Presynaptic and Postsynaptic $\alpha_2$-Adrenergic Receptors in Human Cerebral Arteries and Their Alteration After Subarachnoid Hemorrhage

Tetsuya Tsukahara, MD, Takashi Taniguchi, PhD, Soichi Miwa, MD, Shun Shimohama, MD, Motohatsu Fujiwara, MD, Michio Nishikawa, MD, and Hajime Handa, MD

The nature of $\alpha$-adrenergic receptors in human cerebral arteries was characterized, and alteration of these receptors after subarachnoid hemorrhage was examined using a radioligand binding assay.

Norepinephrine content of control arteries was also analyzed and compared with that of arteries after subarachnoid hemorrhage. Norepinephrine content in human cerebral arteries in cases of subarachnoid hemorrhage was about 5% of the control group. Specific binding of $[^3H]yohimbine$, a selective $\alpha_2$-antagonist, to cerebral arteries of the control group indicated two classes of binding sites: high-affinity sites with $K_D$ of 0.5 nM and $B_{max}$ of 18 fmol/mg protein and low-affinity sites with $K_D$ of 29 nM and $B_{max}$ of 248 fmol/mg protein. In cerebral arteries obtained from the subarachnoid hemorrhage group, $[^3H]yohimbine$ binding sites were of a single class with $K_D$ of 53 nM and $B_{max}$ of 456 fmol/mg protein. These results suggest that sympathetic denervation and subsequent alterations in $\alpha_2$-adrenergic receptors occurred after subarachnoid hemorrhage in human cerebral arteries. These changes in sympathetic innervation to cerebral arteries were considered to be one of the antecedents of delayed vasospasm after subarachnoid hemorrhage. (Stroke 1988;19:80–83)

We have characterized the nature of $\alpha$-adrenergic receptors in human cerebral arteries and examined the alteration of these receptors after subarachnoid hemorrhage (SAH) using a radioligand binding assay, and we have reported that the Scatchard plot of $[^3H]yohimbine$ binding to human cerebral arteries was linear and indicated a single class of binding sites. Recently we examined $[^3H]yohimbine$ binding in dog cerebral arteries after superior cervical ganglionectomy, and the results suggested the presence of two different binding sites, with high and low affinity for $\alpha_2$-adrenergic receptors, that we classify into $\alpha_2H$ and $\alpha_2L$ subtypes, respectively. $\alpha_2H$-Adrenergic receptors are located presynaptically whereas $\alpha_2L$-adrenergic receptors are located postsynaptically. In our previous study on human cerebral arteries, the range of concentrations of $[^3H]yohimbine$ used may not have been sufficiently low to detect a small population of the high-affinity binding sites. Therefore, we have now recharacterized $\alpha_2$-adrenergic receptors in human cerebral arteries and assayed the content of norepinephrine in arteries after SAH compared with control arteries.

Preliminary findings of the present study were reported at the Twelfth International Symposium on Cerebral Blood Flow and Metabolism, Lund/Ronneby, Sweden, June 1985.

Subjects and Methods

Collection of Arteries

Cerebral (mainly basilar, circle of Willis, and middle cerebral) arteries were carefully removed from SAH and control patients at autopsy between 1 and 2 hours after death, immediately washed in saline, and placed in a freezer at $-80^\circ$C. Clinical profiles of the patients are summarized in Table 1. Arterial membrane homogenates were prepared as described.

Assay of Norepinephrine Content in Arteries

Norepinephrine content was estimated using high-performance liquid chromatography with an electrochemical detector as previously described.

Binding Assay

$[^3H]Yohimbine$ binding was performed by incubating aliquots of arterial homogenates at 37°C for 30 minutes in 250 $\mu$L sodium phosphate buffer ($pH$ 7.4) containing $[^3H]yohimbine$ in the presence or absence of high concentrations (100 $\mu$M) of phenolamine. $[^3H]Yohimbine$ bound in the presence of 100 $\mu$M phenolamine (nonspecific binding) was subtracted from that obtained in the absence of 100 $\mu$M phenolamine (total binding) to obtain the specific binding.
Table 1. Clinical Profile of Patients

