Re-evaluation of Amino Acid PET Studies: Can the Protein Synthesis Rates in Brain and Tumor Tissues Be Measured In Vivo?

Kiichi Ishiwata, Kazuo Kubota, Matsutaro Murakami, Roko Kubota, Toru Sasaki, Shinichi Ishii and Michio Senda

Positron Medical Center, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan; Department of Radiology and Nuclear Medicine, The Research Institute for Cancer and Tuberculosis, Tohoku University, Sendai, Japan; and Department of Radiology and Nuclear Medicine, Research Institute for Brain and Blood Vessel-Akita, Akita, Japan

The significance of L-[methyl-^{11}C]methionine (^{11}C-Met), L-[1-^{11}C]leucine (^{11}C-Leu) and L-2-[^{18}F]fluorotyrosine (^{18}F-Tyr) for measuring protein synthesis rates in the brain and in tumors by PET was re-evaluated. Tissue uptake and protein incorporation of ^{3}H-Met, ^{14}C-Leu and ^{18}F-Tyr were investigated in mice bearing the FM3A mammary carcinoma. In the control group, the uptake of all three tracers in the brain and FM3A and their incorporation into the acid-precipitable fraction (APF) increased over 60 min. When the protein synthesis in vivo was inhibited by cycloheximide, the incorporation of all three tracers into the APF was significantly reduced to 6%–32% and 3%–11% of the control in the brain and FM3A, respectively. Under these conditions, total uptake of ^{14}C-Leu in the brain and FM3A decreased rapidly, and most of the ^{14}C in the APF was detected as proteins. On the other hand, ^{3}H-Met and ^{18}F-Tyr uptake continued to increase, and significant amounts of radioactivity were incorporated into nonprotein materials. In mice given ouabain to inhibit amino acid transport, total uptake of all three amino acids by FM3A was reduced to 67%–74% of the control 5 min postinjection. These results demonstrate that uptake of the three amino acids is affected by alterations in the amino acid transport system in the brain and tumor tissues, but that only ^{14}C-Leu uptake reflects protein synthesis rates.

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PET using 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. For this purpose, many positron-emitting amino acids have been prepared (see references in *1*). Among these, L-[methyl-^{11}C]methionine (^{11}C-Met) is widely used for PET studies ((*2–10*) and their references) because it has a high blood-brain barrier permeability and ^{11}C labeling is not too complicated. The preparation is reliable and well suited for routine production. It is suggested that the significance of this tracer is its application to measuring protein synthesis rates (PSR) in tissues. Indeed in the rat brain and in tumors, the tracer was incorporated into acid-precipitable macromolecular materials (*11,12*). It was, however, also incorporated into the lipid fraction (*11,13*) and into nucleic acids (*14*) by transmethylation via S-adenosyl-L-methionine. Carboxylic-labeled amino acids are supposed to be more suitable for measuring PSR because the label is mainly incorporated into proteins or washed out via side reactions such as decarboxylation and oxidation (*15*). A metabolic study of L-[1-^{14}C]tyrosine (^{14}C-Tyr) demonstrated the rationale of using ^{11}C-labeled Tyr for this purpose (*16*). Keen et al. (*17*) reported a more detailed metabolic study using L-[1-^{14}C]leucine (^{14}C-Leu) in rats for tracer kinetic modeling. Recently, a PET study using dogs demonstrated that ^{11}C-labeled CO_2/HCO_3^- is not eliminated from the body rapidly enough to neglect their contribution to PET images (*18*). Therefore, measurement of the PSR in humans with PET using ^{11}C-labeled amino acids could require a kinetic model, including a catabolite-pool (*19,20*). Because of the relatively slow PSR in the primate brain, Coenen et al. proposed that L-2-[^{18}F]fluorotyrosine (^{18}F-Tyr) with a longer lived radionuclide would be a suitable tracer to study PSR (*21*). Brain uptake of ^{18}F-Tyr increased with time and the label was incorporated into cerebral proteins without significant catabolism. These properties could be more promising for measuring PSR and contrast with the properties of the parent amino acid Tyr. Brain uptake of ^{14}C-Tyr decreased over time, probably because of decarboxylation superimposed on increasing radioactivity due to protein incorporation (*16*).

The aim of this study was to investigate whether amino acid uptake by brain and tumor tissues reflects PSR in these tissues. We also discuss the kind of biochemical information that can be extracted from PET studies with the positron-emitting amino acids: ^{11}C-Met, ^{11}C-Leu and ^{18}F-Tyr. A group of mice bearing FM3A mammary carci-

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For correspondence or reprints contact: Dr. Kiichi Ishiwata, Positron Medical Center, Tokyo Metropolitan Institute of Gerontology, 1-1 Naka-cho, Itabashi, Tokyo 173, Japan.

