Investigation of Radiopharmaceuticals for Pancreatic Imaging: Accumulation of Amines in the Pancreas

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To develop radiopharmaceuticals for pancreatic imaging, radioiodinated ethyl benzene derivatives containing various functional groups (amino, carboxyl, and methyl groups) were synthesized and the effects of these functional groups were compared *in vitro* and *in vivo*. At 2 min after intravenous injection, the amino derivative, 2-(4-iodophenyl)-*N*,*N*-dimethyl ethylamine, displayed about twice the pancreatic uptake and a more than 8-fold higher pancreas/liver ratio than the carboxyl and methyl derivatives. This high and selective *in vivo* accumulation on the amino derivative in the pancreas was well supported by *in vitro* studies on the uptake by pancreatic tissue slices. The mechanism promoting pancreatic accumulation of radiopharmaceuticals with an amino group is also discussed.

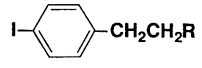
Keywords radioiodinated phenethylamine derivative; radiopharmaceutical; tissue slice; biodistribution; pancreas; pH-shift theory

Introduction

One of the most important unsolved problems in nuclear medicine is the lack of a suitable radiopharmaceutical for pancreatic diagnostic imaging.[1]

We have recently reported on the high pancreatic accumulation of ^{13}N-labeled ammonia[2] and ^{123}I-labeled *N*,*N*,*N'*-trimethyl-*N'*-(2-hydroxy-3-methyl-5-iodobenzyl)-1,3-propane diamine (HIPDM).[3] Polyamines or certain drugs containing an aminophenyl group, such as procainamide, sulfisomidine, and sulfathiazole, have also been reported to show a high level of accumulation in the pancreas.[4] The pancreatic accumulation displayed by these amines suggested that further studies on the amino group would be worthwhile, since if the pancreatic compatibility of this functional group was confirmed, a more pancreatic-specific radiopharmaceutical could be designed.

The present study involved a systematic evaluation of functional groups. Simple radioiodinated ethyl benzene derivatives with attached amino, carboxyl, and alkyl groups were synthesized by taking into account the following factors: (1) ease of synthesis, (2) stability, (3) a compact and uncharged structure, except for the functional group, in order to minimize the stearic and electric perturbations, (4) the usefulness of ^{99m}Tc- and ^{62}Cu-labeled ethyl benzene derivatives as target-specific radiopharmaceuticals,[5] and (5) the decrease of deamination by amine oxidase following the addition of a methyl group to the nitrogen atom of phenylethylamine.[6] Figure 1 shows the chemical structures of the compounds used in this study: 2-(4-iodophenyl)-*N*,*N*-dimethyl ethylamine (IPDMA), 3-(*p*-iodophenyl)propionic acid (IPPA), and 3-(*p*-iodophenyl)propane (IPP). These compounds were labeled with ^{125}I, because of its ready availability and long half-life. Their chemical and biological behavior were compared and the effect of the amino group on pancreatic affinity was determined.



R = $N(CH_3)_2$: IPDMA
COOH : IPPA
CH_3 : IPP

Fig. 1. Chemical Structures of the Compounds Studied

Materials and Methods

[^{125}I]Sodium iodide was purchased from Amersham International Plc. I-U-[^{14}C]leucine was obtained from New England Nuclear and diluted with saline. The other drugs and reagents used were obtained from commercial sources. Male Wistar rats were supplied by Japan SLC Co., Ltd.

Synthesis of [^{125}I]IPDMA Radiolabeling of IPDMA was achieved by a radioisotopic exchange reaction using divalent copper as the catalyst.[7]

IPDMA hydrochloride (1.56 mg), [^{125}I]sodium iodide (1.85 MBq), copper (II) sulfate (100 μg), and ammonium sulfate (2 mg) were dissolved in 0.6 ml of water. The reaction vial was sealed and then the mixture was heated to 130 °C for 2 h. After cooling, the reaction mixture was alkalized with 2N sodium hydroxide and extracted with chloroform. The chloroform extract was evaporated under a stream of nitrogen and the final product was obtained with a yield of 85—95%. The resulting product showed only one radiochemical component on thin-layer chromatography (TLC), which corresponded to unlabeled IPDMA (chloroform : acetone : 28% ammonia aqueous solution = 90 : 10 : 2, Rf = 0.47).

