nC-Iodoantipyrine for the Measurement of Regional Cerebral Blood Flow by Positron Emission Tomography

Validation Studies

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SUMMARY Positron emission tomography (PET) makes it possible to employ an in vivo autoradiographic paradigm to measure regional cerebral blood flow (rCBF) in man. In this study, we synthesized the positron-emitting radiopharmaceutical \(^{11}\)C-iodoantipyrine (\(^{11}\)C-IAP) and validated its suitability as a CBF tracer. \(^{11}\)C (T_1/2 = 20.4 min) was produced by the \((p,\alpha)\) nuclear reaction on \(^{14}\)N. \(^{11}\)C-methyl iodide was used to methylate 3-methyl-1-phenyl-2-pyrazolin-5-one to form \(^{11}\)C-antipyrine, which was iodinated. Radiochemical purity of the \(^{11}\)C-IAP product was 93-98% except as described below. rCBF was measured with \(^{11}\)C-IAP in nitrous oxide-anesthetized Wistar rats by the method of Indicator Fractionation, and values were compared with rCBF values measured with simultaneously administered commercially produced \(^{11}\)C-IAP. rCBF was studied over a range of arterial Pco, values (31-58 mm Hg, mean 43.0 ± 3.5). Mean rCBF data for the 2 tracers agreed to within 4.8% for cerebral hemispheric samples, 3.8% for cerebellum, and 5.3% for brainstem. Mean values (± SEM) for rCBF using \(^{11}\)C-IAP were 1.67 ± 0.20 ml gm⁻¹ min⁻¹ for cerebral hemispheres; 1.31 ± 0.17 for cerebellum; and 1.50 ± 0.21 for brainstem. When chromatographic analysis revealed tracer impurity, rCBF, as measured with \(^{11}\)C-IAP, fell consistently below values obtained with \(^{11}\)C-IAP. The data indicate that \(^{11}\)C-IAP, when properly synthesized and submitted to batch-by-batch quality control, may be suitable for measuring rCBF in man by emission tomography.

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POSITRON EMISSION TOMOGRAPHY (PET) has enabled the neuroscientist to visualize and to quantitate, in transverse section, the distribution of radiopharmaceuticals of biological interest within the living human brain. An important but, as yet, incompletely investigated application of PET technology is the quantitative mapping of regional cerebral blood flow (rCBF) in patients with neurological diseases and in normal subjects during functional activation. An interest of our laboratory is the development of a rigorous "in vivo autoradiographic" paradigm for determining rCBF, analogous to the autoradiographic technique for rCBF which has been widely employed in experimental animals (for recent review, see reference 4). The latter method has been applied to the study of blood flow heterogeneities in the normal brain; \(^{15}\) the relationship of rCBF to local brain metabolism; \(^{9}\) and the investigation of rCBF changes produced by physiological alterations \(^{10}\) and by disease states such as cerebral ischemia. \(^{11-18}\)

Iodoantipyrine (IAP) labelled with carbon-14 has recently been demonstrated by Sakurada and co-workers \(^{9}\) to be a highly suitable tracer for autoradiographic rCBF measurements in experimental animals. IAP is a non-polar, metabolically inert, highly diffusible compound of low molecular weight. It has a high lipid solvent:water partition coefficient and a brain:blood equilibrium partition coefficient that is uniform throughout the brain. \(^{15}\) It is significantly less diffusion-limited and hence more accurate in measuring higher rates of rCBF than antipyrine, which was formerly used for rCBF autoradiography. \(^{9}\)

In the present study, iodoantipyrine labelled with the positron-emitting radionuclide carbon-11 has been synthesized and we have validated the suitability of this product for the measurement of rCBF in the rat as a prelude to its possible application as a blood flow tracer in human studies using emission tomography. Preliminary findings have been reported in abstract form. \(^{14}\)

