Prevention of cerebral vasospasm in the rat by depletion or inhibition of substance P in conducting vessels

Tia Juana Delgado-Zygmunt, M.D., Ph.D., Mohammed Abdul-Rahman Arbab, M.D., Ph.D., Lars Edvinsson, M.D., Ph.D., Inger Jansen, M.D., and Niels Aage Svendgaard, M.D., Ph.D.

Neurosurgical Research Department, Department of Internal Medicine, University Hospital, and Department of Experimental Research, University of Lund, Malmö General Hospital, Lund, Sweden

Cisternal blood injection in the rat induces a biphasic angiographic vasospasm, with a maximal acute spasm at 10 minutes and a maximal late spasm at 2 days after the subarachnoid hemorrhage (SAH). Depletion of substance P-containing sensory nerves to the cerebral arteries with capsaicin prior to SAH prevents the development of both acute and late spasm. Intrathecal administration of the substance P antagonist spantide 2 hours prior to SAH also prevents the development of vasospasm, while spantide administration 1 hour before SAH only hinders the occurrence of late vasospasm. Intracisternal administration of spantide 2 hours post-SAH prevents the development of late vasospasm. This antagonist per se can induce a short-lasting dose-dependent angiographic vasoconstriction. Substance P-containing nerve fibers on the cerebral arteries could constitute the sensory link in a reflex arc system involved in the development of vasospasm in which the presence of blood in the subarachnoid space stimulates sensory substance P-containing nerve fibers on the cerebral arteries inducing a centripetal impulse to the A2-NTS (nucleus tractus solitarius) and setting into motion the events in the brain stem leading to acute and late vasospasm.

KEY WORDS • subarachnoid hemorrhage • vasospasm • substance P • capsaicin • rat

Cerebral vasospasm remains an important cause of morbidity and mortality following aneurysmal subarachnoid hemorrhage (SAH), despite recent encouraging advances made in the management of delayed cerebral ischemia using prophylactic treatment with nimodipine. Lack of knowledge concerning the pathogenesis of vasospasm has, however, prevented the development of a successful mode of therapy.

In investigations of vasospasm in an SAH model in the rat, investigations of the A2 (nomenclature according to Dahlström and Fuxe) and nucleus tractus solitarius (A2-NTS) in the medulla oblongata and its projection site, the median eminence hypothalamus, have been involved in the development of vasospasm. The A2-NTS receives interoceptive sensory signals for transmission to the hypothalamus, where coordination of autonomic and endocrine responses takes place. Signals induced by blood in the subarachnoid space could be transmitted to the A2 by sensory nerve fibers on the cerebral arteries. The anatomical prerequisites exist for the transmission of "information" from the cerebral arteries to the A2-NTS. The cerebral arteries have a substance P-containing trigeminovascular sensory innervation and connections between the A2-NTS and substance P neurons innervating the cerebral arteries from the trigeminal ganglia have recently been identified. Furthermore, clinical and experimental studies indicate that the trigeminovascular sensory innervation can convey nociceptive information to the brain stem and that this innervation is involved in reflex vasomotor function.

The aim of the present study was to investigate the role of the sensory innervation in the development of vasospasm. The effect of lesioning substance P sensory fibers with capsaicin and the therapeutic effect of a substance P antagonist on vasospasm were evaluated in a rat SAH model.

Materials and Methods

Animal Preparation

Male Sprague-Dawley rats (SPF strain), each weighing 240 to 320 gm, were used in this study. The surgical...
and angiographic procedures have been described in detail in an earlier communication and will be mentioned only briefly here. After induction of anesthesia with 4% halothane, the animals were intubated and artificially ventilated. During insertion of catheters, anesthesia was maintained with 75% halothane in 70% N₂O and 30% O₂. After infiltration of the skin with lidocaine hydrochloride, catheters were inserted using a microsurgical technique into the axillary arteries for subsequent angiography. The femoral artery and vein were cannulated for continuous monitoring of blood pressure, blood sampling, and drug infusion. Heparin (75 IU/kg) was given intravenously. Following surgery, the administration of halothane was discontinued and suxamethonium chloride (Celocurin, 3 mg/kg) was given intravenously. Angiography was performed not earlier than 30 minutes following the procedure. The animals were kept on a heating pad and the body temperature was maintained close to 37°C.

