Subarachnoid Haemorrhage in the Rat: Angiography and Fluorescence Microscopy of the Major Cerebral Arteries

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SUMMARY A subarachnoid haemorrhage (SAH) in the rat was produced by the injection of blood via a previously implanted catheter connected to the cisterna magna. Repeated angiographical examinations of the vertebral-basilar arteries revealed a biphasic vasospasm with a maximal acute spasm at ten minutes and a maximal late spasm at two days after cisternal blood injection. Fluorescence microscopic examination of the major cerebral arteries at day two after the SAH revealed a reduction in the fluorescence intensity and in the number of histochemically visible sympathetic nerve terminals.

CEREBRAL VASOSPASM is an angiographically demonstrable, variable arterial constriction that can be clinically asymptomatic or give rise to increasing neurological deficits or death. Vasospasm frequently occurs following a subarachnoid haemorrhage (SAH) secondary to rupture of an intracranial aneurysm. The mechanism underlying the development of spasm is not known.

Research has focused on the discovery of a spasmodicogenic factor. Monoamines and prostaglandins have been proposed as spasm inducing factors. Breakdown products of erythrocytes and free radicals have also been suggested. Correspondingly, monoamine antagonists or inhibitors have been used to prevent or treat vasospasm. The effect of thromboxane synthetase inhibitors and prostacyclin on spasm has also been investigated. However, there is still no definitive treatment for spasm.

In our opinion, there is a need for a simple and inexpensive SAH model giving a reproducible vasospasm in the investigation of the mechanism underlying the spasm syndrome. Therefore, we have developed a SAH model in the rat. This communication presents the angiographical data, and the results of the fluorescence microscopic examinations of the major cerebral arteries following a SAH.

Materials and Methods

Eighty-seven Sprague-Dawley rats weighing between 300 and 425 g were used.

Animal Preparations

Stage I. The animals were anaesthetized with chloral hydrate (250 mg/kg i.p.). An opaque x-ray catheter was inserted into the cisterna magna for subsequent injection of blood. The proximal part of the catheter was blunted and attached to the atlanto-occipital membrane with a purse string suture. The distal tip of the catheter was sealed and sutured subcutaneously to an external skull muscle.

Stage II. Three to seven days after the implantation of the cisternal catheter, the animals were prepared for angiography. The anaesthesia was initiated with 4% halothane. The trachea was exposed and a tube (premature Infant feeding tube size 8 — CR Bard International Ltd., England) was inserted orally and observed entering the trachea. Respiration was controlled in a semiopen circuit using a servoventilator (manufactured at the University Hospital, Lund, Sweden). Anaesthesia was maintained with 70% nitrous-oxide and 30% oxygen.

Catheters were inserted into a femoral artery and vein for continuous blood pressure monitoring and for infusion of drugs, respectively. During the surgical procedure, 0.75% halothane was added to the gas mixture. Heparin (Vitrum, 75 IU/kg) was given intravenously.

The angiography catheter was inserted into the axillary artery bilaterally. By elevating the pectoral muscle, three branches of the brachial-axillary artery were identified. The two proximal branches (deep brachial artery, circumflex-subscapular trunk) were coagulated to reduce the distribution area of the contrast. The distal branch (superficial radial artery) was left intact. The catheter was inserted distal to this branch. The catheter was made of radiopaque polyethylene (Surgimed A/S, Denmark) and the dimensions were OD/ID = 0.9/0.5 mm. The tip of the catheter was tapered to an outer diameter of approximately 0.4 mm. The catheter tip was placed about 2 mm distal to the origin of the vertebral arteries. After the surgical procedure, the halothane was switched off and the animal was placed in a prone position on a plexiglass plate. Suxamethonium chloride (Celocurin, Vitrum; 3 mg/kg) was given and half an hour was allowed to pass before the angiography. Blood samples were drawn periodically for measurement of pH, PaO₂, and PaCO₂.

Following the control angiography, 0.07 or 0.3 ml homologous blood was injected into the cisterna magna via the subcutaneous catheter over about 5 or 30 seconds, respectively. Before the blood injection, cerebrospinal fluid (about 0.3 ml) was gently aspirated. During the injection of blood, the head was lowered by tilting the animal 20°. A transitory increase in blood pressure of five to ten mm Hg lasting about ten seconds.
occurred after injection of 0.07 ml blood. Similarly, injection of 0.3 ml blood caused a transitory increase in blood pressure of 20–30 mmHg lasting about 60 seconds. There was also a decrease in pulse rate in the two groups. Angiography was done at predetermined time points following the cisternal injection (see experimental groups). After the angiography, the catheters were removed and the axillary arteries were ligated distal to the superficial radial artery. The animals were extubated when they were fully awake.

