Subarachnoid Haemorrhage in the Rat: Effect on the Development of Vasospasm of Selective Lesions of the Catecholamine Systems in the Lower Brain Stem

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SUMMARY Intracisternal injection of blood in the rat produces an angiographically demonstrable biphasic vasospasm. Lesioning at the level of the mesencephalon of the ascending catecholamine pathways from locus coeruleus in the pons and the A1 and A2 nuclei in the medulla oblongata prior to cisternal blood injection prevents the development of both acute and late spasm. Selective lesioning in the medulla oblongata of ascending fibres from A1 and A2 also prevents development of spasm, indicating that these nuclei, which project to the hypothalamus-pituitary, are essential for the spasm syndrome. It is suggested that a substance vasospasm is produced by a substance liberated either by the hypothalamus or by the pituitary is involved in the occurrence of spasm.

CEREBRAL ARTERIAL VASOSPASM is one of the major complications of a subarachnoid haemorrhage (SAH) following the rupture of an intracranial aneurysm. Vasospasm can also occur after head injuries and intracranial operations.1, 2 The mechanism underlying the development of spasm is not known.

In a previous communication,3 we presented a vasospasm model in the rat. It was found that intracisternal injection of blood induced a reproducible biphasic spasm that could be evaluated by repeated angiography. The acute spasm was maximal at ten minutes and the late spasm maximal at two days. It has been reported earlier in both experimental and clinical studies that vasospasm has a biphasic time course.4

The role of the sympathetic system in vasospasm has been extensively investigated both in terms of etiology and in the therapy.5-10 However, these studies have not identified the mechanism behind spasm or provided an effective treatment.

The role of the central catecholamine (CA) systems in spasm has not been investigated. In the present study we have examined the effect of lesioning of the central CA systems on the development of vasospasm following a SAH. It is shown that the A1 and A2 (nomenclature according to Dahlström and Fuxe)11 nuclei in the medulla oblongata are essential for the development and maintenance of spasm.

Material and Methods

The experiments were performed on male Sprague-Dawley rats weighing between 300 and 420 g.

Anaesthesia and Surgical Procedures

The anaesthesia was initiated with 4% halothane. The animals were intubated and artificially ventilated. For the surgical procedure, anaesthesia was maintained with 0.75% halothane in a 70% nitrous oxide and 30% oxygen mixture. After infiltrating the skin with lidocain hydrochloride (xylocain®, Astra), a catheter was inserted into the axillary artery bilaterally for subsequent angiography. The femoral artery and vein were cannulated for continuous blood pressure monitoring and for infusion of drugs. (For technical details, see Delgado et al).3 Heparin (Vitrum, 25 IU) was given i.v. After surgery, the halothane was switched off and suxamethonium chloride (celocurin, Vitrum, 1 mg i.v.) was given. Thirty minutes were allowed to pass before the angiography.

Angiography

Vertebro-basilar angiography was performed via bilateral axillary catheters. Metrizamide (Amipaque®, Nyegaard and Co., Oslo, Norway) was used as the contrast medium. The control angiography was followed by the injection of 0.3 ml of homologous blood intracisternally. The blood was injected via a previously implanted catheter connected to the cisterna magna,3

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and repeated angiography was performed. Measurements of the vertebral and basilar arteries were made using a technique similar to that described by Gabrielsen and Greitz.\textsuperscript{12} The diameters at four preselected points within the vertebral-basilar system were averaged and expressed as a percentage of the control values.

**Stereotaxic Lesions**

A Kopf stereotaxic instrument for small animals was used. All lesions were made two to four weeks prior to the angiography. The animals were anaesthetized with Brietal (Lilly; 40 mg/kg i.p.).

**Lesion 1: Bilateral Lesions of the Ascending CA Pathways in the Mesencephalon**

The ascending CA fibres from the medulla oblongata and pons were lesioned chemically in the caudal mesencephalon rostral to the locus coeruleus and the subcoeruleus (fig. 1 and 2, lesion 1). 6-hydroxydopamine (6-OHDA, 8 \( \mu g \) in 4 \( \mu l \) of saline containing 0.2 mg/ml of ascorbic acid) was injected bilaterally close to the dorsal tegmental bundle.\textsuperscript{13} The following coordinates were used: 0.5 mm anterior to the interaural line, 1.1 mm lateral to the midline and 6.5 mm below the dura. The toothbar was kept at zero. The injection time was three minutes followed by another three minutes before withdrawal of the cannula. In sham lesioned animals, a cannula was lowered according to the same coordinates, but only the solvent was injected.

