Blood flow and oxygen consumption of the focally traumatized monkey brain

ALBERT N. MARTINS, COL., MC, USA, AND THOMAS F. DOYLE, B.SC.
Neurosurgery Service, Walter Reed Army Medical Center, Washington, D.C., and Armed Forces Radiobiology Research Institute, Defense Nuclear Agency, Bethesda, Maryland

The exposed left superior frontal gyrus of the anesthetized macaque brain was focally traumatized by a jet of compressed air. Focal blood flow in tissue around the lesion and total cerebral blood flow was determined before and during the 4 hours after trauma by the hydrogen clearance technique. Blood flow fell in tissue adjacent to the injured brain but the reduction was not statistically significant. Total cerebral blood flow, blood flow in the right superior-frontal gyrus, and oxygen consumption of the brain was unaffected by the trauma. The authors conclude that neither spreading ischemia within uninjured tissue surrounding focally traumatized brain nor post-traumatic diaschisis is readily provoked in the anesthetized brain of the monkey.

Key Words - brain injury, acute - cerebral blood flow - cerebral metabolism - cerebral ischemia - hydrogen clearance

Focal cerebral injury may depress cerebral blood flow (CBF) and metabolism globally or at sites contralateral to the area of injury. This type of cerebral shock has been termed "diaschisis" and is thought to be mediated transneurally. The area involved by diaschisis presumably loses facilitation formerly provided by neurons and their connections destroyed by the lesion. Reduced metabolism attends the decreased neuronal activity and blood flow falls as a result. More recently, it has been suggested that diaschisis may result from a generalized release of norepinephrine from presynaptic terminals. Moreover, it has been postulated that an acute lesion of the cerebrum may also be surrounded by a zone of ischemia induced by vasoactive biogenic amines released from within the injured tissue. Whatever mechanism is involved, it is important to know clinically, from both a diagnostic and therapeutic standpoint, whether acute lesions of the brain are to some degree self-propagating.

We started this investigation primarily to document whether ischemia develops in the uninjured tissue surrounding focally traumatized brain. We also wished to learn if focal brain injury is accompanied by a global reduction in CBF and cerebral metabolism, as reflected by oxygen consumption.

Materials and Methods
Animal Preparation
Young adult macaques (Macaca mulatta and Macaca fascicularis) of both sexes, weighing between 3 and 5 kg, were initially anesthetized with 50 mg of ketamine and 0.2 mg atropine sulfate, both given intramuscularly. After being intubated with a cuffed endotracheal tube, they were placed on a volume respirator and paralyzed with an intravenous injection of 0.3 mg pancuronium.
A gas mixture of 70% N\textsubscript{2}O and 30% O\textsubscript{2}, supplemented with a continuous intravenous infusion of ketamine 3 mg/kg/hr, maintained anesthesia for the remainder of the experiment. Through a femoral vein cutdown, lactated Ringer's solution, to which was added 0.8 mg pancuronium/100 ml, was infused continuously at 16 ml/hr in order to maintain fluid and electrolyte balance and muscular paralysis; 100 mg of Evans' blue was given intravenously at the beginning of each experiment as a marker for edema fluid. Moderate hyperventilation was provided by a respiratory rate of 40 to 44/min and a tidal volume of 55 cc. Enough CO\textsubscript{2} was added to the inspired gas mixture to keep the CO\textsubscript{2} pressure (pCO\textsubscript{2}) 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.

The animal was then placed prone with its head held rigidly in a standard stereotaxic device. A rectal thermometer monitored body temperature, which was kept between 37° and 39° C by an externally applied heated pad. A No. 19 needle attached to a polyethylene tube was passed percutaneously into the cisterna magna from which we measured intracranial pressure continuously. Subsequently, using standard microsurgical techniques to avoid trauma to the brain, we performed a 3 × 3 cm craniectomy centered at the bregma, and opened the dura overlying the superior frontal gyri to either side of the midline. 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 O\textsubscript{2} content of the blood draining the brain. Oxygen content of arterial and sagittal sinus blood was measured before each blood flow determination with a Lex-O\textsubscript{2}-Con instrument.* Subsequently, five polarographic electrodes of platinum wire in a constant predetermined array were inserted simultaneously with a stereotaxic micromanipulator through the leptomeninges 3 to 5 mm deep into both superior frontal gyri, 1 cm to either side of the midline. Four of the electrodes were inserted into the left gyrus and a single electrode was inserted into the right gyrus (Fig. 1).

