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Abstract

This study demonstrates that an adrenergic mechanism plays an important role in producing the delayed cerebral vasospasm which follows subarachnoid hemorrhage. Results were as follows:
1. Experimental subarachnoid hemorrhage (SAH) was produced by injection of fresh arterial blood into the cisterna magna in cats. The cerebral vasospasm was shown angiographically to be biphasic in nature: immediate constriction lasting 1 h and marked prolonged spasm occurring between the 3rd and 5th day after SAH. The amount of noradrenaline (NA) and dopamine-beta-hydroxylase (DBH) activity decreased over a period of 24 h both within the wall of the basilar artery and in the locus ceruleus and then gradually increased, reaching a maximum on the 3rd day after SAH.
2. Topical application of spasmogenic substances (NA and blood) produced a marked constriction of the hypersensitive basilar artery on the 3rd day after SAH.
3. 6-Hydroxydopamine (6-OHDA) injection into the cisterna magna produced prolonged vasodilatation. The dilated vessel responded with mild transient constriction after the topical application of NA or fresh blood. DBH activity and NA concentration in the vessels, locus ceruleus and medial hypothalamus decreased markedly on the 3rd day after the cisternal injection of 6-OHDA.
4. Various spasmogenic substances (i.e. serotonin, NA, prostaglandins and methemoglobin) were measured in a mixture of equal volume of CSF and blood in cats. Only the serotonin in the mixed fluid produced vasoconstriction. Spasmogenic substances decreased markedly in the mixed fluid incubated for 3 days at 37 degrees C, and none of these substances apart from methemoglobin was present in a concentration sufficient to produce constriction of vessels.
5. These results suggest that early spasm is induced by serotonin around the arteries of the cranial base, and delayed spasm might be caused by hyperreaction of cerebral vessels to spasmogenic substances such as methemoglobin, during the accumulation of excess NA in the cerebral vessel wall.

KEYWORDS: cerebral vasospasm, dopamine-β-hydroxylase, serotonin, noradrenaline

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CEREBRAL ARTERIAL SPASM.
I. ADRENERGIC MECHANISM IN EXPERIMENTAL CEREBRAL VASOSPASM

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Abstract. This study demonstrates that an adrenergic mechanism plays an important role in producing the delayed cerebral vasospasm which follows subarachnoid hemorrhage. Results were as follows:
1. Experimental subarachnoid hemorrhage (SAH) was produced by injection of fresh arterial blood into the cisterna magna in cats. The cerebral vasospasm was shown angiographically to be biphasic in nature: immediate constriction lasting 1 h and marked prolonged spasm occurring between the 3rd and 5th day after SAH. The amount of noradrenaline (NA) and dopamine-β-hydroxylase (DBH) activity decreased over a period of 24 h both within the wall of the basilar artery and in the locus ceruleus and then gradually increased, reaching a maximum on the 3rd day after SAH. 2. Topical application of spasmogenic substances (NA and blood) produced a marked constriction of the hypersensitive basilar artery on the 3rd day after SAH. 3. 6-Hydroxydopamine (6-OHDA) injection into the cisterna magna produced prolonged vasodilatation. The dilated vessel responded with mild transient constriction after the topical application of NA or fresh blood. DBH activity and NA concentration in the vessels, locus ceruleus and medial hypothalamus decreased markedly on the 3rd day after the cisternal injection of 6-OHDA. 4. Various spasmogenic substances (i.e., serotonin, NA, prostaglandins and methemoglobin) were measured in a mixture of equal volume of CSF and blood in cats. Only the serotonin in the mixed fluid produced vasoconstriction. Spasmogenic substances decreased markedly in the mixed fluid incubated for 3 days at 37°C, and none of these substances apart from methemoglobin was present in a concentration sufficient to produce constriction of vessels. 5. These results suggest that early spasm is induced by serotonin around the arteries of the cranial base, and delayed spasm might be caused by hyperreaction of cerebral vessels to spasmogenic substances such as methemoglobin, during the accumulation of excess NA in the cerebral vessel wall.

