Radioiodinated Phenoxyacetic Acid Derivatives as Potential Brain Imaging Agents. II. Structure–Biodistribution Relationship

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In developing new brain imaging agents for single photon emission computed tomography (SPECT), we synthesized eleven radioiodinated phenoxyacetic acid derivatives and investigated the relationship between the chemical structure and *in vivo* characteristics. Biodistribution studies in mice revealed high initial brain uptake for all the compounds. Blood radioactivity level depended markedly upon the chemical stability of the compound. The α,α-dimethylester derivative (1e), amide derivatives (2a—c) and diamine derivatives (3a, b, 4), which were stable to hydrolysis, showed low blood activity levels following i.v. administration. Disappearance of the ester and amide compounds from the brain was rapid. However, the diamine derivatives displayed improved retention in the brain. Compounds 3a and 4 possessed the best combination of high brain uptake and sufficient retention to be useful as potential brain imaging radiopharmaceuticals with SPECT devices.

Keywords radioiodinated phenoxyacetic acid derivative; brain imaging agent; ^{125}I; ^{123}I; structure biodistribution relationship

In conjunction with the development of single photon emission computed tomography (SPECT) with sensitivity and resolution approaching that of the positron emission tomography (PET), new types of radiopharmaceuticals labeled with a suitable single photon emitting radionuclide such as ^{123}I and ^{99m}Tc for non-invasive examination of brain functions such as regional perfusion, metabolism and receptor function are highly desirable. ^{123}I-Labeled *N*-isopropyl-*p*-iodoamphetamine (IMP)[1] and *N*-(2-hydroxy-5-iodo-3-methylbenzyl)-*N*,*N*′,*N*′-trimethyl-1,3-propanediamine (HIPDM)[2] have generated wide interest in the possibilities for routine imaging of regional cerebral blood flow using SPECT. For brain receptor imaging, ^{123}I-labeled (*R*)-3-quinuclidinyl 4-iodobenzilate (QNB),[3] (*R*)-(+)-2,3,4,5-tetrahydro-8-iodo-3-methyl-5-phenyl-1*H*-3-benzazepin-7-ol (IBZP)[4] and (*S*)-(−)-*N*-[(1-ethyl-2-pyrrolidinyl)methyl]-2-hydroxy-3-iodo-6-methoxybenzamide (IBZM)[5] have been demonstrated to be useful agents for evaluating muscarinic acetylcholine receptor, dopamine D-1 receptor and dopamine D-2 receptor, respectively.

In the initial part of our program to search for efficient brain imaging radiopharmaceuticals, our attention was focused on the chemical structure of meclofenoxate (*N*,*N*-dimethylaminoethyl *p*-chlorophenoxyacetate). Meclofenoxate is currently in clinical use as a brain metabolism stimulant.[6] The drug is well known to penetrate rapidly into the brain immediately after injection and to liberate dimethylaminoethanol, which is subsequently converted into choline derivatives.[7] This might be an appropriate feature for brain imaging within a short time after administration of the imaging agent.

Thus, we designed compounds with the substitution of iodine for chlorine at the aromatic ring of meclofenoxate (compound **1a**, Fig. 1) and its *meta* and *ortho* isomers (**1b**, **c**). Compounds **1a**—**c** might be rather labile to hydrolysis in aqueous solution, since meclofenoxate is easily hydrolyzed to *p*-chlorophenoxyacetic acid and dimethylaminoethanol in an alkaline solution.[8] In order to increase the stability of the ester bond of **1a**, we designed the α-methyl and α,α-dimethyl derivatives (**1d** and **1e**). Moreover, corresponding amide compounds (**2a**—**c**) which seem to be more stable than the esters were also investigated for comparison. Several diamines have been reported to display high brain uptake and long retention in the brain after intravenous injection based on the pH-shift mechanism.[9] Therefore, we further investigated radioiodinated *p*-iodophenoxyethyl ethylenediamine derivatives (**3a**, **b**, **4**).

