A NOVEL ^{123}I-LABELING REAGENT. XIII. SYNTHESIS AND LOADING-DOSE EFFECTS OF ^{123}I-4-IODOPHENYLALANINE AND ^{123}I-5- AND 6-IODOTRYPTOPHAN

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A new iodination reagent—the ^{123}I species resulting from the decay of ^{123}Xe—has been used in the exchange labeling of iodinated radiopharmaceuticals in the melt. Both ^{123}I-4-iodophenylalanine and ^{123}I-5- and 6-iodotryptophan have been labeled by the melt method. A loading-dose effect on the ratio (percent uptake per gram) of pancreas:liver was observed in mice for ^{123}I-4-iodophenylalanine but not for ^{123}I-6-iodotryptophan.

The desirable physical characteristics of ^{123}I, its demonstrated application to nuclear medicine (*1–8*), and the favorable prospects for the general availability of very high purity ^{123}I have motivated us to develop fast and convenient labeling methods for the iodination of radiopharmaceuticals.

We are reporting the development and use of a new labeling reagent, a carrier-free, reactive iodine species (*9*) formed from the ^{123}Xe(β^+,EC)^{123}I nuclear transformation, to prepare ^{123}I-labeled 4-iodophenylalanine and 5- and 6-iodotryptophan by exchange in a melt (*10*). Tissue-distribution studies in mice as a function of loading dose have been made in order to evaluate these amino acids as potential pancreas scanning agents. These model compounds demonstrate a convenient labeling procedure which can be applied to the exchange labeling of other compounds of biologic interest.

METHODS

The carrier-free ^{123}Xe and ^{123}I were obtained as previously described (*1,4–6*). The new iodination reagent (a carrier-free, reactive iodine species), is prepared from the decay of ^{123}Xe to ^{123}I in the absence of air and moisture on a Pyrex surface maintained at 77°K. Xenon-123 ($T_{1/2}$ = 2.1 hr) is allowed to decay for ~6.5 hr. The time interval is an optimum for the highest yield and radiochemical purity of the 13.3-hr half-lived ^{123}I. Subsequent to the formation of the ^{123}I-species, adsorbed on the glass surface, the iodination reagent formed is enveloped with the halogenated liquid or crystalline substrate which is to be labeled. Generally 5–10 mg of crystalline substrate is used. The iodination reagent is kept at liquid nitrogen temperature until its complete immersion in an oil bath at the specified temperature. Vacuum is maintained in the reaction chamber until the labeling process is complete.

A unique feature of the new iodination reagent (the ^{123}I-species) is the ease of preparation for labeling without the addition or removal of solvent. The difficulties of removal of water from Na^{131}I preparations and possible interferences of stabilizer (sodium thiosulfate) for exchange labeling in melts have been discussed (*10,11*). Another advantage of labeling with the ^{123}I-species is the capability of transferring the ^{123}I-species to several reaction vessels through vacuum line manipulations (*12*). Utilization of the iodination reagent for "kit preparations" is envisioned (*13*). The new iodination reagent can be conveniently prepared from ^{123}I, ^{125}I, ^{121}I, and ^{120}I, if the corresponding radioxenon parent is available. A limitation of the present application of the iodination species is that the compound to be labeled must be stable at temperatures near the melting point

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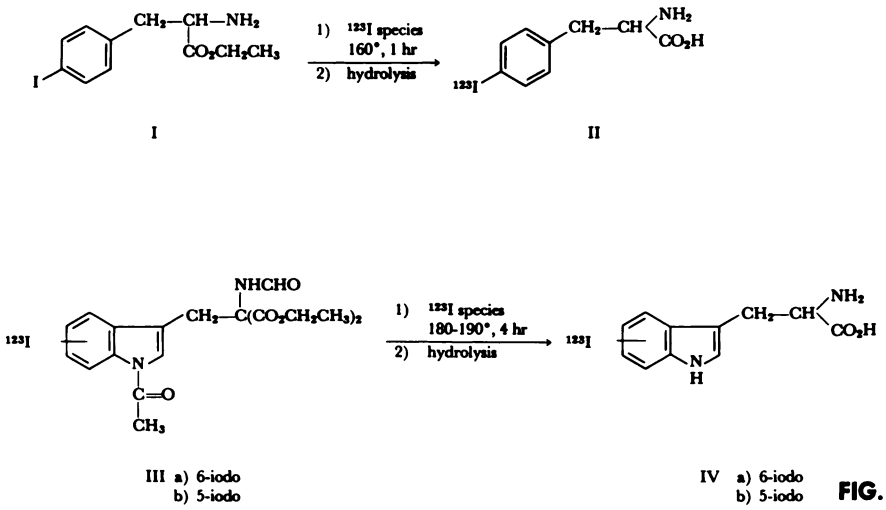

I II

III a) 6-iodo b) 5-iodo IV a) 6-iodo b) 5-iodo

FIG. 1. Synthesis of ^{123}I-labeled amino acids.

though this can be avoided by using low-melting derivatives of compounds that are unstable at their melting points. For example, the compounds that were of interest for this study, 4-iodophenylalanine and 5- and 6-iodotryptophan, are both high-melting solids which melt with decomposition. Therefore the exchange was carried out on low-melting derivatives of these compounds as shown in Fig. 1.

