PANCREATIC CONCENTRATION OF ^{125}I-LABELED PHENYLALANINE IN MICE

Vijay M. Varma, William H. Beierwaltes, Lionel M. Lieberman and Raymond E. Counsell

University of Michigan, Ann Arbor, Michigan

^{14}C- and ^{35}S-labeled amino acids (*1–3*) have been reported to appear in high concentration in the pancreas shortly after being administered intravenously. Blau and Manske (*4*) labeled methionine with ^{75}Se and found that about 6% of the dose administered to dogs was found in the pancreas at 2 hr after administration. The concentration (percent dose per gram) of radioactivity in the pancreas exceeded the concentration in the liver by a factor of 8 or 9. Preliminary experiments suggested that even small modifications of the tryptophane molecule would destroy the pancreas localizing ability (*4*). These experiments suggested that the tagging of an amino acid would have to involve changes less extensive than the addition of even a small external group (*4*).

We report here the specific pancreas localization of ^{125}I-labeled phenylalanine in approximately the same concentrations as the pancreatic concentration of ^{75}Se after the administration of ^{75}Se-selenomethionine (*4*) and after the administration of ^{14}C-labeled DL phenylalanine.

METHOD

^{14}C-labeled-phenylalanine studies. All mice used in this study were male, 3–5 weeks of age, of the C57Bl/6J strain (black) with B-16 melanomas and weighing approximately 15–30 gm. As shown in Table 1, intracardiac injections of 10 μCi of l-^{14}C-phenylalanine, obtained from New England Nuclear Corp. in a solid form with a specific activity of 1–5 mCi/mmole, were given. The labeled amino acid was dissolved in a hydrochloric acid solution with a pH of 1–2 so that the final concentration was 5 μCi/0.1 ml. At intervals of 2, 4 and 24 hr (see Table 1) after injection, the mice were anesthetized with ether. While each animal was still alive but under ether anesthesia, the chest was opened and blood was removed from the cardiac cavity into a 1 ml heparinized syringe. The animal was then killed by cutting out the heart with scissors. Representative 50-mg samples of eye, tumor, skin, liver, kidney, muscle, blood, adrenal, spleen, lung, thyroid gland, pancreas, fat, intestine, heart and bladder were obtained.

Samples were dissected free, cleaned of fat and foreign material and weighed. Samples weighed from 10 to 150 mg. All specimens were placed in counting vials and digested overnight in 0.3 ml of 10% NaOH and heated in near-boiling water for 30 sec. After cooling, four drops of 30% H_2O_2 were added. Ten milliliters of the liquid scintillating solution (*5*) were added, and the radioactivity content was assayed in a liquid scintillation counter. Corrections for quenching were made with an external standard. All results were expressed as cpm/mg ± SEM (standard error of mean) without correction for animal weight.

^{125}I-NaI and ^{125}I-iodophenylalanine studies. Mice of the same strain, weights and ages as above were injected intraperitoneally with 10 μCi of ^{125}I as ^{125}I-NaI or *ortho, meta* or *para* ^{125}I-iodophenylalanine. The specific activity of the iodophenylalanines is stated on each table. The mice were then sacrificed at 2, 4 and 24-hr intervals (Tables 2–4) as described above. Tissue samples were obtained as in the ^{14}C-phenlyalanine experiments. The samples were placed in test tubes and counted in a commercial well counter with correction made for decay.

Radioactivity concentrations were expressed in cpm/mg ± SEM. No corrections were made for body weight.

Intracardiac injection. The possibility that the high concentration of ^{125}I radioactivity in the pancreas was related to contamination of the peritoneal surface of the pancreas by intraperitoneal injection was checked by subsequent experiments in nine mice using *p*-iodophenylalanine but giving 10 μCi of the material by intracardiac injection at intervals of 2 and 4 hr before sacrifice (Table 4).

Synthesis of *o*, *m* and *p* ^{125}I-iodophenylalanine: general procedure. The isomeric iodophenylalanines were prepared according to the method of Redemann and Dunn (*6*) and are described elsewhere (*7*). A 100-mg sample of the appropriate iodophenylalanine was dissolved in 0.5 ml of acetic acid and 3 ml of water containing 2 mCi of ^{125}I-NaI. The

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For reprints contact: W. H. Beierwaltes, University Hospital, Univ. of Michigan Medical Center, Ann Arbor, Mich. 48104.