TABLE 1
Effects of Cycloheximide and Ouabain on Tissue Distribution of Radioactivity in Mice Bearing FM3A Tumors After Intravenous Injection with L-[methyl-^{3}H]methionine

	Treatment	Uptake (%ID/g) 5 min	30 min	60 min
Plasma	Control	1.60 ± 0.08	1.21 ± 0.05	2.41 ± 1.00
	Cycloheximide	2.40 ± 0.30‡	1.40 ± 0.37	1.48 ± 0.09
	Ouabain	1.69 ± 0.21	0.99 ± 0.07‡	1.63 ± 0.15
Brain	Control	1.59 ± 0.11	1.35 ± 0.06	1.85 ± 0.32
	Cycloheximide	1.55 ± 0.34	1.30 ± 0.21	1.39 ± 0.07‡
	Ouabain	1.58 ± 0.29	1.39 ± 0.07	2.01 ± 0.20
FM3A	Control	5.46 ± 1.70	6.07 ± 0.63	7.18 ± 1.31
	Cycloheximide	4.40 ± 1.07	4.98 ± 0.81	5.19 ± 0.65*
	Ouabain	3.68 ± 0.68	4.38 ± 1.08*	7.69 ± 0.44
Pancreas	Control	36.68 ± 4.08	45.75 ± 4.02	38.09 ± 7.61
	Cycloheximide	25.09 ± 4.35†	17.63 ± 1.90‡	13.85 ± 1.44‡
	Ouabain	33.39 ± 6.27	35.74 ± 5.16*	31.88 ± 5.07
Liver	Control	12.92 ± 0.68	15.28 ± 1.22	20.05 ± 1.94
	Cycloheximide	16.61 ± 3.73	18.63 ± 1.81*	33.77 ± 3.04†
	Ouabain	12.03 ± 1.54	7.96 ± 0.23‡	20.30 ± 2.28
Muscle	Control	2.20 ± 0.17	1.54 ± 0.13	1.39 ± 0.09
	Cycloheximide	2.66 ± 0.51	1.81 ± 0.25	2.54 ± 0.60†
	Ouabain	2.37 ± 0.43	1.04 ± 0.09‡	1.72 ± 0.45

Mean ± s.d. (n = 4–5).
Student's t-tests were carried out between the control and the cycloheximide- or ouabain-treated group. *$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$.

noma was treated in vivo with cycloheximide to inhibit protein synthesis in brain and tumor tissues. Another group of mice was given ouabain to block amino acid transport. In these two groups and in the control, ^{3}H-Met, ^{14}C-Leu and ^{18}F-Tyr were co-injected and tissue uptake and the incorporation into the acid-precipitable fraction (APF) were compared. The APF was further divided into proteins, lipids and nucleic acids. In a previous study (*22*), we found that small amounts of ^{3}H-Met and ^{18}F-Tyr were incorporated into nonprotein macromolecules.

MATERIALS AND METHODS

Tritium-3-Met (specific activity of 7.3 GBq/mmol) was purchased from Du Pont-NEN Research Products (Wilmington, DE) and ^{14}C-Leu (specific activity of 1.92 GBq/mmol) from ICN Biomedicals Inc. (Irvine, CA). Fluorine-18-Tyr (specific activity of 12.6–17.4 GBq/mmol) was synthesized in our laboratory (*23*) according to the method of Coenen et al. (*24*). Cycloheximide and ouabain were obtained from Wako Pure Chemical Industries, Ltd. (Tokyo, Japan). C3H/He mice bearing FM3A mammary carcinomas were prepared as described previously (*25*).

Forty mice weighing 26.0 ± 2.0 g were divided into three groups. The control group was injected i.p. with 0.2 ml of physiological saline, whereas the second and the third groups were given cycloheximide (100 mg/kg body weight) or ouabain (1 mg/kg body weight) dissolved in physiological saline, respectively. Thirty minutes later, a mixture of ^{3}H-Met (185 kBq/25.3 nmol), ^{14}C-Leu (37 kBq/19.3 nmol) and ^{18}F-Tyr (1.85 MBq/106–147 nmol) was administered and the mice were killed by cervical dislocation 5, 30 and 60 min postinjection. Blood was removed by heart puncture using a heparinized syringe. Plasma was separated by centrifugation. Brain, pancreas, liver, muscle and FM3A tissues were dissected and divided into three portions. Fluorine-18 in the first portion of weighed tissues (40–120 mg) was counted using a gamma counter. After decay of ^{18}F radioactivity, the tissues were dissolved in tissue solubilizer and counted for ^{3}H and ^{14}C in a liquid scintillation counter. Tissue uptake of radioactivity was expressed as the percent injected dose per gram of tissue (%ID/g). In order to assess the incorporation of radioactivity into macromolecular materials, a second portion of the tissues was homogenized in trichloroacetic acid and divided into the acid-soluble fraction (ASF) and APF as described previously (*16*). The residual part of the brain and tumor tissues was stored at −80°C and used for further experiments. The APF of residual tissues was divided into four fractions: lipids, RNA, DNA and proteins, as described previously (*22*). Briefly, after extracting lipids from the APF with $CHCl_3$-CH_3OH, 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 (alkaline-labile fraction) and precipitate. Thereafter, the precipitate was heated in 0.5 *M* $HClO_4$ at 90°C for 15 min to hydrolyze the DNA. Finally, the solution at ice-cold temperature was divided into supernatant (acid-labile fraction) and the precipitate (protein fraction).

