Regional Cerebral Blood Flow Determination by the Hydrogen Clearance Technique and Comparison With Oxygen Availability in the Rabbit

BY ANDREW D. JAMIESON, M.D., AND JAMES H. HALSEY, JR., M.D.

Abstract: Regional Cerebral Blood Flow Determination by the Hydrogen Clearance Technique and Comparison With Oxygen Availability in the Rabbit

Hydrogen clearance using the tissue polarographical electrode appears to be a potentially useful technique for intermittent measurements of rCBF in relatively small areas. Both chronic and acutely implanted electrodes were placed at different depths in rabbit cerebrum including cortex and subcortical gray and white matter. Polarographical electrodes, oppositely charged, sensing oxygen availability (O_2a) also were implanted proximally for comparison of both rCBF response and O_2a under conditions of normocapnia and hypercapnia. No functional difference was discerned between chronic and acute electrodes. Generally rates of blood flow were faster in gray matter than in white. Both monoexponential and biexponential curves, representing one and two compartments of blood flow, respectively, were observed in both gray and white matter. Some three-compartment curves also were seen. Hypercapnia generally induced faster rCBF in all compartments as well as increased O_2a. Occasionally a change in compartmentalization was recorded from a single electrode during hypercapnia, and two instances of paradoxical decrease in rCBF were observed. A multicompartmental model with an arteriovenous thoroughfare shunt was postulated to account for the wide range of blood flow values recorded: from 5.2+ to 0.2 ml per gram per minute.

Introduction

The study of regional cerebral blood flow (rCBF) has been approached by several different experimental techniques. Direct observation of pial arteriole size, indicator dilution procedures, and analysis of radioactive gas clearance are only some of the methods of previous investigations. The necessity for jugular and/or carotid puncture with some techniques has been circumvented by following the clearance of radioactive gases administered by inhalation. Values recorded from probes external to the brain or skull, however, were taken from relatively large regions. Interfering radioactivity in the airway and scalp and arterial recirculation compounded data analysis, and the relatively heavy molecular weight of the gases made them less than ideal indicators.

Electrochemical methods have provided means of exploration at the tissue level. Hydrogen-sensitive polarographical electrodes placed directly in the brain can measure rCBF in a small region by hydrogen clearance. Hydrogen is a light gas, is readily diffusible, and clears essentially with a single passage of blood through the lungs. The tissue hydrogen electrode was first employed to measure blood flow in muscle and kidney by Aukland, Bower and Berliner in 1964. They suggested its suitability for rCBF studies. Polarographical electrodes for oxygen availability (O_2a) have already been used in animal stroke investigations by Halsey and Clark and Halsey and Capra as an approximate measure of rCBF, but critics have suggested that results could be spurious in pathological states such as the luxury perfusion syndrome.

It was decided to investigate the measurements of rCBF using the polarographical hydrogen electrode in the brain of the rabbit, a relatively inexpensive research animal large enough to accommodate multiple electrodes as well as to permit arterial catheterization. Blood flow rates could then
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be compared in different locations and compared with changes in \( O_{2a} \).

Methods

Adult New Zealand white rabbits weighing 2.5 to 3.2 kg were used for implantation of both chronic and acute electrodes. Electrodes were fashioned according to the sketch in figure 1. The 250\( \mu \) diameter platinum wire was used for electrode tips of 3.0 mm-length in chronic preparations and 0.5 mm in acutely placed electrodes. The wire tip was held by a glass-insulated steel jacket, and the platinum surface was electroplated with a platinum black layer according to procedures described elsewhere. Figure 1 also shows the polarographical circuit pattern used with both \( O_{2a} \) electrodes polarized at \(-0.6 \) V, and hydrogen electrodes polarized at \(+0.2 \) V, each with its separate silver-silver chloride reference electrode. Current tracings from both types of electrodes were recorded using a Model 7 Grass polygraph.

Chronic electrodes were implanted as shown in figure 1 and held firm by dental acrylic and bone screws. They were left in place two weeks before performing blood flow studies. Acute electrodes were placed with the rabbit in a stereotaxic instrument and coordinates selected according to Monnier and Gangloff.

