Cerebral arterial spasm

Part 1: *In vitro* contractile activity of vasoactive agents on canine basilar and middle cerebral arteries

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*In vitro* experiments were performed using a small volume chamber to determine the contractile activity of various vasoactive agents on the canine basilar and middle cerebral arteries. Cumulative dose-response curves were obtained for most of the agents tested including serotonin and three different prostaglandins; many of these curves were found to be similar for segments from both arteries. It was concluded from these curves, and the known concentrations in blood, that serotonin is probably the agent in blood responsible for the cerebral arterial spasm that often follows a subarachnoid hemorrhage. This *in vitro* method is capable of detecting serotonin concentrations as low as $10^{-12}$ gm/ml and may prove useful as a quantitative and well-controlled method for studying the etiology of spasm and the receptor mechanisms present in the cerebral arteries.

**Key Words** - cerebral arterial spasm • vasospasm • serotonin • prostaglandin • basilar artery • middle cerebral artery

Arterial spasm associated with hemorrhage from a cerebral vessel is of paramount clinical importance. This paper reports an *in vitro* study of the major arteries located in the subarachnoid space around the base of the brain. The clinical significance of positive results of such an investigative protocol is apparent to clinicians treating patients with varied types of intracranial hemorrhage.

Echlin demonstrated that the presence of blood in the subarachnoid space produced vasospasm in the large arteries at the base of the brain in experimental animals. This has been demonstrated in dogs, cats, and monkeys. The obvious implication is that some substance in blood must be responsible for this spasm.

Experimental studies on the production and relief of spasm have been done primarily *in vivo*. The techniques of angiography or direct observation of the arteries after surgical exposure have been widely employed to demonstrate spasm. Non-cerebral vascular smooth muscle has been studied *in vitro* for many years and
more recently cerebral arterial preparations have also been studied.\textsuperscript{15}

This report describes the application of an \textit{in vitro} method, utilizing a small volume chamber, to the problem of cerebral arterial spasm.

\textbf{Materials and Methods}

\textit{Chamber Studies}

Twenty dogs of both sexes, weighing between 15 and 26 kg, were sedated with intravenous sodium pentobarbital (32 mg/kg) and sacrificed by rapid exsanguination. The brains were removed and placed in Krebs-Ringer buffer at room temperature. The artery to be studied was then dissected free under magnification. A segment of the artery was obtained by sectioning the vessel with two razor blades fixed in parallel 3 mm apart. This segment was then mounted on rigid parallel prongs similar to those described by Nielsen and Owman\textsuperscript{15} inside the chamber diagrammed in Fig. 1. Segments from the distal centimeter of the basilar artery or the proximal centimeter of the middle cerebral artery were used. The time interval from sacrifice to mounting was never greater than 25 minutes. During mounting, the upper section of the chamber was held several inches above the lower section to facilitate placement of the artery. After the artery was in place, the two sections of the chamber were clamped together. The chamber was then filled with 10 ml of Krebs-Ringer buffer solution of the following composition (mM concentrations): NaCl 120, KCl 4.5, CaCl₂ 2.5, MgSO₄ 1.0, NaHCO₃ 27.0, KH₂PO₄ 1.0, Na₄EDTA 0.01, and glucose 10.0. A mixture of 95% O₂-5% CO₂ was continuously bubbled through both the chamber and the buffer reservoir. Stirring was provided by the vigorous bubbling of the gas

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{chamber_diagram.png}
\caption{Schematic drawing of the chamber used. Insert illustrates the artery mounted on the rigid parallel prongs.}
\end{figure}
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mixture. The buffer was adjusted to pH 7.4 ± 0.05 where it remained throughout the experiments. The jacketed chamber and buffer reservoir were maintained at 37 ± 0.5°C by means of a circulating, constant temperature bath.

Isometric tension was measured using Hewlett-Packard FTA-10-1 and FTA-100-1 transducers with a Model 7858A polygraph; the transducers were held by a fine positioning device (FTA 1011)* to allow the resting tension to be accurately adjusted. This equipment was calibrated before each experiment with standard weights.

All agents to be tested were added by means of a Bioquest† automatic pipette in increments of 50 to 500 μl. The total volume added for one test never exceeded 500 μl, or 5% of the volume of the chamber contents. At the end of a test the chamber and artery were washed with three 100 ml aliquots of 37°C buffer, which were pumped through the chamber utilizing the buffer overflow outlet. Between each wash the contents of the chamber were adjusted to 10 ml using the buffer outflow stopcock (Fig. 1). From 20 to 30 minutes were allowed for washing and stabilizing of the artery between tests.

