Local Cerebral Blood Flow in the Conscious Rat As Measured with $^{14}$C-Antipyrine, $^{14}$C-Iodoantipyrine and $^{3}$H-Nicotine

K. OHNO, M.D., K. D. PETTIGREW, AND S. I. RAPOPORT, M.D.

SUMMARY Local cerebral blood flow (LCBF) in the conscious rat was estimated with one of 3 radio-tracers — $^{14}$C-antipyrine, $^{14}$C-iodoantipyrine or $^{3}$H-nicotine. A tracer was infused intravenously at a constant rate and blood concentration was followed until the animal was killed by decapitation. Tracer concentration was then measured in each of 14 brain regions. The Kety-Schmidt analysis was applied to the data with $^{14}$C-antipyrine and that approximate LCBF's found with an inert gas. LCBF was calculated from the $^{3}$H-nicotine data by assuming complete extraction of tracer by brain. This assumption was approximated for infusion times of 50 sec or less, when LCBF's derived with $^{3}$H-nicotine generally did not differ significantly from LCBF's obtained with $^{14}$C-iodoantipyrine. Fifty-sec $^{3}$H-nicotine-derived flows for the pineal and pituitary glands were, respectively, 2.59 ± 0.36 (SEM) cm$^3$ g$^{-1}$ min$^{-1}$ and 1.28 ± 0.08 cm$^3$ g$^{-1}$ min$^{-1}$. LCBF's calculated for 70 sec to 240 sec of $^{3}$H-nicotine infusion were lower than 50-sec values due to back-diffusion, but nevertheless were as high as $^{14}$C-antipyrine LCBF's due to the marked binding of tracer by brain tissue. The results supported the conclusion of Sakurada et al. (1978) that iodoantipyrine is the non-gaseous agent of choice for measuring LCBF precisely, but also showed that short infusion schedules with nicotine provided good estimates of LCBF.

LOCAL CEREBRAL blood flow (LCBF) can be determined in experimental animals by employing an inert gas tracer, $^{131}$I-trifluoriodomethane, and applying the mathematical principles of Kety to analyze blood-brain exchange.1-4 The principles are valid because diffusional equilibrium between brain and blood is established almost instantaneously.5, 6 Since use of a gas tracer presents technical difficulties, attempts have been made to employ non-gaseous tracers to measure LCBF. $^{14}$C-Antipyrine has been used with some success, but it provides LCBF's which are less than those obtained using inert gases. Transfer of $^{14}$C-antipyrine from blood to brain is limited by the comparatively low diffusion coefficient of that tracer in the brain. The cerebrovascular PS value (permeability × surface area) of $^{14}$C-antipyrine is about 0.020 cm$^3$ sec$^{-1}$ g$^{-1}$, and is not sufficiently high to satisfy the assumptions of the Kety method.5, 6, 7-10 Early work suggested that $^{131}$I-iodoantipyrine might...
be useful for measuring cerebral blood flow, but it was not until recently that Sakurada et al. showed that \(^{131}\)I-iodoantipyrine, which has a higher oil/water partition coefficient than \(^{14}\)C-antipyrine and also is more permeable at the cerebrovascular, provides values of LCBF by the Kety method in the conscious rat that are comparable to those obtained with \(^{131}\)I-trifluoriodomethane. Autoradiography was used to measure local brain concentrations of the \(^{14}\)C-tracer.

In this paper, we compare LCBF's that were obtained with each of 3 tracers — \(^{14}\)C-antipyrine, \(^{14}\)C-iodoantipyryne and \(^{3}\)H-nicotine — in regions of the rat brain that were dissected out according to criteria of Glowinski and Iversen and Chiueh et al. Knowledge of LCBF in these specific regions is required to calculate regional cerebrovascular permeability to a variety of agents.

