Potentiating effects of extraluminal oxyhemoglobin to intraluminal 5-hydroxytryptamine in isolated canine internal carotid arteries

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The interaction between oxyhemoglobin (oxyHb) and 5-hydroxytryptamine (5-HT) was investigated in an experimental model of isolated canine internal and common carotid arteries with insertion of stainless steel cannulae. Extraluminal application of $10^{-5} \text{M}$ oxyHb induced marked and long-lasting vasoconstriction in the internal carotid but not in the common carotid arteries. The 5-HT-induced vasoconstriction was potentiated significantly in both the internal and common carotid arteries. These potentiations were not influenced by the presence or absence of endothelium, a finding which was confirmed by vascular responses to intraluminal acetylcholine. It is concluded that the interaction between extraluminal oxyHb and intraluminal 5-HT may be one of the possible etiological factors behind the chronic phase of vascular spasm following subarachnoid hemorrhage.

Key Words • internal carotid artery • oxyhemoglobin • endothelium • 5-hydroxytryptamine • vasospasm • dog

Although numerous endogenous substances have been considered to participate in causing cerebral vasospasm, it is agreed that hemoglobin is one of the factors responsible for vasospasm following subarachnoid hemorrhage (SAH). This is especially true of oxyhemoglobin (oxyHb), a substance that produces much greater vasoconstriction than does methemoglobin, which is generated by auto-oxidation of oxyHb. On the other hand, there have been few investigations of the interaction between oxyHb and other agonists, even though the potentiating effect of oxyHb to fibrin degradation products has been examined. It has been controversial whether sensitivity to vasoconstrictive agents in arteries exposed to SAH increases or decreases.

This study has attempted to investigate the interaction between extraluminally administered oxyHb and intraluminally infused 5-hydroxytryptamine (5-HT) in an in vitro model of isolated and perfused canine intracranial arteries with insertion of stainless steel cannulae. The study was undertaken because extravascular oxyHb may interact with 5-HT liberated from blood platelets aggregating on the internal surface of the artery after SAH. In addition, the endothelium was removed with intraluminal saponin treatment to study both the influence of the endothelium on the constrictor responses to oxyHb and its interaction with a vasoactive amine.

Materials and Methods

Twenty-four mongrel dogs of either sex, each weighing 8 to 16 kg, were anesthetized with intravenous sodium pentobarbital (30 mg/kg) and sacrificed by rapid exsanguination from the right common carotid artery after treatment with intravenous sodium heparin (200 U/kg). The principles and details of arterial preparation have been described in previous articles. Basically, a cylinder-like arterial vessel was isolated and intraluminally perfused with a modified Krebs solution, the constituents of which are (mM): NaCl 118, KCl 4.7, CaCl$_2$ 2.5, KH$_2$PO$_4$ 1.3, MgCl$_2$ 1.2, NaHCO$_3$ 25, and glucose 5.6 in 1000 ml of distilled water. For this, the internal carotid arteries of the intracranial cavernous segment (1.0 to 2.0 mm in outer diameter and 10 to 18 mm long) and the common carotid arteries (2.5 to 3.2 mm in outer diameter and 25 to 30 mm long) were removed and their branches were ligated. The surrounding connective tissues were carefully dissected. The cavernous segment of the inter-
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FIG. 1. Schematic representation of the perfusion circuit of an isolated vessel with Krebs-Ringer solution. Oxyhemoglobin (oxyHb) is applied extraluminally to the bath, and norepinephrine and 5-hydroxytryptamine are administered intraluminally. This method was modified from that of Hongo and Chiba.12

A stainless steel cannula was inserted carefully into each isolated artery so as to avoid injury to the internal surface of the vessel. Various sizes of cannulae were used such that the outer diameter of each was slightly thinner than the inner diameter of its corresponding artery, yet it had sufficient caliber to obtain an adequate perfusion pressure (30 to 80 mm Hg). Each cannula had three small holes 5 mm from its distal blind end (see Fig. 1). The distal part of each isolated artery was tied with a thin thread to the blind end of the cannula. Thus, the stream of modified Krebs solution, which was bubbled with a mixture of 95% O2/5% CO2, passed through the holes in the cannula and over the intraluminal surface of the isolated artery at a constant flow rate of 1.6 ml/min by means of an infusion pump.*

The isolated arteries were placed in a 100-ml organ bath which, together with the perfusion system, was warmed with a circulator Thermopump† at a constant temperature of 37°C. The perfusion pressure was measured with an electric manometer and a carrier amplifier for monitoring the vasoconstriction in relation to increases in perfusion pressure.‡ A recorder documented the mean value of the perfusion pressure throughout the experiments.§ The equilibration time for the specimens in the organ bath was over 60 minutes.

