A Feasibility Study on L-[1-Carbon-11]Tyrosine and L-[Methyl-Carbon-11]Methionine to Assess Liver Protein Synthesis by PET

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We studied the potential of L-[1-^{11}C]tyrosine ([1-^{11}C]Tyr) and L-[methyl-^{11}C]methionine ([Me-^{11}C]Met) as tracers for measuring protein synthesis rate (PSR) in the liver by PET and proposed their metabolic models. **Methods:** In the liver and plasma of control and cycloheximide-treated mice injected with [1-^{14}C]Tyr and [Me-^{3}H]Met, incorporation of the radioactivity into the acid-soluble fraction and chloroform/methanol-extract (CM), RNA and protein fractions were measured. Data were compared with those from rat studies with ^{11}C-labeled analogs and PET. **Results:** In mice, liver uptake of [Me-^{3}H]Met was over twice as large as that of [1-^{14}C]Tyr. Similar uptake patterns of the ^{11}C-labeled analogs were found in rats by PET. In the mouse liver at 1 to 6 hr after injection, ~69%–73% of the ^{14}C was detected in the protein fraction, whereas ~65%–70% of the ^{3}H was in the CM fraction, which reflected phospholipid synthesis. In plasma, the percentages of the protein fractions were ~73%–76% for ^{14}C and ~36%–46% for ^{3}H. Gel-filtration analysis suggested that 80% of the ^{14}C-labeled plasma proteins was albumin originating from the liver, which corresponds to approximately 25% of the total labeled proteins synthesized in the liver at 6 hr. When protein synthesis was inhibited by cycloheximide, the liver uptake of the [1-^{14}C]Tyr and the protein-incorporation of ^{14}C in the liver and in plasma were decreased dose-dependently. On the other hand, uptake of [Me-^{3}H]Met was significantly enhanced in the liver due to increased incorporation into the CM fraction. **Conclusion:** [1-Carbon-11]Tyr can be used for measuring the PSR in the liver by PET. Liver uptake of [Me-^{11}C]Met mainly reflects phospholipid synthesis through the transmethylation process.

Key Words: protein synthesis; phospholipid synthesis; liver; PET; tyrosine; carbon-11-methionine

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PET using appropriate positron-emitting amino acids is now an established in vivo methodology of measuring amino acid metabolism in tissues such as the brain and tumors. Among many positron-emitting amino acids which have been prepared (see references in *1*), L-[methyl-^{11}C]methionine ([Me-^{11}C]Met) is the most widely used for PET studies (*2–10*). Carbon-11-carboxylic-labeled amino acids, however, are supposed to be more suitable for measuring the protein synthesis rate (PSR) because the label is mainly incorporated into proteins or washed out through side reactions such as decarboxylation and oxidation (*11*). Metabolic studies with L-[1-^{14}C]tyrosine ([1-^{14}C]Tyr) and L-[1-^{14}C]leucine ([1-^{14}C]Leu) demonstrated the usefulness of ^{11}C-labeled analogs for this purpose (*12,13*). Recently, Ishiwata et al. reported criticism on the selection of labeled amino acids for measuring PSR in the brain and tumor tissues by PET (*14,15*). When in vivo, the protein synthesis was inhibited by cycloheximide, the total uptake as well as the protein incorporation of [1-^{14}C]Leu were significantly reduced in the brain and in tumor tissue, whereas the uptake of [Me-^{3}H]Met increased. A significant fraction of the [Me-^{3}H]Met was incorporated into nonprotein materials such as lipids and RNA (*15*).

Many PET studies have been performed to assess amino acid metabolism in the brain and tumor tissues; however, little attention has been paid to metabolism in the liver. The liver has a very active protein synthesis for endogenous use, but the organ is also responsible for supplying plasma proteins such as albumin. After intravenous injection of radiolabeled amino acids into animals or humans, the labeled proteins appear into the circulation. Using [Me-^{11}C]Met in human plasma, a large individual variation was found in the appearance rate and the amounts of the labeled acid-precipitable materials (*16*). This probably reflects a varying ability of the liver to synthesize plasma proteins. These results prompted us to investigate the in vivo protein synthesis in the liver by PET.

Based on the literature, it can be expected that the uptake of carboxylic labeled amino acid reflects protein synthesis in the liver and that the uptake of methyl-labeled methionine mainly shows the transmethylation process into the phospholipid synthesis. In mice treated with cycloheximide to inhibit protein synthesis in vivo, the liver uptake of [1-^{14}C]Leu was significantly reduced; under the same conditions, however, liver uptake of [Me-^{3}H]Met was significantly increased (*15*). A metabolic study with [1-^{14}C]Met and [Me-^{14}C]Met demonstrated that half of the methyl-^{14}C in the rat liver was incorporated into the chloroform/methanol-extracted (CM) lipid fraction at 60 min postinjection, whereas carboxyl-^{14}C was hardly incorporated into this fraction (*17*). This difference can be explained by the metabolism of methionine. The methyl group of methionine is used as a precursor for phospholipid synthesis in the liver by the *S*-adenosyl-L-methionine (*18–21*), while the carboxylic ^{14}C is metabolized to $^{14}CO_2$ and removed from the tissue.

The aim of this study was to investigate the potential of [1-^{11}C]Tyr and [Me-^{11}C]Met to assess the PSR in the liver in vivo by PET. [1-Carbon-11]Tyr is a useful tracer for measuring the PSR in tumor tissues (*22*) because of its high protein-incorporation and low level of nonprotein metabolites in the brain and tumor tissues (*12*). Incorporation of radioactivity from [1-^{14}C]Tyr and [Me-^{3}H]Met into high molecular weight materials such as proteins, RNA and phospholipids was studied using a mice model. The relationship between the protein

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synthesis in the liver and the appearance of labeled proteins in blood was also addressed. Finally, the effects of inhibition of protein synthesis by cycloheximide on liver metabolism were investigated.

