The production of intracranial vascular spasm by hypothalamic extract

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This study reports a new method for producing acute intracranial vascular spasm and compares it with the commonly used technique of injecting blood into the subarachnoid space. The data from this investigation provide angiographic evidence that extracts of hypothalamic tissue when injected into the cisterna magna of mongrel dogs will produce a high incidence (86%) of diffuse spasm in most intracranial vessels. Extracts of cerebral cortical tissue produced no constriction. The onset of spasm initiated by hypothalamic extract was later (1 1/2 hours postinjection) and the duration of spasm longer (up to 5 or 6 hours) than that produced by the presence of blood. This indicates, then, that there are substances in certain brain tissues that could also be released into the cerebrospinal fluid and add to spasm produced by blood in the subarachnoid space.

Key Words • intracranial vasospasm • subarachnoid space • vertebral angiography • hypothalamic extract

The phenomenon of cerebral vasospasm that frequently occurs in patients with subarachnoid hemorrhage has been implicated as one of the major causes of mortality following rupture of intracranial aneurysms.\(^1,5\) Clinical and experimental data now available suggest that cerebral arterial spasm is a response to mechanical,\(^1,16,26\) chemical,\(^2,3,8,18,21,24,31,34,35\) and neurogenic\(^4,7,9,17,26\) factors. Most research has dealt with the effect on cerebral vessels of chemicals or breakdown products released from extravasated blood within the subarachnoid space.\(^6,14,15,29,32\) Very little attention has been given to any possible spasmogenic effects of substances present in brain tissue.

Therefore, the purpose of this investigation was to determine if there are any substances present in brain tissue that could be released into the cerebrospinal fluid and complement spasm produced by the presence of blood. Extracts of tissue prepared from two different regions of the brain, the hypothalamus and cerebral cortex, were used to determine if they were spasmogenic, and, if so, to compare this type of spasm with that known to be produced by subarachnoid blood.

Materials and Methods

Fifty-three mongrel male dogs weighing between 25 and 30 lbs were used. After the animals were anesthetized with sodium pentobarbital (30 to 40 mg/kg), they were intubated endotracheally and allowed to breathe spontaneously.
Vascular spasm was evaluated by means of vertebral angiography following cannulation of the right vertebral artery just as it entered the transverse foramen. Single x-ray films made after 5 ml of Renografin-60 had been injected through the catheter recorded consistently reproducible angiograms of the basilar artery and posterior portion of the circle of Willis. In all animals before any experimental procedures were begun, control angiograms were made to obtain one or more baseline x-ray films for comparison with subsequent angiograms made on the same dog. All substances used in the experiments (listed below) were injected in 5 ml amounts into the cisterna magna through a 20-gauge needle placed in the subarachnoid space at this location. In each case 4 to 5 ml of cerebrospinal fluid were removed before the substances were injected to prevent a high intracranial pressure. Following injection of the test substances into the cisterna magna, experimental angiograms were taken at intervals of every 5 to 15 minutes for the first hour and then every 30 minutes thereafter until the experiments were terminated, usually 5 to 6 hours after injection. The exposure factors for the angiograms were 300 mA, 70 kV at 1/20 sec.

The experimental plan was as follows:

**Group 1 (8 dogs):** These animals were injected with 5 ml of serum from autogenous whole blood incubated at 37°C for 4 days (as described by Wilkins and Levitt).  

**Group 2 (14 dogs):** These animals were injected with 5 ml of hypothalamic extract incubated at 37°C for 4 days.  

**Group 3 (5 dogs):** These animals were injected with 5 ml of non-incubated hypothalamic extract.  

**Group 4 (6 dogs):** These animals were injected with 5 ml of a combination of serum (2.5 ml) from autogenous whole blood and hypothalamic extract (2.5 ml), both incubated at 37°C for 4 days.  

**Group 5 (6 dogs):** These animals were injected with 5 ml of incubated (3 animals) and non-incubated (3 animals) cerebral cortical extract.

