

Radioiodinated *N*-(2-Aminoethyl)-2-chloro-4-iodobenzamide: A New Ligand for Monoamine Oxidase B Studies with Single Photon Emission Computed Tomography

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In developing monoamine oxidase (MAO)-B specific radioligands, *N*-(2-aminoethyl)-2-chloro-4-[¹²⁵I]iodobenzamide [¹²⁵I]FIBA was conveniently synthesized from its tributylstannyl precursor by an iododestannylation reaction using sodium [¹²⁵I]iodide and hydrogen peroxide with high radiochemical yield. The method should be applicable for labeling with ¹²³I, a suitable radioisotope for *in vivo* imaging with single photon emission computed tomography (SPECT). *In vitro* binding studies using mouse brain mitochondrial preparations showed that the specific binding of [¹²⁵I]FIBA was saturable and of high affinity. Calculated values for *K_D* and *B_{max}* were 201 nM and 9.5 pmol/mg protein, respectively. The [¹²⁵I]FIBA binding was effectively prevented by MAO-B specific inhibitors (*l*-deprenyl, Ro 16-6491, FIBA) or substrate (β -phenethylamine). However, MAO-A specific inhibitor (clorgyline) and substrate (serotonin) had no significant effect. After an intravenous injection into mice, [¹²⁵I]FIBA showed high brain uptake (1.64% dose/g at 15–30 min post injection) and long retention (1.11% dose/g at 120 min post injection) in the brain. Good brain-to-blood radioactivity ratios of 2.19 and 2.41 at 60 and 120 min after injection, respectively, were obtained. The *in vitro* binding data and *in vivo* characteristics suggested that [¹²⁵I]FIBA is potentially useful as a probe for biological studies including specific labeling of MAO-B *in vivo* as well as *in vitro*. Moreover, the ¹²³I-labeled counterpart, [¹²³I]FIBA, might be valuable for non-invasive imaging and mapping of MAO-B in the living brain with SPECT.

Keywords monoamine oxidase B; radioiodinated inhibitor; *N*-(2-aminoethyl)-2-chloro-4-iodobenzamide; iodine-125; iodine-123; single photon emission computed tomography

Monoamine oxidase (MAO) [EC 1.4.3.4] is a flavin-containing enzyme¹⁾ located in the outer membrane of mitochondria.²⁾ MAO catalyzes the oxidative deamination of endogenous neurotransmitter amines as well as exogenous amines.³⁾ It exists in two subtypes, MAO-A and MAO-B, on the basis of their different specificities toward substrates and inhibitors.⁴⁾ MAO is of considerable pharmacological interest because of its key role in the metabolism of monoamine neurotransmitters and its possible involvement in certain psychiatric disorders such as Parkinson's disease and depression.⁵⁾

Positron emission tomography (PET) and single photon emission computed tomography (SPECT) have been successfully employed for the noninvasive study of biochemical transformations and physiological processes in the intact living human brain, utilizing organic molecules labeled with a positron emitter or a single photon emitter. For direct and noninvasive mapping and functional studies of MAO activity in the living brain, ¹¹C-labeled clorgyline and *l*-deprenyl,⁶⁾ ¹⁸F-labeled *l*-deprenyl⁷⁾ and radioiodinated clorgyline⁸⁾ have been investigated as radio-ligands for PET and SPECT studies, respectively. Clorgyline and *l*-deprenyl irreversibly and selectively inhibit MAO-A and MAO-B, respectively, by binding covalently to the flavin coenzyme of MAO.⁹⁾ They behave as mechanism-based enzyme inhibitors and are also termed suicide substrates.¹⁰⁾

