AMONG THE MANY FACTORS PROPOSED as causes of prolonged cerebral vasospasm following ruptured intracranial aneurysms, subarachnoid blood is one obvious one.1-3 Recently, free radical pathology has been discussed in the pathogenesis of prolonged cerebral vasospasm.4-6 Subarachnoid hemorrhage (SAH) gives rise to a specific environment around the cerebral arteries. There is an abundance of oxyhemoglobin, which can generate superoxide during autoxidation,7-8 and also of polyunsaturated fatty acids derived from the lysis of erythrocyte membranes; those fatty acids autoxidize readily to form lipid peroxides.9,10 Generation of more active species of oxygen on the surface of leucocytes during their phagocytic activities may also be important.11 Activated products such as active species of oxygen, lipid peroxides and other free radicals are likely to cause functional and morphological damage to cells in the arterial wall through peroxidation of membrane phospholipids or enzymes.12,13

Against such damage, there are a variety of biological protective systems.6,14-16 In normal cells, superoxide radical anion is broken down by superoxide dismutase (SOD), and the hydrogen peroxide that is a product of this reaction is broken down by catalase and glutathione peroxidase (GSH-px). These enzymes may be important protectors against lipid peroxidation damage to cerebral arteries after the onset of SAH.

There are a number of reports in which the peroxidation of polyunsaturated fatty acids by active species of oxygen or hydroxyl radicals and its inhibition by SOD, catalase, and GSH-px were studied. However, there have been no thorough studies of these enzymes protection against peroxidation damage in patients with ruptured intracranial aneurysms.
Next, 2.4 ml of this supernatant and 2.4 ml of 0.67 M thiobarbituric acid were mixed and boiled for 15 minutes. After rapid cooling, the extinction of the solution was read from a spectrophotometer at 535 nm. The amount of malon dialdehyde formed was calculated with 5 nM of tetraethoxypropane as the standard solution.

Determination of SOD activity was assayed by the method of Sun and Zigman. In brief, SOD activity was measured by a spectrophotometer at 320 nm for 10 minutes immediately after 0.02 ml of 50 mM epinephrine was added to a mixture of 0.05 ml of the sample and 2.0 ml of 50 mM sodium bicarbonate buffer at pH 10.0 (A\text{sample}). A matched control without the sample was also measured (A\text{control}). The extinction of the relationship of ΔA = (A\text{sample} - A\text{control}), over time was constant from 2 to 8 minutes after the start of the reaction. By measuring ΔA at various standard concentrations in the same way, we obtained the curve of the relationship of ΔA and concentration; it was linear up to 0.5 μg/ml purified SOD. Thus, SOD activity in the CSF was expressed as the concentration of purified SOD. All measurements were performed below 25°C.

Determination of catalase activity was assayed by the decrease in extinction at 240 nm caused by the decomposition of hydrogen peroxide with catalase. The reaction mixture was composed of 0.9 ml of 50 mM phosphate buffer (pH 7.0) containing 4 mM EDTA, 0.2 ml of 10 mM sodium azide, 0.1 ml of Drapkin’s solution, 0.2 ml of 0.01 M reduced glutathione, 0.04 ml of 7.5 mM NADPH, 0.03 ml of glutathione reductase (6 U/ml), 0.32 ml of distilled water, and 0.1 ml of sample. All components were mixed together and incubated at 25°C for 5 minutes. The reaction was started by adding 1.0 ml of 30 mM hydrogen peroxide in the test cuvette and by adding the same amount of distilled water instead of hydrogen peroxide in the reference cuvette. The decrease in extinction was followed with a recorder for 3 minutes at 20°C. The value of extinction of the reference was subtracted from that of the test cuvette before units of activity were calculated.

Determination of GSH-px activity was assayed by following the oxidation of NADPH at 340 nm in the presence of glutathione reductase, which catalyzes the reduction of oxidized glutathione formed by the peroxidase, in a spectrophotometer. The standard reaction mixture was composed of 1.0 ml of 50 mM phosphate buffer (pH 7.0) containing 4 mM EDTA, 0.2 ml of 10 mM sodium azide, 0.1 ml of Drapkin’s solution, 0.2 ml of 0.01 M reduced glutathione, 0.04 ml of 7.5 mM NADPH, 0.03 ml of glutathione reductase (6 U/ml), 0.32 ml of distilled water, and 0.1 ml of sample. All components were mixed together and incubated at 25°C for 5 minutes. The reaction was started by adding 0.033 ml of 30 mM of hydrogen peroxide in the test cuvette and by adding the same amount of distilled water instead of hydrogen peroxide in the reference cuvette.