The mathematical treatment of Kety was employed to estimate LCBF with both \(^{14}\)C-antipyrine and \(^{14}\)C-iodoantipyrine. The treatment could not be used with \(^{3}\)H-nicotine, however, because this tracer binds to brain and is not freely exchangeable between brain and blood. We estimated LCBF with \(^{3}\)H-nicotine by assuming that all of the tracer was extracted from blood by brain. We chose \(^{3}\)H-nicotine because it, like \(^{14}\)C-iodoantipyrine, is more permeable than \(^{14}\)C-antipyrine at the cerebral vasculature and because its affinity for brain tissue might allow approximately complete extraction at short infusion times.

**Methods**

Male rats (Osborn-Mendel strain), weighing 250 g to 350 g, were anesthetized with Na pentobarbital (35 mg kg\(^{-1}\), i.p.). Polyethylene catheters containing 100 IU Na heparin and 0.009 g NaCl/ml of water were implanted in the left femoral and vein. The skin was sutured at the catheter exit and infiltrated with 2% bupivacaine sulfate (Abbott Labs, N. Chicago, Ill.). The hindquarters of the animals were wrapped in a fast-setting plaster bandage (Johnson and Johnson,  N.J.), with hindlimbs and catheters protruding, after which the bandage was tied down on a lead block. The animals were allowed to recover from anesthesia for 4 hr or more. In their harness, the conscious rats could freely move their forequarters, head and neck, and appeared comfortable.

The catheter in the femoral vein was connected to a 10-ml syringe, which was held in a constant flow pump (Model 255-2, Sage Instruments Inc., White Plains, N.Y.) that was set to deliver fluid at a rate of 0.28 ml min\(^{-1}\). Prior to infusion, a sample of arterial blood was removed for the determination of hematocrit, and for measurement of Pco\(_2\), pH and Pao\(_2\) (pH-Blood Gas Analyzer, No. 213, Instrumentation Labs, Lexington, Mass.). The femoral vein was infused for up to 4 min with isotonic saline containing 20 \(\mu\)C/ml of \(^{14}\)C-nicotine-d-bitartrate (Amersham/Searle, sp. act. = 240 mC/mmold), for 60 sec with isotonic saline containing 4 \(\mu\)C/ml of \(^{14}\)C-antipyrine (Amersham/Searle, sp. act. = 52 mC/mmold) and for 45 sec with isotonic saline containing 9 \(\mu\)C/ml of \(^{131}\)I-iodoantipyrine (New England Nuclear, sp. act. = 50 mC/mmold). The purity of each tracer exceeded 96%, as confirmed by thin layer chromatography. Periodically during infusion, 20 \(\mu\)l samples of arterial blood were collected into heparinized tubes, after which 10 \(\mu\)l aliquots were transferred to scintillation vials.

Animals were decapitated 30 sec to 4 min after infusion began. The skull was opened and the brain was removed and placed on cold filter paper wetted with 0.9% NaCl. Large subarachnoidal and dural blood vessels were removed and discarded. Brain regions then were dissected out, according to the method of Chiueh et al., and placed in tared scintillation vials that immediately were re-weighed.

The pineal and pituitary glands were removed and the brain was hemisectioned in the midline. The caudate nucleus in the anterior horn of the lateral ventricle, and the hippocampus in the posterior-inferior horn were removed with a curved forceps. The hypothalamus and thalamus then were dissected away from the cerebral cortex and midbrain, using landmarks of the anterior commissure, massa intermedia, mamillary body and internal capsule. The cerebellum subsequently was separated from brain stem, and midbrain and brain stem were separated at the level below the inferior colliculi. In addition to sampling occipital and frontal cortical and subcortical regions, which contained gray and white matter, gray matter was separated from white matter in the temporal region and corpus callosum. Reproducibility of the dissection procedure has been published.

Scintillation vials that contained blood or brain samples received 1.5 ml of Soluene 100 (Packard Instrument Co., Downers Grove, Ill.) and were shaken for 6 hr in a water bath at 60°C. Fifteen ml of Dimilume 30 (Packard Instrument Co.), a liquid scintillation fluid, were added before counting in a Packard Tricarb Liquid Scintillation Spectrometer (Model 2405). Counts per minute (cpm) was converted to disintegrations per minute (dpm), a measure of absolute radioactivity, by using external standardization and predetermined efficiency curves.