MATERIALS AND METHODS

Radiopharmaceuticals

[1-^{14}C]Tyr (specific activity of 2.0 GBq/mmole) and [Me-^{3}H]Met (specific activity of 7.2 GBq/mmole) were purchased from NEN Research Products (Wilmington, DE). [1-^{11}C]Tyr (*23*) and [Me-^{11}C]Met (*24*) were prepared as previously described. Cycloheximide was obtained from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan) and Soluene-350 was from Packard Instrument Company, Inc (Meriden, CT).

Tissue Distribution of [1-^{14}C]Tyr and [Me-^{3}H]Met

A mixture of [1-^{14}C]Tyr (46 kBq/23 nmole) and [Me-^{3}H]Met (230 kBq/32 nmole) was injected intravenously into male ddY mice weighing 36.0 ± 1.7 g. They were killed by cervical dislocation at 5, 15 and 30 min and 1, 2 and 3 hr postinjection. Blood was taken by heart puncture using a heparinized syringe and centrifuged to obtain plasma. Cold physiological saline was perfused into the mice through the inferior vena cava to remove blood from the liver. The liver, brain and pancreas were dissected. Part of the dissected, tissues (about 50–100 mg) and 20 μl of the plasma were dissolved in tissue solubilizer (Soluene-350) and counted for ^{14}C and ^{3}H activity using a liquid scintillation counter. The tissue uptake of radioactivity was expressed as the percent injected dose per gram of tissue. Total plasma radioactivity was also calculated, assuming that plasma volume in mice is 48.8 ml/kg body weight.

A second group of mice received cycloheximide dissolved in physiological saline intraperitoneally (1, 10 and 100 mg/kg body weight). Thirty minutes later, a mixture of [1-^{14}C]Tyr and [Me-^{3}H]Met was injected intravenously into the mice. The animals were killed 60 min post-tracer injection and treated as previously described.

Protein Incorporation of [1-^{14}C]Tyr and [Me-^{3}H]Met

Protein incorporation of radioactivity was measured as described previously (*14,15*), with only a slight modification. The liver (50–100 mg) was homogenized in 1 ml ice-cold water. The homogenate was put in a tube with rubber cap. Then, 0.3 ml 1 *M* $HClO_4$ was added to the homogenate, and the generated $^{14}CO_2$ was trapped into 1 ml Soluene 350. The homogenate was divided into the acid-soluble fraction (ASF) and acid-precipitable fraction (APF). The APF was divided into three fractions: lipids [chloroform/methanol-extract (CM) fraction], RNA and proteins as described previously (*14*). Briefly, after extracting lipids from the APF with chloroform/methanol (2/1, v/v), the residual precipitate was incubated in 0.3 *M* KOH at 37°C for 60 min to hydrolyze RNA. The solution was then acidified with $HClO_4$ and divided into supernatant (RNA fraction) and the precipitate (protein fraction).

To analyze the labeled plasma proteins, 50 μl plasma were treated as described above without separating the RNA fraction. To the plasma, 1 ml 0.3 *M* $HClO_4$ was added and the generated $^{14}CO_2$ was trapped into Soluene 350. The solution was then divided into the ASF and APF. The latter was divided into the extract with chloroform-methanol (lipids) and the precipitate (proteins).

HPLC Analysis of Labeled Plasma Proteins

To divide the labeled plasma proteins into albumin and globulin fractions, 50 μl plasma were applied to gel-filtration chromatography. A TSKgel G3000SW column (7.5 mm i.d. × 300 mm length, Tosoh, Tokyo) was used with 50 m*M* sodium phosphate, pH 7.4, containing 0.1 *M* NaCl as eluent at a flow rate of 1.0 ml/min. The elution profile was detected with an UV monitor at 280 nm and the radioactivity in 0.5 ml fractions was measured with a liquid scintillation counter.

PET

The liver uptake of [1-^{11}C]Tyr and [Me-^{11}C]Met was measured tomographically in rats as a function of time using a stationary double-headed positron camera as previously described (*17*). The [Me-^{11}C]Met (2–4 MBq) was injected intravenously into anesthetized male Wistar rats weighing 200–250 g, and the distribution of ^{11}C was measured for 1 hr. Two hours later, a mixture of [1-^{11}C]Tyr (2–4 MBq) and [1-^{14}C]Tyr (37 kBq/100 g body weight) was injected into the same rats, and the distribution of ^{11}C was measured for 1 hr. Arterial blood (0.1 ml) was obtained through a catheter at 2, 5, 10, 15, 30, 45 and 60 min postinjection. Carbon-14 radioactivity in total plasma and in the APF was measured as described above and was expressed as the standardized uptake value [SUV, (activity/ml) × (g body weight/total injected activity)]. Liver uptake of ^{11}C measured by PET was also expressed as the SUV.

