Cerebral Blood Flow in the Monkey after Focal Cryogenic Injury

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SUMMARY. A focal cryogenic lesion was made in the left superior frontal gyrus of the anesthetized macaque brain. Cerebral blood flow (CBF) was determined by the hydrogen clearance technique before and during the 4 hours following trauma. Local CBF in tissue adjacent to the lesion increased in the first half hour after the lesion was made and then decreased during the ensuing 3½ hours. Local CBF in the contralateral superior frontal gyrus, as well as total CBF and oxygen consumption, were unchanged by cryogenic trauma. The spread of vasogenic edema into uninjured tissue probably accounts for the observed decrease in local CBF. This experimental model may assist in discovering therapy to alter favorably the spatial and temporal profile of pathologic CBF changes in tissue surrounding an acute lesion of the brain.

IT HAS BEEN proposed that injury to the brain initiates an autodestructive process that enlarges the lesion beyond its original limits. According to this hypothesis, injured neurons and blood vessels release vasoactive biogenic amines that diffuse into tissue surrounding the lesion where they constrict vessels of the microcirculation enough to decrease blood flow and infarct tissue. If this hypothesis is valid, it would be theoretically possible to block propagation of brain necrosis and thereby minimize the volume of tissue lost. This important therapeutic implication led us at first to study, in an experimental model of brain injury, local cerebral blood flow changes in the superior frontal gyrus of the macaque brain after it had been focally contused and lacerated by a jet of compressed air. We found that CBF usually fell immediately after the lesion was made, but if so, whether it can be prevented, was limited because the lesion produced by the jet of compressed air varied in size from animal to animal. In addition, the interpretation of results was often confounded by the presence of subcortical hematomas of varying size. To overcome these difficulties, in subsequent experiments we employed, instead, the more easily reproducible lesion produced by the application of a cold metal disk adjacent to the lesion. However, the model's value in testing whether acute lesions of the brain enlarge, and, if so, whether it can be prevented, was limited because the spread of vasogenic edema into uninjured tissue probably accounts for the observed decrease in local CBF. This experimental model may assist in discovering therapy to alter favorably the spatial and temporal profile of pathologic CBF changes in tissue surrounding an acute lesion of the brain.

Materials and Methods

Young adult macaques (M. mulatta and M. fascicularis) of both sexes weighing between 3 and 5 kg were given an intramuscular injection of ketamine 50 mg and atropine sulfate 0.2 mg. They were subsequently intubated endotracheally with a cuffed tube, placed on a volume respirator and paralyzed with an intravenous injection of pancuronium 0.3 mg. A gas mixture of 70% N₂O and 30% O₂, supplemented with a continuous intravenous infusion of ketamine 3 mg/kg/hr, maintained anesthesia for the remainder of the experiment. Lactated Ringers' solution, to which was added 0.8 mg/100 ml of pancuronium, was infused continuously through a femoral vein cutdown of 16 ml/hr to maintain fluid and electrolyte balance and muscular paralysis. At the beginning of each experiment, 100 mg of Evan's blue was also given intravenously to serve as a marker for edema fluid. Moderate hyperventilation was provided by a respiratory rate of 40-44 per minute and tidal volume of 55 cc. 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 arterial blood for blood gas analysis before and after each blood flow determination. Rectal temperature was kept between 37° and 39°C with an externally applied, heated pad.

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 from this needle we continuously monitored intracranial pressure. Subsequently, using standard microsurgical techniques to avoid trauma to the brain, we performed a 3 cm by 3 cm craniectomy centered at bregma and opened the dura overlying both superior frontal gyri. A small polyethylene catheter was passed into the anterior end of the sagittal sinus from which we periodically withdrew 0.3 ml of blood to measure O₂ content of the blood draining from the brain. Oxygen content of arterial and sagittal sinus blood was measured before each blood flow determination with a Lex-O₂-Con instrument.* Five polarographical electrodes of glass-insulated platinum wire, in a constant predetermined array, were then inserted simultaneously with a stereotactic micromanipulator through the leptomeninges 3 mm to 4 mm deep into both superior frontal gyri, 1 cm to either side of the midline (fig. 1). Subsequent necropsy of each animal

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revealed that the electrodes were usually recording from tissue to either side of the junction between grey and white matter (fig. 2). Four electrodes were inserted into the left gyrus and a single one was inserted into the right gyrus. The active portion of each platinum wire electrode was 2 mm long, 0.3 mm in diameter, and sharpened to a point. Focal tissue blood flow was determined with these electrodes. Simultaneously, total cerebral blood flow (CBF) was determined with a polarographical electrode in the torcular Herophili. After inserting the electrodes, we covered the exposed leptomeninges and brain with saline-soaked cotton patties. Electrodes were allowed to stabilize for at least 1 hour before use.

