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Carbon-11 labeled ethionine and propionine as tumor detecting agents

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To develop ^{18}F-fluoroalkyl derivatives of methionine (MET) as a tumor detecting agent by mean of clinical PET, a pilot study assessing the potential of their parent compounds, ^{11}C-labeled ethionine (^{11}C-ETH) and propionine (^{11}C-PRO), was performed. ^{11}C-ETH and ^{11}C-PRO were prepared by the reaction of L-homocysteine thiolactone and corresponding ^{11}C-alkyl iodides. After i.v. injection of a mixture of ^{3}H-MET, ^{14}C-ETH and ^{11}C-PRO into mice bearing FM3A mammary carcinoma, the highest FM3A uptake was found in ^{14}C-ETH, followed by ^{3}H-MET and ^{11}C-PRO, while the FM3A-to-brain and FM3A-to-muscle ratios were nearly the same for all three compounds. The FM3A uptake of ^{14}C-ETH and ^{11}C-PRO were nearly equal or slightly higher than the liver uptake. In the pancreas, liver, FM3A and brain tissues, incorporation of ^{14}C-ETH into acid-precipitable materials was much lower than that of ^{3}H-MET, whereas no incorporation of ^{11}C-PRO was found. Brain uptake of all three compounds was significantly reduced by carrier MET-loading (5 min p.i.) or by cycloheximide treatment to inhibit protein synthesis (60 min p.i.), whereas the FM3A uptake was not affected. Incorporation of ^{14}C-ETH into acid-precipitable materials was inhibited by the cycloheximide. The results suggest that ^{11}C-labeled ETH has a similar potential for tumor detection by PET as ^{11}C-MET, and that ^{11}C-PRO has similar properties to those of other artificial amino acids. The development of ^{18}F-fluoroalkyl derivatives of MET is of interest as the next step.

Key words: [^{11}C]ethionine, [^{11}C]propionine, tumor detection, PET

INTRODUCTION

POSITRON EMISSION TOMOGRAPHY (PET) with appropriate positron-emitting amino acids is now an established *in vivo* means of measuring amino acid metabolism in tissues such as the brain and tumors. Among the many amino acids prepared,[1] L-[*methyl*-^{11}C]methionine (^{11}C-MET) is widely used for clinical PET studies.[2–10] Indeed, it is considered that ^{11}C-MET is a useful radiopharmaceutical for tumor diagnosis because of its simple preparation in ^{11}C-labeling as well as favorable biological properties of MET *in vivo*. Rat tumor uptake of ^{11}C-MET was higher than that of other amino acids,[11] resulting in tumor detection with high contrast to surrounding normal tissue such as brain and lung. From the biological point of view, the label of ^{11}C-MET is not only incorporated into proteins but also into non-protein materials such as lipids and RNA fractions.[12,13] The pathway to transmethylation processes possibly indicates advantageous properties in ^{11}C-MET compared with other amino acids. On the other hand, ^{18}F-labeled amino acids have practical benefits for tumor diagnosis in clinical PET studies. Because a large amount can be produced with ^{18}F-anion, single production of the tracer is sufficient for several patients. It is also possible that the radiopharmaceuticals with a longer half-life can be delivered to a distant PET clinic from the production site. The MET analogs which contain the ^{18}F-labeled alkyl group used in the transalkylation process *in vivo* are

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therefore of interest for tumor detection. Methyl-^{18}F-labeled MET [2-amino-4-(fluoromethylthio)butylic acid], is a candidate compound; but is reported to be unstable.[14] Fluoroalkyl derivatives of MET may be another candidate. It is reported that ethionine [2-amino-4-(ethylthio)butylic acid], an *S*-ethyl analog of MET, is incorporated into proteins and that the ethyl group is also used as an ethyl donor via the transethylation process as in the case of the methyl group of MET.[15,16]

The aim of this study is to prepare ^{11}C-labeled ethionine (^{11}C-ETH) and propionine [2-amino-4-(propylthio)butylic acid, ^{11}C-PRO] as parent compounds of ^{18}F-fluoroalkyl derivatives of MET and to assess their potential for tumor detection. The labeled compounds were prepared by the reaction of L-homocysteine thiolactone and corresponding ^{11}C- or ^{14}C- labeled alkyl iodides. In tumor bearing mice, tumor uptake of L-[*methyl*-^{3}H]methionine (^{3}H-MET), L-[*ethyl*-^{14}C]ethionine (^{14}C-ETH) and L-[*propyl*-^{11}C]propionine (^{11}C-PRO) and their incorporation into macromolecular materials were measured. Biological properties of ETH have been reviewed,[15,16] and distribution of L-[*1,2-ethyl*-^{14}C]ethionine in normal rats over three days after the tracer injection was investigated by mean of whole-body autoradiography[17] and tissue sampling,[18] but the tumor accumulation of the compound in animals is unknown. On the other hand, no information is available for PRO.