The agents tested were added in a cumulative log-dose manner;2a 50 μl volumes of test solution were added consecutively, each having a concentration 10 times greater than the preceding solution. After each addition, the artery was allowed to reach a stable state of contraction (Fig. 2). This was continued until a subsequent addition either did not increase the contraction or caused the artery to relax. The maximum contraction at each concentration was recorded and plotted as a cumulative dose-response curve (Figs. 3 and 4).

Serotonin was found to be the most reliable internal standard for each artery. An arterial preparation was not used unless repeated serotonin cumulative dose-response curves were identical within experimental error. Such serotonin internal standard responses were obtained periodically on each artery. Vasoactive agents, including serotonin, were added to the chamber in a different sequence from one arterial preparation to another.

Vasoactive Agent-Induced Contractions

Two parameters are important in defining the response of these arteries to the various agents.4 The \( K_{ED50} \) is the concentration of an agent at which 50% of the maximal contraction obtainable with that agent occurred. The \( K_{ED50} \) is characteristic of the agent-receptor interaction and does not vary with the number of receptor sites or the amount of tissue present. The maximal contraction obtainable with each agent, \( C_{MAX} \), is dependent on the amount of muscle

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*FTA-10-1 and FTA-100-1 transducers, Model 7858A polygraph, and the FTA 1011 fine positioning device manufactured by Hewlett-Packard, Customer Service Parts Center, W-120 Century Road, Paramus, New Jersey 07652.

†Bioquest pipette manufactured by Schwartz-Mann, Orangeburg, New York 10962.
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FIG. 4. Polygraph tracing of the isometric contraction of a basilar artery segment to cumulative additions of serotonin. Numbers at base of chart indicate the gm tension at full scale reading, and the arrows at the base of the tracing show when this was changed. Arrows on the tracing indicate the points where the concentration of serotonin was adjusted to the values shown by addition of 50 μl of stock solution.

tissue and the number of receptors present and therefore will vary depending on the size of the tissue sample studied. Because C_MAX varies with the arterial sample, it was necessary to express C_MAX in terms of one agent if various arterial samples were to be compared and averaged. Serotonin was chosen as the standard since every artery responded to it and it gave more contraction at lower concentrations than the other agents tested.

Compounds Used

The chemicals used to make up the Krebs-Ringer buffer solution and the potassium chloride were all American Chemical Society certified reagent grade. The vasoactive agents* were made up fresh each day in triply distilled water except for the prostaglandins.† All the catecholamine solutions were in 10 μM Na_4EDTA to prevent trace metal catalyzed oxidation.

Results

Determination of Optimal Resting Tension

Three arteries were allowed to stabilize in the chamber for 2 to 3 hours at a resting tension of 200 to 400 mg, and then cumulative dose-response curves to serotonin were obtained at various resting tensions from 400 mg to 10 gm. The arteries gave relatively constant K_EDS0 values from serotonin between 2 and 8 gm of resting tension, but below 2 gm it was found that the K_EDS0 increased (Fig. 5). The maximal contraction obtainable with serotonin was also a function of the resting tension (Fig. 6). Similar results were observed with one artery when epinephrine cumulative dose-response curves were obtained. Cumulative dose-response curves of F_{2α} at 400 mg and 3 gm of resting tension also showed these same results. Three gm of tension were chosen as the resting tension for the remainder of the study.

It was found, however, that if the artery were adjusted to a resting tension of 3 gm immediately after it was placed in the chamber, the K_EDS0 and C_MAX values were

*The following vasoactive agents were obtained from the Sigma Chemical Company, P. O. Box 14508, St. Louis, Missouri 63178: serotonin (5HT) (serotonin creatinine sulfate), epinephrine (Epi) (L-epinephrine bitartrate), norepinephrine (Norepi) (L-arterenol bitartrate monohydrate), histamine (Hist) (histamine dihydrochloride), acetylcholine (Ach) (acetylcholine chloride), isoproterenol (Iso) (L-isoproterenol bitartrate). The following prostaglandins were a gift from Upjohn Company, Kalamazoo, Michigan 49001: prostaglandin E_1 (E_1), prostaglandin A_1 (A_1), prostaglandin F_{2α} (F_{2α}) (tromethamine salt). Angiotensin (Ang) (Hypertensin) was a gift from the CIBA Pharmaceutical Company, Summit, New Jersey 07901. The following were a gift from the Sandoz Pharmaceutical Company, Division of Sandoz-Wander, Inc., E. Hanover, New Jersey 07936: vasopressin (Vp) (Lysine-8-vasopressin) and bradykinin (Bk).