Materials and Methods

Synthesis of \(^{11}\)C-Iodoantipyrine

Carbon-11 (T_1/2 = 20.4 min) was prepared in a CS-30 cyclotron (Cyclotron Corp.) by the \(^{14}\)N(\(p,\alpha\))\(^{11}\)C nuclear reaction. "No-carrier-added" \(^{11}\)CO\(_2\) was synthesized by the method of Finn and Wolf, \(^{15}\) and \(^{11}\)C-methyl iodide was prepared from it by reduction with lithium aluminum hydride and distillation of the labelled methanol through hydriodic acid. This, in turn, was reacted with 3-methyl-1-phenyl-2-pyrazolin-
S-one to produce 14C-antipyrine plus other, undesired, methylated products which were removed on a silica gel column. 14C-iodoantipyrine was then prepared by iodination of 14C-antipyrine and was separated from the latter and from inorganic iodine residues on a silica gel column. The product was sterilized by Millipore filtration. Analyses by means of thin-layer chromatography and high performance liquid chromatography confirmed the product to be 14C-iodoantipyrine with a radiochemical purity of 93-98%, except in the special instances to be described below. Specific activity was approximately 30 Ci/m mole. Campbell and co-workers have presented a complete account of this synthesis.18

Animal Preparation

Wistar rats weighing 300-400 gm were anesthetized with diethyl ether, tracheostomized, immobilized with d-tubocurarine (1.8 mg i.p.), and ventilated mechanically on mixtures of 70% nitrous oxide and 30% oxygen. Catheters were inserted into one external jugular vein and one or both femoral arteries. Mean arterial blood pressure was recorded by a transducer (Statham) and displayed on an analog meter (Stentor). Arterial blood gases were monitored periodically with a multi-electrode analyzer system (Corning). Rectal temperature was maintained at 37°C by a thermostatically controlled heating lamp.

Regional Cerebral Blood Flow (rCBF)

rCBF was measured by the method of indicator fractionation.19 In these studies, we wished to compare rCBF as measured by 14C-IAP synthesized in our laboratory with rCBF measured using commercially prepared 14C-IAP (4-[N-methyl-14C] — iodoantipyrine, New England Nuclear, specific activity 40-60 mCi/m mole). Thus, a mixture of 100-500 µCi of 14C-IAP and 30 µCi of 14C-IAP dissolved in 300 µl normal saline was injected as a bolus into the external jugular vein via a short catheter. An infusion/withdrawal pump (Harvard Apparatus Co.) was used to withdraw an arterial "reference organ" blood sample at a precalibrated, constant rate (approximately 0.15 ml/min) into a 50 cm-long P.E. 50 polyethylene catheter via a 250 µl microliter syringe (Hamilton). The withdrawal of arterial blood was begun just prior to tracer injection. Seven sec after injection, the animal was decapitated, and the "reference organ" arterial catheter was simultaneously extracted from the animal and its contents delivered into a vial containing 500 µl isopropanol-Soluene-350 (Packard) (1:1, v:v), to which 200 µl hydrogen peroxide was added. The brain was removed and dissected into the two cerebral hemispheres, cerebellum, and brainstem. Each cerebral hemisphere was bisected into anterior and posterior quadrants. All brain samples were weighed. A sodium iodide well counter was used to measure 14C activity of the "reference organ" blood sample and brain samples; this instrument detected the 511 keV emission of 14C but was insensitive to the β-emission of 14C. The 14C count rates were corrected for background activity and for 14C decay.

Measurement of 14C activity was delayed by at least one day to permit complete decay of 14C. 14C activity was assessed by measurement of its β-emission in a liquid scintillation spectrometer. Brain samples were solubilized in Soluene-350 and counted in Dimilume-30 (Packard). The blood sample was counted in Instagel (Packard) acidified with HCl. The external standard ratio method of correction for quenching was employed.

For each tracer, rCBF was calculated from the equation:

\[
\text{rCBF (ml gm^{-1} min^{-1})} = \frac{\text{Brain activity per gm} \times \frac{\text{Ref. organ}}{\text{flow (ml min^{-1})}}}{\text{Ref. organ activity}}
\]

Results

Physiological Data

Mean arterial blood pressure in the 9 animals of this series was 141 ± 5 mm Hg (mean ± SEM). Arterial PCO2 was intentionally varied from 31 to 58 mm Hg in individual animals in order to produce a range of rCBF values. Mean arterial blood values (± SEM) were: P02, 115 ± 8 mm Hg; PCO2, 43.0 ± 3.5 mm Hg; pH, 7.357 ± 0.032.