TABLE 1. DISTRIBUTION OF RADIOACTIVITY AFTER ADMINISTRATION OF ^{14}C-DL PHENYLALANINE

No. of mice	Time after injection (hr)	Pancreas	Thyroid	Eye	Tumor	Skin	Liver	Kidney	Muscle	Blood	Adrenal
3	2	8,658.7 ±495.2	1,534.7 ±127.1	454.1 ±34.4	1,711.7 ±256.0	300.3 ±17.5	1,668.0 ±181.8	3,116.1 ±525.7	632.1 ±47.6	501.1 ±49.8	1,441.5 ±142.5
3	4	3,684.6 ±1,303.7	1,055.5 ±753.8	263.1 ±37.8	2,009.3 ±174.1	288.7 ±27.6	1,428.1 ±192.6	2,705.8 ±293.9	514.7 ±167.5	455.8 ±42.6	1,257.1 ±142.8
3	24	638.9 ±281.3	61,344.2 ±21,273.9	514.3 ±309.5	452.2 ±255.8	913.3 ±676.7	1,634.9 ±314.9	1,559.0 ±223.2	262.0 ±123.9	620.0 ±99.3	771.5 ±54.7

* Cpm/mg ± SEM; dose = 10 μCi intracardiac. Concentration = 100 μCi/ml; 8 mg/ml; 0.8 mg injected.

TABLE 2. DISTRIBUTION OF RADIOACTIVITY AFTER ADMINISTRATION OF ^{125}I-O-IODOPHENYLALANINE

No. of mice	Time after injection (hr)	Pancreas	Thyroid	Eye	Tumor	Skin	Liver	Kidney	Muscle	Blood	Adrenal	Spleen
						^{125}I-o-iodophenylalanine						
3†	2	2,176.7 ±620.3	1,358.2 ±652.1	347.1 ±52.8		917.0 ±1,013.1	318.7 ±12.1	674.2 ±150.2	306.1 ±25.0	314.3 ±20.5	269.8 ±52.8	310.2 ±27.9
3†	4	3,822.7 ±681.6	1,664.4 ±549.5	645.8 ±123.3	806.7 ±111.3	337.8 ±71.5	410.7 ±42.2	622.1 ±30.6	678.9	374.1 ±20.4	394.5 ±28.0	351.6 ±36.2
3†	24	834.7 ±402.0	1,982.7 ±451.7	1,038.0 ±673.2	105.7	217.0 ±149.1	293.8 ±251.7	275.5 ±150.9	238.9 ±195.6	246.2 ±194.6	217.6 ±104.6	304.0 ±259.2
						^{125}I-NaI						
3‡	2	286.3 ±33.7	117,800.2 ±37,392.3	138.8 ±11.1		307.1 ±7.8	203.8 ±21.7	416.0 ±37.1	160.4 ±54.8	553.7 ±41.1	181.1 ±32.3	309.3 ±34.9
2‡	4	240.3 ±37.7	408,995.0 ±337.0	193.6 ±49.3	272.9 ±141.4	483.6 ±59.0	308.7 ±19.7	417.1 ±65.0	128.9 ±15.7	471.4 ±159.9	334.2 ±176.6	1,093.8 ±76.2
3‡	24		139,561.0	10.6 ±3.6	12.4 ±4.1	26.6 ±8.6	11.3 ±3.6	17.0 ±4.6	3.9 ±1.5	21.5 ±6.5	10.3	20.0 ±7.1

* Cpm/mg ± SEM; dose = 10 μCi intraperitoneal.
† Concentration = 63 μCi/ml; 6.95 mg/ml; 1.1 mg injected.
‡ Concentration = 100 μCi/ml.

TABLE 3. DISTRIBUTION OF RADIOACTIVITY AFTER ADMINISTRATION OF ^{125}I-*M*-IODOPHENYLALANINE

No. of mice	Time after injection (hr)	Pancreas	Thyroid	Eye	Tumor	Skin	Liver	Kidney	Muscle	Blood	Adrenal	Spleen
3†	2	1,155.8 ±185.2	536.1 ±238.9	244.4 ±24.6	291.0 ±38.1	309.9 ±63.9	417.0 ±71.6	602.3 ±51.6	370.9 ±90.9	525.3 ±120.1	305.1 ±160.3	368.5 ±121.2
6‡	4	1,191.6 ±1,627.8	1,330.8 ±2,184.9	583.3 ±720.7	243.7 ±344.3	162.8 ±151.7	195.8 ±253.4	395.7 ±421.3	167.3 ±186.9	220.4 ±123.1	139.2 ±279.6	223.3 ±360.3
3‡	24	137.4 ±112.7	1,376.7 ±602.7	139.7 ±93.0	30.8 ±20.5	40.3 ±15.1	27.4 ±17.3	77.3	16.6 ±13.1	59.5 ±39.3	46.6 ±18.2	47.0 ±38.5

* Cpm/mg ± SEM; dose = 10 μCi intraperitoneal.
† Concentration = 198.9 μCi/ml; 27.7 mg/ml; 1.4 mg injected.
‡ Concentration = 79 μCi/ml; 10.7 mg/ml; 1.3 mg injected.

