Synthesis and Myocardial Kinetics of N-13 and C-11 Labeled Branched-Chain L-Amino Acids

Jorge R. Barrio, Fritz J. Baumgartner, Eberhard Henze, Martin S. Stauber, James E. Egbert, Norman S. MacDonald, Heinrich R. Schelbert, Michael E. Phelps, and Fu-Tong Liu

University of California, Los Angeles, Los Angeles, and Scripps Clinic and Research Foundation, La Jolla, California

Glutamate dehydrogenase (GDH), immobilized on CNBr-activated Sepharose supports, was used with N-13 ammonia to aminate α-ketoisocaproic acid (KIC), and α-ketoisovaleric acid (KIV) to produce N-13-labeled branched-chain L-amino acids with radiochemical yields ranging from 29% to 35%. From kinetic and practical considerations, pH 7.5–8.0 was established to be optimal for the synthesis of N-13-labeled branched-chain-L-amino acids. Myocardial time-activity curves in dogs at control, during low-flow ischemia, reperfusion, and after transaminase inhibition following intracoronary bolus injection of the N-13-labeled amino acids were biexponential. Higher retention of N-13 activity was observed in ischemic segments both during low-flow ischemia (29.2%) and reperfusion (23.2%) when compared with controls (20.0%), (n = 4). On the other hand, transaminase inhibition decreased residue fractions from 21.0% at control to 13.9% (n = 4). The residual activity with L-[1-^{11}C]leucine allows for the calculation of protein synthesis rates.

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The use of radiopharmaceuticals to image local physiological processes requires an adequate understanding of the metabolism of the labeled compound. This ability to model the activity of a tracer is one of the prerequisites of true "physiologic tomography" (*1*). Central to these concepts of traceable metabolism and physiologic tomography are positron-emitting pharmaceuticals that are metabolically trapped within tissues. The principle of metabolic trapping is very effectively used with 2-deoxy-2-[^{18}F]fluoro-D-glucose in humans for measuring local cerebral (*1*) and myocardial (2) metabolic rates for glucose. In this paper we integrate the application of this principle of metabolic trapping for the in vivo evaluation of myocardial metabolism of branched-chain L-amino acids.

The branched-chain L-amino acids (BCAA), L-leucine (LEU), L-valine (VAL), and L-isoleucine (ILEU), differ from most other amino acids in that they are metabolized mainly in extrahepatic tissues (*3*). The metabolic pathways are shown in Fig. 1. The initial step is transamination of LEU, VAL, and ILEU to α-ketoisocaproic acid (KIC), α-ketoisovaleric acid (KIV), and α-keto-β-methylvaleric acid (KMV), respectively, with subsequent decarboxylation. It was recognized early that appreciable amounts of myocardial CO_2 production are derived from the carbon skeletons of these amino acids (*4–6*). It is this decarboxylation step, rather than transamination, which appears to be rate-limiting in rat myocardium (*4*).

The BCAA are involved in L-alanine homeostasis by donating amino and carbon units to form alanine precursors (i.e., L-glutamate and oxaloacetic-derived phospho-enolpyruvate). The alanine thus formed from tissues such as kidney, gut, brain, and heart (*3*) can enter the liver to serve as a carrier of gluconeogenesis. We note that the oxidation of BCAA increases in diabetes and starvation, and results in increased plasma alanine. This has been suggested to explain increase of plasma glucose in such conditions (*3*). On the other hand, maple syrup urine disease involves a defect in branched-chain ketoacid dehydrogenase, which is often accompanied by episodes of low plasma alanine and glucose.

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For reprints contact: J. R. Barrio, PhD, UCLA School of Medicine, Dept. of Radiological Sciences, Div. of Biophysics, Los Angeles, CA 90024.