†F_{2α} was made up in water and stored in the refrigerator. A_1 and E_1 were made up as the sodium salts as follows: to each 1 mg of prostaglandin was added 0.1 ml of 95% ethanol and 0.9 ml of a 0.2% Na_2CO_3 solution. These concentrated stock solutions were divided into aliquots and frozen. Solutions were made up from a freshly thawed aliquot each day.
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Fig. 5. Relative sensitivity of four canine basilar arteries to vasoactive agents shown as cumulative log-dose, isometric response curves. All data are presented in terms of per cent of maximal response to serotonin. 100% response to serotonin = 7.4 gm. (Serotonin C_{MAX} values for the four arteries were 5.0, 5.8, 6.9, and 11.9 gm.) Each point is the mean value at that concentration of the agent for the number of arteries that responded to the agent.

Similar to those obtained at resting tensions below 1 gm. Therefore, in the following experiments, the artery was allowed to stabilize at a resting tension of 200 to 400 mg for 2 to 3 hours before it was increased to 3 gm. This resting tension was maintained with periodic adjustments of the positioning device throughout the experiment.

Fig. 6. Relative sensitivity of two canine middle cerebral arteries to vasoactive agents shown as cumulative log dose isometric response curves. All data are presented in terms of per cent of maximal response to serotonin. 100% response to serotonin = 5.4 gm. (Serotonin C_{MAX} values for the two arteries were 5.0 and 5.7 gm.) Each point is the mean value at that concentration of the agent.
<table>
<thead>
<tr>
<th>Agent*</th>
<th>$\text{K}_{\text{EDSO}}$ Molar Conc.</th>
<th>$\text{C}_{\text{MAX}}^{**}$</th>
<th>Molar Conc. for $\text{C}_{\text{MAX}}$</th>
<th>Molar Conc. in Platelets/ml Blood†</th>
</tr>
</thead>
<tbody>
<tr>
<td>5HT</td>
<td>$7 \times 10^{-4}$</td>
<td>100%</td>
<td>$5 \times 10^{-7}$</td>
<td>0.6 - 1.6 $\times 10^{-4}$ (14)</td>
</tr>
<tr>
<td>A₁</td>
<td>$8 \times 10^{-7}$</td>
<td>107</td>
<td>$3 \times 10^{-6}$</td>
<td></td>
</tr>
<tr>
<td>E₁</td>
<td>$2 \times 10^{-10}$</td>
<td>49</td>
<td>$1 \times 10^{-7}$</td>
<td>0.4 - 2.9 $\times 10^{-8}$ (18)</td>
</tr>
<tr>
<td>F₂α</td>
<td>$3 \times 10^{-7}$</td>
<td>112</td>
<td>$3 \times 10^{-8}$</td>
<td>0.4 - 3.0 $\times 10^{-8}$ (18)</td>
</tr>
<tr>
<td>Epi</td>
<td>$1 \times 10^{-7}$</td>
<td>59</td>
<td>$5 \times 10^{-6}$</td>
<td>0.5 - 2.0 $\times 10^{-6}$ (14)†</td>
</tr>
<tr>
<td>Norepi</td>
<td>$8 \times 10^{-8}$</td>
<td>46</td>
<td>$5 \times 10^{-6}$</td>
<td>0.5 - 2.0 $\times 10^{-6}$ (14)†</td>
</tr>
<tr>
<td>Hist</td>
<td>$4 \times 10^{-6}$</td>
<td>70</td>
<td>$1 \times 10^{-4}$</td>
<td>4.5 $\times 10^{-9}$ (14)</td>
</tr>
<tr>
<td>Bk</td>
<td>$9 \times 10^{-10}$</td>
<td>37</td>
<td>$4 \times 10^{-7}$</td>
<td>1.4 $\times 10^{-9}$ (21)§</td>
</tr>
<tr>
<td>KCl</td>
<td>$3 \times 10^{-2}$</td>
<td>82</td>
<td>$8 \times 10^{-1}$</td>
<td>3.6 - 5.6 $\times 10^{-3}$ (10)§</td>
</tr>
</tbody>
</table>

* Abbreviations for agents: 5HT = serotonin; A₁ = prostaglandin A₁; E₁ = prostaglandin E₁; F₂ = prostaglandin F₂α (tromethamine salt); Epi = epinephrine; Norepi = norepinephrine; Hist = histamine; Bk = bradykinin; and KCl = potassium chloride.

** $\text{C}_{\text{MAX}}$ of 100% = 7.4 gm.

† Assuming $5 \times 10^4$ platelets per ml blood.