**Mathematical Analysis of Data**

Arterial blood concentrations (\(C_{\text{blood}}\)dpm/ml) that were measured during i.v. infusion of either \(^{14}\)C-antipyrine or \(^{131}\)I-iodoantipyrine were plotted against time and were fit by a non-linear, least-squares procedure with the following equation:

\[
C_{\text{blood}} = A + B e^{-Rt} + D e^{-St}
\]  

A, B, C, R, and S are constants in Eq. 1 and \(A + B + C = 0\), because \(C_{\text{blood}} = 0\) at \(t = 0\) (time when blood concentration starts to rise). Computer fitting provided numerical estimates of the constants from the arterial plasma curve (fig. 1 below).

As shown by Eckman et al., a transfer constant \(K\) can be defined as follows: where \(F = \text{LCBF}, \lambda = \text{steady state, tissue:blood partition coefficient for a
particular tracer, and \( m \) is a constant between 0 and 1 that represents the extent to which diffusional equilibrium is established between tissue and blood,

\[
K = \frac{mF}{\lambda}
\]  

(2)

Local diffusional equilibrium has to be established for the Kety approach to be valid, in which case \( m \) approximates 1 and \( K = F/X \). \( F \) then can be obtained by a least squares fit of the following equation to the data, where \( T = \) time of brain sampling and \( C_{\text{brain}}(T) = \) tracer concentration (dpm/g) in brain parenchyma (excluding intravascular concentration) at time \( T \), and only \( K \) is unknown,

\[
\frac{C_{\text{brain}}(T)}{X} = A - \frac{(A + BK/[K - R] + DK/[K - S])e^{-RT} + (K/[K - R])Be^{-RT} + (K/[K - S])De^{-ST}}{[K - S]} (3)
\]

\( K \) was calculated with Eq. 3 by a non-linear iterative least squares process that employed the MLAB program on a PDP-10 computer. \( F \) was obtained from \( K \) by Eq. 2, letting \( m = 1 \) and \( X = 0.9 \) for \( ^{14} \text{C-antipyrine} \) and 0.8 for \( ^{14} \text{C-iodoantipyrine}. \)

Results

Figure 1 illustrates blood concentrations of \( ^{14} \text{C-iodoantipyrine} \) during 45 sec of infusion, as well as the least squares fit of Eq. 1 to these data. The constants that were derived by the fit were inserted into Eq. 3, together with \( m = 1 \), \( \lambda = 0.9 \) and \( C_{\text{brain}}(T) \). \( C_{\text{brain}}(T) \), which represents intraparenchymal brain concentration of tracer, was obtained by subtracting intravascular from net regional radioactivity when the former quantity was taken as the product of regional blood volume and blood concentration (dpm/ml). Regional blood flows at the pineal and pituitary glands were taken as 5% of wet wt.\(^{15,19} \)

Mean hematocrit, arterial pH, \( P_{\text{aO}_{2}} \) and \( P_{\text{aCO}_{2}} \) did not differ significantly between experimental groups (\( P > 0.05 \)). For 7 animals infused either with \( ^{14} \text{C-antipyrine} \) or \( ^{14} \text{C-iodoantipyrine} \), means \( \pm \) sem's were as follows: hematocrit = 48.0 \( \pm 0.24\% \), \( P_{\text{aO}_{2}} = 38.4 \pm 1.8 \text{ mm Hg} \), \( P_{\text{aCO}_{2}} = 83.8 \pm 3.2 \text{ mm Hg} \) and pH = 7.41 \( \pm 0.013 \) units. For 10 animals administered \( ^{3} \text{H-nicotine} \), means \( \pm \) sem's were: hematocrit = 47.4 \( \pm 0.81\% \), \( P_{\text{aO}_{2}} = 39.2 \pm 0.46 \text{ mm Hg} \), \( P_{\text{aCO}_{2}} = 84.1 \pm 0.81 \text{ mm Hg} \) and pH = 7.41 \( \pm 0.02 \) units.