**Lesion 2: Bilateral Lesions of the Ascending CA Pathways in the Medulla Oblongata**

The ascending CA fibres from A\textsubscript{1} and A\textsubscript{2} were lesioned chemically in the medulla oblongata (fig. 2, lesion 2). 6-OHDA (3 \( \mu g \) in 1.5 \( \mu l \) of saline containing 0.2 mg/ml of ascorbic acid) was injected at two levels: 7.2 and 8 mm below the dura. The other coordinates were: 2.3 mm caudal to the interaural line and 1.1 mm lateral to the midline. The toothbar was kept at zero. In the sham lesioned animals, the cannula was lowered to the same coordinates, but only the solvent was injected.

**Control of the Lesions**

The lesion of the ascending CA pathways in the mesencephalon was evaluated chemically and with fluorescence histochemistry. The frontal cortex and the diencephalon were examined for the content of noradrenaline (NA) using high performance liquid chromatography with electrochemical detection. The NA content of the frontal cortex was reduced by 97 ± 1.7% (mean ± SEM). The NA content in the diencephalon was reduced by 87.3 ± 6.4%. For the fluorescence histochemistry the animals were perfused according to the method of Lorén et al.\textsuperscript{14} The frontal cortex and the diencephalon were examined. There was a complete denervation of the cortex and a marked denervation of the hypothalamus.

The lesion of the ascending CA pathways in the medulla oblongata was controlled by chemical analysis of the NA content in the frontal cortex and the diencephalon, and with fluorescence microscopy of the lesion site. There was no significant reduction in the NA content of the frontal cortex. In the diencepha-
ion the NA content was reduced by 65.9 ± 5.1%. The microscopy confirmed that the lesions were correctly placed and that the locus coeruleus was intact.

**Experimental Design**

All angiographical examinations were carried out between two and four weeks after the stereotaxic lesions. The animals had control angiography followed by the intracisternal injection of blood. Repeat angiography was made ten minutes and two days post SAH.

**Group I. Lesion of the Ascending CA Pathways in the Mesencephalon**

Six animals had angiography pre and post SAH. Three of the six animals were perfused for fluorescence microscopy. In the other three, the NA content in the frontal cortex and the diencephalon was determined.

**Group II. Lesions of the Ascending CA Pathways in the Medulla Oblongata**

Six animals had angiography pre and post SAH. In all six animals, the NA content in the frontal cortex and the diencephalon was measured. Fluorescence microscopy of the lesion site and the locus coeruleus area was made in three of the animals.

**Group III. Sham Lesions**

There were three animals with sham lesions in the mesencephalon and three animals with sham lesions in the medulla oblongata. All animals were examined with angiography, both pre and post SAH. Chemical determination of the NA content in the frontal cortex and the diencephalon was made in all the animals.

**Group IV. Normal Animals**

Six animals had angiography pre and post SAH. Chemical determination of the NA content in the frontal cortex and the diencephalon was made in all the animals.

**Statistical Analysis**

The data were evaluated statistically with the Student t-test.

**Results**

The physiological parameters in the experimental groups are shown in Table 1. There was no inter-group difference in the sham lesioned animals and the data from these animals were therefore pooled. The control mean arterial blood pressure (MABP) in animals with medullary lesions was significantly lower as compared to sham lesioned animals (p < 0.05). Immediately after the intracisternal injection, there was an increase in MABP in all the groups. The MABP was still elevated in the animals with medullary lesions ten minutes after the blood injection. It was significantly higher than the control value (p < 0.01). The pH values were close to 7.4. The PaO₂ values were about 150 mm Hg, and the PaCO₂ values were around 37. The temperature was kept close to 37°C.

In both normal and sham lesioned animals, cisternal blood injection produced an acute spasm of about 40% at ten minutes and a late spasm of about 25% at two days post SAH (fig. 3).

Lesioning of the ascending CA pathways in the medulla oblongata or in the mesencephalon in advance of the SAH completely prevented the development of both the acute and the late spasm (fig. 4A and B and fig. 5). The pre SAH mean vessel diameter of the vertebro-basilar system in the lesioned animals did not differ from that seen in sham lesioned or normal animals.

No paralysis was noted in any of the animals after the SAH. The normal and the sham lesioned animals were noticeably drowsy after blood injection. In contrast, the animals with lesions in the mesencephalon or medulla oblongata seemed unaffected by the SAH.