RESULTS

Organ Uptake

Table 1 summarizes the tissue distribution of [1-^{14}C]Tyr and [Me-^{3}H]Met in the control mice. The pancreas showed the highest uptake of both amino acids: the radioactivity level increased for the first 30 min and decreased gradually. In the liver, [1-^{14}C]Tyr uptake was half that of [Me-^{3}H]Met: after 15 min ^{14}C radioactivity was decreased gradually while ^{3}H radioactivity was increased during the first 30 min and remained constant during the next 5.5 hr. The plasma levels of ^{3}H and ^{14}C

TABLE 1
Tissue Distribution of Radioactivity after Intravenous Injection of L-[Methyl-^{3}H]Methionine and L-[1-^{14}C]Tyrosine into ddY Mice

	Uptake (% ID)/g)					
	5 min	15 min	30 min	60 min	120 min	360 min
L-[1-^{14}C]tyrosine						
Plasma	1.35 ± 0.20	1.08 ± 0.33	0.81 ± 0.06	1.21 ± 0.09	1.21 ± 0.08	1.24 ± 0.14
Liver	2.19 ± 0.39	2.65 ± 0.59	2.40 ± 0.57	2.42 ± 0.19	2.39 ± 0.16	2.29 ± 0.20
Pancreas	17.32 ± 7.33	30.64 ± 1.88	30.75 ± 3.61	28.25 ± 4.44	25.41 ± 3.52	22.58 ± 4.05
Brain	0.97 ± 0.14	0.82 ± 0.10	0.74 ± 0.02	0.73 ± 0.16	0.66 ± 0.09	0.56 ± 0.03
L-[methyl-^{3}H]methionine						
Plasma	1.60 ± 0.24	1.01 ± 0.05	0.99 ± 0.08	1.22 ± 0.04	1.30 ± 0.13	1.44 ± 0.14
Liver	3.56 ± 0.73	5.53 ± 0.73	5.63 ± 1.71	5.60 ± 0.47	5.65 ± 0.47	5.64 ± 0.33
Pancreas	17.16 ± 7.05	29.29 ± 3.19	29.78 ± 3.65	25.68 ± 2.95	22.09 ± 3.01	21.08 ± 3.55
Brain	1.01 ± 0.16	0.97 ± 0.07	1.02 ± 0.09	1.06 ± 0.14	1.01 ± 0.04	0.97 ± 0.08

Values are mean ± s.d. (n = 4).

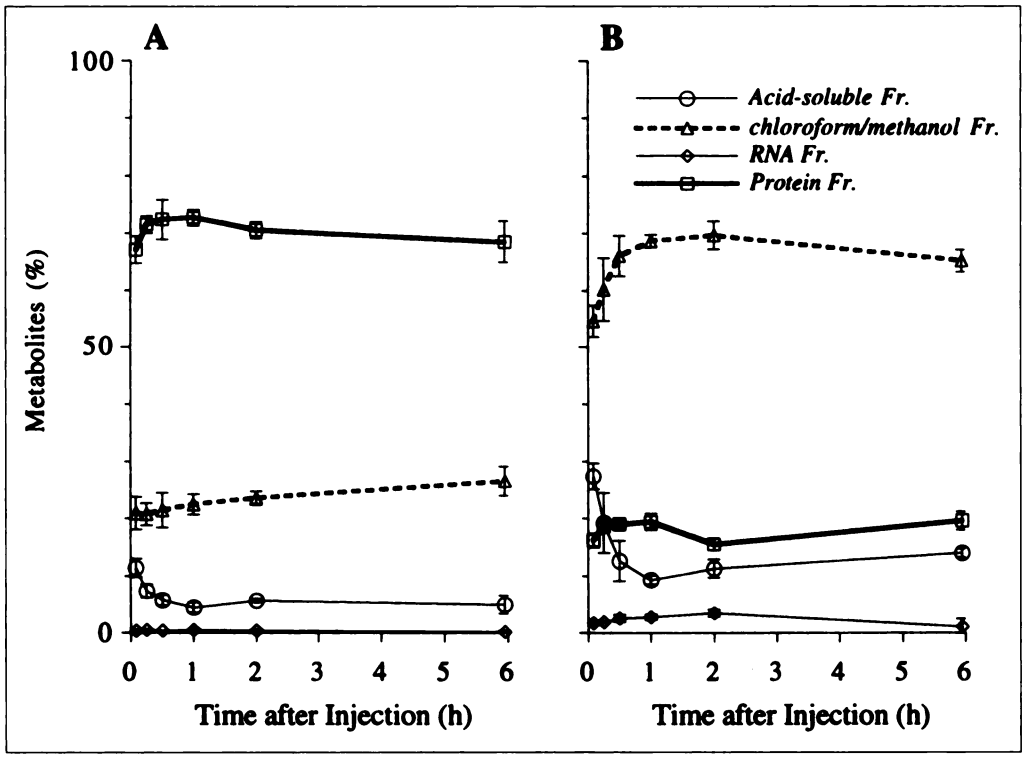

FIGURE 1. Fraction of metabolites in the liver after intravenous injection of L-[1-^{14}C]tyrosine (A) and L-[methyl-^{3}H]methionine (B) into ddY mice. Each fraction was calculated as the percentage of total liver radioactivity at each time point. Mean ± s.d. (n = 4).

decreased during the first 30 min and then increased. In the brain, ^{14}C radioactivity levels decreased gradually, whereas ^{3}H radioactivity levels remained constant until 6 hr.

Metabolism

The metabolites of [1-^{14}C]Tyr and [Me-^{3}H]Met in the liver were investigated. Figure 1 represents the time course of the four fractions of the metabolites in the control liver. The acid-soluble fraction decreased rapidly for the first hour ([Me-^{3}H]Met > [1-^{14}C]Tyr). Among the acid-precipitated fractions of [1-^{14}C]Tyr, the protein fraction was a major component (67%–73%), and the CM fractions were 21%–27%. The RNA fractions (<0.5%) and the bicarbonate fractions (<0.1%) were negligible. On the other hand, the CM fraction was a major component of [Me-^{3}H]Met and reached a maximum of 70% of the total radioactivity 1 hr after injection, whereas the protein fraction was only 16%–20%. The RNA fractions were 1%–3%. The ratios of the CM fraction to the protein fraction were 0.29–0.39 for [1-^{14}C]Tyr and 3.2 to 4.5 for [Me-^{3}H]Met.