Synthesis of [^{125}I]IPPA Radiolabeling of IPPA was also achieved by a radioisotopic exchange reaction.[7]

IPPA (1.38 mg), [^{125}I]sodium iodine (1.85 MBq), copper (II) sulfate (100 μg), and ammonium sulfate (2 mg) were dissolved in 0.6 ml of water. The reaction vial was sealed, and then the mixture was heated to 140 °C for 3 h. After cooling, the reaction mixture was acidified with 1N hydrochloric acid and then extracted with chloroform. After removing the organic solvent, the final product was obtained with a yield of 80—92%. This product showed only one radiochemical component on TLC, which corresponded to unlabeled IPPA (chloroform : acetone : acetic acid = 90 : 10 : 1, Rf = 0.51).

Synthesis of [^{125}I]IPP Since radioiodinated IPP could not be prepared by a radioisotopic exchange reaction, it was synthesized by the Sandmeyer reaction.[8]

p-Aminophenyl propane (8.3 mg) was suspended in 3.5 ml of water and 0.36 ml of sulfuric acid at 0 °C. To the resulting suspension, a solution of sodium nitrite (4.2 mg) in 0.1 ml of water was added dropwise. The reaction mixture was stirred for 30 min and then was treated with 100 MBq of [^{125}I]sodium iodide, 10 μg of potassium iodide, and 0.3 ml of 1×10^{-7} M sodium thiosulfate solution. The reaction mixture was stirred for an additional 60 min at room temperature and then heated on a boiling water bath for 60 min. After cooling, the solution was alkalinized with sodium hydroxide and a small amount of sodium sulfate was added. Then extraction with chloroform was performed and the chloroform extract was evaporated to dryness. The residue was dissolved in chloroform and then subjected to chromatography on silica gel with chloroform. The desired fractions were combined and the final product was obtained by removal of the organic solvent. The yield was about 3.5% and the resulting product showed only one radiochemical component on TLC, which corresponded to the authentic compound prepared according to the method of Meisenheimer[9] (chloroform, Rf = 0.86).

Measurement of Partition Coefficients Measurement of partition coefficients was carried out by a slight modification of the method reported previously.[10] Aliquots (20 μl) of radioiodinated samples were mixed in a test tube with 3 ml each of *n*-octanol and various 0.1 M buffers (pH

5.0—10.0) of acetate, phosphate, and carbonate. This tube was stirred with a vortex mixer (3 × 1 min), incubated for 1 h at room temperature, and then centrifuged for 5 min. Then 500-μl aliquots of each phase were taken out and counted for ^{125}I activity using a well-type NaI scintillation counter. The partition coefficient (P) was determined by calculating the ratio of cpm/ml for octanol to buffer.

Incorporation into Pancreatic and Liver Slices Male Wistar rats (200—250 g) were sacrified by decapitation. The pancreas and liver were rapidly removed from each rat, cut into pieces, and placed into cold saline. Slices were prepared with a conventional Stadie–Riggs slicer[11] and pooled in cold saline. About 100 mg of slices were placed in a vial containing 1.9 ml of *N*-hydroxyethylpiperazine-*N'*-2-ethansulfonate (HEPES) buffer (pH 7.4). Then, 0.1 ml of each ^{125}I-labeled sample or [^{14}C]leucine was added and incubation was performed at 37 °C for specified intervals. If necessary, various concentrations of ouabain were added to the medium 30 min before the addition of the radioactive sample solution. At the end of incubation, the medium was removed and the slices were washed twice with 2 ml of cold saline. The radioactivity of the slices, medium, and washings was then measured and the results were expressed as the % uptake/100 mg slice weight, which was calculated as reported previously.[12]

Biodistribution in Rats An amount of 7.4 kBq of each ^{125}I-labeled sample was injected intravenously into male Wistar rats (150—180 g). At designated time intervals, animals were killed by decapitation and their organs were removed. A blood sample was obtained by cardiac puncture immediately before decapitation. The excised organs and blood samples were weighed and their radioactivity was determined using a well-type NaI scintillation counter. Results were expressed as the % injected dose per gram of tissue.

Results

Partition Coefficients The solubility of the ^{125}I-labeled compounds in *n*-octanol is shown in Fig. 2 as a function of the pH of the aqueous phase of a *n*-octanol-buffer liquid partition system.