Recently, a new generation of short-acting MAO inhibitors with high selectivity toward the two different forms of MAO has been developed, and their usefulness as antidepressant and as anti-Parkinsonian drugs is

currently being evaluated.¹¹⁾ Among the new class of potent and specific MAO-B inhibitors, *N*-(2-aminoethyl)-4-chlorobenzamide (Ro 16-6491, **1**, Chart 1) is a reversible and highly selective MAO-B inhibitor.¹²⁾ It counteracts the neurotoxic effects induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a suspected Parkinsonism-inducing drug.¹³⁾ Moreover, tritium-labeled Ro 16-6491 binds specifically and with high affinity to MAO-B.¹⁴⁾

We have explored the feasibility of using radioiodinated MAO inhibitors for functional MAO studies in the living brain with SPECT. We recently reported the design, synthesis, and biological evaluation of a novel series of iodinated Ro 16-6491 analogues.¹⁵⁾ Among them, *N*-(2-aminoethyl)-2-chloro-4-iodobenzamide [FIBA, **2**, Chart 1] was found to have high inhibitory potency and selectivity toward MAO-B, comparable to those of Ro 16-6491 examined under the same conditions, and its characteristics are very suitable for use as a radio-pharmaceutical.

We report here the synthesis of the radioiodinated

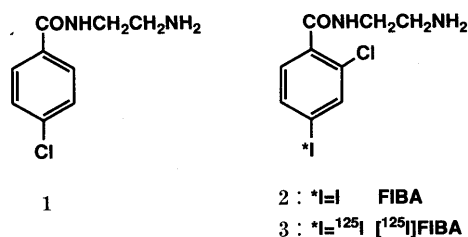


Chart 1

counterpart, *N*-(2-aminoethyl)-2-chloro-4- 125 Iiodobenzamide (125 I]FIBA, **3**, Chart 1). Preliminary studies of *in vitro* binding to mouse brain mitochondrial preparation and *in vivo* tissue distribution in mice were also performed in order to evaluate it as a new radio-ligand for *in vivo* MAO-B studies with SPECT as well as *in vitro* biochemical application.

Synthesis

125 I]FIBA was synthesized by using an iododestannylation reaction with a tributylstannyl precursor as outlined in Chart 2. 2-Chloro-4-iodobenzoic acid was converted to its methyl ester (**5**), which was then reacted with hexa-*n*-butylditin in the presence of a catalytic amount of tetrakis(triphenylphosphine)palladium in anhydrous toluene at 140 °C to afford the arylstannyl ester (**6**) in 63% yield. Subsequent treatment of **6** with ethylenediamine at 120 °C gave the desired key intermediate stannylbenzamide (**7**) in 52% yield. Iododestannylation of **7** was easily accomplished by treatment with iodine in chloroform at ambient temperature to produce FIBA (**2**) in the high yield of 89%.

Radioiodination of **7** was achieved using hydrogen peroxide as an oxidant and sodium 125 I iodide (specific radioactivity 74 GBq/ μ mol) in 0.1 N HCl/ethanol solution at room temperature, followed by high performance liquid chromatographic (HPLC) purification. The radiochemical yield of the product, 125 I]FIBA (**3**), was 85–90% based on sodium 125 I iodide. The radiochemical purity of 125 I]FIBA was higher than 99% as assessed by HPLC

