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KEYWORDS: vasospasm, methemoglobin, spasmolytic agent, ascorbic acid

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CEREBRAL ARTERIAL SPASM
II. ETIOLOGY AND TREATMENT OF EXPERIMENTAL CEREBRAL VASOSPASM

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Abstract. Delayed cerebral vasospasm is caused by excessive accumulation of dopamine-β-hydroxylase (DBH) and noradrenaline in cerebral vessel walls. This study demonstrates the mechanisms of delayed spasm, particularly the role of red blood cell components, and the successful relief of delayed cerebral vasospasm. Spasmogenic substances which contained a heme component, such as methemoglobin, methemalbumin and catalase enhanced DBH activity in human serum as measured by a one step chemical spectrophotometric assay. The concentration which gave the highest DBH activity caused the maximum constriction of the basilar artery, when the substances were applied topically. Among components of red cells, methemoglobin, methemalbumin, catalase and nicotinamid adenin dinucleotide (NADH) caused constriction of basilar artery in cats, when applied topically, whereas hematin, hemin and bilirubin caused no significant spasm. An oxyhemoglobin solution obtained by mixture with methemoglobin and ascorbic acid produced no significant vascular spasm either. Relief of delayed cerebral vasospasm was obtained with topical application of specific alpha adrenergic blocking drug such as phenoxybenzamine, specific inhibitors of DBH such as fusaric acid, o-phenanthroline and \( \alpha \alpha ' \) dipyridyl, \( \beta _2 \) adrenergic stimulants such as salbutamol, and a phosphodiesterase inhibitor, ascorbic acid.

Keywords: vasospasm, methemoglobin, spasmolytic agent, ascorbic acid

The occurrence of both pre-and postoperative spasm is often one of the major factors necessitating delay in definitive surgical treatment (1, 2). Pharmacological treatment to prevent or relieve cerebral vasospasm would facilitate the effective management of patients with intracranial aneurysms.

As mentioned in part I of this study, the elevation of noradrenaline (NA) and increased dopamine-β-hydroxylase (DBH) activity in arterial walls caused long-lasting spasm. In addition, components of red blood cells such as methemoglobin seemed to be concerned with the occurrence of late spasm (3). But it is not known whether this noradrenergic mechanism is affected by spasmogenic substances derived from the components of red cell after subarachnoid hemor-
rhage (SAH).

Therefore, an understanding of these mechanisms is necessary before satisfactory approaches to the therapy of delayed cerebral arterial spasm can be devised. The present experiment was designed to clarify the mechanism of delayed spasm, specifically the role of the components of red cells on the activity of DBH, and to find successful treatment for delayed spasm.

MATERIALS AND METHODS

Adult cats weighing from 3.0 to 3.5 kg were anesthetized with intramuscular Ketamine hydrochloride (2-[0-chlorophenyl]-2-[methylamino] cyclohexane hydrochloride) (20 mg/kg) after having received atropine (0.03 mg/kg). After tracheal intubation, animals were paralyzed with intramuscular succinylcholin chloride at a dosage of 1 to 2 mg/kg and ventilated with a Takaoka respirator at a specified volume and rate of frequency.

Experimental SAH was produced by injection of 2 to 4 ml of fresh autogenous arterial whole blood into the cisterna magna after removal of an equal volume of cerebrospinal fluid.

The exposure of the basilar artery was performed according to the method already described (3).

The exposed vessel was photographed using Fuji color film with a 35 mm Nikon camera attached to the operating microscope. The serial pictures obtained from each experiment were projected in a standard manner with a 35 mm slide projector; this enabled direct measurement of the vessel diameter so that percentage change in vessel diameter could be calculated.

DBH activity in human serum was measured by a modification of the Nagatsu's method (4) which permits an assay of the maximum velocity of reaction at a saturating concentration of substrate under optimum conditions. Human serum with DBH activity of 40 to 50 μmol octopamine formed per min per liter serum at 37°C, measured by the Nagatsu's method, was used as the source of enzyme.

The incubated mixture contained: human serum 200 μl; 1 M sodium acetate buffer, 200 μl (pH 6.0); 0.1 M N-ethylmaleimide, 100 μl; 20 μM CuSO₄, 50 μl; 0.2 M sodium fumarate 50 μl; 0.2 M ascorbic acid, 50 μl; 0.4 M tyramine, 50 μl and various concentrations of methemoglobin or catalase or methemalbumin, 50 μl. A sample of boiled serum (95°C for 5 min) was used as a blank.

