The effect of hemoglobin on vasodilatory effect of calcium antagonists in the isolated rabbit basilar artery

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ALTHOUGH the pathogenesis of cerebral vasospasm after subarachnoid hemorrhage (SAH) remains poorly understood, a large number of agents have been used to prevent or reverse arterial narrowing. Since the constrictor responses of cerebral arteries have been demonstrated to be more dependent on extracellular Ca++ than other vessels, calcium antagonists have received the most attention. Among them, nimodipine has been investigated most intensively. Recent clinical trials suggest that nimodipine reduces the incidence of delayed ischemic neurological deficits, but has no major effect on angiographically visualized vasospasm.

Nimodipine is effective in dilating or preventing cerebral vasoconstriction induced by various agents in vitro. However, in several animal models of vasospasm after SAH, even large doses of nimodipine are not effective in reversing established vasospasm. The reason for the inefficacy of calcium antagonists is unclear, although some putative spasmogens may utilize intracellular Ca++ in their contractile response.

A number of vasoactive substances released from the subarachnoid clot have been postulated as spasmogens. Hemoglobin, one of the most widely studied spasmogens, has also been shown to potentiate vasoconstriction induced by other substances as well as inhibit endothelium-dependent relaxation. However, the sensitivity of calcium antagonists to hemoglobin-induced contraction is controversial, and the efficacy of calcium antagonists on vasoconstriction potentiated by hemoglobin or elicited in endothelium-denuded specimens of cerebral artery has not been reported.

We employed an isometric tension measurement technique in rabbit basilar artery to investigate the influence of hemoglobin on the ability of calcium antagonists, especially nimodipine, to inhibit the contraction elicited by other substances. High K+-depolarization and serotonin (5-hydroxytryptamine, 5-HT) were used as vasoconstrictors since nimodipine was reported to be more effective on vasoconstriction induced by these agents.

Materials and Methods

Animal and Artery Preparation

Adult male New Zealand White rabbits, each weighing 3.2 to 4.2 kg, were anesthetized with an intramus-
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cular injection of a mixture of ketamine (20 mg/kg) and xylazine (5 mg/kg), and were sacrificed by exsanguination from the femoral artery. The brain, with the basilar artery in situ, was removed and placed in a dissecting chamber filled with a modified Krebs bicarbonate solution (see below). The basilar artery was dissected free under magnification, and arterial rings 3 mm in length were cut.

In some rings, the endothelial cells were removed by gentle rubbing with a No. 24 polytetrafluoroethylene catheter* inserted into the lumen of the rings. Fine circular serrated edges had been made on the surface of the catheter using a surgical knife.

Each arterial ring was suspended between two L-shaped stainless-steel rods in an organ bath with a 10-ml working volume of Krebs solution, which was aerated with 95% O2/5% CO2. The pH of the solution ranged between 7.4 and 7.5. Isometric tension was recorded using a force-displacement transducer and was displayed on a chart recorder.† The preparations were allowed to equilibrate at 37°C for 90 minutes before use, and resting tension was adjusted to 400 mg. In order to confirm appropriate contractile activity in each specimen, the contractile response to 40 mM KCl was first obtained in each ring segment. In the endothelium-denuded ring, it was confirmed that 10^{-4} M acetylcholine did not elicit relaxation during contraction with 10^{-6} M of 5-HT.

Experimental Protocol

Effect of Nimodipine on Hemoglobin-Induced Contraction. After the contraction elicited by 10^{-3} M hemoglobin had stabilized, nimodipine (10^{-11} to 10^{-7} M) was added in a cumulative fashion to obtain a dose-response curve. At the end of this experiment, 10^{-4} M papaverine was added, and the relaxation induced by nimodipine was expressed as a percentage of the maximum relaxation elicited by 10^{-4} M papaverine. Rings that did not show a significant contraction to 10^{-3} M hemoglobin were eliminated from this analysis.

Effect of Hemoglobin on Relaxation Elicited by Calcium Antagonists. In the next series of experiments, two arterial rings were selected randomly from the same basilar artery and were subjected to different treatments. In one (control) ring, the effect of the calcium antagonist was investigated. The other ring was treated with hemoglobin (10^{-2}, 10^{-6}, or 10^{-3} M) 20 minutes before receiving the same procedures.