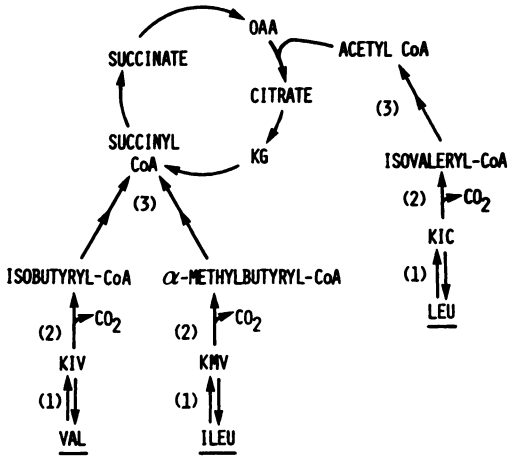

FIG. 1. Metabolic oxidative pathways of branched-chain amino acids (BCAA). (1) Branched-chain amino acid transaminase (BCAT) catalyzes reversible transamination of BCAA with α-ketoglutaric acid to form respective α-ketoacid and L-glutamic acid. (2) Branched-chain ketoacid dehydrogenase (BCKD) catalyzes oxidation of all three BCAA α-ketoacids; in process NAD^+ is reduced to NADH and CO_2 is given off. (3) Represents multistep, enzymatic pathway to produce succinyl-CoA and acetyl-CoA, respectively. Other symbols are: LEU = L-leucine; ILEU = L-isoleucine; VAL = L-valine; KIC = α-ketoisocaproic acid; KIV = α-ketoisovaleric acid; KMV = α-keto-β-methylvaleric acid; OAA = oxaloacetic acid.

In addition to the role of BCAA in alanine and glucose maintenance, there is evidence for their involvement in the control of protein synthesis and turnover. LEU (but not ILEU or VAL) and metabolites of all three BCAA both inhibited protein degradation and accelerated protein synthesis in rat myocardium (*5*).

BCAA metabolism appears to be heavily regulated in myocardium. In rats, free fatty acid stimulate their oxidative metabolism, whether in the presence or absence of insulin, and also inhibit their incorporation into proteins (*6*). Furthermore, glucose and pyruvate inhibit oxidation of branched-chain L-amino acids (*6*).

The importance of BCAA and their regulatory mechanisms in myocardial physiology and pathophysiology cannot be overestimated. The use of BCAA labeled with positron emitters would permit the in vivo evaluation of these biochemical processes, particularly transamination reactions and protein synthesis, using external detection.

Several papers describing enzymatic synthesis of N-13 (*7–17*) and C-11-labeled (*18*) L-amino acids have been published. These methods encompass the use of either batch methods (*7–13*) or enzymes immobilized on solid supports (*14–18*). Immobilized glutamate dehydrogenase (GDH) has permitted the preparation of sterile, pyrogen-free L-[^{13}N]glutamate suitable for human use (*15,16,19*). It has long been known, however, that GDH—which appears to be the only enzyme in mammals that catalyzes a reversible oxidation, linked to pyridine nucleotide (NADH or NADPH), of L-amino acids—shows broad specificity (*20,21*). This makes it convenient for the preparation of various N-13-labeled-L-amino acids including N-13-labeled BCAA (*8,22*). In this paper we report (a) the remote controlled, semiautomated enzymatic synthesis of [^{13}N]LEU, and [^{13}N]VAL, using immobilized GDH; and (b) the application of tracer kinetics to the in vivo evaluation of BCAA metabolism in normal and ischemic myocardium.

MATERIALS AND METHODS

L-[1-^{11}C]leucine was synthesized using the Bucherer-Strecker reaction with subsequent oxidation of the D-isomer using immobilized D-amino acid oxidase/catalase as described by Barrio et al. (*23*).

Production of ^{13}N BCAA. Forty-five milligrams (approximately 2,000 units) of bovine liver GDH (E.C.1.4.1.3.)* were immobilized on 1.5 g CNBr-Sepharose† for 2 hr in the presence of the following substrate-containing coupling buffer: 30 m*M* sodium phosphate pH 7.2, with 1 m*M* α-ketoacid, 0.5 m*M* EDTA, 1 m*M* ADP, 200 μ*M* NADH, and 50 μ*M* GTP. The choice of α-ketoacid to be used in the coupling buffer depended on which BCAA the column was designed to produce. Each particular column was only used in the production of one particular N-13 BCAA. The remaining immobilization procedure was similar to that previously described (*17*). The column was stored at 4°C in 5 m*M* EDTA in 30 m*M* sodium phosphate pH, 7.5.

Before the tagged run, the column was allowed to reach room temperature and was then thoroughly washed: first with 30 ml of 50 m*M* α-ketoacid, 2 m*M* NADH in 30 m*M* sodium phosphate (pH 7.5), then with 140–250 ml 25 m*M* α-ketoacid, 1 m*M* NADH in 30 m*M* sodium phosphate pH, 7.5.