Vertebrobasilar angiography was performed via the bilateral occipital catheters using metrizamide (Amipaque). After control angiography, 0.3 ml homologous blood was injected intracisternally through a previously implanted catheter. Repeat angiography was performed 10 minutes and 2 days after the injection of blood (that is, at the time of maximal acute and late vasospasm in this animal model). Following angiography on Day 0, the axillary and femoral catheters were removed. The animals were extubated and returned to their cages. On Day 2, the animals were anesthetized and reintubated as described, and catheters were inserted into the axillary and femoral vessels for angiography and blood pressure recording.

Measurements of the vertebrobasilar arteries were made from the angiograms, using a technique similar to that described by Gabrielsen and Greitz. The diameters were measured with a 0.05-mm accuracy, which is about 5% of the mean vessel diameter, using a calibrated precision lens with fixed magnification and lens-to-film distance. The values from four preselected points within the vertebrobasilar system were averaged and expressed as a percentage of control values. Each animal served as its own control.

Experimental Design

Group I included six normal animals described in a previous communication. Group IIA included six animals pretreated with capsaicin neonatally and three animals pretreated with capsaicin as adults. Group IIB comprised two animals pretreated with the solvent neonatally and two treated as adults; these animals served as controls. Group III included five animals injected with the substance P antagonist spantide (1 μg) intracisternally 2 hours prior to SAH (Subgroup A); seven animals injected with spantide (1 μg) intracisternally 2 hours after the SAH (Subgroup B); and four animals injected with spantide (1 μg) intracisternally 1 hour prior to SAH (Subgroup C). Group IV included three animals treated with spantide (0.1 μg) intracister-

nally 2 hours prior to SAH. Group VA comprised three animals with control angiography followed by intracisternal injection of spantide (1 μg); angiography was repeated at 10 and 30 minutes postinjection. Group VB included four animals with control angiography followed by intracisternal injection of spantide (0.1 μg); angiography was repeated at 10 minutes postinjection. In Group VI studies, the cerebral arteries of five untreated animals were used for determination of the concentration-response curve to spantide in vitro.

The animals in Groups I to IV were subjected to an SAH as described. In these groups, control angiography was followed by repeat angiography at 10 minutes and 2 days post-SAH.

Capsaicin Treatment

Group IIA animals were treated with capsaicin either neonatally or as adults. The neonatal animals received capsaicin (50 mg/kg dissolved in 10% ethanol and 10% Tween 80 in 0.9% NaCl solution) on the 2nd day after birth. The capsaicin solution was injected subcutaneously under halothane anesthesia. The injection caused respiratory difficulties that were relieved by the administration of oxygen and manually assisted respiration. Two to 3 months later, an SAH was induced and angiography was performed. Adult animals were given capsaicin subcutaneously, 125 mg/kg, under ether anesthesia: 25 and 50 mg/kg on the 1st day and 50 mg/kg on the 2nd day. The animals injected with capsaicin as adults underwent angiography 1 to 3 weeks later. A control group of animals (Group IIB) were treated with solvent either neonatally or as adults. As a control for the lesions, the pial arteries were examined for the presence of substance P-containing fibers with an immunohistochemical technique. In the capsaicin-treated rats, no substance P-containing nerve fibers were seen in the vertebrobasilar system.

Treatment With Substance P Antagonist

The substance P antagonist, spantide (D-arg₁, d-trp⁷⁻Leu¹ substance P), was injected through a previously implanted cisternal catheter. Injections of 1 or 0.1 μg spantide in 0.1 ml saline were performed in Group III, IV, and V animals.