For relaxation studies, precontraction was induced with 40 mM KCl in the control and hemoglobin-treated rings. After the contraction had stabilized, a calcium antagonist was added cumulatively to obtain a dose-response curve. After each concentration of the calcium antagonist, the preparation was allowed to reach equilibration before the next was added. Relaxation was expressed as a percentage of the maximum relaxation obtained with 10^{-4} M papaverine. The following calcium antagonists were used: nimodipine, 10^{-11} to 10^{-5} M; nicardipine, 3 x 10^{-11} to 3 x 10^{-6} M; verapamil, 3 x 10^{-6} to 3 x 10^{-7} M; and diltiazem, 10^{-1} to 10^{-4} M.

Effect of PGF{sub}2{alpha}, Albumin, and Endothelial Removal on Relaxation Elicited by Nimodipine. The effects of prostaglandin F{sub}2{alpha} (PGF{sub}2{alpha}), albumin, and endothelial removal were compared with the effect of hemoglobin on the relaxation elicited by nimodipine. Prostaglandin F{sub}2{alpha} (10^{-8} M), which evoked a mild lasting contraction, or albumin (10^{-5} M), which is a protein with a molecular weight similar to hemoglobin, was administered instead of hemoglobin 20 minutes before application of 40 mM KCl. The relaxing effects of nimodipine were investigated in the pairs of control rings and albumin-treated, PGF{sub}2{alpha}-treated, or endothelium-denuded rings by the same procedures as those in the pair of control and hemoglobin-treated rings.

Effect of Hemoglobin on the Ability of Nimodipine to Inhibit CaCl{sub}2-Induced Contraction. For further analysis of the effect of hemoglobin, vasoconstriction induced by increasing the CaCl{sub}2 concentration following incubation with nimodipine was investigated in control and hemoglobin-treated rings. In this experiment, HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid) solution was used as the bathing medium to prevent precipitation of calcium, and the solution was aerated with 100% O2. At first, a pair of rings was incubated for 10 minutes in a Ca^{2+}-free HEPES solution containing 0.2 mM ethyleneglycolbis(β-aminoethyl ether)-N,N'-tetra-acetic acid (EGTA); the solution was then replaced by 60 mM of a K^{+}-rich, Ca^{2+}-free HEPES solution. At the same time, one paired ring was treated with 10^{-3} M hemoglobin and was designated as the hemoglobin-treated ring. Twenty minutes after incubation, 0.03 to 20 mM CaCl{sub}2 was added cumulatively to establish dose-response curves for CaCl{sub}2 without nimodipine in both paired rings. A 30-minute equilibration in normal HEPES solution followed; these procedures were repeated in the presence of 10^{-10} or 10^{-9} M nimodipine. Nimodipine was administered 15 minutes before the application of 0.03 mM CaCl{sub}2 in each series.

To quantify the antagonistic effect of nimodipine, "inhibitory values" were calculated by the following formula in both the control and hemoglobin-treated rings at each concentration of CaCl{sub}2: inhibitory value (%) = 100 x (1 - (contraction with nimodipine/contraction before nimodipine)).

Effect of Hemoglobin on the Ability of Nimodipine to Inhibit 5-HT-Induced Contraction. In this experiment, three arterial rings were selected randomly from the same rabbit basilar artery. One was used as the control ring, another was denuded of endothelium, and

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* Catheter manufactured by Critikon, Tampa, Florida.
† Force-displacement transducer, Model FT.03, manufactured by Grass Instrument Co., Quincy, Massachusetts; chart recorder, Model 3418, manufactured by Soltec Corp., San Fernando, California.
Fig. 1. Dose-response curve showing the effect of nimodipine on 10^{-3} M hemoglobin-induced contraction of rabbit basilar artery. Relative relaxation was induced by nimodipine in 10 arterial rings; 10^{-4} M papaverine-induced relaxation was considered 100%. Significant relaxation was produced by 10^{-6} to 10^{-7} M nimodipine. Vertical bars indicate standard errors of the mean. * = p < 0.05.

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the third was pretreated with 10^{-5} M hemoglobin 20 minutes before application of 5-HT.

Contractile responses elicited by 10^{-6} M of 5-HT were recorded until the tension stabilized (10 to 15 minutes) in each ring. After a 20-minute equilibration in Krebs solution, the procedures were repeated in the presence of 10^{-10} to 10^{-7} M nimodipine. Nimodipine was administered 15 minutes before the application of 5-HT. Contraction elicited by 10^{-4} M of 5-HT was expressed as a percentage of the 40 mM KCl-induced contraction in the same ring. The contraction of the rings incubated with nimodipine was expressed as a percentage of the contraction in the same rings before incubation.