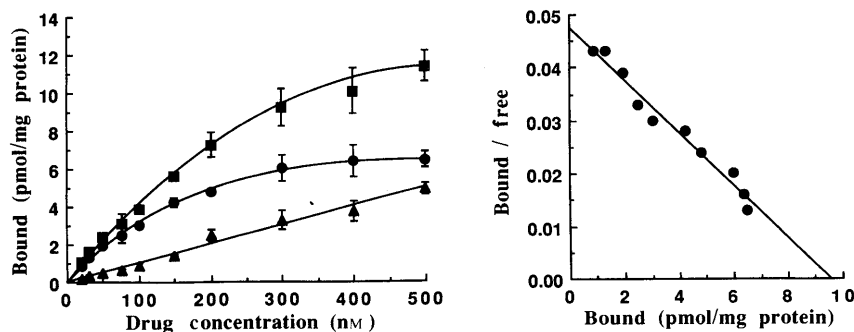
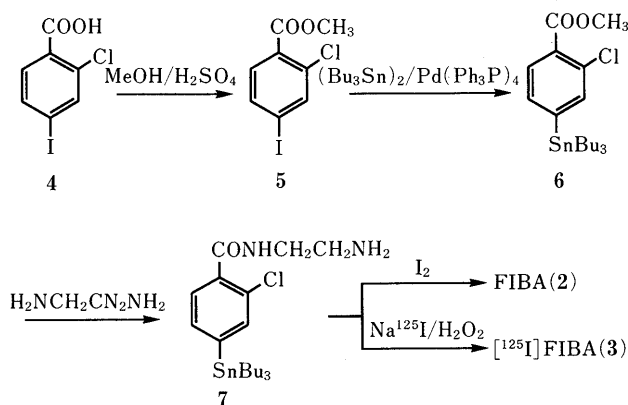


Fig. 1. Equilibrium Binding of 125 I]FIBA to Crude Mouse Brain Mitochondrial Preparations

Mitochondrial preparations were incubated for 1 h at 20 °C with various concentrations of the ligand. Nonspecific binding was determined in the presence of 1 μ M *l*-deprenyl. Left: Binding curves of total ($\blacksquare$), specific ($\bullet$), and nonspecific ($\blacktriangle$) binding of 125 I]FIBA to mitochondrial preparations. All points represent the mean values $\pm$ S.D. of three independent assays performed in duplicate. Right: Scatchard plot from the specific binding data.

analysis, with the specific radioactivity of approximately 74 GBq/ μ mol. This method should be applicable for labeling with 123 I, a very suitable radioisotope (half-life of 13 h and gamma ray energy of 159 keV) for *in vivo* imaging with SPECT.

Biological Results and Discussion

The selectivity and affinity of the new radioligand, 125 I]FIBA, for MAO-B were investigated by *in vitro* binding studies using crude mouse brain mitochondrial preparations. As shown in Fig. 1, the specific binding of 125 I]FIBA to mitochondrial preparations was found to be saturable and of high affinity. Nonspecific binding (determined in the presence of 1 μ M *l*-deprenyl) was linear over the range of ligand concentrations used. Scatchard analysis of the saturation data for 125 I]FIBA showed a single population of binding sites. Calculated K_D and B_{max} values of 125 I]FIBA for crude mouse brain mitochondrial preparations were 201 nM and 9.5 pmol/mg protein, respectively.

Competition of various compounds with 125 I]FIBA for binding to mitochondrial preparations is presented in Fig. 2. The selective and potent MAO-B inhibitors *l*-deprenyl, Ro 16-6491 and FIBA greatly reduced the binding of 125 I]FIBA. Under the same experimental conditions, however, the MAO-A inhibitor clorgyline had no significant effect. A preferential MAO-B substrate β -phenethylamine significantly reduced the 125 I]FIBA binding, whereas the MAO-A selective substrate serotonin was ineffective. These results indicate that the binding site for 125 I]FIBA is on MAO-B and that 125 I]FIBA is very selective toward this form of MAO.

Thus, 125 I]FIBA should be a very effective probe for specific labeling and characterization of MAO-B and for mapping the distribution and abundance of MAO-B in different tissues by quantitative enzyme autoradiography similar to receptor autoradiography.¹⁶⁾ Moreover, the greater specific radioactivity of 125 I]FIBA (74 GBq/ μ mol) compared to 3 H-labeled compounds (theoretical maximum specific radioactivity 1 GBq/ μ mol) and the better radiation characteristics of 125 I than those of 3 H provide an advantage in biological systems where MAO-B concentration is low.