Regional Cerebral Blood Flow

Figure 1A compares rCBF as measured by 14C-IAP and 14C-IAP in 34 cerebral hemispheral samples from 9 rats. There was excellent agreement between the 2 tracers. Statistical analysis of the data was facilitated by computing a weighted mean cerebral hemispheral blood flow for each animal; these data are shown in figure 1B. Again, there was close agreement between tracers. The paired Student's t-value for the data was 2.71 (p < 0.05). rCBF values were well segregated according to ranges of arterial PCO2 (fig. 1B).

Figure 2 displays rCBF values in cerebellum and brainstem; again, values determined by 14C- and 14C-IAP agreed closely. Table 1 summarizes the rCBF data. Mean rCBF data for the 2 tracers agreed to within 4.8% for cerebral hemispheres, 3.8% for cerebellum, and 5.3% for brainstem. As table 1 demonstrates, mean rCBF values obtained with 14C-IAP were lower than 14C-IAP values for all 3 structures. A Student's t-test revealed no significant differences between the respective 14C and 14C mean values. Nonetheless, the rather large variance associated with each

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Regional Cerebral Blood Flow (rCBF)* (ml gm^{-1} min^{-1})</th>
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<tbody>
<tr>
<td></td>
<td>14C-Iodoantipyrine</td>
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<tr>
<td>Cerebral hemispheres</td>
<td>1.67 ± 0.20</td>
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<tr>
<td>Cerebellum</td>
<td>1.32 ± 0.17</td>
</tr>
<tr>
<td>Brainstem</td>
<td>1.50 ± 0.21</td>
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<td>*Mean ± SEM, n = 8-9.</td>
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<tr>
<td>PCO2 = 43.0 ± 3.5 mm Hg; range 31.1-51.0 mm Hg.</td>
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"C-IAP IN EMISSION TOMOGRAPHY FOR rCBF/Ginsberg et al.

CEREBELLUM

\[ {^{11}}\text{C-CBF} = 0.88 - ^{14}\text{C-CBF} + 0.12 \]

FIGURE 1A. rCBF in 34 cerebral hemispheral samples from 9 rats as measured with "C- and "C-IAP. The dotted line is the line of identity; the solid line is the least-squares fit to the data.

CEREBRAL HEMISPHERES

\[ {^{11}}\text{C-CBF} = 0.89 - ^{14}\text{C-CBF} - 0.11 \]

\[ r = 0.934 \]

\[ p < 0.01 \]

FIGURE 1B. Weighted mean cerebral hemispheral CBF as measured in 9 rats with "C- and "C-IAP. Filled circles represent rats with arterial Pco\textsubscript{2} 31-34 mm Hg; open circles, 37-40 mm Hg; and triangles, 50-58 mm Hg.

mean value (caused by the wide range of Pco\textsubscript{2} values intentionally engendered in these animals) may have obscured the statistical significance of the smaller variation between the "C and "C data sets. To examine this further, a ("C-CBF/"C-CBF) ratio was computed for each analyzed brain sample from each animal, and a mean ratio was calculated for each animal. These values are shown in table 2. The series mean for this ratio is 0.96, and its 95% confidence limits are 0.88-1.04. Thus, the tendency for "C-IAP to underestimate rCBF relative to "C-IAP does not attain statistical significance.

rCBF values were highly correlated with arterial Pco\textsubscript{2}. Figure 3 shows this relationship for mean cerebral hemispheral CBF as measured with "C-IAP in the 9 animals of this series. If one assumes a linear relationship between arterial Pco\textsubscript{2} and rCBF within the range studied, these data indicate a CBF responsiveness of 0.048 ml gm\textsuperscript{-1} min\textsuperscript{-1} per mm Hg change of arterial Pco\textsubscript{2}. The corresponding value as measured

BRAINSTEM

\[ {^{11}}\text{C-CBF} = 0.86 - ^{14}\text{C-CBF} + 0.13 \]

\[ r = 0.934 \]

\[ p < 0.01 \]

FIGURE 2. rCBF in cerebellum (fig. 2A) and brainstem (fig. 2B) as measured in 8 rats with "C- and "C-IAP.
with $^{11}$C-IAP was 0.041. These values exceed those reported by other workers in the nitrous oxide-anaesthetized rat$^{18}$ and in other species.$^{19}$ This discrepancy may be due in part to the limited number of data points in our series, particularly in the mid-range of $P_{CO_2}$ (fig. 3).