Cerebral angiography was done twice, as a rule. The first angiogram was made on the day of admission in all patients, and the second was between the seventh and tenth day after surgery to assess the presence or absence of cerebral vasospasm, in patients whose condition allowed it. The severity of vasospasm in postoperative angiography was rated as: none, mild (maximum reduction of less than 50% in the internal carotid artery, the A segment of the anterior cerebral artery, and the M segment of the middle cerebral artery with the affected area being more than 1 cm in length), and severe (reduction exceeding 50%).

Preoperative computerized tomographic scanning (CT scan) was done on the day of admission in all cases, and it was reviewed to estimate the amount of subarachnoid blood from the rupture. Postoperative CT scan was done within 48 hours after the operation in some cases, and this was reviewed to evaluate the reduction of subarachnoid blood by surgery. The amount of subarachnoid blood judged by CT scans was classified into 3 grades according to Fisher’s system. Symptomatic vasospasm was considered to be present in those patients who awoke from anesthesia unchanged from their previous state, and in whom focal neurological deficits developed several days after the operation. These symptoms were generally compatible with the findings on angiographic vasospasm.

Five categories were used for judging the operative results at follow-up at three months: excellent, in which the patient is healthy and fully able to work; good, capable of working but has minor neurological deficits; fair, capable of self-care but has major neurological deficits; poor, incapable of self-care; and dead.

### Results

#### Clinical Data (tables 1 and 2)

Of the 25 patients in this series, 16 patients developed symptomatic vasospasm after the operation. The preoperative neurological status was much more severe in the patients with symptomatic vasospasm. The amount of subarachnoid blood was much greater in those with symptomatic vasospasm; Grade 3 of Fisher’s classification, judged from the CT scans, was found in 14 of the 16 (88%) patients with vasospasm and in 4 of the 9 (44%) without. Severe vasospasm was seen in postoperative angiography in 12 of the 13 patients with symptomatic vasospasm who could be checked, whereas mild vasospasm only was present in only 2 of the 7 patients without symptomatic vasospasm.

### Lipid Peroxides

The values of lipid peroxides measured in cisternal CSF in this study were below 2.0 nmol/ml in almost all of our patients. There were two patterns in the changes in lipid peroxides concentration following the onset of SAH. One was where the concentration of lipid peroxides remained low, less than 1.0 nmol/ml, throughout the period of study; this was the pattern for most patients without symptomatic vasospasm. The other was where the concentration of lipid peroxides increased markedly, to more than 1.0 nmol/ml, during the initial four days after SAH, and continued to increase throughout the study; this was noted in all patients with symptomatic vasospasm (table 2).

To evaluate the difference in these changes between patients with and without symptomatic vasospasm, the mean values of lipid peroxides in each group of
The increase in lipid peroxides in the patients with vasospasm was steep, but lipid peroxides remained as low as on Days 0 to 2 throughout the study in the patients without. The differences in the mean values for lipid peroxides were significant from Days 3 and 4, and then decreased further to the end of the study in the patients without symptomatic vasospasm (table 2). To evaluate differences in these changes between patients with and without symptomatic vasospasm, the mean values for the two groups were plotted from the onset of SAH (fig. 2). SOD activity in the patients with symptomatic vasospasm sharply decreased on Days 3 and 4, and then decreased further to the end of the study in the patients without symptomatic vasospasm (table 2).

**SOD Activity**

The values of SOD activity in cisternal CSF were below 0.3 μg/ml in most patients with ruptured intracranial aneurysms, but the mean ± SEM (n = 5) was 0.30 ± 0.02 μg/ml in the CSF of the nonhemorrhagic patients. There were, again, two patterns in the changes after SAH. One was where the activity started to decrease rapidly until Day 4 after SAH, and remained at a low level, less than 0.1 μg/ml; afterwards, this was observed in almost all patients with symptomatic vasospasm. The second pattern was where SOD activity was maintained at the initial level of Days 0 to 2 until Day 8 after SAH and decreased slightly with some daily fluctuation; this pattern was seen in most patients without symptomatic vasospasm (table 2). To evaluate differences in these changes between patients with and without symptomatic vasospasm, the mean values for the two groups were plotted from the onset of SAH (fig. 2). SOD activity in the patients with symptomatic vasospasm sharply decreased on Days 3 and 4, and then decreased further to the end of the study in the patients without symptomatic vasospasm (table 2).