**Table 1. Physiological Parameters pre and post SAH: Influence of Lesions of the Central CA Systems**

<table>
<thead>
<tr>
<th>Animals</th>
<th>MABP (mm Hg)</th>
<th>Pulse</th>
<th>pH</th>
<th>PaO₂</th>
<th>PaCO₂</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</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>
<td>6</td>
</tr>
<tr>
<td>10 min</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>
<td>6</td>
</tr>
<tr>
<td>2 days</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>
<td>6</td>
</tr>
<tr>
<td>sham</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>126 ± 4</td>
<td>314 ± 11</td>
<td>7.37 ± 0.02</td>
<td>151 ± 7</td>
<td>37.2 ± 1.1</td>
<td>6</td>
</tr>
<tr>
<td>10 min</td>
<td>121 ± 8</td>
<td>317 ± 17</td>
<td>7.34 ± 0.02</td>
<td>150 ± 10</td>
<td>37.7 ± 1.0</td>
<td>6</td>
</tr>
<tr>
<td>2 days</td>
<td>119 ± 7</td>
<td>290 ± 24</td>
<td>7.44 ± 0.03</td>
<td>155 ± 12</td>
<td>36.8 ± 1.3</td>
<td>6</td>
</tr>
<tr>
<td>bilateral lesions in the mesencephalon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>121 ± 4</td>
<td>321 ± 13</td>
<td>7.39 ± 0.03</td>
<td>149 ± 9</td>
<td>37.7 ± 1.7</td>
<td>6</td>
</tr>
<tr>
<td>10 min</td>
<td>120 ± 5</td>
<td>310 ± 10</td>
<td>7.39 ± 0.03</td>
<td>149 ± 9</td>
<td>37.7 ± 1.7</td>
<td>6</td>
</tr>
<tr>
<td>2 days</td>
<td>114 ± 9</td>
<td>293 ± 26</td>
<td>7.43 ± 0.03</td>
<td>159 ± 15</td>
<td>37.3 ± 1.8</td>
<td>6</td>
</tr>
<tr>
<td>bilateral lesions in the medulla oblongata</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>108 ± 6*</td>
<td>295 ± 16</td>
<td>7.38 ± 0.01</td>
<td>154 ± 8</td>
<td>38.3 ± 1.2</td>
<td>6</td>
</tr>
<tr>
<td>10 min</td>
<td>131 ± 5†</td>
<td>255 ± 15</td>
<td>7.39 ± 0.02</td>
<td>146 ± 8</td>
<td>38.2 ± 1.3</td>
<td>6</td>
</tr>
<tr>
<td>2 days</td>
<td>114 ± 8‡</td>
<td>252 ± 12</td>
<td>7.42 ± 0.03</td>
<td>140 ± 5</td>
<td>38.0 ± 1.8</td>
<td>6</td>
</tr>
</tbody>
</table>

Values are means ± SEM. * p < 0.05, † p < 0.01, ‡ p < 0.001
Figure 3. Vertebro-basilar angiography in a sham lesioned animal. A, control. B and C, ten minutes and two days respectively, after cisternal blood injection. Spasm is seen at both ten minutes and two days post SAH.

Figure 4 A and B. Angiographical changes in vertebro-basilar diameter post SAH after lesions in the mesencephalon (A) and medulla oblongata (B). The values are means ± SEM in percent of control. *p < 0.05, **p < 0.01, ***p < 0.001.
FIGURE 5. Vertebro-basilar angiography in an animal with bilateral lesions of the ascending CA pathways in the medulla oblongata. A, control. B and C, ten minutes and two days respectively, after cisternal blood injection. No spasm is seen at ten minutes and two days post SAH.

Discussion

The present study demonstrates that chemical lesioning of the ascending CA pathways in the mesencephalon or in the medulla oblongata in the rat prior to cisternal blood injection completely prevents the development of both acute and late spasm. Repeated angiography after a cisternal blood injection in normal animals has shown an acute spasm maximal at ten minutes and a late spasm maximal at two days post SAH.3

Lesions

The lesions were produced by 6-OHDA. This compound has a selective destructive effect on CA axons and terminals, leaving neurons containing other transmitters unaffected.15,16 Lesioning of the ascending CA fibres in the mesencephalon has been found to cause a reduction in the NA content of the frontal cortex of between 89 and 100%;17,18 in this study the reduction was 97%. Lesioning of the ascending CA pathways in the medulla oblongata has not to our knowledge been described earlier. In our study, there was no significant reduction in the NA content of the frontal cortex with this lesion. In this group, fluorescence microscopy showed that locus coeruleus, which innervates the frontal cortex, was intact.

