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2-^{18}F-PHENYLALANINE AND 3-^{18}F-TYROSINE — SYNTHESIS AND PRELIMINARY DATA OF TRACER KINETICS

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SUMMARY

2-^{18}F-phenylalanine and 3-^{18}F-tyrosine were synthesized by direct electrophilic substitution of L-phenylalanine and L-tyrosine with ^{18}F-acetylhypofluorite. Both compounds were obtained in 30 % radiochemical yield based on ^{18}F-acetylhypofluorite within 60 min after the end of irradiation. In rat brain, 30 % of the radioactivity was fixed into acid-insoluble macromolecules and most of the free radioactivity existed as amino acids at one hour after 2-^{18}F-phenylalanine injection. In the case of 3-^{18}F-tyrosine, significant amounts of radioactivity existed as organic acids. The catecholamine fraction had negligible radioactivity in both cases. These results indicate that 2-^{18}F-phenylalanine is suitable for compartmental analysis for tissue accumulation because of its simple metabolic behavior.

Key words: 2-^{18}F-Phe, 3-^{18}F-Tyr, ^{18}F-AcOF, rat brain

INTRODUCTION

Amino acids labelled with positron emitting nuclides are useful for the investigation of amino acid metabolism in-vivo. At present, carbon-11 labelled amino acids are most used, but the physical half life (20.4 min) of carbon-11 seems to be too short for proper kinetic analysis. Furthermore, the enantiomeric resolution which is necessary in the case of carboxyl

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carbon labelling by the Strecker reaction results in long synthesis times. L-(methyl-^{11}C)-methionine is obtained by a one step methylation in a short time. However, the compartmental analysis of L-(methyl-^{11}C)-methionine is too difficult because the radioactive carbon atom is incorporated rapidly in vivo into many molecules by transmethylation reactions. On the other hand, ^{18}F-fluorinated amino acids might overcome the shortcomings of ^{11}C-labelled amino acids because of the moderately long half life (109.8 min) of fluorine-18 and the strength of the C-F bond. Fluorination of aromatic amino acids by the Shiemann reaction have been reported [1] but the preparation required several hours. Recently, Coenen et al reported the direct fluorination of L-phenylalanine with ^{18}F-F_2 [2,3]. We carried out the comparative synthesis of ^{18}F-phenylalanines by electrophilic substitution with ^{18}F-F_2 and ^{18}F-AcOF [4]. In this paper, we describe the radiosynthesis of 2-^{18}F-phenylalanine (2-^{18}F-Phe) and 3-^{18}F-tyrosine (3-^{18}F-Tyr) and a preliminary study of their kinetics in rats.

METHODS

Radiosynthesis of 2-^{18}F-Phe and 3-^{18}F-Tyr

The target gas, neon containing 0.5 % carrier F_2, was loaded into a 141 ml chamber up to 3.0 kg/cm^2 and was irradiated by 6.8 MeV deuterons. After irradiation, the target gas was recovered through an AcOK/AcOH column [5] by a 500 ml/min He flow and was bubbled into a vessel containing 100 μmole of L-Phe or L-Tyr in 10 ml of CF_3COOH at 0°C. At the end of bubbling, CF_3COOH was evaporated under reduced pressure at 50°C and the radioactive residue was dissolved in 0.5 ∿ 0.8 ml of H_2O. The radioactive solution was applied to HPLC (Waters, μBondapak-C_{18}, 7.6 × 300 mm, 10 % CH_3OH containing 0.1 % AcOH as a mobile phase) and the

desired fraction was collected, evaporated and prepared as an injectable solution of 2-^{18}F-Phe or 3-^{18}F-Tyr. The chemical and radiochemical purity was analyzed by HPLC as above, and by TLC (cellulose, n-butanol 20: AcOH 3: H_2O 5 as a solvent). The identification of the products was carried out by comparison of their peak elution volume and Rf values with those of commercially available authentic samples (Wako Pure Chemical, Ltd).