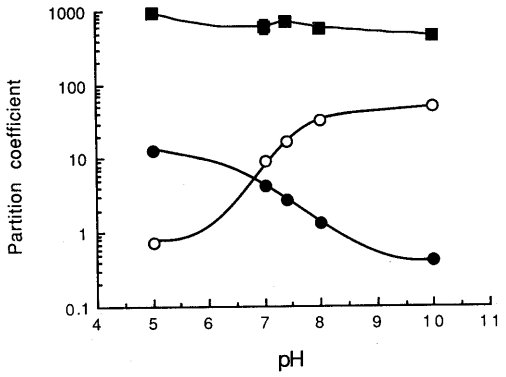


Fig. 2. Partition Coefficient *vs.* pH Profiles for [^{125}I]IPDMA (○), [^{125}I]IPPA (●), and [^{125}I]IPP (■)

Extraction with *n*-octanol was carried out in various buffers (pH 5.0—10.0).

Uncharged [^{125}I]IPP was extracted at an almost constant ratio independent of the pH, while the carboxyl-containing derivative ([^{125}I]IPPA) showed a decrease in *n*-octanol extraction with an increase of the pH. On the other hand, the amino-containing derivative ([^{125}I]IPDMA) showed increased extraction with an increase of the pH and a sharp rise of the partition coefficient in the physiological pH range. Thus, the partition coefficients reflected the charcteristics of the functional groups tested.

Accumulation in Rat Pancreatic and Liver Slices In order to study the *in vitro* pancreatic accumulation of [^{125}I]IPP, [^{125}I]IPPA, and [^{125}I]IPDMA, these compounds were incubated with freshly prepared rat pancreatic slices.

The time course of the accumulation of these three compounds in rat tissue slices is shown in Fig. 3. [^{125}I]-IPDMA showed rapid accumulation in the pancreatic slices and reached the highest level among the compounds tested. Furthermore, [^{125}I]IPDMA showed approximately a three times higher accumulation in pancreatic slices than in liver slices after 60 to 120 min of incubation. The lowest accumulation in pancreatic slices was obtained with [^{125}I]IPPA (30 to 40% of the level for [^{125}I]IPDMA), and [^{125}I]IPP showed intermediate accumulation. For both [^{125}I]IPPA and [^{125}I]IPP, accumulation in the liver was similar to that in the pancreas.

The effects of ouabain on the accumulation of [^{125}I]-IPDMA and *l*-[^{14}C]leucine in pancreatic slices are shown in Fig. 4. Although the accumulation of *l*-[^{14}C]leucine was inhibited dose-dependently by incubation with ouabain, there was no effect on the accumulation of [^{125}I]-IPDMA.

***In Vivo* Biodistribution in Rats** Table I shows the biodistribution data for [^{125}I]IPDMA, [^{125}I]IPPA, and [^{125}I]IPP, expressed as a percentage of the dose administered per gram of wet tissue at 0.5—15 min following intravenous injection. Figure 5 shows the pancreatic radioactivity and the pancreas/liver ratio for each ^{125}I-labeled compound at 0.5—5 min following intravenous administration.

As shown in Table I, [^{125}I]IPDMA exhibited high pancreatic uptake and rapid blood clearance. The radioactivity in the liver, a competing organ that generally produces poor resolution in pancreatic imaging, was rather low and therefore the pancreas/liver ratio was quite high (1.9—3.5) at 0.5—2 min after injection (Fig. 5). This ratio was higher than that obtained with [^{13}N]ammonia (1.3—3.2).[13] A high initial lung uptake was observed, but

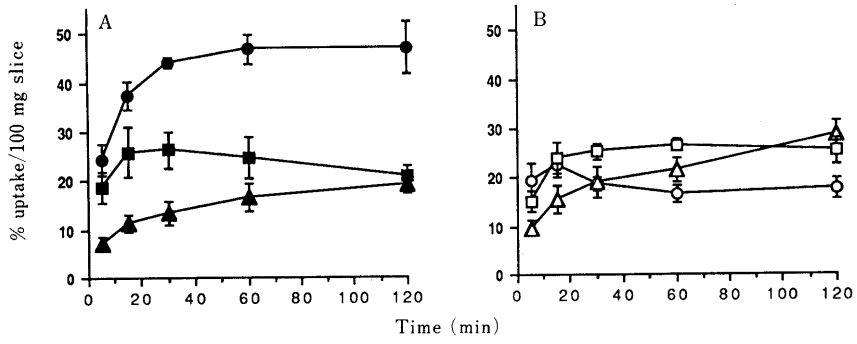


Fig. 3. Accumulation of [^{125}I]IPDMA (●, ○), [^{125}I]IPPA (▲, △), and [^{125}I]IPP (■, □) in Rat Tissue Slices

A, pancreas; B, liver. Each point represents the mean of five experiments.