Gaseous N-13 ammonia (150–200 mCi) produced by the ^{16}O(p,α)^{13}N reaction on H_2O (*24*) was swept with N_2 into 3 ml 30 m*M* sodium phosphate, pH 8.0, containing 1 m*M* NADH and 25 m*M* of the appropriate α-ketoacid. This was transferred to the GDH column and allowed to remain in contact with the enzyme for 10 min. The column was then washed with 8 ml of 30 m*M* sodium phosphate, pH 8.0, and the eluate was forced through an 8.5 by 0.7 cm cation exchange AG 50 W X-8 or X-12 resin‡ equilibrated with 30 m*M* sodium phosphate, pH 6.0. The product was brought to isotonicity and forced through a sterile 0.22 μm pore filter. After the run, the column was stored as described above. Under these conditions the immobilized enzyme columns were reusable for several months.

N-13 BCAA: Product analysis. The purity of the final product was verified using the method of *o*-phthaldialdehyde (OPT) precolumn fluorescence derivatization (*17,18*), and the amino-acid-OPT complex analyzed with reversed-phase HPLC (Ultrasphere ODS, 5 μm, 4.6 × 150 mm i.d.; solvent: 15 min linear gradient from

55% 100 m*M* potassium phosphate buffer, pH 7.0, and 45% MeOH to 100% MeOH; flow rate 1.0 ml/min; fluorescence and radioactivity detectors).

Pyrogenicity testing, using the *Limulus* test as well as rabbit assay, were routinely done in order to assess whether enzymatic leakage had occurred from the column; GDH protein was determined both spectrophotometrically (280 nm) and using solid-phase radioimmunoassay (RIA). Sterility testing was also routinely done.

Rabbit anti-GDH antiserum (RAG) to be used in the RIA was prepared by immunizing New Zealand red rabbits in the footpads with 100 μg of GDH in complete Freund's adjuvant. A booster injection consisting of 100 μg of GDH in incomplete Freund's adjuvant was given subcutaneously 2 wk after primary immunization. The rabbit was bled ten days after the boost. RAG serum was precipitated with ammonium sulfate at 50% saturation at 4°C, redissolved in phosphate-buffered saline (PBS) (0.01 *M* sodium phosphate, pH 7.2, 0.15 *M* NaCl), and precipitated again. The precipitates were redissolved in and dialyzed against PBS. The RAG serum was further purified by affinity binding of the RAG to an immobilized GDH column. The column was washed with PBS until the absorbance at 280 nm was <0.02. Bound RAG was eluted with 0.1 *M* glycine-HCl, pH 2.4, and immediately neutralized with 1 *M* Tris-HCl, pH 8.5. Pooled RAG was then dialyzed against 0.1 *M* sodium phosphate, pH 7.5. Iodine-125 labeled RAG was obtained by adding 1 mCi Na I-125 to 10 μg purified RAG followed by 0.01 mg Chloramine-T. The mixture was placed on ice 1 min, excess Chloramine-T was reduced with 0.02 mg sodium metabisulfite, and the protein peak was eluted with PBS from a Sephadex G-25 column. RIA was performed by coating polyvinyl wells[§] with 5 μg unlabeled RAG at 4°C overnight, washing with PBS, and saturating unattached sites with 5% newborn-calf serum in PBS at room temperature. After 2 hr, the plates were again washed with PBS. Serial dilutions of GDH standards, immobilized GDH column effluent, and a simulated GDH run were applied to the plates and incubated at 4°C for 3 hr or overnight, then washed with PBS. Each well was provided with 50,000 cpm I-125-labeled RAG incubated at 4°C 5 hr, washed with PBS, and counted.

Myocardial metabolism. Myocardial uptake and clearance of ^{13}N-labeled BCAA were studied at control, during low-flow ischemia, reperfusion, and after transaminase inhibition with 2.0 m*M* aminooxyacetic acid (AOA). The experimental protocol using open-chested instrumented dogs was similar to that previously described (*19*).

The methodology and validation of residue fraction and tissue clearance detection have been described (*19,25–29*). A 0.2 ml bolus containing 10–30 μCi of N-13-labeled BCAA was injected into the LAD and the N-13 tracer retention and clearance were followed with a shielded, collimated 7.5- by 5.0-cm NaI(TI) scintillation detector over the heart. A digital computer was used to store data after collection of decay-corrected increments of 0.1 sec. The residue fraction of positron activity retained in myocardium was determined by graphic extrapolation of the second slow-clearance phase (C) back to the time of the maximal peak (A) representing the total amount of activity injected. The residue fraction was computed as the ratio C/A.