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Cause of death</th>
<th>Location of ruptured aneurysm</th>
<th>Day of death</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>44</td>
<td>M</td>
<td>Brain tumor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>51</td>
<td>M</td>
<td>Stomach cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>M</td>
<td>Head injury</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>47</td>
<td>F</td>
<td>Head injury</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>40</td>
<td>M</td>
<td>Lung cancer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subarachnoid hemorrhage group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>42</td>
<td>M</td>
<td>ACOM</td>
<td>Day 2</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>42</td>
<td>M</td>
<td>Basilar head</td>
<td>Day 3</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>71</td>
<td>F</td>
<td>ACOM</td>
<td>Day 5</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>55</td>
<td>M</td>
<td>Right MCA</td>
<td>Day 2</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>55</td>
<td>M</td>
<td>VA-PICA</td>
<td>Day 13</td>
<td></td>
</tr>
</tbody>
</table>

ACOM, anterior communicating artery; MCA, middle cerebral artery; VA, vertebral artery; PICA, posterior inferior cerebellar artery.

The assay was terminated by addition of 3 ml ice-cold buffer and rapid filtration through Whatman GF/B glass fiber filters (Clifton, New Jersey) under suction. After washing twice with 3 ml ice-cold buffer, the filters were dried in an oven, transferred to vials, and 8 ml scintillation fluid was added. Radioactivity was counted in a Packard Model 3255 Tri-Carb scintillation spectrometer (Downers Grove, Illinois).

Drugs

[3H]Yohimbine (specific activity 89.7 Ci/mmol) was purchased from New England Nuclear (Boston, Massachusetts), stored at 5°C in ethanol, and protected from light. Immediately before use, appropriate amounts of stock solution were diluted with water so that ethanol concentrations in the final assay system did not exceed 1.0%. All other chemicals were of reagent grade or the purest grade commercially available.

Computer Analysis

Scatchard plots of [3H]yohimbine binding were curvilinear, suggesting the presence of multiple binding sites. Data obtained from assays were analyzed by a nonlinear least-squares best fit of the specific [3H]yohimbine binding as a function of free [3H]yohimbine in the assay, using a Fujitsu Micro 7 computer (Tokyo, Japan). Data points were fitted to a two-independent-binding-site model described by Olsen et al.:

\[
B = B_{\text{max}1} \left( \frac{S}{K_D1 + S} \right) + B_{\text{max}2} \left( \frac{S}{K_D2 + S} \right)
\]

where \( B \) is the total amount of ligand bound at a free ligand concentration \( S \). \( B_{\text{max}1} \) and \( B_{\text{max}2} \) are the total number of binding sites for two sites having dissociation constants \( K_D1 \) and \( K_D2 \), respectively.

Results

Norepinephrine Content in Cerebral Arteries

Table 2 shows the norepinephrine content in the cerebral arteries of the control and SAH groups. Norepinephrine content was significantly lower in the arteries from the patients with SAH.

Saturability of Specific [3H]Yohimbine Binding

Specific binding of increasing concentrations of [3H]yohimbine (0.6–55 nM) was saturable in cerebral arteries of the control group (Figure 1). Scatchard analysis of the mean saturation curve of the control group was curvilinear and indicated two independent classes of [3H]yohimbine binding sites in the arteries, with \( K_D \) of 0.5 and 29 nM, respectively, and \( B_{\text{max}} \) of 18 and 248 fmol/mg protein, respectively.

Specific binding of increasing concentrations of [3H]yohimbine (0.6–55 nM) was also saturable in the cerebral arteries of the SAH group (Figure 2). Scatchard analysis of the mean saturation curve of the SAH group was, however, linear and indicated a single class of binding sites, with \( K_D \) of 53 nM and \( B_{\text{max}} \) of 456 fmol/mg protein.

Discussion

Norepinephrine content in human cerebral arteries, despite rather large individual differences, was markedly decreased within a few days of the occurrence of SAH. Previous studies in rabbits and cats showed that experimental SAH induces a transient adrenergic denervation of the cerebral arteries. Lobato et al. reported that a decrease in norepinephrine content in cat cerebral arteries was detected 3 days after experimental SAH and that the norepinephrine content gradually recovered within 15 days. Our findings confirm that adrenergic denervation occurred within 3 days of SAH in human cerebral arteries.