Figure 2 summarizes the metabolites of [1-^{14}C]Tyr and [Me-^{3}H]Met appearing in the plasma. The elevated level of total radioactivity after 30 min (Table 1) was accompanied by an increased amount of acid-precipitated materials. Most of the ^{14}C radioactivity in the acid-precipitated materials was recovered in the protein fraction and the CM fraction was minor,

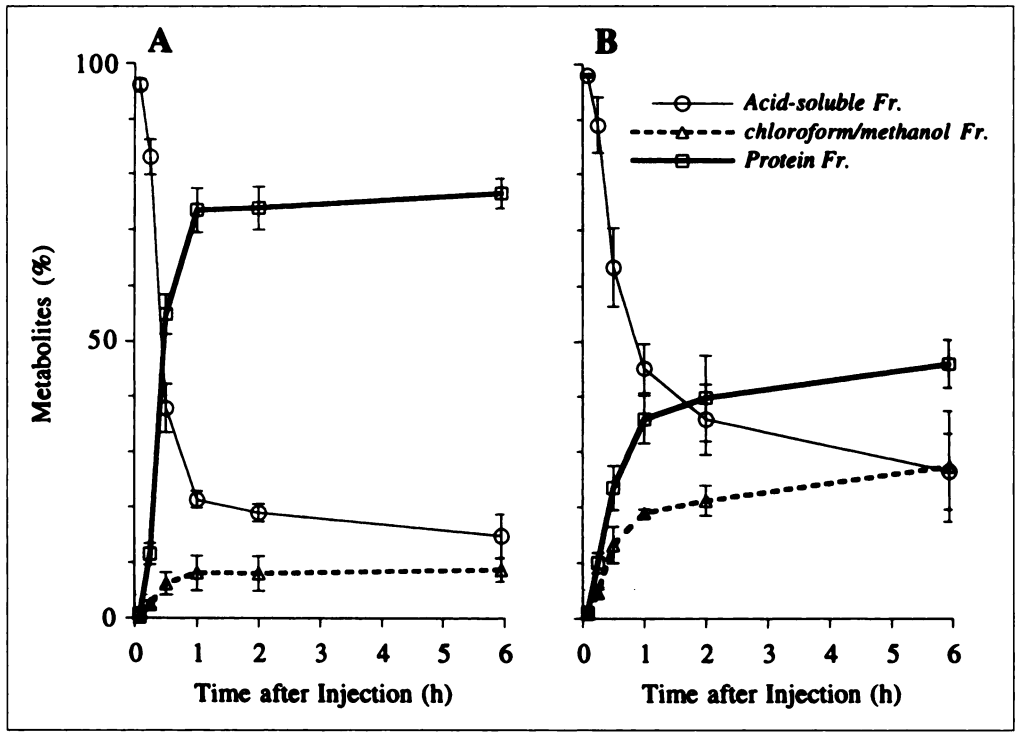

FIGURE 2. Fraction of metabolites in the plasma after intravenous injection of L-[1-^{14}C]tyrosine (A) and L-[methyl-^{3}H]methionine (B) into ddY mice. Each fraction was calculated as a percentage of total liver radioactivity at each time point. Mean ± s.d. (n = 4).

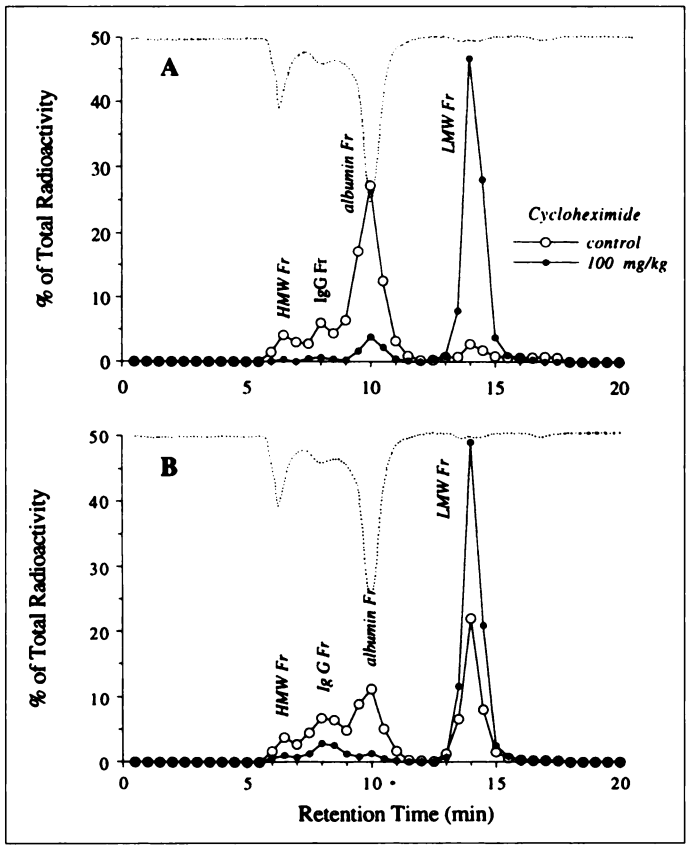

FIGURE 3. Gel filtration elution profiles of plasma obtained 60 min after injection of L-[1-^{14}C]tyrosine (A) and L-[methyl-^{3}H]methionine (B) into ddY mice. Dotted lines show elution profiles at 280 nm. Fractions of albumin, immunogloblin G (IgG), high molecular weight materials (HMW) and low molecular weight materials (LMW), including methionine, are indicated.

whereas for [Me-^{3}H]Met the CM fraction was significantly increased. The ratios of the CM fraction to the protein fraction were 0.11 to 0.22 for [1-^{14}C]Tyr and 0.47 to 0.59 for [Me-^{3}H]Met.