In Vitro Recordings

In Group VI studies, circular segments of rat basilar arteries were placed between two L-shaped metal holders in a temperature-controlled (37°C) 5-ml tissue bath containing a buffer solution. The methodological details are given by Högestätt, et al. The buffer solution was bubbled with a mixture of 95% O₂/5% CO₂ to maintain a pH of 7.3. One of the holders was connected to a force transducer for registration of isometric tension and the other to a movable unit allowing fine adjust-

† Force transducer, Model FT 03C, manufactured by Grass Instrument Co., Quincy, Massachusetts.
Prevention of cerebral vasospasm by substance P depletion

### TABLE 1

<table>
<thead>
<tr>
<th>Group</th>
<th>Time of Study</th>
<th>MABP (mm Hg)</th>
<th>Pulse (/min)</th>
<th>pH</th>
<th>PaO₂ (mm Hg)</th>
<th>PaCO₂ (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I: normal animals (6 rats)</td>
<td>control (pre-SAH)</td>
<td>121 ± 4</td>
<td>305 ± 29</td>
<td>7.41 ± 0.01</td>
<td>156 ± 10</td>
<td>35.7 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>10 min post-SAH</td>
<td>118 ± 8</td>
<td>282 ± 18</td>
<td>7.41 ± 0.01</td>
<td>156 ± 10</td>
<td>35.7 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>2 days post-SAH</td>
<td>111 ± 8</td>
<td>300 ± 35</td>
<td>7.41 ± 0.02</td>
<td>156 ± 15</td>
<td>36.5 ± 1.2</td>
</tr>
<tr>
<td>IIA: animals pretreated with capsaicin as neonates (6 rats)</td>
<td>control (pre-SAH)</td>
<td>104 ± 3†</td>
<td>283 ± 17</td>
<td>7.38 ± 0.01</td>
<td>153 ± 9</td>
<td>39.0 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>10 min post-SAH</td>
<td>122 ± 4‡</td>
<td>288 ± 29 (5)</td>
<td>7.38 ± 0.01</td>
<td>149 ± 13</td>
<td>37.3 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>2 days post-SAH</td>
<td>119 ± 2</td>
<td>300 ± 15</td>
<td>7.37 ± 0.03</td>
<td>169 ± 16</td>
<td>38.2 ± 2.0</td>
</tr>
<tr>
<td>IIA: animals pretreated with capsaicin as adults (3 rats)</td>
<td>control (pre-SAH)</td>
<td>126 ± 2</td>
<td>300 ± 0 (2)</td>
<td>7.39 ± 0.01</td>
<td>138 ± 14</td>
<td>38.0 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>10 min post-SAH</td>
<td>128 ± 13</td>
<td>300 ± 0 (1)</td>
<td>7.39 ± 0.01</td>
<td>138 ± 14</td>
<td>38.0 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>2 days post-SAH</td>
<td>121 ± 3</td>
<td>280 ± 20</td>
<td>7.38 ± 0.03</td>
<td>131 ± 20</td>
<td>35.8 ± 1.3</td>
</tr>
<tr>
<td>IIB: animals pretreated with solvent (4 rats)</td>
<td>control (pre-SAH)</td>
<td>108 ± 10</td>
<td>300 ± 24</td>
<td>7.34 ± 0.02</td>
<td>115 ± 14</td>
<td>38.6 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>10 min post-SAH</td>
<td>117 ± 13</td>
<td>300 ± 0</td>
<td>7.34 ± 0.02</td>
<td>115 ± 14</td>
<td>38.6 ± 2.0</td>
</tr>
<tr>
<td></td>
<td>2 days post-SAH</td>
<td>120 ± 3</td>
<td>300 ± 24</td>
<td>7.35 ± 0.02</td>
<td>136 ± 11</td>
<td>39.4 ± 2.0</td>
</tr>
</tbody>
</table>

* Values are means ± standard error of the means for the number of rats given or the number indicated in parentheses. SAH = subarachnoid hemorrhage; MABP = mean arterial blood pressure.
† Significance of difference from control value in animals treated as adults: p < 0.05.
‡ Significance of difference from control pre-SAH value: p < 0.01.

