Experimental Model for Systematic Study of Impaired Microvascular Reperfusion

JOHN M. HALLENBECK, M.D., AND MARK E. BRADLEY, M.D.

SUMMARY A technique is described for reliably producing quantifiable impairment of microvascular reperfusion of the brain after ischemia in dogs. The technique is derived from an analysis of the cerebrospinal fluid compression ischemia model as a Starling resistor. It is proposed that this model would be useful in a systematic study of post-ischemic impairment of reperfusion.

IN DEATHS due to dybaric cerebral air embolism observed by the authors, a course suggesting progressive impairment of microcirculatory reperfusion has repeatedly occurred. Recompression to a simulated 165 feet in a chamber has usually been delayed for at least 5 minutes in these cases but, when instituted, should have eliminated the mechanical obstruction of cerebral vessels by bubbles. During the 15 to 30 minute interval at 165 feet called for by standard Navy treatment tables, these patients characterizedly regained consciousness and demonstrated varying degrees of recovery. However, beginning about 30 minutes to 2 hours after the initial ictus, the clinical state progressively deteriorated, often with the patient still under pressure and demonstrating varying degrees of consciousness and demonstrated varying degrees of obstruction of cerebral vessels by bubbles.

It follows that an experimental model permitting study of the factors that influence the adequacy of post-ischemic microcirculatory reperfusion would have potential therapeutic relevance. Models of cerebral ischemia in animals abound. Most of these models involve preliminary surgery which ranges from minor to mutilating. The consequent tissue damage introduces the probability of alteration in the organism which could theoretically affect the experimental outcome, a circumstance for which the term “physiologic reactance” has been used. Indeed, Bergstrom has demonstrated circulating platelet aggregates in the hamster cheek-pouch minutes after crush injury to the thigh and has classified into 4 phases the serial response of blood to tissue injury. The latency for all of these changes was much shorter in the vicinity of the tissue damage.

Most of the surgical trauma can be avoided in the cerebrospinal fluid compression ischemia model as a Starling resistor. It is proposed that this model would be useful in a systematic study of post-ischemic impairment of reperfusion.

References


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The experiments reported were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council, DHEW Pub. No. (NIH) 74-23.
licated intravascular blood as a factor that somehow adversely influences post-ischemic reperfusion.6, 18 By subjecting the CSF compression model of ischemia to a Starling-resistor analysis, it was possible to find a relationship between systemic arterial pressure (SAP) and CSFP that would cause global neuraxis ischemia with a cyclic ebb and flow of blood into and out of the cerebral vessels coincident with fluctuations of SAP.

Methods

Five splenectomized, conditioned male mongrel dogs weighing 7–12 kg were anesthetized with intravenous pentobarbital sodium 25–30 mg/kg. A schematic diagram of the preparation is shown in figure 1. The animals were intubated and connected to a Bird respirator. End-tidal CO₂ concentration was continuously monitored by a Beckman LB-2 CO₂ analyzer to assist maintenance of the Pco₂ in the normal range. Core temperature was monitored by means of a tympanic membrane thermistor connected to a YSI telethermometer (Yellow Springs Instrument Company) and the temperature was maintained at 38°C with an infrared light and heating pads. Two catheters were placed in the right femoral artery. One was directed proximally into the aorta and the other was directed into the distal femoral artery. When these two catheters were later joined through a Y-connector, the arterial flow to the right hindleg was externalized permitting rapid sampling of arterial blood, a requirement operation in the ¹⁴C-antipyrine autoradiographic blood flow assay.¹¹ A right ventricular catheter was placed via the right femoral vein. A 1000 ml bottle of Elliott’s solution B was lowered until its line pressure equaled 10 mm Hg and the systolic SAP was kept between 70 and 90 mm Hg range. Subsequently, the mean arterial pressure was maintained between 70 and 90 mm Hg by reinfusing the withdrawn blood and infusing saline or levarterenol bitartrate (Levophed, Winthrop) 1 mg/250 cc of % dextrose in water as necessary. After 35 minutes of CSF compression ischemia the bottle of Elliott’s solution B was lowered so that its line pressure equaled 10 mm Hg and the systolic SAP was kept between 110 and 120 mm Hg for 30 minutes. This represented the post-ischemic recirculation period. At the conclusion of this recirculation period, a ¹⁴C-antipyrine autoradiographic blood flow measurement was performed. Essentially, this required intravenous infusion of 100 µc/kg of ¹⁴C-antipyrine for one minute with serial sampling of arterial blood repeated every 4 to 6 seconds. The cardiac arrest which terminated this procedure was produced by injecting a bolus of 20 cc of saturated potassium chloride through the right femoral vein catheter into the right ventricle. The brain, spinal cord, and heart were then removed, frozen in liquid freon suspended over liquid nitrogen and cut into 20 micron sections. The tissue concentration of the isotope was determined autoradiographically. Local blood flow was calculated from the following formula: 

\[ C_{(T)} = \lambda k_i C_T e^{-\lambda T} \Delta t \]

where \( C_{(T)} \) = the concentration of tracer substance in the tissue at time \( T \); \( \lambda \) = the tissue-blood partition coefficient for the tracer material; \( k_i \) = the rate of blood flow per unit weight of tissue multiplied by the reciprocal of the partition coefficient for that tissue; and \( C_T \) = the concentration of tracer substance in the arterial blood.

