Synthesis and Characterization of Radioiodinated (*S*)-5-Iodonicotine: A New Ligand for Potential Imaging of Brain Nicotinic Cholinergic Receptors by Single Photon Emission Computed Tomography

Hideo SAJI,*[,a] Akira WATANABE,[a] Yasuhiro MAGATA,[b] Yoshiro OHMOMO,[c] Yasushi KIYONO,[a] Yoshihisa YAMADA,[a] Yasuhiko IIDA,[b] Yoshiharu YONEKURA,[b] Junji KONISHI,[b] and Akira YOKOYAMA[a]

Faculty of Pharmaceutical Sciences[a] and School of Medicine,[b] Kyoto University, Yoshida, Sakyo-ku, Kyoto 606–01, Japan and Osaka University of Pharmaceutical Sciences,[c] Takatsuki, Osaka 569–11, Japan.
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(*S*)-5-Iodonicotine (4a), an (*S*)-nicotine analog iodinated at the 5-position of the pyridine ring, was synthesized and evaluated as a potential radiopharmaceutical for investigating brain nicotine receptors by single photon emission computerized tomography (SPECT). [^{125}I]-(*S*)-5-Iodonicotine ([^{125}I]-4a) was synthesized by the iododestannylation reaction under no-carrier-added conditions and purified by high-performance liquid chromatography (HPLC). The binding affinity of 4a for brain nicotine receptors was measured in terms of displacement of [^{3}H]cytisine from binding sites in rat cortical membranes. The binding data revealed that the affinity of 4a was the same as that of (*S*)-nicotine and 80-fold higher than that of the (*R*)-enantiomer (4b). Biodistribution studies in mice disclosed that the brain uptake of [^{125}I]-4a was rapid and profound. Regional cerebral distribution studies in rats by autoradiography disclosed that the accumulation of [^{125}I]-4a was dense in the thalamus, intermediate in the cortex and striatum, and less marked in the cerebellum. Furthermore, the administration of (*S*)-nicotine reduced the uptake of [^{125}I]-4a in the thalamus and resulted in a nearly identical level of radioactivity in the cerebellum. [^{125}I]-(*R*)-5-Iodonicotine ([^{125}I]-4b) showed more rapid washout from the brain and a less extensive regional cerebral distribution than the (*S*)-enantiomer ([^{125}I]-4a). Thus, 4a bound to brain nicotine receptors *in vivo*, and therefore iodine-123-labeled 4a may be a potential radioligand for use in *in vivo* cerebral nicotinic receptor studies by SPECT.

Key words (*S*)-5-iodonicotine; central nicotine receptor; radiopharmaceutical; stereoisomer; single photon emission computed tomography; autoradiography

Changes in the density of nicotine receptors have recently been reported in the brains of patients with various disorders, including Alzheimer's disease and Parkinson's disease.[1–5] An increased density of nicotine receptors has also been reported in the brains of smokers.[6] These observations have stimulated interest in means of imaging the distribution of brain nicotinic receptors noninvasively *in vivo* with external imaging techniques such as positron emission tomography (PET) and single photon emission computed tomography (SPECT). For this purpose, [^{11}C]-(*S*)-nicotine has been recently developed as a radioligand for PET studies.[7–12] The successful imaging and functional studies of nicotine receptors with [^{11}C]-(*S*)-nicotine and the superior radiation properties of ^{123}I for SPECT prompted us to synthesize a radioiodinated nicotine analog with high receptor affinity.

Structure–activity relationship studies of the nicotine molecule have shown that the binding of nicotine to nicotine receptors may be associated with a pyrrolidine nitrogen atom (a cationic center), a pyridine nitrogen atom (an electronegative atom), planarity of the pyridine ring, and the distance between the two nitrogen atoms.[13,14] In addition, modification of the structure of the pyrrolidyl moiety drastically affects the binding affinity of nicotine for its receptor.[15] Nicotine has two stereoisomers, (*S*)- and (*R*)-nicotine, due to the presence of a center of asymmetry at the carbon joining the pyrrolidine ring to the pyridine ring, and the (*S*)-enantiomer has a greater affinity for brain nicotine receptors than dose the (*R*)-enantiomer.[16–18] It is also known that aryl iodides are more stable than alkyl iodides[19–21] and that pyridine derivatives halogenated at position 5 are less reactive than those halogenated at position 2, 4, or 6,[22–24] suggesting that introduction of iodine at position 5 of the pyridine ring should minimize susceptibility to deiodination *in vivo*. Thus, from the viewpoints of minimum disturbance of receptor binding and maximum *in vivo* stability, position 5 of the pyridine ring of (*S*)-nicotine appeared to be the most practical site for iodination.