RESULTS

Tissue distribution of the three tracers and cycloheximide and ouabain effects are summarized in Tables 1 to 3. In the control group, all three tracers showed similar tissue distribution; the highest uptake was observed in the pancreas followed by the liver, FM3A, muscle and brain. In the brain, FM3A and liver, increasing uptake was observed during 60 min after injection. The pancreas showed the highest uptake at 30 min after injection. The brain uptake of ^{3}H-Met was slightly higher than that of ^{14}C-Leu or ^{18}F-Tyr,

TABLE 2
Effects of Cycloheximide and Ouabain on Tissue Distribution of Radioactivity in Mice Bearing FM3A Tumors After Intravenous Injection with L-[1-^{14}C]Leucine

	Treatment	Uptake (%ID/g) 5 min	30 min	60 min
Plasma	Control	1.63 ± 0.16	1.81 ± 0.33	3.26 ± 0.73
	Cycloheximide	3.04 ± 0.50‡	0.94 ± 0.25†	0.48 ± 0.06‡
	Ouabain	1.68 ± 0.24	1.87 ± 0.17	3.38 ± 0.26
Brain	Control	0.91 ± 0.08	0.99 ± 0.10	1.39 ± 0.22
	Cycloheximide	0.60 ± 0.13†	0.26 ± 0.02‡	0.17 ± 0.02‡
	Ouabain	1.16 ± 0.24	1.39 ± 0.07‡	1.68 ± 0.18
FM3A	Control	4.08 ± 1.05	4.38 ± 0.53	4.60 ± 1.05
	Cycloheximide	3.49 ± 0.60	2.51 ± 0.26	1.32 ± 0.20‡
	Ouabain	3.01 ± 0.54	4.38 ± 1.08	5.06 ± 0.65
Pancreas	Control	28.08 ± 4.45	36.11 ± 4.89	29.79 ± 7.10
	Cycloheximide	11.85 ± 2.37‡	5.48 ± 0.65‡	2.68 ± 0.15‡
	Ouabain	24.76 ± 5.60	32.56 ± 0.27	24.96 ± 5.85
Liver	Control	5.86 ± 1.32	5.85 ± 0.88	6.73 ± 0.49
	Cycloheximide	1.23 ± 0.15‡	0.80 ± 0.15‡	0.76 ± 0.08‡
	Ouabain	5.71 ± 0.83	7.96 ± 0.20†	8.84 ± 0.92†
Muscle	Control	1.88 ± 0.10	0.86 ± 0.11	0.92 ± 0.05
	Cycloheximide	2.35 ± 0.34*	0.88 ± 0.10	0.42 ± 0.09‡
	Ouabain	2.06 ± 0.40	1.46 ± 0.09‡	1.11 ± 0.10†

Mean ± s.d. (n = 4–5).
Student's t-tests were carried out between the control and the cycloheximide- or ouabain-treated group. *p < 0.05, †p < 0.01, ‡p < 0.001.

and the tumor uptake of ^{14}C-Leu was slightly lower as compared with the other two amino acids.

Cycloheximide induced a very different tissue distribution. Tumor uptake of ^{3}H-Met increased for 60 min, whereas that of the brain remained nearly constant. Radioactivity levels in these tissues were slightly lower compared with the control. Uptake in the pancreas decreased over time. Liver uptake increased over time and was even enhanced when compared to untreated controls. The amount of ^{14}C-Leu in the brain, FM3A, pancreas and liver decreased rapidly. The level of ^{18}F-Tyr increased over time in the brain and FM3A, but decreased in pancreas and liver.

