Effect of Topically Applied Serotonin on Local Cerebral Blood Flow

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SUMMARY It has been hypothesized that acute lesions of the brain enlarge through an autodestructive process. Serotonin (5HT), a potent cerebral vasocostritor, is believed by some to mediate the process by reducing cerebral blood flow (CBF) in tissue surrounding the lesion. The hypothesis was tested in cynomolgus monkeys anesthetized with ketamine and nitrous oxide. Craniectomies, 7 mm in diameter, were performed in each parietal area. The dura was opened and polarographical electrodes of thin platinum wire were inserted into the parietal lobe cortex of each hemisphere. Mock cerebrospinal fluid (CSF) was irrigated continuously onto the brain surrounding the electrodes, from which local CBF was determined repeatedly by the hydrogen-clearance technique. After baseline CBF was established, solutions of 5HT in mock CSF (in concentrations of 5 x 10^{-4} M, 5 x 10^{-5} M, and 5 x 10^{-6} M) were irrigated onto one hemisphere while the opposite hemisphere served as control. CHT failed to change CBF.

Although 5HT is a potent vasocostritor, under physiologic conditions it appears to have hemodynamically significant constriction of the peripheral cerebral vasculature of the anesthetized monkey brain.

IN RESPONSE to central nervous system injury, biogenic amines with vasocostritor properties are thought to be released from neurons and blood vessels in quantities sufficient to enlarge the original lesion. According to this hypothesis, an ischemic or traumatic lesion of the brain or spinal cord may enlarge because amines released by injury reduce blood flow sufficiently to infarct tissue surrounding the lesion.2-4 Serotonin (5-hydroxytryptamine, 5HT) has been proposed as a mediator of this hypothetical self-propagating zone of spreading ischemia.2-4 Because it is both a potent cerebral vasocostritor2-4 and present in substantial concentrations in brain2-4 and blood.15-18 If the hypothesis is valid, local cerebral blood flow (CBF) should decrease when an appropriately concentrated solution of 5HT is applied topically to the brain. This paper reports the results of an investigation designed to test the hypothesis.

Materials and Methods

Young, adult cynomolgus monkeys (Macaca fascicularis) of both sexes weighing between 3 and 5 kg were initially given 50 mg of ketamine and 0.2 mg of atropine sulfate intramuscularly. They were then intubated with auffed endotracheal tube, placed on a volume respirator, and paralyzed with an intravenous injection of 0.3 mg of pancuronium. Anesthesia was maintained for the remainder of the experiment with an inhaled gas mixture of 70% N2O and 30% O2, supplemented with a continuous intravenous infusion of ketamine at 3 mg/kg/hr. Lactated Ringer’s solution, to maintain fluid and electrolyte balance and muscular paralysis. Rectal temperature was kept between 37°C and 38°C with an externally applied heated pad. The animal was moderately hyperventilated at a respiratory rate of 40 per minute and a tidal volume of 55 cc per minute, which served to clear inspired hydrogen from the lungs rapidly.23 Enough CO2 was added to the inspired gas mixture to keep Pco2 between 30 and 35 mm Hg. From a catheter in the femoral artery, we monitored arterial blood pressure continuously and obtained blood for gas analysis before and after each blood flow determination.

The animal was then placed prone with its head held rigidly in a standard stereotactic frame. A 19-gauge needle attached to a polyethylene tube was passed percutaneously into the cisterna magna, and the cerebrospinal fluid (CSF) was allowed to drain freely from the open end of the tube, which was placed 10 cm below the cisterna magna. This minimized brain pulsation and emptied the convexity subarachnoid space of CSF. Using microsurgical techniques to avoid traumatizing the brain, we performed bilateral parietal craniectomies, each 7 mm in diameter and centered about 1 cm to either side of the midline. The dura was excised to expose the leptomeninges and gyri of the parietal lobes. As soon as the dura was opened, irrigation of the exposed brain was begun with mock CSF23 with a pH of 7.30-7.35. The craniectomies were fashioned so that the mock CSF formed a pool 1-2 mm deep over the exposed brain. An infusion pump delivered the mock CSF continuously to each craniectomy site at a rate of 6 ml/hr for the remainder of the experiment. The pools of mock CSF were heated by lamp to a constant temperature of 35°C, as measured by a thermistor probe.