Stage III. One to seven days later, the animals were again anaesthetized and intubated as described earlier. Catheters were reinserted in the axillary and femoral vessels. The angiography was repeated. In addition to the control angiography and one or two examinations during a 90 minute period after the blood injection, most of the animals were reexamined once between day one and day seven after the SAH. A minor group of animals was examined twice between day one and day seven. After the final examination, the animals were sacrificed.

**Angiography**

An x-ray tube (Opti-100/12/15 HSG, Elema-Siemens, Sweden) with a 0.2 x 0.2 mm focus spot was used; exposure data were 80 mAs, 60 kV and 0.16 sec. The focus-objective distance was 43 cm, and the focus-film distance was 123 cm, giving a linear magnification of 2.86. Angiograms were made on mammographic film (Kodak NMB Film).

For angiography, metrizamide (Amipaque®, Nyegaard and Co., Oslo, Norway), in a concentration of 370 mg I/ml, was simultaneously manually injected into both axillary catheters over approximately three seconds. The total amount of contrast medium injected was 0.7 ml; 0.4 ml on the right and 0.3 ml on the left side. Before injection of contrast, one film was exposed for subtraction. Amipaque was chosen because of its low toxicity; it is nonionic with an osmolality of about one-third that of conventional media, and has less vasodilatory effect than other media. All the angiographies were performed by the same investigator.

**Measurement of Arterial Diameter**

Measurements of the vertebral and basilar arterial diameters before and after subarachnoid blood injection were made using a technique similar to that described by Gabrielsen and Greitz. Using a magnifying glass, the contour of the vessels was enhanced with a sharp soft pencil. The diameter of the vessel was then measured with a precision calibrated measuring lens of fixed magnification and lens film distance. A vascular index representing the mean value of the diameters at four preselected points within the vertebral-basilar system was calculated in an animal both before and after the SAH (fig 1). The degree of vasoconstriction was determined from the ratio of the two indices. The measurements were made in random order without previous knowledge of whether or not the animals had been injected with blood.

**Fluorescence Microscopy**

Under barbiturate anaesthesia (Brietal, Lilly; 40 mg/kg i.p.) vascular perfusion was done with isotonic cold saline. Following brain removal, the major cerebral arteries were extirpated and briefly rinsed in cold saline. Whole-mount preparations of vertebral, basi-

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**FIGURE 1. Bilateral vertebral angiography in the rat showing the points (small arrows) at which vertebral and basilar artery diameters were measured: 0.5 cm proximal to the vertebo-basilar junction on the vertebral arteries; on the basilar artery 0.5 cm above the junction of the vertebral arteries and 0.5 cm below the origin of the posterior cerebral arteries. An index was calculated as the mean value of the measured diameters. Axillary catheters are indicated by large arrows. Axial view, magnification 2.86.**
lar, internal carotid and middle cerebral arteries were dried \textit{in vacuo} in a desiccator over phosphorous pentoxide for at least two hours. They were exposed to formaldehyde gas of optimal humidity at 80\degree C for one hour, based on the method described by Falck et al\(^2\) (for details of the present technique, see Björklund et al, 1975\(^2\)). The preparations were mounted in liquid paraffin and examined in a Zeiss fluorescence microscope equipped with BG 12 (Schott) as mercury lamp filter and Zeiss 47 + 50 as secondary filters.

**Experimental Groups**

In four animals, blood mixed with cardiac green was injected into the cisternal catheter to determine the distribution of blood within the subarachnoid space. The animals were sacrificed right after the injection.

In five normal animals, repeated angiography was done to investigate the spontaneous intra-individual variations in vessel diameter. The animals had angiography twice on day zero. They were reexamined once during the following week.

Fifty-two animals were used to delineate the degree and duration of vasoconstriction following the injection of 0.3 ml blood intracisternally. A control vertebro-basilar angiography was done in all animals before blood injection. Five to ten animals were reexamined at each of the following time points: 5, 10, 15, 30, 60 and 90 minutes and 1, 2, 3, 5, and 7 days following the blood injection. In a number of animals, there was also good filling of the right internal carotid artery (fig. 1). In the five animals used to investigate intra-individual variations, the standard deviation of the mean vessel diameter of the vertebro-basilar system was 1.5\%; the maximal difference was 4\%.

