The Response of Cortical Vessels to Serotonin in Experimental Cerebral Infarction*

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That anatomic cerebral infarction (as distinct from physiological paralysis) does not develop immediately following occlusion of a major cerebral vessel, but rather represents a dynamic process of pathological evolution, gives hope to the clinician and challenge to the investigator. Blood viscosity, cerebral edema, the blood-brain barrier, collateral circulation, and peripheral blood pressure are parameters of known importance in the development of cerebral infarction.\textsuperscript{2,10,12,13,17–19} These, as well as other possible factors related to the gradual spread of cerebral infarction, need clarification.

In a previous work, one of us noted the predictable and reproducible sequence of pathological events following the occlusion of a major cerebral vessel.\textsuperscript{19} These are:

1. Darkening of venous blood draining the area of ischemia
2. Venous sludging in areas of reduced flow
3. Development of small foci of cortical pallor which gradually coalesce
4. Arterial spasm in the areas of cortical pallor
5. Edema in areas of ischemia
6. Venous platelet thrombi which further impede the flow and are not infrequently followed by a perivenular hemorrhage
7. Transformation of dark venous blood to bright red blood in selected areas late in the evolution of the infarction.

The gradual spread and coalescence of small foci of cortical pallor following cerebral vascular occlusion seemingly represent a self-perpetuation of the initial pathological process by the liberation of a factor which is vasoconstrictive. However, no such factor has been identified.

It has been shown that the cortical vessels of the cat and monkey are sensitive to topically applied serotonin. The cortical pallor and arterial spasm in such a preparation bears a striking resemblance to an infarct in evolution. It was postulated that serotonin, or a serotonin-like substance, might be responsible for the spreading and coalescing of the areas of cortical pallor observed in the developing cerebral infarction. Our study was designed to determine if additional investigation was justified relative to the release of serotonin or a serotonin-like agent in areas of cerebral ischemia. Such a vasoconstrictive agent could explain the gradual spread of the cortical pallor with its associated vasospasm. Such a factor might also be related to the alterations in the blood-brain barrier and the development of cerebral edema.

Before postulations for the role of serotonin in the evolution of a cerebral infarction could be formulated, it was important to know if the cortical vessels in an area of ischemia were sensitive to the vasoconstrictive properties of serotonin itself. We have conducted related studies which now form the subject of this report.

Materials and Methods

The experimental animal used was the squirrel monkey (\textit{Saimiri sciureus}), average weight of 0.7 kg. The animals were anesthetized with 0.25 ml of sodium pentobarbital (Nembutal, 50 mg per ml) injected into the intrapleural space with a $\frac{3}{8}$-in. No. 25 hypodermic needle. Tracheotomies were performed with the electrosurgical unit. The femoral vessels on one side were exposed by sharp dissection under the Zeiss operation microscope. Mean systemic blood pressures were measured by a Tyco manometer attached through a bubble trap to a siliconized polyethylene catheter inserted into the exposed femoral artery. The animals were maintained at normotensive levels, the mean blood pressure ranging between levels of 80
and 120 mm Hg. In 17 animals blood samples were taken from the femoral vein prior to the craniectomy and upon completion of the experiment for direct platelet counts and microhematocrits. Peripheral blood smears were also done on these same 17 animals. Body temperature was measured with a rectal thermistor and controlled with hot water bottles. The animal was secured in a Waltz headholder and bilateral scalp flaps reflected utilizing the electrosurgical unit.

Bilateral craniectomies were performed on 35 animals. The origin of the right middle cerebral artery was exposed under the Zeiss operation microscope by the retro-orbital, extradural approach as described by Sundt and Waltz. This vessel was occluded at its origin with a miniaturized Mayfield clip.* In each animal the opposite exposed hemisphere with its intact middle cerebral blood flow served as a control. Following the craniectomy on this side, dissection was carried down the sphenoid ridge in an attempt to equalize the amount of surgical manipulation on both sides. The dura over the hemispheres was excised under microscopic guidance without trauma to the underlying cortex. The exposed cortex was kept moist with gentle irrigations of normal saline at room temperature and covered with Saran Wrap between irrigations and observations. Observations of the cortical vessels were made through the operation microscope, and representative photographs of the brain were taken by a technique previously described in detail. Maximal blood loss from the entire procedure was estimated to be from 3 to 5 cc on each animal.

A solution of 0.1% serotonin creatine sulfate in distilled water with a pH of 6.5 was made up immediately after each experiment was begun. The individual aliquots had been weighed out previously on an analytic balance and stored in a desiccator under refrigeration. Topical application of the serotonin solution was initiated 10 to 15 minutes after middle cerebral occlusion when venous cyanosis and particulate blood flow were well developed but before the spontaneous development of focal areas of cortical pallor and spasm. This interval before testing was not rigidly adhered to but varied 5 minutes or so depending upon the rapidity of the evolution of the infarct.

Only the larger arteries constituting the major division of the middle cerebral complex were tested. The serotonin solution was applied from a tuberculin syringe with a ½-in. No. 25 needle from a distance of a few millimeters above the cortex under direct microscopic observation. The solution was applied at the rate of 1 drop per minute until vasoconstriction was observed. Individual arterial responses were timed with a stop watch. In those instances in which the arteries remained unreactive to multiple drops, the entire field was then irrigated with up to 1 cc of the solution. The total number of arteries tested, the total number of arteries reacting, the time of onset of spasm, and an estimate of the degree of spasm were recorded for each hemisphere.

The total number of individual arterial responses was tabulated and analyzed by a $2 \times 2$ Chi-square test (degree of freedom = 1, no correction factor applied).

Evaluation was made on 35 monkeys. The first 5 monkeys in this group (not appearing in the statistical analysis) served as a pilot study to determine the effects on the pial vasculature of topically-applied distilled water, physiologic saline, and to test various concentrations of serotonin in order to determine which strength solution allowed the most accurate evaluation of individual arterial responses. Following this pilot study, critical observations and data were collected on 15 adult male and 15 adult female squirrel monkeys, which formed the basis of this report.

**Results**

_Cortical Vessel Response to Serotonin._ A summary of the pertinent data on each individual animal is presented in Table 1. In each animal, the following observations were made in each hemisphere: 1) the occurrence of spasm, 2) the ratio of reacting to nonreacting vessels, 3) the time of onset of the spasm, and 4) the degree of the spasm.

By comparing the results of these observations it was possible to subdivide these animals into five groups (Table 2):

Group 1. _Response only on occluded side._ In 17 animals, representing 56.6% of the

* Manufactured by Kees Surgical Specialty Company, 117 West Main Street, Alexandria, Kentucky 41001.