Cerebral Arterial Spasm*

Part 3: Partial Purification and Characterization of a Spasmogenic Substance in Feline Platelets

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From data thus far available, it appears that cerebral arterial spasm must be viewed as a response to multiple stimuli, with spasmogenic substances in the medium surrounding the artery each contributing in some manner and degree to persistent spasm. Both serotonin and angiotensin have been shown to cause spasm when applied topically to cerebral arteries. Angiotensin can probably be dismissed as a cause of persistent spasm, since it is rapidly destroyed by angiotensinases in the blood, and its continued presence in clots or bloody cerebrospinal fluid therefore seems unlikely. We have been able to prepare a spasmogenic fraction from platelets which is heat stable, organic, and adsorbable by cation exchange resin IRC-50; all of these are properties compatible with serotonin.

This study was done to determine if the fraction contained a spasmogenic substance in addition to serotonin, and to characterize the substance thus identified. Solutions were assayed for spasmogenic activity on the basilar artery of the cat. The artery was exposed transorally, as previously described, and continuously irrigated with Ringer's solution at 37°C. Body temperature of the animal was maintained at 37° to 39°C, and arterial pressure was continuously monitored. For assay of solutions for spasmogenic activity, the solutions, warmed to 37°C, were substituted for control Ringer's solution in the gravity irrigation system. Vessel diameter was determined by measuring the column of blood within the lumen of the vessel on color photographs taken through the operative microscope. Results after 1 min of irrigation were expressed as per cent change in vessel diameter relative to the immediately preceding control diameter during irrigation with Ringer's solution.

Preparation of Platelets for Chromatography

Platelets were separated from EDTA-anticoagulated blood by differential centrifugation. The platelets were lysed with a quantity of distilled water equal to ten per cent of the original volume of blood from which they were extracted and boiled 3 to 5 min. The cooled suspension was centrifuged at 28,000 × g for 15 min, and the supernatant fluid was decanted and measured. Radioactive serotonin‡ was added (0.1 μg, 6 μg for platelets from 60 to 100 ml blood, twice this amount for larger volumes), and the solution was diluted to 10% of the original blood volume. An aliquot was removed for vasospasm assay (standard), lyophilized, and diluted to the original blood volume in Ringer's solution. The remainder was lyophilized and diluted to the proper volume for column chromatography.

Sephadex and Biogel Columns. Sephadex G-10§ (fine), and Biogel P-10** (50 to 150 mesh) were hydrated overnight in the solution which was used to elute the column. Columns of various sizes were poured and washed with large volumes of the eluting

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‡ Purchased from New England Nuclear Corp., Boston, Mass. Supplied with radiochromatogram showing homogeneity, found to be homogeneous when rechromatographed in this laboratory on Sephadex G-10 in 0.1 M. acetic acid.
§ Purchased from Pharmacia Fine Chemicals, Inc., Piscataway, N.J.
** Purchased from Bio-Rad Laboratories, Richmond, Calif.
buffer. A sample of this wash was saved and processed to determine if any vasoactive materials were washed from the column before platelets were applied. The solution of lysed platelets and radioactive serotonin in distilled water was applied to the columns in volumes of 2 to 5 ml, depending on the amount of material and column size. The eluting fluid was pumped at a rate of 0.32 ml/min and 4.8 ml fractions were collected. The optical density of each fraction was determined, and 0.1 ml aliquots of alternate fractions were counted in a liquid scintillation spectrometer to locate the serotonin-containing fractions. Fractions were then pooled to include the radio-activity in a single fraction and to separate optical density peaks and interpeaks in other fractions. Fractions eluted in volatile buffers and dilute Ringer's solution were lyophilized, and each pooled fraction or an aliquot thereof was diluted in Ringer's solution to a volume so that if all vasoactive material in the platelets were present in the fraction, the concentration would equal that of the standard. Salt concentrations of fractions eluted in dilute Ringer's solution were adjusted so that the salt content of the assayed fractions was equal to the salt concentration of undiluted Ringer's solution. When fractions were eluted with undiluted Ringer's solution, the volume of each pooled fraction was measured, and the fractions were assayed for vasoconstrictor activity without further dilution. The pH of each pooled fraction was adjusted to 6.5 to 7.0 (the pH of control Ringer's solution) prior to assay.

Sephadex Columns. Boiled, centrifuged platelet extracts containing radioactive serotonin were chromatographed in four instances on columns of Sephadex G-10, of various sizes and in various electrolyte or buffer solutions. For chromatography on three columns, platelet extract was prepared as previously described. For the fourth column, radioactive serotonin was added to the lysed platelets prior to boiling. Per cent light transmission at 254 m\(\mu\) of the column eluate was continuously recorded with an LKB model 4701A uvicorder. The fractions were pooled according to peaks and interpeaks of light absorbance, and the pooled fractions were counted for carbon-14 to locate the serotonin.

A similar pattern of both light transmission and vasoconstrictor activity was found in all columns. Maximum vasoconstrictor activity was found in the first light-absorbing peak. This was followed by one or two smaller peaks and then by a broad peak containing radioactive serotonin. Radioactivity was found only in the last peak in all studies except the one in which the radioactive serotonin was added prior to boiling the platelet extract. In this study, there was an additional earlier peak of radioactivity. This fraction, however, did not produce vasoconstriction. The results of this study are shown in Fig. 1, and data from all sephadex columns are summarized in Table 1. Constriction exceeding one standard deviation of photographic variability was not produced by wash from any column.

Biogel Columns. All Biogel P-10 was equilibrated in and columns were eluted with Ringer's solution, in a cold room at 2°C. Columns of various sizes were used. Flow rate was maintained constant at 0.32 ml/min by a pump. Optical densities at 280 m\(\mu\) of each fraction were measured, and alternate fractions were counted for radioactivity. The fractions were pooled to include the radioactivity in one fraction and to separate optical density peaks and interpeaks in other fractions. Pooled fractions were recounted to confirm localization of radioactivity and to quantitate recovery of radioactivity.

On all Biogel P-10 columns, a fraction with potent vasoconstrictor activity was separated from the fraction containing radioactive serotonin. On earlier columns, two major optical density peaks were eluted. The serotonin-free vasoactive material was found in the first portion of the second optical density peak, and the radioactive serotonin was in the terminal portion of this same peak. As columns were made longer, better separation was obtained. With a 49-cm column, resolution of the second peak into two peaks was accomplished, and the interpeak was shown to be inactive (Fig. 2). The data from all columns are summarized in Table 2.

Some Properties of the Serotonin-Free Fraction

Dialysis. Samples of 5 ml of the serotonin-free active fraction in Ringer's solution,