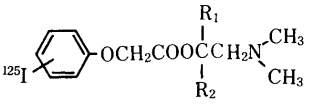
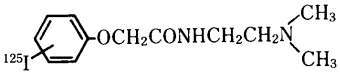
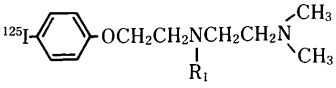
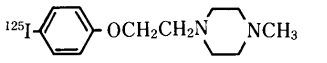
structure	compd. No.	^{125}I	R_1	R_2
	**1a**	*para*	H	H
	1b	*meta*	H	H
	1c	*ortho*	H	H
	1d	*para*	H	CH_3
	1e	*para*	CH_3	CH_3
	**2a**	*para*		
	2b	*meta*		
	2c	*ortho*		
	**3a**	*para*	H	
	3b	*para*	CH_3	
	**4**	*para*		

Fig. 1. Chemical Structures of the Iodinated Phenoxyacetic Acid Derivatives

Prior to the preparation of the above-mentioned radioiodine-labeled compounds, we have attempted to overcome some of the problems associated with the conventional radioiodination methods such as radioiodine exchange reaction and direct electrophilic radioiodination of aromatic compounds. Consequently, we have established the usefulness of radioiodination *via* demetallation of aryltrimethysilyl intermediates. The *ipso* electrophilic substitution reaction was very rapid and regiospecific incorporation of radioiodine could be carried out with high radiochemical yields at carrier-added and no-carrier-added quantities of radioiodine. The identical radiolabeling procedure is readily applicable for the labeling reaction with ^{123}I, which has superior physical properties of a short half-life ($T_{1/2}$ = 13.2 h) and a suitable gamma energy (159 keV) for imaging with SPECT devices. On the basis of our successful utilization of this method in the preparation of

radioiodinated compounds, we extended this approach to the syntheses of radioiodinated phenoxyacetic acid derivatives (**1a—e**, **2a—c**, **3a**, **b** and **4**) as reported in the preceding paper.[10]

We report here the results of preliminary *in vivo* studies on the relationship between the chemical structure and biodistribution in mice of the radioiodinated phenoxyacetic acid derivatives and the evaluation of these compounds as potential brain imaging radiopharmaceuticals.

Results and Discussion

Chemical stability to hydrolysis of the *p*-isomer series of the iodophenoxyacetic acid derivatives (**1a**, **d**, **e**, **2a**, **3a**, **b** and **4**) *in vitro* was studied by incubation in 0.05 M phosphate buffer (pH 7.4) at 37 °C followed by analyses using high-performance liquid chromatography (HPLC). The results are shown in Fig. 2. *N,N*-Dimethylaminoethyl *p*-iodophenoxyacetate (**1a**) was found to be very labile and easily hydrolyzed to *p*-iodophenoxyacetic acid and dimethylaminoethanol. Only 53.6% and 33.8% remained intact after incubation for 5 and 10 min, respectively. No stabilizing effect of monomethylation at the α-position to the ester bond of **1a** was observed. The α-monomethyl derivative (**1d**) was more rapidly hydrolyzed than **1a**, with 27.2% and 9.1% remaining intact after 5 and 10 min, respectively. On the other hand, α, α-dimethylation effectively contributed to the stabilization of the ester bond. The rate of hydrolysis of the α, α-dimethyl derivative (**1e**) was markedly decreased compared with that of the parent compound (**1a**) and monomethyl congener (**1d**), as shown in Fig. 2. *N*-(*N,N*-Dimethylaminoethyl)-*p*-iodophenoxyacetamide (**2a**), *N,N*-dimethyl-*N'*-(*p*-iodophenoxyethyl)ethylenediamine (**3a**) and related diamines (**3b**, **4**) were stable under these conditions and no degradation products were detected within the period of time studied (data for **3a**, **b** and **4** are not included in Fig. 2).

The relationship between the chemical structure and *in vivo* characteristics of the radioiodinated phenoxyacetic acid derivatives (**1a—e**, **2a—c**, **3a**, **b** and **4**) was investigated

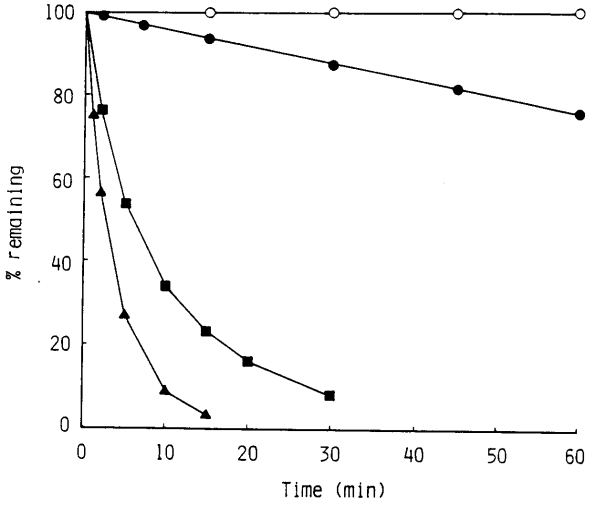
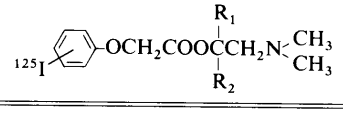


