Regional Cerebral Blood Flow in the Rat as Determined by Particle Distribution and by Diffusable Tracer

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SUMMARY Measurements of total and regional cerebral blood flow in paralyzed rats maintained on 70% N2O/30% O2 obtained by a diffusible tracer technique, iodoantipyrine, and by a particle distribution method, microspheres, have been compared. Total CBF values were in good agreement, 0.86 ± 0.07 ml/g/min (Paco2, 37.3 ± 1.5, iodoantipyrine method) and 0.88 ± 0.02 (Paco2, 36.2 ± 0.8, microsphere method). Regional cerebral blood flows showed good agreement with the 2 methods, with highest flow in the colliculi, striatum and cerebral cortex and lowest flows in the hypothalamus, pons medulla and cerebellum. The iodoantipyrine method is technically easier to perform and had a higher precision.

In experimental animals, cerebral blood flow (CBF) determinations have most commonly utilized tracer diffusion methods with arterial and cerebral venous blood sampling or external sealers for radioactivity determinations. They yield values corresponding approximately to whole brain CBF (tCBF) or to cerebral mantle CBF depending on the sampling procedure. Such measurements may fail to detect changes in relative regional CBF (rCBF) associated with normal or abnormal functional states.

Two basic principles underlie the methods that are presently available for the systematic study of rCBF. These are particle distribution, which is dependent on the physical trapping of labelled particles in the microvasculature of the tissue, and tracer diffusion, utilizing an inert, soluble tracer that very rapidly achieves an equilibrium distribution between blood and brain. The particle distribution method assumes 1) distribution of particles in proportion to local blood flow, 2) no regional changes in flow as a consequence of particle entrapment and 3) particles extracted during one circulation through the organ. It has been claimed that these criteria can be fulfilled by the use of microspheres (ion-exchange beads of uniform size, to which a radionuclide is attached; coated to ensure in-ertness and stability of labelling). Microspheres have been used extensively for determination of the regional distribution of cardiac output in several experimental animals and for rCBF in large mammals, but have been little used for rCBF determination in small laboratory rodents.

The diffusible tracer method depends on the mathematical development of the Fick principle by Kety and Schmidt. Although several tracers have been utilized, gaseous compounds present technical problems and non-gaseous compounds have proved to suffer from diffusional limitations. However, such limitations appear minimal with iodo-13C antipyrine which yields CBF measurements comparable to those determined with gaseous tracers in rats.

We have, therefore, compared rCBF determinations with 131I labelled microspheres, 15µm diameter, and with iodo-13C antipyrine in 13 brain regions of paralyzed rats ventilated with N2O/O2.

Methods

Male Wistar rats (400–500g) were provided with food and water ad libitum until the experiment. They were anesthetized with 2% halothane (Fluothane, I.C.I.), tracheotomized, paralyzed with tubocurarine (1 mg/kg i.m.) and artificially ventilated (tidal volume 3–4 ml, 80 breaths/min) with 30% oxygen/70% nitrous oxide by a mechanical ventilator. Body temperature was maintained at 37°C by an automatic heating blanket coupled to a rectal probe. A catheter (nylon tubing, external diameter 0.63 mm, internal diameter 0.50 mm) was placed in the left femoral artery for the anaerobic removal of blood for Paco2, Pao2 and pH determinations (Radiometer Blood Gas Analyzer) and for the measurement of radioactive iodoantipyrine or collection of the arterial reference sample (microsphere method). The right femoral artery was cannulated (polythene tubing, external diameter 0.61 mm, internal diameter 0.28 mm) for continuous blood pressure recording using a Beckman pressure transducer. The left femoral vein was cannulated for the injection of drugs and for the infusion of iodoantipyrine. The ECG and bilateral EEG were continuously recorded via platinum needle electrodes.

