SUMMARY In the rat an intracisternal injection of blood induces an angiographically demonstrable biphasic cerebral arterial vasospasm. Chemical destruction of the central serotoninergic and dopaminergic pathways prior to the cisternal blood injection does not affect the spasm pattern after the subarachnoid haemorrhage. It is suggested that neither system plays a role in the development of spasm.

STROKE Vol 17, No 1, 1986

Subarachnoid Haemorrhage in the Rat: Effect on the Development of Cerebral Vasospasm of Lesions in the Central Serotonergic and Dopaminergic Systems

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CEREBRAL ARTERIAL VASOSPASM of the major cerebral arteries often develops in patients with a subarachnoid haemorrhage (SAH), and the late phase of spasm can lead to cerebral ischemia.1,2 The etiology of vasospasm remains obscure, and at present there is no effective therapy.

Serotonin, or 5-hydroxytryptamine (5-HT), has a ubiquitous distribution within the brain.3-5 It is a potent vasoconstrictor and has often been suggested as the spasmogenic factor.5-8 However, other investigators have doubted a major role for 5-HT in the development of spasm.9,10

Dopamine (DA) is also widely distributed in the central nervous system.11 It has been demonstrated that DA is able both to constrict and dilate cerebral vessels.12-13 Accordingly, DA has been proposed as a causative factor of vasospasm14 but it has also been suggested in the treatment of spasm.15,16

In order to investigate the mechanism behind vasospasm, we have developed a SAH model in the rat.17 Angiographical examination of the SAH animals revealed a biphasic spasm pattern with a maximal acute spasm at ten minutes and a maximal late spasm at two days post SAH. In the present study we examined the effect of lesioning of the central serotoninergic and dopaminergic systems on the development of both the acute and the late vasospasm following a SAH.

Materials and Methods

Experiments were performed on male Sprague-Dawley rats weighing between 240 and 300 g. The animals had free access to tap water and pellets (San-Bolagen, Malmoe, Sweden). The animals with bilateral lesions of the dopaminergic pathways were fed three times a day via a gastric tube with a nitrogen rich compound (Vivonex®HN, Kela Lab., Belgium). Animals with bilateral lesions of the central DA systems became aphagic and adipsic.

General Procedures

The methods for anaesthesia and surgical procedures have been described in detail in a proceeding report, and will be summarized.17

The anaesthesia was initiated with 4% halothane. The animals were intubated and artificially ventilated. During the surgery, the anaesthesia was maintained with 0.75% halothane in a 70% nitrous oxide and 30% oxygen mixture. After infiltrating the skin with lidocaine hydrochloride (Xylocain®, Astra), catheters were inserted into the axillary arteries for subsequent angiography. Catheters were inserted into a femoral artery and vein for continuous blood pressure monitoring and for infusion of drugs. Heparin (Vitrum 75 IU/kg) was given intravenously. After completion of the surgery, the halothane was disconnected and sufentanil chloride (Sufenta, 3 mg i.v./kg) was given. Thirty minutes were allowed to pass before angiography.

Angiography

An x-ray tube (Opti-100/12/15 HSG, Elema-Sieverts, Sweden) with a 0.2 x 0.2 mm focus spot was used with the following exposure data: 80 mAs, 60 kv and 0.16 sec. The magnification was 2.86. Mammographic film (Kodak NMB) was used.

Vertebral-basilar angiography was carried out via bilateral axillary catheters. As contrast medium, metrizamide (Amipaque®, Nyegaard & Co., Oslo, Norway) was used. Following the control angiography, 0.07 ml of homologous blood was injected into the cisterna magna via a previously implanted catheter.17 Repeat angiography was performed ten minutes and/or two days after the blood injection. Measurements of the vertebral and basilar arteries were made at four selected points using a technique similar to that described by Gabrielsen and Greitz.18 The values were averaged and then expressed as a percentage of control.

Stereotaxic Lesions

A Kopf stereotaxic instrument was used. All lesions were made 2-4 weeks prior to the angiography under barbiturate anaesthesia (Brietal®, Lilly; 40 mg/kg i.p.).
Lesion of the 5-HT Containing Systems

The lesion was produced by a single injection of 150 ug 5,7 dihydroxytryptamine (5,7 DHT) creatinine sulfate (Regis Chemical & Co., USA) containing 0.2 mg/ml of ascorbic acid into one lateral ventricle. The animals were given the uptake blocker desipramine (25 mg/kg i.p.; Ciba-Geigy, Switzerland) to protect the noradrenergic neurons. In the sham lesioned animals, only the solvent was injected into the ventricle.

