Cerebrovascular reactivity to noradrenaline and serotonin following experimental subarachnoid hemorrhage

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This study analyzes the time course of the changes induced by subarachnoid hemorrhage (SAH) in the sensitivity of cat cerebral arteries to noradrenaline and serotonin. Cerebral arteries displayed a supersensitivity to these amines, which was most marked 3 days after the experiment and then gradually disappeared. The supersensitivity to serotonin was greater and longer than the response to noradrenaline. The increase in the vascular contractile response induced by SAH was similar to that seen after superior cervical ganglionectomy or intracisternal injections of 6-hydroxydopamine. It is suggested that supersensitivity to noradrenaline and serotonin induced by SAH may be involved in the production of chronic cerebral vasospasm.

KEY WORDS • subarachnoid hemorrhage • cerebral vasospasm • noradrenaline • serotonin

EXPERIMENTAL evidence showing that noradrenaline and 5-hydroxytryptamine (5-HT, serotonin) are involved in the production of cerebral vasospasm has been obtained in normal animals. It seems appropriate to analyze the spasmogenic ability of these amines on the cerebral arteries of animals previously exposed to subarachnoid hemorrhage (SAH).

The present experiments were designed to study in vitro the effect of noradrenaline and serotonin on the cerebral arteries of cats exposed to SAH. Since the most common pattern found in humans is that of delayed vasospasm, we analyzed the changes in the reactivity of cerebral arteries to noradrenaline and serotonin at various time periods after the hemorrhage. In addition, considering that the pial vessels seem to be denervated after SAH, we also compared the reactivity of the cerebral arteries from cats with SAH and from cats subjected either to removal of the superior cervical ganglia or to intracisternal injections of 6-hydroxydopamine (6-OHDA), an agent that specifically destroys the adrenergic nerve terminals.

Materials and Methods

Cats of either sex, ranging in weight from 1.5 to 4 kg, were anesthetized intraperitoneally with sodium pentobarbital (35 mg/kg). One group of animals was pretreated with 1 to 2 ml of autologous blood injected into the cisterna magna by means of a polyethylene microcatheter. In a second group of cats, both superior cervical sympathetic ganglia were removed. A third group of animals was pretreated with 6-OHDA (5 mg intracisternally). To reduce the rate of oxidation of this drug it was dissolved in 1.0 ml of mock cerebrospinal fluid (composition in mM: Na+, 156; K+, 3; Ca++, 1.5; Cl-, 151) containing 0.01% (weight/volume) of ascorbic acid. An additional group of cats served as untreated controls.

At various time periods after treatment, the cats were killed by bleeding under pentobarbital anesthesia. The brains were carefully removed, and the posterior communicating arteries (PCA's) were cut into cylindrical segments 4 mm in length. Each arterial cylinder was prepared for isometric recording in an organ bath according to the method described by
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FIG. 1. Effect of subarachnoid hemorrhage (SAH) on the dose-response curves to noradrenaline (left) and serotonin (5-HT) (right). Numbers in parentheses indicate the number of arterial segments used. Each point represents the mean ± standard error of the mean.

Nielsen and Owman. The organ bath contained 6 ml of Krebs-Henseleit solution (composition in mM: NaCl, 115; KCl, 4.6; CaCl₂, 2.5; KH₂PO₄, 1.2; MgSO₄·7H₂O, 1.2; NaHCO₃, 25; glucose, 11.1; EDTA, 0.03). This solution, maintained at 37°C, was continuously bubbled with a 95% O₂-5% CO₂ mixture that gave a pH of 7.3 to 7.4. Isometric vascular responses were measured using a Grass force-displacement transducer connected to a Grass polygraph.* A resting tension of 0.5 gm was applied to the arterial segments and readjusted every 15 minutes during an equilibration period of 90 to 120 minutes before cumulative dose-response curves to noradrenaline and serotonin were made in the arteries. The arteries were from cats in four groups as follows: 1) cats exposed to SAH 3, 7, 15, and 30 days before the experiments; 2) cats in which both superior cervical ganglia had been removed 15 days before the experiments; 3) cats pretreated 7 and 15 days before with intracisternal injections of 6-OHDA; and 4) control cats. The median effective doses (ED₅₀) for the agonists were calculated according to the method described by Fleming, et al.

Results

Noradrenaline induced a dose-dependent contractile response of the PCA's of normal cats (Fig. 1). This response was significantly reduced (p < 0.02) in a competitive manner by phentolamine (10⁻⁶M), an alpha-adrenergic blocker.