Blood gases and blood pH were frequently monitored and blood was drawn for hematocrit and platelets initially as a baseline control (C), prior to ischemia (PI), prior to the post-ischemic recirculation period (PR), and just prior to the blood flow study (PF).

Two splenectomized male mongrel dogs were subjected to the same procedure except that after 12 and 15 minutes of CSF compression ischemia respectively, autoradiographic blood flow procedures were performed to measure the extent of blood flow interruption with the CSFP maintained equal to MSAP during the flow study.

Results

The two animals studied during CSF compression ischemia to determine the extent of blood flow interruption...
TABLE 1 Several Variables From the Group of 5 Animals Sampled During the Control Period (C), Prior to Ischemia (PI), Prior to Recirculation (PR), and Prior to the Blood Flow Study (PF) (mean ± SEM).

<table>
<thead>
<tr>
<th>Hematocrit</th>
<th>pH</th>
<th>Systolic BP - mm Hg</th>
<th>Diastolic BP - mm Hg</th>
<th>Heart Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>38 ± 3</td>
<td>93.2 ± 2.3</td>
<td>31.5 ± 1.4</td>
<td>33.3 ± 2.6</td>
</tr>
<tr>
<td>PI</td>
<td>39 ± 3*</td>
<td>86.7 ± 3.6*</td>
<td>35.5 ± 3.2</td>
<td>34.5 ± 2.3</td>
</tr>
<tr>
<td>PR</td>
<td>37 ± 2</td>
<td>55.7 ± 3.2</td>
<td>32.5 ± 1.6*</td>
<td>34.5 ± 2.3</td>
</tr>
<tr>
<td>PF</td>
<td>36 ± 6</td>
<td>55.7 ± 6</td>
<td>32.5 ± 1.6*</td>
<td>34.5 ± 2.3</td>
</tr>
</tbody>
</table>

Platelets/mm³

<table>
<thead>
<tr>
<th>Hematocrit</th>
<th>pH</th>
<th>Systolic BP - mm Hg</th>
<th>Diastolic BP - mm Hg</th>
<th>Heart Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>454,800 ∼ 109,450</td>
<td>454,000 ± 150,260</td>
<td>357,800 ± 87,240</td>
<td>366,920 ± 78,170</td>
</tr>
<tr>
<td>PI</td>
<td>164 ± 11</td>
<td>153 ± 10</td>
<td>115 ± 6</td>
<td>114 ± 2</td>
</tr>
<tr>
<td>PR</td>
<td>101 ± 20</td>
<td>72 ± 6</td>
<td>69 ± 4</td>
<td>67 ± 2</td>
</tr>
<tr>
<td>PF</td>
<td>111 ± 15</td>
<td>73 ± 8</td>
<td>67 ± 4</td>
<td>65 ± 2</td>
</tr>
</tbody>
</table>

PO₂ - mm Hg

<table>
<thead>
<tr>
<th>Hematocrit</th>
<th>pH</th>
<th>Systolic BP - mm Hg</th>
<th>Diastolic BP - mm Hg</th>
<th>Heart Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>315 ± 1.4</td>
<td>93.5 ± 11</td>
<td>53 ± 2</td>
<td>33 ± 14</td>
</tr>
<tr>
<td>PI</td>
<td>101 ± 15</td>
<td>55 ± 8</td>
<td>73 ± 2</td>
<td>67 ± 3</td>
</tr>
<tr>
<td>PR</td>
<td>136 ± 8</td>
<td>69 ± 4</td>
<td>67 ± 3</td>
<td>65 ± 2</td>
</tr>
<tr>
<td>PF</td>
<td>150 ± 14</td>
<td>67 ± 3</td>
<td>65 ± 2</td>
<td>65 ± 2</td>
</tr>
</tbody>
</table>

PCO₂ - mm Hg

Both had absent flow in the parenchyma of the neuraxis. Therefore, setting CSFP equal to MSAP and, by definition, reducing the cerebral perfusion pressure (CPP) to zero produced a state of global central nervous system (CNS) ischemia rather than oligemia in these animals.