Solution and Drugs

The composition of the modified Krebs bicarbonate solution was (mM): NaCl 120; KCl 4.5; MgSO_{4} 1.0; NaHCO_{3} 27.0; KH_{2}PO_{4} 1.0; CaCl_{2} 2.5; and dextrose 10.0. In the HEPES solution, 10 mM HEPES was substituted for 27 mM NaHCO_{3} and 1.0 mM KH_{2}PO_{4} of the modified Krebs solution. The pH of this solution was adjusted to 7.4 with NaOH. The Ca^{2+}-free HEPES solution was made by excluding CaCl_{2} from the HEPES solution (pH 7.4). The K^{+}-rich, Ca^{2+}-free HEPES solution contained 60 mM KCl in equimolar exchange for NaCl of the Ca^{2+}-free HEPES solution (pH 7.4).

The following pharmacological agents were used: 5-HT (5-HT creatinine sulfate), acetylcholine, PGF_{2alpha}, EGTA, papaverine, nicardipine, verapamil, diltiazem, albumin (bovine albumin, free of fatty acid), human hemoglobin, and nimodipine. Nimodipine (10^{-2} M) was dissolved in 95% ethanol and protected from light. Further dilutions were made with distilled water, and the highest ethanol concentration used in the organ bath was 0.001%. The human hemoglobin solution was prepared according to the method of Martin, et al. All other drugs were dissolved in distilled water, such that volumes of less than 0.1 ml were added to the organ bath.

Statistical Analysis

The data were expressed as means ± standard error of the means. In the rings contracted by high K+, statistical comparison between responses of the control rings and the hemoglobin-treated, albumin-treated, PGF_{2alpha}-treated, or endothelium-denuded rings were made using Student's t-test for unpaired observations. Dose-response curves for hemoglobin or 5-HT were analyzed using general linear models of analysis of variance. Scheffé's test was used for multiple comparisons to analyze concentration differences or differences between treatment groups. P values of less than 0.05 were considered to be statistically significant.

TABLE 1

<table>
<thead>
<tr>
<th>Study</th>
<th>No. of Rings</th>
<th>Precontraction (g/m²)†</th>
<th>Maximum Relaxation (%)‡</th>
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<tr>
<td>nimodipine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control rings</td>
<td>6</td>
<td>2.06 ± 0.33</td>
<td>101.77 ± 1.14</td>
</tr>
<tr>
<td>10^{-7} M Hb-treated</td>
<td>5</td>
<td>2.21 ± 0.27</td>
<td>100.86 ± 0.54</td>
</tr>
<tr>
<td>rings</td>
<td></td>
<td>(0.04 ± 0.01)</td>
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</tr>
<tr>
<td>control rings</td>
<td>8</td>
<td>2.16 ± 0.28</td>
<td>101.41 ± 0.80</td>
</tr>
<tr>
<td>10^{-6} M Hb-treated</td>
<td>8</td>
<td>2.45 ± 0.28</td>
<td>100.15 ± 0.51</td>
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<tr>
<td>rings</td>
<td></td>
<td>(0.09 ± 0.02)</td>
<td></td>
</tr>
<tr>
<td>control rings</td>
<td>8</td>
<td>2.05 ± 0.22</td>
<td>100.97 ± 0.84</td>
</tr>
<tr>
<td>10^{-5} M Hb-treated</td>
<td>8</td>
<td>2.30 ± 0.24</td>
<td>98.05 ± 1.04</td>
</tr>
<tr>
<td>rings</td>
<td></td>
<td>(0.13 ± 0.02)</td>
<td></td>
</tr>
<tr>
<td>control rings</td>
<td>8</td>
<td>1.83 ± 0.19</td>
<td>102.34 ± 1.22</td>
</tr>
<tr>
<td>10^{-6} M PGF_{2alpha}</td>
<td>8</td>
<td>2.11 ± 0.22</td>
<td>101.72 ± 0.82</td>
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<tr>
<td>treated rings</td>
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<td>(0.18 ± 0.06)</td>
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<tr>
<td>control rings</td>
<td>8</td>
<td>2.01 ± 0.14</td>
<td>104.08 ± 1.80</td>
</tr>
<tr>
<td>10^{-3} M albumin-</td>
<td>8</td>
<td>1.94 ± 0.11</td>
<td>102.92 ± 1.06</td>
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<tr>
<td>treated rings</td>
<td></td>
<td>(0.12 ± 0.06)</td>
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<tr>
<td>control rings</td>
<td>8</td>
<td>1.99 ± 0.25</td>
<td>103.44 ± 0.80</td>
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<td>endothelium-denuded</td>
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<td>2.28 ± 0.16</td>
<td>106.40 ± 1.37</td>
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<td>rings</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

* Data are expressed as means ± standard error of the means.