MATERIALS AND METHODS

^{3}H-MET (specific activity of 2.59 GBq/μmol) and [1-^{14}C]ethyl iodide (specific activity of 1.85 MBq/μmol) were purchased from American Radiolabeled Chemicals Inc. (St. Louis, MO, USA). Methylmagnesium bromide (3 M solution in diethyl ether) ethylmagnesium bromide (1 M solution in THF), $LiAlH_4$ (1.0 M solution in THF) and THF, which were specially packed in Sure/Seal™ bottles, were purchased from Aldrich Japan Inc. (Tokyo), L-homocysteine thiolactone was from Sigma-Aldrich Japan (Tokyo), L-methionine, L-ethionine and cycloheximide were from Wako Pure Chemical Industries, Ltd. (Tokyo) and Soluen-350 was from Packard Instrument Company, Inc. (Meriden, USA). Male C3H/He mice were supplied by Tokyo Laboratory Animals Co., Ltd. (Tokyo).

Synthesis of ^{11}C-ETH, ^{14}C-ETH and ^{11}C-PRO

Carbon-11 labeled ethyl and propyl iodides were prepared by the method of Längström et al.[19] Briefly, 0.15 mL THF solution of 1 M methylmagnesium or ethylmagnesium bromide was carbonated with $^{11}CO_2$. To the reaction mixture 0.05 mL of 1 M $LiAlH_4$ was added. After dryness of THF, 0.5 mL of HI was added and the solution was heated. The ^{11}C-ethyl iodide or ^{11}C-propyl iodide generated was transferred with a N_2 flow into acetone or ethanol at –40°C. To the solution, aqueous L-homocysteine thiolactone solution and NaOH solution were added. The final 0.5 mL reaction mixture in 75%, 50% or 25% of acetone or ethanol solution contained 2.5 mg of L-homocysteine thiolactone and 0.1 mmol NaOH. The mixture was heated at 60°C for 5 min. One-mL of 0.1 M HCl was then added to the reaction mixture, and the solution was subjected to HPLC separation with a Megapak SIL C18 column (10 mm i.d. × 250 mm, Japan Spectroscopic Co. Ltd., Tokyo). The mobile phase for the separation of ^{11}C-ETH was physiological saline. ^{11}C-ETH and a byproduct ^{11}C-MET were eluted at 5.9–6.9 min and 2.9–3.3 min, respectively, at a flow rate of 10 mL/min. In the case of ^{11}C-PRO the mobile phase was a mixture of CH_3OH and 5 mM HCO_2NH_4 (1/9, v/v), and ^{11}C-MET and ^{11}C-PRO were eluted at 2.5–2.7 min and 6.5–7.5 min, respectively, at a flow rate of 10 mL/min. The ^{11}C-PRO fraction was collected and evaporated to dryness. The ^{11}C-PRO was dissolved in physiological saline followed by membrane filtration and used for the animal study.

Carbon-14 labeled ETH was synthesized by the reaction of L-homocysteine thiolactone and ^{14}C-ethyl iodide in 2 mL of 50% aqueous acetone at 60°C for 5 min. After adding HCl to the reaction mixture, unreacted ^{14}C-ethyl iodide and acetone were removed with a N_2 flow at 60°C. The ^{14}C-ETH was separated under the same HPLC conditions as in the case of ^{11}C-ETH. The radiochemical yield was 67%. The radiochemical and enantiomeric purity analyzed as described below were > 99% and 90%, respectively.

Radiochemical purity was analyzed by HPLC on a Crestpak C18S-10 column (4.6 mm i.d. × 150 mm, Japan Spectroscopic Co. Ltd.). The retention times for ^{11}C-MET and ^{11}C-ETH were 3.0 min and 5.4 min, respectively, with 10 mM HCO_2NH_4 as the elution solution at the flow rate of 1 mL/min. ^{11}C-PRO was eluted at 9.8 min with a mixture of CH_3OH and 10 mM HCO_2NH_4 (5/95, v/v) at a flow rate of 1 mL/min.

Enantiomeric purity was analyzed on a Crownpak CR (+) column (4.0 mm i.d. × 150 mm, Daicel Chemical Industries, Tokyo). The elution solution was 0.01 N $HClO_4$, pH 2.0 and a flow rate was 0.8 mL/min. The retention times were: D- and L-enantiomers of MET, 3.4 min and 5.1 min; D- and L-enantiomers of ETH, 5.4 min and 9.2 min; and D- and L-enantiomers of PRO, 11.4 min and 20.9 min.