### TABLE 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Time of Study</th>
<th>MABP (mm Hg)</th>
<th>Pulse (/min)</th>
<th>pH</th>
<th>PaO₂ (mm Hg)</th>
<th>PaCO₂ (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I: normal animals (6 rats)</td>
<td>control (pre-SAH)</td>
<td>121 ± 4</td>
<td>305 ± 29</td>
<td>7.41 ± 0.01</td>
<td>156 ± 10</td>
<td>35.7 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>10 min post-SAH</td>
<td>118 ± 8</td>
<td>282 ± 18</td>
<td>7.41 ± 0.01</td>
<td>156 ± 10</td>
<td>35.7 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>2 days post-SAH</td>
<td>111 ± 8</td>
<td>300 ± 35</td>
<td>7.41 ± 0.02</td>
<td>156 ± 15</td>
<td>36.5 ± 1.2</td>
</tr>
<tr>
<td>IIA: animals injected with spantide intracisternally</td>
<td>control (pre-SAH)</td>
<td>119 ± 8</td>
<td>330 ± 17 (4)</td>
<td>7.34 ± 0.01</td>
<td>133 ± 11</td>
<td>38.7 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>10 min post-SAH</td>
<td>128 ± 9</td>
<td>323 ± 23 (4)</td>
<td>7.34 ± 0.01</td>
<td>133 ± 11</td>
<td>38.7 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>2 hrs post-SAH (5 rats)</td>
<td>121 ± 7 (4)</td>
<td>270 ± 30 (2)</td>
<td>7.39 ± 0.2 (4)</td>
<td>148 ± 8 (4)</td>
<td>38.7 ± 1.5 (4)</td>
</tr>
<tr>
<td>IIB: animals injected with spantide intracisternally</td>
<td>control (pre-SAH)</td>
<td>121 ± 6</td>
<td>330 ± 17</td>
<td>7.34 ± 0.01</td>
<td>119 ± 8</td>
<td>37.6 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>10 min post-SAH</td>
<td>128 ± 3</td>
<td>310 ± 24 (6)</td>
<td>7.34 ± 0.01</td>
<td>119 ± 8</td>
<td>37.6 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>2 hrs post-SAH (7 rats)</td>
<td>123 ± 8</td>
<td>310 ± 29 (6)</td>
<td>7.39 ± 0.02</td>
<td>149 ± 10</td>
<td>36.6 ± 1.0</td>
</tr>
<tr>
<td>IIC: animals injected with spantide intracisternally</td>
<td>control (pre-SAH)</td>
<td>125 ± 8</td>
<td>276 ± 15</td>
<td>7.36 ± 0.02</td>
<td>132 ± 14</td>
<td>38.4 ± 1.0</td>
</tr>
<tr>
<td></td>
<td>1 hr post-SAH (4 rats)</td>
<td>119 ± 7</td>
<td>300 ± 35</td>
<td>7.35 ± 0.02</td>
<td>123 ± 15</td>
<td>39.0 ± 1.0</td>
</tr>
</tbody>
</table>

* Values are means ± standard error of the means for the number of rats given or the number indicated in parentheses. SAH = subarachnoid hemorrhage; MABP = mean arterial blood pressure.

### Results

#### Physiological Parameters

The physiological parameters at the time of angiography before and after the SAH in Groups I to IV are shown in Tables 1, 2, and 3. The control mean arterial blood pressure (MABP) in the animals treated neonatally with capsaicin was 17% lower than that in the animals treated as adults (p < 0.05; Table 1). At 10 minutes after SAH, MABP in the neonatally treated animals was 18% higher than the pre-SAH values (p < 0.01), while in the adult-treated animals there was little difference in MABP at the two time points. After intracisternal administration of spantide, there was no significant change in MABP (Tables 2 and 3).