Racemic 5-iodonicotine has already been synthesized,[23,24] but no report has appeared on stereoselective synthesis of (*S*)- and (*R*)-5-iodonicotine (**4a** and **4b**). In this study, the stereoisomers **4a** and **4b** were each synthesized, and the binding affinity of the (*S*)-enantiomer (**4a**) for brain nicotine receptors and its biodistribution, including regional cerebral distribution, were compared with those of the (*R*)-enantiomer (**4b**) to assess the feasibility of using it for imaging brain nicotine receptors.

Chemistry

The synthesis of enantiomers of nonradioactive 5-iodonicotine, **4a** and **4b**, is outlined in Chart 1. Racemic 5-bromonicotine (**1**) was synthesized from ethyl 5-bromonicotinate according to the procedure of Leete *et al.*[25] Resolution of racemic 5-bromonicotine (**1**) was carried out by fractional crystallization with (+)- or (−)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) by a modification of the method of Jacob for the resolution of racemic 5-bromonornicotine[26]; *i.e.*, treatment of **1** with (−)- or (+)-MTPA in ethyl acetate gave the MTPA salt of (*S*)-(−)- or (*R*)-(+)-5-bromonicotine, **2a** or **2b**, respectively. The analysis using chiral high-performance

* To whom correspondence should be addressed.

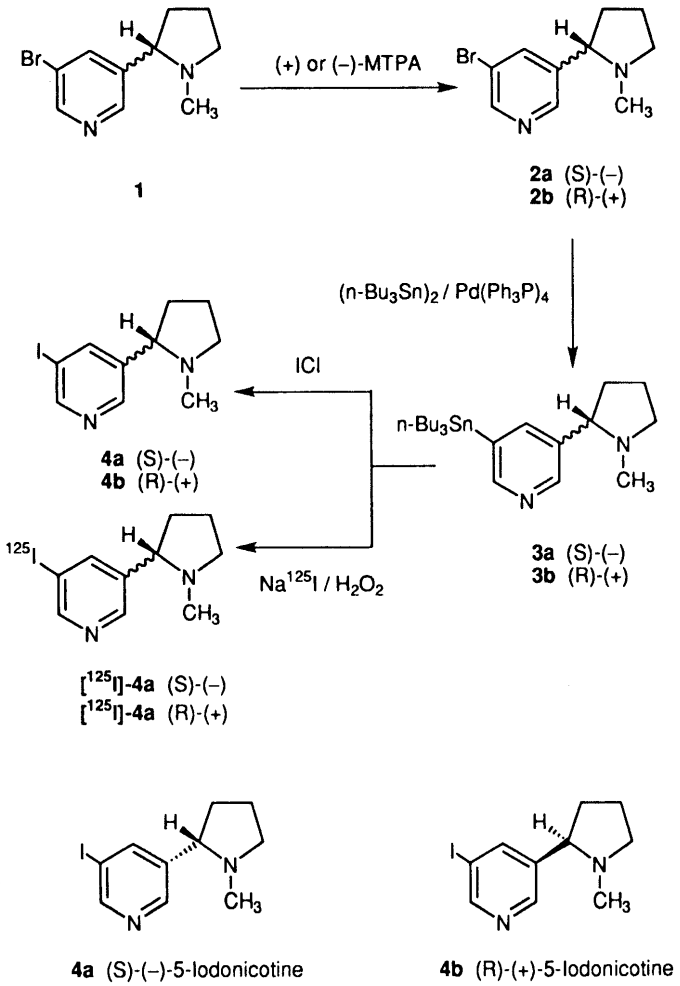

Chart 1

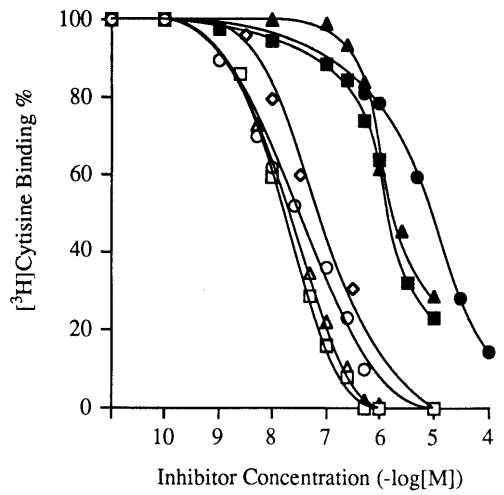

Fig. 1. Inhibition of [^{3}H]Cytisine Binding to Rat Cortical Membranes by Various Drugs

○, **2a** (*S*)-5-bromonicotine; ●, **2b** (*R*)-5-bromonicotine; □, **4a** (*S*)-5-iodonicotine; ■, **4b** (*R*)-5-iodonicotine; △, (*S*)-nicotine; ▲, (*R*)-nicotine; ◇, acetylcholine.