Fig. 2. Mean Percent Remaining as Intact Species after Incubation of Compounds **1a** (■), **1d** (▲), **1e** (●) and **2a** (○) in 0.05 M Phosphate Buffer (pH 7.4) at 37 °C

TABLE I. Biodistribution of Dimethylaminoethyl [^{125}I]Iodophenoxyacetate Derivatives in Mice



Compd. No.	^{125}I	R_1	R_2	Time after injection (min)	Mean % injected dose ± S.D./g tissue[a] Brain	Blood	Liver	Kidneys	Heart	Lungs
1a	*para*	H	H	2	6.47±1.64	16.16±1.19	6.44±0.18	14.92±0.55	7.81±0.47	10.67±1.07
				5	6.36±1.87	15.62±1.14	6.82±0.99	16.39±1.02	6.98±0.99	9.90±2.09
				15	4.57±1.60	14.67±2.77	6.90±1.51	17.14±0.61	6.91±1.28	8.52±1.70
				30	1.98±0.96	13.91±2.79	5.69±1.41	14.39±0.87	5.81±1.21	8.33±2.20
				60	0.48±0.15	12.15±1.20	4.77±0.49	15.60±0.27	5.22±0.69	6.24±1.48
1b	*meta*	H	H	2	7.05±1.12	15.47±2.66	4.65±0.44	18.82±2.71	7.73±1.31	14.52±1.93
				5	4.80±1.17	13.50±1.91	4.07±0.43	19.53±2.47	6.19±0.95	10.38±1.70
				15	1.63±0.72	11.28±2.49	3.51±0.40	18.89±2.68	5.16±0.82	7.70±1.43
				30	0.78±0.15	8.86±1.36	2.64±0.50	18.11±1.93	3.85±0.54	5.45±2.08
				60	0.28±0.07	3.92±0.54	1.13±0.12	9.14±1.67	1.66±0.14	3.00±0.72
1c	*ortho*	H	H	2	6.65±1.05	15.76±2.11	5.75±0.72	15.43±2.85	7.00±0.98	12.24±4.39
				5	3.85±0.70	11.37±1.99	4.32±0.66	12.77±2.50	4.62±0.60	6.80±1.13
				15	2.31±0.28	7.80±2.87	3.10±1.21	12.83±2.88	3.26±1.16	3.95±1.51
				30	0.63±0.08	2.02±1.70	0.83±0.65	8.04±2.77	0.81±0.70	1.04±0.87
				60	0.07±0.03	0.25±0.14	0.11±0.05	4.51±2.71	0.08±0.04	0.15±0.06
1d	*para*	H	CH_3	2	7.98±1.12	15.35±1.22	3.48±0.31	13.16±0.77	6.72±0.85	13.74±1.52
				5	6.01±1.90	16.40±2.82	3.79±1.19	19.27±2.14	6.00±1.71	9.37±2.57
				15	3.74±0.56	16.81±2.19	4.05±1.11	23.59±3.66	5.84±0.58	7.48±1.26
				30	1.48±0.35	14.23±1.87	3.31±1.14	19.40±1.95	4.63±0.59	7.83±1.39
				60	0.24±0.12	11.10±1.88	2.15±0.94	16.04±2.84	3.54±1.34	5.96±2.71
1e	*para*	CH_3	CH_3	2	7.54±1.84	4.40±1.43	2.95±0.89	7.13±2.40	3.79±1.31	11.15±2.39
				5	8.44±1.00	7.93±0.80	4.21±0.37	8.18±0.59	3.98±0.43	13.38±1.41
				15	4.03±1.05	9.55±0.91	3.79±0.57	10.38±2.63	3.40±0.67	7.86±1.49
				30	1.97±0.48	9.68±2.00	2.87±1.02	8.43±2.17	1.52±0.89	5.43±2.38
				60	0.44±0.14	9.05±1.40	2.22±0.14	7.99±0.95	2.70±0.22	5.09±0.93

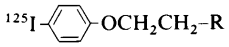
a) Four animals per time point.