In our previous paper, we detected only one class of \( \alpha_2 \)-adrenergic receptors in human cerebral arteries using [3H]yohimbine as a ligand. That previous study may not have fully characterized the receptors because we could not define the location of two types of \( \alpha_2 \)-adrenergic receptors; only low-affinity sites were detected. Agrawal and Daniel also reported the existence of one class of \( \alpha_2 \)-adrenergic receptors with low affinity in rat mesenteric arteries. They proposed that the receptors detected were located postsynaptically since the receptors were not influenced by chemical denervation. More precise analysis of the high-

Table 2. Norepinephrine Content in Human Cerebral Arteries

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Norepinephrine (ng/g wet wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5</td>
<td>247.2 ± 49.7</td>
</tr>
<tr>
<td>Subarachnoid hemorrhage</td>
<td>5</td>
<td>9.8 ± 3.0*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM for 5 determinations. Control group, cases 1–5; subarachnoid hemorrhage group, cases 6–10.

*Significantly different from control group at \( p<0.01 \).
affinity receptor sites enabled us to detect two classes of \( \alpha_2 \)-adrenergic receptors in human cerebral arteries. Two classes of \( \alpha_2 \)-adrenergic receptors were also detected in dog cerebral arteries. Since high-affinity \( \alpha_2 \)-adrenergic receptors disappeared 7 days after superior cervical ganglionectomy in dog cerebral arteries, it was considered that the high-affinity receptors of human arteries are also located presynaptically and that low-affinity receptors are postsynaptic. Scatchard plots of control human cerebral arteries were similar to those of normal dog cerebral arteries, and Scatchard plots of human cerebral arteries after SAH were similar to those after sympathetic denervation. There were two classes of \( \alpha_2 \)-adrenergic receptors in the control human cerebral arteries but only one class of receptors after SAH. Consequently, the absence of high-affinity receptors was considered to represent a dysfunction of presynaptic \( \alpha_2 \)-adrenergic receptors in the arteries after SAH. As functioning presynaptic \( \alpha_2 \)-adrenergic receptors are reported to regulate norepinephrine release, it is possible that SAH induces dysfunction of the regulatory mechanism of norepinephrine release in human cerebral arteries.

**Figure 1.** Saturation of specific \(^3\)H-yohimbine binding to human cerebral arteries of control group (Cases 1–5). Each point represents mean ± SEM for 5 determinations. Inset shows Scatchard plot of mean saturation curve. High-affinity site: \( K_D = 0.5 \text{nM}, B_{\text{max}} = 18 \text{ fmoles/mg protein} \); low-affinity site: \( K_D = 29 \text{ nM}, B_{\text{max}} = 248 \text{ fmoles/mg protein} \).

**Figure 2.** Saturation of specific \(^3\)H-yohimbine binding to human cerebral arteries of subarachnoid hemorrhage group (Cases 6–10). Each point represents mean ± SEM for 5 determinations. Inset shows Scatchard plot of mean saturation curve. \( K_D = 53 \text{nM}, B_{\text{max}} = 456 \text{ fmoles/mg protein} \).
cerebral arteries. It was also observed that the affinity of postsynaptic \( \alpha_2 \)-adrenergic receptors decreased and that \( B_{max} \) of these receptors increased in human cerebral arteries after SAH. We found no alteration of the affinity of postsynaptic \( \alpha_2 \)-adrenergic receptors in dog cerebral arteries after surgical denervation by superior cervical ganglionectomy\(^2\) though \( B_{max} \) of the receptors increased. Therefore, it is considered that SAH not only has a denervation effect on these arteries but also has some direct effect on postsynaptic \( \alpha_2 \)-adrenergic receptors. The role of postsynaptic \( \alpha_2 \)-adrenergic receptors is still controversial. Norepinephrine-induced contraction is weak and mediated by postsynaptic \( \alpha_2 \)-adrenergic receptors in dog cerebral arteries\(^2\); in human cerebral arteries, however, norepinephrine-induced contraction is mediated by \( \alpha_1 \)-adrenergic receptors.\(^{10}\) In such arteries, in which the existence of both \( \alpha_2 \)- and postsynaptic \( \alpha_2 \)-adrenergic receptors are postulated, the role of postsynaptic \( \alpha_2 \)-adrenergic receptors is not clear. Although vasodilatory function of these receptors was also reported,\(^{11}\) further investigations will be needed to clarify the function of the \( \alpha_2 \)-adrenergic receptors and the significance of alterations of such receptors after SAH.

In conclusion, sympathetic denervation of the cerebral arteries and subsequent dysfunction of presynaptic \( \alpha_2 \)-adrenergic receptors were observed in human cerebral arteries after SAH. Dysfunction of the regulatory mechanism of norepinephrine release may be related to the occurrence of delayed vasospasm after SAH.

Acknowledgment

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