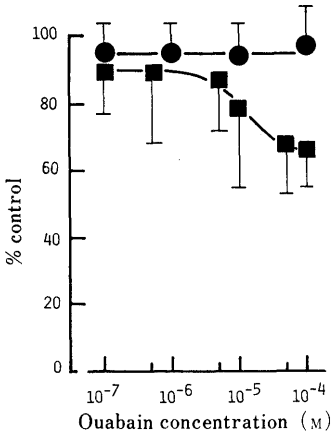


Fig. 4. Effect of Ouabain on Uptake of [^{14}C]Leucine (■) and [^{125}I]IPDMA (●)

TABLE I. Biodistribution of [^{125}I]IPDMA, [^{125}I]IPPA, and [^{125}I]IPP

Organ	Time after injection (min)[a]			
	0.5	2	5	15
[^{125}I]IPDMA				
Blood	0.71 (0.08)	0.56 (0.08)	0.85 (0.08)	1.49 (0.06)
Pancreas	1.25 (0.13)	1.86 (0.37)	2.40 (0.20)	1.63 (0.20)
Intestine	0.61 (0.17)	0.68 (0.23)	0.91 (0.11)	1.00 (0.05)
Liver	0.47 (0.06)	0.55 (0.14)	1.23 (0.18)	1.58 (0.18)
Kidney	2.74 (0.44)	2.76 (0.93)	3.00 (0.25)	2.76 (0.28)
Lung	16.59 (3.08)	8.84 (1.25)	5.15 (1.14)	2.47 (0.43)
Stomach	0.41 (0.02)	0.73 (0.25)	0.94 (0.15)	1.12 (0.27)
Brain	2.07 (0.50)	2.73 (0.79)	1.92 (0.95)	1.01 (0.07)
[^{125}I]IPPA				
Blood	8.16 (0.88)	6.07 (0.52)	5.66 (0.40)	5.11 (0.38)
Pancreas	0.68 (0.13)	0.93 (0.14)	1.02 (0.05)	0.97 (0.13)
Intestine	0.30 (0.05)	0.47 (0.08)	0.51 (0.07)	0.57 (0.04)
Liver	1.74 (0.13)	2.23 (0.63)	2.86 (0.19)	2.74 (0.79)
Kidney	1.93 (0.21)	2.16 (0.30)	1.95 (0.16)	1.94 (0.28)
Lung	4.62 (0.87)	2.50 (0.07)	2.25 (0.34)	2.27 (0.38)
Stomach	0.36 (0.04)	0.39 (0.05)	0.52 (0.04)	0.63 (0.18)
Brain	0.23 (0.03)	0.15 (0.02)	0.14 (0.01)	0.13 (0.01)
[^{125}I]IPP				
Blood	2.27 (0.16)	1.09 (0.04)	1.13 (0.11)	1.16 (0.04)
Pancreas	0.95 (0.10)	1.17 (0.12)	0.97 (0.06)	0.81 (0.17)
Intestine	0.35 (0.04)	0.38 (0.07)	1.07 (0.22)	1.93 (0.29)
Liver	1.77 (0.47)	2.93 (0.43)	2.70 (0.36)	2.34 (0.27)
Kidney	1.96 (0.25)	1.86 (0.13)	1.79 (0.24)	1.73 (0.16)
Lung	6.57 (1.09)	5.57 (0.79)	6.63 (3.87)	5.67 (0.84)
Stomach	0.34 (0.04)	0.43 (0.12)	0.61 (0.09)	1.16 (0.37)
Brain	0.77 (0.04)	0.78 (0.08)	0.48 (0.03)	0.25 (0.04)

a) Each value is the mean (S.D.) for 4 animals.

this cleared rapidly. Release of radioactivity from the lung might have resulted in the steep increase of blood radioactivity after 5 min, a finding also observed with radioiodinated *N*-isopropyl-*p*-iodoamphetamine, an aromatic alkylated amine derivative.[14] A relatively high brain uptake of radioactivity was also noted.