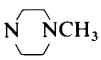
TABLE II. Biodistribution of Dimethylaminoethyl [^{125}I]Iodophenoxyacetamide Derivatives in Mice

Compd. No.	^{125}I	Time after injection (min)	Mean % injected dose ± S.D./g tissue[a] Brain	Blood	Liver	Kidneys	Heart	Lungs
2a	*para*	2	8.69 ± 1.93	4.25 ± 0.43	11.08 ± 1.13	13.67 ± 3.07	6.14 ± 1.16	13.19 ± 2.95
		5	8.74 ± 0.76	4.49 ± 0.55	14.74 ± 1.97	12.15 ± 1.90	4.91 ± 0.50	12.62 ± 1.02
		15	7.09 ± 0.88	5.88 ± 0.45	12.59 ± 0.54	14.00 ± 2.67	4.80 ± 0.26	12.35 ± 1.70
		30	3.38 ± 0.25	5.90 ± 0.44	9.00 ± 0.85	15.85 ± 3.03	3.76 ± 0.24	11.96 ± 2.95
		60	1.58 ± 0.33	6.15 ± 0.89	5.81 ± 1.14	12.48 ± 1.68	3.06 ± 0.57	10.34 ± 1.83
2b	*meta*	2	9.04 ± 1.30	3.35 ± 0.28	11.13 ± 1.19	12.64 ± 4.07	5.99 ± 0.47	11.03 ± 1.91
		5	9.30 ± 0.83	3.72 ± 0.24	14.10 ± 1.17	13.08 ± 4.25	4.65 ± 0.32	13.77 ± 0.63
		15	4.62 ± 0.23	3.67 ± 0.34	10.26 ± 0.73	14.71 ± 4.45	3.15 ± 0.15	10.45 ± 0.93
		30	1.87 ± 0.13	2.88 ± 0.17	5.78 ± 0.22	13.90 ± 3.73	2.04 ± 0.20	6.48 ± 0.30
		60	0.79 ± 0.08	1.63 ± 0.16	2.89 ± 0.37	7.60 ± 3.45	1.06 ± 0.07	4.11 ± 0.23
2c	*ortho*	2	12.58 ± 1.90	2.63 ± 0.29	6.81 ± 1.64	18.12 ± 2.92	5.10 ± 0.43	13.68 ± 0.88
		5	10.44 ± 1.66	3.43 ± 0.43	7.75 ± 1.57	15.42 ± 2.63	4.24 ± 0.62	11.88 ± 1.08
		15	2.96 ± 0.85	2.80 ± 0.56	3.96 ± 0.72	14.20 ± 3.39	2.04 ± 0.35	5.65 ± 1.61
		30	0.73 ± 0.25	1.40 ± 0.20	1.73 ± 0.20	15.98 ± 4.58	0.85 ± 0.13	2.06 ± 0.30
		60	0.14 ± 0.03	0.26 ± 0.06	1.54 ± 0.45	6.89 ± 2.20	0.17 ± 0.04	0.48 ± 0.06

a) Four animals per time point.

TABLE III. Biodistribution of [^{125}I]Iodophenoxyethylethylenediamine Derivatives in Mice