Radioactivity incorporation into biomolecules

The metabolic route of 2-^{18}F-Phe and 3-^{18}F-Tyr was considered to be as shown in Fig. 1.

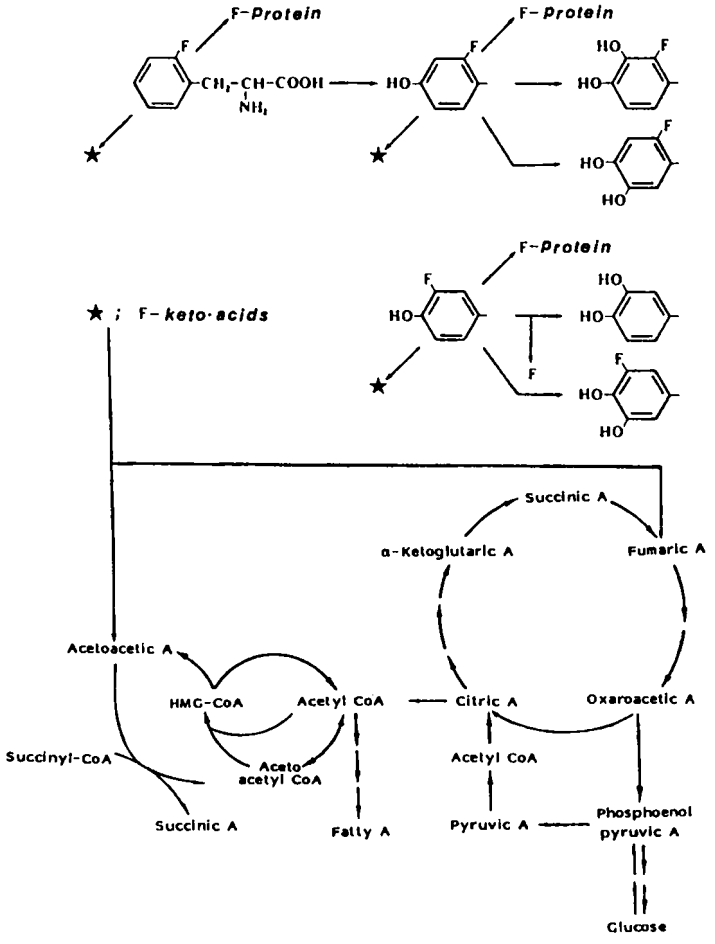

Fig. 1. The scheme of 2-^{18}F-Phe and 3-^{18}F-Tyr metabolism

In the following study, biomolecules were analysed as a study of radioactivity incorporated. Protein (Pro) and protein bound lipids (Lip) were obtained as an acid insoluble fraction. As an acid soluble fraction, free fatty acids (FFA), free $^{18}F^-$ion ($^{18}F^-$), catecholamines (CA), organic acids (OA), free amino acids (AA) and nonionic molecules (NI) were obtained.

Male Wistar rats weighing 250 ∿ 340 g had a polyethylene cannule inserted into the internal jugular vein and were fasted 10 ∿ 18 hrs before experiments. Three mCi of 2-^{18}F-Phe or 3-^{18}F-Tyr was administered to each rat via the cannule, and 60 min later the rats were sacrificed. The duplicate samples (cerebrum and cerebellum, 200 mg each) were homogenized in 2 ml of 5 % TCA and centrifuged. The homogenizing step was carried out twice. The acid insoluble precipitate was extracted with 2 ml of $CHCl_3$- CH_3OH (2:1), and the $CHCl_3$ layer (Lip) and CH_3OH plus protein layer (Pro) were separated. The combined acid soluble supernatant was extracted twice with 4 ml of ether and once with 4 ml of $CHCl_3$. The combined organic layer contained free fatty acids (FFA). The H_2O layer was adjusted to pH 7.0 and 1 ml of portions were applied to columns (0.7 × 3.0 cm) packed with AG 50W × 8 (H^+, 100 ∿ 200 mesh, Bio-Rad) and AG1 × 8 (OH^-, 100 ∿ 200 mesh, Bio-Rad). The remaining H_2O layer was adjusted to pH 8.3 and applied to a column (0.7 × 2.5 cm) packed with alumina (activity II ∿ III, 70 ∿ 230 mesh, E. Merk Darmstadt). Each column was washed with 4.0 ml of H_2O and the washes were collected. The alumina column was further eluted with 3 ml of 0.5 N-HCl.