Figure 4 emphasizes the importance of performing quality control on each batch of $^{11}$C-IAP prior to its use in measuring rCBF. In a study in which thin-layer chromatography of the $^{11}$C-product revealed no IAP to be present, the apparent "rCBF" as measured with this product was markedly reduced compared with values obtained with $^{11}$C-IAP (fig. 4A). In another study, the $^{11}$C-product had been heated to enhance its solubilization; this resulted in heat-decomposition of the product. rCBF values obtained with the $^{11}$C-product were consistently below those obtained with $^{14}$C-IAP (fig. 4B). The mean value for the ($^{11}$C-CBF/$^{14}$C-CBF) ratio was 0.772 ± 0.001 (SEM). These data suggest that the heat-decomposition may have yielded

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

**Figure 3.** Mean cerebral hemispheral CBF as a function of arterial $P_{CO_2}$. rCBF and $P_{CO_2}$ are highly correlated (paired Student $t$ value, 12.30; $p < 0.001$).

**Figure 4.** Apparent "rCBF" as measured with $^{11}$C-products which proved not to be iodoantipyrine, compared with rCBF as measured with $^{14}$C-IAP. 4A: Study in which no IAP was present in the $^{11}$C-product by chromatography. The $^{11}$C-product fails to measure rCBF. 4B: Study in which the $^{11}$C-IAP had heat-decomposed, possibly to $^{11}$C-antipyrine. rCBF values with the $^{11}$C-product are 77.2 ± 0.1% (mean ± SEM) of those obtained with $^{14}$C-IAP. Symbols: filled circles, cerebral hemispheral samples; open circles, brainstem; triangles, cerebellum.

$^{14}$C-antipyrine, which, by virtue of its greater diffusion-limitation, tends to underestimate rCBF of gray-matter structures relative to iodoantipyrine.$^8$

**Discussion**

The data of this study demonstrate that $^{14}$C-iodoantipyrine, when synthesized as described and
subjected to proper quality control, can be used for accurately measuring rCBF in the experimental animal and thus would be expected to be suitable for the emission-tomographic measurement of rCBF in man. Certain qualifications, however, must be appended. As the data of figure 4 suggest, batch-by-batch quality control of the \(^{14}\)C-product is essential if valid rCBF results are to be assured. Antipyrine, the immediate precursor of \(^{14}\)C-IAP, has been shown by several workers to be significantly permeability-limited at normal rates of gray-matter blood flow and to produce a falsely low estimate of rCBF.\(^{10,21}\) The percentage by which antipyrine underestimates rCBF relative to IAP is calculated from the data provided by Sakurada et al. to average 39% for gray-matter structures of the conscious rat.\(^8\) In the study shown in figure 4B, the 23% decrement observed approximates this value and suggests that the \(^{14}\)C-product in that instance may have consisted in part of antipyrine or a related, permeability-limited \(^{14}\)C-labelled substance.

Apart from the necessity for proper quality control, an additional disadvantage of \(^{14}\)C-IAP is the relative difficulty of scaling its synthesis up to the 5-10 mCi amount required for human emission-tomographic imaging. In this respect, \(^{14}\)O-labelled water (T\(_\text{1/2}\) 123 sec), which we are also investigating, appears to be a superior tracer for human rCBF studies inasmuch as it can by synthesized readily with high purity and ample yield (typically, 50 mCi in a volume of 50 \(\mu\)l) by a simple and straightforward strategy.\(^{22}\)

For tomographic studies of rCBF in man employing an autoradiographic paradigm, an estimated dose of 5-10 mCi of \(^{14}\)C-IAP would be required, to be administered by intravenous infusion over 1-2 min. The resulting radiation exposure to the patient is determined by the physical half-life of the radionuclide and the biodistribution of the radiopharmaceutical. IAP has been shown to approach equilibrium in the blood within 10 min of its intravenous administration and to have a calculated distribution volume approximating 65% of body weight.\(^{22}\) Significant amounts of inorganic iodide detach from the IAP molecule within 15 min, and up to 70% of this iodide appears in the urine within 24 h.\(^{22}\) The resulting \(^{14}\)C-antipyrine has a biologic half-life of 7 to 20 h in plasma and is distributed throughout body water.\(^{22}\) Thirty to 40% of the antipyrine is oxidized to 4-hydroxyantipyrine, rapidly conjugated to the glucuronide and possibly sulfate, and excreted. The metabolic fate of the remainder is not known. The physical half-life of \(^{14}\)C (20.4 min) is considerably shorter than the biological half-life of either IAP or its metabolite, antipyrine, and is thus the chief determinant of the radiation dose to the patient. If one assumes a uniform whole body distribution and 100% retention of the tracer, an injected dose of 10 mCi in a 70 kg man would result in an absorbed dose of 112 mRad to the whole body.\(^{22}\) This is an acceptable level to permit its use for rCBF studies in man.