Compd. No.	R	Time after injection (min)	Mean % injected dose ± S.D./g tissue[a] Brain	Blood	Liver	Kidneys	Heart	Lungs
3a	$NHCH_2CH_2N(CH_3)_2$	2	10.67 ± 2.10	3.08 ± 0.35	10.94 ± 0.93	19.29 ± 2.00	5.71 ± 0.61	13.59 ± 1.59
		5	15.00 ± 1.05	3.68 ± 0.43	15.82 ± 1.86	20.91 ± 2.57	4.71 ± 0.46	16.05 ± 1.99
		15	12.06 ± 1.04	3.75 ± 0.11	16.00 ± 1.48	16.65 ± 3.25	3.37 ± 0.17	12.85 ± 2.29
		30	9.38 ± 1.20	4.23 ± 0.41	13.32 ± 0.74	15.09 ± 3.94	3.39 ± 0.24	13.53 ± 2.11
		60	5.72 ± 0.42	4.09 ± 0.32	8.08 ± 0.72	9.90 ± 1.32	3.24 ± 0.63	8.90 ± 1.00
		120	3.15 ± 0.24	2.97 ± 0.26	4.88 ± 0.72	5.83 ± 0.46	2.03 ± 0.84	6.52 ± 0.83
3b	$NCH_2CH_2N(CH_3)_2$ CH_3	2	15.48 ± 2.42	2.87 ± 0.78	6.94 ± 0.89	13.71 ± 1.85	6.17 ± 1.68	15.02 ± 2.12
		5	14.47 ± 1.40	4.24 ± 0.55	10.80 ± 0.89	13.07 ± 1.43	4.45 ± 0.45	13.23 ± 1.47
		15	10.30 ± 0.77	7.01 ± 0.30	9.00 ± 0.75	13.59 ± 1.57	3.90 ± 0.29	11.47 ± 1.75
		30	5.45 ± 0.80	6.50 ± 0.77	6.58 ± 1.17	12.77 ± 1.21	2.93 ± 0.54	8.65 ± 1.74
		60	2.54 ± 0.16	6.87 ± 0.29	5.00 ± 0.33	10.60 ± 1.52	2.87 ± 0.21	6.72 ± 0.45
		120	0.83 ± 0.08	3.65 ± 0.36	2.24 ± 0.06	7.67 ± 1.40	1.37 ± 0.21	3.64 ± 0.30
4		2	16.08 ± 2.87	2.98 ± 0.57	9.33 ± 1.41	17.05 ± 1.16	7.33 ± 0.99	16.40 ± 2.79
		5	16.04 ± 2.91	2.92 ± 0.50	15.03 ± 2.03	13.22 ± 2.88	4.82 ± 0.94	17.18 ± 2.67
		15	9.18 ± 1.25	4.40 ± 0.93	15.04 ± 1.60	13.88 ± 2.82	4.46 ± 0.71	17.81 ± 2.67
		30	5.49 ± 1.20	3.01 ± 0.44	11.38 ± 1.40	13.90 ± 2.59	3.22 ± 0.61	11.99 ± 2.14
		60	4.64 ± 0.28	1.79 ± 0.25	8.31 ± 0.47	8.08 ± 2.08	2.48 ± 0.49	10.25 ± 2.94
		120	4.63 ± 0.18	0.98 ± 0.09	5.37 ± 0.24	6.56 ± 0.98	1.36 ± 0.13	6.97 ± 0.97

a) Four animals per time point.

by means of biodistribution studies in mice. Table I shows the results on the biodistribution of *N*,*N*-dimethylaminoethyl [^{125}I]iodophenoxyacetate derivatives (**1a—e**), expressed as percent of administered dose per gram of wet tissue at 2—60 min following intravenous injection. Each value represents the average of data from four animals. Tables II and III show similar data for *N*-(*N*,*N*-dimethylaminoethyl)[^{125}I]iodophenoxyacetamide derivatives (**2a—c**) and *N*,*N*-dimethyl-*N'*-(*p*-[^{125}I]iodophenoxyethyl)ethylenediamine derivatives (**3a**, **b**, **4**), respectively. Figures 3, 4 and 5 present the radioactivity in the brain (solid bar) and blood (open bar) for ester compounds (**1a**, **d**, **e**), the amide compound (**2a**) and diamine compounds (**3a**, **b**, **4**), respectively, at 2—60 min following i.v. administration. Each point represents the average of data from four mice, expressed as percent of administered dose per gram of wet brain or whole blood.

The *para*-isomer of *N*,*N*-dimethylaminoethyl iodophenoxyacetate (**1a**) exhibited a high initial brain uptake of 6.47%/g at 2 min and 6.36%/g at 5 min, but underwent rapid clearance from this organ. The radioactivity in blood retained rather high, 16.16%/g at 2 min to 12.15%/g at 60 min. This is presumably due to the hydrolysis of **1a** *in vivo*, as *in vitro* (Fig. 2), followed by long retention of the resulting *p*-iodophenoxyacetic acid in the blood. Similar phenomena were reported in the biodistribution of ^{14}C-labeled dimethylaminoethyl *p*-chlorophenoxyacetate.[7a,b] The *meta* and *ortho* isomers of dimethylaminoethyl iodo-