TABLE 3
Effects of Cycloheximide and Ouabain on Tissue Distribution of Radioactivity in Mice Bearing FM3A Tumors After Intravenous Injection with L-[2-^{18}F]Fluorotyrosine

	Treatment	Uptake (%ID/g) 5 min	30 min	60 min
Plasma	Control	3.14 ± 0.20	2.99 ± 0.24	4.38 ± 0.38
	Cycloheximide	4.02 ± 0.48†	2.61 ± 0.35	2.65 ± 0.32‡
	Ouabain	2.96 ± 0.33	2.89 ± 0.15	4.70 ± 0.43
Brain	Control	0.78 ± 0.14	1.18 ± 0.52	1.49 ± 0.33
	Cycloheximide	0.98 ± 0.16	1.07 ± 0.24	1.25 ± 0.25
	Ouabain	0.98 ± 0.29	1.00 ± 0.11	1.03 ± 0.03*
FM3A	Control	4.67 ± 0.91	6.11 ± 0.38	6.89 ± 1.03
	Cycloheximide	4.31 ± 0.95	6.00 ± 0.96	6.53 ± 0.62
	Ouabain	3.46 ± 0.49*	5.02 ± 0.72*	6.24 ± 0.65
Pancreas	Control	25.33 ± 3.29	30.70 ± 2.70	30.27 ± 3.00
	Cycloheximide	20.06 ± 3.40*	11.88 ± 2.04‡	10.38 ± 1.09‡
	Ouabain	21.69 ± 1.95	18.19 ± 0.56‡	21.31 ± 3.04†
Liver	Control	8.75 ± 1.01	8.93 ± 1.20	9.85 ± 0.71
	Cycloheximide	8.27 ± 1.83	5.33 ± 1.84*	3.34 ± 0.58‡
	Ouabain	9.38 ± 1.25	11.64 ± 2.23	11.71 ± 1.49*
Muscle	Control	2.53 ± 0.15	1.38 ± 0.15	1.03 ± 0.09
	Cycloheximide	2.86 ± 0.38	2.26 ± 0.26‡	2.51 ± 0.46‡
	Ouabain	2.59 ± 0.49	1.37 ± 0.16	0.99 ± 0.14

Mean ± s.d. (n = 4–5).
Student's t-tests were carried out between the control and the cycloheximide- or ouabain-treated group. *p < 0.05, †p < 0.01, ‡p < 0.001.