Synthesis of PRO

PRO was synthesized by the reaction of 10 mg (65 μmol) of L-homocysteine thiolactone and 60 μL (620 μmol) of propyl iodide in 1 mL of 50% aqueous acetone at 60°C for 5 min. After adding HCl, the reaction mixture was evaporated to dryness, and the residue was dissolved in 2 mL of water. The solution was loaded onto a YMC-pack ODS-A column (20 mm i.d. × 150 mm, SH-342-5, S-5 120 Å, YMC Co. Ltd., Kyoto), and eluted with a mixture of CH_3OH and 5 mM HCO_2NH_4 (1/9, v/v) at a flow rate of 10

Table 1 Tissue distribution of radioactivity after intravenous injection of L-[*methyl*-^{3}H]methionine, L-[*ethyl*-^{14}C]ethionine and L-[*propyl*-^{11}C]propionine into FM3A bearing mice

	Uptake (%ID/g)			
	5 min	15 min	30 min	60 min
L-[*methyl*-^{3}H]methionine				
Plasma	4.36 ± 0.78	2.05 ± 0.25	2.67 ± 0.16	4.78 ± 1.10
Liver	14.78 ± 4.90	24.86 ± 4.80	18.99 ± 1.07	22.15 ± 4.05
Pancreas	42.57 ± 2.47	74.07 ± 10.56	97.10 ± 34.78	90.00 ± 22.03
Kidney	12.89 ± 2.61	11.11 ± 1.60	10.92 ± 1.36	10.84 ± 0.82
Muscle	3.64 ± 0.72	3.61 ± 0.67	3.13 ± 0.84	2.45 ± 0.21
Brain	2.89 ± 0.82	2.79 ± 0.29	2.47 ± 0.18	2.53 ± 0.20
FM3A	5.11 ± 2.26	9.03 ± 1.11	5.88 ± 2.62	6.00 ± 2.33
L-[*ethyl*-^{14}C]ethionine				
Plasma	9.08 ± 0.80	6.13 ± 0.32	5.69 ± 0.36	5.87 ± 0.54
Liver	7.52 ± 2.22	11.69 ± 2.68	9.41 ± 1.54	10.50 ± 1.77
Pancreas	31.70 ± 1.81	39.78 ± 1.33	40.42 ± 13.34	37.67 ± 8.71
Kidney	15.28 ± 2.29	13.01 ± 1.25	13.23 ± 2.95	12.10 ± 1.26
Muscle	4.96 ± 1.18	6.30 ± 1.51	6.23 ± 1.40	5.53 ± 0.83
Brain	3.53 ± 0.32	4.59 ± 0.53	3.83 ± 0.45	4.08 ± 0.49
FM3A	5.50 ± 2.42	11.33 ± 0.89	9.43 ± 4.22	10.26 ± 4.42
L-[*propyl*-^{11}C]propionine				
Plasma	5.23 ± 0.43	3.17 ± 0.14	2.27 ± 0.17	1.44 ± 0.28
Liver	5.14 ± 0.35	3.85 ± 0.23	3.09 ± 0.39	2.57 ± 0.74
Pancreas	11.70 ± 2.10	7.39 ± 0.67	4.95 ± 0.92	4.01 ± 0.46
Kidney	40.24 ± 2.01	33.09 ± 3.09	21.83 ± 3.02	16.33 ± 1.92
Muscle	3.17 ± 0.23	2.88 ± 0.27	2.52 ± 0.28	1.69 ± 0.09
Brain	2.63 ± 0.14	2.05 ± 0.10	1.35 ± 0.14	1.03 ± 0.27
FM3A	3.20 ± 0.53	4.78 ± 0.64	2.59 ± 0.44	3.84 ± 1.76

Mean ± S.D. (n = 4–5)

mL/min. The PRO fraction was collected and evaporated to dryness. After this purification procedure was repeated once, the PRO fraction was lyophilized to remove concomitant HCO_2NH_4.

PRO was identified by ^{1}H-NMR spectroscopy. ^{1}H-NMR spectra were recorded with a JNM-EX90A spectrophotometer (JEOL, Tokyo) on 89.45 MHz. Chemical shifts (δ) referred to the external TMS standard were expressed as ppm. Samples were dissolved in D_2O containing a few drops of DCl. ^{1}H-NMR data: PRO δ 0.89 (t, J = 7.1 Hz, 3H), 1.42–1.74 (m, 2H), 2.12–2.31 (m, 2H), 2.44–2.77 (m, 4H), 4.19 (t, J = 6.1 Hz, 1H); ETH δ 1.18 (t, J = 7.4 Hz, 3H), 2.14–2.80 (m, 6H), 4.21 (t, J = 6.4 Hz, 1H); and MET δ 2.09 (s, 3H), 2.13–2.77 (m, 4H), 4.23 (t, J = 6.2 Hz, 1H).