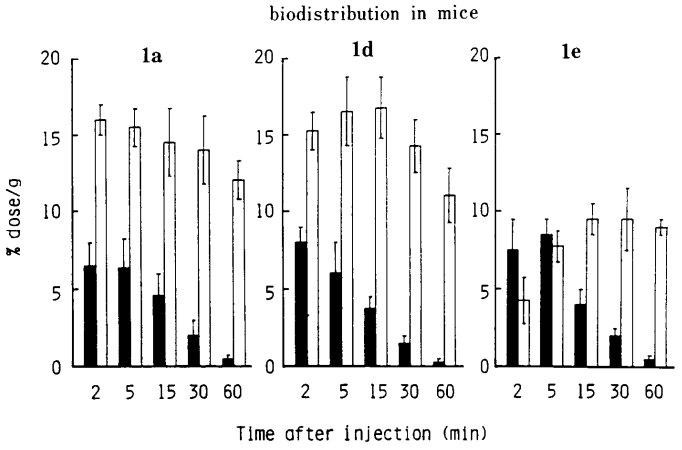


Fig. 3. Comparison of the Relative Brain (■) and Blood (□) Radioactivity Levels (Mean Percent Dose/g±S.D. of Four Animals) Following Administration of Compounds **1a** (Left), **1d** (Middle) and **1e** (Right) to Mice

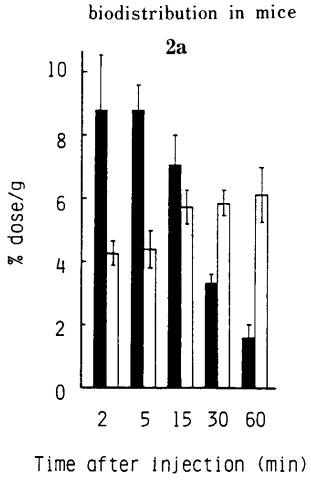


Fig. 4. Comparison of the Relative Brain (■) and Blood (□) Radioactivity Levels (Mean Percent Dose/g±S.D. of Four Animals) Following Administration of Compound **2a** to Mice

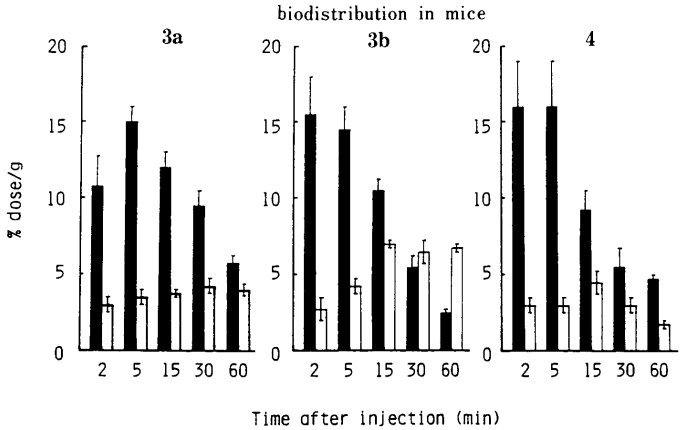


Fig. 5. Comparison of the Relative Brain (■) and Blood (□) Radioactivity Levels (Mean Percent Dose/g±S.D. of Four Animals) Following Administration of Compounds **3a** (Left), **3b** (Middle) and **4** (Right) to Mice

phenoxyacetate were eliminated faster from brain, blood and other organs than the *para* compound (**1b** and **1c** as opposed to **1a** in Table I).

Monomethylation at the α-position had little effect on the biodistribution (compound **1d** as compared to **1a**). However, α,α-dimethylation remarkably lowered the blood radioactivity level. The activity in blood gradually increased from 4.40%/g at 2 min to 9.05%/g at 60 min following i.v. injection of compound **1e**, as opposed to 16.16%/g at 2 min and 12.15%/g at 60 min after administration of **1a** (Table I, Fig. 3). Compound **1e** showed the high initial brain uptake and subsequent rapid clearance as seen with **1a**. These results indicated that stabilization of the ester bond to hydrolysis by α,α-dimethylation was very effective to decrease the blood activity level while maintaining the ability to penetrate the blood–brain barrier.