#### Effect of Capsaicin Treatment on Vasospasm

In the normal animals (Group I), SAH induced an acute vasoconstriction of about 36% and a late vasospasm of about 23%. Pretreatment with solvent (Group IIB) did not affect the degree of acute or late vasospasm. Pretreatment with capsaicin (Group IIA) prevented both acute and late vasospasm (Fig. 1). The capsaicin-treated group consisted of six animals treated neonatally and three animals treated as adults. Since there was no difference in the angiographic findings in the neonatal or adult treatment group, the data were pooled. The mean vessel diameter pre-SAH was the same in normal, solvent-treated, or capsaicin-treated animals.
TABLE 3
Physiological parameters pre- and post-SAH: influence of intracisternal injection of 0.1 \( \mu \text{g} \) spantide*

<table>
<thead>
<tr>
<th>Group</th>
<th>Time of Study</th>
<th>MABP (mm Hg)</th>
<th>Pulse (/min)</th>
<th>pH</th>
<th>PaO2 (mm Hg)</th>
<th>PaCO2 (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control (pre-SAH)</td>
<td>121 ± 4</td>
<td>305 ± 29</td>
<td>7.41 ± 0.01</td>
<td>156 ± 10</td>
<td>35.7 ± 1.3</td>
</tr>
<tr>
<td>I: normal animals (6 rats)</td>
<td>10 min post-SAH</td>
<td>118 ± 8</td>
<td>282 ± 18</td>
<td>7.41 ± 0.01</td>
<td>156 ± 10</td>
<td>35.7 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>2 days post-SAH</td>
<td>111 ± 8</td>
<td>300 ± 35</td>
<td>7.41 ± 0.02</td>
<td>156 ± 15</td>
<td>36.5 ± 1.2</td>
</tr>
<tr>
<td>IV: animals injected with spantide intracisternally</td>
<td>control (pre-SAH)</td>
<td>129 ± 6</td>
<td>310 ± 26</td>
<td>7.39 ± 0.02</td>
<td>133 ± 9</td>
<td>38.8 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>10 min post-SAH</td>
<td>132 ± 4</td>
<td>345 ± 15 (2)</td>
<td>7.39 ± 0.02</td>
<td>133 ± 9</td>
<td>38.8 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>2 days post-SAH</td>
<td>122 ± 6</td>
<td>390 ± 30 (2)</td>
<td>7.35 ± 0.04</td>
<td>167 ± 30</td>
<td>40.0 ± 1.3</td>
</tr>
</tbody>
</table>

* Values are means ± standard error of the means for the number of rats given or the number indicated in parentheses. SAH = subarachnoid hemorrhage; MABP = mean arterial blood pressure.

Effect of Spantide Treatment on Vasospasm

Intracisternal administration of spantide (1 \( \mu \text{g} \)) 2 hours before SAH (Group IIIA) prevented both acute and late spasm (Fig. 2). Intracisternal administration of spantide (1 \( \mu \text{g} \)) 2 hours after SAH (Group IIIB) prevented the development of late vasospasm (Fig. 2). The degree of acute vasospasm in this group 10 minutes after the SAH was similar to the degree seen in untreated SAH animals. The animals receiving spantide (1 \( \mu \text{g} \) ) 1 hour before SAH (Group IIIC) demonstrated a degree of acute vasospasm equivalent to that seen in untreated SAH animals (Group I) (Figs. 2 and 3); however, the development of late vasospasm was prevented in Group IIIC animals. Intracisternal injection of 0.1 \( \mu \text{g} \) spantide 2 hours before the SAH (Group IV) prevented the development of both acute and late vasospasm (Fig. 4).

Effect of Spantide Treatment on Cerebral Arterial Diameter

Intracisternal administration of 1 \( \mu \text{g} \) spantide (Group VA) caused a mean constriction of the vertebrobasilar system of 27% at 10 minutes. The constriction disappeared by 30 minutes. In Group VB, the mean arterial diameter was not affected by the administration of 0.1 \( \mu \text{g} \) of the antagonist (Table 4).

