Experimental Study on the Genesis of Cerebral Vasospasm

BY RICHARD P. WHITE, PH.D., A. AINSWORTH HAGEN, PH.D., HOWARD MORGAN, M.D., WILLIAM N. DAWSON, M.D., AND JAMES T. ROBERTSON, M.D.

Abstract:
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The cerebral vasospasm produced by blood, fractions of blood, and blood-borne agents administered intracisternally was studied arteriographically to attain a better understanding of the genesis of vasospasm. The results indicate this phenomenon is multifarious in origin, involving a number of spasmogens. Whole blood, platelets, platelet extracts, some isolated components of platelets, plasma, thrombin, histamine, serotonin and prostaglandins F\textsubscript{10}, E\textsubscript{2} and F\textsubscript{2a} produced a significant incidence and duration of spasm. Norepinephrine and prostaglandin E\textsubscript{1} were inactive. Spasm produced by arachidonic acid and red blood cells was of questionable significance. Compared to whole blood, thrombin usually produced spasm which was more delayed in onset while most other active substances produced a shorter-lived spasm. However, among the pure substances tested, serotonin, prostaglandin E\textsubscript{2} and prostaglandin F\textsubscript{2a} induced spasm in small doses which most nearly resembled that observed with whole blood.

The hypothesis that the course of spasm depends upon synthesis of spasmogens by brain and blood is advanced. Prostaglandin synthesis plays a major role in this concept.

Additional Key Words: platelets, prostaglandins, spasmogens

Introduction

In attempts to study experimentally mechanisms involved in the cerebral vasospasm which follows intracranial bleeding in humans, many investigators have given blood intracisternally to animals and noted the presence of vasospasm by arteriography. Others have applied blood topically to exposed cerebral vessels and recorded vascular changes photographically. Reports agree that blood can produce cerebral vasospasm in a wide variety of species. However, there is diverse opinion as to what specific factor(s) in blood might be primarily responsible for the spasmogenic phenomenon. In most studies the range of substances examined were so select that the inferences drawn have excluded large numbers of possible agents and mechanisms which may be involved in vasospasm. An exception to this has been the report of Kapp et al.\textsuperscript{1} (1968) who tested experimentally a wide variety of blood components and blood-borne agents suspected of causing vasospasm. Their results clearly showed that a number of factors in blood, especially platelets, are spasmogenic when washed topically onto an exposed basilar artery in cats.

The present study was performed to ascertain whether substances found to be spasmogenic by Kapp et al.\textsuperscript{1} in cats might apply to another species when introduced into the cisterna magna, where the milieu more nearly resembles the clinical setting. Moreover, additional substances were tested for spasmogenicity.

Methods

Mongrel dogs of both sexes, weighing from 14 to 29 kg, were anesthetized with pentobarbital sodium (30 mg per kilogram) given intravenously and maintained with supplemental amounts. Tracheostomy was routine. The right vertebral artery was cannulated at the base of the neck in order to obtain arteriograms of the basilar artery by injecting rapidly 5 ml of Hypaque 60% (meglumine diatrizoate). The experimental substances were administered into the cisterna magna as follows. With the animal on its side the cisterna magna was tapped with an 18-gauge spinal needle atraumatically (confirmed by cell count of the cerebrospinal fluid and at autopsy). In experiments in which plasma or blood was given, 2 ml of cerebrospinal fluid was collected and discarded prior to injection. In others, the substance tested was mixed in the cerebrospinal fluid (CSF) and the mixture injected into the cisterna magna. Harvested platelets, however, were given in 0.9% saline (volume 2 ml) to avoid aggregation by calcium in CSF. The animal was then placed on its back for the remainder of the experiment in all cases except those given blood. They were placed on their ventral surface with the head tilted 15° lower for ten minutes to enhance contact of blood around the basilar artery as suggested by other investigators.\textsuperscript{2,3}

Arteriograms were obtained with the x-ray unit at a fixed distance from the head, prior to and usually at 5, 15, 30 and 60 minutes after the injection of the experimental substance and hourly thereafter for at least three hours. Control procedures consisted of the collection and injection of CSF alone, the injection of CSF containing substances such as...
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heparin used in the fraction of blood, and CSF containing the vehicle for some of the drugs administered (details of which will be given in Results). Systemic blood pressure (femoral), heart rate, ECG, and respiratory rate were monitored routinely; blood gases, platelet count, and pH values were obtained in some animals (presented in Results).