Bilateral Lesions of the Nigrostriatal DA Pathways

The lesion was made rostro-medially to the substantia nigra where the nigrostriatal, mesolimbic and mesocortical DA fibres assemble. The lesions were produced by injecting 6-hydroxydopamine (6-OHDA, Sigma Chemical Co., USA; 8 ug in 4 ug of saline containing 0.2 mg/ml ascorbic acid) at the following coordinates: 4.5 mm behind the bregma, 1.1 mm lateral to the midline and 8 mm below the dura. The toothbar was 3 mm below the interaural line. The injection time was three minutes followed by another three minutes before withdrawal of the cannula. In the sham lesioned animals, only the solvent was injected.

Control of Lesions

The lesion of the 5-HT containing systems was evaluated chemically using high performance liquid chromatography with electrochemical detection. The frontal cortex and the pons-medulla oblongata were examined for 5-HT. The animals included in the study had a 84.8 ± 3.0 (mean ± SEM) percent reduction of 5-HT in the cortex and a 57.7 ± 6.2 percent reduction of 5-HT in the pons-medulla oblongata.

The lesions of the nigrostriatal DA pathways were evaluated chemically by examining the caudate putamen for the DA content. There was a decrease in the DA content of 90.6 ± 3.4 percent in the animals included in the study.

Statistics

The data were analyzed using the Student t-test.

Experimental Design

All examinations were carried out between two and four weeks after the stereotaxic lesions. The animals had angiography at ten minutes and/or two days post SAH, i.e., the time points for the maximal acute and late spasm, respectively. In both lesioned groups, there were six animals examined at ten minutes and six animals examined at two days post SAH.

There were three animals with sham lesions of the 5-HT system and three with sham lesions of the DA system. These animals had angiography at both time points.

There were six normal animals examined with angiography at ten minutes and two days post SAH.

Results

The physiological parameters before and after the SAH are shown in table 1. The mean arterial blood pressure (MABP) before the cisternal blood injection was similar in all the experimental groups. There was a

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Physiological Parameters Pre- and Post SAH in Animals with Lesions of the Central 5-HT Systems or Bilateral Lesions of the Central DA Pathways</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>MABP (mm Hg) 122±4, 290±15, 7.45±0.02, 137±7, 36.3±1.4, 6</td>
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<tr>
<td>10'</td>
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<td>2D</td>
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<tr>
<td>Sham lesions</td>
<td></td>
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<tr>
<td>control</td>
<td>MABP (mm Hg) 127±7, 300±27, 7.40±0.02, 157±16, 34.2±1.1, 6</td>
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<tr>
<td>10'</td>
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<td>2D</td>
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<tr>
<td>Lesion of 5-HT containing systems</td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>MABP (mm Hg) 125±4, 312±22, 7.38±0.02, 134±5, 38.1±1.2, 9</td>
</tr>
<tr>
<td>10'</td>
<td></td>
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<td>2D</td>
<td></td>
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<tr>
<td>Bilateral lesions of ascending DA pathways</td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>MABP (mm Hg) 118±5, 278±15, 7.39±0.02, 140±10, 35.5±1.2, 9</td>
</tr>
<tr>
<td>10'</td>
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<tr>
<td>2D</td>
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*p < 0.05.
MABP = mean arterial blood pressure.
The values are means ± SEM.
( ) Indicates numbers of measurements.

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significant decrease in MABP at two days post SAH in the animals with a lesion of the central 5-HT systems \( (p < 0.05) \). The pH values were close to 7.4. The \( \text{pO}_2 \) values were about 140 mmHg and the \( \text{pCO}_2 \) values were around 37 mmHg. The temperature was kept close to 37°C.

The baseline vessel diameter of the vertebro-basilar system in lesioned animals did not differ from that seen in normal and sham lesioned animals. The sham lesioned animals showed the same degree of spasm (about 36% at ten minutes and about 23% at two days) post SAH as the normal animals. The values from the sham lesioned animals were pooled. Lesioning of the 5-HT or DA systems did not change the degree of spasm at either ten minutes or two days as compared to sham lesioned animals. (fig. 1, 2, 3.)