SYNTHESES

^{123}I-4-iodophenylalanine. Twelve milligrams (0.035 mmoles) of 4-iodo-DL-phenylalanine ethyl ester I (*14*), a thick colorless oil, was injected through a Burrell seal onto the ^{123}I-species contained in an evaculated glass reaction vessel. The sealed vessel was heated at 155°C for 1 hr. Approximately 0.1 mg of KI and two drops of 4 *N* NaOH were added and the contents heated ~2 min in a boiling water bath until solution was complete. The solution was cooled in an ice bath; two drops of glacial acetic acid and two drops of water were added to precipitate the amino acid. Then the mixture was centrifuged and the mother liquor discarded. This step was followed by two washings with water and one with methanol. The solid was again dissolved in two drops of 4 *N* NaOH and precipitated and washed as described. The solid was recrystallized from glacial acetic acid and ether. This gave 5.22 mg (43.5%) of 4-^{123}I-iodo-DL-phenylanine II [m.p. 234°–237°C, Lit. (*14*) 270°C].

The infrared spectrum was identical to an authentic sample of 4-iodophenylalanine. Thin-layer chromatography on silica gel with butanol:acetic acid:water (5:2:1) as the eluant indicated all the ^{123}I-activity to be in the spot containing the 4-iodophenylalanine. It should be noted that the 4-iodophenylalanine ethyl ester slowly solidifies to another compound (probably its dimer) on standing and therefore should be used soon after its preparation.

The total synthesis time required to prepare ^{123}I-4-iodophenylalanine was 2 hr. The specific activity was 0.014 mCi/mg (based on 1 mCi of ^{123}I species).

^{123}I-6- and 5-iodo-DL-tryptophan. The quantities and conditions used in the synthesis of the 6- and 5-isomers are included in the same procedure. The minor variations for the 5 isomer are indicated in parentheses. Sixty milligrams of IIIa (IIIb) (see below) and the ^{123}I-species were placed in vacuum in a sealed Pyrex tube. The solid was melted and maintained at 180°C (190°C) for 4 hr (6 hr). The tube was opened, solid was dissolved in hot acetone, transferred to a flask, and the solvent removed using a stream of nitrogen. To the residue, 2.0 ml of 2.5 *N* NaOH were added, the mixture was refluxed for 1 hr, the pH was adjusted to 1 with 6 *M* sulfuric acid, and the mixture was refluxed for an additional 10 min. The solution was then neutralized to pH = 5–6 with saturated sodium bicarbonate, the solvent was evaporated, and 2.5 ml of acetic acid was added to the residue. The mixture was heated at 100°C and centrifuged. Ethanol (0.5 ml) and ether (4 ml) were added to the mother liquor and then the solution was cooled. The precipitated solid was heated at 190°C and recrystallized from 65% ethanol to produce 12 mg (7 mg) of IVa (IVb) [m.p. 268–270°C (265°C) (Lit. (*15*) 265–267°C)].

Analysis. Calculated for $C_{11}H_{11}O_2N_2I$:

	C 40.01%	H 3.33%	N 8.48%
Found (6-):	C 40.55%	H 3.27%	N 8.13%
Found (5-):	C 41.13%	H 3.83%	

Thin-layer chromatography on silica gel using butanol:acetic acid:water (5:2:1) indicated the concentration of ^{123}I activity in the spot corresponding to the iodotryptophan. The total synthesis time required to prepare ^{123}I-labeled 5- and 6-iodotrypto-

phan using this method was 6 and 8 hr, respectively. Specific activities of 0.015 mCi/mg were obtained (based on 1 mCi of ^{123}I species).