Then, we studied the amide derivatives, which were more stable than the ester derivatives to hydrolysis (Fig. 2). Compound **2a**, the *para*-isomer, showed a low blood radioactivity level of 4.25%/g at 2 min and 6.15%/g at 60 min; these values are lower than those of the esters **1a** and **1e**, as expected (Table II, Fig. 4). Initial brain uptake of **2a** was 8.69%/g at 2 min and 8.74%/g at 5 min. Brain retention was slightly improved, resulting in the brain activity of 7.09%/g at 15 min as opposed to 4.57%/g of **1a** and 4.03%/g of **1e** at 15 min after injection. Nevertheless, the disappearance from the brain was still rapid. The accumulation of **2a** in the lungs and liver was notably higher than that of the esters **1a**—**e**. As regards other isomers of the amide derivatives, iodination in the *ortho* position resulted in increased intitial brain uptake (12.58%/g at 2 min) but more rapid clearance from the brain, blood and other organs than iodination in the *para* position (**2c** as opposed to **2a** shown in Table II).

Based on the results obtained with the radioiodinated phenoxyacetic acid esters (**1a**—**e**) and amides (**2a**—**c**), we designed and studied diamine derivatives to improve the brain retention. It is apparent from the data shown in Table III and Fig. 5 that initial brain uptake was increased for the diamine compounds as compared with the amide derivatives, and retention of the radioactivity in the brain was improved. Consequently, the brain maintained a higher activity level than the blood following i.v. administration of **3a** or **4**, as shown in Fig. 5. However, **3b** displayed a higher level in blood than brain at 30—120 min after injection due to the elevation of the blood activity. The reason for this discrepancy between **3a** or **4** and **3b** is not clear, and was not further investigated in the present study.

The brain uptake of many compounds can be explained, by and large, in terms of their lipid solubility. Compounds with high lipid solubility penetrate the blood–brain barrier easily in both directions. However, if there is a sharp decrease in lipid solubility at the lower intracellular brain pH (pH = 7.0), then exit from the brain is inhibited.[9] This pH-shift mechanism conceivably contributed in part to the superior brain retention of the present diamine compounds, since the partition coefficient between octanol and buffer, which represents the lipid solubility of the diamines, was 4.4 at pH 7.0 and 10.1 at pH 7.4 for **3a**, 9.8 at pH 7.0 and 22.4 at pH 7.4 for **3b**, and 39.6 at pH 7.0 and 79.5 at pH 7.4 for **4**. In order to retain the activity for a long time in the brain, it might be necessary to increase the ratio of the lipid solubility at pH 7.4/lipid solubility at pH 7.0, as with HIPDM.[2]

In conclusion, compounds **3a** and **4** possessed the best

combination of high brain uptake and sufficient retention to obtain brain images. In addition, these agents can be labeled with ^{123}I by a simple reaction. Thus, these agents labeled with ^{123}I can be used as potential brain imaging agents in conjunction with SPECT. Further studies are in progress to clarify the regional cerebral perfusion and metabolism of these radiopharmaceuticals.

Experimental

Compounds All iodinated and [^{125}I] radioiodinated phenoxyacetic acid derivatives (**1a—e**, **2a—c**, **3a—b**, **4**) were synthesized from the corresponding trimethylsilylphenoxyacetic acid intermediates according to the previously described methods.[10] Specific radioactivity of the ^{125}I-labeled compounds for animal studies was adjusted to 1 μCi/mg by addition of the non-labeled authentic compound. Radiochemical purity was greater than 99% as assessed by HPLC.

HPLC HPLC (Waters HPLC system) analyses were performed using a C-18 reversed-phase column (4.6 × 150 mm; Nacalai Tesque), a ultraviolet (UV) detector (model 490, Waters) set at 254 nm and elution with a solvent mixture of methanol : 1% acetic acid = 55 : 45 at a flow rate of 1 ml/min. The retention time of each compound was given in the preceding report.[10]

Chemical Stability *in Vitro* Each iodinated compound (3 mg) was dissolved in 3 ml of 0.05 M phosphate buffer (pH 7.4) and incubated at 37 °C. At the desired time interval following incubation, an aliquot of the sample was withdrawn and assayed by HPLC. The data were expressed as mean percentage of the intact species remaining from five experiments.