Key words: cerebral vasospasm, dopamine-β-hydroxylase, serotonin, noradrenaline

It is well known that a marked decrease in arterial diameter can lead to brain ischemia and infarction (1), and that cerebral vasospasm after subarachnoid hemorrhage (SAH) due to the rupture of an intracranial aneurysm plays an
important role in the outcome of both the natural and the postoperative courses. Angiographic examination has proved that the cerebral arterial spasm may be biphasic, with the first phase beginning immediately after SAH and lasting for min, and the second phase beginning from 4 to 10 days following SAH with a duration of several weeks (2, 3). This delayed or persistent vasospasm appears to be associated with the mortality and morbidity of patients with SAH.

Postoperative vasospasm can frequently be demonstrated angiographically after surgery performed within 3 to 7 days of the rupture of an intracranial aneurysm not present in the preoperative angiogram (1, 3). It is important to decide whether patients should be treated surgically or conservatively at this time.

Although many clinical and experimental studies have been performed on vasospasm, its pathogenesis has not been well clarified. The presence of an adrenergic nerve plexus within the walls of cerebral vessels in animals has been demonstrated by the fluorescence staining method (4, 5). By means of immunofluorescent techniques, Hartman and Udenfriend showed that the enzyme dopamine beta hydroxylase (DBH) was localized in nerve endings at the base of the brain in rats (6, 7). This enzyme is essential to the synthesis of noradrenaline (NA).

Recently the author demonstrated that both DBH activity and the concentration of NA were markedly elevated in human cerebrospinal fluid (CSF) after SAH (8). The present study was designed to clarify the relationship of the noradrenergic system in cerebral vessels to cerebral vasospasm.

MATERIALS AND METHODS

Adult cats weighing 3.0 to 3.5 kg were anesthetized with intramuscular Ketamine hydrochloride (2-[0-chlorophenyl]-2-[methylamino] cyclohexanone hydrochloride) (20 mg/kg). After tracheal intubation, the animals either were allowed to breathe spontaneously or were paralyzed with intramuscular succinylcholine chloride (1 to 2 mg/kg periodically as needed) and ventilated with a Takaoka respirator at a specified volume and rate of respiration. The head was immobilized in a stereotaxic instrument. Experimental SAH was produced by injection of 2 to 4 ml of fresh autogenous arterial whole blood into the cisterna magna after removal of the equivalent volume of cerebrospinal fluid. Changes in vessel caliber were studied at short intervals by angiography. This was achieved with a polyethylene catheter placed in the aortic arch by a transfemoral approach. A volume of 8 ml of 60% Conray (iodoalamide meglumine) was injected as the contrast medium.

Changes in vessel caliber were also inspected directly through an operative microscope. The basilar artery was exposed transclivally (9). Hemostasis was meticulously obtained before the dura was opened. The arachnoid adjacent to the basilar artery was opened, care being taken not to strip the arachnoid from the vessels.
Isotonic saline at body temperature was used to cover the artery during dissection. Sufficient time was allowed for all mechanically produced spasm to disappear. Then, photographs of the vessels were taken at 25× magnification, using Fuji color film with a 35 mm Nikon camera attached to the operative microscope. Color slides were later projected on to a screen so that the inner diameter of the basilar artery could be measured at three points. The average was calculated. DBH activity in the tissue was measured by a modified Nagataz method (Table 1) (10). Animals were sacrificed in a cold room after brain irrigation with cold saline (0°C). The cannula was positioned in the thoracic aorta. The frozen brain which was stored in a refrigerator was later chiseled out in the frozen state and the major vessels at the base of brain, the locus ceruleus and the medial hypothalamus were separated.