TABLE 2 Mean Local Blood Flows in Various Neuroanatomic Areas and Heart in ml/100gm/min From the Group of 5 Animals

<table>
<thead>
<tr>
<th>Area</th>
<th>Flow (ml/100gm/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anterior Centrum Ovale</td>
<td>13 ± 4*</td>
</tr>
<tr>
<td>Auditory Cortex</td>
<td>46 ± 11</td>
</tr>
<tr>
<td>Caudate Nucleus</td>
<td>51 ± 16</td>
</tr>
<tr>
<td>Corpus Callosum</td>
<td>16 ± 6</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>30 ± 9</td>
</tr>
<tr>
<td>Internal Capsule</td>
<td>19 ± 6</td>
</tr>
<tr>
<td>Middle Centrum Ovale</td>
<td>12 ± 4</td>
</tr>
<tr>
<td>Optic Radiations</td>
<td>13 ± 4</td>
</tr>
<tr>
<td>Sensorimotor Cortex</td>
<td>36 ± 2</td>
</tr>
<tr>
<td>Thalamus</td>
<td>44 ± 12</td>
</tr>
<tr>
<td>Visual Cortex</td>
<td>40 ± 10</td>
</tr>
<tr>
<td>Posterior - Middle Cerebral Watershed Cortex</td>
<td>15 ± 7</td>
</tr>
<tr>
<td>Anterior - Middle Cerebral Watershed Cortex</td>
<td>31 ± 6</td>
</tr>
<tr>
<td>Anterior Association Cortex</td>
<td>38 ± 7</td>
</tr>
<tr>
<td>Spinal Cord Gray</td>
<td>48 ± 17</td>
</tr>
<tr>
<td>Spinal Cord White</td>
<td>18 ± 2</td>
</tr>
<tr>
<td>Heart - Left Ventricle</td>
<td>281 ± 88</td>
</tr>
</tbody>
</table>

*Mean ± standard error of mean.

The average local blood flows for various neuroanatomic areas and the free wall of the left ventricular myocardium are shown in table 2. The average blood flow for each brain structure was somewhat low relative to flows measured in barbiturate anesthetized animals and no tendency toward reactive hyperemia was induced by the state of temporary ischemia. The major contrast with the uniform depression of blood flow caused by anesthesia alone was the extreme heterogeneity of blood flows in post-ischemic animals with large focal variations often encountered within single neuroanatomic structures. The focal variations are displayed in table 3. Foci of extremely low flow, which are felt to correspond to zones of impaired microvascular perfusion noted in other models, ranged in incidence from infrequent and confined to several structures (figs. 2-4) to ubiquitous (fig. 5). No focal areas of flow heterogeneity were noted in spinal cord gray matter, spinal cord white matter or free wall of the left ventricular myocardium.

TABLE 3 Focally Heterogeneous Flows Within Neuroanatomic Areas Categorized into Low, Intermediate and High Levels From the Group of 5 Animals

<table>
<thead>
<tr>
<th>Area</th>
<th>Flow (ml/100gm/min)</th>
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</thead>
<tbody>
<tr>
<td>Anterior Centrum Ovale</td>
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<td>96 ± 16</td>
</tr>
<tr>
<td>Corpus Callosum</td>
<td>23 ± 8</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>73 ± 4</td>
</tr>
<tr>
<td>Internal Capsule</td>
<td>27 ± 6</td>
</tr>
<tr>
<td>Middle Centrum Ovale</td>
<td>17 ± 5</td>
</tr>
<tr>
<td>Optic Radiations</td>
<td>18 ± 4</td>
</tr>
<tr>
<td>Sensorimotor Cortex</td>
<td>58 ± 6</td>
</tr>
<tr>
<td>Thalamus</td>
<td>59 ± 11</td>
</tr>
<tr>
<td>Visual Cortex</td>
<td>53 ± 3</td>
</tr>
<tr>
<td>Posterior - Middle Cerebral Watershed Cortex</td>
<td>38 ± 18</td>
</tr>
<tr>
<td>Anterior - Middle Cerebral Watershed Cortex</td>
<td>40 ± 2</td>
</tr>
<tr>
<td>Anterior Association Cortex</td>
<td>60 ± 8</td>
</tr>
</tbody>
</table>

*Mean ± standard error of mean.