The *in vivo* tissue distribution of 125 I]FIBA was examined in male ddY mice at 5, 15, 30, 60 and 120 min

TABLE I. Tissue Distribution of [125 I]FIBA in Mice^{a)}

Tissue	Time after injection				
	5 min	15 min	30 min	60 min	120 min
Blood	2.32 ± 0.21	1.59 ± 0.09	1.22 ± 0.11	0.69 ± 0.05	0.46 ± 0.04
Liver	11.11 ± 1.20	9.38 ± 0.69	6.78 ± 0.37	6.03 ± 0.51	4.91 ± 0.63
Kidney	30.59 ± 3.52	24.69 ± 2.84	14.41 ± 2.76	9.21 ± 4.92	3.48 ± 0.68
Heart	4.52 ± 0.38	2.55 ± 0.12	1.56 ± 0.14	0.98 ± 0.03	0.57 ± 0.05
Lung	13.11 ± 2.26	12.95 ± 2.29	8.40 ± 1.17	4.58 ± 0.83	1.95 ± 0.55
Pancreas	6.23 ± 0.52	4.95 ± 0.13	2.61 ± 0.30	1.69 ± 0.10	1.08 ± 0.09
Spleen	4.79 ± 0.21	4.07 ± 0.05	1.96 ± 0.23	1.08 ± 0.06	0.44 ± 0.04
Stomach	4.85 ± 0.56	5.65 ± 0.11	6.57 ± 0.36	2.36 ± 0.93	2.73 ± 0.57
Brain	1.30 ± 0.12	1.64 ± 0.09	1.64 ± 0.06	1.51 ± 0.09	1.11 ± 0.17
Brain/Blood	0.56	1.03	1.34	2.19	2.41

a) Mean % injected dose ± S.D. per gram tissue of four animals.

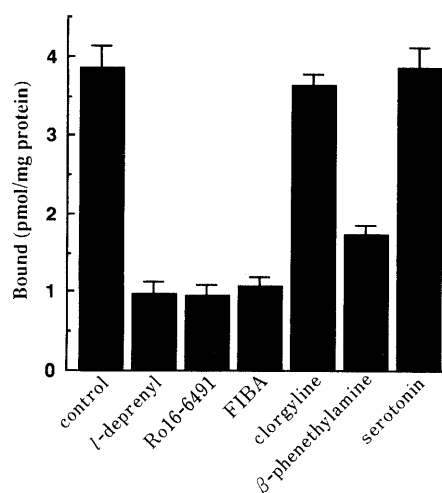


Fig. 2. Effects of Various Compounds on the [125 I]FIBA Binding to Crude Mouse Brain Mitochondrial Preparations

Mitochondrial preparations were incubated for 1 h at 20°C with [125 I]FIBA (100 nM) in the presence of competing ligands. Data are shown as means ± S.D. of three independent assays performed in duplicate.

after intravenous administration. As summarized in Table I, [125 I]FIBA was transported well into various tissues. The initial level of accumulation of [125 I]FIBA in the brain was high, 1.30% dose/g at 5 min after injection, and then the brain radioactivity level increased gradually to 1.64% dose/g at 15–30 min after injection. [125 I]FIBA exhibited the desired prolonged retention in the brain (1.11% dose/g at 120 min after injection). In contrast to the high brain uptake and retention, the disappearance of radioactivity from the blood was very rapid, 2.32, 0.69 and 0.46% dose/g at 5, 60 and 120 min post injection, respectively, resulting in good brain-to-blood radioactivity ratios of 2.19 and 2.41 at 60 and 120 min after administration, respectively.

In conjunction with the binding characteristics, the tissue distribution data suggested that [125 I]FIBA is a potentially useful radioligand for *in vivo* as well as *in vitro* studies of MAO-B in both normal and pathological conditions in which alterations of monoamine neurotransmitter metabolism have been reported.^{5,17} The 123 I-labeled counterpart may be suitable for non-invasive imaging of MAO-B in the living brain with SPECT.