Gel filtration chromatography according to the molecular weight was used to further separate the plasma protein fraction into albumin and immunogloblin G (IgG) fractions (Fig. 3). The elution profile of ^{14}C radioactivity was similar to that of proteins measured at 280 nm (Fig. 3A). Most of the ^{14}C was detected in the albumin fraction, while IgG and high-molecular weight fractions were minor. On the other hand, the elution profiles of the ^{3}H (Fig. 3B) showed significantly smaller incorporation into the albumin fraction. Relatively high activity was observed in the IgG and high molecular weight fractions.

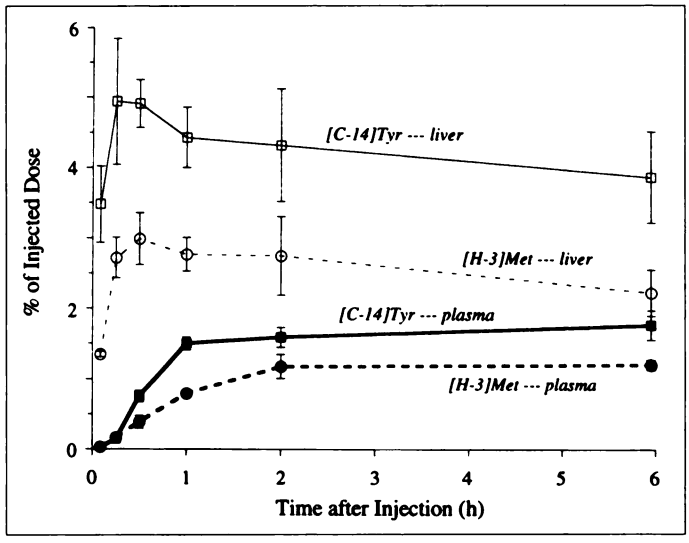

FIGURE 4. Total amount of protein-incorporated radioactivity in the liver and plasma after intravenous injection of L-[1-^{14}C]tyrosine and L-[methyl-^{3}H]methionine into ddY mice. The amount was expressed as the percent injected dose. Total liver radioactivity was calculated from the percent injected dose per gram of tissue multiplied by the weight of the whole liver. Mean ± s.d. (n = 4).

Amount of Protein Incorporation

The incorporation of the labeled amino acids into protein fractions in the whole liver and in total plasma was estimated as percentages of the injected dose (Fig. 4). The amounts of ^{14}C in liver and plasma were significantly larger than those of ^{3}H. In the liver, ^{14}C- and ^{3}H-labeled proteins increased during the first 30 min and then decreased until 1 hr postinjection. During the next 5 hr, the levels slightly decreased further. In contrast to the liver, the labeled protein fraction in plasma increased rapidly for the first hour and gradually during the next 5 hr. Assuming that the plasma albumin fraction had been synthesized in the liver, the ratios of the labeled proteins excreted into plasma to the total labeled proteins synthesized in the liver were comparable for the two amino acids and increased with time. The percentages [100 × (amounts in the albumin fraction in plasma)/(summed amounts of protein fraction in liver and the albumin fraction in plasma)] were: 10.9%, 21.0%, 20.8%, and 25.2% for [1-^{14}C]Tyr and 9.5%, 18.2%, 23.4% and 28.6% for [Me-^{3}H]Met at 0.5, 1, 2 and 6 hr, respectively.

Effect of Cycloheximide on Organ Uptake, Metabolism and Protein Incorporation

Table 2 represents the effect of cycloheximide treatment on the distribution of the two amino acids. Pancreatic uptake of both amino acids showed a dose-dependent decrease, whereas the uptake pattern in the liver and plasma was different. Liver uptake of [1-^{14}C]Tyr decreased in a dose-dependent manner similar to pancreatic uptake. The level of ^{14}C in the plasma decreased. On the other hand, liver uptake of [Me-^{3}H]Met was enhanced, and the level of ^{3}H in plasma was elevated.

Table 3 summarizes the effect of cycloheximide on the metabolism of the two amino acids in the liver and plasma. In plasma for both amino acids, cycloheximide treatment significantly reduced the percentages of the protein and CM fractions. In the liver, the percentages of the protein fraction also decreased, whereas those of the CM fraction slightly increased. In all groups, the CM-to-protein fraction ratio for [Me-^{3}H]Met in the liver was more than ten times larger than that for [1-^{14}C]Tyr. Cycloheximide treatment significantly increased the CM-to-protein ratios for [Me-^{3}H]Met (3.54 at 0 mg/kg to 23.1 at 100 mg/kg in the liver, and 0.53 at 0 mg/kg to 1.76 at 100 mg/kg in the plasma). This effect was not observed for [1-^{14}C]Tyr.

By gel filtration chromatography (Fig. 3), the albumin, IgG and high molecular weight fractions of ^{14}C were decreased and the relative ratios of the three fractions remained constant in the mice treated with cycloheximide. On the other hand, the relative ratio of the albumin fraction of ^{3}H was smaller than those of the IgG and high molecular weight fractions (considered to be lipid as discussed later).