In Vitro Measurements

The effect of various concentrations of spantide on the rat basilar artery in tissue-bath experiments (Group VI) is demonstrated in Fig. 5. The degree of contraction is expressed as a percentage of the contraction induced by 60 mM K⁺. The constriction was maximal at 10⁻⁴ M of spantide.

Morbidity and Mortality

All animals treated in adulthood with capsaicin or spantide survived. Following SAH, the animals were drowsy, with very little spontaneous activity. No paralysis was noted in any animal group. The animals treated neonatally with capsaicin were fragile and one-third of the animals died within 48 hours post-SAH.
Prevention of cerebral vasospasm by substance P depletion

FIG. 3. Vertebrobasilar angiography in an animal receiving spantide (1 μg) 1 hour prior to subarachnoid hemorrhage, before (A), 10 minutes after (B), and 2 days after (C) cisternal blood injection. There is marked acute vasospasm but no late vasospasm.

FIG. 4. Angiographic changes in mean vertebrobasilar diameter in rats treated intracisternally with spantide (0.1 μg) 2 hours prior to subarachnoid hemorrhage (Group IV). The values are means ± standard error of the means. Double asterisks: p < 0.01; triple asterisks: p < 0.001.

TABLE 4
Effect of intracisternal spantide on vertebrobasilar artery vessel diameter

<table>
<thead>
<tr>
<th>Time After Injection (min)</th>
<th>No. of Rats</th>
<th>Dose (μg)</th>
<th>Vessel Diameter*</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>3</td>
<td>1</td>
<td>73.3 ± 8.9</td>
</tr>
<tr>
<td>30</td>
<td>3</td>
<td>1</td>
<td>100.0 ± 7.6</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>0.1</td>
<td>102.5 ± 3.6</td>
</tr>
</tbody>
</table>

* Values are expressed as a percentage of control.

Discussion

Cisternal injection of blood in the rat induces a biphasic reproducible vasospasm that can be measured with angiographic evaluation of the vertebrobasilar system. The acute vasospasm is maximal at 10 minutes and the late vasospasm is maximal at 2 days post-SAH. Treatment with capsaicin prior to SAH prevents the development of both the acute and late vasospasm. Intracisternal injection of the substance P antagonist spantide (1 μg or 0.1 μg) 2 hours before SAH also prevents the occurrence of acute and late vasospasm, while treatment 1 hour before SAH only hinders the development of late vasospasm. Injection of the antagonist (1 μg) 2 hours post-SAH prevents the development of late vasospasm. The intracisternal injection of 1 μg spantide induces a short-lasting angiographic vasoconstriction of about 27%; injection of 0.1 μg does not affect the mean vascular diameter. An in vitro evaluation of spantide revealed a similar dose-dependent vasoconstriction.

J. Neurosurg. / Volume 72 / June, 1990
Capsaicin Treatment

The sensory innervation of the cerebral arteries was lesioned with capsaicin, a neurotoxin which acts on primary afferent sensory neurons. The sensory perivascular nerve fibers originate in the trigeminal ganglia with a contribution to the innervation of the posterior circulation from the upper two spinal ganglia. Immunohistochemical and lesioning studies indicate that the neurons in the trigeminal and spinal ganglia innervating the cerebral arteries contain substance P. Capsaicin depletes substance P from primary sensory afferents, leaving substance P levels in central nervous system areas not receiving input from sensory afferents unchanged. In addition to the decrease in substance P levels in sensory nerves to the cerebral blood vessels and in the sensory ganglia, substance P levels are decreased in primary sensory neurons in the dorsal spinal cord and in the medulla oblongata. The depletion of substance P is permanent in neonatally treated animals, while in adult animals the depletion is often reversible (see review by Buck and Burks). In the rat, capsaicin depletes substance P in the posterior circulation but depletion is less complete in the anterior circulation. The action of capsaicin on cerebral arteries appears to be relatively specific for substance P. Capsaicin does not affect vasoactive intestinal peptide (VIP), norepinephrine, choline acetyltransferase, or acetylcholinesterase. Calcitonin gene-related peptide (CGRP) co-localization with substance P in the trigeminal ganglia and the perivascular nerve fibers is reduced in the cerebral arteries after neonatal treatment with capsaicin. In the spinal cord sensory afferents, the effect of capsaicin is less selective. In neonatally treated animals cholecystokinin, VIP, and somatostatin are moderately reduced, while in adult animals there is a reduction in cholecystokinin and somatostatin.