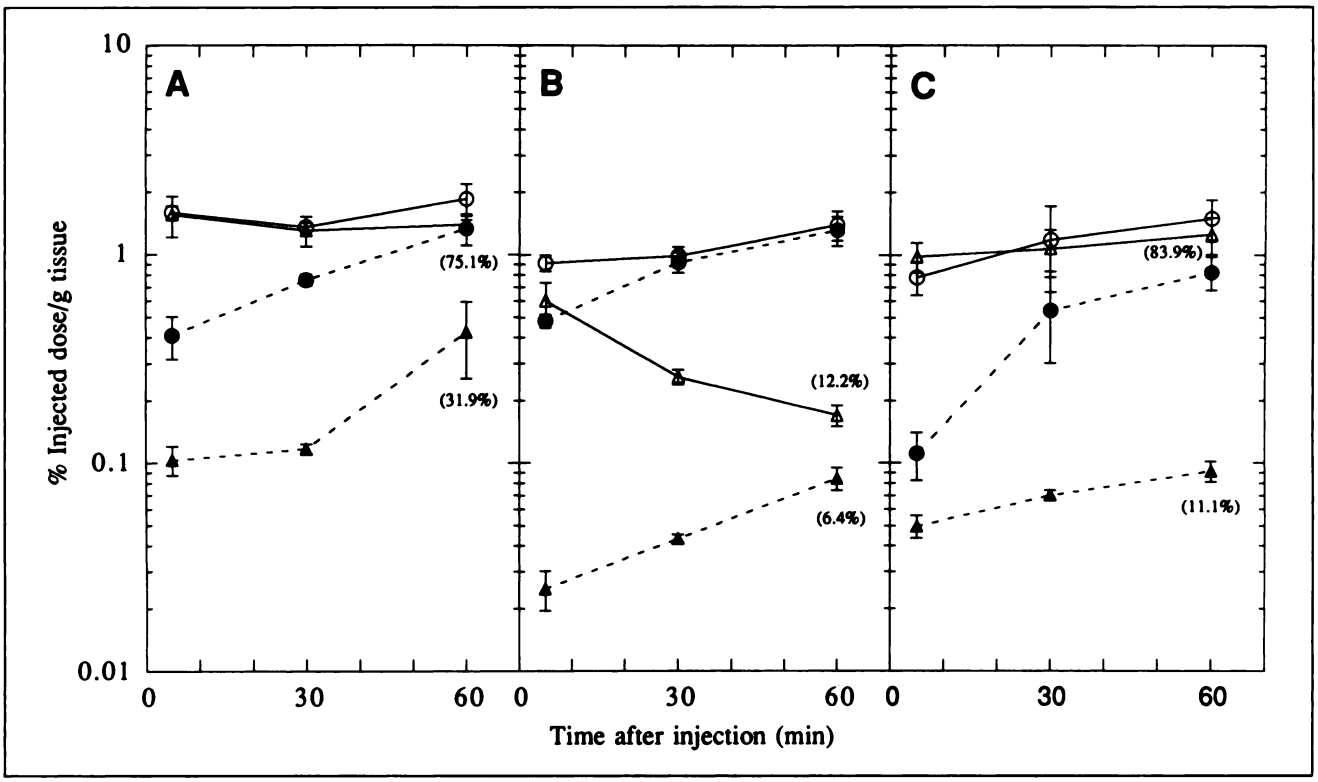

FIGURE 1. Radioactivity uptake of ^{3}H-Met (A), ^{14}C-Leu (B) and ^{18}F-Tyr (C) by the brain. Uptake was expressed as the %ID/g of tissue. In controls, total uptake is denoted by (——○——) and the acid-precipitable fraction by (- - -●- - -). In the cycloheximide-treated group, total uptake is denoted by (——△——) and the acid-precipitable fraction by (- - -▲- - -). Numbers in parentheses show the ratios of the cycloheximide group to controls for uptake in total tissue and the acid-precipitable fraction.

Ouabain inhibited initial uptake (5 min after the injection) of the three amino acids to 67%–74% of the control in FM3A and to 86%–91% in the pancreas. No significant effect was found in the brain and liver. In a later stage, pancreatic uptake of all three amino acids was also reduced, whereas the effect on amino acid uptake in the other tissues was minor.

Incorporation of the three tracers into AFP increased with time in the control group. The highest incorporation was found for ^{14}C-Leu, followed by ^{3}H-Met and ^{18}F-Tyr. The mean APF percentages at 5, 30 and 60 min, respectively, were in the brain, 54.0%, 92.6% and 94.4% for ^{14}C-Leu, 25.5%, 56.1% and 72.0% for ^{3}H-Met and 14.2%, 45.7% and 55.6% for ^{18}F-Tyr. In the FM3A tumor, the ratios were 58.6%, 92.0% and 92.4% for ^{14}C-Leu, 25.5%, 69.2% and 77.4% for ^{3}H-Met and 12.5%, 33.4% and 52.8% for ^{18}F-Tyr. After cycloheximide treatment, the ratios also increased but to a significantly lower level when compared with the control values. On the other hand, the effect of ouabain on the ratios was negligible. Total uptake in the brain and FM3A tissues and the APF expressed as %ID/g tissue are summarized in Figures 1 and 2. After cycloheximide treatment, the APF of ^{14}C-Leu in the brain was reduced to only 6% of the control 60 min after injection, while the reduction was lower for ^{18}F-Tyr (11%) and ^{3}H-Met (32%). The reduction was larger in the FM3A tumor than in the brain: corresponding figures in the FM3A were 3% for ^{14}C-Leu, 6% for ^{18}F-Tyr and 11% for ^{3}H-Met. Only the uptake of ^{14}C-Leu decreased in the total amino acids. The rate of decrease in radioactivity levels in the brain was faster than in the FM3A tumor, due to a reduction in APF incorporation in the brain and FM3A tumor. On the other hand, total uptake of ^{3}H-Met or ^{18}F-Tyr was high and increased with time despite low APF incorporation rates.

APFs of the brain and FM3A were further divided into four fractions: lipids, alkaline-labile and acid-labile fractions as well as proteins (Table 4). In the control group, the protein fraction was the major component for all three amino acids (^{14}C-Leu > ^{18}F-Tyr > ^{3}H-Met). The amounts of ^{14}C in the lipids and alkaline-labile fraction were negligible both in brain and tumors; however, some ^{3}H was detected in these two fractions and ^{18}F was detected in the lipid fraction. Comparable percentages in the acid-labile fractions were measured for the three amino acids. On the other hand, in the cycloheximide-treated mice, the protein fraction remained a major component for ^{14}C-Leu, whereas proportions of the lipid and alkaline-labile fractions for ^{3}H-Met and ^{18}F-Tyr were significantly enhanced.