Subsequently, polarographical electrodes of fine platinum wire (0.18 mm in diameter), insulated with Teflon, were inserted through the pool of mock CSF and leptomeninges into the exposed gyri of each parietal lobe to a depth of 1-2 mm. The active portion of each electrode was needle sharp and approximately 1.5 mm long. Two electrodes were usually inserted into each parietal lobe within a few mm of each other. The operating microscope at 40X magnification was used during the insertion of the electrodes in order to avoid impaling small surface vessels. After the electrodes had stabilized, local CBF was determined from each area by measuring clearance of hydrogen from tissue.23-25 A CBF determination was begun by adding 5-10 volumes percent of hydrogen to the inspired gas mixture for 3-5 minutes, after which it was stopped abruptly. The first minute of the clearance curve after hydrogen cleared from the lungs was
considered to be monoexponential and analyzed as such to give CBF values as we have previously described in detail.\textsuperscript{20-22} Baseline CBF was determined for each area during the first hour. The sides were then designated at random as either control or experimental. On the control side, irrigation with mock CSF continued without interruption for the remainder of the experiment. At this point on the experimental side, a solution containing 5 \times 10^{-8} \text{ M} 5\text{HT} in mock CSF was substituted for plain mock CSF. (All solutions of 5\text{HT} in mock CSF were prepared from the creatinine sulfate salt immediately before use, adjusted to a pH of 7.30-7.35, and used completely within 1 hour of preparation.) Irrigation of both sides proceeded at 6 ml/hr for another hour, during which 2 or 3 CSF determinations were made. Then the solution irrigating the experimental side was changed to one containing 5 \times 10^{-6} \text{ M} 5\text{HT}, and CBF determinations were repeated 2 or 3 times during the ensuing hour. The procedure was repeated for the final hour with a solution of 5 \times 10^{-6} \text{ M} 5\text{HT} in mock CSF. At the end of 4 experiments and while irrigation with 5 \times 10^{-6} \text{ M} 5\text{HT} continued, vascular reactivity to hypocapnia (Pco2 of 15 mm Hg) and hypercapnia (Pco2 of 50 mm Hg) was tested.

Assay of Vasoconstrictor Activity of 5\text{HT}

At the conclusion of 2 experiments, we measured the ability of aliquots of our stock of 5\text{HT}, in mock CSF, to induce contraction of vascular smooth muscle. Fresh, spirally cut strips of rabbit aorta were employed as described in detail by Furchgott and Bhdarakom,\textsuperscript{22} except that a force displacement transducer measured contraction of smooth muscle isometrically.

During some experiments we photographed the surface of the brain through the operating microscope at 40X magnification before and during irrigation with 5 \times 10^{-6} \text{ M} 5\text{HT}. The change in calibre of vessels was then determined directly from the photograph.

Determination of Tissue Levels of Exogenous 5\text{HT}

The extent to which 5\text{HT} in solution in mock CSF was able to penetrate leptomeninges and diffuse into cerebral tissue was determined in 2 experiments at the end of 1 hour of irrigation with a mock CSF solution containing 5 \times 10^{-10} \text{ M} of tritium-tagged 5\text{HT} (equivalent to 880 \mu g/ml of free base). The irradiated surface of brain was blotted dry with filter paper, the animal killed, the brain quickly removed and immersed in liquid nitrogen until firm. A coronal section of brain 5 mm thick was cut from the section to include the site of irrigation. A cube 5 mm on the edge was cut from the section to include the irradiated surface and brain that had surrounded the electrodes, and subsequently sliced on a plane parallel to the surface into blocks of tissue 1 mm thick. Each block was placed into a tared counting vial and weighed. One ml of NCS solubilizer was added to each vial and the tissue allowed to digest overnight at 45°C. The specimens were then cooled and 10 ml of Liquiflor scintillant was added to each vial. The $^3$H activity per mg of tissue was determined with a liquid scintillation counter. Samples of tissue from the control side as well as aliquots of irrigation fluid were similarly processed. The amount of exogenous 5\text{HT} (or its metabolite, 5-hydroxyindole acetic acid) in the brain was calculated from the following formula:

$$\text{exogenous 5HT \mu g/gm of tissue =}$$

$$\frac{\text{\textsuperscript{3}H activity/gm tissue}}{\text{\textsuperscript{3}H activity/gm mock CSF \times 880}}$$

At the end of each experiment the animal was killed with a rapid intravenous injection of a saturated solution of MgSO4. The brain was removed, sectioned, and examined for evidence of trauma or hemorrhage at the site of electrode insertion. Data from electrodes within hemorrhagic brain were discarded.