We suggest that capsaicin prevents the development of vasospasm through its effect on substance P sensory afferents to the cerebral arteries which terminate in the brain-stem nucleus of the A2-NTS. The A2-NTS, which integrates afferent impulses and acts as a relay station, is involved in the development of both acute and late experimental vasospasm via its ascending projections to the median eminence hypothalamus. Arbab, et al., demonstrated that pretreatment with capsaicin decreased the density of labeled neurons and terminals in A2-NTS areas receiving projections from trigeminal and upper spinal ganglia nerve fibers innervating the cerebral arteries. Other anatomical studies have provided evidence of synaptic interaction between substance P terminals and catecholaminergic neurons of the A2 region, and physiological studies have revealed an excitatory effect of substance P on vasomotor and respiratory centers in the medulla oblongata. Furthermore, the reduction in substance P levels in cerebral arteries following SAH could reflect enhanced release or depletion from sensory nerve terminals. Capsaicin treatment also induces a decreased reflex release of vasopressin, and vasopressin has been shown to underlie the development of acute spasm in our rat SAH model.

Substance P could also have local vasomotor effects if stimulation of cerebral arteries results in antidromic as well as orthodromic transmission. Substance P causes an endothelium-dependent relaxation of cerebral arteries and has been shown to increase vascular permeability when administered intracerebrally. If antidromic stimulation does occur, it could play a role in the inflammatory response noted in cerebral arteries after SAH. Stimulation of cerebral arteries could also induce local release of CGRP which is co-localized with substance P and is a more potent vasodilatory than substance P with a prolonged action. It has been suggested that the local release of CGRP after SAH could act to restore normal vascular diameter. However, in these studies lesioning of sensory substance P and CGRP fibers with capsaicin prevented both acute and late vasospasm and there was no evidence of a more prolonged initial constriction in the angiographically visible arteries.

Spantide Treatment

Spantide is one of the recently developed substance P analogues with substance P antagonistic effect. The mechanism of action is thought to be competitive inhibition. Spantide is a potent antagonist with a weak spasmodic activity, possibly related to its ability to release histamine. Substance P antagonists can block the cerebrovascular dilatation induced by substance P, and when administered intrathecally can exert antinociceptive action and inhibit vasomotor responses elicited from the ventral medulla. Motor dysfunction in rats following intrathecal administration in the lumbar subarachnoid space has been reported with doses slightly above those exerting antinociceptive effects but less than that used to inhibit vasomotor responses. The motor dysfunction is dose-dependent and associated with histopathological changes in the spinal cord consistent with ischemia. Matsumura, et al., showed that the intrathecal administration of 1 µg of (D-pro2-d-trp7,9)-substance P in 10 µl saline at the L2-3 vertebral level did not cause motor dysfunction, while 2 µg injected in the same manner gave rise to transitory mild hindlimb dysfunction in 40% of the animals and 4 µg caused hindlimb paralysis in 80%. Post and Paulsson evaluated the effect of administration into the lumbar subarachnoid space of spantide. They found that while 1 µg spantide in 15 µl saline produced a transient slight motor dysfunction in some animals with no histopathological changes in the spinal cord, 2 µg spantide in 15 µl saline induced in all animals a profound and persistent hindlimb motor impairment with extensive necrosis of neuronal bodies in both the ventral and dorsal horns with axonal sparing. The motor dysfunction induced by substance P antagonists is most likely secondary to vasoconstriction in spinal cord vessels. Cox, et al., evaluated the effect of the substance P antagonist (D-arg1-d-pro2-d-trp7,9-
Prevention of cerebral vasospasm by substance P depletion

