jnm/ PRELIMINARY NOTE

ENZYMATIC SYNTHESIS AND USE OF ^{13}N-LABELED L-ASPARAGINE FOR MYOCARDIAL IMAGING

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A method has been described for the enzymatic synthesis of ^{13}N-labeled L-asparagine. In vivo scanning studies have characterized the high uptake of the labeled amino acid in the myocardium and the results suggest that ^{13}N-asparagine may be a useful myocardial scanning agent.

As part of a study concerned with the enzymatic formation of ^{13}N-labeled compounds of biologic interest and with the evaluation of these labeled compounds as organ and tumor-scanning agents, the amino acid L-asparagine has been synthesized with ^{13}N labeled in the amide position. This report describes the synthetic procedure as well as the measurement of high uptake of ^{13}N-asparagine in the heart of a normal dog. These results suggest that this labeled compound may be superior to the usual myocardial scanning agents, the isotopes of the alkaline metals, potassium, rubidium, and cesium (*1–3*) or to ^{13}N-labeled ammonia (*4,5*).

METHODS

Nitrogen-13-labeled asparagine was synthesized by the amidation of L-aspartic acid by ^{13}N-labeled NH_3 in a reaction catalyzed by the enzyme asparagine synthetase (L-aspartate: L-glutamine amido-ligase (AMP), E.C. 6.3.5.4.). Nitrogen-13-NH_3 was produced with the Sloan-Kettering cyclotron by the ^{16}O (p,α) ^{13}N reaction on H_2O and the subsequent reduction of the generated ^{13}N-labeled NO_2 and NO_3. The H_2O was bombarded with 14-MeV protons at a beam current of 25 μA for 30 min to produce $^{13}NO_2$ and $^{13}NO_3$. These compounds were reduced to $^{13}NH_3$ by the action of sodium hydroxide on Devarda's alloy. The vaporized $^{13}NH_3$ was condensed and passed through a capillary pipet and bubbled through a solution containing 3 ml of 0.01 *M* Tris buffer of pH 8.5. An enzyme reaction mixture containing (in μmoles): aspartic acid (20), adenosine triphosphate, (15), $MgCl_2$, (15), ammonium acetate, (1), and 5 mU of asparagine synthetase (the enzyme was prepared from extracts of Novikoff hepatoma (*6*) and was the generous gift of MK Patterson) was immediately added to the $^{13}NH_3$ solution and incubated at 37°C for 10 min. The synthesized ^{13}N-labeled asparagine was separated from the unreacted $^{13}NH_3$ by elution of the amino acid from a Dowex 50 cation-exchange column equilibrated with 0.01 *M* Tris-acetate buffer of pH 4.0. The eluate containing the formed ^{13}N-asparagine was neutralized with sodium hydroxide and made isotonic by the addition of sodium chloride. The solution was then passed through a Millipore filter and was suitable for animal injection and scanning experiments. The procedure of enzymatically incorporating $^{13}NH_3$ into asparagine and preparing the labeled amino acid for in vivo scanning experiments was carried out in 20 min. Three millicuries of labeled asparagine were produced from 150 mCi of $^{13}NH_3$ for a yield of about 10%. The product of the reaction was characterized as asparagine by its R_f value on cellulose thin-layer chromatography developed in methanol:water:pyridine (80:20:4) (*7*); by its elution with H_2O from acid-washed alumina (*8*); and by its elution from a strong cation-exchange column at low pH.

RESULTS

The organ metabolism of ^{13}N-asparagine was evaluated in two normal dogs under sedation by an initial dynamic measurement of the radionuclide accumulation in the heart and liver after intravenous injection. Figure 1 shows a rapid accumulation of ^{13}N-asparagine in the heart and liver. No subsequent washout of activity was observed.