The radioactivity compounds distributed as shown in Table 1. The radioactivity percentages of the columns, washes and eluents were calculated as fractions of the total amount of activity applied.

Table 1. Distribution of compounds in H_2O layer to each compartment

Packings	Column	Washate	Eluate
AG 50W (H^+)	AA, CA	OA, F^-, NI	
AG1 (OH^-)	AA, CA, OA F^-	NI	
Al_2O_3	F^{-*}	AA, OA, NI	CA

* *The data was corrected by the leakage rate from Al_2O_3 column to wash (15.4 ± 3.2 %) and eluent (16.3 ± 1.6 %) that was shown by six measurements using $^{18}F^-$ solutions.*

Then, the incorporation ratio into compounds in the brain tissue were obtained by multiplication of the ratio of H_2O layer (0.67 ± 0.05, n = 8) in the brain to following fractions in H_2O layer.

$^{18}F^-$; Al_2O_3 column (%)

CA; Al_2O_3 eluate (%)

NI; AG1 wash (%)

AA; AG 50W column (%) − Al_2O_3 eluate (%)

OA; AG 50W wash (%) − Al_2O_3 column(%) − AG1 wash(%)

RESULTS

Radiosynthesis of 2-^{18}F-Phe and 3-^{18}F-Tyr

The elution pattern of the compounds used in this study is shown in Fig. 2. 3-^{18}F-Phe and 4-^{18}F-Phe had the same retention time with these HPLC conditions. The chromatogram of the reaction mixture in the 3-^{18}F-Tyr synthesis is shown in Fig. 3. The significant increase in the radioactive peak just before

3-^{18}F-Tyr was observed with ^{18}F-F_2 as a precursor and resulted in a decrease in 3-^{18}F-Tyr yield. The chromatogram of the 2-^{18}F-Phe synthesis was shown in our previous paper [4].

A summary of 2-^{18}F-Phe and 3-^{18}F-Tyr synthesis is shown in Table 2. Both compounds were obtained in 30 % radiochemical yield based on ^{18}F-AcOF within 60 min after the end of irradiation.

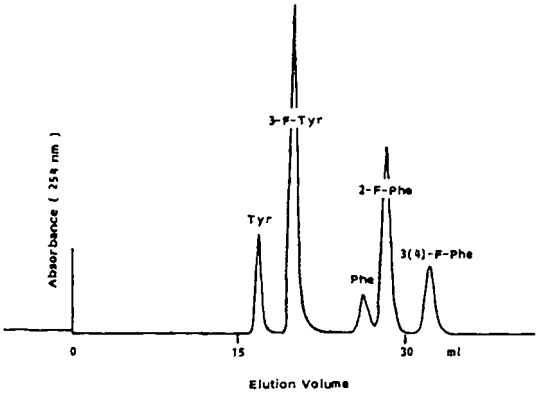

Fig. 2
The elution pattern of starting materials and product

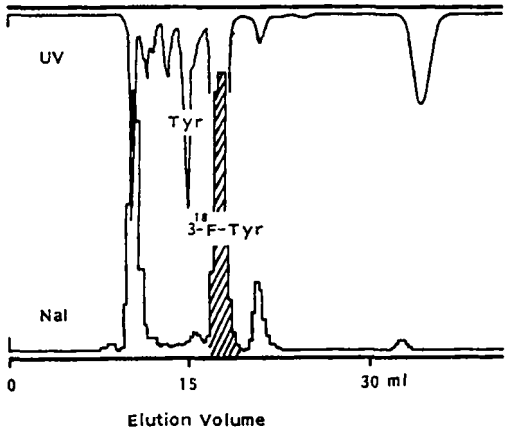

Fig. 3
The chromatogram of reaction mixture in 3-^{18}F-Tyrosine synthesis

The specific activities determined by HPLC were around 300 mCi/m mole. The UV absorbances of these compounds showed no change 12 hrs after the synthesis.