Drug injection was performed after the perfusion pressure returned to the basal resting level. At least 5 minutes was allowed before drug injection to prevent tachyphylaxis. The volume of a single dose of the drug solution injected by microsyringe into the rubber tubing (which was closely connected to the shank of the specimen) was 0.01 to 0.03 ml, and the injection time was 4 seconds.

The data are presented as means ± standard error of the means. An analysis of variance with replicates was used to evaluate any differences in the responses among the study groups. Student’s t-test was used, and a p value of 0.05 was considered significant. Drugs injected intraluminally were 5-HT creatinine sulfate, acetylcholine chloride (ACh), imipramine hydrochloride, and human hemoglobin. The oxyHb was prepared in the

* Microtube pump, Model MP-3A, manufactured by Tokyo Rikakikai, Tokyo, Japan.
† Thermopump manufactured by Haake Buchler Instruments, Saddle Brook, New Jersey.
‡ Manometer, Model MPU-0.54A, manufactured by Nihon Kohden Kogyo, Tokyo, Japan; amplifier, a biophysiograph 180 system, manufactured by Sanei Sokki Co., Tokyo, Japan.
§ Rectigraph, Model WT-645G, manufactured by Nihon Kohden Kogyo, Tokyo, Japan.
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FIG. 2. Effects of extraluminal oxyhemoglobin (oxyHb) and intraluminal saponin on vasoconstrictor responses to 5-hydroxytryptamine (5-HT) in isolated canine internal carotid arteries (left) and common carotid arteries (right).

Fig. 3. Effects of intraluminal acetylcholine (ACh) on internal carotid artery preparations without saponin treatment (upper tracing) and those treated with 1 mg of saponin (lower tracing). Intraluminally administered ACh clearly induced vasodilation of the nontreated preparation that was preconstricted by extraluminal 5-hydroxytryptamine (5-HT) at 0.67 × 10⁻⁴ M.

Reduced form as described by Martin, et al., and applied extraluminally to the bath. Saponin for removal of endothelium was dissolved in 0.9% saline.

Results

When 10⁻⁵ M oxyHb was administered extraluminally to the internal carotid artery of the dog, the perfusion pressure gradually increased, reaching a maximum level within approximately 15 minutes which continued for over 1 hour. At 30 minutes after administration of oxyHb, the perfusion pressure in 14 specimens had increased 32 ± 5.7 mm Hg (range 32 ± 2.8 to 63 ± 5.9 mm Hg) (Fig. 2 left). On the other hand, in the common carotid arteries, oxyHb caused no significant vasoconstriction at 10⁻⁵ M (Fig. 2 right).

When 1 or 3 mg of saponin was given intraluminally, vasoconstrictor responses immediately occurred in the internal carotid arteries but not in the common carotid arteries. As has been reported by others, the endothelium had entirely disappeared after treatment with 1 or 3 mg of saponin. In the present study, ACh clearly induced vasodilation when administered intraluminally to the nontreated preparation preconstricted by extraluminal 5-HT. Figure 3 shows the effects of intraluminal ACh on the internal carotid artery. After saponin treatment, ACh did not produce vasodilation, indicating disappearance of the endothelium. Figure 4 shows the effects of intraluminal ACh on saponin-treated and nontreated common carotid arteries. Following a 1- or 3-mg dose of saponin administered intraluminally to the internal and common carotid arteries, the basal perfusion pressure was usually increased 4 to 25 mm

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Hg and became stable within 30 minutes at a higher level than the control value. The basal perfusion pressure of eight internal carotid arteries treated with saponin increased 31 ± 3.9 mm Hg (range 49 ± 9.8 to 81 ± 9.6 mm Hg) after extraluminal administration of 10⁻⁵ M oxyHb, while that of the common carotid arteries did not increase.

In internal carotid arteries, 5-HT produced vasoconstriction in a dose-related manner in the dose range of 0.001 to 0.3 μg (Fig. 2 left). At 0.3 μg, 5-HT usually induced a maximum increase in perfusion pressure over 150 mm Hg (Fig. 5). The 5-HT-induced vasoconstrictions were prominently potentiated after extraluminal application of oxyHb. Saponin treatment did not significantly modify 5-HT-induced responses, as shown in Fig. 2 left. The potentiating effect of oxyHb to 5-HT was also demonstrated after removal of the endothelium. A summary of the data is shown in Fig. 5 left.