The degree of spasm of the basilar artery in a given experiment was independently assessed by at least two of the authors, quantified by using a standard comparator (Edmund Scientific Co., Barrington, New Jersey) and with a surgical microscope.

Platelets were obtained from heparinized blood (5 units per milliliter of blood) or blood treated with K3 ethylenediaminetetraacetic acid (EDTA): final concentration 1% in blood. This blood was centrifuged for 15 minutes at 600 RPM after which the plasma containing most of the platelets was removed. The plasma was then centrifuged at 3,000 RPM for 15 minutes to collect the platelets, the plasma supernatant being removed. This supernatant was used in some experiments. The platelets were suspended in 2 ml saline for injection or after boiling used to make aqueous extracts as described by Kapp et al. Further isolation of spasmyogenic substance from platelets was accomplished on Sephadex G-10 and G-25 columns. The substance was proved to be free of serotonin, using serotonin 4-C as an indicator of retention time on the column. In seven experiments, 2 ml of red blood cells were obtained by centrifugation and suspended in isotonic saline prior to injection into the cisterna magna.

The commercial chemicals tested for spasmyogenicity were arachidonic acid, norepinephrine bitartrate, histamine dihydrochloride, serotonin creatinine sulfate (Nutritional Biochemicals Corporation), thrombin (Upjohn and Parke-Davis Companies: 1,000 NIH units/vial) and prostaglandins E1, E2, F1α, F2α, and F3α, tromethamine (courtesy Dr. John Pike, Upjohn Pharmaceutical Co.). All doses were computed as the active base. The tromethamine salt of F2α is water soluble; the remaining prostaglandins were dissolved in 95% ethanol (5,000 µg per milliliter) with the maximum volume added to 2 ml of CSF being 0.05 ml. This vehicle of ethanol was inactive. A 20 mg per kilogram dose of indomethacin was dissolved in 10 ml of 0.1 M phosphate buffer (pH 8.0) and infused intravenously at a rate of 1 ml per minute. This dose should be more than sufficient to inhibit significantly the synthesis of prostaglandins by platelets and other peripheral tissue. It did not change respiratory rate, ECG, or blood pressure at any time during or after the infusion. Similarly, blood gases, pH and platelet count taken at 20 and 40 minutes from the start of the infusion were not altered. One hour after indomethacin, 4 ml of blood from one femoral artery was removed and injected into the cisterna magna.

Results

Table 1 compares results obtained with blood and various fractions of blood. As shown, all of the major components of blood tested produced spasm in some animals. However, the incidence and duration of spasm obtained with red blood cells were extremely low, suggesting that a contaminant from the extraction procedure might be responsible. Compared to whole blood, platelets and platelet extracts produced an early short-lived (less than 60 minutes) spasm, whereas thrombin induced spasm that usually appeared later but persisted throughout the observation period. It is also obvious that (1) none of the agents studied, including blood, always produced spasm and (2) in some animals recovery occurred

| TABLE 1 |
| Incidence of Cerebral Vasospasm Induced by Blood and Blood Components Injected into the Cisterna Magna in Dogs |