DISCUSSION

The aim of this study was to investigate whether uptake of ^{14}C-Leu, ^{3}H-Met or ^{18}F-Tyr by the brain and tumor tissues reflects PSR in vivo. In control mice, the increasing uptake of the three amino acids should reflect PSR in the brain and tumor tissues since most of the radioactivity was

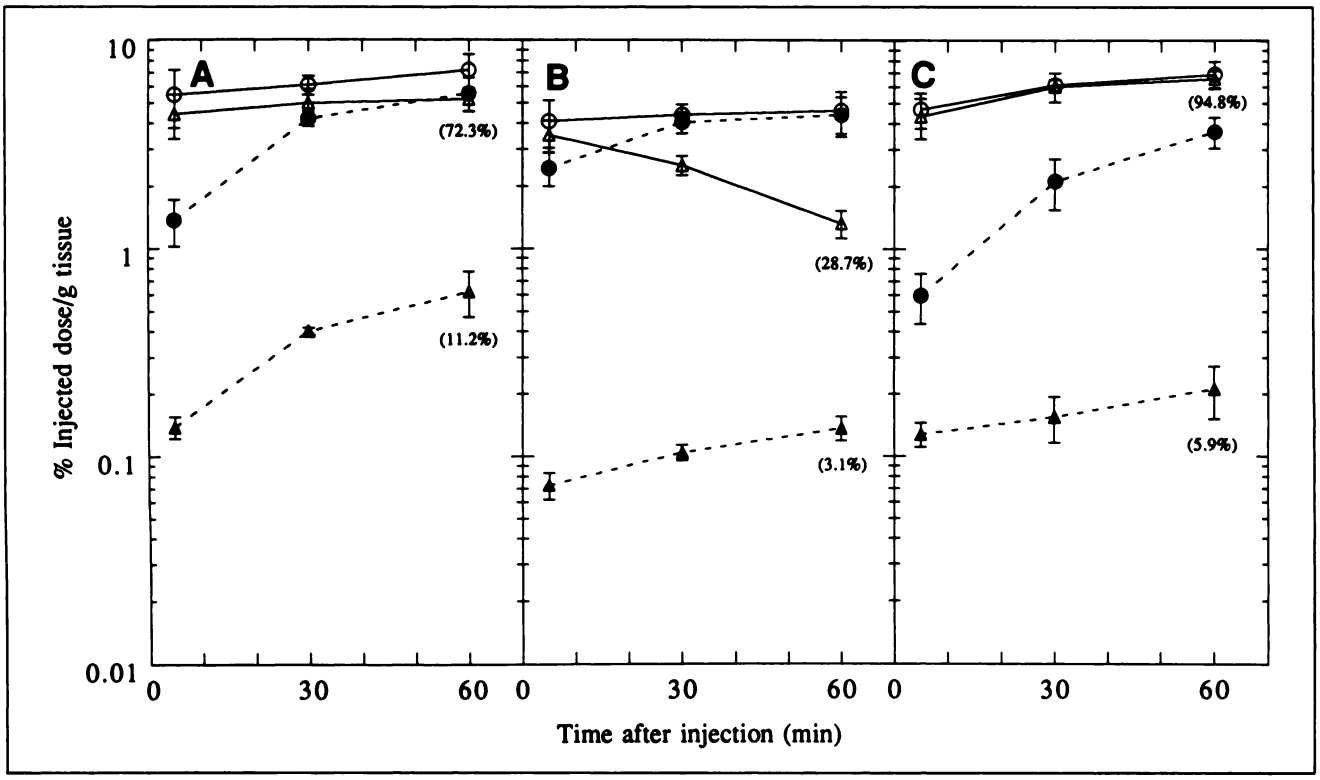

FIGURE 2. Radioactivity uptake of ^{3}H-Met (A), ^{14}C-Leu (B) and ^{18}F-Tyr (C) by the FM3A. Uptake is expressed as the %ID/g of tissue. In the control, total uptake is (——○——) and the acid-precipitable fraction is (- - -●- - -). In the cycloheximide-treated group, total uptake is (——△——) and the acid-precipitable fraction is (- - -▲- - -). Numbers in parentheses denote the ratios of the cycloheximide group to the control calculated for uptake in total tissue and the acid-precipitable fraction.

incorporated into the APF. When cycloheximide was given to inhibit PSR, the incorporation of ^{14}C-Leu into the APF was significantly reduced to 6% of the control in the brain and to 3% in the tumor, and the total uptake was decreased. However, significant amounts of ^{3}H-Met signals were found in the APF in the brains (32%) and tumors (11%) of cycloheximide-treated mice. The effect of cycloheximide on APF incorporation of ^{18}F-Tyr was intermedi-

TABLE 4

Effects of Cycloheximide on the Incorporation of Radioactivity into High-Molecular Weight Materials in Mice Bearing FM3A Tumors 60 Minutes After Intravenous Injection with L-[Methyl-^{3}H]Methionine, L-[1-^{14}C]Leucine and L-[2-^{18}F]Fluorotyrosine

