Imaging of the Human Heart after Administration of L-(N-13)Glutamate

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In normal volunteers and cancer patients, studies using L-(N-13)glutamate as an imaging agent showed localization of N-13 activity in the heart. Other organs that were well visualized include the liver, pancreas, and salivary glands. In ten subjects the average myocardial uptake after intravenous injection of labeled glutamate was (5.7 ± 0.39)% (s.e.m.) of injected dose, as determined by a quantitative scanning system. The concentration of N-13 activity in the human heart could not be predicted from previous studies involving myocardial uptake in dogs and rodents after administration of L-(N-13)glutamate.

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We have been using L-(N-13)glutamate as an imaging agent for bone tumors in patients (*1*) and have been evaluating its uptake as an indicator of the early response of these tumors to chemotherapy (*2*). In these patients as well as in normal volunteers, we noted that the heart was well visualized after i.v. administration of L-(N-13)glutamate. This finding differed from its failure to concentrate in the dog heart (*3*) and its lower uptake in rodent myocardial tissue (*4*), compared with N-13 ammonia. The present report quantitates the uptake of N-13 in the human heart after injection of labeled L-glutamate.

MATERIALS AND METHODS

The cyclotron production of N-13 ammonia and its subsequent use in the enzymatic conversion to L-(N-13)glutamate have been described (*3*). To make the labeled amino acid suitable for patient studies, this synthesis has been modified by immobilizing glutamate dehydrogenase (L-glutamate: NAD(P) oxidoreductase (deaminating) EC 1.4.1.3) on an activated Sepharose support (*5*). The immobilization procedure prevents the entry of potential pyrogenic or antigenic enzyme protein into the labeled product. To immobilize the enzyme, 10 mg of bovine-liver glutamate dehydrogenase* is dialyzed overnight against 0.05 *M* sodium phosphate buffer (pH 8.0) and is then mixed with 15 μmoles α-ketoglutarate, 15 μmoles adenosine diphosphate, 2 μmoles reduced nicotinamide adenosine dinucleotide, and 0.50 g CNBr-activated Sepharose. The mixture is brought to 15 ml by the addition of 0.05 *M* sodium phosphate buffer (pH 8.0) and is shaken overnight at 4°C with a wrist-action shaker.† The suspension is poured into a glass column and unbound enzyme is removed by passage of 500 ml of the phosphate buffer through the column.

A reaction mixture containing 100–200 mCi of N-13 ammonia, 5 μmoles of α-ketoglutarate, and 3 μmoles of reduced nicotinamide adenine dinucleotide in 3 ml of 0.05 *M* sodium phosphate (pH 8.0) is passed through the column containing the bound enzyme. The column is then washed with 3 ml of the phosphate buffer. To separate the labeled L-glutamate from unreacted N-13 ammonia, the combined effluents are then passed through an AG-50 ion-exchange column equilibrated at pH 4.0. The eluate of the ion-exchange column is passed through a Millex filter with 0.22 μm pore size into a sterile vial, is made isotonic with NaCl, and is ready for intravenous injection. In a typical preparation, 80 mCi of L-(N-13)glutamate is synthesized, in a volume of 6 ml, 4 min after collection of 150 mCi of N-13 ammonia.

The radiopurity of L-(N-13)glutamate was determined by analyzing the product with high-pressure liquid chromatography. A 20-μl sample was injected onto a Whatman SAX anion column (10 μm diameter, 25-cm

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length). Elution with a 5 m*M* potassium phosphate-HCl buffer (pH 3.5), at 22°C and 750 psi resulted in a single radioactive peak with a retention time the same as that of a known sample of glutamate (*6*).

Samples of L-(N-13)glutamate were routinely tested and found to be sterile and free of pyrogens. No adverse reactions have been observed in patients. The procedures were reviewed and approved by the Center's Clinical Investigations Committee and the Committee on Radiation.