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 measured from these electrodes. Simultaneously, total CBF was measured with a polarographic electrode in the torcular herophili. After electrode insertion, the exposed leptomeninges and brain were covered 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 30-minute and 1-hour intervals by measuring the clearance of hydrogen from tissue and from torcular blood. The 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

*Lex-O\textsubscript{2}-Con manufactured by Lexington Instrument Corp., Waltham, Massachusetts.
TABLE 1

Baseline local cerebral blood flow (ml/100 gm/min)*

<table>
<thead>
<tr>
<th>Electrode No.</th>
<th>Control Group</th>
<th>Experimental Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>1</td>
<td>52 ± 9</td>
<td>52 ± 13</td>
</tr>
<tr>
<td>2</td>
<td>41 ± 13</td>
<td>42 ± 8</td>
</tr>
<tr>
<td>3</td>
<td>34 ± 8</td>
<td>45 ± 9</td>
</tr>
<tr>
<td>4</td>
<td>59 ± 6</td>
<td>58 ± 13</td>
</tr>
<tr>
<td>5</td>
<td>52 ± 12</td>
<td>45 ± 11</td>
</tr>
</tbody>
</table>

*Measurement made in eight control and 12 experimental animals. The differences are not statistically significant (unpaired t-test).

Remainder analyzed as we have previously described in detail. Oxygen consumption (CMRO₂) was computed from the total CBF and the cerebral arteriovenous oxygen (AVO₂) difference.

Experimental Protocol

The animals were divided into a control group and an experimental group. Repeated CBF determinations were performed in the control group during the ensuing 5 hours. After baseline CBF and AVO₂ difference values were established, the experimental group was subjected to cerebral trauma. The brain was uncovered. With all electrodes remaining undisturbed, the tissue surrounding electrode No. 2 was traumatized by the blast of a single jet of compressed air delivered by a spring-driven air pistol. Variables that determined the force with which the jet of air impacted upon the brain were kept as constant as possible. After the trauma, the exposed brain was covered with cotton patties. When the electrodes stabilized again, measurement of CBF and cerebral AVO₂ difference was resumed for the next 4 hours.

After the last measurement, each animal was killed with a rapid intravenous injection of a saturated solution of MgSO₄. The calvaria was removed and the brain examined in situ with the electrodes in place. Color photographs were made for subsequent correlation of lesion size with flow data. If an electrode other than electrode No. 2 was within hematoma, its data was discarded.

Results

Each experiment usually required approximately 7 hours to complete. During this time vital signs, intracranial pressure (ICP), and blood gases of all animals of which the data were used for analysis remained normal and stable. For the two groups, mean arterial blood pressure ranged between 98 and 105 mm Hg, mean pCO₂ between 32 and 35 mm Hg, mean pH between 7.38 and 7.43, mean pO₂ between 135 and 150 mm Hg, mean ICP between 3 and 6 mm Hg, and mean rectal temperature between 38° and 39° C. There were no statistically significant differences in these variables between the control and the experimental group (unpaired t-test). Baseline flows and CMRO₂ were the same for both groups (Tables 1 and 2).

TABLE 2

Total cerebral blood flow, arteriovenous oxygen difference, and oxygen consumption in control and experimental animals*