In conclusion, [125 I]FIBA was conveniently synthesized

from its tributylstannyl precursor by an iododestannylation reaction using sodium [125 I]iodide and hydrogen peroxide with high radiochemical yield. *In vitro* binding studies using mouse brain mitochondrial preparations showed that the specific binding of [125 I]FIBA was saturable and of high affinity. The binding of [125 I]FIBA to mitochondrial preparations was effectively prevented by inhibitors (*l*-deprenyl, Ro 16-6491, FIBA) or substrate (β -phenethylamine) selective for MAO-B. However, MAO-A specific inhibitor (clorgyline) and substrate (serotonin) had no significant effect. After an intravenous injection into mice, [125 I]FIBA showed high brain uptake and long retention in the brain. The brain-to-blood radioactivity ratio was high. The *in vitro* binding data and *in vivo* characteristics suggested that [125 I]FIBA is potentially useful as a probe for biological studies including specific labeling of MAO-B *in vivo* as well as *in vitro*. Moreover, the 123 I-labeled counterpart, [123 I]FIBA, might be suitable for non-invasive imaging and mapping of MAO-B in the living brain with SPECT.

Experimental

Melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Infrared (IR) spectra were taken on a JASCO IR-700 spectrometer. Proton nuclear magnetic resonance (1 H-NMR) spectra were recorded on a Varian Gemini-200 (200 MHz) spectrometer and the chemical shifts are reported in ppm downfield from an internal tetramethylsilane standard. High-resolution mass spectra (HRMS) were obtained on a Hitachi M-80 instrument. The HPLC system used included a Waters M-600 pump, a Lambda-Max 481 ultraviolet detector, a Beckman 170 NaI radioactivity detector, and a Cosmosil 5C18-AR column (10 × 250 mm, Nacalai Tesque). Radioactivity was measured using an Aloka ARC-300 NaI(Tl) gamma scintillation counter.

Materials Ro 16-6491 (1),¹⁵ 2-chloro-4-iodobenzoic acid (4),¹⁸ and clorgyline hydrochloride^{8a)} were synthesized by the reported methods. *l*-Deprenyl hydrochloride, serotonin creatinine sulfate, and β -phenethylamine hydrochloride were purchased from Research Biochemicals Inc., E. Merck, and Nacalai Tesque, respectively. The other chemicals used were of reagent grade. Male ddY mice were supplied by Japan SLC Co., Ltd.

Methyl 2-Chloro-4-iodobenzoate (5) A mixture of 2-chloro-4-iodobenzoic acid (4) (1.5 g, 5.3 mmol), methanol (20 ml) and concentrated sulfuric acid (0.5 ml) was refluxed for 3 h. After cooling, the solvent was removed *in vacuo*. The residue was dissolved in chloroform (20 ml) and washed successively with water (10 ml), saturated aqueous sodium hydrogen carbonate solution (10 ml) and water (10 ml). The organic layer was dried over sodium sulfate, filtered and then evaporated *in vacuo*. Column chromatography on silica gel eluting with chloroform afforded 5 (1.45 g, 92%) as a colorless liquid. IR (CHCl₃): 3018, 1730, 1575, 1290, 1217, 1123, 1051 cm⁻¹. 1 H-NMR (CDCl₃) δ : 3.93 (3H, s, CH₃), 7.55

(1H, d, $J=8.3$ Hz, aromatic H-6), 7.67 (1H, dd, $J=8.3, 1.6$ Hz, aromatic H-5), 7.85 (1H, d, $J=1.6$ Hz, aromatic H-3). Chemical ionization (CI)-HRMS Calcd for $C_8H_7ClO_2$ (MH^+) m/z : 296.9178. Found: 296.9175.