Except for the blood and brain uptake, both [^{125}I]IPPA and [^{125}I]IPP showed a similar biodistribution, and they were markedly different in behaviour from [^{125}I]IPDMA (Table I). In particular, both [^{125}I]IPPA and [^{125}I]IPP showed 1.3—2.5 times lower uptake by the pancreas and 2.2—5.3 higher uptake by the liver when compared with [^{125}I]IPDMA. Consequently, the pancreas/liver ratios for [^{125}I]IPPA and [^{125}I]IPP were 5—8.4 times less than that

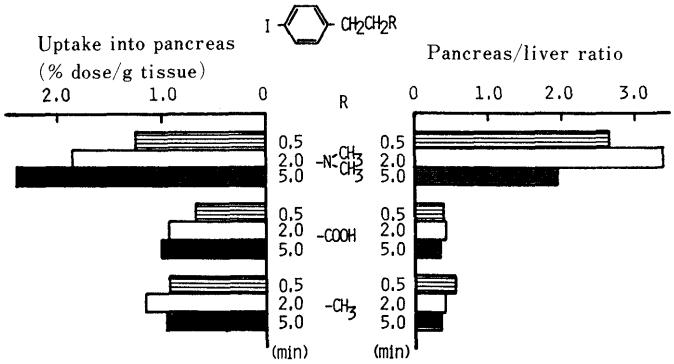


Fig. 5. Pancreatic Uptake and Pancreas/Liver Ratio of [^{125}I]IPDMA, [^{125}I]IPPA, and [^{125}I]IPP

for [^{125}I]IPDMA (Fig. 5). In the blood, a very high level of radioactivity was observed with [^{125}I]IPPA, while [^{125}I]IPP showed rather rapid blood clearance. [^{125}I]IPP was incorporated into the brain, although its uptake was lower than that of [^{125}I]IPDMA, but [^{125}I]IPPA showed no brain uptake.

All three compounds showed a low uptake by the stomach. Since accumulation of radioactivity in the stomach after a injection of a radioiodinated compound serves as a practical screening index for deiodination in small animals,[7,15] the results obtained in this study suggested the *in vivo* stability of these compounds.

Thus, [^{125}I]IPDMA with an amino group showed the highest affinity for the pancreas and the highest pancreas/liver ratio, both characteristics which are necessary for desirable pancreatic imaging.

Discussion

The comparative *in vivo* biodistribution study showed that IPDMA with an amino group had a higher pancreatic uptake than IPPA with a carboxyl group or IPP with a methyl group (Table I, Fig. 5). This higher affinity of IPDMA for the pancreas was well supported by the *in vitro* study using pancreatic slices, which are a good working model for screening the pancreatic affinity of a compound[12] (Fig. 3). Thus, the results obtained clearly demonstrate that an amino group increases the pancreatic accumulation of compounds.

The brain uptake of amines such as HIPDM, di-β-(piperidinoethyl)selenide (PIPSE), di-β-(morpholinoethyl)selenide (MOSE), and ammonia is explained by the pH-shift theory in relation to regional pH differences between blood and the brain.[14a,16] At blood pH (7.4), these compounds are neutral and lipid-soluble and can diffuse freely into the brain, while at the lower intracellular brain pH (7.0) they pick up a hydrogen ion, become charged, and can not diffuse out of the brain again. This pH-shift theory can also explain the accumulation of amines in other organs with an intracellular pH that is significantly lower than blood pH.[16a]

The uptake of pH-shift agents is closely related to their lipid solubility at blood pH and to the shape of the partition coefficient *vs.* pH curve. As shown in Fig. 2, IPDMA has a high lipid solubility at pH 7.4 ($P=17.1$) and a steep lipid solubility *vs.* pH curve between pH 6.5 and 8.0.

There are no reports available on the pH of pancreatic

tissue, but the tissue pH can be calculated from the tissue–blood partition coefficient of dimethyloxazolidinedione (DMO) using the Waddell–Buther equation.[17] The pancreatic tissue water content has been reported to be 74.8%,[18] so by assuming a tissue water content of 0.748 ml/g, the pancreatic tissue pH is estimated as 7.07 using the [^{11}C]DMO biodistribution data reported by Kearfott *et al.*[19] This calculated pH value is considerably lower than that of the blood and almost the same as that reported for the brain.[19]

Thus, it seems that the pH-shift may contribute to the pancreatic uptake of amino compounds such as IPDMA. Furthermore, we found that ouabain, an inhibitor of cationic membrane transport, had no effect on the accumulation of IPDMA in pancreatic slices. This suggested that IPDMA passed through the cell membrane in a nonionic form, which is a fundamental requirement for the pH-shift theory.

In conclusion, our findings provide evidence that an amine group effectively promotes the uptake of drugs by the pancreas. Thus, the present study offers a good basis for the design of new radiopharmaceuticals with a higher pancreatic affinity. Using the data obtained in this study, a bifunctional radiopharmaceutical labeled with ^{99m}Tc and ^{62}Cu is now being developed for pancreatic imaging diagnosis.

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