Table 1 lists mean LCBF's that were calculated by Eqs. 1 and 3 from data obtained with \( ^{14} \text{C-antipyrine} \) and \( ^{14} \text{C-iodoantipyrine} \). The results confirm the original findings of Sakurada et al.\(^{4} \) that \( ^{14} \text{C-iodoantipyrine} \) provides about 2-fold higher values of LCBF than does \( ^{14} \text{C-antipyrine} \). Furthermore, the LCBF's in

**TABLE 1**

<table>
<thead>
<tr>
<th>Structure</th>
<th>Local Cerebral Blood Flow, cm(^3)g(^{-1})min(^{-1}) ( ^{14} \text{C-Antipyrine} )</th>
<th>( ^{14} \text{C-Iodoantipyrine} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olfactory bulb</td>
<td>0.59 ( \pm 0.03 )(6)(^{a,b} )</td>
<td>1.02 ( \pm 0.10 )(6)</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>0.62 ( \pm 0.04 )^{b}</td>
<td>1.80 ( \pm 0.19 )</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>0.60 ( \pm 0.05 )^{a}</td>
<td>1.38 ( \pm 0.16 )</td>
</tr>
<tr>
<td>Frontal lobe</td>
<td>0.73 ( \pm 0.05 )^{a}</td>
<td>1.68 ( \pm 0.29 )</td>
</tr>
<tr>
<td>Occipital lobe</td>
<td>0.70 ( \pm 0.06 )^{a}</td>
<td>1.80 ( \pm 0.29 )</td>
</tr>
<tr>
<td>Thalamus + hypothalamus</td>
<td>0.62 ( \pm 0.05 )^{b}</td>
<td>1.50 ( \pm 0.17 )</td>
</tr>
<tr>
<td>Superior colliculus</td>
<td>0.67 ( \pm 0.05 )^{b}</td>
<td>1.68 ( \pm 0.17 )</td>
</tr>
<tr>
<td>Inferior colliculus</td>
<td>0.80 ( \pm 0.08 )^{a}</td>
<td>2.04 ( \pm 0.25 )</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.58 ( \pm 0.04 )^{b}</td>
<td>1.02 ( \pm 0.11 )</td>
</tr>
<tr>
<td>Pons</td>
<td>0.56 ( \pm 0.04 )^{b}</td>
<td>1.26 ( \pm 0.12 )</td>
</tr>
<tr>
<td>Medulla</td>
<td>0.49 ( \pm 0.03 )^{b}</td>
<td>1.20 ( \pm 0.13 )</td>
</tr>
<tr>
<td>Gray matter, parietal</td>
<td>0.86 ( \pm 0.08 )^{b}</td>
<td>2.40 ( \pm 0.37 )</td>
</tr>
<tr>
<td>White matter, corpus callosum</td>
<td>0.43 ( \pm 0.04 )^{a}</td>
<td>1.14 ( \pm 0.14 )</td>
</tr>
<tr>
<td>Pineal gland</td>
<td>0.61 ( \pm 0.40 )</td>
<td>—</td>
</tr>
<tr>
<td>Pituitary gland</td>
<td>0.74 ( \pm 0.16 )</td>
<td>1.04 ( \pm 0.15 )</td>
</tr>
</tbody>
</table>

\(^{a}\text{Mean} \pm \text{SEM (NO. of experiments in column is in parenthesis).} \)

\(^{b}\text{Differ significantly from} ^{14} \text{C-iodoantipyrine mean (} \text{p <0.05).} \)
The Kety analysis, which assumes diffusional equilibrium between plasma and brain, cannot be employed if the tracer is bound or incorporated within the brain as is \(^2\)H-nicotine.\(^{10,17}\) We therefore estimated LCBF with \(^2\)H-nicotine when assuming complete extraction by brain, and evaluated this assumption at different durations of i.v. infusion. LCBF was calculated as the ratio of net measured regional brain \(^3\)H content at the time of death (T), dpm/g [parenchymal tracer \(C_{brain} (T)\) plus intravascular tracer (\(C_{blood} (T) \times \text{Blood Volume}\))], divided by the integrated blood concentration curve up to this time,

\[
LCBF = \frac{\text{Net brain concentration (T)}}{\int_0^T C_{blood} dt} \tag{4}
\]