		High-molecular weight materials (%)*			
		Lipid	Alkaline-labile	Acid-labile	Protein
Brain					
Control	^{3}H-Met	7.1 ± 1.8‡	4.5 ± 0.7‡	5.3 ± 1.1	82.9 ± 1.6‡
	^{14}C-Leu	1.2 ± 0.3	0.7 ± 0.3	3.9 ± 0.8	94.3 ± 1.2
	^{18}F-Tyr	5.4 ± 2.1†	0.8 ± 0.4	3.0 ± 0.7	90.8 ± 2.3†
Cycloheximide	^{3}H-Met	14.7 ± 2.2‡	13.8 ± 4.0‡	4.6 ± 2.3	67.1 ± 4.9‡
	^{14}C-Leu	5.3 ± 2.2	bg	4.4 ± 3.0	90.4 ± 3.0
	^{18}F-Tyr	21.3 ± 2.8‡	3.9 ± 0.7‡	9.4 ± 2.0†	65.4 ± 1.9‡
FM3A					
Control	^{3}H-Met	2.4 ± 0.2‡	5.6 ± 0.4‡	7.2 ± 1.6	84.6 ± 1.8‡
	^{14}C-Leu	0.9 ± 0.3	1.2 ± 0.1	5.0 ± 1.3	93.1 ± 1.2
	^{18}F-Tyr	5.1 ± 1.5‡	3.0 ± 1.7	4.9 ± 1.2	87.0 ± 3.3†
Cycloheximide	^{3}H-Met	12.2 ± 0.5‡	22.8 ± 1.3‡	12.3 ± 2.2‡	52.7 ± 1.7‡
	^{14}C-Leu	6.7 ± 0.6	5.4 ± 0.4	3.1 ± 2.0	84.8 ± 2.1
	^{18}F-Tyr	38.7 ± 3.5‡	7.8 ± 0.9†	9.1 ± 1.9†	44.4 ± 2.3‡

Mean ± s.d. (n = 4).

*Percentages against the total acid-precipitable radioactivity.

bg: background radioactivity level.

Student's t-tests were carried out between ^{14}C-Leu and the other amino acids. *$p < 0.05$, †$p < 0.01$, ‡$p < 0.001$.

ate. When compared with the control group, ^{3}H-Met and ^{18}F-Tyr showed a slightly lower but similarly increasing uptake.

Amino acids transfer to the brain or tumor tissue across the blood-brain barrier or plasma membranes and enter the free amino acid pool in tissue. Most free amino acids are used in protein synthesis via amino acyl-tRNA, and a minor portion is degraded by catabolic pathways, such as decarboxylation, oxidation and transamination. The transfer of Leu and Met, and probably the fluorinated analog of Tyr, are mediated by the same cerebrovascular neutral amino acid transport system (*26,27*). In many peripheral tissues and tumors, Leu and Tyr are taken up by the L (leucine-preferring) transport system and Met is taken up by both the L and A (alanine-preferring) systems (*28,29*). The transport system is inhibited by ouabain, which is not a specific inhibitor of amino acid transport, but of Na^{+}, K^{+}-ATPase (*30,31*). When ouabain was given, uptake of all three amino acids (91%–64% of the control) in tumors and pancreas decreased soon after injection. Under these conditions, compared with the control group, there was no significant difference in APF incorporation of radioactivity of all three amino acids. Further uptake reduction by ouabain was not achievable because of its toxicity [in a preliminary study LD_{50} (i.p.): 5–10 mg/kg]. Although in the brain in vitro ouabain certainly binds to Na^{+}, K^{+}-ATPase (*32*), no decreased uptake of amino acids was observed in vitro. This can probably be explained by transport restriction of ouabain across the blood-brain barrier.

It is known that cycloheximide, a specific inhibitor of protein synthesis, reduces cerebral protein synthesis rates in mice in vivo (*33*). Assuming that the radioactivity incorporation rate into the APF reflects PSR in tissues, the PSRs in the brain and in tumors of mice given cycloheximide are reduced significantly (Figs. 1 and 2). Carbon-14-Leu was taken up by the brain and tumor tissues, but was rapidly washed out from the tissue into plasma. In contrast, the radioactivity of ^{3}H-Met and ^{18}F-Tyr in both the brain and tumors still increased with time. These time-radioactivity curves are quite different. The effect of the influx rates may be minor. As discussed above, the transfer of the three amino acids into tissues is mediated by the same carrier system, and cycloheximide does not affect the amino acid transport systems (*31*). A second explanation is that alterations in amino acid pool sizes by cycloheximide does influence the time-radioactivity curve. According to the literature (*12,17,34,35*), the concentrations of Leu in mice and rats are higher in plasma than in the brain, whereas those of Met are the reverse. Concentrations of Tyr are comparable in plasma and brain. In this study, the administered mass of ^{18}F-Tyr was 4.2–7.6 times higher than the mass of ^{14}C-Leu and ^{3}H-Met. The ^{18}F-Tyr dose results in a concentration comparable to the plasma tyrosine concentration. These facts partially explain the different time curves of the three radioactive amino acids in the brain. The following two possibilities may explain the difference in the ^{14}C-Leu uptake in the brain and tumor tissues: total uptake and APF incorporation in the brain were reduced to 12% and 6% of the control at 60 min, while the corresponding figures in the tumor were 29% and 3%.

