Cerebral arterial spasm

Part 3: *In vivo* intracisternal production of spasm by serotonin and blood and its reversal by phenoxybenzamine

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*In vivo* experiments in dogs demonstrated that physiological concentrations of serotonin, when injected intracisternally, caused cerebral arterial spasm that lasted for at least 3 hours. Comparable spasm was produced by the injection of blood containing approximately the same amount of serotonin. Phenoxybenzamine reversed both the spasm produced by pure serotonin and that produced by blood. A hypothesis of the etiology of cerebral arterial spasm is proposed based on the experimental results of the entire study.

**Key Words** cerebral arterial spasm • vasospasm • serotonin • subarachnoid hemorrhage • phenoxybenzamine

The results of the *in vitro* experiments reported in Parts 1 and 2 of this study (pp. 433-450) have demonstrated that serotonin plays a major role in the production of cerebral arterial spasm. In addition, phenoxybenzamine was shown to irreversibly block the basilar artery's serotonin receptors even in the presence of high physiological concentrations of serotonin. It remained to be demonstrated *in vivo* that serotonin in physiological concentration would produce prolonged spasm, and that phenoxybenzamine would reverse this spasm. In this report it will be shown that these earlier *in vitro* results can be reproduced in intact animals.

**Materials and Methods**

Fifteen dogs of both sexes, weighing 14 to 32 kg, were used for these experiments. The dogs were sedated with intravenous sodium pentobarbital (32 mg/kg) and allowed to breathe without assistance. Percutaneous puncture of the left femoral artery was performed utilizing the Seldinger technique. A cordis catheter, shaped with a slight curve at its tip, was placed in the femoral artery and passed up the aorta. The catheter size varied from French No. 5 to 7 depending on the size of the dog. The left vertebral artery was then selectively catheterized and the catheter firmly fixed within the artery. The dog was then turned from the supine to the
prone position, and the head was fixed in a position of extension that would allow the best visualization of the basilar artery. A control angiogram, performed with a hand injection, was obtained using 4 to 6 ml of Renografin 60. Filming was done on an Amplatz Serial Film Changer (not available commercially) and radiographs were taken at a rate of 2/sec for 3 sec. Eastman Kodak RP Royal Film and 90-sec developing were employed. A magnification factor of 2.0 was obtained with an x-ray tube having a 0.3 mm focal spot. The radiographic exposure factors were: 100 MA, 1/30 sec, and kV ranging from 84 to 92. Between angiograms, the catheter was flushed continuously with a small volume of normal saline.

After the control angiogram was obtained and examined for positioning and technique, a small catheter or needle was inserted into the subarachnoid space by the cisternal approach. In some dogs, a second control angiogram was obtained after insertion of the subarachnoid catheter, and no difference in the diameter of the arteries was found when compared to the initial control angiogram. Fresh venous blood from the dog being studied was used for the cisternal injection of blood. Serotonin (5HT) (serotonin creatinine sulfate) and phenoxybenzamine (Phb) (phenoxybenzamine hydrochloride)* were made up fresh in 0.9% NaCl prior to cisternal injection.

Autopsies were performed on all dogs. Three of the 15 dogs were excluded from the study: two because the vertebral artery could not be catheterized, and one because of difficulty in obtaining a cisternal puncture.

The diameters of the arteries visualized on the angiograms were measured by first marking the edges of the arteries under 2X magnification. Then the diameters were measured with a 7X Bausch and Lomb measuring instrument† with a metric reticle graduated in 0.1 mm increments.

**Results**

The autopsies performed on all dogs demonstrated bloody CSF and blood clots at the base of the brain only in those dogs given intracisternal blood. The CSF was always crystal clear at autopsy in those dogs that had not received intracisternal blood.

All differences in the diameters of the distal centimeter of the basilar arteries were grouped according to time intervals and what had been injected. Student t tests were done, and all group differences were significant with p < 0.01.

The results of these experiments are shown in Figs. 1-3 as bar graphs representing changes in the cross sectional area of the basilar artery. The distal centimeter of the basilar artery was always selected for measurements, although this area did not always represent the greatest spasm or relief of spasm. This is the same segment of the basilar artery used in the earlier in vitro experiments. Serotonin consistently caused spasm that lasted at least 3 hours, after 2 ml at concentrations approximating those of blood were injected into the cisterna magna. This was approximately the amount of spasm produced by the injection of 2 ml of blood.