Data Analysis

For each experiment, the average CBF determined at each site during irrigation with 5HT solution was compared with baseline CBF using the paired t-test. In addition, the change in CBF observed at the experimental site during irrigation with 5HT solution was compared with the change that took place at the control site during the same period (unpaired t-test).

Results

Data suitable for analysis were obtained from 9 animals; in 6 all 3 concentrations were tested in succession as described. The response of CBF to a solution 5 \times 10^{-6} \text{ M} 5\text{HT} only was determined in an additional 3 animals. Since the results were the same, all data were analyzed together.

Experiments in which 3 concentrations of 5\text{HT} were tested usually required 7 hours or more to complete. During each individual experiment, mean arterial blood pressure, rectal temperature, and blood gases remained stable and normal. For all animals, mean arterial blood pressure ranged between 70 and 130 mm Hg, rectal temperature between 37°C and 38°C, Pco2 between 29 and 35 mm Hg, pH between 7.38 and 7.49, and PO2 between 130 and 150 mm Hg. 5\text{HT} irrigation produced no discernible systemic effects.

Baseline local CBF was 54 ± 10 ml/100 gm/min (mean ± SD; n = 9). Local CBF during irrigation with each of the 3 concentrations of 5\text{HT} did not differ significantly from baseline CBF (table 1). The magnitude of change from baseline CBF values observed on the experimental side during application of 5\text{HT} did not differ significantly from changes observed on the control side during continuous irrigation with mock CSF (P > 0.15, unpaired t-test) (fig. 1). When Pco2 was reduced

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<tr>
<th>Exp. No.</th>
<th>Baseline</th>
<th>$5 \times 10^{-8}$ M</th>
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<th>$5 \times 10^{-4}$ M</th>
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<td>9</td>
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<tr>
<td>Mean SD</td>
<td>54 ± 10</td>
<td>58 ± 8</td>
<td>52 ± 10</td>
<td>59 ± 11</td>
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</table>

$p**$ > .2 > .4 > .1

$\text{ml/100 gm/min.}$

$p**$Probability that difference from baseline CBF would have occurred by chance (paired t-test).
from a mean of 33 mm Hg to 14 mm Hg, CBF decreased 13% on both control side and experimental side during irrigation with $5 \times 10^{-8}$ M 5HT. When PCO$_2$ was increased from a mean of 33 mm Hg to 48 mm Hg, CBF increased 55% on control side and 70% on the experimental side during irrigation with $5 \times 10^{-8}$ M 5HT.

In the assay for vasoconstrictor activity, our stock 5HT in mock CSF produced a measurable contraction (0.1 gm force) of the rabbit aorta preparation when the final concentration of 5HT in the muscle bath reached $10^{-7}$ M. Maximal contraction (2.5 gm force) was produced by $6 \times 10^{-8}$ M. Color photographs of the irrigated surface showed that $5 \times 10^{-3}$ M narrowed small (40-100$\mu$ diameter) pial arteries by 10% and produced a mild pallor of the cortical surface.

Experiments to determine if 5HT penetrated the leptomeninges and diffused into brain surrounding the electrodes showed the following: After 1 hour of irrigation the mean concentration of tritium-tagged 5HT in the first 1 mm of brain and meninges that had been subjacent to the pool of irrigation fluid was approximately 8% of the concentration of 5HT in solution. The concentration of 5HT in tissue samples decreased by approximately one-half for each additional 0.6 mm that the samples were located from the leptomeningeal surface (fig. 2).

**Discussion**

The results of this investigation contradict the hypothesis that by virtue of its vasoconstrictor properties, 5HT plays an important role in the progression of ischemic necrosis following cerebral infarction, hemorrhage, or trauma. We observed that focal CBF remained unaffected by topically applied 5HT that had been demonstrated as (1) biologically active as a constrictor of smooth muscle and (2) having attained within the meningeovascular surface membrane and subjacent cerebral tissue a concentration 100 to 1000 times greater than that which produces a maximal contraction of cerebral arteries in vitro. However, our results do not rule out the possibility that, under special circumstances, elevated levels of 5HT in brain and CSF are capable of producing cerebral ischemia. For example, it is conceivable that by constricting, even slightly, collateral Anastomotic vessels to an infarcted area, 5HT may contribute to the factors acting to enlarge the lesion. Moreover, there is some experimental evidence to suggest that cerebral arteries in an area of ischemia are more sensitive to 5HT. Finally, as yet unidentified substances, which are released into tissue and CSF by trauma and infarction, may potentiate the vasoconstrictor effect of 5HT.