Table 2 presents mean LCBF's that were calculated by Eq. 4 at infusion times of 30 to 240 sec, and figure 2 presents these means when normalized to the maximal mean for a specific region, taken at 100% and usually at 50 sec of infusion. In no instance did the 30-sec mean differ significantly from the 50-sec mean LCBF, although LCBF's derived at infusion times of 70 sec or more generally were significantly less than respective 50-sec values (\(P < 0.05\)). With some few exceptions (cf. table 1), 30-sec and 50-sec LCBF's derived with \(^2\)H-nicotine did not differ from LCBF's derived with \(^1\)C-iodoantipyrine but did exceed \(^1\)C-antipyrine LCBF's.

As illustrated by figure 3, binding of \(^3\)H-nicotine by brain was markedly evident at infusion times of 70 sec to 240 sec, when brain concentration of tracer exceeded blood concentration by as much as 4-fold. For infusion times of 50 sec or less, blood concentration usually exceeded brain concentration. This inequality and the large binding of intracerebral tracer reduced back-diffusion from brain to such an extent that Eq. 4 could be used to afford LCBF's that approximated those obtained with \(^1\)C-iodoantipyrine.

**Discussion**

These findings on dissected brain regions confirm conclusions obtained by autoradiographic procedures that \(^1\)C-iodoantipyrine provides higher values of LCBF than does \(^1\)C-antipyrine in the conscious rat.

**Table 2** Calculated LCBF with \(^3\)H-nicotine in Relation to i.v. Infusion Time. Mean LCBF's at Infusion Times of 30, 70, 180, 240 sec were Compared to LCBF's at 50 sec by a Multiple Comparisons Procedure, Using Bonferroni T Statistics.\(^{38}\)