No paralysis was noted in any of the animals after the SAH. The lesioned animals were noticeably more drowsy after the SAH as compared to normal or sham lesioned animals injected with blood. There was a high mortality in the lesioned groups in relation to the cisternal blood injection and angiography. There was no mortality in the sham lesioned animals.

**Discussion**

The present data demonstrate that lesioning of the central serotoninergic or dopaminergic systems prior to cisternal blood injection does not alter the spasm pattern. The lesioned animals develop the same degree of both acute and late spasm post SAH as normal animals. A period of at least two weeks between the lesion and the angiography was chosen. Examination at an earlier time point of the DA lesioned animals was found to give varying, but mostly more marked spasm, and a greater mortality.

The lesion of the central serotoninergic system was produced by 5,7 DHT. This compound causes selective degeneration of the 5-HT and to a lesser extent the noradrenaline containing axons. The damage to the noradrenergic neurons was avoided by pretreatment of the animals with desipramine. A single intraventricular injection of 150 \( \mu \)g 5,7 DHT has been found to induce a long-lasting decrease in the content of serotonin in the cortex of 90% and in the pons-medulla of about 60%. Similar reductions were seen in the present study.

Several investigations have suggested the presence of a serotoninergic innervation of the cerebral vessels. Chan Palay demonstrated the innervation autoradiographically, while Griffith and Burnstock and Edvinsson et al. visualized the 5-HT containing fibres with an immunohistochemical technique. The latter authors also demonstrated that intraventricular injection of 5,7 DHT decreased the 5-HT content of the pial arteries.

The fact that the animals with destruction of the central serotoninergic pathways and serotoninergic vascular innervation had the same degree of spasm as normal animals, suggests that the central serotoninergic systems are not involved in the development of spasm. However, this finding does not eliminate the possibility that 5-HT in the platelets and mast cells could be involved in spasm. Allen et al proposed that vasospasm after a SAH was produced by 5-HT released into CSF from platelets in the blood clots. Recent studies have provided evidence against this theory. Boullin et al. showed that blood induced acute spasm was not reversed by the 5-HT antagonist, BW501C67. Further, measurements of CSF concentrations of 5-HT or its metabolite 5-hydroxyindole acetic acid from patients with a SAH have demonstrated levels similar to control values. Finally, injections
into the cisterns or subarachnoid space of unphysiological, large amounts of 5-HT only produced a moderate and shortlasting spasm.12-16

It is interesting that Tagari et al recently have found a 5-HT like vasoconstrictor activity in CSF collected peroperatively from SAH patients.60 This factor might represent 5-HT in association with another compound. The vasoconstrictor activity was antagonized by ketanserin which acts as a specific 5-HT2 antagonist.31

The lesions of the central dopaminergic systems were produced by 6-OHDA. This compound has a selective destructive effect on catecholamine axons and terminals, leaving neurons containing other transmitters unaffected.32-33 Lesioning of the ascending DA fibres rostromedial to the substantia nigra has been shown to cause a reduction in the DA content of the caudate-putamen of between 86 and 95%.34-35 In the present study, there was a 90% reduction.

There exists a close association between the dopaminergic terminals and the parenchymal vessels in the brain, especially in the caudate-putamen. However, a true dopaminergic vascular innervation has not been shown.

The present study suggests that the central DA systems are not involved in the development of spasm. It has been proposed that DA released from the brain parenchyma could cause vasospasm.36 White et al found that DA injected intracisternally in dogs produced a short-lasting spasm which was delayed in onset.14 On the other hand Boullin et al showed that intracranial DA perfusion in SAH patients with spasm could generate a short-lasting reversal of the vasoconstriction.15 In vitro13 and in vivo16 studies have demonstrated a dose dependent vascular effect of DA with lower dosages causing dilatation and higher dosages producing constriction. It has been suggested that the vasoconstrictive effect of DA is mediated either by the alpha-adrenergic or the serotoninergic receptor, while the dilatory effect is mediated by a specific DA receptor.12-16

In conclusion, the study suggests that the central serotoninergic and dopaminergic systems are not involved in the development and maintenance of vasospasm after a SAH in the rat.

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Subarachnoid haemorrhage in the rat: effect on the development of cerebral vasospasm of lesions in the central serotoninergic and dopaminergic systems.
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Stroke. 1986;17:86-90
doi: 10.1161/01.STR.17.1.86
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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