Preparation of diethyl formamido (1-acetyl-6-iodoindol-3-yl-methyl malonate (IIIa) and diethyl formamido (1-acetyl-5-iodoindol-3-yl-methyl) malonate (IIIb). A mixture of 1.874 gm diethyl formamido (1-acetyl-6-aminoindol-3-yl-methyl) malonate or diethyl formamido (1-acetyl-5-aminoindol-3-yl-methyl) malonate (*16*), concentrated hydrochloric acid 3.75 ml (4.0 ml), and 7.5 ml (8.0 ml) H_2O at ~5 —10°C was treated dropwise with a solution of 0.348 gm $NaNO_2$ in 0.72 ml H_2O to a positive starch iodine test. To this was added a solution of 0.84 gm KI in 0.82 gm H_2O and the mixture was allowed to warm to room temperature. The mixture was heated to 40°C and evaporated to dryness. The residue was dissolved in 30 ml acetone, passed through a (4.5 × 4 cm) bed of alumina (activity III), and eluted with 250 ml chloroform in benzene (1:4). The eluate was evaporated to dryness and the residue was dissolved in 10 ml chloroform and again passed through the alumina bed. The eluate (500 ml) was evaporated to dryness and the residue recrystallized from 25 ml ethanol to give 0.52 gm of IIIa (0.43 gm of IIIb) [m.p. (IIIa) 190–195°C; m.p. (IIIb) 170–173°C].

Analysis. Calculated for $C_{19}H_{21}N_2O_6I$:

C 45.6% H 4.2% I 25.38%

Found (IIIa): C 45.72% H 4.37% I 25.04%

Found (IIIb): C 46.15% H 4.26% I 24.32%

ANIMAL EXPERIMENTS

The ^{123}I-iodinated amino acids were given intravenously to mice. The loading dose of ^{123}I-4-iodophenylalanine was varied by a factor of 333 from 0.012 mg to 4.0 mg/kg of body weight. Mice were sacrificed at 0.5 and 4.0 hr and the results are summarized in Tables 1 and 2. Iodine-123-6-iodotryptophan was investigated as a function of loading dose in mice sacrificed at 0.5 hr and the results are summarized in Table 3. The percent uptake of the administered dose in the organ of interest and the

TABLE 1. EFFECT OF LOADING DOSE ON THE DISTRIBUTION OF ^{123}I-4-IODOPHENYLALANINE IN MICE 30 MIN AFTER IV ADMINISTRATION*

Loading dose, mg kg^{-1}	Pancreas		Kidney		Intestine		Liver		Carcass	Total retained	Pancreas / Liver
	% admin dose	% admin dose, gm^{-1}	% admin dose	% admin dose, gm^{-1}	% admin dose	% admin dose, gm^{-1}	% admin dose	% admin dose, gm^{-1}	%		
0.012	3.18	16.92	2.82	—	8.79	—	6.56	4.06	48.42	69.83	4.17
	±0.85	±0.87	±0.14	—	±0.97	—	±0.56	±0.18	±3.79	±5.03	±0.15
0.056	3.64	21.50	1.85	4.97	8.03	2.68	9.64	7.55	41.31	64.45	2.84
	±0.57	±4.65	±0.22	±0.31	±0.55	±0.38	±0.74	±1.33	±2.88	±3.19	±0.43
0.42	1.76	11.41	1.50	4.50	6.94	2.42	5.80	4.58	41.32	57.32	2.48
	±0.24	±1.93	±0.08	±0.30	±0.47	±0.22	±0.39	±0.17	±5.03	±4.31	±0.37
0.8	0.67	2.44	0.72	1.30	1.91	0.58	2.14	1.08	50.93	56.37	2.22
	±0.37	±0.63	±0.42	±0.57	±0.36	±0.13	±0.28	±0.14	±4.85	±5.41	±0.37
4.0	1.39	5.54	1.21	2.14	4.60	1.60	3.83	2.61	34.82	45.87	2.10
	±0.43	±1.34	±0.65	±0.82	±0.82	±0.45	±0.45	±0.54	±3.98	±5.10	±0.20

* Five mice in each group. The error is given as the standard deviation.

TABLE 2. EFFECT OF LOADING DOSE ON THE DISTRIBUTION OF ^{123}I-4-IODOPHENYLALANINE IN MICE 4 HR AFTER IV ADMINISTRATION*

Loading dose, mg kg^{-1}	Pancreas		Kidney		Intestine		Liver		Carcass	Total retained	Pancreas / Liver
	% admin dose	% admin dose, gm^{-1}	% admin dose	% admin dose, gm^{-1}	% admin dose	% admin dose, gm^{-1}	% admin dose	% admin dose, gm^{-1}	%		
0.26	0.62	4.89	0.75	1.99	2.67	0.94	2.41	1.67	16.82	23.37	2.75
	±0.31	±2.78	±0.08	±0.36	±0.80	±0.37	±0.76	±0.61	±4.71	±6.08	±0.65
0.52	0.65	4.43	0.65	1.84	2.86	0.88	2.62	1.94	5.79	12.57	4.75
	±0.27	±1.91	±0.22	±0.76	±1.51	±0.37	±2.13	±1.29	±2.47	±6.57	±0.57

* Five mice in each group. The error is given as the standard deviation.