Focal (tissue) and total (torcular) CBF was then determined at ½- or 1-hour intervals by measuring the clearance of hydrogen from tissue and torcular blood. CBF determinations were begun by adding hydrogen (5 to 10 Vol %) to the inspired gas mixture for 10 minutes, after which it was stopped abruptly. The first 40 seconds of the clearance curve was discarded and the remainder analyzed as we have previously described in detail. Oxygen consumption (CMRO₂) was computed from the total CBF and the cerebral arterio-venous oxygen difference (A-VO₂).

The animals were divided into control and experimental groups. The control group was subjected to repeated CBF and A-VO₂ determinations during the ensuing 5 hours. After baseline CBF and A-VO₂ values were established, the experimental group was subjected to focal cryogenic cerebral trauma as follows. The left superior frontal gyrus was uncovered. With the electrodes remaining undisturbed, we created a cryogenic lesion by placing the flat end of a slotted brass cylinder, previously cooled in liquid nitrogen, in contact with the surface of the brain surrounding electrode §2 and allowing it to remain on the brain for 2 min (fig. 1). The cylinder was 8.8 mm in diameter, 8 mm in height, and weighed 4.6 gm; its base had a surface area of 53 mm². After the lesion was made, the exposed brain was again covered with saline-soaked cotton patties. When the electrodes had restabilized, we resumed measurement of CBF and A-VO₂ for the next 4 hours.

Focal (tissue) CBF data suitable for analysis were obtained from an untraumatized control group of 17 animals and a cryogenically traumatized experimental group of 14 animals. Total CBF was determined in 14

**FIGURE 1.** Electrode montage and slotted brass cylinder used to make the cryogenic lesion. After immersion in liquid nitrogen, the cylinder is applied as illustrated to the area surrounding electrode §2. Electrode §3 is 5 mm from §2; §1 is 7 mm from §2; §4 is 10 mm from §2; and §5 is in the same relative position as §2 in the contralateral gyrus.

**FIGURE 2.** A typical cryogenic lesion approximately 4 hours old. (Top) Circumscribed hemorrhagic lesion. (Middle) Position of electrodes relative to the lesion. From left to right, electrode §1 through §4. Although electrode §2 was at the center of each application, the lesion's center was consistently 2 or 3 mm farther posterior. (Bottom) Parasagittal section through lesion with electrodes in place. Not visible on this black-and-white photograph is the blue discoloration of the subcortical white matter caused by the Evan's blue edema marker dye.
control and 13 experimental animals. CMRO$_2$ was determined in 6 control and 6 experimental animals.

After the last measurement, each animal was killed with a rapid intravenous injection of a saturated solution of MgSO$_4$. The calvarium was removed and the brain examined in situ with the electrodes in place. Data from electrodes surrounded by hematomas were discarded. Color photographs were made for later correlation of flow data with lesion size and extent of edema.

Data Analysis

Previous experience with acutely implanted polarographic electrodes of the type employed in this experiment revealed that local CBF, on the average, gradually decreased spontaneously during the last 5 hours of a 7-hour experiment to about 75% of the initial value. To correct for this in the present investigation, we compared changes in local CBF after trauma to changes in local CBF in untraumatized controls at the same period in the experiment (unpaired t-test). Changes in total CBF and CMRO$_2$ produced by trauma were analyzed by using animals as their own controls (paired t-test).

Results

Experiments usually required approximately 7 hours to complete. During each individual experiment, mean arterial blood pressure, rectal temperature, blood gases, and intracranial pressure remained stable and normal. For all animals, mean arterial blood pressure ranged between 80 and 125 mm Hg, rectal temperature between 37° and 38°C, PCO$_2$ between 29 and 35 mm Hg, pH between 7.37 and 7.49, PO$_2$ between 130 and 160 mm Hg, and intracranial pressure between 2 and 8 mm Hg. There were no statistically significant differences in these variables between control and experimental groups (unpaired t-test).

Mean baseline values (± standard deviation) for all experiments were as follows: local CBF in tissue to either side of the junction between grey and white matter 34 ± 15 ml/100gm/min; total CBF 63 ± 12 ml/100gm/min; total CMRO$_2$ 4.6 ± 1 ml/100gm/min.

Application of the cold brass cylinder to the brain produced a circumscribed hemorrhagic lesion approximately 12 mm in diameter in the sagittal plane and 9 mm in the coronal plane (fig. 2). Although electrode #2 was at the center of the area to which the cylinder was applied, the center of the lesion was invariably 2 or 3 mm farther posterior. The appearance of the lesion varied little from animal to animal. The hemorrhagic zone was always confined to the most superficial 4 or 5 mm of cerebral mantle. The deeper layers of cortex and subjacent white matter were edematous and discolored varying shades of blue. At times, the hemorrhagic zone extended deep enough to involve the tip of electrodes #2 and #3. The brain surrounding electrode #2 and #3 was always discolored by the Evan's blue marker dye. There was a rough correlation between the degree to which blood flow fell at a particular electrode site and the amount of edema fluid in surrounding brain, as judged by the intensity of blue discoloration.