The myocardial uptake of activity from intravenously administered L-(N-13)glutamate was studied in ten subjects, each receiving up to 10 mCi. These included two normal subjects (ages 57 and 70) and eight patients (ages 10–23) with osteogenic sarcoma, Ewing's sarcoma, or benign ganglioneuroma. Normal subject WM (age 70) was imaged with L-(N-13)glutamate and N-13 ammonia on succeeding weeks. In some subjects, blood clearance data for the first 20 min after injection were obtained from venous blood samples counted in a gamma scintillation spectrometer. In a normal subject, the dynamic uptake of N-13 activity in the thoracic and abdominal regions was measured for the first 3 min after injection of glutamate by means of our Total Organ Kinetic Imaging Monitor (TOKIM) gamma camera (*7*). This system enables one to integrate regions of interest and thus to yield time-dependent organ uptake data.

Quantitative whole-body scans were begun 5 min after injection with the High-Energy Gamma (HEG) dual-detector rectilinear scanner. This instrument is equipped with digital data recorders and constant-response collimators (*8*). The procedure for calculating in vivo organ uptake from the HEG scan data has been described (*9*). Briefly, the calculation of myocardial uptake is performed as follows: the scan data are obtained in digital form on magnetic tape and are then read by computer and corrected for the physical decay of the N-13 label during the scanning period. A boundary-detecting algorithm is applied to the image to obtain the silhouette of the heart, and a second algorithm is applied to obtain the total count rate within that silhouette. By means of data points in regions adjacent to the heart, the contribution of activity in overlying muscle and skin is estimated and subtracted from the heart data. Finally, the net count rate is normalized to the subject's body thickness and compared with the count rate derived from a phantom of similar size containing a known amount of activity. The results are expressed as percent of administered activity. No correction was made for activity contained in the cardiac blood pool; for L-(N-13)glutamate this quantity is estimated to be less than 0.5% of the administered activity.

RESULTS

Figure 1 shows the clearance of N-13 activity from the blood of three subjects after i.v. injection of L-(N-13)-glutamate. About 95% of the injected activity clears from the blood with a half-time of 0.4–1.6 min and ~5% remains in the vascular compartment for a time that is long compared with the period of observation. The count rates in the heart, liver, and pancreas, measured with the TOKIM system as a function of time, are shown in the same figure. Figure 2 shows anterior images of normal subject WM, taken with the HEG rectilinear scanner.

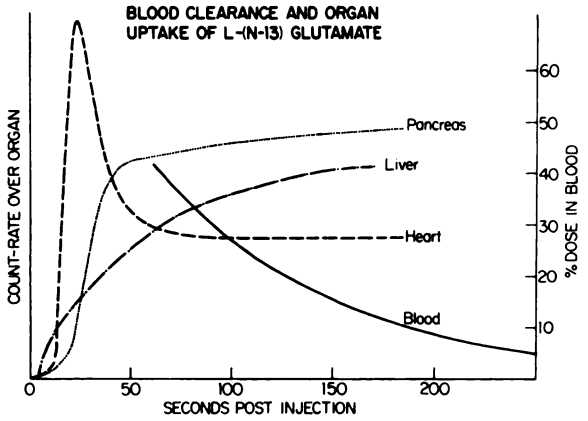

FIG. 1. Venous blood clearance and dynamic uptake curves for heart, pancreas, and liver in human subjects. Data for blood were not obtained before 50 sec. Organ curves are intended to demonstrate time course of distribution and not relative amount of N-13 uptake, since only portions of liver and pancreas were monitored.

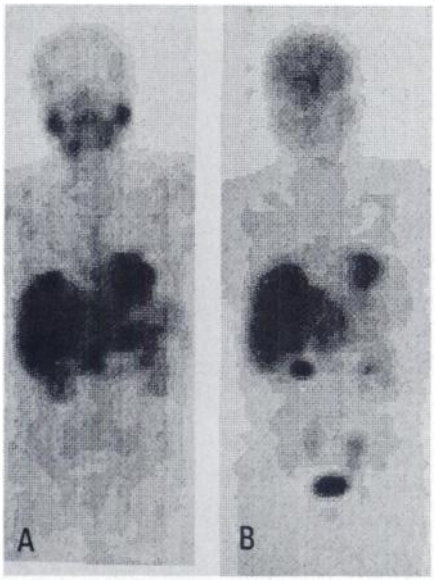

FIG. 2. Organ distribution studies with (A) L-(N-13)glutamate and (B) N-13 ammonia. Both studies were performed with digital rectilinear scanning system designed for quantitative studies. Imaging was begun 5 min after i.v. injection and required 25 min to complete. The L-(N-13)glutamate scan visualizes the heart, liver, pancreas, and salivary glands. N-13 ammonia scan shows localization of activity in brain, heart, liver, kidneys, and urinary bladder.

