Measurement of regional cerebral blood flow with antipyrine-14C in awake cats.
M Reivich, J Jehle, L Sokoloff and S S Kety

This article has been cited by 14 other HighWire-hosted articles:
http://jap.physiology.org/content/27/2/296.citation#cited-by

Updated information and services including high resolution figures, can be found at:
http://jap.physiology.org/content/27/2/296.citation.full

Additional material and information about Journal of Applied Physiology can be found at:
http://www.the-aps.org/publications/jappl

This information is current as of February 28, 2013.
Measurement of regional cerebral blood flow with antipyrine-14C in awake cats

MARTIN REIVICH, JANE JEHLE, LOUIS SOKOLOFF, AND SEYMOUR S. KETY
Laboratory of Clinical Science, National Institute of Mental Health, National Institutes of Health, Bethesda, Maryland 20014

Reivich, Martin, Jane Jehle, Louis Sokoloff, and Seymour S. Kety. Measurement of regional cerebral blood flow with antipyrine-14C in awake cats. J. Appl. Physiol. 27(2): 296-300. 1969.—Antipyrine-14C was utilized for the quantitative measurement of regional cerebral blood flow in cats by means of an autoradiographic technique. The advantages of this technique over a previously described method are the uniformity of the brain-blood partition coefficient for antipyrine, the better resolution of the autoradiograms, the ability to make permanent 14C standards, the ability to obtain adjacent sections for histological studies, and the exposure of the autoradiograms at room temperature rather than at -70 C. Errors in the determination of the tissue concentration of antipyrine-14C can arise from several sources. Those due to errors in replicate optical density measurements plus variations in section thickness were found to be 3.6%. The standards used introduced only a small error since the standard deviation of replicate standards was no greater than 1.1%. Regional cerebral blood flow was measured in a series of six awake, unrestrained resting cats. The values varied from 0.21 ml/g per min in the cerebral white matter to 1.74 ml/g per min in the inferior colliculus. Arterial Pco2 was 34 ± 2.9 mm Hg, PO2 was 102 ± 8.4 mm Hg, pH was 7.44 ± .01, and hematocrit was 29 ± 6.6%.

Antipyrine-14C was used as the inert tracer in the present studies for several reasons. Antipyrine is freely diffusible in total body water and its distribution in the body is proportional to the water content of the various tissues (1). This suggested that the brain-blood partition coefficient might be similar for all regions of the brain. The compound is very slowly metabolized in the liver (6% per hr) so that the possible error from this source would be negligible in the 1 min duration of these studies.

The use of a 14C label has several advantages. It is a pure beta-emitter of low energy (0.155 Mev) and therefore produces autoradiograms of better resolution than 131I, which, in addition to having a higher energy beta-emission (0.608 Mev), is also a gamma-emitter. Furthermore, the long half-life of 14C enables permanent standards to be made. The long half-life also allows a smaller dose of isotope to be used since longer exposure times are possible.

The use of a nongaseous tracer obviated its possible loss from the surface of the sections thus making it possible to use a cryostat to cut 20-μ-thick sections. This allowed serial sections to be obtained so that histological studies could be undertaken along with the autoradiographic studies. Since the tracer was nongaseous, the autoradiograms could be made at room temperature instead of -70 C. Further modifications included the blood sampling technique and the use of a liquid scintillation counter for determining the 14C concentration in the blood. In addition a program was written for a digital computer to perform the calculations necessary in computing the regional blood flow values.

METHODS

The technique like its predecessor, is based on the Fick principle, which for a biologically inert diffusible tracer substance may be stated as follows: the time rate of change of the amount of tracer substance within the tissue is equal to the difference between the rate at which the substance is brought to the tissue in the arterial blood and removed from it in the venous blood. It can be shown that this can be expressed mathematically as follows (4):

\[ C_s(T) = \frac{1}{V_s} \int_0^T C_a e^{-k(T-t)} \, dt \]  

where \( C_s(T) \) is the concentration of tracer substance in the tissue at time \( T \); \( \lambda \) is the tissue-blood partition coefficient for the tracer material; \( k_1 \) is the rate of blood flow per unit weight of tissue multiplied by the reciprocal of the partition coefficient for that tissue; and \( C_a \) is the concentration of tracer substance in the arterial blood. Thus, to calculate the blood flow to a given homogenous region of the brain one must know: 1) the tissue-blood partition coefficient of the tracer substance for that region of the brain, 2) the concentration of the tracer substance in that region of the brain at some time, \( T \), which in these studies was 1 min after the start of the infusion, and 3) the time course of change of arterial concentration of tracer material.