Methyl 2-Chloro-4-(tri-*n*-butylstannyl)benzoate (6) Compound **5** (0.3 g, 1.0 mmol) and hexa-*n*-butylditin (1.74 g, 3.0 mmol) were dissolved in dry toluene (10 ml), and a catalytic amount of tetrakis(triphenylphosphine)palladium (20 mg, 0.017 mmol) was added. The mixture was heated at 140°C and stirred for 15 h under an argon atmosphere. After cooling, the reaction mixture was filtered through Celite. The filtrate was concentrated *in vacuo* and the oily residue was purified by column chromatography on silica gel eluting with chloroform-hexane (1:1), affording **6** (0.29 g, 63%) as a colorless oil. IR ($CHCl_3$): 3016, 2958, 2926, 1726, 1287, 1217, 1050 cm^{-1} . 1H -NMR ($CDCl_3$) δ : 0.85–1.58 (27H, m, $SnBu_3$), 3.93 (3H, s, CH_3), 7.39 (1H, d, $J=7.5$ Hz, aromatic H-6), 7.52 (1H, s, aromatic H-3), 7.71 (1H, d, $J=7.5$ Hz, aromatic H-5). CI-HRMS Calcd for $C_{20}H_{34}ClO_2Sn$ (MH^+) m/z : 461.1269. Found: 461.1261.

***N*-(2-Aminoethyl)-2-chloro-4-(tri-*n*-butylstannyl)benzamide (7)** A mixture of compound **6** (0.29 g, 0.63 mmol) and ethylenediamine (2 ml) was stirred at reflux for 2 h. The excess ethylenediamine was removed under reduced pressure. The resulting oil was chromatographed on silica gel with methanol-chloroform (1:1) to give **7** (0.16 g, 52%) as a pale yellow oil. IR ($CHCl_3$): 3016, 2960, 2926, 1656, 1521, 1213, 1047 cm^{-1} . 1H -NMR ($CDCl_3$) δ : 0.85–1.57 (29H, m, $SnBu_3$, NH_2), 2.96 (2H, t, $J=5.8$ Hz, CH_2NH_2), 3.53 (2H, q, $J=5.8$ Hz, $NHCH_2$), 6.65 (1H, br, NH), 7.39 (1H, d, $J=7.3$ Hz, aromatic H-6), 7.45 (1H, s, aromatic H-3), 7.59 (1H, d, $J=7.3$ Hz, aromatic H-5). CI-HRMS Calcd for $C_{21}H_{38}ClN_2OSn$ (MH^+) m/z : 489.1694. Found: 489.1682.

***N*-(2-Aminoethyl)-2-chloro-4-iodobenzamide (FIBA, 2)** A 0.1 M solution of iodine in chloroform was added at ambient temperature to a solution of **7** (0.48 g, 1.0 mmol) in chloroform (10 ml) until a pink color remained. The reaction mixture was washed with 10% aqueous sodium thiosulfate solution (10 ml) and water (10 ml). The organic phase was extracted with 1 N HCl solution (10 ml $\times$ 3). The combined aqueous layers were made basic with a 5 N NaOH solution and extracted with chloroform (10 ml $\times$ 3). The combined organic layers were washed with water (10 ml), dried over sodium sulfate and the solvent was removed *in vacuo* to give the free base, which was then converted to its hydrochloride salt. Recrystallization from methanol-ether afforded **2** as a hydrochloride salt (0.32 g, 89%). mp 238–240°C. (lit.¹⁵) mp 238–240°C. The spectral data were consistent with those previously reported.¹⁵

***N*-(2-Aminoethyl)-2-chloro-4-[^{125}I]iodobenzamide ([^{125}I]FIBA, 3)** Aqueous hydrogen peroxide solution (10 μ l, 30% (w/v)) was added to a mixture of **7** (10 μ l, 1.0 mg/ml in ethanol), 0.1 N HCl (0.1 ml), and sodium [^{125}I]iodide (10 μ l, 7.4 MBq, specific activity 74 GBq/ μ mol) in a sealed vial. The reaction was allowed to proceed for 15 min at room temperature, then terminated by the addition of aqueous sodium bisulfite solution (0.1 ml, 10% (w/v)). The product was isolated by HPLC using 0.01 M sodium dihydrogenphosphate-methanol (3:7, v/v) as an eluent at a flow rate of 3.0 ml/min. The fractions corresponding to FIBA ($t_R=5.6$ min) were collected and the solvent was removed *in vacuo*. The final product, [^{125}I]FIBA, was taken up in an isotonic saline solution and passed through a 0.22 μ m filter. The radiochemical yield was 85–90%. The radiochemical purity was more than 99% as determined by HPLC using the same elution conditions described above. The specific radioactivity was about 74 GBq/ μ mol.