Subarachnoid hemorrhage induced a great increase in the contractile response of the PCA to noradrenaline. This response reached a peak 3 days after SAH, and was significantly increased at all doses used (p < 0.001) (Fig. 1). Thereafter, a tendency to normalization in the sensitivity of the PCA to noradrenaline was observed, and 7 days after the hemorrhage the contractile vascular responses were not significantly different from those found in control animals.

The removal of both superior cervical ganglia performed 2 weeks prior to the experiments induced an increase in the contractile response of the PCA to noradrenaline, which was significant at all doses used (p < 0.025) (Fig. 2). Intracisternal injections of 6-OHDA 7 and 15 days before the experiment also in-
duced an increase in the contractile response of the PCA to noradrenaline, which was significant even after 2 weeks of the administration of 6-OHDA (p < 0.05) (Fig. 3).

Serotonin induced a dose-dependent contractile response of the PCA in normal cats (Fig. 1). This response was significantly diminished (p < 0.02) in the presence of $6 \times 10^{-9}$M lysergic acid diethylamide (LSD), a blocker of the tryptaminergic receptors.

Subarachnoid hemorrhage induced a great increase in the contractile response of the PCA to serotonin. This increase reached a peak 3 days after SAH, and was significant at all doses used (p < 0.001) (Fig. 1). Thereafter, a decrease in the vascular contractile response to serotonin was observed. However, 30 days after the hemorrhage the vasoconstriction induced by serotonin was still significantly increased in comparison with controls (p < 0.05) (Fig. 1). The addition of LSD ($6 \times 10^{-9}$M) significantly diminished the contractile response induced by serotonin in the PCA of cats exposed to SAH 15 days before the experiment (p < 0.02). The effect of LSD on the contractile response of the PCA from treated animals was similar to that found in controls.

The removal of both superior cervical sympathetic ganglia performed 15 days before the experiments also elicited an increase in the contractile response of the PCA to serotonin that was significant even after 2 weeks of the administration of 6-OHDA (p < 0.05) (Fig. 3).

The EDso values and the maximum responses of the PCA to noradrenaline and serotonin for each experimental situation are shown in Table 1. The EDso values were significantly diminished only for noradrenaline in ganglionectomized cats (p < 0.02), and for serotonin in cats pretreated 7 days before the experiment with intracisternal injections of 6-OHDA (p < 0.001). The maximum responses of the PCA to both amines were significantly increased in all these experimental situations, except the response to noradrenaline 7, 15, and 30 days after SAH.

Discussion

In a previous paper, we demonstrated that SAH induces a decrease in the noradrenaline content and the dopamine beta-hydroxylase activity of cat cerebral arteries. In the present work, we have shown that SAH induces a supersensitivity of these vessels to noradrenaline which peaks 3 days after the hemorrhage and then gradually disappears. Taking into account these findings and those reported by other authors, it seems conceivable that the supersensitivity observed in the present experiments is of a postdenervation type. This hypothesis is supported by the finding that the supersensitivity induced...
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![Graph showing the effect of intracisternal injections of 6-hydroxydopamine (6-OHDA) on the dose-response curves to noradrenaline (left) and serotonin (5-HT) (right). Numbers in parentheses indicate the number of arterial segments used. Each point represents the mean ± standard error of the mean.](image)

**TABLE 1**

Vasoconstrictor effects of noradrenaline and serotonin on the posterior communicating artery of the cat

<table>
<thead>
<tr>
<th>Treatment &amp; Days After Treatment</th>
<th>No. of Arterial Segments</th>
<th>Noradrenaline</th>
<th>Serotonin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>ED_{50} (M) (95% confidence interval)</td>
<td>Maximum Responses (mg ± SE)</td>
</tr>
<tr>
<td>none</td>
<td>13</td>
<td>6.7 × 10^{-7} (1.4-32)</td>
<td>298 ± 39</td>
</tr>
<tr>
<td>ganglionectomy (15 days)</td>
<td>12</td>
<td>3.6 × 10^{-8} (1.3-9.6)</td>
<td>508 ± 81</td>
</tr>
<tr>
<td>6-OHDA 7 days</td>
<td>8</td>
<td>1.9 × 10^{-7} (0.2-17)</td>
<td>852 ± 104‡</td>
</tr>
<tr>
<td>15 days</td>
<td>5</td>
<td>4.8 × 10^{-7} (1.4-17)</td>
<td>567 ± 92†</td>
</tr>
<tr>
<td>SAH 3 days</td>
<td>5</td>
<td>3.1 × 10^{-7} (0.4-20)</td>
<td>778 ± 97‡</td>
</tr>
<tr>
<td>7 days</td>
<td>15</td>
<td>6.6 × 10^{-7} (1.8-23)</td>
<td>431 ± 90</td>
</tr>
<tr>
<td>15 days</td>
<td>21</td>
<td>7.5 × 10^{-7} (3.7-15)</td>
<td>412 ± 58</td>
</tr>
<tr>
<td>30 days</td>
<td>7</td>
<td>7.8 × 10^{-7} (2.4-18)</td>
<td>397 ± 52</td>
</tr>
</tbody>
</table>