Determination of brain-blood partition coefficient. Since antipyrine is both excreted unchanged in the urine and metabolized in the
REGIONAL CEREBRAL BLOOD FLOW IN CATS

liver (l), to obtain a steady state for the determination of the brain-blood partition coefficient the arterial blood supply to the kidneys and the venous outflow from the liver was occluded by ligatures in a series of 15 rats. Antipyrine-14C was then injected intravenously. At the end of 1 hr, when equilibrium was reached, a blood sample was obtained by cardiac puncture and the animal was decapitated and the brain removed and frozen in a mixture of acetone and Dry Ice at -30 C. The frozen brain was then cut in half sagitally. From one-half of the brain 20-μ sections were cut in a cryostat to be used for the preparation of autoradiograms as described below. From the other half of the brain samples of white and gray matter were obtained in which the concentration of antipyrine-14C was determined directly by counting techniques. The latter samples were weighed, lyophilized in a freeze-dry apparatus, dissolved in Hyamine, and counted in a toluene-ethanol phosphor solution in a liquid scintillation counter. The blood samples were counted as described below.

The means and standard errors of 17 determinations of the brain-blood partition coefficient in gray and white matter were 1.00 ± 0.026 and 0.97 ± 0.026, respectively. There was no significant difference between these values (0.4 > P > 0.3). Therefore, the average, 0.99, was used as the value of the brain-blood partition coefficient. There was no difference for the value of the partition coefficient among various white or gray structures throughout the entire brain, as demonstrated by the autoradiograms which showed a homogeneous density throughout (Fig. 1).

Collection and counting of blood samples. Arterial blood samples were obtained via a PE-50 polyethylene catheter placed in the femoral artery of the cat and advanced into the descending aorta. An automatic sampling device consisting of a rotating stopcock arrangement shown in Fig. 2 allowed us to collect 0.04-ml samples every 6 sec. These were collected consecutively as individual spots of blood on a strip of no. 3 Whatman filter paper. Each spot was cut out and placed in a counting vial containing 10 ml of naphthalene-dioxide phosphor solution and 1 ml of water. The vials were placed on a mechanical shaker for 24 hr and then counted in a liquid scintillation counter. This period of time is more than adequate since studies of the time course of elution of the antipyrine-14C from the filter paper in 11 samples revealed a time constant whose mean and standard error were 6.2 ± 0.2 hr even without shaking. Therefore, in 24 hr at least 98% of the antipyrine-14C is eluted. The process of elution probably is even faster when a shaker is used. Further studies showed that the presence of the filter paper itself in the counting vial did not cause quenching. However, the blood spot did cause a slight amount (4%) of quenching. Therefore, standards were made up containing a known amount of antipyrine-14C in the blood spot and were counted with the unknown samples. Alternatively, an internal 14C standard can be used.

Removal and sectioning of brain. At the appropriate time, the animals were killed by the rapid intravenous injection of saturated KCl via a catheter placed far up the inferior vena cava. Blood pressure dropped within 5 sec to levels inadequate to sustain any cerebral perfusion. The brains were then rapidly removed and frozen in acetone-Dry Ice at -30 C. Studies have shown that there is no detectable loss of resolution in the autoradiograms by removing the brain first and then freezing it rather than freezing the whole head and then removing the frozen brain. The frozen brain was then cut grossly into two coronal sections and frozen to microtome chucks with 5% gum tragacanth. Twenty-micron sections were then cut in a cryostat at -19 C. Every 25th section was taken for autoradiograms and every 26th for histology. The sections were picked up from the knife with a coverslip at room temperature and immediately placed on a hot plate at 60 C (7). The sections dried within 2-3 sec. Studies were performed comparing the autoradiograms obtained by this method with those obtained from sections which were kept frozen at all times. There was no apparent loss of resolution with this quick-drying method. The coverslip containing the dried section was then affixed to a glass slide with Krylon (Krylon no. 1303, Krylon, Inc., Norristown, Pa.) so that the section was exposed. In a dark room, sections were placed face down on Kodak single-emulsion medical X-ray film in X-ray cassettes. A set of standards containing known concentrations of antipyrine-14C was also placed on each film so that a calibration curve could be obtained for each film relating optical density to 14C concentration. These standards corrected for any differences in exposure and developing that occurred between films. In Fig. 3 are illustrated some autoradiograms obtained with this technique.