§ Total catecholamine level.

§ Plasma concentrations.

Relative Sensitivity of Canine Basilar and Middle Cerebral Arteries to Vasoactive Agents

A typical polygraph tracing of the tensions developed in response to cumulative additions of serotonin (Fig. 2) demonstrates the sensitivity of the artery, rapidity of the response, and accuracy of tension measurements using this method. Within 3 to 5 minutes of initiation of the washing procedure, the artery had returned essentially to its baseline resting tension. The mean cumulative dose-response curves of four basilar arteries to the various agents examined (Fig. 3) demonstrates that some agents never gave much contraction regardless of the concentration added, while other agents gave a large contraction, but only at concentrations of the agent that are not likely to be present in blood (Table 1). Serotonin, in contrast, was unique in that 90% of its maximal contraction was obtained with a concentration 10 to 30 times less than that present in blood. Its $\text{K}_{\text{EDSO}}$ was the lowest of those agents giving a $\text{C}_{\text{MAX}}$ of over 50% of the value of serotonin (Table 1).

Prostaglandin E₁ gave some contraction at very low concentrations, but above $1.25 \times 10^{-7}$ M it caused the artery to relax (Figs. 3 and 4). Prostaglandin F₂α and A₁ gave the highest contractions of all the agents tested but at concentrations which are not likely to be present in the blood (Table 1). Potassium chloride initially either gave a slight relaxation of the artery (Fig. 3) or a small contraction (Fig. 4) at a total concentration of 10 mM, but then gave a very steep dose-dependent contraction.

Not every arterial preparation responded to norepinephrine, epinephrine, bradykinin, and histamine (Table 2). Isoproterenol and L-8-vasopressin did not cause any of the arteries to contract and often caused them to relax below the resting tension of 3 gm.

Two middle cerebral arteries were tested with several of the agents (Fig. 4), and their dose-response curves were very similar to the curves obtained from the basilar arteries (Fig. 3). It should also be noted that arterial preparations were kept in the chamber for up to 18 hours without any change in their responsiveness. Only one arterial preparation was discarded, and its lack of responsiveness was thought to be due to technical problems encountered while mounting the artery in the chamber.

Discussion

The $\text{K}_{\text{EDSO}}$ values for many of the agents reported in this paper are 10 to 100 times smaller (greater sensitivity) than those
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TABLE 2
Response of four basilar arteries to agents tested

<table>
<thead>
<tr>
<th>Agent</th>
<th>Molar Conc. Range in Chamber</th>
<th>Response Ratio*</th>
</tr>
</thead>
<tbody>
<tr>
<td>5HT</td>
<td>5 × 10^{-12} to 5 × 10^{-4}</td>
<td>4/4</td>
</tr>
<tr>
<td>A2</td>
<td>2.5 × 10^{-12} to 2.5 × 10^{-4}</td>
<td>4/4</td>
</tr>
<tr>
<td>E1</td>
<td>1.25 × 10^{-12} to 1.25 × 10^{-6}</td>
<td>4/4</td>
</tr>
<tr>
<td>Fa</td>
<td>2.5 × 10^{-12} to 2.5 × 10^{-4}</td>
<td>4/4</td>
</tr>
<tr>
<td>Epi</td>
<td>5 × 10^{-12} to 5 × 10^{-5}</td>
<td>2/4</td>
</tr>
<tr>
<td>Norepi</td>
<td>5 × 10^{-11} to 5 × 10^{-6}</td>
<td>2/4</td>
</tr>
<tr>
<td>Bk</td>
<td>4.3 × 10^{-12} to 4.3 × 10^{-7}</td>
<td>3/4</td>
</tr>
<tr>
<td>Hist</td>
<td>1 × 10^{-11} to 1 × 10^{-4}</td>
<td>2/4</td>
</tr>
<tr>
<td>Ang</td>
<td>5 × 10^{-12} to 5 × 10^{-5}</td>
<td>4/4</td>
</tr>
<tr>
<td>Ach</td>
<td>5 × 10^{-12} to 5 × 10^{-6}</td>
<td>4/4</td>
</tr>
<tr>
<td>Iso</td>
<td>5 × 10^{-12} to 5 × 10^{-5}</td>
<td>0/4</td>
</tr>
<tr>
<td>Vp</td>
<td>1.78 × 10^{-11} to 1.78 × 10^{-7}</td>
<td>0/4</td>
</tr>
<tr>
<td>KCl</td>
<td>4.5 × 10^{-5} to 7.9 × 10^{-5}</td>
<td>4/4</td>
</tr>
</tbody>
</table>

* Response ratio = number of arteries responding/number of arteries testing.

reported by Neilsen and Owman\textsuperscript{15} for the feline middle cerebral artery in vitro. Contractions could be routinely detected with concentrations of many agents as low as 5 × 10^{-12} M. Part of the greater responsiveness of the arterial preparation reported here may be the result of using the optimal resting tension. Conditioning the arteries by allowing them to stabilize at a very low resting tension for 2 to 3 hours before the final resting tension was applied also increased their sensitivity. It is possible that by stretching the arteries, slight conformational changes in the receptors take place which increase their affinity (lower \textit{K}_{ED50}) for the agent.