We are not the first to study the response of local CBF to exogenous 5HT. Rosendorff injected concentrated solutions of 5HT in unbuffered saline ($5 \times 10^{-4}$ M to $2 \times 10^{-3}$ M) directly into the hypothalamus of the conscious rabbit. He found that hypothalamic blood flow increased after the injection. It is difficult to assess the significance of this finding since Rosendorff used unbuffered saline solutions of 5HT, which have a pH of less than 4 (personal observation). Stein et al. presented a brief report of an investigation in which they irrigated $1.2 \times 10^{-4}$ M 5HT in mock CSF onto the cortex of cats for 1 hour and then determined local CBF by the $^{14}$C-antipyrine method. Although pial arteries constricted when 5HT was applied, they showed no changes in local CBF. Stein et al. did not estimate the extent to which 5HT was able to penetrate cerebral tissue in their cat model.

How do we account for the fact that although 5HT has been demonstrated to be a potent vasoconstrictor of extra-parenchymal cerebral vessels (both large and small) it produced no change in CBF? A number of explanations come to mind. First, cerebral vasculature of the cynomolgus monkey may be relatively resistant to the effects of 5HT. This is unlikely since others have demonstrated that arteries of the brain from diverse species, including man, are...
very sensitive to 5HT. Second, our choice of anesthetic agents and muscle relaxants may have muted the response to 5HT. Third, 5HT may have produced a transitory fall in CBF not detectable by the hydrogen-clearance technique. Any physiologically significant fall in CBF that would lead to infarction should have been of sufficient magnitude and duration to be readily detected by the hydrogen-clearance technique. Fourth, the concentration of 5HT employed was insufficient to achieve within tissues the concentration achieved at the receptor sites under natural circumstances. This explanation seems untenable in view of our experiments with tritium-tagged 5HT which proved that pial vessels plus the entire volume of cerebral tissue (with its contained receptor sites) were exposed to a concentration of 5HT far in excess of the $5 \times 10^{-7} \text{M}$ reported by Allen et al. to have maximum contractile activity on smooth muscle of human cerebral arteries. Finally, and most likely, resistance offered by the intraparenchymal arterioles decreased sufficiently through metabolically controlled autoregulatory mechanisms to neutralize the increase in resistance in larger, principally extraparenchymal vessels induced by 5HT. In this regard, Bohr et al. have noted that vasoconstrictor effects on the larger arteries cannot be construed as representative of the characteristics of smooth muscle concerned with control of most of the resistance of the brain's arterial network. By increasing $\text{PCO}_2$ while irrigating with $5 \times 10^{-7} \text{M}$ 5HT, we hoped to detect vasoconstriction that could be unmasked only during high-flow states. But even under these conditions, 5HT failed to produce hemodynamically significant constriction of the peripheral cerebral vasculature of the monkey cortex. The fact that CBF was maintained throughout the experiment despite very high tissue levels of 5HT suggests that the intraparenchymal resistance vessels of the cerebral cortex are relatively insensitive to 5HT.

Because epinephrinoid application of 5HT did not affect CBF as predicted by current theory, we were required to prove that 5HT reached the target vessels of the pia and brain in physiologically significant concentrations within the 1-hour period of irrigation. The extent to which arachnoid acts as a barrier to diffusion of 5HT has been ignored in previous investigations. Although our experiments have shown that 5HT reached pial vessels and brain, they effects give an accurate measure of the degree to which the intact leptomeninges act as a barrier to diffusion of 5HT. It will be recalled that the leptomeninges and brain were punctured at two sites by electrodes. Doubtless, these holes facilitated the penetration of 5HT into the subarachnoid space and underlying brain.

In conclusion, we failed to find evidence to support the hypothesis that abnormal levels of 5HT in cerebrospinal fluid or brain reduces cerebral blood flow.

References

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