FIGURE 2. An autoradiogram showing low blood flow in the dorsolateral cortex and a region of impaired perfusion in the caudate nucleus. The animal received 35 minutes of CNS ischemia followed by 30 minutes of recirculation.
Discussion

The results indicate that this technique for producing global CNS ischemia does produce focal areas of extremely low blood flow after an ischemic interval of 35 minutes followed by 30 minutes of general recirculation. We have noted that dogs anesthetized with pentobarbital sodium without exposure to transient CNS ischemia have uniformly reduced brain blood flows relative to awake, unrestrained animals\textsuperscript{13} that contrast with the present series by the absence of focal areas of extremely low flow. However, such dogs constitute an inappropriate comparison group for the present series since their brain blood flows do not reflect the potentially large influence of prior ischemia. A more appropriate comparison would be made between two groups exposed to CSF compression ischemia as outlined but differing with regard to some operation designed to prevent impaired microvascular reperfusion. One such comparison, in which one group was subjected to glass-wool filtration of each animal's blood and the other group was handled in an otherwise identical manner, revealed greatly increased post-ischemic brain flows in the filtered group.\textsuperscript{18}
that antipyrine is not an ideal tracer for blood flow measure-
ment because its uptake in brain is diffusion limited par-
icularly at high flow rates, leading to systematic underesti-
mates of local CBF. However, the method is more accurate at 
low flows such as those in question and does permit 
assessment of patterns of heterogeneous flow change. Until 
a better tracer is developed, the ability of the 14C-antipyrine 
autoradiographic technique to detect a pattern of flow 
change characterized by very low flows interspersed with 
normal or high flows warrants its use in this type of study.

There is good evidence that in global ischemia, definite 
near cerebral damage occurs after 5–15 minutes in the absence 
of impaired microvascular reperfusion. This renders 
unlikely the suggestion that the first irreversible change during 
global cerebral ischemia occurs in the vessels with irrevers-
ible neuronal damage following secondarily. However, the 
possibility remains that a common mechanism is shared by 
impaired microvascular reperfusion occurring after 
relatively long periods of ischemia and progressive deteriora-
tion of microcirculatory flow in regions of acute tissue 
damage. The fundamental fact may be that a blood flow 
of less than some critical value in a zone of acute tissue 
damage tends to shut itself off by some mechanism other 
than clot formation. Such a mechanism could explain the 
observed failure over time of collateral circulation in focal 
cerebral ischemia.

The mechanism underlying the phenomenon of impaired 
microvascular reperfusion should be worked out whether or 
not one believes it has an important role in the process of 
ischemic tissue damage. The ultimate demonstration that 
impaired microvascular perfusion is irrelevant to the genesis 
of neuronal infarction in cerebral ischemia would be to pre-
vent impaired perfusion and document that it fails to in-
fluence the course and outcome of graded ischemic insults. 
If, however, prevention of impaired perfusion should prove 
beneficial, a new approach to therapy could emerge.

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SUMMARY  The effect of respiration on the cerebrovascular response to elevated intracranial pressure (ICP) was studied in anesthetized dogs. Total and regional cerebral blood flows were measured using labelled microspheres. In spontaneously breathing dogs total and regional cerebral blood flows increased when cerebral perfusion pressure was reduced to 20 mm Hg. The increase in cerebral flow in spontaneously breathing dogs was associated with the development of hypoxemia and respiratory acidosis since cerebral vessels retain reactivity to increased Paco3 when the vessels are dilated due to respiratory acidosis. Therefore, the potential exists for the alteration in the cerebrovascular response to elevated ICP in the controlled ventilated dogs with a 5% CO2 in air gas mixture, reversed the decrease in cerebral flows. The results suggest that the increase in cerebral blood flow during elevated ICP in spontaneously breathing dogs is secondary to the development of hypoxemia and respiratory acidosis since cerebral vessels retain responsiveness to increased Paco3 when the vessels are diluted due to elevated ICP. The results also indicate that the total and regional cerebral blood response to elevated ICP is non-uniform.

ELEVATION IN THE intracranial pressure (ICP) in spontaneously breathing animals results in hypoventilation which is induced by the mediatory respiratory centers. The decrease in ventilation is associated with decreases in arterial PO2 and pH, and increase in arterial CO2. The blood gas and pH alterations may affect the cerebrovascular response to intracranial hypertension. Data from patient and animal studies suggest that cerebral vessels retain their reactivity to carbon dioxide during cerebral vasodilation and reduced blood flow. Therefore, the potential exists for the alteration in the cerebrovascular response to elevated ICP due to the development of hypoxemia and respiratory acidosis. The purpose of the study was to determine the role of blood gas and pH alterations occurring during elevated ICP in spontaneously breathing dogs on the total and regional cerebrovascular response to elevated ICP.

Methods
Fifteen mongrel dogs (mean weight 11.2 ± 1.5 kg) were anesthetized with 30 mg/kg sodium pentobarbital and intubated. Teflon catheters were positioned in a femoral artery from the 8th intercostal space, and a small incision was made on the left side of the neck to introduce the cannula into the carotid artery.
Experimental model for systematic study of impaired microvascular reperfusion.
J M Hallenbeck and M E Bradley

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