Hydrogen was introduced into the cerebral circulatory system by injection of a 2 to 3 ml bolus of hydrogen-saturated normal saline at 39\(^\circ\)C via polyethylene catheter through the right axillary artery into the aortic arch. Also, frequently hydrogen was administered separately by inhalation for comparison with clearance curves following intra-arterial injection. Hydrogen clearance curves were replotted on a semilog of current versus time graph to facilitate determination of half-time (\( T_\frac{1}{2} \)) and compartmental analysis by curve stripping based on the principles of the Kety-Schmidt technique as described by Lassen and Høedt-Rasmussen. Line slopes were checked by a Wang computer program using a least squares plot, and blood flow then calculated from \( T_\frac{1}{2} \) according to the formula:

\[
F = \frac{0.693 \times \lambda}{T_\frac{1}{2}},
\]

where \( F \) = blood flow, and \( \lambda \) is the blood-tissue partition coefficient for hydrogen, taken at unity based on the work of others.

Rabbits were anesthetized for experimental procedures using 50 to 75 mg ketamine intramuscularly, combined with carefully administered intravenous pentobarbital. Continued anesthesia and immobility were maintained with pentobarbital and gallamine. During rCBF studies, blood pressure was monitored via femoral artery catheter, and expiratory carbon dioxide levels (\( eCO_2 \)) were monitored at the tracheal cannula to which a respirator was attached for control of breathing mixture. EEG also was monitored in multielectrode preparations. Hypercapnia was induced by adding \( CO_2 \) to the breathing mixture to elevate \( eCO_2 \) from 4% to 10%. Hypercapnia is known to cause vasodilation with a usual increase in cerebral blood flow. Animals were sacrificed following a series of blood flow determinations, and both gross and microscopic studies were done in an attempt to ascertain electrode location.

Results

Successful recordings of hydrogen clearance curves were obtained from both chronic and acute electrodes. \( O_{2a} \) electrodes were placed adjacent to hydrogen electrodes. An example of tracing obtained is given in figure 2. \( O_{2a} \) current increased with hypercapnia and \( H_2 \) current was greater with a faster clearance curve. A semilog graph of the normocapnic curve is presented in figure 3, which shows that two blood flow compartments were represented. Examination of the rabbit brain revealed the cortex to average 2.3 mm in thickness, thus 3.0 mm electrodes were in contact with both gray and white matter. Generally the curves obtained from chronic electrodes presented this biexponential pattern representative of two blood flow compartments, as has been seen by other investigators and ascribed to gray matter (fast flow) and white matter (slow flow). A summary of results from chronic electrodes is given in table 1. There was generally an increase in rCBF in both compartments during hypercapnia. Exceptions in this table include electrode 2 in rabbit A, which manifested three blood flow compartments during normocapnia, but resolved to two compartments during hypercapnia. Rabbits C and D each had an electrode which presented a single blood flow compartment. These electrodes were subsequently shown to be located inferior to cortex in white matter. Electrode 3 in rabbit D had been stereotaxically aimed at the
caudate nucleus, and it repeatedly manifested a large increase in blood flow with hypercapnia. Confirmation of its location, however, was not histologically demonstrated, so it may have been in the internal capsule or even in globus pallidus.

Studies with acute electrodes were then executed in an effort to elucidate blood flow compartments. Stereotaxic coordinates were selected to place the electrodes directly over the caudate nucleus; 0.5 mm electrodes were then gradually lowered by manipulator, first into cortex and then into lower subcortical levels. The results from these studies are shown in figures 4 through 6.

Figure 4 shows repeated curves at various levels under conditions of normocapnia and hypercapnia. At some levels the acute O₂a electrode (known to be less stable than chronic preparations⁶) gave a measurable response with hypercapnia for comparison with rCBF. Single blood flow compartments predominate in figure 4, and flows tended to

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**FIGURE 1**

Typical tracings are shown for hydrogen clearance and O₂a during normocapnia (eCO₂ = 4%) and hypercapnia (eCO₂ = 10%). At time zero, a 2 to 3-ml bolus of hydrogen-saturated saline was given intra-arterially.