<table>
<thead>
<tr>
<th>Incubation mixture</th>
<th>Supernatant</th>
<th>400 µl</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 M sodium acetate buffer, pH 5.0</td>
<td>200 µl</td>
<td></td>
</tr>
<tr>
<td>0.1 M N-ethylmaleimide</td>
<td>100 µl</td>
<td></td>
</tr>
<tr>
<td>20 µl CuSO4</td>
<td>50 µl</td>
<td></td>
</tr>
<tr>
<td>0.2 M sodium fumarate</td>
<td>50 µl</td>
<td></td>
</tr>
<tr>
<td>0.2 M ascorbic acid</td>
<td>50 µl</td>
<td></td>
</tr>
<tr>
<td>1500 units catalase</td>
<td>50 µl</td>
<td></td>
</tr>
<tr>
<td>0.4 M tyramine</td>
<td>50 µl</td>
<td></td>
</tr>
</tbody>
</table>

Incubation for 60 min at 37°C

The incubation was stopped by adding 0.2 ml of 3 M trichloroacetic acid, after which the mixture was centrifuged at 2500 rpm for 10 min.

Separation of octopamine by Dowex-50W-X4 (H+, 200-400 mesh)

Octopamine was converted to p-hydroxybenzaldehyde by adding 10 µl of 2% NaIO₄ solution. After adding NaIO₄, excess NaIO₄ was reduced by adding 10 µl of 10% Na₂S₂O₅.

Spectrophotometry at 330 nm.

Tissues to be used for the assay of DBH activity were homogenized in 0.1 M potassium phosphate buffer (pH 7.4) containing 0.1% Triton-X 100 in an ice bath. The homogenated specimen was centrifuged at 23,000 g for 30 min. Lipids were removed by aspiration and the supernatant was used as the source of enzyme. Supernatant fluid (400 µl) was added to a 20 ml centrifuge tube, then 200 µl of 1 M sodium acetate buffer (pH 5.0), 100 µl of 0.1 M N-ethylmaleimide, and 50 µl of 20 µl CuSO₄ were added. The solution was mixed to inactivate endogenous inhibitors in the enzyme preparation.

The following reaction reagents were added: 50 µl of 0.2 M ascorbic acid; 1,500 units of catalase in 50 µl of aqueous solution; and 50 µl of 0.4 M tyramine. A sample of boiled enzyme preparation (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. The mixture was centrifuged at 2,500 rpm for 10 min. The supernatant
was transferred to a small glass column (0.5 x 10 cm) of Dowex 50 W x 4 (H+, 200-400 mesh) (packed Vol. 0.2 ml) and absorbed amines were eluted with 1.0 ml of 3N NH₄OH into a 20 ml centrifuge tube. Octopamine in the eluate was converted to p-hydroxybenzaldehyde by adding 10 μl of 2% NaIO₄ solution. After adding NaIO₄, excess NaIO₄ was reduced by adding 10 μl of 10% Na₂S₂O₅ solution. The absorbances at 330 nm was measured with a Hitachi single wave length spectrophotometer.

NA concentration was measured by the gaschromatography method (11). Assay of serotonin was based on the fluorometric method (12) and assays of prostaglandin E₁ (PGF₁α) and PGF₂α were based on the radioimmunoassay method (13). Methemoglobin was measured by the photometric method (14).

RESULTS

Production of biphasic cerebral vasospasm. In this study of early spasm, fresh autogenous whole blood was injected into the cisterna magna of 5 cats. The consequent vasospasm was studied by angiography every 15 min. An immediate marked constriction in the basilar artery and circle of Willis was demonstrated 5 to 10 min after the cisternal injection of blood and lasted for a period of 60 to 90 min. The caliber of the artery decreased to between 50 and 60% of its original diameter. Tonic-clonic convulsive seizures were usually seen in the cats allowed to breathe spontaneously after the cisternal injection of blood.

In this study of late spasm, experimental SAH was produced in 21 cats. The posthemorrhagic course was classified into 3 groups (Fig. 1). The changes

![Fig. 1. Grading after experimental subarachnoid hemorrhage in cats. 11 cases had full recovery within 2 days and 6 cases recovered from grade IV within 7 to 10 days after the hemorrhage. 4 cases expired within 5 days. GI alert, active (normal); GII mildly obtunded (able to walk); GIII moderately obtunded (unable to walk); GIV severely obtunded (unable to move); GV moribund.](http://escholarship.lib.okayama-u.ac.jp/amo/vol32/iss1/3)
expired within 5 days. The 6 cats of Group 3 lay motionlessly and never took food. This latter condition continued with little change for the first 2 to 3 days, but gradually improved within 7 to 10 days of the hemorrhage. These symptoms correlated well with the degrees of spasm demonstrated angiographically. Sequential angiography showed that the most severe spasm occurred between 3 and 5 days, and that relief of spasm followed at from 7 to 10 days after SAH (Fig. 2).