TABLE 3. EFFECT OF LOADING DOSE ON THE DISTRIBUTION OF ^{123}I-6-IODOTRYPTOPHAN IN MICE AT 30 MIN AFTER IV ADMINISTRATION

Loading dose, mg kg^{-1}	Pancreas		Kidney		Intestine		Liver		Carcass %	Total retained	Pancreas / Liver
	% admin dose	% admin dose, gm^{-1}	% admin dose	% admin dose, gm^{-1}	% admin dose	% admin dose, gm^{-1}	% admin dose	% admin dose, gm^{-1}			
0.18	—	10.83	—	5.46	—	1.92	—	3.86	49.95	72.02	2.81
	—	±1.40	—	±0.39	—	±0.22	—	±0.32	—	±0.45	±0.24
1.8	—	14.59	—	4.73	—	1.76	—	3.24	42.30	66.62	4.52
	—	±1.79	—	±0.20	—	±0.16	—	±0.27	—	±7.13	±0.55
2.0	1.00	4.55	0.97	2.10	4.96	1.39	5.51	2.88	40.73	52.17	1.57
	±0.21	±0.81	±0.09	±0.25	±0.25	±0.19	±0.38	±0.33	±1.42	±1.98	±0.33

* Five mice in each group. The error is given as the standard deviation.

percent uptake per gram of tissue are reported. The assay procedures were similar to those used previously. The pancreas-to-liver ratios were calculated from the data for the percent uptake per gram of tissue.

RESULTS AND DISCUSSION

^{123}I-4-iodophenylalanine. There have been a number of studies on the tissue distribution of iodine-labeled iodophenylalanines (*17–23*) and their potential use as pancreas and tumor localization or radiopaquing agents. In view of the striking loading-dose effect which we have observed in the tissue distribution of ^{18}F-labeled 6-fluorotryptophan (*16*), it was appropriate to investigate the effect of this variable on the tissue distribution of iodophenylalanine.

Tables 1 and 2 summarize the results of tissue-distribution studies with ^{123}I-4-iodophenylalanine in mice. Some new observations have been made. A loading-dose effect on the tissue specificity and body retention is apparent. As the loading dose is decreased, the total retention increases and the organ distributions change. The total retention increases from 46% to 70% as the loading dose is reduced from 4.0 mg/kg to 0.012 mg/kg. At 30 min the pancreas-to-liver (P/L) ratio increases from 2.10 ± 0.20 at a loading dose of 4.0 mg/kg to 4.17 ± 0.11 at a loading dose of 0.012 mg/kg. The variation in the P/L ratio is gradual and not a step function, but is rather abrupt at the lowest loading doses. The loading-dose effect, although in a much lower range, is similar to that observed with 6-fluorotryptophan-^{18}F in mice (*16*).

The total-body retention at 4 hr is about 25% of that observed at 30 min with a nearly comparable loading dose. We have verified that the pancreas-to-liver ratio is greater at the longer time interval (*17*) even though the maximum activity appears in the organs about 5 min after administration (*19*). Unfortunately, owing to a lower retention from clearance from the organ, at the longer time periods in a clinical application the reduction of radioactivity in the organ may reduce counting statistics below that obtained with a 30-min uptake. The question is academic with respect to 4-iodophenylalanine. The iodinated amino acid does not appear to localize selectively enough in higher species (*20*) to find diagnostic applications either as a pancreas scanning agent or as a radiopaquing agent (*23*).

^{123}I-6-iodotryptophan. The tissue distribution of ^{123}I-6-iodotryptophan as a function of loading dose is reported in Table 3. We found that the selective uptake of the iodinated amino acid by the pancreas is low (P/L $\simeq$ 2) and is apparently nearly independent of the loading dose. The result is in contrast to 6-fluorotryptophan-^{19}F which resulted in a P/L ratio of up to 16 in mice and in which the P/L ratio was quite sensitive to the loading dose. Although the total retention of 6-iodotryptophan-^{123}I is high, we did not observe tissue specificity. Costello (*24–26*) has found that labeled tryptophan concentrated in zones of metastates in patients with advanced carcinoid syndrome. Blau (*27*) observed a poor concentration of N-iodoacetyltryptophan-^{131}I in the pancreas. The radioiodinated tryptophans may be of value in brain research (*28,29*) since the product amines are thought to serve as transmitters for neuronal pathways.

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