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**FIGURE 2**

This is a semilog graph of the normocapnia hydrogen clearance curve shown in figure 2. The early points of the curve are not linear and values must be subtracted from the extrapolated linear portion and replotted. Two blood flow compartments are represented.
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TABLE 1
Compartmental Blood Flow Changes With Hypercapnia

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Electrode</th>
<th>Compartment</th>
<th>Blood flow with normocapnia (ml/gm/min)</th>
<th>Blood flow with hypercapnia (ml/gm/min)</th>
<th>Change in blood flow with hypercapnia, %</th>
<th>One change (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>Fast</td>
<td>2.46 ± 0.66</td>
<td>3.31 ± 1.14</td>
<td>↑ 35</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Slow</td>
<td>0.304 ± 0.127</td>
<td>0.638 ± 0.20</td>
<td>↑ 27</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Fast</td>
<td>5.19 / 0.620</td>
<td>2.97</td>
<td>↑ 4.2 + 0.09</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Slow</td>
<td>0.246 ± 0.035</td>
<td>0.470 ± 0.121</td>
<td>↑ 91</td>
<td>(81%)</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>Fast</td>
<td>3.79 ± 1.94</td>
<td>4.67 ± 1.00</td>
<td>↑ 23</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Slow</td>
<td>0.357 ± 0.117</td>
<td>0.423 ± 0.116</td>
<td>↑ 17</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Fast</td>
<td>1.98 ± 0.055</td>
<td>2.99 ± 1.85</td>
<td>↑ 51</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Slow</td>
<td>0.292 ± 0.030</td>
<td>0.315 ± 0.054</td>
<td>↑ 8</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Fast</td>
<td>2.34 ± 0.38</td>
<td>2.89 ± 0.063</td>
<td>↑ 24</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Slow</td>
<td>0.302 ± 0.038</td>
<td>0.368 ± 0.124</td>
<td>↑ 22</td>
<td>22</td>
</tr>
<tr>
<td>C</td>
<td>1</td>
<td>Fast</td>
<td>4.76 ± 1.34</td>
<td>5.19 ± 0.23</td>
<td>↑ 9</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Slow</td>
<td>0.401 ± 0.042</td>
<td>0.560 ± 0.059</td>
<td>↑ 40</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Fast</td>
<td>0.260 ± 0.032</td>
<td>0.400 ± 0.064</td>
<td>↑ 54</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Slow</td>
<td>0.390 ± 0.037</td>
<td>0.619 ± 0.030</td>
<td>↑ 59</td>
<td>(57%)</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Fast</td>
<td>3.68 ± 0.85</td>
<td>2.68 ± 0.37</td>
<td>↓ 27</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Slow</td>
<td>0.274 ± 0.065</td>
<td>0.458 ± 0.044</td>
<td>↑ 67</td>
<td>67</td>
</tr>
<tr>
<td>D</td>
<td>1</td>
<td>Fast</td>
<td>0.582 ± 0.148</td>
<td>1.120 ± 0.300</td>
<td>↑ 92</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Slow</td>
<td>0.396 ± 0.066</td>
<td>0.840 ± 0.010</td>
<td>↑ 112</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Fast</td>
<td>2.98 ± 0.21</td>
<td>4.14 ± 0.086</td>
<td>↑ 39</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Slow</td>
<td>0.396 ± 0.066</td>
<td>0.840 ± 0.010</td>
<td>↑ 112</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Fast</td>
<td>0.433 ± 0.030</td>
<td>5.08 ± 1.02</td>
<td>↑ 1,070</td>
<td>(103%)</td>
</tr>
</tbody>
</table>

Four rabbits (A and D) had two to four functioning chronic electrodes for rCBF measurements by hydrogen clearance. Values given are averages of five or six repeated curves with standard deviation. Values separated by a diagonal (/) are for two separate components. The values in the right column are average O2a observations from adjacent electrodes with current in millamps.

be faster in gray matter than in white. Hypercapnia increased blood flow and O2a. Figure 5 shows replication of this experimental protocol, but here two-compartment areas were recorded in both cortex and subcortex. One electrode position shown here repeatedly demonstrated a decrease in rCBF with hypercapnia. Blood samples taken for blood gas determinations during normocapnia (at eCO2 = 3.8%) gave values: pH 7.42, P02 125 mm Hg, and Pco2 28 mm Hg, while during induced hypercapnia (eCO2 = 9%), the blood gases were pH 7.30, P02 160 mm Hg, and Pco2 48 mm Hg. Figure 6 also shows a repeat of this type of experiment, and again the values for rCBF are variable with both single and double compartments. One three-compartment region was observed which hypercapnia reduced to two effective compartments. Again, there was a general increase in both rCBF and O2a with hypercapnia.