Tissue distribution study

FM3A mammary carcinoma-bearing mice weighing 24.3 ± 1.1 g were prepared as described previously.[20] A mixture of ^{3}H-MET (150 kBq/0.058 nmol), ^{14}C-ETH (30 kBq/16 nmol) and ^{11}C-PRO (5 MBq) was injected intravenously into the mice. They were killed by cervical dislocation 5, 15, 30 and 60 min post injection. Blood was removed by heart puncture with a heparinized syringe. Plasma was separated by centrifugation. Brain, liver, pancreas, muscle and FM3A tissues were dissected. The tissue uptake of the three radionuclides was expressed as the % injected dose per g of tissue (%ID/g), as described previously.[12,13]

The second group of mice were injected with a mixture of three amino acids together with carrier MET (25 μmol/animal), and killed 5 min post injection.

The third group of mice were given intraperitoneally cycloheximide (100 mg/kg body weight) dissolved in physiological saline.[12,13] Thirty minutes later, the mixture of labeled amino acids was injected into the mice, which were killed 60 min post injection. The tissue uptake of three radionuclides was measured as described above.

The animal studies were approved by the Animal Care and Use Committee of Tokyo Metropolitan Institute of Gerontology.

Incorporation of ^{3}H-MET, ^{14}C-ETH and ^{11}C-PRO into acid-precipitable materials

The incorporation of ^{3}H- and ^{14}C-radioactivity into acid-precipitable materials was measured in samples of brain,

Table 2 Uptake ratios of tumor to tissue after i.v. injection of L-[*methyl*-^{3}H]methionine, L-[*ethyl*-^{14}C]ethionine and L-[*propyl*-^{11}C]propionine into FM3A bearing mice

	Ratio			
	5 min	15 min	30 min	60 min
L-[*methyl*-^{3}H]methionine				
FM3A/Plasma	1.13 ± 0.40	4.41 ± 0.39	2.19 ± 0.92	1.14 ± 0.31
FM3A/Liver	0.38 ± 0.21	0.38 ± 0.09	0.31 ± 0.14	0.26 ± 0.09
FM3A/Pancreas	0.11 ± 0.06	0.13 ± 0.01	0.06 ± 0.02	0.07 ± 0.02
FM3A/Kidney	0.45 ± 0.29	0.83 ± 0.14	0.55 ± 0.27	0.55 ± 0.22
FM3A/Muscle	1.57 ± 0.97	2.61 ± 0.61	1.80 ± 0.29	2.55 ± 1.16
FM3A/Brain	2.03 ± 1.13	3.26 ± 0.48	2.35 ± 0.89	2.33 ± 0.75
L-[*ethyl*-^{14}C]ethionine				
FM3A/Plasma	0.59 ± 0.24	1.85 ± 0.15	1.65 ± 0.68	1.69 ± 0.66
FM3A/Liver	0.75 ± 0.29	1.01 ± 0.17	1.04 ± 0.52	0.90 ± 0.25
FM3A/Pancreas	0.17 ± 0.09	0.30 ± 0.01	0.23 ± 0.05	0.29 ± 0.09
FM3A/Kidney	0.39 ± 0.23	0.88 ± 0.10	0.73 ± 0.32	0.84 ± 0.35
FM3A/Muscle	1.27 ± 0.81	1.92 ± 0.50	1.45 ± 0.29	1.95 ± 1.05
FM3A/Brain	1.62 ± 0.82	2.52 ± 0.46	2.39 ± 0.76	2.64 ± 1.09
L-[*propyl*-^{11}C]propionine				
FM3A/Plasma	0.62 ± 0.10	1.52 ± 0.24	1.16 ± 0.27	2.88 ± 1.13
FM3A/Liver	0.62 ± 0.06	1.26 ± 0.21	0.87 ± 0.26	1.71 ± 0.74
FM3A/Pancreas	0.24 ± 0.03	0.65 ± 0.08	0.54 ± 0.11	0.96 ± 0.35
FM3A/Kidney	0.08 ± 0.02	0.16 ± 0.02	0.12 ± 0.04	0.24 ± 0.10
FM3A/Muscle	1.01 ± 0.19	1.68 ± 0.29	1.04 ± 0.17	2.31 ± 1.01
FM3A/Brain	1.22 ± 0.21	2.33 ± 0.26	1.97 ± 0.53	4.15 ± 1.50

Mean ± S.D. (n = 4–5)