TABLE 1

L-[^{13}N]amino acid	Yields*	Radiochemical purity	mCi Product†
GLU‡	80	>99	60
LEU	29	>99	20
VAL	35	>99	25

* Yields are decay-corrected percentages of total N-13 activity trapped when ^{13}N-ammonia was bubbled through the appropriate substrate-containing solution. The protocol described in the Material and Methods section was used for the preparation of [^{13}N]GLU, except for the concentration of KG (5 mM) in the buffered (pH 7.5) substrate-containing solution.

† Solution activity of ^{13}N-ammonia trapped in the substrate-containing solution was typically 150–200 mCi.

‡ Symbols are: GlU = L-glutamic acid; LEU = L-leucine; VAL = L-valine.

RESULTS

Production of N-13-labeled BCAA and product analysis. Table 1 lists radiochemical yields, purities, and final product radioactivities of N-13-labeled BCAA compared with L-[^{13}N]glutamate. We found that unless the GDH columns were extensively washed with the appropriate α-ketoacid, the labeled BCAA product would be contaminated with L-[^{13}N]glutamate. This contamination is probably due to residual KG remaining attached to the enzyme active site. We therefore modified the protocol of Baumgartner et al. (*17*) to include immobilization of GDH in, and subsequent washing with, the appropriate α-ketoacid. In addition, substrates and co-factors are used to protect the enzyme catalytic and regulatory binding sites during immobilization (*30*). The product is sterile and pyrogen-free, and essentially protein-free as determined spectrophotometrically at 280 nm. Moreover, the results of solid-phase RIA revealed that effluents from immobilized GDH columns, as well as a simulated GDH run, gave I-125 counts at background level. Since the sensitivity of the assay is about 2 ng/ml, the GDH content in these samples must have been below this concentration.

TABLE 2. EFFECT OF TRANSAMINASE INHIBITION ON CLEARANCE AND RESIDUE FRACTION OF [^{13}N]BCAA

L-[^{13}N] amino acid	Clearance half-time of phase I (sec)		Clearance half time of phase 2 (min)		Residue fraction %	
	Control	AOA	Control	AOA	Control	AOA
LEU	37.0(35.75 to 38.25)	62.4(59.1 to 65.7)	141.1(134 to 148.2)	20.0(17 to 23)	25.5(24.75 to 26.25)	15.4(14.7 to 16.1)
VAL	42.7(41.8 to 43.6)	53.7(50.35 to 57.05)	63.5(60.15 to 66.85)	11.3(9.25 to 13.35)	16.6(16.05 to 17.15)	12.4(12.2 to 12.6)

Data are given as mean (range) (two experiments each).

TABLE 3. EFFECT OF ISCHEMIA ON CLEARANCE AND RESIDUE FRACTION OF [^{13}N]BCAA

L-[^{13}N] amino acid	Clearance half-time of phase 1 (sec)			Clearance half-time of phase 2 (min)			Residue fraction (%)		
	Control	Low-flow ischemia	Reperfusion	Control	Low-flow ischemia	Reperfusion	Control	Low-flow ischemia	Reperfusion
LEU	32.1(31.9 to 32.3)	35.5(32.35 to 38.65)	45.0(35.6 to 54.4)	156.6(148.15 to 165.05)	134.2(111.75 to 156.65)	126.6(122.65 to 130.55)	23.9(22.8 to 25.0)	34.4(33.2 to 35.6)	27.7(25.6 to 29.8)
VAL	43.4(40.65 to 46.15)	42.3(39.35 to 45.25)	41.0(36.8 to 45.2)	78.7(76.05 to 81.35)	58.4(55.9 to 60.9)	71.0(62.75 to 79.25)	16.2(15.95 to 16.45)	24.0(23.0 to 25.0)	18.8(18.75 to 18.85)

Data are given as mean (range) (two experiments each).