Certainly there was a wide range of rCBF rates observed. A compilation of 299 flow compartments obtained from 178 clearance curves in 14 rabbits (both chronic and acute electrodes) is given in the bar graph in figure 7. The cluster of values in the T1 range of 10 to 20 seconds represents a prominence of rCBF rates between 2 and 4 ml per gram of tissue per minute. Slower compartments are distributed over a wide range. Repeated curves from the same electrode under identical conditions often exhibited changes in flow rate and rarely even changes in compartmentalization.

Clearance curves were obtained after hydrogen inhalation and compared to previous and subsequent curves following intra-arterial injection of hydrogen-saturated saline. Values were generally 30% slower by inhalation technique in the faster compartments, but without significant difference in slower rCBF compartments. An intra-arterial electrode (in a femoral artery) may be used to monitor hydrogen clearance from blood and allow correction for recirculation.

Discussion
The hydrogen clearance technique appeared to be a feasible method for determination of rCBF with both acute and chronic polarographical electrodes. With hypercapnia there was a general but not predictable increase in blood flow rate. The paradoxical decrease in rCBF with hypercapnia as well as the presence of multiple compartments in gray and white matter might be explained according to the scheme shown in figure 8. If electrode A is placed in a single...
A hemi-cross-section of rabbit brain is represented at a level posterior to the optic chiasm. NC = caudate nucleus, Ci = internal capsule, GP = globus pallidus, Pu = putamen, A = amygdala, and ot = optic tract. Values reported are averages of three repeated observations with standard deviation. Single compartments predominate with faster flow rates in gray matter than in white.

In this animal two flow compartments are noted at most levels, in both gray and white matter. At one position near the cortex-white matter junction flow consistently decreased at hypercapnia.
Two compartments are present at most levels. Note three compartments at one cortical locus (two fast, one slow) which become two (one fast, one slow) with hypercapnia.

Two blood flow compartments are present. Note three compartments at one cortical locus (two fast, one slow) which become two (one fast, one slow) with hypercapnia.

Could be that the implantation of the electrode caused microhemorrhage or edema; thus CO₂ inhalation would possibly produce further edema or brain compression with resulting flow reduction.

An electrode B in contact with two independent blood flow compartments might record two blood flow rates if tone in the precapillary sphincters were different during normocapnia, but as both approached maximal vasodilation during hypercapnia, the compartments would be diverted away from compartment 1 as a sort of intratissue steal. An explanation for this could be that the implantation of the electrode caused microhemorrhage or edema; thus CO₂ inhalation would possibly produce further edema or brain compression with resulting flow reduction.

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The cerebrovascular circulation appears to be in a dynamic state of flux with a wide range of flow rates. This re-emphasizes a multicompartment model with many different perfusion rates as determined by autoradiographical studies. The incidence of flow rates more rapid than those reported by other investigators may have been due in part to species differences among animals. Ketamine has been reported to produce increased rCBF in dogs, but no increase was noted in rabbits following intravenous administration in our laboratory.

In calculation of rCBF there became evident an increased liability to error in flow rates at brief Ti's (rapid flow rates). The magnitude of this liability is shown in figure 9. With a Ti smaller than eight seconds (rCBF 5 ml per gram per minute), a one-second error in Ti determination might cause a 20% error in flow rate, whereas a one-second Ti error at slow flow may cause flow to be 1% off. Half-time determinations, therefore, were very carefully made.

Plans are to continue utilization of the hydrogen electrode for stroke research in animals. Certainly it would be interesting to examine rCBF with accurate stereotaxic placement of electrodes in subcortical structures such as thalamic, caudate, and lentiform nuclei, internal capsule, and amygdala to compare changes in vasodilation with anatomical vascularity. Miniaturization of electrodes could minimize the trauma of implantation. Elucidation of inhalation clearance curves would obviate arterial catheterization for saline injection. There is potential for application of this technique to monitoring rCBF during neurosurgical procedures and for determining postoperative progress.

References

If a one-second error was made in Ti determination, the magnitude of rCBF error is shown for increasing Ti values.
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