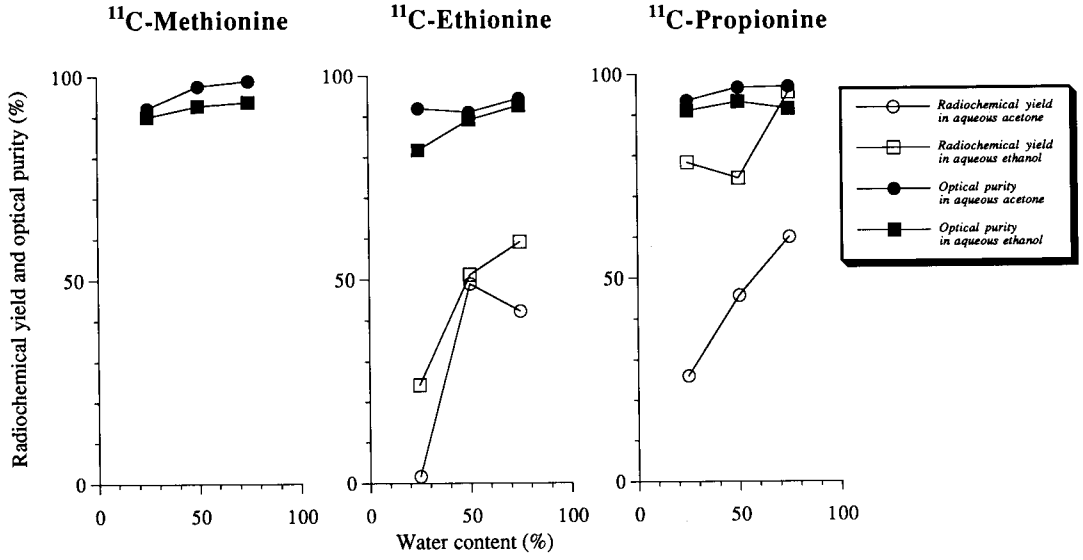

Fig. 1 Radiochemical yields and enantiomeric purity in the synthesis of L-[*ethyl*-^{11}C]ethionine, L-[*propyl*-^{11}C]propionine and L-[*methyl*-^{11}C]methionine. The data for L-[*methyl*-^{11}C]methionine was based on the enantiomeric purity of the L-[*methyl*-^{11}C]methionine obtained as a byproduct in the synthesis of L-[*ethyl*-^{11}C]ethionine. Radiochemical yields were based on the corresponding ^{11}C-labeled alkyl iodides. Data show means of 1–3 experiments. Open symbols, radiochemical yields; solid symbols, optical purity; circle, reaction in aqueous acetone; and square, reaction in aqueous ethanol.