† Precontraction was induced by 40 mM KCl with and without pretreatment. Data in parentheses represent contractile force induced by pretreatment (Hb or PGF_{2alpha}).

‡ Maximum relaxation was induced by 10^{-4} M papaverine. Data were expressed as percentage of the precontraction.

*Data are expressed as means ± standard error of the means.

Hb = hemoglobin; PG = prostaglandin. There was no significant difference in either precontraction or relaxation values between control and pretreated rings.

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Fig. 2. The effect of nimodipine on 40 mM KCl-induced contraction in control and hemoglobin (Hb)-treated rings of rabbit basilar artery. a: Typical traces of the responses of control (left) and 10^{-5} M Hb-treated (right) rings. b, c, and d: Dose-response curves showing the relative relaxation induced by nimodipine in control (closed circles) and 10^{-7} M (b), 10^{-6} M (c), and 10^{-5} M (d) Hb-treated (open circles) rings. Relaxation induced by 10^{-4} M papaverine was considered 100%. Vertical bars indicate standard errors of the mean. *=p<0.05 and **=p<0.01 (control vs. Hb-treated rings).

Results

Effect of Nimodipine on Hemoglobin-Induced Contraction

Nimodipine (10^{-9} to 10^{-7} M) relaxed the rabbit basilar artery precontracted by 10^{-7} M hemoglobin. The relaxation produced by the highest concentration of nimodipine (10^{-7} M) was 62.31\% ± 6.01\% in 10 arterial rings (Fig. 1).

Effect of Hemoglobin on the Relaxation Elicited by Calcium Antagonists

Although hemoglobin induced mild contractions, there was no significant difference in amplitude of precontractions between the control and hemoglobin-treated rings. There was also no significant difference in 10^{-4} M papaverine-induced relaxation between the control and hemoglobin-treated rings (Table 1). The cumulative addition of nimodipine at 10^{-12} to 10^{-8} M caused a dose-dependent relaxation in both rings. However, pretreatment with hemoglobin reduced the relaxation in a dose-related manner. The relaxation induced by 10^{-10} to 10^{-8} M nimodipine was significantly attenuated by pretreatment with 10^{-6} or 10^{-5} M hemoglobin (Fig. 2).

The relaxing responses induced by the other calcium antagonists were also less in the 10^{-5} M hemoglobin-treated rings than in the control rings. The difference was statistically significant in the presence of 3 \times 10^{-10} to 3 \times 10^{-9} M nicardipine, 3 \times 10^{-6} to 3 \times 10^{-7} M verapamil, and 10^{-7} to 10^{-6} M diltiazem (Fig. 3).

Effect of PGF_{2\alpha}, Albumin, and Endothelial Removal on Relaxation Elicited by Nimodipine

Although 10^{-6} M PGF_{2\alpha} induced mild contraction, there was no significant difference in amplitude of precontractions between the control and PGF_{2\alpha}-treated rings (Table 1). There was also no significant difference between the relaxations induced by nimodipine in the control and PGF_{2\alpha}-treated rings (Fig. 4a). Relaxation induced by nimodipine in the albumin-treated rings and endothelium-denuded rings did not differ from that in the control rings (Fig. 4b and c).