Fig. 2. Angiograms in cat. The left angiogram (a) is control, and center (b) and right (c) show angiograms 5 and 7 days after the cisternal injection of blood. Marked vasospasm involving the circle of Willis (upper arrow) and the basilar artery (lower arrows) is seen in the center. The right angiogram shows recovery of vessel size with full recovery of symptoms.

Concentration of various spasmogenic substances in the mixture of fresh blood and CSF in cats. Various spasmogenic substances (serotonin, NA, prostaglandin E1 (PGE1), and prostaglandin F2α (PGF2α)) in the mixture of fresh blood (50%) and CSF (50%) were determined before, and 3 days after, incubation at 37°C. The results are shown in Fig. 3 and Table 2.

In the mixture before incubation, the average content of serotonin (189 ng/ml) was sufficient to produce constriction of cerebral vessels, whereas levels of NA, PGE1 and PGF2α were much less than that required to produce vascular constriction. Of all these substances, serotonin was the only one found capable of producing early spasm. In the 3-days incubated material, serotonin was less
Table 2. Changes in vasoconstrictor materials in the mixture of blood and CSF

<table>
<thead>
<tr>
<th>Vasoconstrictor</th>
<th>Control</th>
<th>3 days after incubation</th>
<th>7 days after incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotonin (ng/ml)</td>
<td>189.12 ± 32.04</td>
<td>0.91 ± 0.75</td>
<td>—</td>
</tr>
<tr>
<td>NA (ng/ml)</td>
<td>3.15 ± 1.13</td>
<td>0.98 ± 0.65</td>
<td>—</td>
</tr>
<tr>
<td>PGE₁ (ng/ml)</td>
<td>5.90 ± 1.82</td>
<td>4.09 ± 0.86</td>
<td>—</td>
</tr>
<tr>
<td>PGF₂α (ng/ml)</td>
<td>1.53 ± 1.29</td>
<td>2.42 ± 0.70</td>
<td>—</td>
</tr>
<tr>
<td>Methemoglobin (%)</td>
<td>0</td>
<td>36.2 ± 4.1</td>
<td>81.1 ± 4.8</td>
</tr>
</tbody>
</table>

Each result is the mean of 5 determinations ± S.E.

Fig. 3. Changes in vasoconstrictor materials in the mixture of blood and CSF. The concentrations of noradrenaline (NA), prostaglandin E₁ (PGE₁) and F₂α (PGF₂α) were less than 10 ng per ml of the mixed fluid with blood and CSF [control]. These vasoactive substances decreased to less than 5 ng per ml of the fluid after 3 days prolonged incubation of the mixed fluid at 37°C. Methemoglobin increased during the first 7 days. Each point in the mean value of 2 cases.

than 1.7 ng/ml. Other spasmogenic substances were less than 10 ng/ml in concentration. These substances (serotonin, NA, PGE₁, PGF₂α), except methemoglobin which had increased up to 30% of the total hemoglobin, were not concentrated enough to produce vessel constriction. The content of methemoglobin increased to approximately 81% of the total hemoglobin 7 days after incubation.

DBH activity and NA concentration in major vessels at the base (basilar artery and circle of Willis), locus ceruleus, and medial hypothalamus. Experimental subarachnoid hemorrhage produced a biphasic change in DBH activity within the vascular wall and in the locus ceruleus (Fig. 4, Table 3).
Adrenergic Mechanism in Cerebral Vasospasm

![Graph showing DBH activity following experimental subarachnoid hemorrhage](image)

Fig. 4. DBH activity following experimental subarachnoid hemorrhage. Subarachnoid hemorrhage caused a decrease in the DBH activity of intracranial major vessels up to 24 h, which was followed by a marked increase at 3 days. DBH activity of the locus ceruleus region showed a biphasic change.

