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Problems of [S-methyl-^{11}C]-L-methionine as a protein synthesis marker in the pancreas

Yasuhisa FUJIBAYASHI*, Keichi KAWAI**, Yoshiharu YONEKURA*, Kazuya MATSUMOTO*
Junji KONISHI* and Akira YOKOYAMA*

*Faculty of Pharmaceutical Sciences and School of Medicine, Kyoto University, Kyoto, Japan
**Faculty of Pharmaceutical Sciences, Science University of Tokyo, Tokyo, Japan

To evaluate the possibility of [S-methyl-^{11}C]-L-methionine as a protein synthesis marker in the pancreas, the effect of various labeling positions in the accumulation and metabolism of ^{14}C-labeled L-methionines (S-methyl-^{14}C, 1-^{14}C and 3, 4-^{14}C) was studied. In mouse biodistribution studies, the methionines showed differing patterns of labeling position-dependent pancreatic accumulation. In the case of [S-methyl-^{14}C]-L-methionine, protein-incorporation and methyl-transformation equally served as retention mechanisms in the pancreas, indicating [S-methyl-^{11}C]-L-methionine's unsuitability as a pancreatic protein synthesis marker. For such purposes, [1-^{11}C]-L-methionine is considered more suitable.

Key words: [S-methyl-^{11}C]-L-methionine, ^{14}C-methionines, labeling position, pancreas, protein synthesis, amino acid metabolism

INTRODUCTION

THE PANCREAS is the most active organ for protein synthesis, producing its own weight in protein in less than 24 hrs.[1] Very probably, it is this activity that induces the surprisingly high amino acid accumulation in the pancreas. That pancreatic amino acid catabolism is negligible in comparison with protein synthesis seems a plausible hypothesis. If so, radiolabeled amino acids can be used to measure pancreatic protein synthesis rates.[2] [S-Methyl-^{11}C]-L-methionine is a widely used amino acid radiopharmaceutical in the pancreas,[3] brain[4] and in tumors,[5–7] etc. In our hospital, [S-methyl-^{11}C]-L-methionine is routinely employed in clinical practice.[8] If [S-methyl-^{11}C]-L-methionine can be used as a protein synthesis marker in the pancreas, the methodology of pancreatic diagnosis might be significantly changed. Indeed, while various PET studies have indicated that the uptake of [S-methyl-^{11}C]-L-methionine in the pancreas reflects exocrine pancreatic functions,[3] the precise relationship between pancreatic [S-methyl-^{11}C]-L-methionine accumulation and protein synthesis is still being debated.

In the present study, the accumulation and metabolism of [S-methyl-^{14}C]-L-methionine in the pancreas was studied in order to clarify whether or not [S-methyl-^{11}C]-L-methionine can be used as a protein synthesis marker in the pancreas. To elaborate on past findings regarding [S-methyl-^{14}C]-L-methionine, comparative studies with [1-^{14}C]-L-methionine and [3,4-^{14}C]-L-methionine were performed. [1-^{14}C]-L-methionine has been reported as a suitable position labeled methionine for the measurement of brain protein synthesis rates[9,10] and [3,4-^{14}C]-L-methionine was selected as a control. Our data elucidated the consequences of differing labeling positions for the accumulation of radiolabeled methionine in the pancreas. In particular, we found [S-methyl-^{14}C]-L-methionine to be unsuitable as a pancreatic protein synthesis marker.

MATERIALS AND METHODS

[S-methyl-^{14}C]-L-methionine (1.89 GBq/mmole), [1-^{14}C]-L-methionine (1.91 GBq/mmol) and [3,4-^{14}C]-L-methionine (2.18 GBq/mmol) were obtained from

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For reprints contact: Akira Yokoyama, Department of Radiopharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto, 606, JAPAN.