Table 1. Inhibition by Various Compounds of [^{3}H]Cytisine Binding to Rat Cortical Membranes

Compound	K_i (nM)
2a	9.73±0.85
2b	1893±161
4a	3.36±0.22
4b	253±53
(*S*)-Nicotine	3.68±0.40
(*R*)-Nicotine	297±89
Acetylcholine	13.7±1.77

Each value represents the mean±S.E.M. of three independent experiments.

liquid chromatography (HPLC) disclosed that the enantiomeric purities of the resolved **2a** and **2b** were greater than 98%. The stereoisomers, **2a** and **2b**, were reacted with hexa-*n*-butylditin in the presence of a catalytic amount of tetrakis(triphenylphosphine)palladium to produce the corresponding tributylstannyl derivative, **3a** or **3b**, in moderate to high yield (45—94%). Compounds **4a** and **4b** were synthesized by iododestannylation of the corresponding tri-*n*-butylstannyl derivatives, **3a** or **3b**, with iodine monochloride in an inert solvent at room temperature, followed by HPLC purification (9—40% yield). The enantiomeric purity of each enantiomer obtained, **4a** and **4b**, was greater than 98% as assessed by chiral HPLC analysis.

Radiolabeling The electrophilic iododestannylation reaction offers several advantages for radioiodination, since it is performed under very mild conditions and with very high regional selectivity, as well as affording a high specific radioactivity. Thus, the preparation of ^{125}I-labeled **4a** and **4b** was performed by iododestannylation under no-carrier-added conditions, and the product was purified by reverse-phase HPLC. Analysis of the final product by chiral HPLC revealed that each of [^{125}I]-**4a** and [^{125}I]-**4b** was enantiomerically pure, since only a single peak was found at the same retention time as that of the corresponding nonradioactive enantiomer. The total radiochemical yield of the synthesis of [^{125}I]-**4a** or [^{125}I]-**4b** was approximately 40 or 20% after HPLC purification, respectively. The radiochemical purity of the product was greater than 98% as determined by HPLC and thin layer chromatography (TLC), and the specific activity was determined from the UV absorbance at 254 nm as more than 55 TBq/mmol (the detection limit of this method). The product remained stable for at least one month after labeling.

***In Vitro* Binding** Using the reference compounds (*S*)-nicotine, (*R*)-nicotine and acetylcholine, we measured the affinities of (*S*)- and (*R*)-5-iodonicotine (**4a** and **4b**) and (*S*)- and (*R*)-5-bromonicotine (**2a** and **2b**) for brain nicotine receptors by examining competition with [^{3}H]cytisine for binding sites in rat cortical membranes. Figure 1 illustrates representative competitive binding curves of these compounds, and the K_i values determined from IC_{50} are summarized in Table 1. The order of potency was **4a** ≃ (*S*)-nicotine > acetylcholine > **2a** > (*R*)-nicotine ≃ **4b** > **2b**. The results indicate that **4a** showed high affinity, essentially the same as that of (*S*)-nicotine. In addition, the affinity of **4a** was 80 times greater than that of the (*R*)-enantiomer **4b**, indicating that **4a** binds to [^{3}H]cytisine recognition sites, *i.e.*, neuronal nicotinic acetylcholine receptors, with high stereoselectivity. Furthermore, **4a** showed higher affinity than the brominated derivative **2a**. The relatively high affinity of **4a** in spite of the great steric effect of the bulky iodine atom might be due to its lower electron-

Table 2. Biodistribution of Radioactivity after Administration of [^{125}I]-**4a** and [^{125}I]-**4b** in Mice[a]