### Table 3. DBH activity following experimental subarachnoid hemorrhage

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>Six h after SAH</th>
<th>One day after SAH</th>
<th>Two days after SAH</th>
<th>Three days after SAH</th>
<th>Seven days after SAH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vessels</td>
<td>9.73±3.07</td>
<td>6.78±1.84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.82±1.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.50±1.53</td>
<td>25.01±3.08&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13.76±2.46&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Locus ceruleus</td>
<td>8.84±1.25</td>
<td>1.92±1.00&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3.46±1.12&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.38±0.75&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.46±2.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.15±1.30</td>
</tr>
<tr>
<td>Medial hypothalamus</td>
<td>5.02±1.53</td>
<td>5.30±1.87</td>
<td>2.32±1.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.84±1.05</td>
<td>4.23±1.55</td>
<td>5.38±0.95</td>
</tr>
</tbody>
</table>

Fresh blood was injected into the cisterna magna of cats. The cats were sacrificed at 6 h, and 1, 2, 3 and 7 days. Each result is the mean of 5 determinations±SE. DBH activity (unit) is expressed as nanomoles of octopamine/min. at 37°C/g tissue. Probabilities were estimated by Student's t-test. <sup>a</sup>p<0.05, <sup>b</sup>p<0.01, <sup>c</sup>p<0.001.

DBH activity in the vessel wall decreased to 63% of the control level during the first 24 h, then increased markedly to 271% of the control level, reaching a maximum at 3 days after SAH and recovering to the control level by 7 days after SAH. The change of DBH activity in the locus ceruleus was similar to that in the vessels.

On the other hand, DBH activity in the medial hypothalamus decreased by 53% of the control level during the first 24 h, then gradually returned to the control level by 7 days. Changes in NA content in the vessels, locus ceruleus and medial hypothalamus following SAH were similar to those for DBH activity (Fig. 5). The elevation of NA concentration and the increased DBH activity within arterial walls on the 3rd day after SAH coincided with the occurrence of late spasm.
The effects of various spasmogenic substances on the diameter of the basilar artery. In this study 36 cats were used. The basilar artery was exposed transclavically with an operative microscope. Spasm was produced by topical application of various spasmogenic substances (serotonin, PGE$_1$, PGF$_2\alpha$, NA, fresh blood, and incubated blood). The incubated blood was obtained as a mixture of an equivalent volume of blood and CSF incubated at 37°C for 3 days.

After 2 min application, these substances were removed by gentle aspiration and photographs were taken for the measurement of the sequential change of the basilar artery diameter.

Group 1. Effects of various spasmogenic substances on the basilar artery in untreated cats. Results are shown in Fig. 6. The basilar arteries in 6 cats were maximally constricted about 5 min after topical application of various spasmogenic substances, and returned to normal size within 15 min.

Serotonin (100 ng/ml) or fresh arterial blood produced the most intense constriction. Following the topical application of fresh arterial blood for two min, the diameter of the basilar artery decreased in all 6 cats with an average reduction of 45% (S.D. ± 10%). The reduction in the vessel caliber produced by topical application of serotonin (100 ng/ml) ranged from 35 to 52% with an average of 43.2% (± 4.8%).

A concentration of NA more than 100 ng/ml produced vasospasm, whereas less than 50 ng/ml of NA caused no significant constriction. With topical application of NA (100 ng/ml), reduction of the caliber ranged from 10 to 38% with an average of 28.0% (± 7.2%). Further prominent reduction of the caliber did not occur even when 500 ng/ml of NA was applied.
Adrenergic Mechanism in Cerebral Vasospasm

The degree of reduction in the caliber produced by prostanglandins was similar to that produced by NA (100 ng/ml).

The spasm produced by incubated blood had a tendency to persist much longer than that produced by other substances.

Fig. 6. Percent change in vessel diameter. Vasospasm in normal cats [controls] was induced by 2 min topical application of various substances and blood. Vessel diameter is shown as a percentage of the vessel diameter before application.