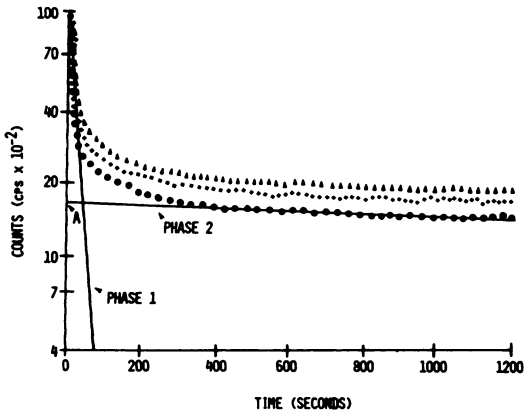

FIG. 2. Dog myocardial time-activity curves subsequent to intracoronary bolus injection of 20 μCi of [^{13}N]VAL under control conditions (● ● ●), after 20 min of induced low-flow ischemia (▲ ▲ ▲) and at reperfusion (induced by 20-min flow reduction but normal flow at data acquisition) (+ + +). Activity peaks were normalized to 10^4 counts in all cases. Computer-calculated slopes corresponding to components of clearance curves are represented by solid lines. Both types of ischemia caused increase of residue fraction.

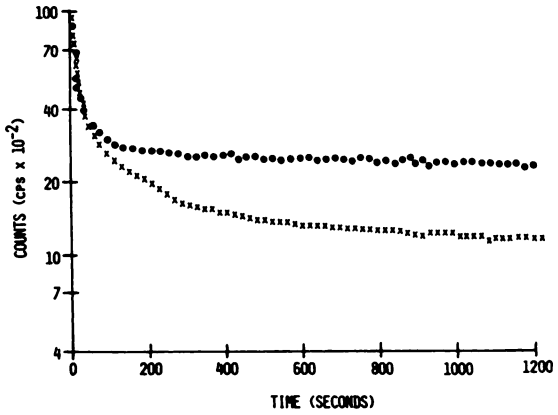

FIG. 3. Typical time course of activity in dog myocardium subsequent to bolus injection of 0.2 ml of [1-^{11}C]LEU (xxx) or [^{13}N]LEU (● ● ●) into left anterior descending coronary artery. Activity peaks were normalized to 10^4 counts in both cases.

Myocardial metabolism. The results of AOA transaminase inhibition as well as low-flow ischemia and reperfusion are shown in Tables 2 and 3, respectively. All L-[^{13}N]amino acids revealed biexponential time-activity curves with $t_{1/2}$ averaging 37.7 sec for phase 1 and 117.8 min for phase 2. Low-flow ischemia and reperfusion had no effect on $t_{1/2}$, but the residue fraction indicating metabolic trapping increased (n = 4) from control (20.0%) to low-flow ischemia (29.2%). An increase (n = 4) was also noted for reperfusion experiments (23.2%). Typical time-activity curves for [^{13}N]VAL, under control conditions as well as both types of ischemia, are shown in Fig. 2.

Transaminase inhibition prolonged $t_{1/2}$ of phase 1 from 39.8 sec to 58.0 sec (n = 4), shortened $t_{1/2}$ of phase 2 from 102.3 min to 15.6 min (n = 4), and decreased residue fractions from 21.0% to 13.9% (n = 4). Unlike L-[^{13}N]amino acids, L[1-^{11}C]LEU demonstrated three-phasic time-activity curves under control conditions with half-times of 9.9 sec, 85.7 sec, and 51.0 min. The residue fraction of [1-^{11}C]LEU was 14.6%, which is comparable to the residue fraction of N-13-labeled BCAA after transamination inhibition. Figure 3 shows time-activity curves for [^{13}N]LEU and [1-^{11}C]LEU.

DISCUSSION

Preparation of L-[^{13}N]amino acids. From kinetic and practical considerations we have selected pH 7.5–8.0 for the immobilized enzyme production of [^{13}N]GLU, [^{13}N]LEU, and [^{13}N]VAL. The kinetic parameters of free GDH have been extensively studied (*20,23,31,32*); however, it is apparent that kinetic results obtained using free GDH cannot be readily extrapolated to the immobilized enzyme (*33*). Immobilization reduces GDH activity and decreases the higher V_{max} observed at high pH values for the free enzyme, with the possible exception of KG (*20,33,34*).

We are well aware of the limitations of selection of pH 7.5–8.0 for production of radiolabeled amino acids using only kinetic considerations. L-[^{13}N]amino acids were prepared in this and previous work (*15–17,19,22,33*) from no-carrier-added N-13-ammonia and, as pointed out by Cooper and Gelbard (*22*), the "ammonia effect" modifies free GDH activity (*31,32*). Whether or not this effect occurs with immobilized GDH has yet to be demonstrated.