Hypothalamic and cerebral cortical extracts were prepared from porcine tissue obtained from a local slaughterhouse. The hypothalamic tissues consisted of the pituitary stalk and the hypothalamus extending from the optic chiasma to the middle of the mammillary bodies. The pieces of tissue measured approximately 10 mm rostro-caudally, 10 mm laterally, and 6 or 7 mm dorsoventrally. Similar size samples of the cerebral cortex were taken from the surface of the frontal lobe. All these tissues were collected within 30 to 45 minutes after death, immediately frozen with solid CO₂, and kept frozen until time for preparation of the extracts. The tissues were allowed to thaw slightly before being ground in a blender with 125 ml of normal saline per 100 gm of tissue. The homogenate was centrifuged at 10,000 rpm for 20 minutes, and then the supernatant was injected into the subarachnoid space of the dogs.

**Results**

**Controls**

Four ml of normal saline were injected into the cisternae magnae of four normal dogs. Subsequent angiograms at 5, 15, 45, 60, 90, and 150 minutes showed no changes in the diameter of the basilar arteries or posterior portions of the circle of Willis. In two other dogs repeated injections of Renografin-60 had no effect on vessel diameter.

**Group 1: Serum from Incubated Whole Blood**

The injection of incubated serum into the cisterna magna produced bilateral diffuse intracranial arterial spasm in six of eight dogs (Table 1). A maximum degree of spasm of the basilar and other arteries was usually seen within 5 to 10 minutes post-injection (Fig. 1 center) and lasted approximately 20 to 30 minutes. Within 1 to 1½ hours, the vessels had largely recovered (Fig. 1 right) although in three animals a limited degree of spasm lasted for several hours. Similar results have been shown by a number of other investigators.
TABLE 1

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Substance Injected into Cisterna Magna</th>
<th>Percent Animals Showing Spasm</th>
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<tbody>
<tr>
<td>1</td>
<td>5 ml serum from whole blood incubated for 4 days</td>
<td>75% (6/8)</td>
</tr>
<tr>
<td>2</td>
<td>5 ml hypothalamic extract incubated for 4 days</td>
<td>86% (12/14)</td>
</tr>
<tr>
<td>3</td>
<td>5 ml non-incubated hypothalamic extract</td>
<td>20% (1/5)</td>
</tr>
<tr>
<td>4</td>
<td>combination of serum from whole blood and hypothalamic extract, incubated 4 days</td>
<td>83% (5/6)</td>
</tr>
<tr>
<td>5</td>
<td>5 ml incubated (3 animals) and non-incubated (3 animals) cerebral cortical extract</td>
<td>0 (0/6)</td>
</tr>
</tbody>
</table>

Groups 2 and 3: Hypothalamic Extract, Incubated and Non-Incubated

The injection of incubated hypothalamic extract (Group 2) resulted in a significant incidence of vasospasm in 12 of 14 dogs (Table 1). However, the onset of spasm occurred much later than that observed with the injection of blood. A limited degree of spasm of the basilar artery and the circle of Willis appeared at 1½ hours following hypothalamic injection, and maximal constriction was not present until 3 to 3½ hours (Fig. 2 center) and then lasted 30 to 45 minutes. At 6 hours post-injection (end of the experiment) the vessels exhibited a less intense spasm (Fig. 2 right), primarily of the basilar artery. In other words, hypothalamic extract produced a more chronic spasm than did blood injected into the subarachnoid space. In Group 3 (animals receiving non-incubated hypothalamic extract) only one of the five dogs showed any spasm, and this was only a minimal amount of constriction of the basilar artery.

Group 4: Combination of Serum and Hypothalamic Extract, Both Incubated

When the blood and hypothalamic extract were administered together, obvious intra-
cranial spasm developed in 83% of the dogs (Table 1). Again, as in Group 2, limited spasm did not occur until 1 1/2 to 2 hours post-injection, with maximum spasmogenetic activity occurring at 3 to 3 1/2 hours (Fig. 3 center). Various degrees of constriction were still present at 5 to 6 hours post-injection (Fig. 3 right). Since this sequence of events resembles that when hypothalamic extract was given alone (Group 2), it appears that when blood and hypothalamic extract are given simultaneously the effects of hypothalamic extract override those produced by blood alone (Group 1).

**Group 5: Cerebral Cortex, Incubated and Non-Incubated**

Neither incubated nor non-incubated samples of cerebral cortex produced any observable changes in the diameter of the
Production of intracranial vascular spasm by hypothalamic extract

vessels when angiograms were made at the same time intervals as in Groups 1-4.