Effect of Hemoglobin on the Ability of Nimodipine to Inhibit CaCl_2-Induced Contraction

In Ca^{2+}-free HEPES buffer containing 60 mM KCl, the addition of increasing concentrations of CaCl_2 up to 10 mM produced concentration-dependent increases in the tension of the rings. Nimodipine displaced the CaCl_2 curves to the right and at the same time dimin-
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FIG. 3. Dose-response curves showing the effect of nicardipine (a), verapamil (b), or diltiazem (c) on 40 mM KCl-induced contraction in control (closed circles) and hemoglobin (Hb)-treated (open circles) rings of rabbit basilar artery. Data are expressed as a percentage of the relaxation induced by 10^{-4} M papaverine. Eight arterial rings were tested in each group. Vertical bars indicate standard errors of the mean. * = p < 0.05 and ** = p < 0.01 (control vs. Hb-treated rings).

ished the maximum responses to CaCl_{2} in both the control and the hemoglobin-treated rings. This displacement of the curves suggests noncompetitive antagonism by nimodipine. Hemoglobin-treated rings showed an increase in the contraction. Without nimodipine, however, the difference was not statistically significant, except for the contraction elicited by 0.03 mM CaCl_{2}. On the other hand, with nimodipine, the contraction induced by 0.3 to 20 mM CaCl_{2} in the hemoglobin-treated rings was significantly different from that of the control rings (Fig. 5a and b).

The “inhibitory values” for each concentration of CaCl_{2} are shown in Fig. 5c. The difference between the values of the control and hemoglobin-treated rings increased at the higher concentrations of CaCl_{2}. The difference was statistically significant at 10 and 20 mM CaCl_{2} with 10^{-9} M nimodipine and at 1 to 20 mM CaCl_{2} with 10^{-8} M nimodipine.

**Effect of Hemoglobin on the Ability of Nimodipine to Inhibit 5-HT-Induced Contraction**

The application of 10^{-6} M of 5-HT produced a steep rise in tension (phasic contraction), sometimes followed by transient relaxation, with a subsequent slow ascent and descent in tension to a sustained plateau (tonic contraction) (Fig. 6a).

The phasic contraction elicited by 10^{-6} M of 5-HT was 44.96% ± 5.25%, 62.62% ± 1.93%, and 75.14% ± 4.49% in eight control, endothelium-denuded, and hemoglobin-treated rings, respectively. The tonic contrac-

![Dose-response curves showing the effect of nimodipine on 40 mM KCl-induced contraction in control (closed circles) and pretreated (open circles) rings of rabbit basilar artery. Prostaglandin F_{2alpha} (PGF_{2alpha})-treated rings (a), albumin-treated rings (b), and endothelium-denuded rings (c) were used as pretreated rings. Data are expressed as a percentage of the relaxation induced by 10^{-4} M papaverine. Eight arterial rings were tested in each group. Vertical bars indicate standard errors of the mean. There was no significant difference in nimodipine-induced relaxation between the control and the pretreated rings.]

![Dose-response curves showing the effect of nicardipine (a), verapamil (b), or diltiazem (c) on 40 mM KCl-induced contraction in control (closed circles) and hemoglobin (Hb)-treated (open circles) rings of rabbit basilar artery. Data are expressed as a percentage of the relaxation induced by 10^{-4} M papaverine. Eight arterial rings were tested in each group. Vertical bars indicate standard errors of the mean. * = p < 0.05 and ** = p < 0.01 (control vs. Hb-treated rings).]

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Fig. 5. The effect of nimodipine on 0.03 to 20 mM CaCl2-induced contraction (in a K+-rich, Ca++-free HEPES solution) in control and hemoglobin (Hb)-treated rings of rabbit basilar artery. a: Experimental sequence and typical traces of the responses of control (upper) and Hb-treated (lower) rings. b: Contractile force (gm) induced by CaCl2 in control (closed symbols) and Hb-treated (open symbols) rings incubated with 10^{-12} M nimodipine (hexagons), 10^{-9} M nimodipine (triangles), or before incubation (circles). c: The ability of 10^{-10} M nimodipine (upper) and 10^{-9} M nimodipine (lower) to inhibit the contraction at each concentration of CaCl2 in control (closed bars) and Hb-treated (open bars) rings. Inhibitory value (%) = 100 \times (1 - \text{ contraction without nimodipine/contraction before nimodipine}). Eight arterial rings were tested in each group. Vertical bars indicate standard errors of the mean. * = p < 0.05; ** = p < 0.01; *** = p < 0.001 (control vs. Hb-treated rings).