In experiments in which microspheres were used, the left ventricle was cannulated via the right brachial artery, using polythene tubing (Portex PP20), drawn out to an external diameter of 0.335–0.345 mm (0.014–0.016") for the terminal 4–5 cm. The catheter was inserted in the brachial artery, immediately proximal to the trifurcation of the vessel where the brachial plexus crosses the artery, and advanced for 4 cm (distance determined in preliminary studies). Three criteria helped to confirm correct placement: 1) that
the catheter was inserted the full 4 cm (resistance before this point was due to obstruction at the ribcage, at the junction with the carotid artery or at the aortic valve), 2) premature ventricular contraction which stopped on slight withdrawal of the catheter, and 3) withdrawal of blood through the catheter (failure was usually due to the tip of the catheter lodging in the aortic valve).

At the end of the surgical procedure (duration 45–75 min) the animal was heparinized (1,000 iu/kg), the halothane withdrawn and the ventilator flushed. The animal was maintained on 70 % N₂/30 % O₂. The respiratory pump was adjusted to give a Paco₂ of 35–45 mm Hg. A minimum of 30 min was allowed to elapse after withdrawal of halothane before the determination of flow, since halothane induces a loss of cerebral autoregulation and a fall in cerebral oxygen consumption. PaO₂, Paco₂ and arterial pH were measured just prior to each flow determination.

Microspheres

Microspheres (15 ± 3 μ diameter, labelled with ¹³¹Ce to a nominal specific activity of 10 mCi/g, suspended in saline containing 0.01 % Tween 80) (New England Nuclear, W. Germany) were used. The microspheres were vigorously shaken for 5 min and an aliquot (50–200 μl containing approximately 50,000–250,000 spheres) withdrawn into a Hamilton microsyringe. Samples were periodically checked microscopically to confirm the absence of clumping. The ventricular cannula was pierced with the tip of the microsyringe and the microspheres injected slowly (20 sec) into a moving stream of saline. The cannula was flushed for a further 10 sec with saline. Total injection volume was 0.4–0.5 ml. An arterial reference sample was withdrawn at a rate of 0.3 ml/min using a pump, starting 30 sec before and stopping 90 sec after the infusion of microspheres. In some experiments a further arterial blood sample was taken for gas determinations after the infusion of microspheres. The animal was killed by an i.v. injection of saturated KG, which stopped on slight withdrawal of the catheter, and the position of the ventricular cannula below the aortic valve verified. The brain was removed and dissected (see below) with the aid of a microscope. The brain samples were frozen in liquid nitrogen, weighed and solubilized by means of 0.8 ml saline containing 0.01 % Tween 80. Blood and brain samples were counted in a well type scintillation counter using a toluene-based medium and counting efficiency determined by internal standardization (¹⁴C toluene).

Brain Dissection

The hypothalamus (mean wt (mg) ± S.E.M. (n = 14) 43.2 ± 1.8) was scooped out with curved forceps. Left and right olfactory tubercles were dissected out and combined (45.9 ± 2.3). The cerebellum (wt 279.5 ± 5.3) was removed intact by blunt dissection. A transverse section caudal to the posterior colliculus yielded the 'pons-medulla' (245.3 ± 15.0). The corpus callosum was cut and the cerebral mantles deflected laterally. The left and right hippocampi were peeled away (combined wt 128.6 ± 4.5). Blunt dissection separated the left and right striata including each globus pallidus (combined wt 67.0 ± 3.5) from the septal area (wt 41.2 ± 3.5). The left and right colliculi were removed (combined wt 60.5 ± 3.0). Vertical cuts separated the midbrain (wt 108.5 ± 5.2) from the thalamus which was divided into left and right halves (combined wt 168.3 ± 10.2). The olfactory bulb was rejected and the left and right cortex divided into anterior, middle and posterior thirds (combined wt: anterior 234.4 ± 9.1; middle 271.4 ± 10.2; posterior 202.2 ± 8.9).