***In Vitro* Binding Studies** Brains of male ddY mice weighing 20–25 g were homogenized with 10 volumes of ice-cold 0.25 M sucrose and 2 mM Tris-HCl buffer (pH 7.4). The homogenates were centrifuged at 1000 g for 10 min at 4°C to remove cell debris. The supernatants were centrifuged at 7500 g for 10 min at 4°C. The resultant pellets were suspended in 50 mM Tris-HCl buffer (pH 7.4) containing 130 mM NaCl, 5 mM KCl, 0.5 mM EGTA, and 1 mM $MgCl_2$ and then centrifuged at 15000 g for 10 min at 4°C. The mitochondrial pellets were resuspended in the same buffer to a protein concentration of 5 mg/ml.

The binding assays were performed by incubating 50 μ l of crude mitochondrial preparation containing 250 μ g of protein with various concentrations of [^{125}I]FIBA in a total volume of 250 μ l of 50 mM Tris-HCl buffer (pH 7.4) containing 130 mM NaCl, 5 mM KCl, 0.5 mM EGTA, and 1 mM $MgCl_2$. After an incubation period of 1 h at 20°C, the samples were rapidly filtered under reduced pressure through glass fiber filters (Whatman GF/B) which were then washed twice with 3 ml of the ice-cold buffer. The nonspecific binding was obtained in the

presence of 1 μ M *l*-deprenyl. The radioactivity of the filters was measured using a well-type NaI(Tl) gamma scintillation counter. The results were analyzed by a computer-assisted nonlinear least-squares regression method.

Competition for [^{125}I]FIBA Binding Crude mitochondrial preparations (250 μ g protein/sample) were incubated for 1 h at 20°C with [^{125}I]FIBA (100 nM) in a total volume of 250 μ l of the assay buffer in the presence of a competitor, *l*-deprenyl (1 μ M), Ro 16-6491 (1 μ M), FIBA (1 μ M), clorgyline (1 μ M), β -phenethylamine (100 μ M), or serotonin (100 μ M). The competitive binding assays were performed by the same procedures as described above.

***In Vivo* Tissue Distribution Studies in Mice** Groups of four male ddY mice (20–25 g) were injected intravenously through a lateral tail vein with [^{125}I]FIBA (37 kBq) in 0.1 ml of saline solution. At the desired time interval after administration, the animals were sacrificed. Samples of blood and organs of interest were excised and weighed. The radioactivity was measured using a well-type NaI(Tl) gamma scintillation counter. The results were expressed in terms of the percentage of the injected dose per gram of blood or organ.