Our final radiochemical yields of [^{13}N]GLU, [^{13}N]LEU, and [^{13}N]VAL at pH 7.5–8.0 are in the same range as those reported by Cooper and Gelbard (*22*) at higher pH (Table 1). However, an additional factor helped us to select pH 7.5–8.0—immobilized GDH seems to be unstable at pH >8.0. For this reason a higher pH for production of radiolabeled L-amino acids using immobilized GDH was considered impractical, since enzyme columns could not be reused due to rapid loss of enzymatic activity. This inconvenience is fully appreciated when multiple production runs per day/week are required.

Our remote, semiautomated system using immobilized GDH (*17*) has permitted the rapid and reliable synthesis of N-13-labeled BCAA. The risk of microbiological and protein contamination in the product is negligible, as determined by sterility and pyrogen testing, spectrophotometric assay, and solid-phase RIA. Samples were routinely found to be sterile. These immobilized enzyme columns have been used for up to 3 mo at a time, and the use of either 2 M KCl or 5 mM EDTA in 30 mM sodium phosphate, pH 7.5, performs quite effectively as an antimicrobial.

N-13-labeled BCAA in normal myocardium. The results of AOA administration on myocardial time-activity curves for N-13-labeled BCAA are an indication of the importance of transamination reactions in BCAA me-

tabolism. Branched-chain amino acid transaminase (BCAT) is responsible for the reversible transamination of the three BCAA with KG (*35,36*). This myocardial enzyme is fairly specific for the above-mentioned amino acids, and it transaminates VAL, ISOLEU, and LEU with KG with high activities. Interestingly, both cytosolic and mitochondrial aspartate- and alanine-aminotransferases (GOT and GPT, respectively) from myocardium do not seem to show any activity for BCAA (*35*).

Caution should be exercised, however, in the interpretation of the results with AOA because of its lack of specificity as a BCAT inhibitor (*18,37*). AOA, also an inhibitor of GOT and GPT, produces a significant disruption of the malate-aspartate shuttle. For example, earlier studies from our laboratories with L-[4-^{11}C]-aspartic acid and [4-^{11}C]oxaloacetic acid (*18*) showed their rapid oxidation in myocardium, with the peak of $^{11}CO_2$ production at approximately 100 sec after intracoronary administration. When transamination was inhibited, $^{11}CO_2$ production was abolished after administration of L-[4-^{11}C]aspartic acid, whereas the kinetics of [4-^{11}C]oxaloacetic acid were not affected by AOA.

Therefore, modifications of the ^{13}N-labeled BCAA tissue clearance curves upon myocardial transaminase inhibition with AOA do not result from the specific inhibition of BCAT with AOA, but rather from the nonspecificity of AOA as an inhibitor of pyridoxal 5′-phosphate-dependent transaminases, which, in turn, may affect the flow of nitrogen from the branched-chain amino acids to other amino acids. More detailed studies must await the discovery of a more selective inhibitor of the branched-chain amino-transferases.

Most probably the metabolic trapping of the N-13 label (residue fraction) after administration of N-13-labeled BCAA reflects both incorporation of the amino acid(s) into proteins and their compartmentalized metabolism initiated by their reversible transamination with KG to form L-[^{13}N]glutamate and the corresponding α-ketoacid. Since no significant breakdown or back diffusion of proteins could be expected during the experiment(s), the $t_{1/2}$ of phase 2 is mainly an indication of the diffusion out of the tissue of labeled L-glutamate or other free L-amino acids (e.g. L-alanine or aspartate) that could be eventually formed from L-[^{13}N]glutamate by transaminase reactions. This diffusion process is slow ($t_{1/2}$ = 117.8 min). The entire kinetic process, if dependent upon transamination reactions, could be significantly altered as expected by transaminase inhibition with AOA (Table 2) or during ischemia (Table 3).

