ADRENERGIC INNERVATION OF CEREBRAL ARTERIES is considered to play an important role in controlling cerebral blood flow, by exerting vasomotor effects on cerebral blood vessels. Chemical and histochemical studies revealed high concentrations of norepinephrine and a rich adrenergic innervation from the superior cervical ganglion to the adventitia and the outer border of the medial layer of pial arteries, in humans and other species. Isolated cerebral arteries, contract in the presence of alpha adrenergic agonists, and this contraction is dose-dependent and is blocked by alpha adrenergic antagonists. Adrenergic receptors in vascular smooth muscles have been subclassified into alpha 1 and alpha 2, according to the relative potencies of selective agonists and antagonists. Sakakibara et al suggested that contractions of isolated dog basilar artery are mediated mainly by alpha 2 adrenergic receptors. In a previous paper, we demonstrated the existence of alpha 2 adrenergic receptors in bovine cerebral arteries and characterized their nature in radioligand binding assays. Other investigators reported that the responsiveness of human cerebral arteries to norepinephrine exceeds that of other species. Differences between these physiological events in human and other species may relate to postsynaptic adrenergic receptors.

Summary

The nature of alpha adrenergic receptors in human cerebral arteries was characterized and alteration of these receptors after subarachnoid hemorrhage (SAH) was examined using a radioligand binding assay. The specific 3H-prazosin binding to human cerebral arteries was saturable and of high affinity (Kd = 4.1 nM) with a Bmax of 92 fmol/mg protein. Specific 3H-yohimbine binding to these tissues was also saturable and of high affinity (Kd = 23 nM) with a Bmax 250 fmol/mg protein. IC50 values of adrenergic agents for 3H-prazosin binding were as follows: prazosin, 1.2 x 10-7M; phenolamine, 1.3 x 10-7M; yohimbine, 1.2 x 10-5M; norepinephrine, 4.9 x 10-5M; epinephrine >1 x 10-3M. IC50 values of adrenergic agents for 3H-yohimbine binding were as follows: phenolamine, 1.7 x 10-7M; yohimbine, 4.2 x 10-7M; prazosin, 1.9 x 10-7M; epinephrine, 4.4 x 10-7M; norepinephrine, 7.9 x 10-4M.

Kd and Bmax of 3H-prazosin and 3H-yohimbine binding after SAH were compared with findings in the non-SAH group. Kd and Bmax of 3H-prazosin binding of SAH group were 6 ± 3 nM and 90 ± 10 fmol/mg protein, respectively (N = 3). Kd and Bmax of 3H-yohimbine binding of SAH group were 42 ± 6 nM and 460 ± 30 fmol/mg protein, respectively (N = 5). On the other hand, Kd and Bmax of 3H-prazosin binding in the non-SAH group were 4 ± 1 nM and 90 ± 20 fmol/mg protein, respectively (N = 5). Kd and Bmax of 3H-yohimbine binding of non-SAH group were 20 ± 5 nM and 260 ± 30 fmol/mg protein, respectively (N = 6). These results suggest that alpha 1 and alpha 2 adrenergic receptors exist in human cerebral arteries and that both Kd and Bmax of 3H-yohimbine binding sites in the SAH group are larger than those of the non-SAH group. This means that the relatively high responsiveness of human cerebral artery to norepinephrine is due to the presence of alpha 1 adrenergic receptors and that this responsiveness may change after SAH.

Vasospasm of cerebral arteries in cases of subarachnoid hemorrhage (SAH) presents severe clinical problems. The pathogenesis of vasospasm is still poorly understood. Hemoglobin, a major component of red blood cells, and its metabolites are thought to play an important role in the genesis of vasospasm, since hemoglobin has contractile activity on the cerebral arteries of the dog and other species, in vitro and in vivo. The contractile response of human cerebral arteries to hemoglobin seems to be smaller, and that to norepinephrine larger than the responses to either agent in other species. Thus, the role of alpha adrenergic receptors has to be given attention when considering the genesis of human cerebral vasospasm after SAH.