Organ	Time after injection (min)			
	5	15	30	60
[^{125}I]-**4a**				
Blood	1.98±0.20	2.39±0.18	2.16±0.16	1.87±0.33
Liver	10.65±1.78	9.59±2.00	7.34±0.70	5.26±0.54
Kidney	9.13±0.40	8.21±1.04	5.20±2.83	5.74±1.69
Stomach	3.92±1.02[c]	3.74±0.25	3.84±0.22	3.91±0.34
Lung	4.87±0.37[c]	3.47±0.69	3.29±0.25	2.10±0.53
Heart	3.27±0.31	2.69±0.17	2.35±0.16	1.80±0.25
Thyroid[b]	0.05±0.04	0.07±0.02	0.10±0.03	0.09±0.01
Brain	3.69±0.47	2.07±0.14[d]	1.66±0.20[d]	1.18±0.06[d]
[^{125}I]-**4b**				
Blood	2.08±0.10	2.34±0.20	2.11±0.09	1.91±0.21
Liver	11.58±1.07	9.32±1.29	8.48±1.65	5.55±0.66
Kidney	8.48±0.55	7.03±1.30	7.72±1.58	5.45±1.19
Stomach	2.47±0.22	3.54±0.28	3.92±0.75	3.77±0.22
Lung	4.20±0.35	3.96±0.24	3.27±0.41	2.25±0.20
Heart	2.65±1.16	2.60±0.22	2.31±0.22	1.62±0.13
Brain	3.36±0.44	1.51±0.15	1.14±0.08	0.70±0.07

a) Percent of injected ^{125}I dose/g of organ; average±S.D. for four mice. *b*) Percent of injected ^{125}I dose/organ; average±S.D. for four mice. *c*) $p<0.05$ as compared with [^{125}I]-**4b**. *d*) $p<0.01$ as compared with [^{125}I]-**4b** (Student's *t* test).

withdrawing effect, since different halogen substituents at the 5-position affect the basicity of the pyridine nitrogen,[23] an important factor in the binding of (*S*)-nicotine to its receptors.[13,14]

Biodistribution The results of the radioactivity distribution studies in mice after intravenous administration of [^{125}I]-**4a** and [^{125}I]-**4b** are summarized in Table 2. [^{125}I]-**4a** rapidly entered the brain, and a high uptake of radioactivity (3.7%/g) was observed at the initial sampling time of 5 min, after which, the radioactivity declined with time. This high brain uptake was in accordance with the high brain uptake index (*BUI*), *i.e.*, [^{125}I]-**4a** showed a high *BUI* (mean±S.D.: 137±19 with respect to the tritiated water reference), which was comparable to that reported by Oldendorf for nicotine (131±7)[27] and higher than that of [^{99m}Tc]-HM-PAO (90±12), a cerebral blood flow agent. The cause of the finding that the *BUI* value of **4a** exceeds 100 may be the use of tritiated water as an internal reference which is not freely diffusible in the brain (the maximal extraction of tritiated water into the brain is reported to be 75%) and the difference of efflux rate constant between water and nicotine.[28]

On the other hand, the radioactivity in the blood was little changed at about 2%/g during the experimental period. The TLC analysis of radioactivity in the blood showed that more than 85% of the original radioactivity was observed at the position of the intact compound at 2 min after the injection of [^{125}I]-**4a**, but this gradually decreased to 45—55% at 15 min and to less than 40% at 30 min in both cases. Thus, the brain-to-blood ratio of the original compound, which was calculated by taking into account the fraction of the original compound in the blood, remained almost constant after 2 min (2.0 at 2 min, 1.8 at 15 min), suggesting that an apparent equilibrium is reached in those two compartments after this time.

High initial uptake was also observed in the kidneys and liver, but the radioactivity was cleared rapidly from these organs. The accumulation of radioactivity in the thyroid was low, which indicated the high stability of **4a** to *in vivo* deiodination. Compound [^{125}I]-**4b** showed a high *BUI* (119±19 with respect to the tritiated water reference) and high initial uptake by the brain (3.4%/g), which were similar to those for the (*S*)-enantiomer **4a**, but the radioactivity subsequently declined more rapidly. Consequently, the uptake of [^{125}I]-**4a** by the brain at and after 15 min postinjection was significantly higher than that of [^{125}I]-**4b**, although the two compounds showed similar radioactivity levels in the blood and other organs.

Table 3. Cerebral Regional Distribution of Radioactivity after Administration of [^{125}I]-**4a** in Rat[a]

Region	Time after injection (min)			
	2	10	30	60
Frontal cortex	0.58±0.04	0.25±0.02	0.23±0.04	0.20±0.01
Parietal cortex	0.53±0.05	0.29±0.05	0.26±0.03	0.21±0.04
Temporal cortex	0.42±0.01	0.24±0.05	0.21±0.02	0.18±0.03
Caudate putamen	0.64±0.01	0.24±0.04	0.19±0.03	0.17±0.01
Thalamus	0.94±0.02	0.45±0.05	0.27±0.04	0.20±0.04
Hypothalamus	0.41±0.03	0.22±0.06	0.18±0.02	0.15±0.03
Cerebellum	0.38±0.08	0.16±0.03	0.16±0.01	0.18±0.01
TH/CE[b]	2.55±0.45	2.87±0.61	1.67±0.33	1.16±0.22

a) Percent of injected ^{125}I dose/g of tissue; average±S.D. for three rats. *b*) TH/CE = thalamus-to-cerebellum ratio.