In common carotid arteries, 5-HT produced vasoconstriction in a dose-related manner in the dose range of 0.003 to 0.3 μg. The 5-HT-induced vasoconstrictions were usually enhanced by 10⁻⁵ M of extraluminally administered oxyHb as shown in Fig. 2 right. After 3 mg of saponin, 5-HT-induced vasoconstrictor responses were enhanced significantly by extraluminal oxyHb at doses of 0.1 and 0.3 μg. The data are summarized in Fig. 5 right. Intraluminal injection of 0.1 mg of imipramine, which was an adequate dose to inhibit neuronal uptake of amines, did not significantly potentiate vasoconstrictor responses to 5-HT in the internal and common carotid arteries in each of the five experiments.

**Discussion**

In the present study, the following points were confirmed. 1) Persistent vasoconstrictor responses of the internal carotid arteries were produced by 10⁻⁵ M of extraluminally administered oxyHb in vessels with and without endothelium, while in common carotid arteries the same concentration of oxyHb failed to produce clear vasoconstriction. 2) Vasoconstrictor responses to 5-HT both in the internal and common carotid arteries were potentiated significantly by oxyHb without regard to the presence of endothelium. 3) Removal of the endothelium did not significantly influence the responses to 5-HT.

It has been shown that products of the hemolysis of erythrocytes, especially oxyHb, may play an important role in the development of chronic cerebral vasospasm.
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spasm. In previous investigations of vasocostriction responses to oxyHb performed with helically cut or ring-form arteries in vitro, oxyHb could not be administered selectively to the outer surface of the vessels, as occurs after SAH. The present studies have demonstrated that selective administration of extraluminal oxyHb at a concentration of $10^{-5}$ M induced vasoconstriction that persisted more than 1 hour in the intracranial internal carotid arteries in vitro. Martin, et al., reported that oxyHb at $10^{-6}$ M reduced and at $10^{-3}$ M abolished the endothelium-dependent relaxation induced by ACh in rabbit aortal arterial rings, suggesting that oxyHb inhibited the relaxation by binding the endothelium-derived relaxing factor (EDRF). Moreover, selective blockade of the endothelium-dependent relaxation by oxyHb was reported to be one of the possible causes of cerebral vasospasm, because EDRF is spontaneously released to maintain the basal tone of the artery. The slightly elevated perfusion pressure of the internal carotid arteries after removal of the endothelium in our present experiments may be explained by a lack of the basal liberation of EDRF. Removal of the endothelium, however, did not modify the oxyHb-induced vasoconstriction. It is likely that oxyHb-induced constrictions might occur independent of the presence of EDRF. The finding that $10^{-3}$ M oxyHb strongly constricted the internal carotid arteries but not the common carotid arteries is in agreement with a previous report that cerebral arteries are much more strongly contracted by oxyHb than are arteries from other anatomical locations. The lower sensitivity of the common carotid artery to the concentration of oxyHb used may relate to properties of the blood vessel wall, such as rates of diffusion of hemoglobin molecules, wall thickness, and sensitivity to hemoglobin and/or its derivatives.

When oxyHb was administered extraluminally, arterial constriction began immediately and reached its maximum level within 15 minutes in spite of the large molecular size of oxyHb. This may indicate that a low-molecular product of oxyHb, one of which may be a superoxide anion or a metabolite of arachidonic acid, contributes to the development of vasospasm.

Cerebral vessels in contact with SAH have an increased sensitivity to several vasoactive agents, including K⁺, Ca²⁺, norepinephrine (NE), and 5-HT. However, Toda, et al., have presented evidence that these vessels become hyposensitive to NE and 5-HT. In the present study, the potentiating effects of hemoglobin to 5-HT were observed both in the intra- and extracranial arteries regardless of whether the endothelium was present or absent. Lobato, et al., have suggested that supersensitivity of the cerebral arteries might be explained on the basis of the transient denervation of the cerebral arteries induced by SAH. We demonstrated that vasoconstriction of the isolated perfused arteries by 5-HT was not significantly influenced by imipramine, which blocked the uptake of amines into the nerve terminal, suggesting that the potentiating effect of oxyHb to 5-HT-induced vasoconstriction is dependent mainly on a postsynaptic mechanism.

Although 5-HT, which is liberated from subarachnoid clots, is one of the most potent vasocostricter amines in the cerebral arteries, Osaka suggested that 5-HT plays a role in developing only early vasospasm and not chronic spasm. On the other hand, 5-HT is known to be liberated from blood platelets aggregated at the sites of intimal damage and to produce vasoconstriction. Morphological changes of the endothelium begin to be observed at 2 hours after SAH by scanning electron microscopy, and adherence and aggregation of circulating platelets subsequently takes place at the denuded regions and separated intercellular junction of the endothelium. It is concluded that the interaction between oxyHb and 5-HT released from platelets may act as an etiological factor in the chronic phase of vascular spasm following SAH.

References

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