Table 2. Radiosyntehsis of ^{18}F-Phenylalanine and ^{18}F-Tyrosine

Starting Material	L-Phenylalanine		L-Tyrosine
^{18}F-Precursor	^{18}F-AcOF		^{18}F-AcOF
^{18}F-Trapping Yield (%)	96.0 ± 2.6		99.2 ± 0.4
Radiochemical Yield (%)	2-^{18}F-Phe	3(4)-^{18}F-Phe	3-^{18}F-Tyr
	28.4 ± 18.5	3.7 ± 1.0	28.8 ± 4.1

The yields based on ^{18}F-AcOF were shown as the mean ± S.D. of four experiments at the end of irradiation.

Radioactivity incorporation into biomolecules

The uptakes of ^{18}F in the rat brain after 60 min were 0.22 ± 0.04 % and 0.10 ± 0.02 % of the injected dose of 2-^{18}F-Phe and 3-^{18}Tyr, respectively. The radioactivity incorporation into acid insoluble macromolecules and acid soluble biomolecules is shown in Fig. 4. The incorporation ratio of 2-^{18}F-Phe into protein and protein-bound lipid showed a tendency to be larger than that of 3-^{18}F-Tyr. As an acid soluble fraction, most of the radioactivity existed as amino acids in the case of 2-^{18}F-Phe. In the case of 3-^{18}F-Tyr, more than 20 % of the organic acids existed in the acid soluble fraction. 3-^{18}F-Tyr resulted in more free fatty acids and $^{18}F^-$ion than did 2-^{18}F-Phe, but these were 10 % or less. Catecholamines and nonionic molecules were negligible in both cases.

DISCUSSION

In this study, the production ratio of 3(4)-^{18}F-Phe to 2-^{18}F-Phe by the ^{18}F-AcOF method was nearly one to ten, and

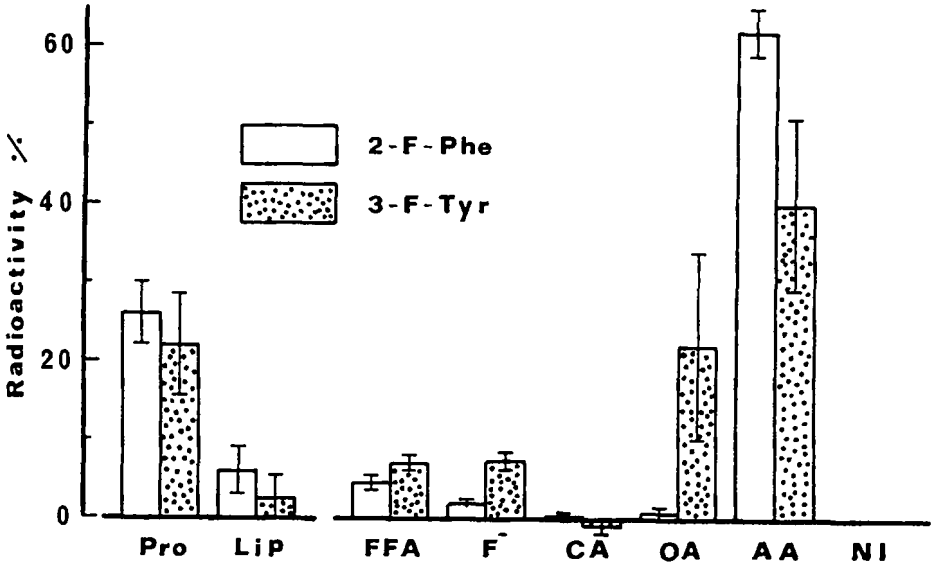

Fig. 4. *^{18}F-radioactivity incorporation into biomolecules. The data is shown as the mean ± S.D. obtained from four rats. The open and dotted columns represent 2-^{18}F-Phe and 3-^{18}F-Tyr, respectively.*