IN BLACK MICE WITH MELANOMAS*

Spleen	Lung	Fat	Intestine	Heart	Bladder
1,558.6 ±157.2	1,008.9 ±172.3	157.0 ±60.5	1,847.7 ±232.8	692.4 ±103.8	1,889.3 ±394.2
1,699.2 ±200.8	858.7 ±103.8	82.5 ±23.3	2,502.7 ±586.4	691.4 ±112.0	1,475.3 ±593.1
953.2 ±335.3	701.7 ±103.0	1,332.4 ±1,158.6	1,379.0 ±55.8	363.4 ±65.6	2,571.7 ±1,953.0

AND ^{125}I-NaI IN BLACK MICE WITH MELANOMAS*

Lung	Fat	Intestine	Heart	Bladder	Brain	Stomach
260.7 ±22.0	241.5 ±7.8	740.9 ±391.8	315.5 ±9.7	2,679.5 ±1,850.5	125.7 ±13.8	409.0 ±77.7
323.7 ±14.0	387.5 ±423.7	519.6 ±212.4	383.9 ±15.8	5,623.4 ±3,987.6	256.3 ±42.7	278.1 ±248.3
129.0 ±76.2	581.1 ±106.5	201.5 ±66.7	239.9 ±171.6	1,786.0 ±2,036.3	151.3 ±118.9	420.3 ±316.6
844.7 ±159.2	176.6 ±24.2	176.6 ±24.2	174.2 ±31.7	1,158.8 ±130.2		
476.8 ±82.2	111.8	298.1 ±121.2	179.9 ±13.0	1,430.4 ±392.4		
14.9 ±5.2			8.8 ±3.1			

IN BLACK MICE WITH MELANOMAS*

Lung	Fat	Intestine	Heart	Bladder	Brain	Stomach
392.9 ±100.7	429.4 ±151.5	360.9 ±106.1	410.1 ±94.7	545.3 ±93.0		
189.6 ±283.4		260.9 ±281.4	177.1 ±180.3	2,484.0 ±2,925.5	96.9 ±120.0	312.3 ±256.9
36.4 ±23.0		43.5 ±43.4	29.9 ±26.0	107.0 ±69.5	14.9 ±6.8	124.1 ±44.8

mixture was stirred and heated at 110°C in a nitrogen atmosphere for 24 hr. The solvent was removed using a rotary evaporator and the residue suspended in 2 ml of cold water. The solid was collected by filtration and recrystallized from aqueous ethanol. The chemical purity of the radioiodinated amino acid was verified by thin-layer chromatography (*n*-butanol:water:acetic acid—3:1:1) and comparison of the infrared spectra with that of an authentic sample. Radiochemical purity was confirmed by radioscanning the above chromatograms. The product was dissolved in 0.1 *N* hydrochloric acid for determination of specific activity and for animal experiments. The specific activity in the initial syntheses ranged between 12 and 17 μCi/mg, and the percent exchange varied between 20 and 30%.

RESULTS

1-^{14}C-DL phenylalanine. Table 1 gives the concentration of ^{14}C radioactivity in blood and organs 2, 4 and 24 hr after intracardiac injection of 1-^{14}C-DL phenylalanine in cpm/mg ± SEM. ^{14}C radioactivity was found in highest relative and absolute concentration in the pancreas 2 hr after i.c. injection. At this time, the ^{14}C radioactivity concentration in pancreas was three times that in kidney and five times that in liver. The high concentration in the pancreas was not found at later intervals of sacrifice. As the pancreatic radioactivity concentration decreased at later intervals, the thyroid-gland concentration of radioactivity rose to higher levels than those observed in all other organs. No other organ assayed showed unusually high concentrations.