Biodistribution in Mice Male ddY mice (20—25 g) were injected intravenously through a lateral tail vein with 0.1 ml of a saline solution containing the appropriate ^{125}I-labeled compound (50 mg/kg, 1.0—1.25 μCi). At the desired time interval after administration, the animals were sacrificed. Samples of blood and tissues of interest were excised, and weighed, and the radioactivity was counted with a Aloka automatic gamma counter (model AR-300). Data were expressed as mean percentage of the administered dose per gram of blood or tissue ± S.D. of each group of four mice.

Partition Coefficient The partition coefficient was measured by mixing the radioactive diamines with 3 ml each of *n*-octanol and 0.05 M phosphate buffer at a desired pH in a test tube. This test tube was vortexed (3 × 5 min), then two samples (0.5 ml each) from the *n*-octanol and buffer layers were counted with a gamma counter. The partition coefficient was determined by calculating the ratio of counts per minute per milliliter of octanol to that of the buffer. This measurement was repeated five times.

References and Notes

1) *a*) H. S. Winchell, R. M. Baldwin and T. H. Lin, *J. Nucl. Med.*, **21**, 940 (1980); *b*) H. S. Winchell, W. D. Horst, L. Braum, W. H. Oldendorf, R. Hattner and H. Parker, *ibid.*, **21**, 947 (1980).
2) *a*) K. M. Tramposch, H. F. Kung and M. Blau, *J. Med. Chem.*, **26**, 121 (1983); *b*) H. F. Kung, K. M. Tramposch and M. Blau, *J. Nucl. Med.*, **24**, 66 (1983).
3) *a*) W. J. Rzeszotarski, W. C. Eckelman, B. E. Francis, D. A. Simms, R. E. Gibson, E. M. Jagoda, M. P. Grissom, R. R. Eng, J. J. Conklin and R. C. Reba, *J. Med. Chem.*, **27**, 156 (1984); *b*) W. C. Eckelman, R. C. Reba, W. J. Rzeszotarski, R. E. Gibson, T. C. Hill, B. L. Holman, T. Budinger, J. J. Conklin, R. R. Eng and M. P. Grissom, *Science*, **223**, 291 (1984); *c*) B. L. Holman, R. E. Gibson, T. C. Hill, W. C. Eckelman, M. Albert and R. C. Reba, *J. Am. Med. Assoc.*, **254**, 3063 (1986).
4) H. F. Kung, J. J. Billings, Y-Z. Guo, M. Blau and R. Ackerhalt, *Nucl. Med. Biol.*, **15**, 187 (1988).
5) *a*) H. F. Kung, Y-Z. Guo, J. Billings, X. Xu, R. H. Mach, M. Blau and R. E. Ackerhalt, *Nucl. Med. Biol.*, **15**, 195 (1988); *b*) H. F. Kung, R. Kasliwal, S. Pan, M-P. Kung, R. H. Mach and Y-Z. Guo, *J. Med. Chem.*, **31**, 1039 (1988).
6) *a*) J. Thuillier, G. Thuillier and P. Rumpf, *Nature* (London), **188**, 152 (1960); *b*) J. Nickel, U. Breyer, B. Claver and G. Quadbeck, *Arzneim.-Forsch.*, **13**, 881 (1963); *c*) H. D. Herrmann and J. Dittmann, *ibid.*, **21**, 984 (1971).
7) *a*) Y. Mitta, M. Kishi and Y. Minaki, *Radioisotopes*, **16**, 29 (1967); *b*) H. Miyazaki, A. Kagemoto, M. Ishii, Y. Minaki and K. Nakamura, *Chem. Pharm. Bull.*, **19**, 1681 (1971); *c*) P. L. Wood and A. Peloquin, *Neuropharmacology*, **21**, 349 (1982).
8) *a*) E. Felder, D. Pitre and A. Rescigno, *Arzneim. -Forsch.*, **12**, 931 (1962); *b*) T. Yamana, F. Ichimura and K. Yokogawa, *Yakuzaigaku*, **32**, 204 (1972).
9) *a*) H. F. Kung and M. Blau, *J. Nucl. Med.*, **21**, 147 (1980); *b*) H. F. Kung and M. Blau, *J. Med. Chem.*, **23**, 1127 (1980); *c*) K. M. Tramposch, H. F. Kung and M. Blau, *ibid.*, **25**, 870 (1982).
10) Y. Ohmomo, S. Okuyama, Y. Magata, Y. Ueno, C. Tanaka and A. Yokoyama, *Chem. Pharm. Bull.*, **37**, 2276 (1989).