We have now characterized alpha adrenergic receptors in human cerebral arteries obtained at autopsy and analyzed the changes in the alpha adrenergic receptors after SAH.

Materials and Methods

Collection of Arteries

Cerebral arteries (mainly basilar, circle of Willis and middle cerebral) were carefully removed at autopsy between 1 and 2 hours after death. A record was kept on each patient, including the past medical history and cause of death. Immediately after the arteries were removed from the brain, they were washed in saline and placed in a freezer (−80°C). Clinical profiles of SAH patients are summarized in table 1.

Membrane Preparation

The arteries were minced with scissors and homogenized in 10 volumes of ice-cold 50 mM sodium phosphate buffer (pH 7.4) with a glass homogenizer. The homogenates were filtered through two layers of...
TABLE 1  Clinical Profile of SAH Patients

<table>
<thead>
<tr>
<th>Patients</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Ruptured aneurysm</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 7</td>
<td>50</td>
<td>M</td>
<td>Right-ACOM</td>
<td>Operation on Day 0 Died on Day 14 of progressive delayed ischemic deficit</td>
</tr>
<tr>
<td>Case 8</td>
<td>70</td>
<td>M</td>
<td>Left-MCA</td>
<td>No operation Died on Day 1 of acute neurological deficit</td>
</tr>
<tr>
<td>Case 9</td>
<td>51</td>
<td>F</td>
<td>Left-MCA</td>
<td>No operation Died on Day 2 of acute neurological deficit</td>
</tr>
<tr>
<td>Case 10</td>
<td>72</td>
<td>M</td>
<td>Right-IC-PC</td>
<td>Operation on Day 0 Rapid deterioration from Day 7 Severe angiographic spasm on bilateral MCA on Day 8 Died on Day 13 of delayed ischemic deficit</td>
</tr>
<tr>
<td>Case 11</td>
<td>83</td>
<td>M</td>
<td>Left-MCA</td>
<td>No operation Died on Day 11 of progressive neurological deficit and pneumonia</td>
</tr>
</tbody>
</table>

The day was counted from the onset of SAH. ACOM = anterior communicating artery; MCA = middle cerebral artery; IC-PC = internal carotid artery posterior communicating artery.

The artery were added to the tubes containing the adrenergic agonists or antagonists at various concentrations and 5.6 nM 3H-prazosin or 22 nM 3H-yohimbine.

Drugs Used

The following drugs were used: 1-epinephrine bitartrate, yohimbine hydrocholoride (Nakarai Chemicals Co., Kyoto, Japan); 1-norepinephrine bitartrate (Sigma, St. Louis, USA); prazosin hydrochloride (Ciba-Geigy, Basel, Switzerland). All other chemicals were of reagent grade or the purest grade commercially available.

Stock solutions of norepinephrine and epinephrine were prepared daily in 0.5% ascorbic acid and further diluted with 0.05% ascorbic acid to the appropriate concentrations to avoid auto-oxidation. After dilution in the incubation mixture, this concentration of ascorbic acid was shown not to interfere with the binding assay.

Radioligands

3H-prazosin (specific activity, 17.4 Ci/mmoles) was purchased from New England Nuclear, Boston, MA, USA, stored at -20°C in ethanol and protected from light. 3H-yohimbine (specific activity, 89.7 Ci/mmoles) was also purchased from the same source, stored at 5°C in ethanol and protected from light. Immediately prior to use, appropriate amounts of stock solutions were diluted with water so that the ethanol concentrations in the final assay system did not exceed 1%.

Results

Saturability of Specific 3H-prazosin Binding

Specific binding of increasing concentrations of 3H-prazosin (1.1 to 14 nM) was saturable (fig. 1). Scatchard analysis indicated a single class of binding sites with an apparent equilibrium dissociation constant (Kd) and maximum binding capacity (Bmax) (fig. 2).