The reaction mixtures were incubated at 37°C for 60 min in air with continuous shaking. The incubation was stopped by adding 0.2 ml of 3 M trichloroacetic acid and the mixture was centrifuged at 2,500 rpm for 10 min. The supernatant fluid was transferred to a small glass column (0.5-10 cm) of Dowex 50W-X4 (H⁺200-400 mesh) (packed vol, 0.2 ml) and the absorbed amines were eluted with 1.0 ml of 3 N NH₃OH into a 20 ml centrifuge tube. Octopamine in the eluate was converted to p-hydroxybenzaldehyde by adding 10 μl of 0.2% NaIO₄ solution. Excess NaIO₄ was reduced by adding 10 μl of 10% Na₂S₂O₅ solution.
Experimental Cerebral Vasospasm

The absorbance at 330 nm was measured with a model Hitachi single wavelength spectrophotometer.

RESULTS

Effects of methemoglobin, catalase and methemalbumin on dopamine-β-hydroxylase in human serum. As shown in Fig. 1 and Table 1, DBH in human serum was most active after incubation with methemoglobin at a concentration of 0.25 mg/ml, whereas higher concentrations of methemoglobin (more than 0.5 mg/ml) were inhibitory.

The effect of catalase as well as methemalbumin on DBH activity in human serum was similar to that of methemoglobin.

Thus the optimum concentration of catalase which gave maximum DBH activity was 1000 units and that of methemalbumin was 0.25 mg/ml.

![Graphs showing effects of various concentrations of methemoglobin, catalase and methemalbumin on DBH activity.](image)

Fig. 1. Effects of various concentrations of methemoglobin, catalase and methemalbumin. DBH activity in human serum was the most active with the following concentrations: methemoglobin (0.25 mg/ml), catalase (1000 units) and methemalbumin (0.25 mg/ml). Higher concentrations of methemoglobin (more than 0.5 mg/ml), catalase (more than 2000 units) and methemalbumin (more than 0.5 mg/ml) inhibited DBH activity.
TABLE 1. Effects of various concentrations of methemoglobin, catalase and methemalbumin on DBH activity in human serum.

<table>
<thead>
<tr>
<th>Methemoglobin (mg/ml)</th>
<th>DBH Activity (%)</th>
<th>Catalase (units)</th>
<th>DBH Activity (%)</th>
<th>Methemalbumin (mg/ml)</th>
<th>DBH Activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.125</td>
<td>113±0.45*</td>
<td>500</td>
<td>107±0.36*</td>
<td>0.125</td>
<td>104±0.73*</td>
</tr>
<tr>
<td>0.250</td>
<td>120±0.32*</td>
<td>1000</td>
<td>110±0.52*</td>
<td>0.250</td>
<td>107±0.87*</td>
</tr>
<tr>
<td>0.375</td>
<td>109±0.93*</td>
<td>1500</td>
<td>105±0.89*</td>
<td>0.375</td>
<td>101±0.42</td>
</tr>
<tr>
<td>0.500</td>
<td>85±0.56*</td>
<td>2000</td>
<td>90±0.34*</td>
<td>0.500</td>
<td>92±0.35*</td>
</tr>
<tr>
<td>0.750</td>
<td>82±0.78*</td>
<td>3000</td>
<td>87±0.52*</td>
<td>0.750</td>
<td>90±0.85*</td>
</tr>
<tr>
<td>1.000</td>
<td>80±0.73*</td>
<td>4000</td>
<td>85±0.78*</td>
<td>1.000</td>
<td>87±0.76*</td>
</tr>
</tbody>
</table>

The enzyme activities are expressed as a percentage of (mean±SE) of the control (100%). *Significantly different from control (p<0.001).

The effects of various components of red cell on the basilar artery. Basilar arteries were exposed transectively under a microscope in 6 cats.

Basilar arteries in all the animals were maximally constricted 5 min after a 2 min topical application of various substances (methemoglobin (less than 0.5 mg/ml), methemalbumin (less than 0.5 mg/ml), catalase (less than 2000 units), reduced nicotinamide adenine dinucleotide (NADH) (1 mg/ml). No significant spasm was observed with the topical application of hematin (5 mg/ml), hemin (5 mg/ml), or bilirubin (5 mg/ml). An oxyhemoglobin solution obtained by mixture with methemoglobin (0.25 mg/ml) and ascorbic acid (50 μg/ml), produced no significant vascular spasm (Fig. 2).