rCBF Calculation

rCBF by the microsphere method was calculated from the equation

\[
\frac{\text{rCBF}_{\text{Regional brain radioactivity}}}{\text{reference sample flow}} = \frac{\text{rCBF}_{\text{reference sample radioactivity}}}{\text{reference sample flow}}
\]

rCBF by the iodoantipyrine method was based on the Fick principle and can be expressed mathematically as

\[
\text{Ci}(T) = \lambda K_i \int_0^T C_i e^{-K_i(T-t)} dt
\]

where Ci(T) equals the tissue concentration of ¹⁴C iodoantipyrine at a given time (T) after its introduction into the circulation; λ is the tissue-blood partition coefficient for iodoantipyrine; Ki, is defined as

\[
K_i = \frac{mF}{W}
\]

where W is the blood flow per unit
mass of tissue, \( m \) equals a constant between 0 and 1 that represents the extent of diffusional equilibrium (in the absence of diffusional limitations \( m = 1 \)); and \( C_x \) is the arterial concentration of iodoantipyrine. With the aid of a PDP12 computer, the above equation was solved to calculate rCBF from 1) the concentration of tracer in the brain region as determined at time \( T \) (30 sec), 2) the time course of change of arterial concentration of iodoantipyrine and 3) the brain to blood coefficient \( (\lambda) \) for iodoantipyrine. The value for \( \lambda \) of 0.8 as determined by Sakurada et al.\cite{1978} was used. Total CBF (by both methods) was calculated by summation of the regional radioactivities and brain weights.

### Statistical Methods

Statistical significance of differences between physiological variables, tCBF and rCBF and regional distribution of CBF between the 2 methods were determined by unpaired Student's \( t \)-test. Significant differences between left and right flow to paired brain areas were determined by paired Student's \( t \)-test.

### Results

The physiological variables measured just prior to blood flow determination in the 2 groups of rats are shown in table 1. Mean BP, \( \text{PaO}_2 \) and \( \text{PaCO}_2 \) did not differ significantly between the 2 groups but the iodoantipyrine group had a significantly higher arterial pH than the microsphere group.

Total CBF showed good agreement between the 2 techniques, 0.88 ± 0.02 ml/g/min (microspheres) and 0.86 ± 0.07 ml/g/min (iodoantipyrine) (table 2). The rCBF in 13 brain regions as determined by the 2 techniques is shown in table 2. rCBFs were in good agreement except that flows in the hypothalamus and tuberculum olfactorium were apparently higher and the posterior cortex apparently lower as determined by the iodoantipyrine method compared to the microsphere method.

The percentage distribution of flow (i.e. the flow to a particular brain region expressed as a % of the total CBF (100%) in the same animal) is shown in the figure. The agreement was good except that the iodoantipyrine method gave apparently higher % distribution of flow to the hypothalamus, tuberculum olfactorium, hippocampus, septum, and mid-brain than the microsphere method, whereas the converse was true for the cerebellum and posterior cortex.

Flow to the left and right sides of paired brain areas was compared to assess the reproducibility of the 2 methods (table 3). There were no significant left vs right differences for any of the paired brain areas with the iodoantipyrine method. There were no overall left vs right differences with the microsphere method but flow was significantly different in paired striatal and posterior cortical samples with this method (table 3).

### Discussion

A comparison of 2 techniques for the measurement of total and regional CBF, based on different principles, has been performed in the rat. Total and cortical CBF measured by the 2 methods were in good agreement with each other (table 2) and with previously reported values in the rat (table 4). The values for tCBF with the microsphere method is higher than previous estimates with this technique in the rat, although bilateral carotid ligation was used in one study\cite{29} and barbiturate anesthesia, which has been shown to reduce CBF,\cite{30, 31, 32} in the other.