![FIGURE 1. Saturation of specific 3H-prazosin binding to human cerebral arteries. Specific 3H-prazosin binding, determined as described in the text, is plotted for increasing concentrations of added 3H-prazosin. Each point represents the mean ± S.E.M. for three cases (N = 5).](http://stroke.ahajournals.org/)

Binding Assay

3H-prazosin and 3H-yohimbine bindings were performed by incubating aliquots of the cerebral artery homogenates at a temperature of 37°C for 20 min in 250 μl of sodium phosphate buffer, containing 3H-prazosin or 3H-yohimbine, in the absence or presence of high concentrations of phentolamine (100 μM). The binding in the presence of 100 μM phentolamine was termed "nonspecific" and was subtracted from that obtained in the absence of 100 μM phentolamine "total binding," to obtain the binding termed "specific binding." The assay was terminated by the addition of 3 ml of the ice-cold buffer and rapid filtration through Whatman GF/C glass fiber filters under suction. After washing twice with 3 ml of the buffer, the filters were dried in an oven, transferred to counting vials and 8 ml of scintillation fluid added. Radioactivity was counted in a Packard Tri-Carb scintillation spectrometer (Model 3255). Scatchard analysis was performed according to Bennett.21
Figure 2. Scatchard plot derived from the specific $^3$H-prazosin binding data of figure 1. The slope of the plot was determined by linear regression analysis ($r = 0.98$). Dissociation constant $K_D = 4.1 \text{nM}$; $B_{\text{max}} = 92 \text{fmol/mg protein}$.

$K_D$ and $B_{\text{max}}$ calculated from non-SAH group were 4.1 nM and 92 fmol/mg protein, respectively ($N = 5$).

Specificity of $^3$H-prazosin Binding
The specificity of $^3$H-prazosin binding was studied using alpha adrenergic agonists and antagonists. Prazosin, a specific alpha 1 antagonist, and phentolamine gave IC$_{50}$ values of $1.2 \pm 0.2 \times 10^{-10}$M, $1.3 \pm 0.2 \times 10^{-6}$M, respectively. Yohimbine, a specific alpha 2 antagonist, gave a higher IC$_{50}$ value of $1.1 \pm 0.1 \times 10^{-5}$M. The adrenergic agonist norepinephrine had IC$_{50}$ value of $4.9 \pm 2.3 \times 10^{-4}$M, and the IC$_{50}$ value of epinephrine was larger than $1 \times 10^{-3}$M (table 2).

Table 2. IC$_{50}$ Values of Various Drugs Inhibiting $^3$H-prazosin and $^3$H-yohimbine Binding to Human Cerebral Arteries

<table>
<thead>
<tr>
<th>Drug</th>
<th>$^3$H-Prazosin site IC$_{50}$ (M)</th>
<th>$^3$H-Yohimbine site IC$_{50}$ (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phentolamine</td>
<td>$1.3 \pm 0.2 \times 10^{-6}$</td>
<td>$1.7 \pm 0.3 \times 10^{-7}$</td>
</tr>
<tr>
<td>Prazosin</td>
<td>$1.2 \pm 0.2 \times 10^{-10}$</td>
<td>$1.9 \pm 0.8 \times 10^{-5}$</td>
</tr>
<tr>
<td>Yohimbine</td>
<td>$1.1 \pm 0.1 \times 10^{-5}$</td>
<td>$4.2 \pm 0.6 \times 10^{-7}$</td>
</tr>
<tr>
<td>L-norepinephrine</td>
<td>$4.9 \pm 2.3 \times 10^{-4}$</td>
<td>$7.9 \pm 1.0 \times 10^{-4}$</td>
</tr>
<tr>
<td>L-epinephrine</td>
<td>$&gt;1 \times 10^{-3}$</td>
<td>$4.4 \pm 1.2 \times 10^{-3}$</td>
</tr>
</tbody>
</table>

Each value is the mean ± SEM of three separate experiments. IC$_{50}$ is the concentration of drugs that reduces the specific $^3$H-prazosin or $^3$H-yohimbine binding by 50%.