Anatomy

The CA containing nuclei in the pons and medulla oblongata giving rise to ascending projections in the brain stem include locus coeruleus or A6 and A1 and A2.11,19 The locus coeruleus is located dorsomedially in the floor of the rostral part of the fourth ventricle in the pons. These neurons, probably all NA containing, project via the dorsal tegmental bundle to the hypothalamus, cortex and hippocampus.20 A1 is an elongated accumulation of neurons located ventrolaterally in the medulla oblongata at the level of the lateral reticular nucleus. A2 is a V-shaped accumulation of cells situated dorsomedially in the medulla oblongata. The base of the V is located caudal to the obex in the commissural nucleus, and the sides project rostrally within the medial part of the nucleus tractus solitarius. The A1 and A2 nuclei are predominantly NA containing. However, immunofluorescence studies indicate that adrenaline producing cells are present in the rostral part of these cell groups.21 Dopamine producing cells have also been described in A2.22,23 The A1 and A2 nuclei project to the diencephalon, providing the main CA innervation to the hypothalamus-pituitary.24,25 Finally, the three nuclei are interconnected and they probably all send fibres to the spinal cord.19

Physiology

The functional significance of the CA containing nuclei in the lower brain stem is only poorly understood. Locus coeruleus has been suggested to participate in the cardiovascular regulation either via its descending projections to the nucleus tractus solitarius and the spinal cord26 or via ascending projections to the hypothalamus.24,26 Also, it has been shown to have an inhibitory effect on the neurons in the cortex,27 cerebellum28 and hippocampus.31 In addition, fibres originating in the locus coeruleus have been seen in close association with the microvessels in the brain parenchyma.32-34 This has led to investigations into the role of the locus coeruleus in cerebral blood flow and metabolism. To date, studies have not revealed a major influence of the locus coeruleus on cerebral blood flow and metabolism under normal conditions.35,36

The connections of the A1 and A2 nuclei suggest an involvement in autonomic regulation, especially in the regulation of systemic blood pressure.37-39 The adrenaline containing neurons in the rostral part of A1 have been shown to exert a tonic vasomotor control on the peripheral vasculature through their connections to the thoracic intermediolateral cell column.38 On the other hand, the NA containing neurons in the caudal part of A1 have been found to inhibit this vasomotor tone.40 The NA containing neurons in A1 have been found to modulate the baroreceptor reflex.39,41 Animals with lesions of A1 demonstrate an increased lability and reactivity in blood pressure to stimuli.39 Stimulation within the dorsal reticular formation has been found to induce alterations in cerebral blood flow.42,43

Finally, NA neurons in A1, A2 and A6 have been suggested to influence the release of hormones from the anterior and posterior pituitary.24,44

Possible Mechanisms behind Vasospasm

The changes in MABP seen both before and ten minutes after the SAH in animals with medullary lesions are not responsible for the absence of vasospasm.
Blood pressure changes of this magnitude do not alter the degree of angiographical spasm (unpublished observations). An explanation for the lower blood pressure pre SAH in these animals is that 6-OHDA affects the rostral part of the A1 nuclei which is known to be involved in the maintenance of peripheral vascular tone. The increase in MABP seen ten minutes after the SAH might reflect an exaggerated response of the blood pressure to a stimulus. This exaggerated response could be due to an impairment of the connection between the rostral A1 and A2.

It has been widely believed that blood or its degradation products directly produce vasospasm, in particular the acute phase after a SAH. The absence of spasm in the lesioned animals after a cisternal blood injection, speaks against this assumption. The present data suggest that a neural or neurohormonal mechanism is involved.

Cerebral vasospasm was prevented by both the mesencephalic and the medullary lesions. The lesions in the mesencephalon destroy the ascending fibres from locus coeruleus running in the dorsal tegmental tract, and the ascending fibres from A1 and A2 projecting via the central tegmental tract. The lesions in the medulla oblongata only destroy the ascending fibres from A1 and A2. Accordingly, the ascending projections from A1 and/or A2 are those involved in the development of spasm.

How are the A1 and/or A2 nuclei involved in the development of spasm? To discuss this question one could consider the afferents and efferents of these nuclei. However, nothing is known about the afferents to A1. On the other hand afferents from the trigeminal and facial nerves are known to terminate in an area of the nucleus tractus solitarius containing A2 neurons. It is interesting that a trigeminal innervation of the circle of Willis and the anterior and middle cerebral arteries has been described. Similar observations have been made in our laboratory using antero- and retrograde tracing techniques (to be published). Subarachnoid blood might stimulate sensory nerves on the cerebral vessels or cerebral tissue setting in motion the events which lead to development of spasm.

There are efferents from A1 and A2 projecting to the hypothalamus-pituitary. The hypothalamus could be involved in the development of spasm in three different ways: Via (1) the autonomic nervous system or (2) the anterior or posterior pituitary or (3) the release of hor- mones into the cerebrospinal fluid from hypothalamic neurons. A role for the hypothalamus in the spasm syndrome has been suggested earlier.

An autonomic mechanism mediated via the adrenal medulla or via the superior cervical ganglion is unlikely. In separate experiments we have injected blood intracisternally in animals with bilateral adrenal de- medullation or cervical ganglionectomy and found that both acute and late spasm still developed.

In conclusion: The A1 and/or A2 cell groups are essential for the development and maintenance of vasospasm after a SAH via their projections to the hypothalamus-pituitary.
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