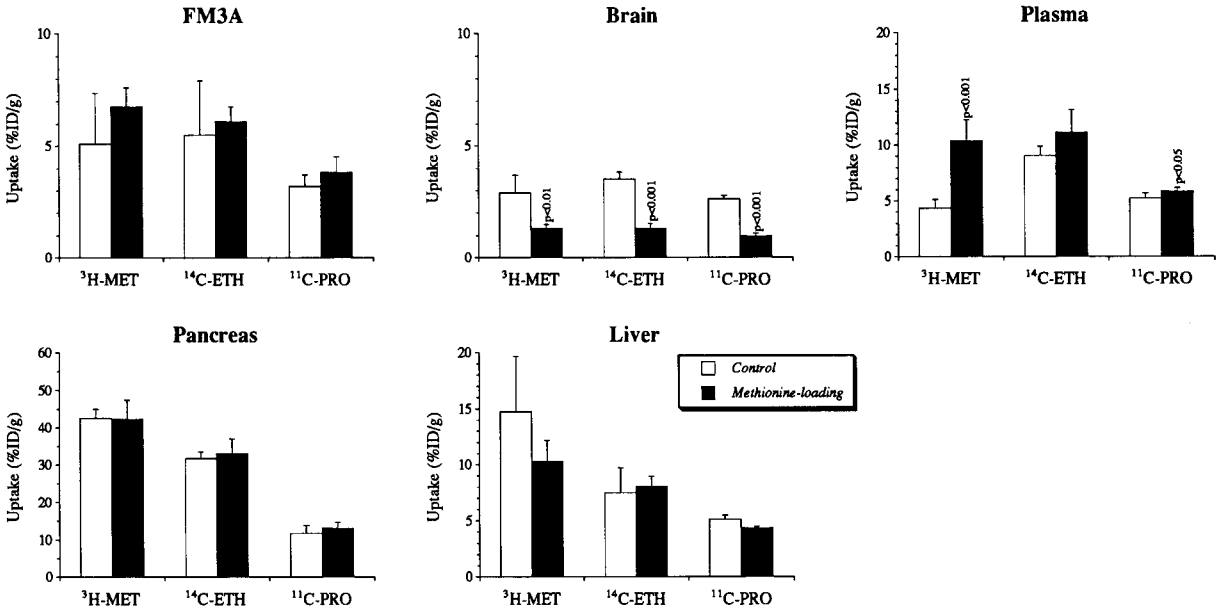

Fig. 2 Effect of carrier methionine-loading on the tissue distribution of radioactivity 5 min after i.v. injection of L-[*methyl*-^{3}H]methionine, L-[*ethyl*-^{14}C]ethionine and L-[*propyl*-^{11}C]propionine into FM3A bearing mice. Open columns, control; and solid columns, methionine-loading group. Mean ± S.D. (n = 5). Student's t-tests were carried out between two groups.

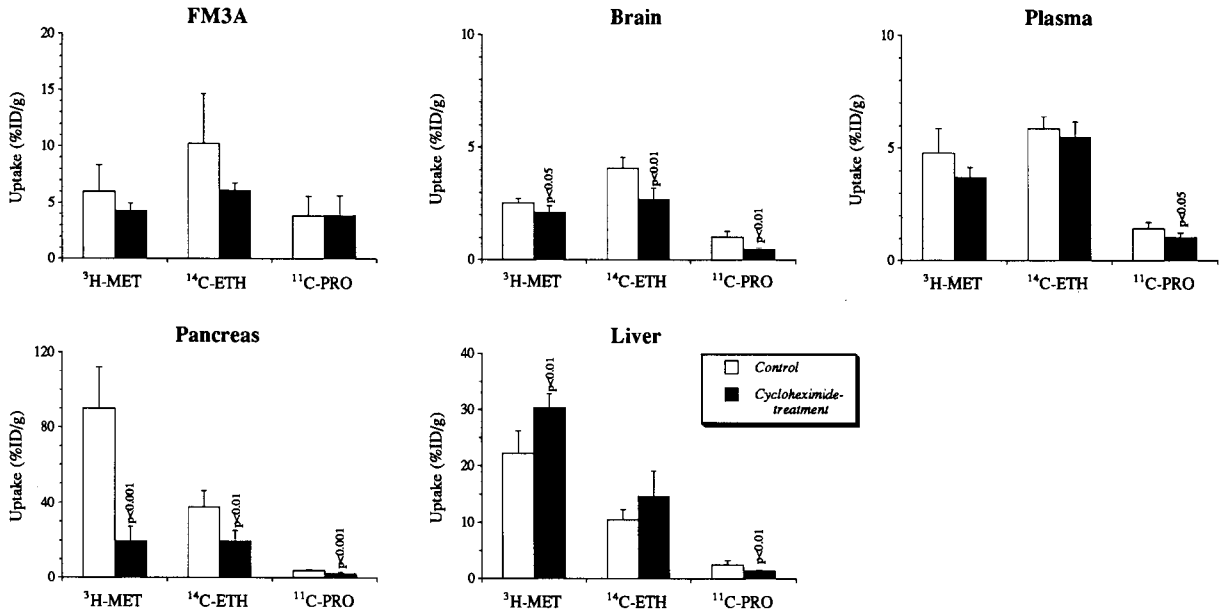

Fig. 3 Effect of cycloheximide-treatment on the tissue distribution of radioactivity 60 min after i.v. injection of L-[*methyl*-^{3}H]methionine, L-[*ethyl*-^{14}C]ethionine and L-[*propyl*-^{11}C]propionine into FM3A bearing mice. Open columns, control; and solid columns, cycloheximide-treated group. Mean ± S.D. (n = 5). Student's t-tests were carried out between two groups.

tumor, pancreas and liver tissues and plasma as described previously.[12,13] To measure incorporation of ^{11}C-radioactivity, only ^{11}C-PRO (5 MBq) was injected intravenously into another group of mice (n = 3). The incorporation of ^{11}C-radioactivity into acid-precipitable materials was measured as described previously.[12,13]

RESULTS

Figure 1 summarizes the results of the radiochemical synthesis. The radiochemical yields of ^{11}C-ETH and ^{11}C-PRO increased with the increasing water contents. The yields were higher in the aqueous ethanol than in the