Preparation of autoradiographic standards. The standards which were placed on each film were made from the brains of rats that had been prepared as described above for the determination of the brain-blood partition coefficient. Rat-brain standards were used because less isotope was required to prepare them and the absorption characteristics should be very similar to that of brain from other species. In a series of 15 animals, various amounts of antipyrine-14C were injected intravenously, so that the concentration in the brain at the end of 1 hr varied from 25 to 2,100 mcg/g. The brains were removed and frozen 1 hr after the intravenous injection and cut in half sagitally. From one-half brain, 20-μ sections were cut and used as standards, and from the other half samples of brain tissue were taken and assayed for antipyrine-14C concentration as described above. The standards prepared in this manner are good for only 4-6 weeks since, being unfixed tissue, they begin to deteriorate and changes in the antipyrine-14C concentration occur. Therefore, permanent standards were made of plastic containing antipyrine-14C which were calibrated against freshly pre-

FIG. 1. Autoradiograms prepared from coronal sections of a rat brain 1 hr after the intravenous injection of antipyrine-14C. A: through the anterior frontal lobes; B: through the posterior frontal lobes at the level of the optic chiasm; C: through the cerebellum and brain stem. Dark areas at the edges in A and B are artifacts due to folding over of the section.
pared rat-brain standards to correct for differences in the absorption characteristics between plastic and brain tissue. The values of the plastic standards ranged from 190 to 1,990 mpc/g in units equivalent to brain concentration. A blank was also placed on each film which consisted of a plastic standard which contained no radioactivity.

Errors in determination of tissue concentration of antipyrine-14C. Several factors may contribute to errors in the determination of the tissue concentration of antipyrine-14C from autoradiograms. Since the sections are less than infinite thickness for 14C, variation in the thickness of each 20-μ section will produce apparent changes in antipyrine-14C concentration. Also, the variability in duplicate readings of the optical density of the autoradiograms contributes an error. The magnitude of the error from these two sources was determined by making multiple readings on a series of 16 sections cut from one of the rat-brain standards. The variation within sections was 1.9% of the mean optical density value, and the variation between sections was 5.6%. The variation within sections is probably due mainly to errors in making duplicate optical density measurements. The variation between sections includes this error plus the error due to variations in the section thickness. All optical density measurements were made with a Photovolt densitometer model 501 A.

Variability in replicate samples of the standards is another source of error. This was reduced by exposing all the standards on X-ray film and reading their optical density. The most divergent standards in each group were discarded so that the resulting standard deviation of each mean value was no greater than 1.1% of that value.

Catheter smearing correction. The theoretical basis of the method requires that the shape of the curve of arterial concentration of isotope from time zero to the time the tissue concentration is measured be known.

The arterial blood samples are drawn through a PE-50 catheter which can cause distortion of the shape of the arterial curve. Studies were therefore done to determine the smearing characteristics of the catheter and sampling system. A step function change in the concentration of antipyrine-14C was introduced into the catheter as follows. A section of catheter, approximately 15 cm longer than the length to be investigated was attached to the sampler with the other end in a beaker of blood. At a point 15 cm from the end, the catheter was placed under the surface of blood containing antipyrine-14C. As the nonradioactive blood was being pulled through the catheter, the radioactive blood was introduced without any intervening air bubble by cutting the catheter at the 15-cm point. The results of such an experiment are shown in Fig. 4. Similar studies were performed with blood of varying hematocrit since this was found to alter the smearing characteristics of the sampling system. The resulting curves could all be described by an equation of the form (see APPENDIX):

\[
 f_o(t) = 1 - Ae^{-rt_1} + Ae^{-rt_2}
\]

Fig. 3. Autoradiograms prepared from coronal sections of the brain of an awake unrestrained cat. A: at the level of the caudate nucleus, septal area, and the putamen; B: at the level of the lateral geniculate body, cerebral peduncle, optic tract, and hippocampus; C: through the inferior colliculi, aqueduct, pons, and posterior suprasylvian gyrus; D: through the cerebellum and medulla oblongata. The darker the region the higher is the regional blood flow.

\[
 G(t) = \frac{C_i(T)}{A_i(T)}
\]
REGIONAL CEREBRAL BLOOD FLOW IN CATS

The values for these functions are shown in Table 2 and compared with the only other available data for awake intact cats (2). Table 3 shows the values obtained for the flow to various regions of the brain. They are compared with the values obtained with the original method as described by Landau et al. Of the 23 regions compared, in only 3 was there a significant difference between the two methods of measuring regional cerebral blood flow, and the present technique offers numerous advantages in convenience, accuracy, and amount of information obtainable.

TABLE 2. Arterial blood constituents

<table>
<thead>
<tr>
<th>Component</th>
<th>Present Study</th>
<th>Fink and Schoolman (2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PO2 (mm Hg)</td>
<td>34±2.9 28±0.7</td>
<td>102±6.4 7.44±0.01 7.38±0.01</td>
</tr>
<tr>
<td>pH</td>
<td>29±6.0 29±1.8</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE.