**Summary of Clinical Data**

**TABLE 1** Summary of Clinical Data for Patients with Ruptured Intracranial Aneurysms Treated by Early Surgery. Lipid Peroxides Concentrations and Enzyme Activities Were Measured in CSF

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age &amp; sex</th>
<th>Site of aneurysm</th>
<th>Pre-operative* grade</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>62 F</td>
<td>lt. MCA</td>
<td>IV</td>
<td>good</td>
</tr>
<tr>
<td>2.</td>
<td>68 F</td>
<td>Acom</td>
<td>IV</td>
<td>poor</td>
</tr>
<tr>
<td>3.</td>
<td>41 F</td>
<td>lt. ACA</td>
<td>I</td>
<td>good</td>
</tr>
<tr>
<td>4.</td>
<td>35 F</td>
<td>lt. ACA</td>
<td>III</td>
<td>excellent</td>
</tr>
<tr>
<td>5.</td>
<td>48 M</td>
<td>lt. IC-PC</td>
<td>II</td>
<td>fair</td>
</tr>
<tr>
<td>6.</td>
<td>50 M</td>
<td>rt. MCA</td>
<td>III</td>
<td>good</td>
</tr>
<tr>
<td>7.</td>
<td>55 F</td>
<td>rt. IC-Ach</td>
<td>III</td>
<td>good</td>
</tr>
<tr>
<td>8.</td>
<td>68 F</td>
<td>rt. MCA</td>
<td>IV</td>
<td>poor</td>
</tr>
<tr>
<td>9.</td>
<td>60 F</td>
<td>rt. MCA</td>
<td>IV</td>
<td>good</td>
</tr>
<tr>
<td>10.</td>
<td>67 M</td>
<td>Acom</td>
<td>II</td>
<td>fair</td>
</tr>
<tr>
<td>11.</td>
<td>58 M</td>
<td>Acom</td>
<td>IV</td>
<td>dead</td>
</tr>
<tr>
<td>12.</td>
<td>71 F</td>
<td>lt. MCA</td>
<td>IV</td>
<td>poor</td>
</tr>
<tr>
<td>13.</td>
<td>57 F</td>
<td>lt. MCA</td>
<td>IV</td>
<td>good</td>
</tr>
<tr>
<td>14.</td>
<td>52 F</td>
<td>lt. IC-PC</td>
<td>IV</td>
<td>fair</td>
</tr>
<tr>
<td>15.</td>
<td>75 F</td>
<td>lt. IC-PC</td>
<td>IV</td>
<td>dead</td>
</tr>
<tr>
<td>16.</td>
<td>60 F</td>
<td>rt. IC-PC</td>
<td>III</td>
<td>fair</td>
</tr>
</tbody>
</table>

**TABLE 2** Summary of Clinical Data

<table>
<thead>
<tr>
<th>Patient</th>
<th>CT grade</th>
<th>Pre-operative SOD activity</th>
<th>Post-operative SOD activity</th>
<th>Lipid peroxides</th>
<th>Angiography†</th>
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</thead>
<tbody>
<tr>
<td>1.</td>
<td>3</td>
<td>3</td>
<td>S</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>3</td>
<td>3</td>
<td>S</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>3</td>
<td>3</td>
<td>S</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>3</td>
<td>2</td>
<td>S</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>3</td>
<td>2</td>
<td>S</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>3</td>
<td>3</td>
<td>S</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>3</td>
<td>3</td>
<td>S</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>3</td>
<td>3</td>
<td>S</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>3</td>
<td>3</td>
<td>S</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>3</td>
<td>3</td>
<td>n.d.</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td>3</td>
<td>3</td>
<td>n.d.</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td>3</td>
<td>3</td>
<td>S</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>13.</td>
<td>3</td>
<td>3</td>
<td>n.d.</td>
<td>↑</td>
<td></td>
</tr>
<tr>
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<td>2</td>
<td>2</td>
<td>S</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>15.</td>
<td>3</td>
<td>3</td>
<td>n.d.</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>16.</td>
<td>2</td>
<td>2</td>
<td>S</td>
<td>↑</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** Acom = anterior communicating artery; IC-PC = internal carotid-posterior communicating artery; IC-Oph = internal carotid-ophthalmic artery; IC-Ach = internal carotid-anterior choroidal artery; MCA = middle cerebral artery; ACA = anterior cerebral artery.