**Hypothalamic Hormones**

Since the techniques used in these experiments produced a crude hypothalamic extract, two hormones, oxytocin and vasopressin, known to be present in hypothalamic tissue, were injected into the subarachnoid space of eight dogs. However, oxytocin in dosages of 0.25, 0.5, 1.0, and 5.0 IU and vasopressin in dosages of 0.5, 1.0, 5.0, and 10.0 IU did not have any effect on the caliber of any intracranial vessels.

**Discussion**

The data presented in this study clearly show that there are substances present in the hypothalamus capable of consistently producing intracranial spasm similar to that induced by blood placed in the subarachnoid space. Many workers have shown that several agents in blood, such as serotonin, produce spasm. To our knowledge no one has demonstrated the direct production of spasm by injecting extracts of brain tissue into the subarachnoid space.

Osterholm and Meyer and Wilkins and Odom have suggested the possibility that substances present in damaged brain tissues could be released into the cerebrospinal fluid and complement spasm produced by subarachnoid blood. It is known that subarachnoid blood can erode brain tissue and produce local brain pathology. The frequent occurrence of aneurysms on the circle of Willis surrounding the hypothalamus and their rupture and subsequent hematoma formation with associated parenchymal damage lend practicality to the concept that chemicals released from damaged brain tissue, in this instance the hypothalamus, may contribute to cerebrovascular spasm.

Type F prostaglandins, which have been shown to be spontaneously released into the cerebral ventricles, may be one such agent that should be considered in this regard. Some investigators have shown that Type F prostaglandins are a causitive agent for spasm, and others have suggested a spasmylic role for prostaglandins of the E type. Holmes has shown that serotonin increases the release of prostaglandins from brain tissue. This information suggests, then, that during the incubation period of these experiments unknown chemicals and possibly prostaglandins may have been released from the tissue and accumulated in significant amounts in the subarachnoid space to produce spasm.

The increased incidence of spasm found in animals receiving incubated extracts of hypothalamic tissue over those receiving non-incubated extracts is consistent with the clinical observation that spasm usually occurs several days after bleeding has occurred. It further suggests that time is needed for the development of a spasmodenic agent of sufficient strength to produce, maintain, and intensify the spasm. Chemicals or a chemical of this type could not wholly reside in fresh blood since none enters the subarachnoid space following the initial hemorrhage until a second rupture occurs. From experimental data we know that blood does not produce lasting spasm. In addition, experimental data on the duration of spasm induced by extracts of blood and hypothalamus injected into the cerebrospinal fluid are still incomplete. It is expected that spasm could be indefinitely maintained by repeated re-introduction of this combination. However, in the clinical setting a constant high titer of the spasmodenic agent in spinal fluid may occur because of the presence of the clot which could cause a physiological interruption or even necrosis of the adjacent brain tissue. Also, the interaction of products released from the blood and hypothalamus may be a continuing source of spasmodgenic agents. Therefore, surgery may be implicated once spasm has occurred in order to remove the source of the spasmodenic agent. Most surgeons prefer to wait until spasm disappears. An alternative to a surgical removal of the cerebral clot and surrounding damaged tissue is the periodic introduction of an antagonist either into the subarachnoid space or the vascular system to counteract the intracranial vascular spasm.

The absence of spasm in animals injected with cerebral cortical extract is difficult to explain. One explanation may be that the mean concentration of Type F prostaglandins is higher in the hypothalamus (200...
ng/gm tissue) than in the cerebral cortex (147 ng/gm tissue). Another hypothesis considers the fact that the hypothalamus contains a larger number of adrenergic innervated blood vessels than most other regions of the brain. Since a more important role for the adrenergic innervation and control of intracranial arteries has recently been suggested, the higher concentration of catecholamines in the hypothalamic region may play a role in the production of vasospasm.

We feel that an experimental model has been developed which, so far, evaluates two of the possible factors that may be responsible for cerebrovascular spasm following aneurysmal rupture, i.e., blood and necrotic or damaged tissue. Cerebrospinal fluid and damaged arteries are two other factors that need to be considered. This model implicates new areas concerned with the production of vasospasm following rupture of intracranial aneurysms and may help develop a spasmylytic agent that could be introduced at the time of the initial lumbar puncture or later via subcutaneous reservoirs leading to the subarachnoid space.

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References

Production of intracranial vascular spasm by hypothalamic extract


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