Fig. 6. The effect of nimodipine on 10^{-9} M serotonin (5-HT)-induced contraction in control, hemoglobin (Hb)-treated, and endothelium-denuded rings of rabbit basilar artery. a: Experimental sequence and typical traces of the responses of control (upper), endothelium-denuded (center), and Hb-treated (lower) rings. Responses of the rings to 10^{-10} M nimodipine are not included. b: Contraction induced by 10^{-6} M of 5-HT consisting of a phasic contraction (left) and a tonic contraction (right). Data are expressed as a percentage of the contraction induced by 40 mM KCl. Hemoglobin and removal of endothelium enhanced both phases of the contraction. NS = not significant; * = p < 0.05. c: Contraction induced by 10^{-6} M of 5-HT with nimodipine (10^{-10} to 10^{-7} M) in control (closed circles), Hb-treated (open circles), and endothelium-denuded rings (triangles). Data are expressed as a percentage of the contraction without nimodipine. Significant inhibition of the phasic contraction (left) was induced by 10^{-8} and 10^{-7} M nimodipine in control and endothelium-denuded rings. Tonic contraction (right) was inhibited by 10^{-6} to 10^{-5} M nimodipine in all three groups. Eight arterial rings were tested in each group. Vertical bars indicate standard errors of the mean.
The tonic contraction was sensitive to nimodipine, being significantly inhibited by \(10^{-10}\) M nimodipine in every ring type; the phasic contraction was less sensitive to nimodipine, but was significantly inhibited by \(10^{-8}\) and \(10^{-7}\) M nimodipine in the control and endothelium-denuded rings. On the other hand, in the hemoglobin-treated rings, the concentrations of \(10^{-10}\) to \(10^{-7}\) M nimodipine tested in this experiment did not inhibit the phasic contraction induced by \(10^{-6}\) M of 5-HT (Fig. 6c).

Discussion

Effect of Hemoglobin on the Cerebral Artery

The effects of hemoglobin demonstrated in this study are summarized as follows: 1) hemoglobin reduced the ability of nimodipine to reverse or prevent contraction induced by high K⁺ depolarization; 2) hemoglobin enhanced both the nimodipine-sensitive tonic contraction and the less sensitive phasic contraction elicited by 5-HT; and 3) hemoglobin abolished the nimodipine-induced inhibition of the phasic contraction elicited by 5-HT. These results indicate that the efficacy of nimodipine decreases in the presence of hemoglobin.

The mechanism of the vasoconstriction induced by hemoglobin has not been clearly understood. While processes mediated by the production of free radicals and stimulation of prostaglandin synthesis have been postulated, there has been little investigation of the mobilization of \(Ca^{++}\) induced by hemoglobin. The sensitivity of the contraction to calcium antagonists is also controversial. In our experiments, rabbit basilar arteries in which hemoglobin elicited significant contraction were partially relaxed by nimodipine. Prostaglandin \(F_2\alpha (10^{-6} \text{M})\) induced almost the same degree of vasoconstriction as hemoglobin, but did not significantly alter the effect of hemoglobin. Hemoglobin is also known to potentiate vasoconstriction induced by other vasoactive agents and to inhibit the endothelium-derived relaxing factor (EDRF). Using isolated rabbit basilar artery, Hongo, et al., showed that the ability of hemoglobin to augment contraction induced by KCl and 5-HT was almost abolished in endothelium-denuded rings. They suggested that the augmentation observed in intact rings was possibly mediated by an inhibition of spontaneously released EDRF. Our results, however, indicate that the effect of hemoglobin on the artery is not identical to endothelial removal.

It may also be noted that the potency of calcium antagonists in endothelium-denuded artery has been controversial. Further investigations using cerebral arteries are required because endothelial damage and the impairment of endothelium-dependent relaxation of the artery after SAH have been reported.

Effect of Hemoglobin on Nimodipine

The mechanism by which hemoglobin attenuates the effect of nimodipine has not been resolved by the present experiments. However, we considered two major possibilities for the mechanism: 1) hemoglobin inactivates nimodipine by direct interaction, and 2) the influence of hemoglobin on \(Ca^{++}\) regulation of vascular smooth-muscle cells causes reduction in the effect of nimodipine.

Although albumin is reported to bind some calcium antagonists, it did not alter the effect of nimodipine at the same concentration as hemoglobin. Hemoglobin reduced the effect of the other calcium antagonists, nicardipine (dihydropyridines), verapamil (phenylalkylamines), and diltiazem (benzothiazepines), which have different chemical structures and physical characteristics. These findings suggest that the effect of hemoglobin results from a mechanism other than binding to nimodipine. However, nimodipine is also considered to be highly protein-bound in serum. The possibility cannot be excluded that hemoglobin binds nimodipine with much more affinity than albumin.