![Figure 2: Percent change in vessel diameter. The most prominent spasm was induced by 2 minutes' long topical application of methemoglobin (0.25 mg/ml) which also gave the highest DBH activity, whereas no significant spasm was demonstrated with the topical application of hematin, hemin, bilirubin and oxyhemoglobin which was obtained after the mixture of methemoglobin (0.25 mg/ml) and ascorbic acid (50 μg/ml).](http://escholarship.lib.okayama-u.ac.jp/amo/vol32/iss1/4)
The most prominent spasm was demonstrated with the topical application of methemoglobin (0.25 mg/ml) which gave the highest DBH activity in the human serum. The percent reduction in the caliber produced by topical application of methemoglobin (0.25 mg/ml) ranged from 43 to 60% with an average of 52% (S. D. ± 5.3%). Higher concentrations of methemoglobin (more than 1.0 mg/ml) inhibited DBH activity and caused no significant constriction of the basilar artery.

Methemalbumin (0.25 mg/ml) as well as catalase (1000 units) also caused prominent spasm with the concentration which gave the highest DBH activity in human serum.

From these results it was deduced that the intensity of spasm was related to the elevation of DBH activity in the vessel wall caused by the concentration gradient of each substance in red cell components.

The relief of the experimental late spasm produced with or without noradrenaline. A solution of phenoxybenzamine (POB) and fusaric acid was applied directly to the constricted basilar artery in 6 cats 3 days after SAH. A specific alpha-adrenergic blocking drug such as POB (1 to 5 mg/ml, pH 1.5) and a specific inhibitor of DBH such as fusaric acid (10 mg/ml, pH 2) caused relief of vasospasm (Fig. 3).

With POB (5 mg/ml), the magnitude of the vascular dilatation was as pronounced as with fusaric acid (10 mg/ml).

![Fig. 3. Effects of various drugs on cerebral vasospasm 3 days after subarachnoid hemorrhage. Specific alpha-adrenergic blocking drugs such as POB and specific inhibitors of DBH such as fusaric acid not only caused relief of vasospasm, but also dilatation of vessels. No significant spasm of these dilated vessels was demonstrated with a topical application of various spasmogenic substances.](image)
The increase in the vessel diameter 20 min after the topical application of POB (5 mg/ml) ranged from 31 to 50% with an average of 40% (S. D. ± 4.8%).

POB (5 mg/ml) as well as fusaric acid (10 mg/ml) caused dilatation of vessels which lasted longer than 4 h. With topical application of various spasmodogenic substances (serotonin (100 ng/ml), NA (10 µg/ml), PGE$_1$ (10 µg/ml), PGF$_2$α (10 µg/ml), fresh and incubated blood), significant constriction of the dilated vessels was not observed (Fig. 3).

Vasospasm was produced by the topical application of NA (5 to 10 ng/ml) for 2 min, to the basilar artery in 5 cats, 3 days after SAH. The basilar artery was observed microscopically after the irrigation of the artery with physiological saline (Fig. 4). The basilar artery constricted maximally 5 min after the topical application of NA and returned to normal size within 15 min.

![Diagram showing effects of various drugs on cerebral vasospasm 3 days after experimental subarachnoid hemorrhage. A 40% decrease of basilar artery diameter was produced by topical application of noradrenaline (NA: 5-10 ng/ml). Topical application of several agents for 5 min produced relief of spasm within 10 min. Topical application of mixed fluid with noradrenaline (NA) and phenoxybenzamine (POB) produced no change in vessel diameter.]

Various vasodilating agents were applied topically 5 min after topical application of NA (5 to 10 ng/ml), when the basilar artery demonstrated maximum constriction.

POB (5 mg/ml), fusaric acid (10 mg/ml), dopamine (100 mg/ml) and salbutamol (1 mg/ml) which is a beta$_2$-adrenergic stimulant, caused relief of this vasospasm.

With topical application of POB (5 mg/ml) as well as fusaric acid (10 mg/ml), the average magnitude of the vascular dilatation that lasted longer than 4 h, was approximately 40% of the original caliber before topical application of
NA. With the topical application of dopamine (100 mg/ml), the average magnitude of the vascular dilatation lasting more than 1 h was approximately 20% of the original caliber.

With salbutamol (1 mg/ml), the magnitude of the vascular dilatation was not as pronounced as with POB (5 mg/ml). Salbutamol (1 mg/ml) caused dilatation that lasted about 30 min. Topical application of the solution with NA (5 ng/ml) and POB (0.5 mg/ml) produced no change in vessel diameter.

The relief of the experimental spasm produced by methemoglobin. Results were obtained from 7 cats 3 days after SAH.