Figure 3. Saturation of specific $^3$H-yohimbine binding to human cerebral arteries. Specific $^3$H-yohimbine binding is plotted for increasing concentrations of added $^3$H-yohimbine. Each point represents the mean ± S.E.M. for six cases (case 1—case 6, table 3).

Saturability of Specific $^3$H-yohimbine Binding
Specific binding of increasing concentrations of $^3$H-yohimbine (4.4 to 55 nM) was saturable (fig. 3). Scatchard analysis indicated a single class of binding sites with $K_D$ and $B_{\text{max}}$ (fig. 4). $K_D$ and $B_{\text{max}}$ calculated...
TABLE 3  Clinical Findings in 11 Patients and KD and Bmax of Specific 3H-yohimbine Binding in Each Case

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age</th>
<th>sex</th>
<th>Cause of death</th>
<th>KD (nM)</th>
<th>Bmax (fmol/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>M</td>
<td>paranasal sinus cancer</td>
<td>22</td>
<td>140</td>
</tr>
<tr>
<td>2</td>
<td>77</td>
<td>M</td>
<td>pneumonia</td>
<td>43</td>
<td>370</td>
</tr>
<tr>
<td>3</td>
<td>79</td>
<td>F</td>
<td>pneumonia</td>
<td>18</td>
<td>220</td>
</tr>
<tr>
<td>4</td>
<td>58</td>
<td>M</td>
<td>pneumonia</td>
<td>19</td>
<td>260</td>
</tr>
<tr>
<td>5</td>
<td>77</td>
<td>F</td>
<td>pontine hemorrhage</td>
<td>15</td>
<td>300</td>
</tr>
<tr>
<td>6</td>
<td>49</td>
<td>M</td>
<td>lung cancer</td>
<td>5</td>
<td>250</td>
</tr>
<tr>
<td>7</td>
<td>50</td>
<td>M</td>
<td>SAH</td>
<td>38</td>
<td>550</td>
</tr>
<tr>
<td>8</td>
<td>70</td>
<td>M</td>
<td>SAH</td>
<td>28</td>
<td>470</td>
</tr>
<tr>
<td>9</td>
<td>51</td>
<td>F</td>
<td>SAH</td>
<td>46</td>
<td>480</td>
</tr>
<tr>
<td>10</td>
<td>72</td>
<td>M</td>
<td>SAH</td>
<td>38</td>
<td>340</td>
</tr>
<tr>
<td>11</td>
<td>83</td>
<td>M</td>
<td>SAH</td>
<td>62</td>
<td>480</td>
</tr>
</tbody>
</table>

KD and Bmax were derived from the slopes of Scatchard plots.

from the non-SAH group (table 3, case 1–case 6) were 23 nM and 250 fmol/mg protein, respectively.

Specificity of 3H-yohimbine Binding

The specificity of 3H-yohimbine binding was studied using alpha adrenergic agonists and antagonists. Yohimbine and phentolamine gave IC50 values of 4.2 ± 0.6 × 10⁻⁷M and 1.7 ± 0.3 × 10⁻⁷M, respectively. Prazosin gave a higher IC50 of 1.9 ± 0.8 × 10⁻⁷M. Norepinephrine and epinephrine had IC50 values 7.9 ± 1.0 × 10⁻⁷M and 4.4 ± 1.2 × 10⁻⁷M, respectively (N = 3) (table 2).

3H-prazosin Binding of Each Case of Non-SAH Group and SAH Group

KD and Bmax of 3H-prazosin binding were examined in each case. There were no remarkable differences between the non-SAH and SAH groups. Mean value and S.E.M. of KD and Bmax were 4 ± 1 nM and 90 ± 20 fmol/mg protein in the non-SAH group (N = 5), and 6 ± 3 nM and 90 ± 10 fmol/mg protein in the SAH group (N = 3). The corresponding values of non-SAH and SAH groups were not significantly different.