The effect of cycloheximide on protein incorporation of [1-^{14}C]Tyr and [Me-^{3}H]Met in the liver and plasma are represented as percentages of the injected dose (Fig. 5). In the liver and plasma, protein incorporation decreased in a dose-dependent fashion. Similar behavior was observed for the two amino acids. On the other hand, cycloheximide treatment affected the incorporation of the two compounds in the CM fractions

TABLE 2
Effect of Cycloheximide Treatment on Tissue Distribution of Radioactivity 60 Minutes after Intravenous Injection of L-[Methyl-^{3}H]Methionine and L-[1-^{14}C]Tyrosine into ddY Mice

Tracer	Uptake (% ID/g)			
	Cycloheximide			
	0 mg/kg	1 mg/kg	10 mg/kg	100 mg/kg
L-[1-^{14}C]tyrosine				
Plasma	1.21 ± 0.09	1.26 ± 0.06	0.82 ± 0.08†	0.70 ± 0.13†
Liver	2.42 ± 0.19	2.27 ± 0.41	1.62 ± 0.26*	0.57 ± 0.18†
Pancreas	28.25 ± 4.44	19.75 ± 1.77*	15.54 ± 2.63*	7.08 ± 1.63†
Brain	0.73 ± 0.16	0.63 ± 0.03	0.62 ± 0.12	0.60 ± 0.06
L-[methyl-^{3}H]methionine				
Plasma	1.22 ± 0.04	1.41 ± 0.10*	1.38 ± 0.21	1.53 ± 0.10†
Liver	5.60 ± 0.47	7.17 ± 1.84	7.18 ± 1.71	8.51 ± 1.65*
Pancreas	25.68 ± 2.95	23.27 ± 2.03	20.12 ± 0.58*	12.03 ± 1.78†
Brain	1.06 ± 0.14	0.97 ± 0.13	1.05 ± 0.19	1.16 ± 0.11

Values are mean ± s.d. (n = 4).
Student's t-tests were performed between the control and the cycloheximide-treated group. *$p < 0.01$ and †$p < 0.001$.

differently (Fig. 6). The CM fraction in the liver was significantly increased for [Me-^{3}H]Met, whereas it decreased for [1-^{14}C]Tyr. In plasma, the CM fraction for the two amino acids was decreased.

In Vivo Study

In vivo PET studies were performed with [Me-^{11}C]Met and [1-^{11}C]Tyr. High accumulation of ^{11}C was observed in the liver, especially for [Me-^{11}C]Met, as shown previously (*17*). Time-activity curves in the liver and plasma are presented in Figure 7. The hepatic level of radioactivity increased for the first 60 min after injection of [Me-^{11}C]Met. When ^{11}C- and ^{14}C-labeled Tyr were co-injected, the uptake level of ^{11}C increased for the first 30 min and thereafter remained constant. The level of labeled plasma proteins measured with ^{14}C increased after 15 min.

DISCUSSION

This study demonstrated the potential of [1-^{11}C]Tyr as a tracer for measuring PSR in the liver by PET. The significance of [Me-^{11}C]Met in PET studies of the liver is probably for the assessment of phospholipid synthesis by transmethylation processes. Several findings in the present study support these views:

1. Uptake of [Me-^{3}H]Met in the liver was significantly higher than that of [1-^{14}C]Tyr, which was also demonstrated in PET studies of rats using the ^{11}C-labeled analogs.
2. Proteins were the predominant metabolites of [1-^{14}C]Tyr in the liver, whereas those of the [Me-^{3}H]Met were acid-precipitable but chloroform/methanol-extracted materials, probably phospholipids.

TABLE 3
Effects of Cycloheximide on Metabolism of Two Amino Acids in Plasma at 60 Minutes Postinjection

Tracer		Metabolites (%)		
		Acid-soluble Fr.	$CHCl_3$/MeOH Fr.	Protein Fr.
L-[1-^{14}C]tyrosine				
Liver	0 mg/kg	4.4 ± 0.9	22.4 ± 1.8	72.7 ± 1.4
	1 mg/kg	4.7 ± 1.1	21.7 ± 3.5	73.2 ± 4.3
	10 mg/kg	9.6 ± 3.6*	24.1 ± 2.7	65.8 ± 3.1†
	100 mg/kg	24.0 ± 5.7‡	29.8 ± 1.6‡	45.1 ± 6.9‡
Plasma	0 mg/kg	21.3 ± 1.5	8.1 ± 3.1	73.4 ± 3.9
	1 mg/kg	18.0 ± 0.7	10.1 ± 1.4	71.7 ± 1.7
	10 mg/kg	26.7 ± 7.4	6.4 ± 1.2	63.1 ± 8.4
	100 mg/kg	85.8 ± 2.7‡	2.7 ± 1.0*	14.2 ± 6.2‡
L-[methyl-^{3}H]methionine				
Liver	0 mg/kg	9.3 ± 0.8	68.7 ± 1.1	19.4 ± 1.4
	1 mg/kg	12.7 ± 3.1	67.7 ± 8.5	18.0 ± 5.6
	10 mg/kg	12.7 ± 2.7*	75.8 ± 3.9†	9.8 ± 2.3‡
	100 mg/kg	14.4 ± 1.4‡	80.2 ± 1.7‡	3.6 ± 0.8‡
Plasma	0 mg/kg	45.2 ± 4.5	18.9 ± 0.7	35.9 ± 4.3
	1 mg/kg	44.3 ± 1.4	22.9 ± 2.6	32.8 ± 2.0
	10 mg/kg	54.4 ± 5.3*	22.0 ± 1.8	24.0 ± 4.1†
	100 mg/kg	82.9 ± 2.1‡	10.6 ± 0.9‡	6.6 ± 2.3‡

Values mean ± s.d. (n = 4)
Student's t-tests were carried out between the control and the cycloheximide-treated group. *$p < 0.05$, †$p < 0.01$ and ‡$p < 0.001$.