<table>
<thead>
<tr>
<th>Structure</th>
<th>30</th>
<th>50</th>
<th>70</th>
<th>120</th>
<th>240</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(cm³ g⁻¹ min⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Olfactory bulb</td>
<td>1.11 ± 0.12(6) b,c</td>
<td>1.23 ± 0.08(10) b</td>
<td>0.87 ± 0.06(6) b</td>
<td>0.78 ± 0.08(3) b</td>
<td>0.73 ± 0.07(3) b</td>
</tr>
<tr>
<td>Caudate nucleus</td>
<td>1.17 ± 0.11d</td>
<td>1.28 ± 0.08c,d</td>
<td>1.01 ± 0.10c</td>
<td>0.77 ± 0.19b</td>
<td>0.51 ± 0.08b</td>
</tr>
<tr>
<td>Hippocampus</td>
<td>1.04 ± 0.10b</td>
<td>1.14 ± 0.08b</td>
<td>0.84 ± 0.07</td>
<td>0.85 ± 0.11</td>
<td>0.71 ± 0.06b</td>
</tr>
<tr>
<td>Frontal lobe</td>
<td>1.74 ± 0.16a</td>
<td>1.82 ± 0.17a</td>
<td>1.23 ± 0.15</td>
<td>1.04 ± 0.28</td>
<td>0.95 ± 0.07b</td>
</tr>
<tr>
<td>Occipital lobe</td>
<td>1.57 ± 0.15b</td>
<td>1.80 ± 0.12c</td>
<td>1.44 ± 0.13b</td>
<td>0.85 ± 0.17b</td>
<td>0.54 ± 0.07b</td>
</tr>
<tr>
<td>Thal. + hypothal.</td>
<td>1.03 ± 0.08c,d</td>
<td>1.23 ± 0.07e</td>
<td>0.87 ± 0.06b</td>
<td>0.83 ± 0.15b</td>
<td>0.77 ± 0.04b</td>
</tr>
<tr>
<td>Sup. colliculus</td>
<td>1.08 ± 0.12d</td>
<td>1.26 ± 0.08d</td>
<td>0.86 ± 0.05b</td>
<td>1.04 ± 0.16</td>
<td>0.78 ± 0.04b</td>
</tr>
<tr>
<td>Inf. colliculus</td>
<td>1.28 ± 0.16c</td>
<td>1.36 ± 0.07c</td>
<td>1.03 ± 0.06</td>
<td>1.03 ± 0.11</td>
<td>0.80 ± 0.04b</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>0.79 ± 0.09</td>
<td>0.91 ± 0.04e</td>
<td>0.71 ± 0.04b</td>
<td>0.64 ± 0.08b</td>
<td>0.58 ± 0.05b</td>
</tr>
<tr>
<td>Pons</td>
<td>0.95 ± 0.07a</td>
<td>1.11 ± 0.07c</td>
<td>0.78 ± 0.05b</td>
<td>0.80 ± 0.07b</td>
<td>0.62 ± 0.03b</td>
</tr>
<tr>
<td>Medulla</td>
<td>0.85 ± 0.09e</td>
<td>1.10 ± 0.06e</td>
<td>0.75 ± 0.05b</td>
<td>0.81 ± 0.05c</td>
<td>0.62 ± 0.04b</td>
</tr>
<tr>
<td>Gray matter</td>
<td>2.38 ± 0.19c</td>
<td>2.81 ± 0.21c</td>
<td>1.72 ± 0.16c</td>
<td>1.23 ± 0.37b</td>
<td>1.08 ± 0.03b</td>
</tr>
<tr>
<td>White matter</td>
<td>0.59 ± 0.05c,d</td>
<td>0.73 ± 0.06c,d</td>
<td>0.53 ± 0.05c</td>
<td>0.44 ± 0.12b</td>
<td>0.51 ± 0.03</td>
</tr>
<tr>
<td>Fisure gland</td>
<td>4.16 ± 1.54</td>
<td>2.50 ± 0.36</td>
<td>1.63 ± 0.53</td>
<td>1.91 ± 1.04</td>
<td>1.57 ± 0.21</td>
</tr>
<tr>
<td>Pituitary gland</td>
<td>1.21 ± 0.24</td>
<td>1.28 ± 0.08</td>
<td>0.96 ± 0.08</td>
<td>1.03 ± 0.19</td>
<td>0.99 ± 0.15</td>
</tr>
</tbody>
</table>

*Mean ± SEM (No. of experiments in column is in parenthesis); \(b\) Differs significantly from 50-sec \(^3\)H-nicotine mean (\(p < 0.05\)); \(c\) Differs significantly from \(^1\)C-antipyrine mean in table 1 (\(p < 0.05\)); \(d\) Differs significantly from \(^1\)C-iodoantipyrine mean in table 1 (\(p < 0.05\)), where only 30-sec and 50-sec \(^3\)H-nicotine means are considered.
...those obtained with $^{131}$I-trifluoriodomethane. 4 $^{14}$C-iodoantipyrine would appear to be the non-gaseous agent of choice for exact measurement of LCBF, not only because it gives values comparable to those with $^{131}$I-trifluoriodomethane, but also because it is long-lived within blood, not significantly metabolized within 1 min, commercially available and exhibits a tissue-blood partition coefficient that is uniform throughout the brain and is independent of hematocrit. 4

Due to its possible back-diffusion from brain to blood, $^3$H-nicotine cannot be used with certainty to obtain LCBF. However, the equivalent values of LCBF as derived with 30 or 50 sec of infusion of $^3$H-nicotine, as compared to those provided by $^{14}$C-iodoantipyrine and the Kety analysis, demonstrate that back-diffusion was minimal for infusion times of up to 50 sec, and that Eq. 4 then can be used to derive LCBF precisely. The lower LCBF's obtained with $^3$H-nicotine than with $^{14}$C-iodoantipyrine in occasional regions (thalamus + hypothalamus at 30 sec of infusion; superior colliculus, caudate nucleus and white matter at 30 and 50 sec), may have been due to incomplete extraction of $^3$H-nicotine by those regions or to some back-diffusion into blood.