Fig. 7. Percent change in vessel diameter (3 days after subarachnoid hemorrhage). Slight spasm of the basilar artery was usually seen 3 days after subarachnoid hemorrhage. Zero percent line (dotted line) shows the diameter of the basilar artery just before topical application of noradrenaline and blood. Ordinate shows the vessel diameter as a percentage of the vessel diameter before application.
Group 2. Effects of various spasmogenic substances on the basilar artery 3 days after SAH. The intensity and duration of spasm produced by topical application of various spasmogenic substances to the basilar artery 3 days after SAH, increased markedly compared with that obtained in untreated cats (Fig. 7). Topical application of incubated blood for 2 min induced marked constriction for more than 60 min. With the topical application of even a low concentration of NA (5 ng/ml), the diameter of the basilar artery decreased in all 6 cats. The reduction in the caliber caused by NA (10 ng/ml) ranged from 35 to 65% with an average of 50.3% (± 8.2%) (Fig. 8).

The duration of basilar artery constriction produced by fresh blood was longer in cats with SAH than in control cats.

![Images of blood vessels](image_url)

Fig. 8. Photographs of the basilar artery in a cat exposed via a transclival approach 3 days after the cisternal injection of blood. a: Slight spasm of vessels is seen just before application of noradrenaline. b: Vessels 5 min after topical application of noradrenaline (5 ng/ml) for 2 min. c: Vessels 30 min after topical application of noradrenaline (5 ng/ml) for 2 min. Vasospasm was produced by very low concentration of noradrenaline and lasted for a longer length of time in the cat 3 days after subarachnoid hemorrhage.

Group 3. Effects of various spasmogenic substances on the basilar artery after cisternal injection of 6-OHDA. 6-OHDA (0.5 mg/kg) was injected into the cisterna magna of 6 cats. The resulting changes in the basilar artery were studied by angiography and direct inspection with an operative microscope. The vessel diameter decreased to less than 30% of the original caliber within 30 to 40 min.
after the injection of 6-OHDA. Thereafter a marked increase of more than 30% of the original caliber had occurred by 120 min after the administration of 6-OHDA (Fig. 9). This degree of dilatation of the vessel persisted for more than 3 days.

Fig. 9. Photographs of the basilar artery in a cat exposed via a translcal approach. Basilar artery just before application of 6-OHDA (control). The diameter of basilar artery was markedly decreased at 30 min after topical application of 6-OHDA (0.5 mg/kg) (center). The diameter of basilar artery was markedly dilated at 120 min after topical application of 6-OHDA (right).

The effects of various spasmogenic substances on the basilar artery after the cisternal injection of 6-OHDA were studied in 6 cats. The reduction in the caliber after the topical application of various spasmogenic substances was mild and transient.

The dilated basilar artery constricted after the topical application of small amounts of NA (10 ng/ml), a condition which might be called denervation hypersensitivity. However, the caliber decreased by only 25 to 35% with an average of 30% (±3.2%) and returned to the original size within 5 min after the topical application. The degree of reduction in the caliber produced by other substances (serotonin 100 ng/ml, fresh blood, incubated blood) was similar to that produced by NA (10 ng/ml) (Fig. 10).

DBH activity and NA concentration in the vessels, locus ceruleus and medial hypothalamus decreased markedly on the 3rd day after cisternal injection of 6-OHDA (Table 4).
Fig. 10. Percent change in vessel diameter of a 6-OHDA treated cat. A. The vessel diameter decreased within about 30 to 40 min after the administration of 6-OHDA, then increased markedly until about 120 min. B. Three days after cisternal injection of 6-OHDA (0.5 mg/kg) the dilated basilar artery was constricted by topical application of a low concentration of NA (10 ng/ml). Reductions in this dilated caliber after the topical application of various spasmogenic substances were mild and transient. ---, NA (10 ng/ml); ----, Fresh blood; ----, NA (100 ng/ml).