TABLE 3. Regional cerebral blood flow in awake cats

<table>
<thead>
<tr>
<th>Region</th>
<th>Landau et al. (5) 10 Cats</th>
<th>Present Study 6 Cats</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebellum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nuclei</td>
<td>0.79±0.05</td>
<td>0.84±0.03</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Cortex</td>
<td>0.69±0.04</td>
<td>0.83±0.03*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>White matter</td>
<td>0.24±0.01</td>
<td>0.24±0.01</td>
<td></td>
</tr>
<tr>
<td>Medulla</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vestibular nuclei</td>
<td>0.91±0.04</td>
<td>0.92±0.03</td>
<td></td>
</tr>
<tr>
<td>Cochlear nuclei</td>
<td>0.87±0.07</td>
<td>0.95±0.11</td>
<td></td>
</tr>
<tr>
<td>Pyramid</td>
<td>0.26±0.02</td>
<td>0.25±0.02</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± SE, given in ml/g per min. * Significant differences. All other pairs of values not significantly different.

APPENDIX

The relationship between the true arterial time course of isotope concentration, $c_a(t)$, and this function after it has been smeared on the input function produces the output function $c_a'(t)$. The function $f_a(t)$ was calculated with the IBM 1620 computer to calculate by means of the trapezoid rule the value of $C_i(T)$ for various values of $k$; from 0 to 3.8 ml/min per g. This table was then used to find the flow for given $C_i(T)$.

RESULTS

With the technique as described above, regional cerebral blood flow was measured in six awake unrestrained resting cats. The animals were kept in a lighted box 1 x 2 ft square and 2 ft high containing a window at each end and which was ventilated from a compressed air source. Catheters had been placed in the femoral artery and vein 4-7 days before the experiment and kept open with a heparin solution. Immediately before the regional blood flow measurement, an arterial blood sample was drawn and analyzed for PO2, PCO2, pH, and hematocrit.

The determinations of pH, PCO2, and PO2 were made by means of an Instrumentation Laboratory system (model 113-S2) utilizing a glass pH electrode (no. 11070) with a calomel reference electrode (no. 13077), a Severinghaus PO2 electrode, and a Clark PO2 electrode. The electrodes were contained in a water bath at 37 C which was thermostatically controlled to within ±0.02 C. The electrodes were calibrated before each determination and an appropriate standard was read after each determination. National Bureau of Standards buffers were used to calibrate the pH electrode. The PO2 electrode was calibrated with gas mixtures containing known concentrations of CO2 which had been determined with a Scholander apparatus (6). The PO2 electrode was calibrated with blood samples that had been tonometered with gas mixtures of known CO2 concentrations which had been determined with a Scholander apparatus. The hematocrit was measured by means of a Guest-Weichselbaum micro hematocrit apparatus.

The values for these functions are shown in Table 2 and compared with the only other available data for awake intact cats (2). Table 3 shows the values obtained for the flow to various regions of the brain. They are compared with the values obtained with the original method as described by Landau et al. Of the 23 regions compared, in only 3 was there a significant difference between the two methods of measuring regional cerebral blood flow, and the present technique offers numerous advantages in convenience, accuracy, and amount of information obtainable.
is described by the equation

\[ T = 1 - A_1 \frac{s}{s + r_1} + A_2 \frac{s}{s + r_2} \]

or since \(1 - A_1 + A_2 = 0\)

\[ T = \frac{A_1 r_1}{s + r_1} - \frac{A_2 r_2}{s + r_2} \]

Assuming the first derivative of \(f_o(t)\) is zero at time zero, then

\[ A_1 r_1 - A_2 r_2 = 0 \]

and

\[ T = \frac{r_1 r_2}{s^2 + (r_1 + r_2)s + r_1 r_2} \]

or in the time domain

\[ C_i(t) = \left[ \frac{1}{r_1 r_2} D^2 + \frac{r_1 + r_2}{r_1 r_2} D + 1 \right] c_o(t) \]

where \(C_i(t)\) is the input function and \(c_o(t)\) the output function. Substituting equation 11 into equation 1 above

\[ C_i(t) = \lambda_i K_i \int_0^T \left[ \frac{1}{r_1 r_2} D^2 + \frac{r_1 + r_2}{r_1 r_2} D + 1 \right] c_o(t) e^{-\lambda_i (T-t)} \, dt \]  

(12)

which after separation into three integrals and integrating by parts produces the final solution:

\[ C_i(t) = \frac{\lambda_i K_i}{r_1 r_2} \int_0^T c_o(t) e^{-\lambda_i (T-t)} \, dt \]

(13)

In testing the operator shown in equation 11 by operating on the generalized function:

\[ f(t) = 1 + Ae^{-\lambda_1 t} + Be^{-\lambda_2 t} \]

it can be shown that the operator converts this function to a step function.

This work was supported, in part, by Public Health Service Research Grant NB-06314.

M. Reivich is the recipient of Public Health Service Career Research Development Award K3-HV-11896. Present address: Dept. of Neurology, School of Medicine, University of Pennsylvania, Philadelphia, Pa. 19104.

Received for publication 31 January 1969.

REFERENCES