Cisternal injection of blood produced a constriction of the vertebro-basilar arteries in all the animals receiving 0.3 ml blood. Figure 2 shows an angiography with spasm of the vertebro-basilar system ten minutes after cisternal blood injection. The spasm had disappeared by day five. Figure 3 shows spasm at two days that had disappeared by day seven. There was a biphasic pattern of vasospasm (fig. 4). During the acute phase,
there was a maximal mean degree of spasm of 40% occurring at ten minutes after blood injection. During the late phase, there was a maximal mean degree of spasm of 27% occurring at day two. A slight dilatation of the vessels was noted at day five and day seven. The spasm was diffuse in most of the animals. In a few animals, bead like irregularities on the narrowed vessels were seen during the late phase. The degree of vasoconstriction in the animals with cisternal injection of 0.07 ml blood was slightly less than that seen in the animals receiving 0.3 ml blood (mean vessel diameter in percent of control ± SEM: at ten minutes 66.4 ± 4.8 for animals receiving 0.07 ml blood versus 60.7 ± 3.8 for animals receiving 0.3 ml blood; at two days 77.5 ± 6.0 versus 73.1 ± 2.4). The six animals injected with saline did not develop vasoconstriction. (Mean vessel

### Table 2. Physiological Parameters in Animals with a Cisternal Injection of 0.3 ml Saline or 0.07 ml Blood

<table>
<thead>
<tr>
<th>Time</th>
<th>Control (mMg)</th>
<th>10 minutes after saline injection</th>
<th>Control (mMg)</th>
<th>Day 2 after saline injection</th>
<th>Control (mMg)</th>
<th>10 minutes after blood injection</th>
<th>Control (mMg)</th>
<th>Day 2 after blood injection</th>
</tr>
</thead>
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<tr>
<td>MABP (mmHg)</td>
<td>116 ± 8</td>
<td>131 ± 5</td>
<td>119 ± 7</td>
<td>112 ± 5</td>
<td>125 ± 5</td>
<td>127 ± 7</td>
<td>125 ± 5</td>
<td>110 ± 9</td>
</tr>
<tr>
<td>Pulse (beat/min)</td>
<td>295 ± 13</td>
<td>305 ± 13</td>
<td>285 ± 15</td>
<td>319 ± 17</td>
<td>336 ± 26</td>
<td>285 ± 15</td>
<td>336 ± 26</td>
<td>282 ± 32</td>
</tr>
<tr>
<td>pH</td>
<td>7.40 ± 0.03</td>
<td>7.40 ± 0.03</td>
<td>7.42 ± 0.02</td>
<td>7.42 ± 0.02</td>
<td>7.44 ± 0.03</td>
<td>7.44 ± 0.03</td>
<td>7.44 ± 0.03</td>
<td>7.44 ± 0.04</td>
</tr>
<tr>
<td>PaO₂ (mmHg)</td>
<td>165 ± 3</td>
<td>165 ± 3</td>
<td>150 ± 11</td>
<td>148 ± 9</td>
<td>156 ± 13</td>
<td>156 ± 13</td>
<td>156 ± 13</td>
<td>150 ± 17</td>
</tr>
<tr>
<td>PaCO₂ (mmHg)</td>
<td>36.6 ± 2.1</td>
<td>36.6 ± 2.1</td>
<td>36.3 ± 1.5</td>
<td>38.0 ± 1.8</td>
<td>35.4 ± 1.6</td>
<td>35.4 ± 1.6</td>
<td>35.4 ± 1.6</td>
<td>35.1 ± 1.0</td>
</tr>
<tr>
<td>n</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>5</td>
<td>6</td>
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<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

MABP = mean arterial blood pressure.  
The values are means ± SEM.
CEREBRAL VASOSPASM IN THE RAT/Delgado et al

FIGURE 3. Vertebro-basilar angiography in the rat. A, control, B and C, two and seven days, respectively, after blood injection. The spasm seen at day two is not seen at day seven. Arrows indicate basilar artery.

The blood injected animals were noticeably drowsy the first two–three days after the SAH. No paralysis was noted in any of the animals. There was a weight loss of about 10% during the first week after the SAH. The behavior of the saline injected animals was normal.

Six of the 52 animals undergoing cisternal blood injection and angiography died, i.e. a mortality of 11.5%. The main cause of death was respiratory failure secondary to obstruction of the tracheal tube. There was no mortality in the six animals receiving saline or in the animals receiving 0.07 ml blood.