References

- 1) E. B. Kearney, J. I. Salach, W. H. Walker, R. L. Seng, W. Kenney, E. Zeszotek, T. Singer, *Eur. J. Biochem.*, **24**, 321 (1971).
- 2) J. W. Greenawalt, *Adv. Biochem. Psychopharmacol.*, **5**, 207 (1972).
- 3) a) P. Dostert, M. Strolin-Benedetti, K. F. Tipton, *Med. Res. Rev.*, **9**, 45 (1989); b) M. Strolin-Benedetti, P. Dostert, K. F. Tipton, *Prog. Drug Metab.*, **11**, 149 (1988).
- 4) a) D. L. Murphy, *Biochem. Pharmacol.*, **27**, 1889 (1978); b) C. J. Fowler, L. Orelund, B. A. Callingham, *J. Pharm. Pharmacol.*, **33**, 341 (1981); c) C. J. Fowler, S. B. Ross, *Med. Res. Rev.*, **4**, 323 (1984).
- 5) M. Strolin-Benedetti, P. Dostert, *Biochem. Pharmacol.*, **38**, 555 (1989).
- 6) a) R. R. MacGregor, C. Halldin, J. S. Fowler, A. P. Wolf, C. D. Arnett, B. Langstrom, D. Alexoff, *Biochem. Pharmacol.*, **34**, 3207 (1985); b) J. S. Fowler, R. R. MacGregor, A. P. Wolf, C. D. Arnett, S. L. Dewey, D. Schlyer, D. Christman, J. Logan, M. Smith, H. Sachs, S. M. Aquilonius, P. Bjurling, C. Halldin, P. Hartvig, K. L. Leenders, H. Lundqvist, L. Orelund, C. G. Stalnacke, B. Langstrom, *Science*, **235**, 481 (1987); c) C. D. Arnett, J. S. Fowler, R. R. MacGregor, D. J. Schlyer, A. P. Wolf, B. Langstrom, C. Halldin, *J. Neurochem.*, **49**, 522 (1987).
- 7) A. Plenevaux, J. S. Fowler, S. L. Dewey, A. P. Wolf, M. Guillaume, *Appl. Radiat. Isot.*, **42**, 121 (1991).
- 8) a) Y. Ohmomo, M. Hirata, K. Murakami, Y. Magata, C. Tanaka, A. Yokoyama, *Chem. Pharm. Bull.*, **39**, 1038 (1991); b) *Idem, ibid.*, **39**, 3343 (1991).
- 9) a) R. R. Rando, *Science*, **185**, 320 (1974); b) J. Nagy, J. I. Salach, *Arch. Biochem. Biophys.*, **208**, 388 (1981).
- 10) a) C. T. Walsh, *Ann. Rev. Biochem.*, **53**, 493 (1984); b) L. E. Richards, A. Burger, *Prog. Drug Res.*, **30**, 205 (1986).
- 11) K. F. Tipton, P. Dostert, M. Strolin-Benedetti (eds.), "Monoamine Oxidase and Disease," Academic Press, New York, 1984.
- 12) a) R. Kettler, H. H. Keller, E. P. Bonetti, P. C. Wyss, M. Da Prada, *J. Neurochem.*, **44** (Suppl.), S94 (1985); b) H. H. Keller, R. Kettler, G. Keller, M. Da Prada, *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **335**, 12 (1987).
- 13) M. Da Prada, R. Kettler, H. H. Keller, E. P. Bonetti, R. Imhof, "Advances in Neurology," Vol. 45, Raven Press, New York, 1986, pp. 175–178.
- 14) a) A. M. Cesura, M. D. Galva, R. Imhof, M. Da Prada, *J. Neurochem.*, **48**, 170 (1987); b) A. M. Cesura, R. Imhof, B. Takacs, M. D. Galva, G. B. Picotti, M. Da Prada, *ibid.*, **50**, 1037 (1988); c) A. M. Cesura, R. Imhof, D. Muggli-Maniglio, G. B. Picotti, M. Da Prada, *Biochem. Pharmacol.*, **39**, 216 (1990).
- 15) Y. Ohmomo, K. Murakami, M. Hirata, A. Sugimoto, Y. Magata, N. Miyahara, C. Tanaka, A. Yokoyama, *Chem. Pharm. Bull.*, **40**, 1789 (1992).
- 16) M. J. Kuhar, J. R. Unnerstall, "Methods in Neurotransmitter Receptor Analysis," Raven Press, New York, 1990, pp. 177–218.
- 17) L. Orelund, Y. Hiraga, S. S. Jossan, B. Regland, C. G. Gottfries, "The Early Markers in Parkinson's and Alzheimer's Diseases," Springer-Verlag, New York, 1990, pp. 267–286.
- 18) I. G. Farbenind, Brit. Patent 353537 (1930) [*Chem. Abstr.*, **26**, 5311 (1932)].