*ED_{50} = molar concentration of agonists at which half-maximum responses occur; 6-OHDA = 6-hydroxydopamine; SAH = subarachnoid hemorrhage; SE = standard error.
†p < 0.05.
‡p < 0.001.

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by bleeding resembles that seen after superior cervical
ganglionectiony or intracisternal injection of 6-
OHDA. These two denervating procedures produce a
nonspecific supersensitivity that may involve both
presynaptic (loss of the uptake of noradrenaline) and
postsynaptic mechanisms.\textsuperscript{10,12} We believe that a sim-
ilar type of supersensitivity develops after SAH.

Moreover, it may be argued that, if vasospasm were
due to the release of serotonin and/or other vasoactive
agents from the extravasated coagulus, it would be at
a maximum level immediately after the bleed and not
delayed in onset.

As occurred with noradrenaline, most of the ex-
periments analyzing the action of serotonin on
cerebral arteries have been performed in normal
animals, and the effect of this amine was only recently
tested on the cerebral arteries of animals exposed to
SAH.\textsuperscript{24} In this study, we have found that SAH induces
a supersensitivity of cat cerebral arteries to serotonin.

This supersensitivity, as with that displayed in re-
sponse to noradrenaline, was maximum 3 days after
SAH, and slowly decreased afterward. However, the
contractile response to serotonin was still significa-
tly different from that found in controls as long as 4
weeks after the hemorrhage. A similar supersensi-
tivity to this amine occurred after superior cervical
ganglionectiony or intracisternal injection of 6-
OHDA. The increase in the maximal responses of the
PCA to serotonin induced by ganglionectiony, 6-
OHDA pretreatment, and SAH indicates that a post-
synaptic supersensitivity is produced by these three
experimental procedures. The ED\textsubscript{50} values for serotonin
were significantly reduced 7 days after an intraciste-
ral injection of 6-OHDA, suggesting that this was the
sole instance in which the affinity of tryptaminergic
receptors was increased and a clear-cut presynaptic
supersensitivity occurred. As ED\textsubscript{50} values were also
decreased (although not significantly), indicating that a
presynaptic component was also involved in some
degree.

The proposition that the supersensitivity of the
cerebral arteries to noradrenaline seen after SAH is
related to the adrenergic denervation of these vessels is
also supported by the fact that the time course of the
changes occurring in the arterial sensitivity after SAH
closely resembles the time course of the changes
induced by this condition in 1) the histochemical fluo-
rescence of the sympathetic cerebrovascular nerves,\textsuperscript{13}
2) the noradrenaline content of cerebral arteries,\textsuperscript{16,23}
and 3) the ability of these vessels to take up and retain
radioactive noradrenaline.\textsuperscript{16,23}

Serotonin is one of the most potent vasoconstrictor
agents of the cerebral arteries hitherto known (com-
pare the ED\textsubscript{50} values and the maximum responses to
noradrenaline and serotonin in Table 1), and there is
experimental evidence favoring this amine as the
causative agent of the cerebral vasospasm that occurs
after SAH.\textsuperscript{2} Allen et al.,\textsuperscript{5} according to Allen, et al.,
serotonin would be released from platelets of the ex-
travasated blood during the chronic phase of the
cerebral vasospasm. However, \textit{in vivo} experiments in-
jecting physiological concentrations of serotonin in
the cerebral subarachnoid space have failed to induce
chronic vasospasm similar to that seen in patients.\textsuperscript{7,17}

Moreover, it may be argued that, if vasospasm were
due to the release of serotonin and/or other vasoactive
agents from the extravasated coagulus, it would be at
a maximum level immediately after the bleed and not
delayed in onset.

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