(A) is a scan after administration of 10 mCi of L-(N-13)glutamate. High uptake is seen in the heart, liver, pancreas, and salivary glands. Little N-13 activity is concentrated in the brain. A different organ distribution is seen in Fig. 2B, which is a scan made after administration of 10 mCi of N-13 ammonia. Areas of high radioactive concentration appear in the brain, heart, liver, kidneys, and bladder. There was 7.7% of the total injected dose in the heart region of this subject after administration of L-(N-13)glutamate. In ten subjects, the average myocardial uptake of N-13 activity after injection of labeled glutamate was 5.7 ± 0.39% (s.e.m.) of dose.

DISCUSSION

The present study demonstrates that L-(N-13)glutamate is taken up at a high concentration in the human heart. These results differ from that found in the dog (*3*) where myocardial uptake was less than 0.5% of the total dose, compared with 3.6% of dose after administration of N-13 ammonia. Ten minutes after i.v. injection of L-(N-13)glutamate, ~0.5% of the dose concentrates in the myocardium of mice and rats. This is one-half (calculated from Ref. *4*) and one-third (B. R. Freed and A. S. Gelbard, unpublished data) of the percent dose in these species, respectively, after administration of N-13 ammonia. Species-related variation in myocardial uptake of labeled amino acids has been observed previously. Nitrogen-13 activity from asparagine, labeled in the amide position, localized in the dog heart at a much higher concentration than did N-13 activity from ammonia (*10*), but did not localize more effectively than N-13 from ammonia in the myocardium of the rabbit or of man (*11*). The N-13 of L-glutamate and L-glutamine was found in the myocardium of a monkey at a higher level than in that of a dog (*12*). Similar species differences have been noted when L-(H-3)arginine localized in the mouse myocardium, whereas L-(C-14)asparagine concentrated in the dog heart (*13*). Whether species specificities in myocardial uptake of amino acids reflect differences in enzyme levels, in intracellular amino acid transport systems, or in cardiac amino acid pools, are areas for further study.

The use of amino acids as myocardial imaging agents has not been extensively studied. Their role as nutritional or energy sources in myocardial metabolism is considered to be limited in comparison with that of fatty acids or of carbohydrates. Amino acids account for only 5.6% of the nutritional supply for the oxidative metabolism in the heart of a fasting human being (*14*). The intramolecular transfer of the amino group of amino acids in the myocardium is high, however, because of the presence of high levels of glutamate α-keto acid transaminases. Thus, amino acids may be taken up in the myocardium and their carbon skeletons metabolized to components of the citric acid cycle to supply energy through subsequent oxidation. Indeed, when Mudge et al. (*15*) measured arteriovenous differences of all naturally occurring amino acids in normal subjects and in patients with coronary artery disease, they found that glutamate was the only amino acid extracted by the heart, and that more glutamate was taken up by the myocardium of patients with coronary artery disease than by normal myocardium.

Two case studies have indicated uptake of N-13 activity in the human heart after the injection of L-(N-13)amino acids. Lathrop et al. (*4*) imaged the heart after the injection of L-(N-13)glutamate, and Cohen (*16*) demonstrated myocardial localization of N-13 after administration of L-(N-13)alanine. In neither study was the quantity of N-13 label in the myocardium reported. The metabolic fate in the myocardium of the N-13 label was not determined in these studies, nor in our work. Thus it is not yet known whether the labeled amino group of alanine must be transaminated to glutamate to be taken up by the heart, as suggested by the results of Mudge et al. (*15*).

The present study shows that N-13 of glutamate is concentrated in the human heart. L-(N-13)glutamate may thus prove useful for imaging the heart and for studying the higher extraction by patients with coronary artery disease (*15*). Nitrogen-13-labeled amino acids may also be useful for studying the observed increase and subsequent decrease in uptake of amino acids in ischemic and failing hearts in animal model systems (*17*).

FOOTNOTES

* Boeringer-Mannheim, Indianapolis, IN.
† Burrell Corp., Pittsburgh, PA.

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