The observation that not all of the arterial preparations would respond to some agents is similar to the results reported by Altura, \textit{et al.},\textsuperscript{3} with human umbilical arteries. It is not surprising that isoproterenol, primarily a beta adrenergic agent, would not cause the arteries to contract. This has also been shown for other smooth muscle preparations,\textsuperscript{20} although the feline middle cerebral artery has been reported to respond to this agent.\textsuperscript{15}

From the experiments reported here, both prostaglandin E\textsubscript{1} and serotonin if present in very low concentrations in the CSF would be expected to affect the cross-sectional area of the large arteries at the base of the brain. It has been reported that both serotonin\textsuperscript{8} and prostaglandin E\textsubscript{1}\textsuperscript{11} are present in the CSF collected by cannulation from the cerebral ventricles of cats and dogs respectively. Care must be taken in interpreting these results, however, since the introduction of blood into the CSF or damage to the brain tissue surrounding the ventricles would be expected to release these substances into the CSF. Norepinephrine, if released from sympathetic nerve terminals, would also be expected to cause a change in the cross-sectional area of the arteries studied, especially since release from the nerve terminals might result in a relatively high concentration of norepinephrine near the alpha adrenergic receptors. From our data it seems possible that serotonin and prostaglandin E\textsubscript{1} are the chemical regulators which, along with the sympathetic nerves to the arteries, control the flow of blood through the circle of Willis under normal physiological conditions.

Potassium ion, even when its concentration was 10 mM, did not cause the arteries to contract. At the levels in the CSF following subarachnoid hemorrhage reported by Wilkins and Levitt,\textsuperscript{25} none of the cerebral arterial spasm following such
hemorrhage should be due to increased potassium in the CSF. From the curves in Figs. 3 and 4 it is apparent that of the agents tested, serotonin is the best candidate as the agent in blood responsible for cerebral arterial spasm following a subarachnoid hemorrhage. Serotonin has long been suspected of playing a role in the genesis of cerebral arterial spasm following a subarachnoid hemorrhage and has previously been shown to cause cerebral arterial spasm.\textsuperscript{1,5,6,17,22} Several other vasoactive agents have also been shown to produce spasm when injected intra-arterially or into the subarachnoid space.\textsuperscript{0,16,24} These studies with serotonin and other agents including prostaglandins have often used large, pharmacological doses of these agents. The \textit{in vitro} data presented here show that any of a number of agents will produce contraction of the canine basilar and middle cerebral arteries providing these agents are present in sufficiently high concentration. In the present report, serotonin produced a 90% maximal contraction of the artery at a concentration 10 to 30 times less than that of blood and was the only agent tested that would produce a maximal contraction at a concentration known to be present free in clotted blood.

With these data established, it was apparent that further \textit{in vitro} studies were required to determine whether serotonin was indeed the agent responsible for the vasospasm observed in patients after subarachnoid hemorrhage.\textsuperscript{2}

The \textit{in vitro} method, with its small volume chamber, described here should prove useful in further studies of the etiology of spasm and the receptor mechanisms present in the cerebral arteries. Further studies are also in progress in this laboratory to see if a synergistic effect of various agents can be observed \textit{in vitro}. It should be noted that this \textit{in vitro} preparation provides an extremely sensitive bioassay, which will estimate serotonin concentrations as low as 10\textsuperscript{-12} gm/ml.

Acknowledgments

Grateful acknowledgment is made to Carol Gross and Connie Willett for technical assistance.

References

15. Neilsen KC, Owman C: Contractile response and amine receptor mechanisms in isolated
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This investigation was supported in part by USPHS Training Program in Cerebrovascular Disease Grant 5-TO1-NSO5625, and USPHS Grant NBO5546.

This paper was presented in part at the meeting of the American Academy of Neurological Surgery, Pasadena, California, November, 1973.

This work is being submitted in partial fulfillment for a Ph.D. degree in neurosurgery by Dr. Allen.

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