3H-yohimbine Binding in the Non-SAH and SAH Groups

KD and Bmax of 3H-yohimbine binding were examined in each case (table 3). Mean value and S.E.M. of KD and Bmax were 20 ± 5 nM and 260 ± 30 fmol/mg protein in the non-SAH group (table 3, case 1–6), 42 ± 6 nM and 460 ± 30 fmol/mg protein in SAH group (table 3, case 7–11) (table 4). The differences of KD and Bmax are statistically significant (p < 0.01). Scatchard plots of 3H-yohimbine binding of typical cases are shown in figure 5.

Discussion

Using 3H-prazosin and 3H-yohimbine, we found that both alpha 1 and alpha 2 adrenergic receptors are present in human cerebral arteries and we characterized these receptors.

Langer and Shepperson suggested that contractile responses to nerve stimulation in peripheral arteries are mediated mainly by alpha 1 adrenergic receptors, though alpha 2 adrenergic receptors also mediate contractile activity in response to circulating catecholamines. Sakakibara et al., however, noted a low response of the dog basilar artery to norepinephrine and suggested that the contraction is mediated by postsynaptic alpha 2 adrenergic receptors. Skárby et al. also noted the contraction mediated by alpha 2 adrenergic receptors in isolated cat middle cerebral arteries. Medgett and Langer found that in the middle cerebral artery of cats, the contractile responses to exogenous norepinephrine are predominantly mediated by alpha 2 adrenergic receptors. We reported the existence of alpha 2 adrenergic receptors in bovine cerebral arteries and clarified their characteristics using 3H-yohimbine. Cerebral arteries are, thus, thought to be typical of tissues in which postsynaptic alpha 2 adrenergic receptors play an important role in vasoconstriction. This is one of the possible causes of the low responsiveness of cerebral arteries of these species to norepinephrine. In the present experiment, both alpha 1 and alpha 2 adrenergic receptors were detected in human cerebral arteries. KD and Bmax of 3H-yohimbine binding site of human cerebral arteries are not so different from those of bovine cerebral arteries. The most important qualitative differences between alpha adrenergic receptors of bovine and human cerebral arteries

Fig 5. Scatchard plot of 3H-yohimbine binding of typical cases.
may be the existence of alpha 1 adrenergic receptors in the latter. Recent data indicated that the contraction of human cerebral arteries, as induced by norepinephrine is blocked by prazosin. The presence of alpha 1 adrenergic receptors would explain the higher responsiveness to norepinephrine.

We also looked at changes in alpha adrenergic receptors in the cerebral arteries after SAH. There were no remarkable changes in the H-prazosin binding site, compared with evidence in the non-SAH group. The H-prazosin used in our study had a specific activity of 17.4 Ci/mmoles. The H-prazosin binding site was smaller than 100 fmol/mg protein. In this situation, our experimental procedure might not be sufficiently sensitive to detect small changes on the H-prazosin binding sites.

K_0 and Bmax values of H-yohimbine binding sites of SAH group were more than twice as large as those in the non-SAH group. We detected alpha adrenergic receptors in both pre- and postsynaptic sites in the present study. Alpha 2 adrenergic receptors mediate contractile responses in the postsynaptic sites and inhibit norepinephrine release in the presynaptic sites in the cerebral artery. Lobato et al reported that the maximum contractile response of the cat cerebral artery to norepinephrine and serotonin was increased after experimental SAH or superior cervical ganglionectomy and that such increases were caused by denervation supersensitivity of postsynaptic mechanisms. Receptor sites become sensitive to circulating or locally released catecholamines after sympathetic denervation. At the postsynaptic receptor site, denervation induces some increase in Bmax. The altered Bmax value of H-yohimbine binding in the present study may also be caused by denervation occurring in the human cerebral arteries after SAH. The increase in Bmax of the SAH group results in enhancement of maximum contractile responses or in a leftward shift of the dose-response curve, when the receptors are exposed to high concentrations of norepinephrine and other adrenergic agents.