Three animals developed an infection following the implantation of the cisternal catheter. They were not included in the experimental groups.

Gross pathological examination of the brains revealed a complete absence of cisternal blood by day three after the SAH. There was a xanthochromic discoloration of the basal surface of the brain that was most pronounced at day two and gradually disappeared over the following five days. There were no signs of brain stem damage secondary to the intracisternal catheter.

In the fluorescence microscopical examination of the cerebral arteries from the animals receiving intracisternal saline, the adrenergic transmitter in the sympathetic nerves was displayed as a bright yellow-green fluorescent network of beaded fibers (fig. 5 upper). In the animals receiving intracisternal blood, there was a marked reduction in the fluorescence intensity and a substantial reduction in the number of histochemically visible nerve terminals (fig. 5 lower).

diameter in percent of control ± SEM, ten minutes: 103.7 ± 2.9; two days: 102 ± 2.8).

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FIGURE 4. Changes in mean vertebro-basilar diameter (percent of control ± SEM) at various time periods after intracisternal blood injection in the rat; number of animals studied within brackets.
Pattern of vasospasm in the rat. The duration of spasm was three days. It is noteworthy that the occurrence of vasospasm surpassing 8% as vasospasm, ie. twice the maximal constriction of 10% as required by Peerless et al, 28 occurs earlier (on day two) in the rat as compared to other species. In the rabbit, the late spasm reaches a maximum three to five days after cisternal blood injection. 29 In the dog 30 and in the monkey 31 a late spasm phase has been demonstrated with maximum between the fifth and the seventh day after the SAH.

Clinically, the existence of an acute spasm phase has not definitely been proven. In patients, the late spasm appears angiographically two to four days after the hemorrhage and reaches a maximal around day seven. It is during the late phase that the ischemia and the neurological deficits develop.

The animals were apathetic for two to three days after the cisternal blood injection. No focal neurological deficits were noted in any of the animals. However, a neurological evaluation is difficult in small animals. The animals receiving 0.07 ml blood intracisternally had less spasm than the group receiving 0.3 ml. Also, the reproducibility of the late spasm was somewhat less in the group receiving 0.07 ml; one of the animals in this group did not have spasm at two days. Although a greater difference in the degree of spasm could have been expected, the data are in keeping with findings from both clinical and experimental investigations that a larger SAH increases the degree and the probability of developing vasospasm. 28, 36

The degree and duration of increase in blood pressure following intracisternal injection of 0.07 ml or 0.3 ml blood, indicate that there is a substantial difference in the intracranial pressure between the two groups. The minor difference in spasm in the two groups therefore suggests that it is the blood per se and not the combination of blood and a marked increase in intracranial pressure that induces the spasm.

The fluorescence microscopical examination showed changes in the perivascular sympathetic nerves similar to the changes noted in vessels from monkeys, 37 rabbits 3 and cats 4 after a SAH.

Barry et al 39 induced vasospasm in Sprague-Dawley rats by puncturing the basilar artery via a transcervical and transclival approach. The basilar artery diameter was measured photographically. The animals were examined on two separate occasions over a seven day period. Similar to our findings, the spasm peak was seen on day two. The acute phase was not documented in their series. Ohta et al 40 using a similar approach and photographic technique as that of Barry et al, 39 induced vasospasm in Wistar rats by irrigating the basilar artery with barium chloride. In a few animals, whole blood or blood components were used to induce spasm. The observation time was six hours. Whole blood produced a severe vasospasm that disappeared spontaneously within 30 minutes. In a comparative study between Wistar and Sprague-Dawley rats, we have found that vasospasm is less pronounced and of shorter duration in Wistar rats (unpublished observations).

Fein 41 induced a SAH in Sprague-Dawley rats via
the injection of whole blood into the cisterna magna. However, the objective of his study was to investigate the acute metabolic changes after a SAH.

Boulin et al. presented an angiographical model in the rat for studying the aetiology of cerebral arterial constriction. The angiography was performed via catheterization and ligation of one common carotid artery.

In conclusion: The present investigation has demonstrated that a reproducible biphasic vasospasm can be induced in the rat and evaluated with repeated angiographical examinations. The model can be used to investigate spasm induced ischemia with cerebral blood flow and metabolic studies. Moreover, the model is suitable in the study of the basic mechanism of vasospasm and in therapeutic trials.

References
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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.

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). Heparin (Vitrum, 25 IU) was given i.v. After surgery, the halothane was switched off and suxamethonium chloride (clocurin, 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.
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