Values for rCBF with the 2 techniques were comparable although there were some apparent discrepancies. Regional flow was highest in the colliculi, striatum and cerebral cortex and lowest in the hippocampus, cerebellum and pons-medulla. Flow to the tuberculum olfactorium and hypothalamus was apparently lower and to the posterior cortex apparently higher with the microsphere method compared to the iodoantipyrine method. However, the lack of a strict independence of the regional measurements means

### Table 1 Comparison of Physiological Variables

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Iodoantipyrine method ( (n = 6) )</th>
<th>Microsphere method ( (n = 8) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>MABP (mm Hg)</td>
<td>124 ± 10</td>
<td>126 ± 5</td>
</tr>
<tr>
<td>( \text{PaO}_2 ) (mm Hg)</td>
<td>178 ± 12</td>
<td>151 ± 10</td>
</tr>
<tr>
<td>( \text{PaCO}_2 ) (mm Hg)</td>
<td>37.3 ± 1.5</td>
<td>36.2 ± 0.8</td>
</tr>
<tr>
<td>pH</td>
<td>7.50 ± 0.03</td>
<td>7.42 ± 0.02*</td>
</tr>
</tbody>
</table>

Arterial blood samples were taken just prior to flow determination. Values are means ± SEM for the number of estimations indicated. Significant differences between means (Student's \( t \)-test) are indicated by \( *p < 0.05 \).
that independent t-testing overestimates the significance of differences. Correction for this indicates that only the lower flow rate to the hypothalamus with the microsphere method is significant (p < 0.001 on individual t-test and an overall probability of (1-0.99915) = 0.014). Microspheres (15μ diameter) probably underestimate hypothalamic flow.

There have been few studies previously reported on rCBF in the rat, making comparisons with the present data difficult. The best study for comparison is that of Abdul-Rahman et al.30 in which rCBF was determined in paralyzed rats maintained on N2O/O2 using iodooantipyrine. The relative distribution of flow is similar in the 2 studies, although our absolute values are lower in most areas (for example 13% lower in the hippocampus and striatum and 30% lower in the cerebellum and cerebral cortex). One possible explanation may be the method of detection of the iodooantipyrine, since we have used dissection followed by scintillation counting, which gives a mean flow to the dissected area, whereas Abdur-Rahman et al.30 used autoradiography and densitometry, which, although capable of greater anatomical discrimination, tends to give higher flow rates because the darkly staining (high flow) areas are selectively measured. Our present results give values that are generally lower in most brain areas than in the lightly restrained conscious rat using the same technique,14,16 although in some regions (for example the septum and hypothalamus) the values were very similar. A differential effect on blood flow to various brain regions due to N2O analgesia or a reduction in CBF due to effect of halothane31 cannot be excluded.

**Table 3** Comparison of rCBF to the Left and Right Sides of Brain Using Iodoantipyrine and Microspheres

<table>
<thead>
<tr>
<th></th>
<th>Iodoantipyrine (n = 6)</th>
<th>Microspheres (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean difference ± SEM</td>
<td>% of mean flow</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Colliculi</td>
<td>-0.055 ± 0.047</td>
<td>-4.4</td>
</tr>
<tr>
<td>Striatum</td>
<td>-0.031 ± 0.026</td>
<td>-3.0</td>
</tr>
<tr>
<td>Anterior cortex</td>
<td>-0.015 ± 0.028</td>
<td>-1.9</td>
</tr>
<tr>
<td>Mid cortex</td>
<td>-0.073 ± 0.051</td>
<td>-8.3</td>
</tr>
<tr>
<td>Posterior cortex</td>
<td>-0.016 ± 0.048</td>
<td>-1.6</td>
</tr>
<tr>
<td>Thalamus</td>
<td>+0.028 ± 0.025</td>
<td>+3.2</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>-0.008 ± 0.009</td>
<td>-1.1</td>
</tr>
</tbody>
</table>

|                  | Mean difference ± SEM  | % of mean flow       |
|                  |                        |                      |
|                  | -0.102 ± 0.113         | -8.9                 |
|                  | +0.256 ± 0.136*        | +21.9                |
|                  | +0.030 ± 0.070         | +3.0                 |
|                  | -0.021 ± 0.091         | -2.2                 |
|                  | -0.290 ± 0.107**       | -22.7                |
|                  | +0.014 ± 0.059         | +1.7                 |
|                  | +0.076 ± 0.063         | +11.6                |