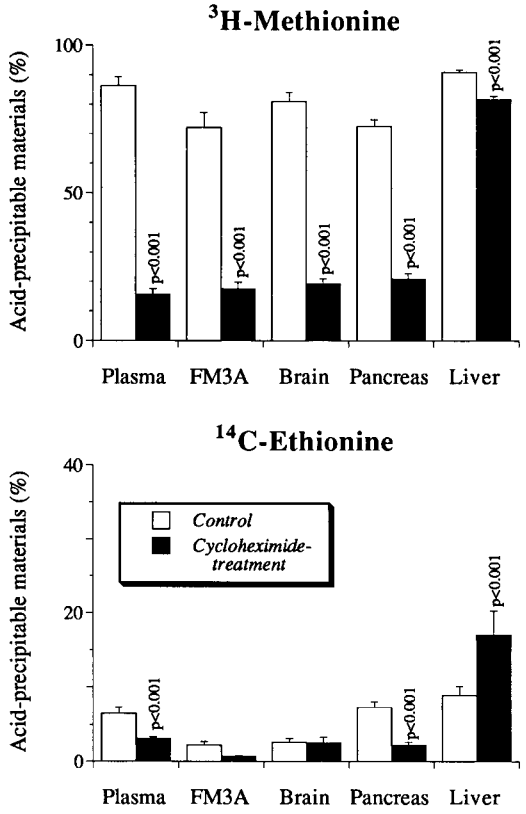

Fig. 4 Effect of cycloheximide-treatment on the incorporation of radioactivity into the acid-precipitable materials 60 min after i.v. injection of L-[*methyl*-^{3}H]methionine, L-[*ethyl*-^{14}C]ethionine and L-[*propyl*-^{11}C]propionine into FM3A bearing mice. Open columns, control; and solid columns, cycloheximide-treated group. Mean ± S.D. (n = 2–5). Student's t-tests were carried out between two groups.

aqueous acetone. Radiochemical purity was > 99%. The enantiomeric purity of the three amino acids also decreased with the decreasing water content, and was slightly higher in the aqueous acetone than in the aqueous ethanol.

The tissue distribution of ^{3}H-MET, ^{14}C-ETH and ^{11}C-PRO in the tumor bearing mice is summarized in Table 1. In the plasma, the levels of the ^{3}H- and ^{14}C-radioactivity decreased for the first 30 min, after which the level of the ^{3}H increased, but the level of the ^{14}C remained constant. The level of ^{11}C-radioactivity decreased with time. The pancreas showed the highest uptake of ^{3}H-MET and ^{14}C-ETH, whereas the highest uptake of ^{11}C-PRO was found in the kidneys. The liver uptake of ^{14}C-ETH and ^{11}C-PRO was significantly lower than that of ^{3}H-MET. The uptake of all three tracers is higher in the tumor than in the brain and muscles. In these three tissues, the highest uptake was found in ^{14}C-ETH, followed by ^{3}H-MET and ^{11}C-PRO, but the tumor to brain and tumor to muscle uptake ratios were similar for all three tracers, and only the tumor to brain and tumor to plasma ratios for ^{11}C-PRO at 60 min were larger than that for ^{3}H-MET (Table 2). The tumor to liver ratios for ^{14}C-ETH and ^{11}C-PRO were nearly unity.

The effects of carrier MET-loading and cycloheximide-treatment on the tissue distribution of the three tracers are summarized in Figs. 2 and 3, respectively. Only the brain uptake of all three tracers was significantly decreased by the co-injection of carrier MET. Following the cycloheximide treatment, the uptake of all three tracers significantly decreased in the brain and pancreas. In the liver, the uptake of ^{3}H-MET and ^{14}C-ETH was enhanced by cycloheximide, but that of ^{11}C-PRO decreased. Neither treatment affected the tumor uptake of any of the three tracers.

Figure 4 shows the incorporation of ^{3}H-MET and ^{14}C-ETH into the acid-precipitable materials at 60 min after injection. Pretreatment with cycloheximide greatly reduced the acid-precipitable fraction of ^{3}H-MET in the brain, tumor, pancreas and plasma. This fraction of ^{14}C-ETH was lower than that of ^{3}H-MET, but it decreased after the pretreatment with cycloheximide. In the liver, the cycloheximide slightly reduced the acid-precipitable fraction of ^{3}H-MET, and enhanced that of ^{14}C-ETH. No incorporation of ^{11}C-PRO into the acid-precipitable fraction was found in the brain, tumor or plasma.

DISCUSSION

In the reaction of L-homocysteine thiolactone and ^{11}C-methyl iodide, D-enantiomer of ^{11}C-MET was produced.[21] Similar results were also found in the synthesis of ^{11}C-ETH and ^{11}C-PRO. Aqueous acetone was preferable in order to maintain high enantiomeric purity. We found that the D-form of ^{11}C-ETH reached 43% in the DMF solution (data not shown). In HPLC separation, physiological saline was successfully used as an eluting solution to separate ^{11}C-ETH as in the case of ^{11}C-MET,[22] but, this was not applicable to the separation of ^{11}C-PRO (retention time, 17–19.5 min).