Table 4. Effect of 6-hydroxydopamine (6-OHDA) on DBH activity and the concentration of catecholamine in cats

<table>
<thead>
<tr>
<th>Tissue</th>
<th>DBH activity (units)</th>
<th>NA (ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vessels</td>
<td>0.16±0.02</td>
<td>3.4±1.5</td>
</tr>
<tr>
<td>Locus ceruleus</td>
<td>1.99±0.75</td>
<td>2.0±1.0</td>
</tr>
<tr>
<td>Medial hypothalamus</td>
<td>1.33±0.64</td>
<td>4.3±0.3</td>
</tr>
</tbody>
</table>

6-OHDA was injected into the cisterna magna of cats. The cats were sacrificed 3 days later. Each result is the mean of 6 determination±S.E. for vessels, locus ceruleus and medial hypothalamus.

DISCUSSION

Both adrenergic and cholinergic nerve fibers along intracranial arteries have been demonstrated by fluorescence histochemical techniques (4, 5, 15). A recent study has demonstrated central adrenergic fibers on microvasculatures in the brain parenchyma. These fibers originate from the locus ceruleus and are considered to regulate intra-cerebral microcirculation (6, 7). The basilar artery, the circle of Willis and the superficial arteries of the cortex derive their noradrenergic innervation mainly from the superior cervical ganglion (16). It has also been reported that the distribution of noradrenaline (as determined by the formaldehyde fluorescence method) corresponds well with the distribution of the DBH.
enzyme (as determined by the immunofluorescence technique (4–7)).

The enzyme DBH is considered to play an important role in neurotransmission in the sympathetic nervous system because of its localization and its catalyzed reaction. This enzyme which hydroxylates dopamine on the beta carbon to form noradrenaline has a molecular weight of 290,000 (17), and contains about 2 μmoles of Cu²⁺ per μmol of enzyme (18).

DBH is formed in the Golgi apparatus of a cell body and is localized in granules containing noradrenaline, ATP and chromogranin. It is present in a bound and soluble form (18). Large granular vesicles are carried to axon terminals via rapid axonal transport on the neurotubules, then preferentially used for discharge of their contents when the nerve ending is depolarized (19). Once soluble DBH and noradrenaline are discharged from sympathetic nerve terminals by a process of exocytosis (20, 21), they may be transformed into small storage vesicles and be concerned primarily with synthesis and storage of the transmitter reserve (22).

Experimental subarachnoid hemorrhage produced a biphasic response in the DBH activity and NA concentration in vessels and the locus ceruleus. This response consisted of a reduction during the first 24 h followed by a marked increase at 3 days.

Prominent spasm demonstrated angiographically concurred with aggravation of symptoms at 3 days after the hemorrhage. These findings suggest that a high level of DBH activity may induce a greater production of NA in the vessel wall, with consequent prolonged vasoconstriction. Delayed spasm is considered to be triggered by the sympathetic discharge resulting from NA accumulation in the vessel walls beyond a normal physiological level.

Direct application of spasmogenic substances produced constriction of the basilar artery on the third day after SAH which was severe and long-lasting in comparison with that which occurred in the untreated cat. On the other hand, 6-OHDA injection into the cisterna magna produced marked dilatation of the basilar artery. The reduction in the caliber was mild and transient after the topical application of various spasmogenic substances.

6-OHDA has been found to induce a long-lasting depletion of NA in sympathetically innervated organs and brain as a result of degeneration of noradrenergic nerve terminals (23, 24). 6-OHDA also causes a fall of ganglia DBH and an almost complete disappearance of the enzyme in tissue (25). From this finding, it seems that the response of the NA receptor is related to the concentration gradient of the transmitter substance.

It has been documented that extravasated blood from a ruptured aneurysm may act as a chemical irritant. In the present study, serotonin was the only substance capable of producing early spasm, and methemoglobin, a component of lysed red cells, was shown to be a possible spasmogenic substance related to the
onset of late spasm. However, more direct evidence is required to clarify this hypothesis, since the biological action of methemoglobin is unknown.

These results suggest that the prolonged late spasm following experimental SAH may be caused by the elevation of NA concentration and the increased DBH activity within arterial walls, as indicated by a greater production of granular vesicles in adrenergic terminals and cell bodies at sites such as the locus ceruleus and the superior cervical ganglion.

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