*Clinical Grade of Hunt and Hess.

**Abbreviations:** lipid peroxides = concentration of lipid peroxides estimated amount of malondialdehyde: f = increase more than 0.1 μg/ml; —» = more than 0.1 μg/ml. SOD activity: I = decrease less than 0.1 μg/ml; —» = more than 0.1 μg/ml.

*According to Fisher’s CT grading.

†Within 48 hours after operation: S = severe > 50% reduction; M = mild < 50% reduction; N = none; n.d. = not determined.
study; SOD activity in the patients without symptomatic vasospasm tended to decrease slightly over the entire study.

The differences were significant from Days 3 and 4 to Days 7 and 8 (p < 0.05). Figure 3 shows the relationship between the values for SOD activity and the concentrations of lipid peroxides for each sample taken from Days 3 to 6, when a sharp decrease in SOD activity and a steep increase in lipid peroxides occurred. The relationship was linear. These results suggest that when SOD activity is more than 0.1 μg/ml, the production of lipid peroxides is inhibited to less than 1.0 nmol/ml; when SOD activity falls below 0.1 μg/ml, lipid peroxides are likely to be generated in greater amounts.

Catalase Activity

To evaluate the differences in changes in catalase activity after the onset of SAH for patients with and without symptomatic vasospasm, the values for these 2 groups were plotted from the onset of SAH (fig. 4).

Mean values for catalase activity gradually increased up to Days 9 and 10 in the patients with symptomatic vasospasm, whereas there was a gradual decrease in catalase activity up to the end of the study in the patients without symptomatic vasospasm, the differences being significant from Days 7 and 8 to Days 9 and 10.

GSH-px Activity

The values of GSH-px activity in our 2 groups were plotted to evaluate the differences in the changes (fig. 5). Mean values of GSH-px activity for both groups gradually increased until Days 9 and 10 without any significant differences between them.

Discussion

Although there is no direct evidence that free radical reactions cause cell damage to the cerebral arteries, thereby causing prolonged cerebral vasospasm, a variety of phenomena in the genesis of cerebral vasospasm can be attributed to the pathological condition caused by such reactions. In dogs, cisternal injection of 15-
200

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GSH-P* (U/ml)

0-6

vaiospasm (+)

0-2

O

12.

5.6 7 8.

9.10.

11.12.

FIGURE 5. Changes in activity of glutathione peroxidase (GSH-px) following the onset of SAH in patients with and without symptomatic vasospasm (Mean ± SEM). There are no significant differences.

hydroperoxy arachidonic acid causes prolonged cerebral vasospasm after the remission of the initial spasm, with morphological changes of the basilar artery similar to those observed in experimentally induced subarachnoid hemorrhage.6 It is likely that subarachnoid hemorrhage provides conditions favoring lipid peroxidation around the cerebral arteries. The superoxide radical anion, O2−, is produced by the spontaneous oxidation of oxyhemoglobin (oxyHb) by Reaction 1 below; O2− produces more methemoglobin (metHb) by Reaction 2, which is slowed down by Reaction 3 when an excess of metHb is present during autoxidation. However, in the presence of SOD, Reaction 4 prevents Reaction 2, causing slower metHb formation. Hydrogen peroxide (H2O2) is broken down by catalase (5) and GSH-px (6).

OxyHb ➔ metHb + O2− (1)

OxyHb + O2− + 2H+ ➔ metHb + H2 O2 (2)

MetHb + O2− ➔ oxyHb (3)

2O2− + 2H+ ➔ superoxide dismutase ➔ O2 + H2 O2 (4)

2H2 O2 ➔ 2H2 + O2 (5)

2GSH + H2 O2 glutathione peroxidase ➔ GSSG + H2 O (6)

More active radicals, such as singlet oxygen (1'O2) or hydroxy radical, are produced by the Haber-Weiss reaction (7) if any of the protective mechanisms are defective or if chelate iron salts are present.5,8

O2− + H2 O2 + H+ ➔ OH− + 1'O2 + H2O (7)

Therefore, it is possible that biological protective mechanisms including the activities of SOD, catalase, and GSH-px are inadequate for scavenging the active species of oxygen in patients with SAH when vasospasm occurs through irreversible cell damage by free radical reactions.15

