![Graph 3](image3.png)

**Figure 3.** Effect of blood treatment on response to 5-hydroxytryptamine (5-HT) of dog basilar arteries before and 1, 4, and 8 hours after exposure to blood. Vertical bars represent standard errors. *p<0.05, **p<0.01, ***p<0.005 different from control.

![Graph 4](image4.png)

**Figure 4.** Effect of blood treatment on response to potassium chloride (KCI) of dog basilar arteries before and 1, 4, and 8 hours after exposure to blood. Vertical bars represent standard errors. *p<0.05, **p<0.01, ***p<0.005 different from control.
provides a much closer approximation of the situation in vivo than preparations in which both drugs and spasmogens have access to both surfaces. In addition, the vessel can be perfused at significant pressures, and vasodilator action can be assessed without the need for preconstriction. The advantages of the intraluminal cannula perfusion method have been discussed by Hongo and Chiba.

We show increased vascular reactivity to 5-HT and decreased reactivity to KCl in dog basilar arteries after exposure to blood. In addition, pretreatment with nimodipine was found to be less effective in antagonizing 5-HT–induced vasoconstriction than KCl–induced vasoconstriction in dog basilar arteries both before and after treatment with blood. This is in agreement with previous findings in rat coronary arteries and dog middle cerebral and basilar arteries.

Nimodipine is relatively long-acting in an isolated tissue experiment, and after a single administration nimodipine attenuates the response of agonists for >1 hour. In our experiments much less time elapsed before tests with the agonists were conducted. It is difficult to compare doses administered in the experimental arrangement we have employed with those in other systems, but we observed reversal of potassium-induced contractions of approximately 50% with 0.1 μg nimodipine, whereas in a conventional organ bath arrangement using arterial rings, this occurs at approximately 10⁻⁹ M. This is approximately the concentration of nimodipine in the cerebrospinal fluid of patients receiving nimodipine in the first clinical trial of nimodipine in cerebrovascular spasm (mean concentration 1.8×10⁻⁹ M). Little is known about the effects of fluctuating plasma concentrations on the effects of these drugs on vascular reactivity.

5-HT

KCl

FIGURE 5. Effect of exposure to blood on response to 5-hydroxytryptamine (5-HT) and potassium chloride (KCl) of dog basilar arteries pretreated with 10 μg nimodipine. O, before; ●, 4 hours after treatment. Vertical bars represent standard errors. *p<0.05, **p<0.01, ***p<0.005 different from before.

FIGURE 6. Effects of nimodipine before and 4 hours after exposure to blood on vasoconstriction induced by 1 μg 5-hydroxytryptamine (5-HT) in dog basilar arteries. Vertical bars represent standard errors. *p<0.05, **p<0.01 different from preparations without pretreatment with nimodipine before application of blood and 4 hours thereafter.

FIGURE 7. Effects of nimodipine before and 4 hours after exposure to blood on vasoconstriction induced by 3 mg potassium chloride (KCl) in dog basilar arteries. Vertical bars represent standard errors. ***p<0.005 different from preparations without pretreatment with nimodipine before application of blood and 4 hours thereafter.
Calcium antagonists exert their actions mainly by blocking the entry of calcium ions from the extracellular space into the smooth muscle cell, although they may have some intracellular effects. Since 5-HT depolarizes the membrane of dog basilar arteries, 5-HT-induced cerebral vasoconstriction may be due to the influx of extracellular calcium ions through potential-operated channels (POCs) as well as through receptor-operated channels (ROCs). Depolarization of the cell membrane by KCl causes contraction of the smooth muscle cells by enhancing an influx of calcium ions from the extracellular space through POCs.

The actions of nimodipine alone include a small vasodilation and a partial inhibition of both the transient elevation of perfusion pressure that immediately follows the application of blood and the prolonged rise in baseline perfusion pressure that occurs over the first 4 hours. These effects of nimodipine are probably mediated by its role in inhibiting calcium entry.

There is no clear evidence as to what proportion of the response of cerebral blood vessels depends on calcium entry and what proportion arises from activation of intracellular calcium stores. In the case of KCl, it would appear that the great majority of the effects arise from entry of calcium through nimodipine-sensitive POCs, but the effects of 5-HT may show species dependence. Tsuji and Chiba concluded that 5-HT-induced vasoconstriction of dog basilar arteries might arise in part from activation of an intracellular calcium pool rather than by causing the influx of calcium from the extracellular space.

After exposure to blood the effect of 5-HT was considerably enhanced, whereas nimodipine did not comparably enhance blocking activity against the 5-HT-induced vasoconstriction. Thus, nimodipine reduces the perfusion pressure by the same amount before and after exposure to blood (viz: approximately 15 mm Hg). This implies that the increase in response to 5-HT seen during the first 4 hours is likely to arise from changes in the availability of intracellular calcium stores or from entry of calcium through nimodipine-insensitive channels. Indeed, the decreased response to KCl may reflect a decreased ability of the cell membrane to transport calcium into the cell through POCs.

While there is little evidence to support the view that late vasospasm is caused solely by 5-HT, it may be that this agent plays an early role. Furthermore, if the changes in the response to 5-HT represent changes in the nature of the steps in the activation of the contractile machinery rather than merely changes at the level of the 5-HT receptor, as seems likely from our studies, a number of other agonists will be similarly affected.

Recently, Rosenwasser et al observed that the calcium antagonist lidoflazine prevented the contraction of rat basilar artery induced by in vivo subarachnoid perfusion with 5-HT or whole blood. On the other hand, McCalden et al showed that in vivo, the cerebral vessels of baboons showed increased sensitivity to infused 5-HT after SAH and that nimodipine produced cerebral vasodilatation after SAH but did not significantly reduce the vasoconstrictor response to 5-HT. Furthermore, Sahlin et al reported that the contractile response of baboon cerebral arteries to 5-HT was increased after SAH and that the dilating effect of nimodipine to contraction by 5-HT was less pronounced following SAH. Tanaka and coworkers have also reported that in cats the inhibition by nimodipine of the response to blood shortly after SAH seems to be smaller, although a direct comparison was not made.

We have shown that oral treatment with nimodipine had little effect on the reactivity of isolated monkey cerebral arteries in chronic vasospasm but that it did enhance the reactivity of contralateral control arteries to 5-HT, KCl, and norepinephrine. The discrepancies among changes in cerebrovascular reactivity to nimodipine after SAH might occur from differences among species, type and dose of calcium antagonist, time after SAH, measuring techniques, and so on. Further experimental studies may help to clarify the mechanisms of enhanced vasoconstriction to 5-HT and reduced vasoconstriction to KCl in dog basilar arteries several hours after exposure to blood. The changes in the artery that result from application of blood are key factors in our understanding of the cause of vasospasm. While surgical manipulation and clot removal at present offer the best options for patients with a SAH, the hope remains that an understanding of the changes in the artery that result in spasm will lead to a specific pharmacologic therapy.

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