Phenoxybenzamine reversed the spasm produced by serotonin as well as that produced by whole blood. Phenoxybenzamine appeared to take longer to act in vivo than in vitro, which may be due to a delay in its getting to the arteries involved. It was observed that after blood and then phenoxybenzamine had been injected, parts of the artery were rapidly relieved of spasm and other parts only slowly or not at all. In one case, in which spasm following the injection of blood was treated with phenoxybenzamine, a large clot was found covering a segment of the basilar artery at autopsy. This segment had not been relieved of spasm as observed angiographically. In other areas of this basilar artery not covered by clot, the spasm was reversed by phenoxybenzamine.

Angiograms in Dog 5 illustrate the spasm induced by serotonin and its relief by

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*Serotonin (5HT) (serotonin creatinine sulfate) was a gift from Sigma Chemical Company, P. O. Box 14508, St. Louis, Missouri 63178, and phenoxybenzamine (Phb) (phenoxybenzamine hydrochloride) was a gift from Smith, Kline & French Laboratories, Philadelphia, Pennsylvania 19101.

†Bausch and Lomb, Inc., P. O. Box 542, Rochester, New York 14602.
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Fig. 1. Dogs 1-4. Changes in the cross-sectional area (radius²/control radius²) of the distal centimeter of 16 basilar arteries after cisternal injection of serotonin (5HT). Time (minutes) when angiogram was taken after serotonin injection is given above vertical bars. Volume of injections = 2 ml. Concentrations of serotonin are shown in parentheses. Control diameters were 2.0, 2.2, 2.7, and 2.0 mm for Dogs 1-4 respectively.

Phenoxybenzamine; after phenoxybenzamine, blood was given and produced no spasm (Fig. 4). The angiograms of Dog 6 (Fig. 5) demonstrate the spasm 1 and 3 hours after the injection of serotonin and its relief by phenoxybenzamine. A control angiogram of Dog 12 was not obtained due to technical problems but a cisternal puncture was traumatic with grossly bloody CSF. An angiogram following this puncture showed obvious spasm (Fig. 6 left), and this spasm was reversed by the administration of phenoxybenzamine (Fig. 6 right).

Discussion

The data presented in this report conclusively demonstrate that the in vitro results¹,² are applicable in the intact animal. One injection of serotonin, in a concentration approximating that in blood, produced spasm for at least 3 hours. Serotonin is not,

Fig. 2. Dogs 5-8. Changes in cross-sectional area (radius²/control radius²) of the distal centimeter of the basilar artery after cisternal injection of serotonin (5HT) and phenoxybenzamine (Phb). Time (minutes) when angiogram was taken after 5HT or Phb injections is given above vertical bars. Volume of injections = 2 ml. Concentrations of Phb = 10⁻² M. Concentrations of serotonin are given in parentheses. Control diameters were 1.9, 2.4, 2.2, and 2.4 mm for Dogs 5-8 respectively.
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Fig. 3. Dogs 9-11. Changes in the cross-sectional area (radius²/control radius²) of the distal centimeter of the basilar artery after the cisternal injection of blood and phenoxybenzamine (Phb). Time (minutes) when angiogram was taken after blood and Phb injections is given above vertical bars. Volume of injections = 2 ml. Concentration of Phb = $10^{-4}$ M. Control diameters were 2.1, 2.3, and 2.3 mm for Dogs 9-11 respectively.

drastically, rapidly inactivated in the CSF. This is not surprising since the main enzyme responsible for its metabolism is monoamine oxidase which is an intracellular enzyme and thus would not be expected to come in contact with free serotonin in the CSF. No correlation was found between the concentration of serotonin injected ($1 - 10 \times 10^{-6}$ M) and the amount of spasm produced. Variations in the concentration of serotonin in the fluid in contact with the basilar artery would be expected to vary with the volume of injected solution that passed along the spinal cord or over the cerebellum. Also, the variability among dogs in the sensitivity of their basilar arteries to serotonin is another factor that might account for the lack of correlation.