Our patients seemed to have a much worse preoperative neurological status, by Hunt and Hess' classification, than such patients reported by others.21-23 Of our groups of patients, those with symptomatic vasospasm had more subarachnoid blood than those without, though only a rough estimation was possible using CT scans, where we found Grade 3 according to Fisher's classification (localized clots, vertical layers of blood 1 mm or greater, or both) in 88% of the vasospasm group and in 44% of the other group. Moreover, we often noted severe vasospasm in postoperative angiography of our patients with symptomatic vasospasm. Only 44% of the patients with symptomatic vasospasm had a satisfactory surgical outcome (excellent or good), but the surgical outcome was satisfactory in 78% of the other patients. The same surgeon (SS) operated on all of the patients, trying for minimum manipulation of the brain and vessels, in order to reduce the influence of operative intervention on this study. Since January, 1981, we have operated within 72 hours after SAH on 75 patients with ruptured intracranial aneurysms of the anterior circle of Willis in preoperative neurological grades I-IV of Hunt and Hess. With these patients, an attempt was made to remove as much of the subarachnoid clot as possible, and simultaneously, ventricular and cisternal catheters were introduced.

In this study, we evaluated the role of SOD, catalase, and GSH-px activities in free radical reactions as a cause of prolonged vasospasm. It seems to be more reliable to take samples of CSF from the basal cistern than via lumbar puncture, because CSF from the basal cistern should reflect more directly the environment in which free radicals can act on the cerebral arteries. However, there was an unavoidable problem in our sampling, that bloody CSF was drained from the cisternal catheter continuously throughout this study to remove harmful substances from the subarachnoid space, despite possible sacrifice of the biochemical properties of CSF for the purposes of the study. To see whether this was a serious problem, we examined the daily amount of CSF drained from the cistern in relation to SOD activity, concentration of lipid peroxides, and the presence of absence of symptomatic vasospasm, but there were no significant relationships.24

The most striking finding in our study was that the concentration of lipid peroxides in CSF increased markedly during the first 4 days after the onset of SAH and continued to increase gradually in all patients with symptomatic vasospasm. However, lipid peroxides levels remained low throughout the study in most patients without symptomatic vasospasm. There was a significant difference between the patients with and without symptomatic vasospasm. It is also of interest that the SOD activity started to decrease rapidly until Day 4 after SAH and persisted at a low value afterwards in almost all patients with symptomatic vasospasm; however, in most patients without vasospasm, SOD activity maintained the control value up to Day 8.
after SAH and then slightly decreased. There was a significant difference in SOD activity, as well, between these groups of patients.

There was clearly a close relationship between the increase in lipid peroxides and the decrease in SOD activity. This suggests that the production of lipid peroxides is inhibited when SOD maintains its activity at over 0.1 μg/ml throughout the 10-day period after SAH. As for other enzymes as scavengers of active species of oxygen in CSF, catalase activity increased gradually up to Days 9 and 10 in the patients with symptomatic vasospasm. Catalase activity decreased gradually to near zero, throughout the period of study in the patients without vasospasm, unlike SOD activity; there was a significant difference in changes in catalase activity between the groups. GSH-px activity gradually increased during the study in both groups of patients.

These enzyme studies suggested the following assumption about the pathogenesis of prolonged cerebral vasospasm. In patients with symptomatic vasospasm after SAH, SOD activity around the cerebral arteries, which are covered by thick blood clots, decreases rapidly due to excessive consumption for scavenging superoxide radical anions, though catalase and GSH-px maintain enough activity to scavenge hydrogen peroxide. As a result, active species of oxygen are generated in abnormally high amounts, while there is a relative decrease in SOD activity, which allows lipid peroxidation damage to the cells of the arterial walls through oxidation of SH-radicals or inhibition of prostacyclin synthesis. We speculate that this pathological radical reaction depends on the quantity of blood clot in the subarachnoid space and may vary among individuals.

The differences in the enzyme activities in CSF may be due to different susceptibilities of each enzyme; a large amount of these enzymes should appear in CSF because of lysis of erythrocytes. We showed here that the SOD activity in CSF stayed at about 0.3 μg/ml in our non-hemorrhagic patients, even though CSF was continuously drained; however, catalase and GSH-px activities did not appear in the CSF of non-hemorrhagic patients. This means that there is some mechanism to maintain SOD activity in the CSF in normal individuals. Moreover, we showed elsewhere that SOD in erythrocytes loses activity rapidly when a mixture of arterial blood and CSF is incubated in sterile conditions at 37°C for 5 days. SOD activity appears to be susceptible to enzymatically generated superoxide; no increase in SOD activity occurs in the mixture even after complete hemolysis. On the other hand, catalase and GSH-px activities seem to be so stable that they increased gradually in proportion to the lysis of erythrocytes in the patients with symptomatic vasospasm. However, the reason why there is a discrepancy between catalase and GSH-px activities in the patients without symptomatic vasospasm is not known.