Table 1 Labeling position effect on methionine biodistribution in ddY male mice*

[S-methyl-^{14}C]-Methionine					
Tissue	5 min	10 min	15 min	30 min	60 min
Pancreas	24.20 (2.84)	30.31 (5.86)	32.67 (9.11)	36.74 (10.85)	41.44 (4.67)
Plasma	2.41 (0.46)	1.33 (0.17)	1.25 (0.35)	1.50 (0.49)	1.91 (0.31)
Liver	9.54 (1.17)	10.45 (1.26)	14.41 (1.29)	13.56 (5.47)	14.98 (3.51)
Kidney	7.86 (1.54)	6.68 (0.77)	7.73 (1.30)	6.85 (1.66)	7.80 (0.61)
[1-^{14}C]-Methionine					
Tissue	5 min	10 min	15 min	30 min	60 min
Pancreas	20.82 (5.45)	27.31 (9.12)	34.46 (10.73)	29.87 (7.24)	27.77 (3.78)
Plasma	1.14 (0.13)	1.28 (0.08)	2.07 (0.54)	1.63 (0.37)	2.00 (0.08)
Liver	6.30 (2.75)	4.97 (0.47)	7.87 (2.34)	7.14 (1.36)	5.28 (0.73)
Kidney	6.04 (0.82)	6.79 (0.34)	6.97 (1.10)	6.76 (0.42)	5.53 (0.30)
[3, 4-^{14}C]-Methionine					
Tissue	5 min	10 min	15 min	30 min	60 min
Pancreas	35.38 (5.02)	28.07 (2.25)	30.10 (10.14)	31.78 (3.53)	33.40 (3.58)
Plasma	6.46 (0.70)	4.65 (0.30)	3.92 (0.62)	3.03 (0.23)	2.87 (0.37)
Liver	4.69 (0.66)	4.39 (0.54)	5.51 (0.43)	6.41 (0.91)	6.60 (0.45)
Kidney	14.09 (0.92)	12.66 (0.75)	14.46 (2.16)	19.01 (1.39)	19.78 (3.51)

* % dose/g tissue, average for 3 animals (1 s.d.).

the Commissariat A L'Energie Atomique (France) and diluted with saline. S-adenosyl-L-methionine was obtained from Sigma Chemical Co., Ltd. Other reagents were of reagent grade.

In-vivo Mouse Biodistribution Studies

Radioactive samples (7.4 KBq, 3.39×10^{-9}-3.92×10^{-9} mol) in 0.1 m*l* were injected into male ddY mice (25 g body weight) through the tail vein. After various time intervals, the mice were killed by ether anesthesia, and dissected. Tissue samples of 50 mg were collected, placed into vials and treated with NCS tissue solubilizer (Amersham), and their radioactivity was measured.

To measure the protein incorporation rate, the 50 mg tissue samples were weighed and homogenized with 450 μl of 0.15 N bicarbonate and mixed with 4.5 m*l* of 5.5% trichloroacetic acid (TCA) (final TCA conc.=5%). Precipitated protein fractions were trapped on a 0.45 μm glass filter (TOYO GC-50, Japan) and washed with cold 5% TCA solution, denaturated by heat treatment (150°C, 1 hr) and their radioactivity measured. Protein incorporation ratio (% of uptake) was calculated as follows:

$$\text{Protein incorporation ratio} = \frac{\%\ \text{protein incorporated dose/g}}{\%\ \text{dose/g}}$$

Thin Layer Chromatographic Analysis

A radioactive sample of 0.1 m*l* (185 KBq, 8.47×10^{-8}—9.80×10^{-8} mol) was injected into a male ddY mouse (25 g body weight) through the tail vein. Fifteen minutes after injection, the mouse was killed by ether anesthesia and dissected. Pancreas tissue (125 mg) was homogenized with 375 μl of 0.15 N bicarbonate, following that 25 μl of 100% TCA was added (final TCA conc.=5%). The supernatant was collected by centrifugation and analyzed by thin layer chromatography (Merck Art. 5553, n-BuOH:

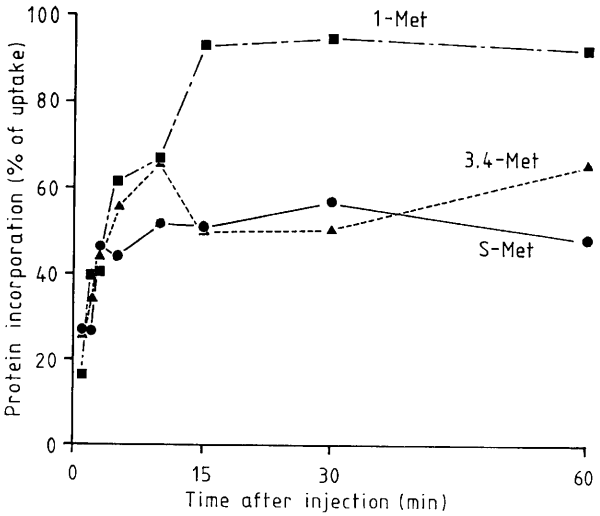

Fig. 1 Protein incorporation of methionines in the pancreas. Each value represents the average for 3 animals.