The increase in K_0 value of H-yohimbine binding of the SAH group in the present study, however, indicates a decreased affinity of alpha 2 adrenergic receptors to norepinephrine. In the presynaptic site, decreased affinity of alpha 2 adrenergic sites induces a collapse of the inhibition mechanism of norepinephrine release. Consequently, excessively released norepinephrine can induce vasoconstriction mediated by unaffected alpha 1 and postsynaptic alpha 2 adrenergic receptors. These changes in alpha 2 adrenergic receptors may be caused by a direct effect of the bloody cerebrospinal fluid in the subarachnoid space and following pathological changes, such as increased intracranial pressure or by degenerative changes in cerebral arteries. Decrease in cerebral blood flow after vasospasm leads to morphological changes in cerebral arteries including myonecrosis of the media and intimal thickening. Much of the arterial tissue which we used was obtained during the degenerative period and the cause of the decrease in affinity of the receptors in the cerebral blood vessels after SAH would thus be explained.

Investigations on the responsiveness to humoral and neurogenic stimuli of cerebral blood vessels after SAH, especially in human cases, are not performed frequently enough. Further pharmacodynamic studies are required to clarify the pathogenesis.

Acknowledgments

We thank the staff in the Departments of Neurosurgery and Pathology of Shizuoka Rosai Hospital for providing the arterial tissues and M. Ohara for reading the manuscript.

References

Decrease in Cerebral Blood Flow in Rats After Experimental Subarachnoid Hemorrhage: A New Animal Model

ROBERT A. SOLOMON, M.D., J. LOBO ANTUNES, M.D., RICHARD Y.Z. CHEN, M.D., LINDA BLAND, B.S., AND SHU CHIEN, M.D., PH.D.

SUMMARY There continues to be a need for good animal models of experimental subarachnoid hemorrhage (SAH). The rat would be an ideal subject in which to study SAH since it is inexpensive and easier to use than the larger laboratory animals. The present study was undertaken to determine if alterations of cerebral blood flow could be produced in the rat after experimental SAH, and thereby justify using the rat as a model for further study of SAH.

Rats weighing between 450 and 500 grams underwent insertion of a cannula into the cisterna magna at least 5 days prior to physiological testing. One group of rats then received a 0.3 cc injection of fresh autologous arterial blood into the cisterna magna to simulate a SAH. Another group of rats received injection of an equal volume of mock CSF (buffered saline) into the cisterna magna. A third group of rats had no subarachnoid injections. In all three groups, blood flow to the cerebral hemispheres was measured with the labeled microsphere technique.

Rats with experimental SAH showed a 40% decrease of cerebral blood flow, whereas rats with saline injections showed only a 15% decrease. Control rats had no changes of cerebral blood flow. These studies demonstrate that the rat is a potential experimental model for investigations into SAH.

RUPTURE OF AN INTRACRANIAL ANEURYSM or arteriovenous malformation produces a subarachnoid hemorrhage which may have devastating secondary effects on the cerebral circulation. Manifestations of these effects range from mild functional and metabolic disturbances of diencephalic structures to massive cerebral infarction produced by delayed vasospasm. Experimental designs in dogs, cats, and primates have been used to reproduce, in a laboratory setting, the blood flow changes seen in humans. These models, however, tend to be cumbersome and expensive and do not exactly mimic the clinical situation under investigation. Furthermore, these large laboratory animals do not lend themselves easily to studies using the newer techniques of quantitative autoradiography.

There continues, therefore, to be a need for improved animal models of subarachnoid hemorrhage. The rat would be an ideal species in which to study this entity since it is relatively inexpensive, easy to use, and is already the preferred model in most current studies of neuroanatomy, neurophysiology, and neuropharmacology. As well, detailed studies of brain me-
Alterations in alpha adrenergic receptors in human cerebral arteries after subarachnoid hemorrhage.

T Tsukahara, T Taniguchi, M Fujiwara, H Handa and M Nishikawa

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