We have recently developed and validated an "in vivo autoradiographic" strategy for measuring regional cerebral blood flow by positron emission tomography.\(^{22}\) In this method, blood flow is computed from the time-integrated cerebral activity curve resulting from a modified ramp intravenous infusion of a positron-emitting blood flow tracer such as \(^{18}\)O-water or \(^{14}\)C-iodoantipyrine administered over an interval of one min or more. This method holds promise for the measurement of rCBF in man by positron emission tomography.

Acknowledgment

The authors are indebted to Cathy M. Butler, Ignacio E. Cendan, Mari T. Gutierrez, and Mercedes Santiso for their expert technical assistance, and to Tracey Moffitt, the typist.

References

Incidence of Asymptomatic Extracranial Arterial Disease

MICHAEL HENNERICI, M.D., ALBRECHT AULICH, M.D., WILHELM SANDMANN, M.D., AND HANS-JOACHIM FREUND, M.D.

SUMMARY Investigations of the incidence and the extent of the asymptomatic early stages of extracranial arterial disease (EAD) have been restricted for methodical reasons. Direct Continuous Wave-Doppler examination has given highly accurate results in the location and correct estimation of the degree of EAD both for the carotid (97%) and the vertebral arteries (90%), as shown from a detailed comparison with carotid (n = 604) and vertebral (n = 426) angiograms. Compared with this degree of reliability, the validity of normal auscultation for the diagnosis of EAD is shown to be poor: if bruits are taken as the only signs of associated carotid (97%) and vertebral (90%) stenosis, there is a 22.6% frequency of false-positives in patients with normal results. The frequency and degree of EAD was studied by the use of direct Doppler examination in 2009 neurologically asymptomatic patients admitted either with severe vascular (n = 375) or coronary atherosclerosis (n = 264) or with high-risk factors (n = 1370). The frequency was significantly higher (32.8%) in patients with peripheral vascular disease than in those with coronary artery disease (6.8%) and in risk-factor patients (5.9%). The combination and degree of vessel involvement are presented in detail and their possible prognostic significance discussed.

A VARIETY of non-invasive techniques have been developed for the detection of extracranial arterial disease (EAD): Some of these methods, such as the supraorbital Doppler, 1, 2 ophthalmodynamometry 3 and ocular pneumoplethysmography 4 are inexpensive and easily performed. Their validity is, however, limited to the detection of high-grade stenosis or occlusion of the carotid arteries. 5, 6 An accurate differentiation between stenosis and occlusion of the carotid arteries is impossible and the exact site of obstruction cannot be localized.

The more sophisticated application of the Continuous Wave (CW)-Doppler technique for direct insonation of the carotid arteries in the neck has considerably improved the value and reliability of the non-invasive examination, particularly for the carotid artery system. 7-12 By continuous scanning of the course of the extra-cranial arteries from the suprACLavicular to the submandibular region it is possible to detect with accuracy the size, extent and location of lesions producing an obstruction of the lumen of about 50% or more. The method can be further refined by spectrum analysis of the Doppler signals 13 allowing the additional detection of lesions producing obstructions of even less than 50% due to atherosclerotic changes in the normal laminar flow within the vessels. 14 With the new combination of real-time B-scan imaging and Doppler systems 15-17 it may become possible to achieve a degree of resolution sufficient for the detection of even small ulcerated lesions of the arterial wall.

It has been reported that use of the Doppler technique to detect flow abnormalities in the vertebral arteries (flow reduction or steal phenomena) provides information on the adequacy of the posterior brain circulation. 16, 11, 18 Although CW-Doppler does not as yet yield quantitative information about cerebral perfusion, the evidence it provides of significant variations in blood flow velocity between the carotid and vertebral artery systems — as well as knowledge of the collaterals — may be of substantial help in making an
11C-Iodoantipyrine for the measurement of regional cerebral blood flow by positron emission tomography. Validation studies.
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