<table>
<thead>
<tr>
<th>Agent</th>
<th>Spasm per N</th>
<th>Incidence of spasm by serial arteriograms (minutes)</th>
<th>Degree of maximum spasm*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Platelets†</td>
<td>5/6</td>
<td>3 4 2 0 0 0</td>
<td>Mild 2 3</td>
</tr>
<tr>
<td>Platelets‡</td>
<td>4/4</td>
<td>4 2 2 0 0 0</td>
<td>Severe 1 3</td>
</tr>
<tr>
<td>Platelets§</td>
<td>5/7</td>
<td>3 3 3 5 1 1</td>
<td></td>
</tr>
<tr>
<td>+ plasma§§</td>
<td>5/6</td>
<td>1 1 3 2 2 1</td>
<td></td>
</tr>
<tr>
<td>Plasma§</td>
<td>3/4</td>
<td>3 3 3 2 1 0</td>
<td></td>
</tr>
<tr>
<td>Platelet extract†</td>
<td>5/6</td>
<td>5 1 0 0 0 0</td>
<td></td>
</tr>
<tr>
<td>Platelet Sephadex</td>
<td>3/3</td>
<td>0 0 2 3 1 0</td>
<td></td>
</tr>
<tr>
<td>RBC**</td>
<td>3/7</td>
<td>1 2 1 0 0 0</td>
<td></td>
</tr>
<tr>
<td>Thrombin</td>
<td>1,000 u</td>
<td>9/10</td>
<td>1 2 4 8 9 7</td>
</tr>
<tr>
<td>100 u</td>
<td>6/6</td>
<td>2 4 6 6 1 1</td>
<td>(1) 2</td>
</tr>
<tr>
<td>Blood, 4 ml</td>
<td>13/16</td>
<td>1 1 1 8 6 6</td>
<td>(3) (3)</td>
</tr>
<tr>
<td>Blood, 2 ml</td>
<td>7/7</td>
<td>6 6 6 4 4</td>
<td>(1) (2)</td>
</tr>
</tbody>
</table>

*Mild (10% to 30% reduction), Severe (>30% reduction).
†Obtained from 10 ml blood (EDTA).
‡Obtained from 20 ml blood (EDTA).
§Times at which arteriograms were occasionally taken; numbers in parentheses represent selected additional determinations.
**Two milliliters of heparinized platelet-poor plasma.
††Two milliliters of red blood cells, heparinized or EDTA.
regardless of the agent used to induce spasm. Moreover, doubling the amount of blood present did not increase the incidence or duration of the spasm generated. These findings clearly indicate that blood contains a number of spasmogenic agents, that some (thrombin) may act largely indirectly, and that the duration of the spasm depends upon the maintenance of a critical level of the spasmogen around the vasculature.

Table 2 summarizes a dose-response study with substances known to be present in blood or CSF and which might play a role in the genesis of vasospasm. Of the prostaglandins tested, PGE1 was inactive whereas PGE2 and PGF2α were active over a wide range of doses. They also produce only a monophasic response (contraction).

Serotonin was active in lower doses (about 0.2 μg per kilogram), but in higher doses (10 μg per kilogram or more) it failed to produce constriction, and often dilated (5 of 16 animals). This dilation is not likely due to a biphasic effect of serotonin because it occurred early and was transient. It may be due to inhibition of enzymes or receptors by the salt moiety; it is not due to a change in CSF pH. The higher doses were given because of reports that serotonin will enhance prostaglandin release from the brain and because it is a precursor for PGE2 and F2α synthesized by platelets and it might be likewise in the brain, thereby increasing to CSF levels of prostaglandins. The short-lived spasm caused by arachidonic acid suggests, again, that critical levels in CSF are necessary to maintain spasm.

Indomethacin pretreatment (20 mg per kilogram i.v.) failed to prevent 4 ml of autogenous blood from generating spasm in four of six animals. The incidence of spasm was only slightly less than in the control group (13 of 16, table 1) and the percent with spasm at 120 minutes was comparable in both groups (33% and 46%, respectively). Moreover, whatever amount of indomethacin was present from the intracisternal blood injection of PGF2α into the cisterna magna.