agrees with our previous report [4]. From ^{18}F-AcOF, 2-^{18}F-Phe and 3-^{18}F-Tyr were obtained in 30 % yield based on ^{18}F-AcOF within 60 min after the end of irradiation, but the yield might be increased by improvement in reaction and preparative HPLC conditions. Therefore, the fluorination of aromatic amino acids using ^{18}F-AcOF is thought to be very convenient for clinical application because of its high yield, simplicity and short time requirement.

The investigation of radioactivity incorporation into biomolecules is necessary before clinical application, because metabolic tracers such as amino acids, especially these ketogenic and glucogenic types, may be incorporated into many biological molecules via complex metabolic pathways. For example, in the case of 2-^{18}F-Phe, the ^{18}F-atom may not be

affected by phenylalanine hydroxylase and tyrosine hydroxylase. So, it may show the same behavior as endogenous phenylalanine. In the case of 3-^{18}F-Tyr, we may detect the tyrosine hydroxylase activity, which is the rate limiting enzyme in the catecholamine synthetic pathway, as the amount of $^{18}F^-$ion eliminated. However, this study revealed that 2-^{18}F-Phe and 3-^{18}F-Tyr converted minimally to 2-^{18}F-Dopa and 3-^{18}F-Dopa, respectively. This indicates either that tyrosine hydroxylase acts on the C_3-F bond specifically or that a smaller amount of conversion to catecholamines than that detectable in this study has occurred. In fact, the fraction of $^{18}F^-$ ion was greater (7.56 ± 1.15 %) after 3-^{18}F-Tyr administration than that in 2-^{18}F-Phe (1.83 ± 0.37 %). Nevertheless, another experiment such as that described below may be needed to determine whether the amount of $^{18}F^-$ion had a linear correlation with tyrosine hydroxylase activity or not. In-vitro enzymatic studies and a study similar to the present one but where the tyrosine hydroxylase activity is changed by a norepinephrine depleting agent, such as reserpine, should solve this question, because the enzyme is susceptible to feedback inhibition by norepinephrine, the end product of the catecholamine synthetic sequence.

The organic acid fraction in the 3-^{18}F-Tyr case was significantly greater than in the 2-^{18}F-Phe case. This fact is thought to be accounted by the differences of the metabolic route and the metabolic speed of the tracers. The metabolic pathways of m-fluorotyrosine (3-F-fluorotyrosine) was reported by Weissman and Koe [6]. According to their pathways, the relatively nontoxic compound, ^{18}F-fluorofumaric acid, generation and ^{18}F-elimination resulting in nonfluorinated acetoacetic acid generation would be occurred after 2-^{18}F-Phe administration, while in the case of 3-^{18}F-Tyr, the very toxic compound, ^{18}F-fluoroacetoacetic acid, besides ^{18}F-fluorofumaric acid would be

generated. However, in our study, the fraction not only of ^{18}F-organic acid but of $^{18}F^-$ ion was significantly low after 2-^{18}F-Phe injection as compared with that after 3-^{18}F-Tyr injection. This data indicates that the metabolic speed of 2-^{18}F-Phe is very slow at the homogentisic acid generation or the earlier steps.

In conclusion, 2-^{18}F-Phe and 3-^{18}F-Tyr can be obtained in 30 % radiochemical yields within 60 min by electrophilic substitution with ^{18}F-AcOF. 2-^{18}F-Phe is more suitable than 3-^{18}F-Tyr in the quantitative compartment analysis of its transportation across the blood-brain-barrier and accumulation in the brain because of its simple metabolic route slow metabolism and low toxicity.

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