**Differences in flow to left and right regions of each brain area were assessed using paired t-test. Significant differences are denoted by *p < 0.05; **p < 0.01. The % of mean flow is the mean difference between left and right flow as a percentage of the mean flow to that area (table 2). + values indicate right flow > left flow; — values indicate left flow > right flow.**
Thus both methods we have used give values comparable to those reported in the literature. Both methods have the advantage over the $^{133}$Xe inhalation technique that measurements can be carried out quickly (30 sec) and are thus more applicable to changing physiological states.

The present results with microspheres demonstrate that this technique is applicable to regional flow determination in the rodent brain. However, it has disadvantages compared to the iodoantipyrine method. It is technically more difficult to perform since it is essential that the microspheres are thoroughly mixed with the blood which requires left ventricular cannulation. This is tedious to perform in the rat, particularly without some form of visualization, but a satisfactory success rate can be achieved with practice (70%). The most common reason for failure was due to the tip of the catheter becoming lodged in an aortic valve leaflet. Premature ventricular contraction, presumably caused by irritation of the ventricular wall by the catheter, was sometimes observed but could usually be reversed by slight withdrawal of the catheter or by infusion of 50–100 $\mu$l of 1% xylocaine through the ventricular catheter. If cannulation of the ventricle resulted in abnormal heart rate or fall in BP which could not be quickly reversed, the experiment was terminated. We estimate that the cannula occluded less than 5% of the lumen of the right common carotid artery and when inserted via the brachial artery obviates the need to ligate or occlude the carotid artery. Direct cardiac injection of microspheres was, in our hands, too often associated with irreversible changes in heart rate and BP to offer a satisfactory alternative. The present results in the rat brain confirm adequate mixing since there were no overall systematic left vs right differences for paired brain areas (table 3).

The precision of the microsphere method is dependent upon the number of microspheres in the reference and tissue samples, and not on the amount of radioactivity. Calculated theoretically and confirmed experimentally that errors due to non-randomness were minimized if at least 400 spheres were present in each tissue and reference sample. The number of spheres in a particular sample is dependent upon the number injected, the flow to the particular organ or region of organ, the size of the tissue and the rate of withdrawal of the reference sample. The number of spheres we have injected and the withdrawal of the reference sample did not normally result in changes in BP or heart rate (the experiments in which changes were observed were rejected) or in arterial blood gases (in those experiments where blood gases were measured following microsphere administration). The estimated number of spheres in the reference samples (mean ± SEM 1425 ± 205, range 700–2500) were all in excess of the minimal recommendation of 400. In some brain areas the small number of spheres may be responsible for larger left vs right differences (–8.9% to +21.9%) of paired organs such as the kidneys, left and right brain hemispheres, and paired regional cerebral cortical samples. The present results in the rat brain confirm adequate mixing since there were no overall systematic left vs right differences for paired brain areas (table 3).

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Thus both methods we have used give values comparable to those reported in the literature. Both methods have the advantage over the $^{133}$Xe inhalation technique that measurements can be carried out quickly (30 sec) and are thus more applicable to changing physiological states.

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### Table 4 Total or "Cortical" CBF in the Rat