There was a delay of 2 to 5 days in the onset of symptoms after the start of reduction of SOD activity with increasing of lipid peroxides, in agreement with the results of Sasaki et al. They studied the daily levels of GSH-px activity and alphatocopherol in CSF from patients with ruptured intracranial aneurysms and found that these levels decreased markedly before the development of cerebral vasospasm. Thus, it is likely that normal arterial wall cells can repair peroxidized phospholipids in cell membranes when they are exposed to injury by an active species of oxygen or lipid peroxides. However, continuous exposure of the arteries to vigorous free radicals gradually results in instability of the cell membranes or a reduction of enzyme activities within the cells.

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Characterization of Beta Adrenergic Receptors in Human Cerebral Arteries and Alteration of the Receptors After Subarachnoid Hemorrhage

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SUMMARY The nature of beta adrenergic receptors in human cerebral arteries was characterized and alteration of these receptors after subarachnoid hemorrhage was examined using a radioligand binding assay. The specific $^3$H-dihydroalprenolol, a beta adrenergic antagonist, binding to human cerebral arteries was saturable and of high affinity ($K_D = 12.3 \text{ nM}$) with a Bmax of 790 fmol/mg protein. Ki values and Hill coefficients of adrenergic agents for $^3$H-dihydroalprenolol were as follows; propranolol, $4.1 \times 10^{-7}$M, 1.01; isoproterenol, $1.7 \times 10^{-7}$M, 0.80; epinephrine, $8.3 \times 10^{-7}$M, 0.48; norepinephrine, $2.3 \times 10^{-7}$M, 0.45; metoprolol, $6.8 \times 10^{-7}$M and $7.9 \times 10^{-7}$M, 0.62; butoxamine, $2.2 \times 10^{-7}$M and $2.1 \times 10^{-7}$M, 0.43. The analysis of inhibition of specific $^3$H-dihydroalprenolol binding by these adrenergic agents suggests that human cerebral arteries contain a high density of beta adrenergic receptors and that the receptors are classified into two types, namely beta 1 and beta 2 adrenergic receptors. The calculated beta 1/beta 2 ratio from Hofstee plots was approximately 4/6.

$K_D$ and Bmax of $^3$H-dihydroalprenolol binding to the cerebral arteries after subarachnoid hemorrhage were compared with those of control group. $K_D$ and Bmax of $^3$H-dihydroalprenolol binding of subarachnoid hemorrhage group were $13.9 \text{ nM}$ and $1140 \text{ fmol/mg protein}$, respectively. The calculated beta 1/beta 2 ratio was approximately 6/4. These data suggest that the density of total beta adrenergic receptors increased without any significant change in the affinity after subarachnoid hemorrhage and that the increase of beta 1 adrenergic receptors was dominant.

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to their agonists and antagonists, in various tissues. Pharmacological studies suggested that beta 1 adrenergic receptors mediated relaxations in human and cat cerebral arteries, whereas in other peripheral vessels, beta 2 adrenergic receptors seemed to mediate relaxations. It was reported that blood flow in the caudate nucleus of rabbit brain was increased by isoproterenol and that the increase was blocked by a selective beta 1 adrenergic antagonist, practolol. On the contrary, biochemical studies suggested the predominant existence of beta 2 adrenergic receptors in the cat cerebral microvessels. Thus subtypes of beta adrenergic receptors on the cerebral arteries were not clearly characterized.

Vasospasm of cerebral arteries in the case of subarachnoid hemorrhage (SAH) frequently presents severe clinical problems, as a result of cerebral ischemia. The pathogenesis of vasospasm is still poorly understood. The level of circulatory catecholamines often increases after SAH. In addition, the contractile response of human cerebral arteries to norepinephrine
Biological defence mechanism in the pathogenesis of prolonged cerebral vasospasm in the patients with ruptured intracranial aneurysms.

S Sakaki, H Kuwabara and S Ohta

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