Leu$^{13}$-substance P on cerebral pial arterioles and found that the antagonist administered in concentrations from 0.1 to 1 μg/μl produced vasoconstriction. Helke, et al., 28 found that administration of the same substance P antagonist (5 μg in 7.5 μl phosphate-buffered saline) at T9–10 significantly decreased blood flow in the thoracic and lumbosacral spinal cord 15 to 20 minutes after administration. Freedman, et al., 18 found similar reductions in spinal cord blood flow after injection of 2 μg spantide in 10 μl saline, followed by gradual recovery. Injection of 1 μg spantide also caused a reduction in blood flow, but with more rapid normalization. The animals with blood flow reductions also demonstrated severe impairment of motor function in the hindlimbs. 18

We found that injection into the cisterna magna of 1 μg spantide in 100 μl of saline (about 6 × 10$^{-6}$ M) caused a 27% constriction of the vertebrobasilar arteries that had disappeared at 30 minutes after the injection. Injection of 0.1 μg did not affect the mean vascular diameter. In vitro analysis revealed a similar dose-dependent constriction. Although no paralysis was noted in the animals, a transient motor dysfunction could have been masked by the anesthesia. The mechanism behind the vasoconstriction is not clear. It could be due to a specific interaction with substance P receptors, a nonspecific membrane effect, or release of a spasmodenic substance. The correlation between the angiographic and the in vitro data in the present study could suggest that the vasoconstriction is receptor-mediated. On the other hand, Matsumura, et al., 41 showed that motor dysfunction after intrathecal administration of (D-pro$^2$,D-trp$^{7,9}$)-substance P was not reversed by intrathecal substance P. Also, Freedman, et al., 18 found no evidence for a protective effect of substance P against the decrease in blood flow induced by spantide.

Spantide might prevent the development of vasospasm by blocking substance P receptors on the cerebral arteries or by acting on substance P terminals in the A2-NTS. If spantide has a capsaicin-like effect, it could induce a depletion of substance P from afferents to the cerebral arteries and/or their brain-stem terminations in the A2-NTS. There is a reduction in substance P levels in the cerebral arteries following intracisternal administration of spantide (unpublished data). The reduction could reflect an enhanced release of substance P from sensory nerve terminals and is consistent with either a receptor interaction or a capsaicin-like effect. It is difficult to explain why there is no effect on acute spasm when spantide is administered 1 hour prior to SAH, while administration 2 hours before SAH completely prevents vasospasm. This speaks against a conventional antagonist-receptor interaction.

Although spantide can prevent the development of vasospasm, its ability to induce vasoconstriction makes it perhaps more relevant as a tool in establishing the basic mechanisms underlying vasospasm than as a clinically useful treatment. Further investigation is warranted to evaluate the feasibility of developing a substance P analogue capable of preventing vasospasm and lacking a vasoconstrictive action.

Conclusions

The prevention of vasospasm by capsaicin treatment or intracisternal administration of the substance P antagonist spantide indicates that substance P-containing sensory afferents play a role in the development of vasospasm following SAH. The prevention of vasospasm suggests that SAH induces a centripetal impulse from afferents of the cerebral arteries to the A2-NTS, setting in motion the events in the brain stem that lead to acute and late vasospasm.

References

13. Duckles SP, LeVitt B: Specificity of capsaicin treatment
22. Haecusler G, Osterwalder R: Evidence suggesting a transmitter or neuromodulatory role for substance P at the first synapse of the baroreceptor reflex. Naunyn Schmiedebergs Arch Pharmacol 314:111–121, 1980
Prevention of cerebral vasospasm by substance P depletion


Address reprint requests to: Tia Delgado-Zygmunt, M.D., Neurosurgical Research Department, University Hospital, 22185 Lund, Sweden.