In the tumor-bearing mice, the pattern of distribution of ^{14}C-ETH in the normal organs was similar to that in normal rats described previously.[16,17] As for the tumor uptake of ^{14}C-ETH and ^{11}C-PRO, several interesting characteristics as methionine analogs were found. Although the tumor uptake increased in the order of ^{11}C-PRO, ^{3}H-MET and ^{14}C-ETH, the tumor to brain and tumor to muscle uptake ratios were similar for all three tracers. The radioactivity levels of ^{14}C-ETH and ^{11}C-PRO in the brain and tumor may depend on the corresponding protein-free radioactivity in the plasma. An advantage of ^{14}C-ETH and ^{11}C-PRO is the low uptake by the liver and pancreas compared with ^{3}H-MET: the order of the liver to tumor uptake ratio, ^{11}C-PRO ($\geq$1), ^{14}C-ETH ($\fallingdotseq$1) and ^{3}H-MET ($1 \gg$), suggesting practicability for tumor imaging in the abdominal region. Since ^{11}C-PRO did not follow the metabolic pathways of methionine as described below, it was cleared from the liver and other organs and excreted into urine via the kidneys, but the ^{11}C-PRO was retained in the amino acid pool of tumor tissues. A similar charac-

teristic was also found in other artificial amino acids.[11]

The reduced brain uptake of the three tracers caused by carrier methionine loading suggests these three amino acids are taken by the same transport system across the blood-brain barrier. On the other hand, the tumor uptake was not affected. These results also suggest that the contrast between the tumor and the surrounding normal brain tissue is enhanced by the methionine-loading.

It has been reported that the ^{14}C-ETH was incorporated into proteins and other macromolecules via the transethylation process.[15,16] As shown in Fig. 4, we confirmed the incorporation of ^{14}C-ETH into acid-precipitable materials, but the fraction was small compared with that of ^{3}H-MET. The cycloheximide treatment, which inhibited protein synthesis *in vivo* (Fig. 4 and references 12 and 13), reduced the brain uptake of all three tracers but not the tumor uptake (Fig. 3). A similar conflicting response to the cycloheximide treatment between the brain and tumor is also observed for other natural amino acids including L-leucine[12,13] and L-tyrosine.[23] Furthermore, the cycloheximide-treatment significantly reduced the incorporation of ^{14}C-ETH into acid-precipitable materials in the pancreas and tumor. In the liver the cycloheximide-treatment enhanced the incorporation, suggesting the incorporation of the radioactivity into non-protein materials such as phospholipids, as shown in the case of ^{3}H-MET.[23] These facts demonstrate that the ^{14}C-ETH was incorporated not only into proteins but also into non-protein materials via the transmethylation process. On the other hand, ^{11}C-PRO was not incorporated into the acid-precipitable materials.

It is pointed out that ETH induces liver cancer as chronic biological effects, although it shows signs of similar metabolism to that of MET and inhibits tumor growth.[16] Nevertheless, the addition of extra methionine completely counteracts the carcinogenic activity and every other biochemical and morphologic effect of the ETH.[16] In PET studies, the estimated injected dose of ^{11}C-ETH is very low in inducing even acute biological effects[16] because of the high specific activity of the ^{11}C. In addition, the PET studies are usually limited within a few times. ^{11}C-ETH would therefore be used for the tumor diagnosis under careful supervision in which subjects are given a sufficient amount of the natural amino acid containing MET immediately after the PET studies.

The present study shows that ^{14}C-ETH but not ^{11}C-PRO has similar *in vivo* characteristics to ^{3}H-MET, suggesting that ^{11}C-labeled ETH possibly has a similar potential for tumor detection by PET as ^{11}C-MET. ^{11}C-PRO showed signs of similar properties to other artificial amino acids[11] reflecting the amino acid transport. Both ^{11}C-ETH and ^{11}C-PRO may be of interest for tumor imaging in the abdominal region from the point of view of the tumor-to-organ uptake ratios. ^{18}F-fluoroalkyl derivatives of MET are therefore of interest as a next step in the development of ^{18}F-labeled amino acids for tumor detection in clinical PET studies. In view of the similar van der Waals radiuses of fluorine and hydrogen, a ^{18}F-fluoroethyl derivative of ETH [^{18}F-2-amino-4-(fluoroethylthio)butylic acid] is an ETH analog, as in the case of ^{18}F-2-deoxy-2-fluoro-D-glucose as a 2-deoxy-D-glucose analog. On the other hand, when taking account the electronegativity of fluorine, methyl-^{18}F-labeled MET [^{18}F-2-amino-4-(fluoromethylthio)butylic acid] and ethyl-^{18}F-labeled ETH may correspond to ETH and PRO, respectively, as in the case of ^{18}F-5-fluoro-2′-deoxyuridine as a thymidine analog.

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