However, other factors are necessary to explain the differences between ^{14}C-Leu and the two other amino acid studies. The most likely explanation is that protein synthesis with the alternative metabolic pathways for ^{3}H-Met or ^{18}F-Tyr are important in the brain and tumors. The results shown in Table 4 support this explanation. From a theoretical perspective (*15*), Leu labeled with ^{14}C in its carboxyl group is incorporated into protein, whereas other ^{14}C-metabolites are removed from tissues. No incorporation of the ^{14}C-label in the carboxyl group of Leu into DNA is considered. Indeed, most ^{14}C-Leu was incorporated into proteins both in the control and cycloheximide-treated mice. Although small amounts of ^{14}C were detected in the acid-labile fraction, similar proportions of radioactivity were also found for ^{3}H-Met or ^{18}F-Tyr. Therefore, the ^{14}C detected in the acid-labile fraction is probably derived from basic proteins, such as chromosomal histones, but not from DNA, as previously discussed (*22*). The lesser effect of cycloheximide on the incorporation of ^{3}H from ^{3}H-Met into the APF suggests that it is also incorporated into nonprotein macromolecules. Possible pathways are transmethylation to lipids, nucleic acids, sarcosine and methylthioadenosine (in polyamine synthesis process) via S-adenosyl-L-methionine (*13,14,36,37*). After giving [methyl-^{14}C]Met to rats, S-adenosyl-L-[^{14}C]methionine and radiolabeled lipids have been found in brain and tumor tissues (*11*). In tumor-bearing rats given S-adenosyl-L-[methyl-^{11}C]methionine, a considerable amount of radioactivity was incorporated into the APF of the tumor (*38*).

In this study, small amounts of radioactivity were detected in the lipid and alkaline-labile fractions of the control mice, and the proportions were enhanced by cycloheximide. The presence of ^{3}H in the alkaline-labile fraction demonstrates incorporation of ^{3}H-Met to RNA via S-adenosyl-L-[^{3}H]methionine. The effects of cycloheximide treatment on APF incorporation of ^{18}F-Tyr were smaller than in the ^{14}C-Leu study, but larger than in the ^{3}H-Met study. On the other hand, the effect on total uptake of ^{18}F-Tyr was smaller even when compared with the effect on ^{3}H-Met.

When protein synthesis was inhibited, incorporation of ^{18}F-Tyr into the lipid fraction was important, especially in tumor tissue. Since Coenen et al. (*21*) did not detect ^{18}F-fluorodopa as a metabolite of ^{18}F-Tyr in the brain, a significant dopamine pathway has to be considered (*16*). Murakami et al. found that radioactivity of 3-^{18}F-fluoro-L-tyrosine, an isomer of ^{18}F-Tyr, was not only incorporated into proteins but also into protein-bound lipids and into a free fatty acid pool (*39*). So, to understand the time-radioactivity curves of ^{3}H-Met and ^{18}F-Tyr, other metabolic pathways besides protein synthesis have to be considered. This is even true in the pancreas, which most actively synthesizes protein. In the control group, this organ showed the highest uptake for all three amino acids. The

uptake of ^{14}C-Leu by the pancreas was most drastically reduced in mice given cycloheximide, to less than 10% at 60 min, whereas under the same conditions one-third of ^{3}H-Met or ^{18}F-Tyr uptake was retained. In this study, we used ^{3}H-labeled Met to assess the potential of ^{11}C-Met used in PET studies. Although the ^{3}H-label is exchangable in vivo, tissue distribution and incorporation into the APF in this study were comparable with results using ^{14}C-labeled Met in rats (*11*). We do not consider that the ^{3}H exchange in vivo influenced the results during the 60 min after tracer injection.

CONCLUSION

Using the tumor-bearing mouse model in which PSR was inhibited by cycloheximide, uptake of ^{14}C-Leu in the brain and tumor corresponded well with PSR in vivo. However, in studies with ^{3}H-Met and ^{18}F-Tyr, an uncoupling was observed between these two parameters. We conclude that ^{11}C-Met and ^{18}F-Tyr should be used to assess the amino acid transport system by PET (*6,40*). For this application, the PET study should be performed for a short period of time because the effect of metabolic changes of ^{11}C-Met in human plasma (*7,41,42*) on the kinetic analysis will be minor. Of the three amino acids investigated, ^{11}C-Leu is the best choice for measuring PSR in vivo with PET.

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