For 120 sec or 240 sec of $^3$H-nicotine infusion, delivery rate may be less important than tissue uptake in calculating LCBF. The higher calculated LCBF of gray as compared to white matter reflects in part specific, reversible uptake of $^3$H-nicotine by cell bodies and nerve endings, in part at cholinergic receptors. Uptake is lowered in vivo by pentobarbital anesthesia, and is reduced in brain slices by lowering temperature. 16, 22-27 Most of the $^3$H in the brain at 240 sec is associated with $^3$H-nicotine; less than 3% is due to $^3$H-cotinine, a metabolic derivative of $^3$H-nicotine that is produced in liver and kidney and appears in blood about 180 sec after an intravenous injection, but is poorly permeable at the blood-brain barrier. 26, 28

The pineal and pituitary glands are outside of the blood-brain barrier system. 29 Local blood flows in these glands, as measured with $^{14}$C-iodoantipyrine and $^3$H-nicotine, are comparable to flows derived by analyzing the distribution of cardiac output to these glands — 2 to 3.43 cm$^3$g$^{-1}$min$^{-1}$ at the pineal gland, 1.01 cm$^3$g$^{-1}$min$^{-1}$ at the anterior pituitary and 3.74 cm$^3$g$^{-1}$min$^{-1}$ at the posterior pituitary. 18, 30, 31 $^{125}$I-antipyrine gives flows of 1.2 cm$^3$g$^{-1}$min$^{-1}$ at the anterior pituitary and 2.36 cm$^3$g$^{-1}$min$^{-1}$ at the posterior pituitary of the conscious rat. 29

LCBF closely correlates with regional cerebral metabolism under many physiological conditions in the same animal, and when comparing brains of different species. 23 Both LCBF and cerebral energy metabolism are higher, for example, in the rat than in many other species, possibly because cellular packing is greater in the rat brain. 4, 34-36 If $^3$H-nicotine binding by brain is a measure of density of cells and nerve endings, then short-term experiments (50 sec or less) with nicotine might be used to estimate LCBF and long-term experiments (5 min or more) to estimate density of cells and nerve endings. 18, 22-27 Nicotine, when incorporating the positron emitter $^{13}$N, might also be used in man to measure regional blood flow by means of emission computed tomography. 27

![Figure 3](http://stroke.ahajournals.org/)

**Figure 3.** $^3$H concentration (dpm/ml) in whole blood of rats during i.v. infusion of $^3$H-nicotine for 30 sec to 240 sec, as well as regional brain parenchymal concentrations, $C_{brain}$ dpm/g, at time of death in individual experiments. Concentrations were normalized to the 30-sec blood concentration (taken as 1).

$^{14}$C-iodoantipyrine also furnishes higher LCBF's than does the hydrogen clearance method. For example, at the frontal cortex of the conscious rat, LCBF equals 0.79 ± 0.22 (SEM) cm$^3$g$^{-1}$min$^{-1}$ with the hydrogen clearance method, 31 but equals 1.68 ± 0.29 cm$^3$g$^{-1}$min$^{-1}$ with $^{14}$C-iodoantipyrine (table 1).

The Kety method 1, 2 was designed for use with an inert gas, such as $^{131}$I-trifluoriodomethane, that can reach almost instantaneous equilibrium between brain and blood. This condition is not satisfied by $^{14}$C-iodoantipyrine, which provides LCBF's that compare with those obtained with $^{131}$I-trifluoriodomethane. 4 $^{14}$C-iodoantipyrine would appear to be the non-gaseous agent of choice for exact measurement of LCBF, not only because it gives values comparable to those with the inert gas, but also because it is long-lived within blood, not significantly metabolized within 1 min, commercially available and exhibits a tissue-blood partition coefficient that is uniform throughout the brain and is independent of hematocrit. 4
Acknowledgment

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References

Local cerebral blood flow in the conscious rat as measured with 14C-antipyrine, 14C-iodoantipyrine and 3H-nicotine.
K Ohno, K D Pettigrew and S I Rapoport

Stroke. 1979;10:62-67
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