During the first half hour after trauma, local CBF in areas closest to the lesion (i.e., areas monitored by electrode #2 and #3) increased significantly. Thereafter, blood flow fell in those areas as well as in the area of electrode #4. Local CBF changes anterior (electrode #1) and contralateral (electrode #5) to the lesion did not differ significantly from those which occurred spontaneously in the control group. The graph depicted in fig. 3 summarizes the effect of cryogenic trauma on local CBF as a function of time.

Total CBF and CMRO$_2$ did not change significantly from baseline values in either traumatized or control group (p > 0.05, paired t-test).

Discussion

A brief period of hyperemia was observed in the rim of tissue closest to the lesion. Within an hour, it was replaced by progressive ischemia. These results are in agreement with the results of earlier investigations into the effect of cryogenic trauma on CBF. The mechanism by which blood flow falls around injured brain cannot be discerned from this investigation. The role, if any, of vasospasm produced by biogenic amines released from injured tissue, increasing tissue pressure, or traumatic edema remains to be defined.

Cryogenic trauma produces a lesion that is reproducible from one animal to the next, while the hydrogen clearance technique permits one to measure CBF repeatedly in small volumes of tissue with a degree of resolution not readily obtainable with other methods. However, this model may be criticized on the grounds that cryogenic trauma lacks clinical relevance. It is by no means certain that the physiologic state of uninjured tissue surrounding a lesion is the same irrespective of whether the brain had been contused and lacerated, infarcted, or injured cryogenically. Indeed, the unusually great edemagenic potential of cryogenic injury has already been mentioned and is evidence to the contrary. Another negative attribute of this model is that with the hydrogen clearance technique, one may observe large differences among baseline local CBF values with electrodes ostensibly recording from the same tissue. This has been commented upon also by other investigators and is presumably related to the fine resolution of the hydrogen clearance technique reflecting actual fluctuations in local CBF in response to changing regional metabolic rates. Techniques that sample larger volumes would tend to average out these fluctuations. We circumvented these difficulties by normalizing values and by comparing changes in local CBF in control and experimental groups. A third difficulty with this model is the tendency for local CBF recorded from acutely implanted electrodes of the type employed in this investigation to fall an average
of 25% in control animals over a 5-hour period. The reason for the fall is not readily apparent. We suspect that it is probably related to the development of focal edema around the electrode caused by the trauma of insertion. We have recently observed that when we employ either acutely implanted electrodes of smaller diameter (0.18 mm) or electrodes implanted 1 week or more before use, local CBF does not fall (unpublished observations).

Cryogenic injury to the left superior frontal gyrus affected neither blood flow in the right superior frontal gyrus nor total CBF and CMRO₂. Frei et al. 11 likewise noted that CBF did not fall in the uninjured hemisphere of cats 24 hours after making a cryogenic lesion in the contralateral hemisphere. On the contrary, others have found that infarction in 1 hemisphere may be accompanied by a substantial fall in CBF in the contralateral hemisphere, 18-22 a phenomenon termed "diaschisis." And Bruce et al. 23 found that CBF fell in the contralateral hemisphere within 4 hours of making a large cryogenic lesion in the occipital lobe of rhesus monkeys. The discrepancy between our results and those of Bruce et al. 23 may be related to the larger lesion they produced, to the fact that the calvarium was intact in their animals or to both. Tissue pressure increase contralateral to the lesion from brain herniation could account for the fall in CBF they observed.

The area of cryogenically induced hemorrhagic necrosis spread eccentrically more posteriorly than anteriorly (fig. 2). Although electrode #2 was at the center of each application, the lesion's center was consistently 2 or 3 mm farther posterior. The anterior extent of the lesion, which was usually about 4.5 mm anterior to electrode #2, coincided closely to the surface that was actually in contact with the cylinder. The posterior extent, however, extended more than 9 mm from electrode #2 and was more than 4 or 5 mm beyond the area of brain that had been in contact with cold metal. The eccentric spread of the area of necrosis posteriorly is most likely related to the fact that the principal source of blood to the superior frontal gyrus is from anterior via the anterior cerebral arteries. These observations correlate well with flow data, which show that CBF was unaffected anterior to the lesion, but fell posterior to it.

We have concluded that the spatial and temporal profile of local CBF changes around an acute lesion of the brain can be readily and reliably studied with this model. It may, therefore, be of some value in assessing the efficacy of therapies aimed at reducing the volume of brain lost from injury.

References

1. Meyer JS, Welch KMA, Okamoto S et al: Disordered neurotransmitter function. Demonstration by measurement of norepinephrine and 5-hydroxytryptamine in CSF of patients
with recent cerebral infarction. Brain 97: 655-664, 1974
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