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Baseline</th>
<th>0.5</th>
<th>1</th>
<th>1.5</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>total CBF (ml/100 gm/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control (8)</td>
<td>84 ± 8</td>
<td>81 ± 8</td>
<td>83 ± 7</td>
<td>73 ± 8</td>
<td>81 ± 9</td>
<td>71 ± 6</td>
<td>67 ± 6</td>
</tr>
<tr>
<td>experimental (12)</td>
<td>80 ± 8</td>
<td>82 ± 8</td>
<td>83 ± 7</td>
<td>83 ± 8</td>
<td>81 ± 11</td>
<td>68 ± 8</td>
<td>82 ± 14</td>
</tr>
<tr>
<td>AVO₂ difference (vol.%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control (7)</td>
<td>5.3 ± 0.1</td>
<td>5.3 ± 0.9</td>
<td>6.0 ± 0.7</td>
<td>6.1 ± 0.7</td>
<td>6.6 ± 0.8</td>
<td>6.4 ± 0.6</td>
<td>6.1 ± 0.2</td>
</tr>
<tr>
<td>experimental (8)</td>
<td>5.6 ± 0.5</td>
<td>6.0 ± 0.7</td>
<td>6.0 ± 0.6</td>
<td>6.1 ± 0.7</td>
<td>5.7 ± 0.1</td>
<td>6.7 ± 1.1</td>
<td>7.1 ± 1.3</td>
</tr>
<tr>
<td>CMRO₂ (ml/100 gm/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control (7)</td>
<td>4.6 ± 0.7</td>
<td>4.0 ± 0.5</td>
<td>4.5 ± 0.5</td>
<td>3.9 ± 0.3</td>
<td>4.1 ± 0.2</td>
<td>4.3 ± 0.4</td>
<td>3.9 ± 0.2</td>
</tr>
<tr>
<td>experimental (8)</td>
<td>4.0 ± 0.3</td>
<td>4.6 ± 0.4</td>
<td>4.7 ± 0.4</td>
<td>4.6 ± 0.4</td>
<td>4.4 ± 0.5</td>
<td>4.1 ± 0.5</td>
<td>4.2 ± 0.7</td>
</tr>
</tbody>
</table>

*Mean ± standard error; number of experiments is given in parentheses. Differences not statistically significant (p > 0.1, unpaired t-test).

†Traumatized at zero hours.
CBF in experimental focal trauma

The jet of air lacerated and contused the cortex and underlying white matter surrounding electrode No. 2 (Fig. 2). The volume of the lesion was usually between 1 and 2 cc. Large differences in the size of the lesion from animal to animal were common and mainly due to the variability in the size of the intracerebral hematoma produced by the jet of air. The subcortical white matter was often stained with Evans' blue, but the extent and intensity of color were variable.

After trauma, blood flow recorded by electrode No. 2 decreased significantly. Blood flow also fell in electrodes adjacent to the trauma (Nos. 1 and 3), but the magnitude did not reach statistical significance. At sites farther away, and contralateral to the site of trauma, blood-flow changes in the control group and the experimental group after trauma did not differ significantly. Likewise, there were no significant differences in total CBF and CMRO₂ between the control group and the experimental group after trauma (Fig. 3, Table 2).

The CBF values recorded from tissue electrodes in the control group tended to fall with time so that on the average, flows at the end of the experiment were about 25% less than flows recorded at the start. Total CBF and CMRO₂ in the controls also tended to fall, but to a lesser degree.

Discussion

These experiments have failed to prove that ischemia develops acutely in the uninjured tissue surrounding focally traumatized brain. There was a pronounced tendency for blood flow to fall close to the lesion, but it did not reach statistical significance. However, these negative results must be interpreted with caution because of the potential sources of error inherent in the design of this experimental model. One obvious defect is that the brain was anesthetized. Because general anesthetics alter brain function, cerebrovascular and neural responses of the anesthetized brain to trauma may be different from those that take place in the awake brain. We sought to minimize the effect of anesthesia by using nitrous oxide supplemented with ketamine, which seems to render the brain more vulnerable to an insult. A second potential source of error is that only acute effects of trauma were studied. Any change occurring after 4 hours was missed. Third, the amount of tissue destroyed by trauma was small, often involving less than 3% of the mass of the monkey brain. Perhaps a larger traumatic lesion would have had global or distant effects not provoked by a small hemispheric lesion. Fourth, the absolute CBF values recorded at the same electrode site in different animals varied widely, which is characteristic of the hydrogen clearance technique. Consequently, recognition of statistically significant differences in absolute CBF requires a large number of experiments. We minimized this deficiency by comparing changes in CBF between the controls and the experimental group after trauma. Finally, conclusions can only apply to trauma to the cerebral hemispheres, which contain relatively small concentrations of vasoactive amines compared with other regions such as the diencephalic gray matter and the basal ganglia.