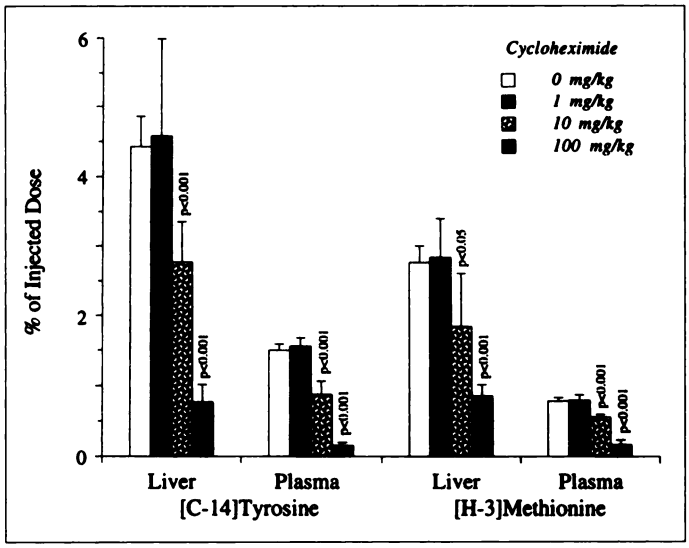

FIGURE 5. Effect of cycloheximide on the amount of radioactivity incorporated into the protein fraction after intravenous injection of L-[1-^{14}C]tyrosine and L-[methyl-^{3}H]methionine into ddY mice. Student's t-tests were conducted between the control and the cycloheximide-treated group. Mean ± s.d. (n = 4).

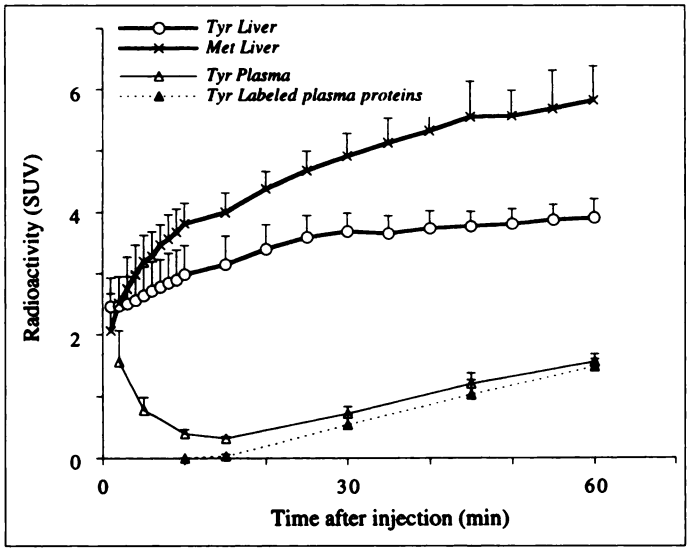

FIGURE 7. Time-activity curves in the liver and plasma. Liver activity was measured with ^{11}C-labeled tracers and PET; the plasma curve was measured with ^{14}C-labeled tracer and liquid scintillation counting. (n = 4–5).

3. The protein fractions labeled by either amino acid in the liver increased during the first 30 min after injection and then gradually decreased, after which labeled proteins were observed in plasma.
4. In plasma, the amounts of ^{14}C-labeled proteins, originating from [1-^{14}C]Tyr, were significantly higher than those of ^{3}H-labeled proteins arising from [Me-^{3}H]Met, whereas the reverse relationship was found for the CM fraction.
5. Gel filtration analysis showed about 80% of the ^{14}C-labeled plasma proteins to be present in the albumin fraction during the first hour. This analysis also suggested the presence of ^{3}H-labeled lipoproteins.
6. When in vivo protein synthesis was inhibited by cycloheximide, total uptake of [1-^{14}C]Tyr by the liver and the labeled protein fraction is decreased in a dose-dependent fashion. On the other hand, total uptake of [Me-^{3}H]Met and the ^{3}H-labeled CM fraction were significantly increased, while the amount of the ^{3}H-labeled protein fraction was decreased.

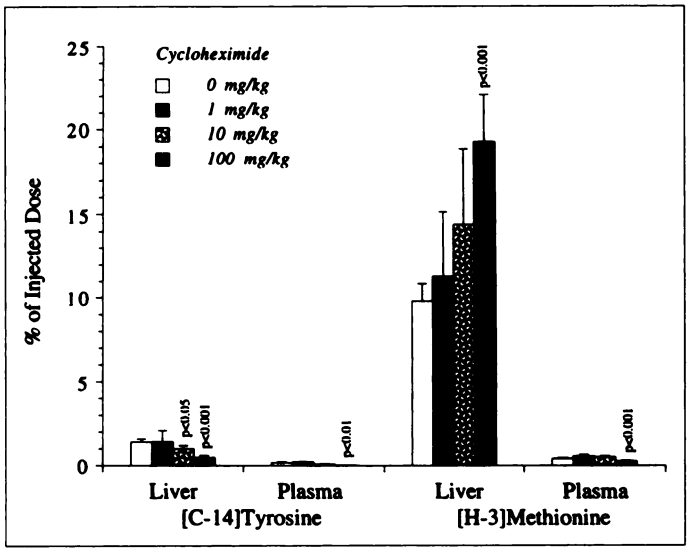

FIGURE 6. Effect of cycloheximide on the amount of radioactivity incorporated into the CM fraction after intravenous injection of L-[1-^{14}C]tyrosine and L-[methyl-^{3}H]methionine into ddY mice. Student's t-tests were performed between the control and the cycloheximide-treated group. Mean ± s.d. (n = 4).

Usually, lipids are extracted from a tissue homogenate with chloroform/methanol. In the present study, the ^{3}H-labeled CM fraction was significantly increased in the liver after cycloheximide treatment. It was decreased in the plasma, but the effect was smaller compared with [1-^{14}C]Tyr. The ratio of the CM fraction to the protein fraction was much larger for [Me-^{3}H]Met than for [1-^{14}C]Tyr at 1 hr in the control and became higher after cycloheximide treatment: 6.5 times in the liver and 3.3 times in the plasma. These results show that most of the ^{3}H-labeled CM fraction in the liver represents phospholipids labeled by [Me-^{3}H]Met by transmethylation, as suggested in previous studies (*18–21*). The newly labeled phospholipids in the liver are possibly excreted into the blood stream as low-density lipoproteins.