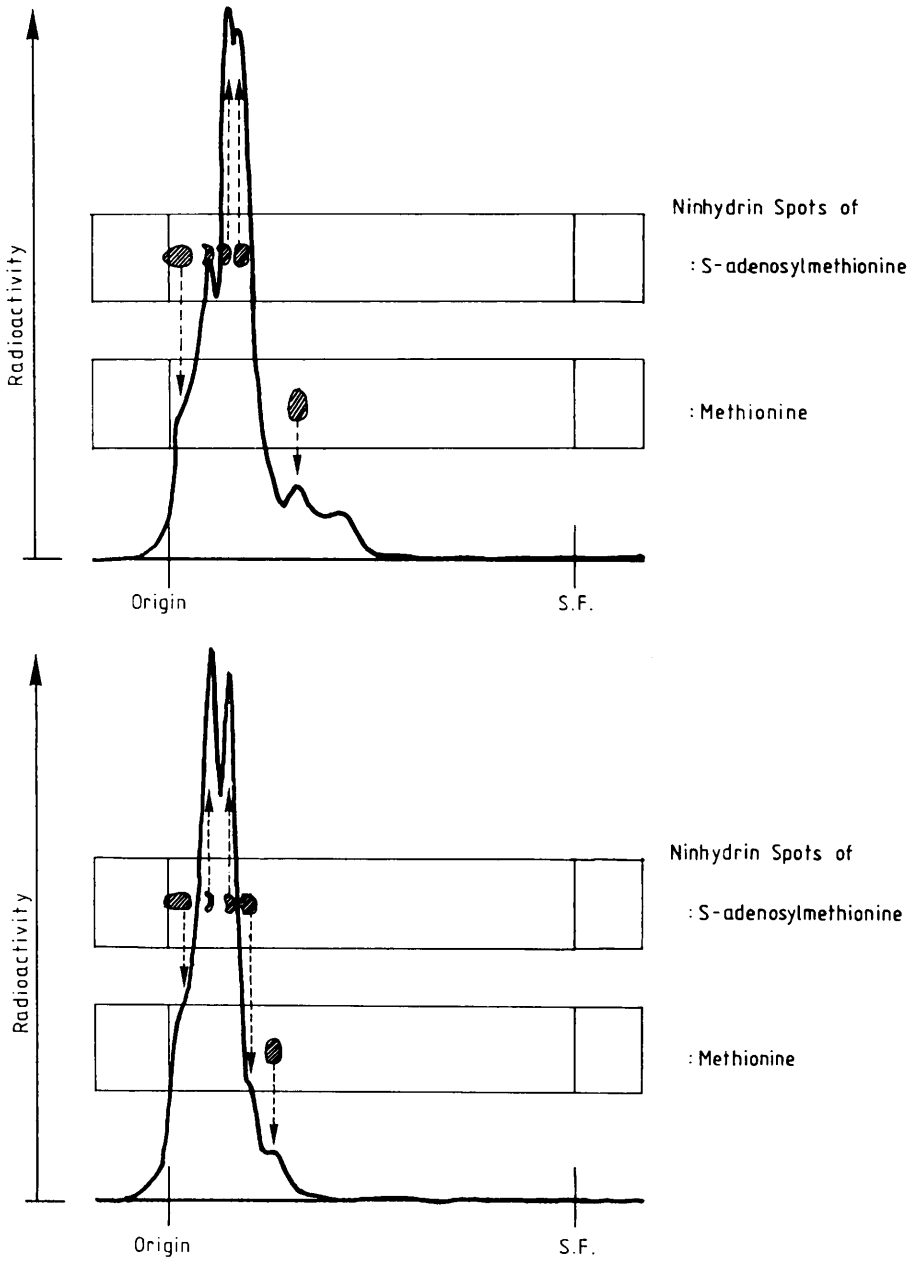

Fig. 2 Thin layer chromatographic profiles of the radioactivity in the TCA-soluble fraction of [S-methyl-^{14}C]-L-methionine injected mouse pancreas (Upper: acidic solvent, Lower: basic solvent).

CH_3COOH: H_2O=4: 1: 1, or CH_3OH: NH_4OH (25%)=70: 1 as solvents). The radioactivity profile was obtained with a radiochromatoscanner (Radiochromanizer, Aloka, Japan). Spots of standard samples were made visible by ninhydrin treatment.