Experimental vasospasm was produced by applying a small pledget soaked with methemoglobin (0.25 mg/ml) for a period of 2 min. Immediately after removal of the pledget photographs were taken and various solutions were applied topically. The initial vasoconstriction was stabilized at the end of 3 min after topical application of methemoglobin (0.25 mg/ml). Ascorbic acid (10 mg/ml, pH 7.0), o-phenanthroline (1 µg/ml), αα'-dipyridyl (1 µg/ml) and fusaric acid (10 mg/ml) caused relief of vasospasm (Fig. 5). With the topical

![Graph showing the effects of various drugs on cerebral vasospasm. A 40% decrease of basilar artery diameter was produced by topical application of methemoglobin (0.25 mg/ml). Topical application of several agents for 5 min produced relief of spasm. Ascorbic acid as well as fusaric acid not only caused relief of spasm, but also dilatation of vessels. The initial dilatation with O-phenanthroline as well as αα'-dipyridyl was the most rapid among these substances.](image)

application of ascorbic acid (100 mg/ml), the magnitude of the vascular dilatation was as pronounced as that with fusaric acid (10 mg/ml). The increase in the vessel diameter 20 min after the topical application of ascorbic acid (10 mg/ml) ranged from 20 to 40% with an average of 30% (± 5.3%) (Fig. 6).
Ascorbic acid as well as fusaric acid caused vasodilatation that lasted more than 4 h and the constricted vessel returned to the original caliber after topical application of o-phenanthroline (1 μg/ml) or aa'-dipyridyl (1 μg/ml). o-phenanthroline and aa'-dipyridyl made constricted vessels recover more rapidly than ascorbic acid or fusaric acid.

The dilatation with ascorbic acid was initiated earlier than that with fusaric acid.

Fig. 6. Photographs of the basilar artery in cat through a translcal approach. Left: Basilar artery just before application of methemoglobin (control). Center: The diameter of basilar artery markedly decreased after topical application of methemoglobin (0.25 mg/ml). Right: The diameter of basilar artery after topical application of methemoglobin (0.25 mg/ml) markedly increased following topical application of ascorbic acid (10 mg/ml).

DISCUSSION

From the author's analysis of the probable causes of vasospasm, the mechanism of early and late spasm is summarized as follows;

Early spasm may be produced by soluble DBH and NA discharge from sympathetic nerve terminals by a process of exocytosis when the nerve ending is depolarized by stimulation of spasmogenic substances such as serotonin (5, 6).

The reaction of NA receptor to extravascular serotonin is the essential process of early spasm. The depletion of NA and DBH from nerve terminals may result in a greater amount of granular vesicles in cell bodies such as the locus
The increase in DBH activity which produced elevations of NA concentration at the nerve terminals on the 3rd day following SAH, may be not only a consequence of increased formation of DBH in the perikaryon, but also a result of activating effects of substances which have heme components of the ferri type such as methemoglobin, methemalbumin and catalase.

Thus, late spasm is considered to be a consequence of the excessive and long-lasting release of increased NA from nerve terminals (Fig. 7).

Fig. 7. Proposed mechanism of cerebral arterial spasm following subarachnoid hemorrhage.

On the basis of the author's data, successful approaches to the treatment of delayed cerebral arterial spasm may be as follows: 1. Specific alpha-adrenergic blocking drugs such as phenoxybenzamine, appear to reduce or abolish the spasm consistently only when applied topically (7, 8). However, phenoxybenzamine has been shown to produce chemical arachnoiditis when given intracisternally (9). 2. Specific inhibitors of dopamine-β-hydroxylase such as fusaric acid (10, 11), o-phenanthroline, and αα'-dipyridyl completely relieved the delayed spasm (12). The effectiveness of these drugs is dependent on the chelating properties of the compounds. Chelating agents such as o-phenanthroline and αα'-dipyridyl chelate with the iron of heme components and eliminate the biological actions of vasoconstrictors such as methemoglobin, methemalbumin and catalase. Although we observed that the topical application of o-phenanthroline and αα'-dipyridyl strikingly relieved arterial spasm, further studies are necessary before their clinical use in humans for the treatment of cerebral vasospasm. 3. β2-adrenergic stimulators such as salbutamol (13) act to enhance the activity of adenyl cyclase, an enzyme necessary for conversion of adenosine triphosphate (ATP) to cyclic 3'-5'-adenosine monophosphate (cyclic AMP), an increase of which seems to cause the relaxation of smooth muscle (14–20). However, the magnitude of the vascular dilatation with salbutamol was not so pronounced as that with fusaric.
acid. 4. Phosphodiesterase inhibitors such as ascorbic acid (21–23) caused vasodilation in cats. In contrast to $\beta_2$-adrenergic stimulators, ascorbic acid increases cyclic AMP accumulation by blocking the activity of phosphodiesterase, an enzyme degrading cyclic AMP to 5'-AMP (21–23). Ascorbic acid, which is well known as a reducing agent, also may act to convert heme component of the ferri type to that of the ferro type thus eliminating the possible action of spasmodic genic substances. Ascorbic acid may well prove useful for the treatment of patients with delayed spasm following subarachnoid hemorrhage.

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