Assuming that the fall in flow adjacent to the lesion represents a reproducible phenomenon, one cannot conclude from these data that the reduction in flow is mediated by vasospasm produced by biogenic amines released from damaged tissue. One may argue that the reduction in flow was due to traumatic edema, or increasing tissue pressure, or both.

Trauma to the left superior frontal gyrus did not affect blood flow in the right superior frontal gyrus, nor did it affect total CBF or CMRO₂. In contrast, others have found that infarction in one hemisphere may be accom-
FIG. 3. Graph summarizing the effect of trauma on local CBF as percentage change from initial baseline value (see Table I), in 12 traumatized monkeys (broken line), and eight controls (continuous line). Trauma at arrow. Values are mean ± standard deviation. Asterisk (*) = statistically significant difference, p < 0.0001 (unpaired t-test).

panied by a substantial fall in blood flow in the other hemisphere. Our failure to detect diaschisis may reflect a fundamental difference in the way the brain reacts to traumatic and ischemic injury. On the other hand, diaschisis may occur with trauma, but was not detected because of the potential sources of error previously noted in this experimental model.

The results of this investigation differ from those of a previously reported investigation from this laboratory in which blood flow was measured in and around a traumatic lesion of the spinal cord. Blood flow temporarily increased significantly in the white matter surrounding the cord lesion. Why blood flow tends to fall around a traumatic lesion of the cerebral hemisphere but increases around a similar lesion of the cord is unclear. It may reflect a difference in endogenous biogenic amines liberated by the trauma. Or it may imply that vasomotor tone of the microvascular bed is greater in the cord than the cerebrum, in which case hyperemia would be
CBF in experimental focal trauma

more likely in the cord during the period of posttraumatic vasoparalysis. Additional studies are required to clarify this point.

The jet of air produced a lesion of variable size, depending on the number and caliber of vessels torn by the blast. Consequently, this method of inflicting trauma to the brain cannot be used when the size of the lesion must be kept constant. Others have used cryogenic trauma to study the effect of brain injury on CBF and metabolism.1,3 Although cryogenic trauma obviously lacks the clinical relevance of the laceration-contusion type of injury produced by a jet of air, its magnitude is easier to control and reproduce. Nevertheless, our results agree with those reported for cryogenic trauma. In focally frozen cat brain, CBF fell adjacent to the lesion, CBF being a reciprocal function of the increased water content (edema) accompanying the lesion. Diaschisis was not observed in the cryogenically traumatized brain.a

The hydrogen clearance technique permitted us to measure blood flow repeatedly in small volumes of tissue before and after trauma. We were able to resolve differences in blood flow of adjacent tissue to a degree unattainable by most other clearance techniques. But there were difficulties with the technique. For unexplained reasons, mean blood flow recorded by electrodes in the subcortical white matter of control monkeys fell unexpectedly as the experiment progressed. Flows at the beginning of the experiment averaged 25% higher than at the end. Whether this is an artifact of our model, or a real fall in blood flow to the tissue surrounding these acutely implanted electrodes is unknown. Additional experiments are under way to study this phenomenon. In any case, these spontaneous changes in blood flow of the control group complicated data analysis. In order to determine whether or not trauma changed blood flow significantly, we found it necessary to compare changes in blood flow after trauma to changes in blood flow recorded in the control group during the same epoch in the experiment.

Summary

Global CBF and oxygen consumption of the anesthetized monkey brain was unaffected by a focal lacerating injury to the superior frontal gyrus produced by a jet of air. Focal blood flow in the contralateral superior frontal gyrus likewise was unaffected by the trauma. Blood flow fell in tissue adjacent to the injured brain, but the reduction did not reach statistical significance. The relative importance of biogenic amines, edema, and increasing tissue pressure in determining blood flow around the injured brain remains to be determined.

We conclude that posttraumatic cerebral diaschisis and spreading ischemia within uninjured tissue surrounding focally traumatized brain is not readily provoked in the anesthetized brain of the monkey.

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References


Address reprint requests to: Albert N. Martins, COL, MC, USA, Neurosurgery Service, Walter Reed Army Medical Center, Washington, D.C. 20012.