^{125}I-DL iodophenylalanine. The next three tables give the relative tissue concentrations of ^{125}I in blood and various organs mentioned above from ^{125}I-*o*-iodophenylalanine and ^{125}I-NaI (Table 2), ^{125}I-*m*-iodophenylalanine (Table 3) and ^{125}I-*p*-iodophenylalanine (Table 4).

^{125}I-o-iodophenylalanine. The mean concentration of ^{125}I in the pancreas after ^{125}I-*o*-iodophenylalanine was highest at 4 hr, next highest at 2 hr and lowest at 24 hr. The pancreatic concentration at 4 hr was nine times that in liver and six times that in kidney. At 2 hr the concentration was about seven times that in liver and three times that in kidney. The concentration of radioactivity in the bladder after it was emptied and the inside was washed was slightly greater than in the pancreas at 4 hr and roughly equal at 2 hr and two times that in pancreas at 24 hr. Thyroid counts were lower than those of the pancreas at 2 and 4 hr but two times that of the pancreas at 24 hr. Thyroid counts were ninety times greater at 2 hr after the administration of an equal amount of ^{125}I as ^{125}I-NaI than when given as the

TABLE 4. DISTRIBUTION OF RADIOACTIVITY AFTER ADMINISTRATION OF ^{125}I-*P*-IODOPHENYLALANINE

No. of mice	Time after injection (hr)	Pancreas	Thyroid	Eye	Tumor	Skin	Liver	Kidney	Muscle	Blood	Adrenal
						Intraperitoneal injection					
3†	2	812.8 ±337.9	1,616.9 ±1,401.4	306.7 ±37.6	371.1 ±63.9	118.1 ±10.5	189.3 ±23.8	353.4 ±18.8	561.3 ±397.5	373.0 ±48.2	254.4 ±38.6
3†	4	496.4 ±91.4	213.2 ±96.6	113.6 ±10.9	132.2 ±14.8	60.3 ±7.6	90.7 ±10.5	205.3 ±20.3	75.9 ±16.8	174.1 ±32.8	147.1 ±17.0
3†	24	3.2 ±0.8	417.6 ±281.1	24.5 ±17.9	5.5 ±1.4	8.0 ±2.5	7.3 ±1.8	8.4 ±5.9	3.9 ±0.2	9.7 ±7.1	8.9 ±1.9
						Intracardiac injection					
3‡	2	2,306.2 ±246.7	355.2 ±77.8	195.6 ±25.2	477.2 ±49.1	216.1 ±61.2	363.3 ±81.2	809.9 ±180.1	271.6 ±18.8	473.8 ±124.7	245.3 ±20.8
3‡	4	2,997.0 ±598.1	308.9 ±40.5	230.8 ±30.4	394.5 ±1.0	272.8 ±29.8	465.7 ±34.8	776.7 ±42.3	337.5 ±48.1	510.2 ±19.1	352.4 ±165.5

* Cpm/mg ± SEM; dose = 10 μCi.
† Concentration = 115.5 μCi/ml; 8.7 mg/ml; 0.11 mg injected.
‡ Concentration = 27 μCi/ml; 1.8 mg/ml; 0.72 mg injected.

^{125}I-*o*-iodophenylalanine (Table 2). Radioactivity of intestine (opened, emptied and washed) was one-third that of pancreas at 2 hr and then fell to values below that of most other tissues by 24 hr. The concentration of ^{125}I in the melanoma was almost two times that in liver at 4 hr.

^{125}I-m-iodophenylalanine. Uptake of ^{125}I from ^{125}I-*m*-iodophenylalanine was similar to that after the *ortho* compound. Concentration of radioactivity in the pancreas at 4 hr was about six times that in liver and three times that in kidney. The pancreatic concentration was not remarkable at 24 hr. Bladder concentration of ^{125}I was again high (about two times that in pancreas) at 4 hr but relatively low at 24 hr. Thyroid counts roughly equalled pancreas counts at 4 hr but exceeded pancreas counts by a factor of 10 at 24 hr. Again the mean concentration of radioactivity in malignant melanoma exceeded that in liver at 4 hr. Thyroid counts were again about ninety times greater after equivalent doses of ^{125}I-NaI than after *m*-iodophenylalanine.

^{125}I-p-iodophenylalanine. Uptake of ^{125}I from ^{125}I-*p*-iodophenylalanine was similar to that after the *ortho* and *meta* labeled compounds. The pancreas showed the highest radioactivity concentration (other than bladder) at 4 hr, being five times that in liver and almost three times that in kidney. The concentration in melanoma, however, did not exceed that in blood. Thyroid counts were again about eighty times greater after equivalent doses of ^{125}I-NaI. The pancreatic concentration of ^{125}I after intracardiac injection of ^{125}I-*p*-iodophenylalanine was almost three times greater than that after intraperitoneal administration.