Other observations were as follows. Nine dogs given CSF alone failed to manifest spasm. Four of these were observed for three hours and the remaining for two hours. The spasm seen may be segmental and usually, but not always, involves branches of the basilar and the anterior spinal artery. In retrospect, such variation may be due, in part, to the beveling of

### Table 2

**Dose-Response Study of Spasmogenicity Caused by Naturally Occurring Compounds Injected Intracisternally in Dogs**

<table>
<thead>
<tr>
<th>Agent</th>
<th>Dose (μg/kg)</th>
<th>Incidence of spasm/total number</th>
<th>Spasm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostaglandins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E1</td>
<td>0/3</td>
<td>0/5</td>
<td>No</td>
</tr>
<tr>
<td>F1α</td>
<td>8/10</td>
<td>0/5</td>
<td>Yes</td>
</tr>
<tr>
<td>F2α</td>
<td>16/16</td>
<td>5/5</td>
<td>Yes</td>
</tr>
<tr>
<td>E2</td>
<td>11/13</td>
<td>5/5</td>
<td>Yes</td>
</tr>
<tr>
<td>Arachidonic acid</td>
<td>500</td>
<td>100</td>
<td>Yes</td>
</tr>
<tr>
<td>Norepinephrine</td>
<td>100†</td>
<td>2/5</td>
<td>No</td>
</tr>
<tr>
<td>Serotonin</td>
<td>500</td>
<td>20</td>
<td>Yes</td>
</tr>
<tr>
<td>Histamine</td>
<td>0/5</td>
<td>0/2</td>
<td>Yes</td>
</tr>
</tbody>
</table>

*Originally administered as micromolar solutions (1 X 10^-6 M or less).
†Constriction of basilar artery transient and mild.
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TABLE 3

<table>
<thead>
<tr>
<th>Agent</th>
<th>Incidence of spasm/serial arteriogram (minutes)</th>
<th>Degree of maximum spasm</th>
<th>Spasm per N</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mild</td>
<td>Severe</td>
</tr>
<tr>
<td>Histamine</td>
<td>6/6</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>Serotonin</td>
<td>7/7</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>
| PGF
\(_{2\alpha}\) | 7/7                                           | 5   | 4     |

*Numbers in parentheses represent selected additional determinations.
†Mild (10% to 30% reduction), severe (>30% reduction).
‡Numbers in parentheses represent selected additional determinations.

Discussion

The results clearly indicate that the origin of experimental vasospasm is multifarious and support the findings of Kapp et al.\(^2\) (1968) in another species using different procedures. The fact that some agents injected into the cisterna magna only produced a short-lived spasm does not eliminate them as important factors in the genesis of spasm. Under proper conditions, they may be synthesized by elements in blood and by brain tissue in sufficient amounts to induce spasm. Concerning the latter, norepinephrine, histamine, prostaglandins and serotonin are among substances known to be synthesized in the brain, some of which may account for the recent finding\(^11\) that extracts of hypothalamic tissue produce spasm when given intracisternally to dogs. Vasopressin and oxytocin were not responsible for this spasm.\(^11\) The present study also would rule out norepinephrine, prostaglandin \(E\_1\), red blood cells, and certain components of platelets as likely spasmogens.

By any standard of comparison, prostaglandins \(E\_2\) and \(F\_2\alpha\) must be considered as spasmogens involved in the genesis of cerebral vasospasm. In minute amounts they generated spasm of long duration. Both are known to be synthesized by platelets; thrombin stimulates this synthesis.\(^8\) Both are known to be synthesized by brain.\(^6\) The concept that the brain may be a source for spasmogens was first clearly stated by Schmidt\(^12\) and more recently used to explain vasospasm seen clinically\(^13\) and produced experimentally.\(^14\)\(^,\)\(^15\) Moreover, prostaglandin \(E\_2\) is associated with and causes inflammation; it may be responsible for the report\(^2\) that the severest vasospasm produced by intracisternal blood was in one dog with meningitis. Since prostaglandin synthesis by brain normally varies some fivefold,\(^1\) this concept may be linked to prostaglandin synthesis to explain why not all patients incur cerebral vasospasm after hemorrhage, why spasm is often delayed in onset, why head trauma may generate spasm\(^14\) and why indomethacin failed to prevent vasospasm in the present study, assuming adequate dosage. This drug enters the brain poorly and does not inhibit brain prostaglandin synthetase activity.\(^16\) Also, the thrombin is an antagonist of indomethacin\(^8\) which in CSF may readily negate the latter's effect. Moreover, preliminary experiments indicate that thrombin alone given intracisternally dramatically increases prostaglandin levels in the CSF\(^17\) and can stimulate platelet synthesis of prostaglandins in CSF.\(^17\) This agent, therefore, may play a dual role in the genesis of vasospasm. Lastly, CSF obtained from lumbar puncture in patients with cerebral vasospasm have abnormal quantities of prostaglandin \(F\_2\alpha\),\(^18\) which the present study indicates is only one of several which could be responsible for this condition.