Phenoxybenzamine does block the spasm produced by pure serotonin and by serotonin released from blood. In the intact animal, it is reasonable to expect that in those areas where the arteries are intimately covered with blood clot, phenoxybenzamine may be unable to reach the arterial receptors. Theoretically, it would seem better to inject the blocking agent intra-arterially, but when using an alkylating agent such as phenoxybenzamine, there are major problems with this route of administration. There is blockage of other noncerebral arterial receptors, with resulting side effects such as severe hypotension. In addition, it is difficult to get a sufficiently high concentration of the blocking agent at the cerebral arterial receptors because of the greater volume into which it is injected and the greater number of proteins (plasma proteins) with which the blocking agent might react.

CSF samples are being collected and assayed in our laboratory for serotonin, using the in vitro method described earlier. There may be a correlation between the amount of serotonin in the CSF and the degree of spasm, although the CSF collected is from the lumbar subarachnoid space and therefore not directly in contact with the large arteries at the base of the brain.

Clinically, it may prove beneficial to replace as much of the CSF as possible with a serotonin-free substitute and to remove the clot from around the clipped aneurysm at the time of surgery. In addition, treatment of the preoperative patient with reserpine would insure that no additional serotonin gained access to the CSF at the time of surgery. Phenoxybenzamine may prove useful in the prevention and reversal of spasm, but its toxicity when injected into the subarachnoid space needs to be ascertained before clinical trials are begun.
Hypothesis: Etiology of Cerebral Arterial Spasm Following Subarachnoid Hemorrhage

It is proposed that spasm is related directly to the amount of free serotonin present in the CSF and in the clot surrounding the cerebral arteries. Serotonin is also present bound in the platelets, which are both in the blood clots and suspended in the CSF. This bound serotonin is released over a period of several days as the clots lyse and as the suspended platelets break down. Presumably, it is this continued release of additional free serotonin from platelets that prolongs the spasm for several days. The variation in the degree of spasm from patient to patient would then be dependent upon: 1) the amount of blood released into the subarachnoid space; 2) the amount of serotonin present in their blood at the time of subarachnoid hemorrhage (the range of serotonin reported in human blood is 0.025 to 0.65 μg/ml; 3) the sensitivity of their cerebral blood vessels to serotonin (10 canine basilar arteries gave a range for serotonin \( K_{\text{ED50}} \) of 1.5 to
FrG. 5. Dog 6. Vertebral angiograms showing the longevity of spasm of the basilar artery following injection of serotonin and the relief of spasm after phenoxybenzamine. Top Left: Control angiogram. Top Right: 60 min following injection of 2 ml of serotonin (10^{-6} M). Bottom Left: 180 min following serotonin injection. Bottom Right: 60 min following the injection of 2 ml phenoxybenzamine (10^{-2} M) (250 min following injection of serotonin).

9.5 \times 10^{-6} M;^2 and 4) the extent of impairment of normal CSF circulation since such impairment might be expected to prolong the half-life of free serotonin and suspended platelets in the CSF and allow locally high concentrations of these to occur.

The variation of spasm with time in a particular patient could be a result of the noncontinuous release of free serotonin from the pool of bound serotonin in the platelets. It should be noted that the concentrations of free serotonin found in the CSF from three patients following sub-
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Fig. 6. Dog 12. Vertebral angiograms showing spasm of the basilar artery following traumatic cisternal puncture resulting in bloody CSF and the relief of spasm with phenoxybenzamine. Left: 20 min following traumatic puncture. Right: 30 min following injection of 2 ml of phenoxybenzamine (10⁻² M) (60 min following the traumatic puncture).

Arachnoid hemorrhage were on the vertical portion of the serotonin dose-response curve.² Therefore, small changes in the concentration of free serotonin would be expected to produce large changes in the degree of spasm.

The site of spasm should be related to the free serotonin concentration in the CSF surrounding the arteries. Thus, spasm could occur in arteries some distance from the aneurysm as the platelets containing bound serotonin and the free serotonin spread throughout the subarachnoid space. This has certainly been observed clinically.

The findings of the present study certainly support this hypothesis. Further studies to determine the chemical nature of the serotonin cerebral arterial receptor are in progress in our laboratory.

Acknowledgments

Grateful acknowledgment is made to Stephen Durst and Bradley Johnson for technical assistance.

References

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This investigation was supported in part by USPHS Training Program in Cerebrovascular Disease Grant 5-TO1-NS05625-05, and by 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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