DISCUSSION

The external iodination of phenylalanine has been achieved without loss of the pancreas specificity characteristic of ^{14}C-phenylalanine. The pancreatic concentration of ^{125}I after administration of these iodinated amino acids is 5–9 times that in the liver 4 hr after administration. Furthermore, the specific concentration in the pancreas indicates that deleterious deiodination does not occur in the mouse during a 2–4 hr interval after administration of the iodinated compound. The fact that deiodination does not occur to any marked degree is further demonstrated by the 80–90 times higher concentration of ^{125}I in the thyroid gland after an equivalent amount of ^{125}I as ^{125}I-NaI at 4 hr. Specific concentration of ^{125}I in the malignant melanoma under these circumstances may be related to the fact that phenylalanine is a precursor in melanin biosynthesis. In a previous publication (*8*), we found that the next precursor (3,4-dihydroxyphenylalanine) labeled with ^{14}C concentrated in the same mouse melanoma and even more in the Syrian hamster melanomas.

The nine-fold rise in thyroidal ^{14}C radioactivity concentration at 4 hr and the additional six-fold rise in radioactivity concentration at 24 hr is presumably associated with the precursor relationship of this amino acid to tyrosine. This increasing radioactivity concentration from ^{14}C, however, raises the possi-

IN BLACK MICE WITH MELANOMAS*

Spleen	Lung	Fat	Intestine	Heart	Bladder	Brain
142.9 ±15.5	141.3	394.3 ±185.5	217.3 ±18.2	187.0 ±25.6	2,483.4 ±148.1	98.4 ±11.4
60.5 ±6.5	124.6 ±17.7	56.6 ±5.4	81.3 ±12.6	113.8 ±21.1	1,474.5 ±187.9	46.0 ±5.8
2.6 ±0.5	9.5 ±1.1	6.2 ±1.3	3.8 ±0.5	5.6 ±1.2	43.1 ±13.8	1.6 ±0.6
248.5 ±37.0	392.9 ±87.6	44.2 ±0.9	254.5 ±28.7	367.1 ±110.1	738.2 ±337.1	
329.9 ±42.0	448.9 ±20.2	146.6 ±83.0	302.2 ±36.5	464.7 ±49.6	410.2 ±15.3	

bility that the rising thyroidal ^{125}I activity after iodophenylalanine is not entirely related to deiodination.

After the present manuscript was written, Ullberg and Blomquist (*9*) confirmed that *p*-iodophenylalanine concentrated specifically in the pancreas of the mouse. 3,4-Diiodophenylalanine showed somewhat lower concentration, and 3-hydroxy-4-iodophenylalanine showed no specificity of concentration in the pancreas. Garzo, Hansson and Ullberg (*10*) also showed that 4-fluorophenylalanine followed the distribution pattern of natural amino acids with similar concentration in the pancreas.

The demonstration that a radioiodinated amino acid will concentrate in the pancreas of the mouse raises the possibility of using the radioiodinated amino acids for scintillation scanning at 2–4 hr after administration for diagnosis of pancreatic lesions in the human. Ullberg and Blomquist (*9*) suggested two advantages of the use of this amino acid over ^{75}Se-selenomethionine:

1. Higher degree of specificity of concentration in the pancreas.
2. More rapid renal excretion with short biologic half-life.

In Ullberg's experiments the iodinated phenylalanine did not appear to be incorporated into pancreatic proteins. Moreover, it appeared to be transported across the pancreatic cell membrane like the natural amino acids, but not accepted by the protein synthesizing system, with a resultant rapid return to the blood. The pancreatic uptake peaked between 2 and 6 hr.

To these two advantages over ^{75}Se-selenomethionine might be added:

3. The physical half-life can be markedly shortened using ^{123}I.

It is unknown at present, however, whether the human pancreas will concentrate phenylalanine in a manner similar to the mouse pancreas. Sherman *et al* (*11*) injected mice, rabbits and dogs with α-aminocyclopentane carboxylic acid (carboxyl-^{14}C) and the distribution of the drug was studied by tissue analysis. Concentration of the isotope was observed in mouse pancreas but not in that of dogs and rabbits. Our present mouse experiments must therefore be repeated in monkey and man.

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