The spasm caused by thrombin is of special interest because it has not been heretofore hypothesized in the genesis of vasospasm. The use of this agent in neurosurgery may be ill-advised, especially at closure because the spasm generated was usually delayed in onset (table 1). The results with histamine and prostaglandin \(E\_2\) were also interesting because both substances given intravenously are depressor agents in dogs but were constrictor when present in the CSF. Histamine dihydrochloride has been shown by others to cause constriction when applied topically to the basilar artery in cats and dogs, exposed\(^9\) or isolated.\(^9\) Norepinephrine creatinine sulfate also constricts basilar arteries when applied topically,\(^1\) but was inac-
tive in the present study. Perhaps norepinephrine failed because it is normally rapidly taken up by sympathetic nerve endings in the area\(^1\) as well as being washed away by the normal flow of CSF. Indeed, the latter may be responsible for the spontaneous reversal of the spasm produced by the various substances tested. In this regard, Echlin\(^2\) has shown in monkeys that a saline wash will reverse spasm produced by subarachnoid blood.

The present study and others clearly indicate that the genesis of cerebral vasospasm is complex, as it appears to be clinically. Blood does not always generate spasm\(^3\) and the spasm generated may spontaneously lyse and reappear.\(^4\) Pure compounds given intracisternally may induce a spasm which is mainly segmental\(^5\) or may affect some arteries more than others. The presence of blood for 24 or more hours in the cisterna magna, when spasm is absent, will increase the sensitivity of the basilar artery to PGF\(_2\alpha\).\(^6\) Among the many spasmogens studied, it is likely that some act synergistically, an unexplored possibility. Experimentally the anesthetic employed might also alter the vascular responses produced by a given spasmogen, though in our experience (unpublished data) penthrane does not prevent or relieve spasm induced by blood or prostaglandin F\(_2\alpha\). Also, some of the pure compounds studied (histamine, serotonin, and prostaglandin F\(_2\alpha\)) are spasmongenic when applied to the isolated basilar artery of dogs where, presumably, the pentobarbital used initially would not be active.\(^7\) The vascular spasmogen serotonin will also increase the release of prostaglandins from brain.\(^8\) Reserpine pretreatment may prevent vasospasm\(^9\) by reducing levels of serotonin, histamine, and norepinephrine,\(^9\) by inhibiting prostaglandin release from tissue,\(^10\) by inhibiting platelet function,\(^11\) and by inhibiting phosphodiesterase activity.\(^12\) The latter effect apparently interferes with the contraction process (which all spasmogens cause) and promises to be of value in the reversal of spasm.\(^13\) Indeed, the variety of substances reported to be effective in reversing spasm suggests that in suitable dosage they have a common mode of action, e.g., nitroglycerin,\(^14\) phenoxybenzamine,\(^15\) phentolamine,\(^16\) theophylline,\(^17\) and choline.\(^18\) Nevertheless, the fact that spasm can be reversed by saline wash\(^19\) indicates that spasmogens are responsible for this contraction. The present study and others\(^14, 15, 17, 18\) indicate that prostaglandins could be of paramount importance in the genesis of cerebral vasospasm and that a suitable inhibitor of prostaglandin activity or synthetase could significantly alter its course.

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**References**


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