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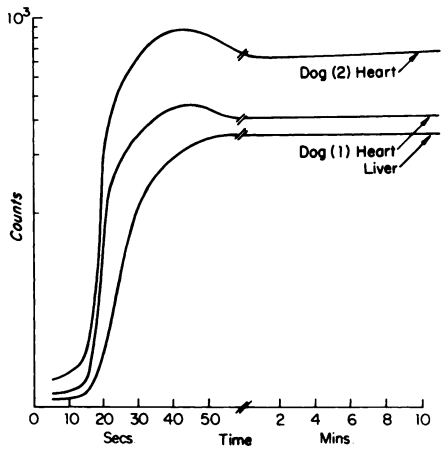

FIG. 1. Dynamic measurement of ^{13}N-asparagine uptake in heart and liver using collimated probe detectors. Dogs had been sedated with Sedasol prior to injection.

A quantitative whole-body lateral scan of one dog 10 min after injection of 1 mCi of ^{13}N-asparagine using our dual detector scanner (*9*) is shown in Fig. 2A. The whole-body scan indicated a rapid clearance from the blood and a distinct visualization of the heart. Activity could be seen in the liver, brain, and salivary glands. An incomplete clearance of activity from the kidneys to the bladder was indicated. The calculated percent dose in each organ derived from two quantitative scans on the same dog was determined by methods previously described (*10,11*). The organ uptake data were calibrated by phantom measurements and were corrected for isotope decay, lateral dog thickness, and surrounding blood or tissue background. Similar uptake of ^{13}N-asparagine in the myocardium was calculated for both scans with an average value of 13.7% of injected dose (0.11%/gm of tissue). The concentration of radioactivity in the liver averaged 13.7% (0.02%/gm of tissue). About 2% of the injected dose of ^{13}N-asparagine appeared in the bladder. A different pattern of organ distribution after injection of ^{13}N-ammonia is shown in Fig. 2B. This labeled compound is taken up in the heart (3–4% of injected dose), liver, brain, kidney, and bladder.

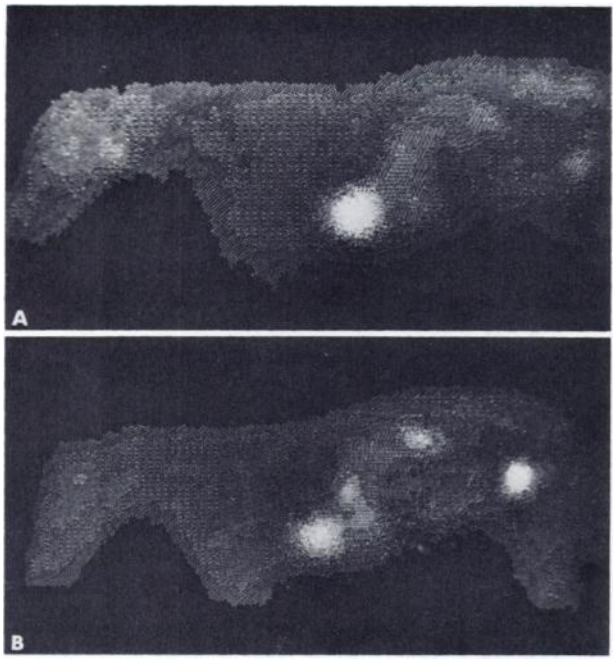

FIG. 2. Whole-body scan of normal dog showing distribution of (A) ^{13}N-asparagine and (B) ^{13}N-ammonia. Whole-body scan time was 15 min.

DISCUSSION

The myocardial uptake of ^{13}N-asparagine after intravenous injection is significantly higher than that reported for the alkaline metals (*1,12,13*) where an uptake of 3–5% of injected dose was determined by in vitro organ counting or for ^{13}N-ammonia which was measured by quantitative scanning. The preferential uptake of ^{13}N-asparagine may offer a better method of visualizing the myocardium and the in vivo quantitative assay of myocardial uptake suggests that a noninvasive method for measuring myocardial blood flow is possible.

The high uptake of ^{13}N-asparagine in the myocardium was not expected. Unlike glutamine, the other monoamide ester of a dicarboxylic amino acid that is centrally involved in cellular nitrogen metabolism and which acts as a storage nitrogen moiety, asparagine does not appear to have a function in normal animal tissues other than that of a protein constituent. The reason for the high uptake of labeled asparagine in the myocardial region is thus obscure but our results indicate that asparagine may play a significant role in myocardial metabolism.

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