RESULTS

Table 1 shows the biodistribution of methionines in the mice. The methionine analysed showed preferential accumulation in the pancreas in comparison with the liver or plasma. However, time-activity profiles of radioactivity in the pancreas showed different patterns. Namely, that of [S-methyl-^{14}C]-L-methionine showed the most stable retention, followed by [3, 4-^{14}C]-L-methionine. In the case of [1-^{14}C]-L-methionine, on the other hand, the radioactivity had cleared within 15 minutes after the injection. Thus, differing positions of methionine labeling resulted in remarkable changes in the retention of radioactivity in the pancreas.

These results inspired further analysis of the pancreatic retention mechanism for each methionine. Fig. 1 shows the in vivo protein incorporation ratio

in the pancreas of mice. Protein incorporation fractions of [S-methyl-^{14}C] and [3,4-^{14}C]-L-methionine were relatively constant 10 minutes after the injection, and amounted to 50–60% of the total accumulated activity. In the case of [1-^{14}C]-L-methionine, on the other hand, more than 90% of the accumulated radioactivity was found in the protein fraction 15 minutes after the injection. The absolute radioactivity of protein incorporated in the pancreas was similar to the values for the three methionines. These results indicate that radiolabeled TCA-soluble metabolites originate from the S-methyl-carbon and those from the 3,4-carbon have higher retention characteristics than those from the 1-carbon.

In order to evaluate the radiolabeled metabolites from [S-methyl-^{14}C]-L-methionine, thin layer chromatography profiles of radioactivity in the TCA-soluble fraction of [S-methyl-^{14}C]-L-methionine injected mouse pancreas were studied (Fig. 2). The S-methyl-carbon of methionine is a universal source of methyl transformation.[11] Thus, S-adenosyl-L-methionine, the most important metabolic intermediate for methyl transformation, was selected as our reference. In the early stages of biodistribution (15 minutes after the injection), a large proportion of the radioactivity in the pancreas was found in the same fractions as the S-adenosyl-L-methionine, by both acidic and basic solvent systems. Thus, we consider methyl transformation to be one of the metabolic pathways contributing to the retention of [S-methyl-^{14}C]-L-methionine.

DISCUSSION

The importance of the labeling position of the amino acid in the measurement of protein synthesis activity is usually discussed vis-à-vis the brain. In the brain, amino acids are used as substrates for protein synthesis, neurotransmitters, energy production, etc. S-methyl residue, in particular, due to the unique labeling position of [S-methyl-^{11}C]-L-methionine, is a universal source of methyl transformation. ^{11}C injected as [S-methyl-^{11}C]-L-methionine in the brain is not only incorporated into proteins, but also transferred to various methyl acceptors (neurotransmitters, metabolic intermediates etc.) via S-adenosyl-L-methionine[11] and retained. The present results indicate that methyl transformation processes were significant in the pancreas as well. In particular, differences in the labeling position of methionines resulted in different distributions of radioactivity in the pancreas. These differences were mainly dependent on the character of TCA-soluble metabolites and not on the protein incorporated fraction. In the case of [S-methyl-^{14}C]-L-methionine, in particular, a large contribution of the TCA-soluble fraction in radioactive retention was seen. When [S-methyl-^{14}C]-L-methionine was injected, methyl transformation was one of the important mechanisms contributing to the retention of radioactivity in the pancreas.

Thus, in the pancreas, retention of [S-methyl-^{11}C]-L-methionine might be derived from the sum of protein synthesis and retentive methyl transformation processes. When [S-methyl-^{11}C]-L-methionine was used as a tracer, the measurement of the pancreatic protein synthesis rate was difficult since radiolabeled metabolites should be eliminated from the target tissue in order to permit calculation of protein synthesis rates from the residual activity in the tissue.[2]

For the measurement of protein synthesis rates in the brain, Sokoloff et al. proposed the use of 1-labeled amino acid as a tracer.[12] In the pancreas as well, more than 90% of the accumulated radioactivity is protein-incorporated 15 minutes after [1-^{11}C]-L-methionine injection, indicating protein synthesis selective retention. Thus, in the pancreas too, [1-^{11}C]-L-methionine was considered to be more suitable than [S-methyl-^{11}C]-L-methionine.

In conclusion, it became clear that [S-methyl-^{11}C]-L-methionine could not be used as a true protein synthesis marker in the pancreas. Rather, it seemed to be a "methionine metabolism marker" approximating the sum of protein synthesis and methyl transformation.

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