Effect of Hemoglobin on the Regulation of Intracellular \(Ca^{++}\)

Calcium antagonists relax vascular smooth-muscle cells by restricting \(Ca^{++}\) influx via calcium channels. Therefore, if hemoglobin is able to increase the permeability of the plasma membrane, reduce the function of the \(Ca^{++}\) extrusion system, or increase the utilization of intracellularly stored \(Ca^{++}\) by other substances, the effectiveness of calcium antagonists is likely to decrease. There are some studies supporting this possible mechanism.

It is well known that production of lipid peroxides may be initiated by activated oxygen produced during auto-oxidation of oxyhemoglobin. This process can cause cell damage by involving the cellular membrane. Recently, Schachter, et al., showed that even low concentrations of oxygen free radicals increased intracellular \(Ca^{++}\) in cultured vascular smooth-muscle cells. This rise in \(Ca^{++}\) was mostly due to \(Ca^{++}\) influx from the extracellular medium, which was insensitive to nifedipine and diltiazem. Their results may be consistent with our findings that hemoglobin restored the \(CaCl_2\)-induced contraction inhibited by nimodipine in the presence of higher concentrations of \(Ca^{++}\).

Hemoglobin inhibits the relaxation induced by nitrovasodilators and EDRF. The relaxation is known to be associated with increasing levels of cyclic guanosine monophosphate (cGMP) in vascular smooth-muscle cells. Recently, hemolysate has been reported to inhibit the cerebrovascular relaxation induced by vasodilator nerve activation. This inhibition was also suggested to result from decreased cGMP production. Although the mechanism by which cGMP produces the relaxation of vascular smooth muscle remains undetermined, it has been suggested that cGMP primarily decreases the intracellular-free \(Ca^{++}\) concentration. Cyclic GMP has been shown to activate the plasma membrane \(Ca^{++}\)-adenosine triphosphatase, reduce the release of intracellularly stored \(Ca^{++}\), and inhibit \(Ca^{++}\) influx. Hemoglobin may potentiate contraction or
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inhibit relaxation by the inhibition of these molecular events. If this is true, the ability of calcium antagonists is likely to be attenuated in the presence of hemoglobin.

Onoue, et al., investigated the effect of prolonged exposure of canine cerebral arterial segments to oxyhemoglobin, and reported impaired activity of the electrogenic sodium pump. This may also alter the regulation of intracellular Ca++ concentration mediated by both membrane polarization and the Ca++ extrusion mechanism.38

Contribution to Delayed Vasospasm

In the monkey model or canine multiple-hemorrhage model of vasospasm, chronic arterial narrowing has been reported to have either reduced or no sensitivity to nimodipine.12,13 The arterial segments from these animal models have been reported to have decreased distensibility and contracility.10 In the baboon model of vasospasm, nimodipine was shown to be less efficient in vessel segments obtained after SAH.27 These studies suggest that some pathological changes in the arterial wall in chronic vasospasm prevent the effect of calcium antagonists. On the other hand, several drugs, such as intracellular calcium antagonists, calmodulin antagonists, or a myosin light chain kinase inhibitor, have been shown to induce significant dilation of vasospasm in the canine two-hemorrhage model.11,13,15 This suggests that vasospasm is, to some extent, smooth-muscle cell contraction.

Recently, Takenaka, et al.,22 reported a sustained increase in the concentration of cytosolic calcium ([Ca++]i) induced by exposure to oxyhemoglobin using cultured vascular smooth-muscle cells. The [Ca++]i elevation was considered to be caused by the influx of extracellular Ca++, but was insensitive to verapamil. They mentioned that oxyhemoglobin may contribute to damage and abnormal contraction in vascular smooth muscle cells. Wellum, et al.,39 suggested that the alteration of intracellular free Ca++ regulation of arterial smooth-muscle cells might be involved in delayed vasospasm. Hemoglobin may contribute to such an alteration of Ca++ regulation.

Conclusions

The results of these experiments demonstrate that hemoglobin decreases the vasodilatory effect of calcium antagonists in isolated rabbit basilar artery. This finding may be one reason why the calcium antagonists are not more successful in the management of angiographic vasospasm. It should be noted that our results do not disaffirm any possible therapeutic action of the calcium antagonists on the spastic artery. Rather, we suggest that more attention be given to the administration of the drugs (including dosage and route) in order to maximize the therapeutic effect.

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