<table>
<thead>
<tr>
<th>Method</th>
<th>Anaesthetic state</th>
<th>PaCO$_2$</th>
<th>Blood flow</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{133}$Xe breathing (transverse sinus)</td>
<td>N$_2$O</td>
<td>35.6 ± 1.0</td>
<td>0.79 ± 0.03</td>
<td>17</td>
</tr>
<tr>
<td>$^{133}$Xe breathing (transverse sinus)</td>
<td>N$_2$O</td>
<td>34.9 ± 0.6</td>
<td>0.80 ± 0.02</td>
<td>18</td>
</tr>
<tr>
<td>$^{133}$Xe breathing (sagittal sinus)</td>
<td>N$_2$O</td>
<td>37.6 ± 0.6</td>
<td>1.00 ± 0.03</td>
<td>19</td>
</tr>
<tr>
<td>$^{133}$Xe breathing (transverse sinus)</td>
<td>N$_2$O</td>
<td>40.0 ± 1.3</td>
<td>0.98 ± 0.06</td>
<td>20</td>
</tr>
<tr>
<td>$^{133}$Xe breathing (sagittal sinus)</td>
<td>N$_2$O</td>
<td>40.0 ± 1.0</td>
<td>1.06 ± 0.07</td>
<td>21</td>
</tr>
<tr>
<td>$^{133}$Xe breathing (sagittal sinus)</td>
<td>N$_2$O</td>
<td>36.2 ± 2.9</td>
<td>1.03 ± 0.22</td>
<td>21</td>
</tr>
<tr>
<td>$^{133}$Xe iv infusion + decapitation</td>
<td>N$_2$O</td>
<td>37.7 ± 0.2</td>
<td>0.86 ± 0.03</td>
<td>22</td>
</tr>
<tr>
<td>$^{14}$C antipyrine</td>
<td>conscious</td>
<td>1.02 ± 0.04</td>
<td></td>
<td>23</td>
</tr>
<tr>
<td>$^{14}$C antipyrine</td>
<td>N$_2$O</td>
<td>36.7 ± 0.5</td>
<td>0.95 ± 0.04</td>
<td>23</td>
</tr>
<tr>
<td>$^{14}$C antipyrine</td>
<td>N$_2$O</td>
<td>37.3 ± 0.7</td>
<td>0.95 ± 0.04</td>
<td>23</td>
</tr>
<tr>
<td>$^{13}$H water</td>
<td>N$_2$O</td>
<td>38.1 ± 0.7</td>
<td>1.06 ± 0.14</td>
<td>23</td>
</tr>
<tr>
<td>$^{133}$Xe inhalation</td>
<td>N$_2$O</td>
<td>38 ± 0.8</td>
<td>0.97 ± 0.07</td>
<td>23</td>
</tr>
<tr>
<td>$^{14}$C antipyrine</td>
<td>N$_2$O</td>
<td>37.3 ± 1.5</td>
<td>1.27 ± 0.05</td>
<td>23</td>
</tr>
<tr>
<td>Microspheres</td>
<td>N$_2$O</td>
<td>36.2 ± 0.8</td>
<td>1.07 ± 0.03</td>
<td>23</td>
</tr>
</tbody>
</table>

Adequate mixing of microspheres following ventricular administration has been based on measurements of equal flow to paired organs such as the kidneys, left and right brain hemispheres, and paired regional cerebral cortical samples. The present results in the rat brain confirm adequate mixing since there were no overall systematic left vs right differences for paired brain areas (table 3).

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such as thalamus (640 ± 100), anterior cortex (1070 ± 140) and mid cortex (1230 ± 200) (table 3). It is difficult to explain the left vs right difference for the posterior cortex since a large number of spheres were present (1260 ± 230). The difference is unlikely to be related to a systematic dissection difference since the mean weights were not significantly different (left 101.3 ± 6.7 mg; right 109.9 ± 8.2 mg).

The agreement between the 2 methods reported here is for tCBF and rCBF with normal flow rates. Further experiments are required to determine if the agreement is maintained under conditions producing very high or low flow rates.

Acknowledgment

This investigation was supported by grants from the Medical Research Council (RH and BSM) and by USPHS grant NS 11075 from NINCDS (TAP).

The authors thank Miss J. R. McWilliam for excellent technical assistance and Drs. Abdul-Rahman and M. Ingvar of the Laboratory for Experimental Brain Research, Lund, Sweden, for a copy of their computer program for flow determination with iodoantipyrine and H. C. Bertoya of our department for adapting this program for the PDP 12 computer.

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Stroke. 1980;11:39-44
doi: 10.1161/01.STR.11.1.39

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