On the other hand, after intracoronary bolus administration of L-[1-^{11}C]LEU, a different clearance of positron activity from cardiac tissue was observed: the tracer clearance kinetically resembled that reported for L-[4-^{11}C]aspartic acid (*18*). In this case the metabolic pathway, which competes with protein synthesis, results in the rapid removal of the label as $^{11}CO_2$. Our preliminary experiments in the heart with [1-^{11}C]LEU—as well as those already in progress in the human brain—indicate that residual activity in the tissue allows for the calculation of protein synthesis rates using intravenous administration and positron emission tomography (PET). We have performed initial studies revealing that transamination is important in BCAA metabolism in brain as well as in myocardium. As expected, [^{13}N]LEU and [1-^{11}C]LEU both reveal similar extraction fractions upon intracarotid bolus injections in a monkey, but the slow phase of the clearance curve reveals much longer half-time for the N-13 label compared with the C-11 label. This longer retention reflects incorporation of LEU into proteins as well as transamination of the N-13 label to form GLU which, due to its poor permeability across the blood-brain barrier, is retained. This makes [1-^{11}C]LEU more attractive for the quantification of protein synthesis rates.

N-13-labeled BCAA in ischemic myocardium. Control of branched-chain amino acid oxidation appears to rest at the oxidative decarboxylation step subsequent to the initial reversible transamination (Fig. 1). We found that myocardial ischemia has no effect on $t_{1/2}$ of phases 1 and 2, whereas the residue fractions increased from 20.0% at control to 29.2% during low-flow ischemia and to 23.2% during reperfusion, and this cannot be attributed to increased protein synthesis rate (*3*) but rather to metabolic alterations. In myocardial ischemia, blood flow and oxidative metabolism are impaired, and it is observed that both uptake and oxidation of free fatty acids are depressed in ischemic myocardium, with a concomitant increase in glucose utilization. Although the precise cause of this metabolic alteration is not known, a declining TCA cycle activity has been observed.

Rau et al. (*38*) observed that several L-amino acids were effective in the prevention of ischemic damage and enhancement of mechanical recovery in rabbit myocardium. L-Glutamate in cardioplegic solutions has been used to reverse ischemic myocardial damage in dogs (*39*). The protective effect of the amino acids seems to arise from their ability to form different TCA-cycle intermediates. Since α-ketoacids formed from transamination of BCAA are known to increase the level of TCA-cycle intermediates in the heart, increased utilization of α-ketoacids would stimulate enzymatic transamination of the ^{13}N-labeled branched-chain L-amino acids. This could be one of the mechanisms for increased N-13 residue fractions observed upon administration of N-13-labeled branched-chain L-amino acids in myocardial ischemia.

CONCLUSIONS

N-13-labeled L-amino acids are amenable to tracer kinetic modeling and have already shown their potential

value for PET imaging in derangement of human myocardial metabolism (*19*). The intricate metabolic regulation of branched-chain amino acid oxidation has certain physiological implications in normal myocardium and, although no experimental evidence has yet been offered, could contribute to the recovery of ischemic myocardium. The involvement of these amino acids in energy-producing schemes in the heart as well as their incorporation into proteins explains the retention of the N-13 label in myocardium.

FOOTNOTES

* Sigma Chemical Co., St. Louis, Mo.
† Pharmacia.
‡ Bio-Rad Laboratories.
§ Microtiter plates, Dynatech Laboratories, Inc., Alexandria, Va.

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Announcement of Berson-Yalow Award

The Society of Nuclear Medicine invites abstracts for consideration for the Sixth Annual Berson-Yalow Award. Work will be judged on originality and contribution to the fields of basic or clinical radioassay. The abstract will be presented at the 31st Annual Meeting of the Society of Nuclear Medicine in Los Angeles, CA, June 5–8, 1984, and a suitably engraved plaque will be awarded to the authors by the Education and Research Foundation of the Society of Nuclear Medicine.

The abstract should be submitted on the official abstract form with a letter requesting consideration for the award.

Deadline for receipt of manuscripts: Thursday, January 12, 1984.

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Southern California Chapter Society of Nuclear Medicine and Santa Barbara Cancer Foundation Cardiovascular Nuclear Medicine Symposium

December 2–4, 1983 **Santa Barbara Biltmore** **Santa Barbara, California**

Announcement

The Southern California Chapter of the Society of Nuclear Medicine and the Santa Barbara Cancer Foundation announce a Cardiovascular Nuclear Medicine Symposium to be held December 2–4, 1983, at the Santa Barbara Biltmore Hotel, Santa Barbara, California (guaranteed rates $59.00 single or double effective December 1–5, 1983).

Members of the Organizing Committee for the Symposium are James McClintock, MD, Ken Lyons, MD, Dennis O'Grady, Alan Waxman, MD, and Daniel Berman, MD.

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