In contrast with the ^{3}H-labeled CM fraction arising from [Me-^{3}H]Met, most of the [1-^{14}C]Tyr-labeled CM fraction in the liver and plasma probably represents labeled proteins. The amounts of CM fraction decreased depending on the administered dose of cycloheximide (Fig. 6), and the CM-to-protein fraction ratio in control mice was not affected after cycloheximide treatment. This may indicate that some labeled protein such as lipoproteins is also extracted with chloroform/methanol, which means that both protein and CM fractions (over 90% of ^{14}C in Figs. 1 and 2 and Table 3) represent the ^{14}C-labeled proteins. It is also possible that a small amount of [1-^{14}C]Tyr was incorporated into lipids.

The amount of labeled plasma proteins excreted by the liver or lymphoid tissues can be estimated by gel filtration analysis. Coincidence of the elution patterns of ^{14}C and proteins as detected by UV absorption (Fig. 3) and the cycloheximide-dependent decrease of the ^{14}C-labeled fraction (Fig. 5) clearly show that the major metabolite of [1-^{14}C]Tyr in plasma is protein. Among the labeled proteins, the albumin fraction, which is about 80% of the labeled plasma proteins at 1 hr, is synthesized in the liver and excreted into plasma. The lymphoid tissues probably delivered the IgG fraction and the high molecular weight materials. The distribution patterns of ^{3}H-labeled metabolites of [Me-^{3}H]Met on gel filtration chromatography were very different from those of the ^{14}C-labeled metab-

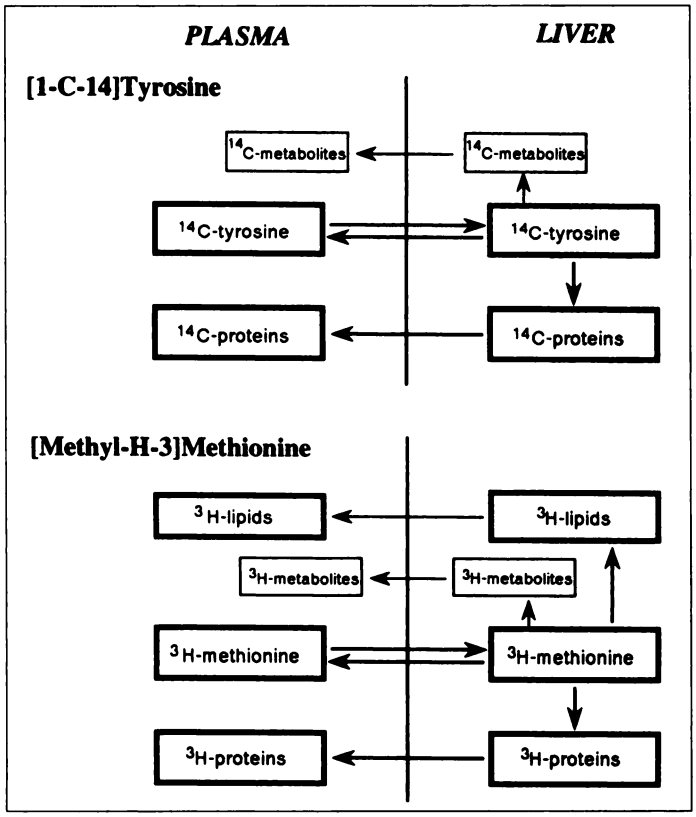

FIGURE 8. Metabolic models for L-[1-^{14}C]tyrosine and L-[methyl-^{3}H]methionine.

olites. This finding and the metabolite analysis, as discussed above, probably indicate that ^{3}H-labeled materials such as low-density lipoproteins are included in the IgG fraction and high molecular weight materials.

As far as the labeled protein fractions, the two amino acids showed comparable results. After an initial increase, the amount of labeled proteins in the liver decreased gradually. During this decrease, the labeled proteins appeared in the plasma. Approximately 80% of labeled plasma proteins were excreted within 1 hr (Fig. 4).

The metabolic models for both amino acids are summarized in Figure 8. Because of biological interest of the fate of labeled liver proteins, we have investigated the metabolism for 6 hr after injection of the amino acids. During the first 2 hr after injection, the metabolism reached a nearly equilibriated state. Within the first hour, over 90% of the [1-^{14}C]Tyr accumulating in the liver is used for protein synthesis. Approximately 20% of the labeled proteins appear as labeled plasma proteins. About 70% of [Me-^{3}H]Met is incorporated into the lipid fraction and only 20% into proteins. About 5% of the ^{3}H-labeled lipids was excreted into plasma (Fig. 6). The fraction of the metabolites in the ASF may be minor, especially for [1-^{14}C]Tyr (*12*).

High and selective protein incorporation of [1-^{14}C]Tyr as discussed above has demonstrated the potential of [1-^{11}C]Tyr as a tracer for measuring PSR in the liver by PET. Because of the potential application for [1-^{11}C]Tyr in PET studies, assessment of liver function (e.g., after transplantation) is of great interest. On the other hand, [Me-^{11}C]Met does not reflect protein synthesis but phospholipid synthesis in the liver. Enomoto et al. (*25*) studied this compound in patients with jaundice. Also, assessing the regeneration processes of the liver by PET with [Me-^{11}C]Met is possible because phospholipids are essential components of the cellular membrane (*21*).

CONCLUSION

[1-Carbon-11]Tyr has potential as a tracer for measuring the PSR in the liver by PET. The significance of [Me-^{11}C]Met in PET studies of the liver may be the characterization of phospholipid synthesis by transmethylation.

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