The cerebral regional distribution of radioactivity after injection of [^{125}I]-**4a** was determined in rats using *ex vivo* quantitative autoradiography (Table 3). At 10 min after injection, marked differences in the regional distribution of radioactivity were observed: *i.e.*, the thalamus showed the highest uptake, followed by the cortex, striatum and cerebellum in that order (Fig. 2a). At this time, the thalamus-to-cerebellum (TH/CB) ratio (*i.e.*, specific *versus* nonspecific uptake) was 2.7, but it declined after 30 min. Analysis of brain homogenates was carried out at 10 and 30 min after injection of [^{125}I]-**4a**. At 10 min, about 80% of the radioactivity in the homogenates could be extracted with methanol, and the extracted material displayed a single peak on TLC with the same *Rf* value as that of cold **4a** added simultaneously. On the other hand, about 60% of the radioactivity was in the original form at 30 min after injection. Thus, the cause of the lower cerebral localization of radioactivity at 30 min after injection of [^{125}I]-**4a** may be the presence of metabolites in the brain. Considering that a pseudoequilibrium in the brain and the blood compartments was observed after 2 min in the biodistribution studies and that the highest cerebral localization of radioactivity (TH/CB ratio) was obtained at 10 min, subsequent studies were carried out at 10 min after injection.

The regional distribution of specifically bound [^{125}I]-**4a** at 10 min, obtained by subtracting the cerebellar values from those for each region, correlated well with the distribution of nicotine receptors reported from *in vitro* studies ($r^2=0.99$) (Fig. 3).[12,18,29-33] Figure 2b shows the effect of (*S*)-nicotine on brain distribution of [^{125}I]-**4a**. Treatment with (*S*)-nicotine reduced the radiotracer levels in the thalamus, cortex, and striatum by 53, 19, and 19%, respectively, and resulted in similar levels of

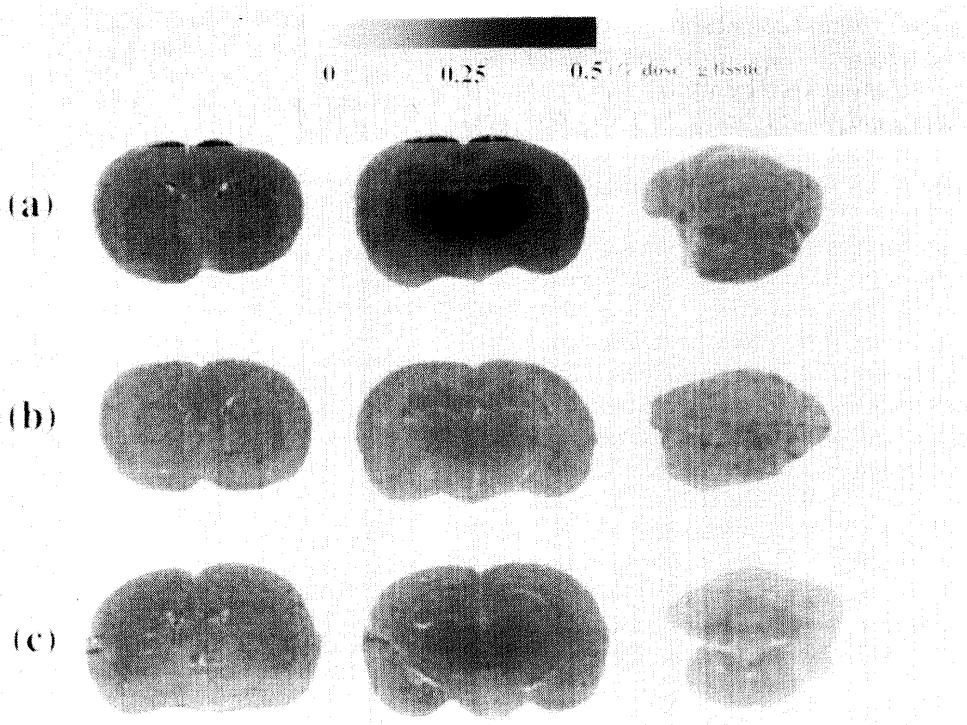

Fig. 2. *Ex Vivo* Autoradiography of Rat Coronal Brain Sections at 10 min after Injection of Each Radioligand
(a) [^{125}I]-**4a**. (b) (*S*)-nicotine (60 μg/kg) administered simultaneously with [^{125}I]-**4a**. (c) [^{125}I]-**4b**.

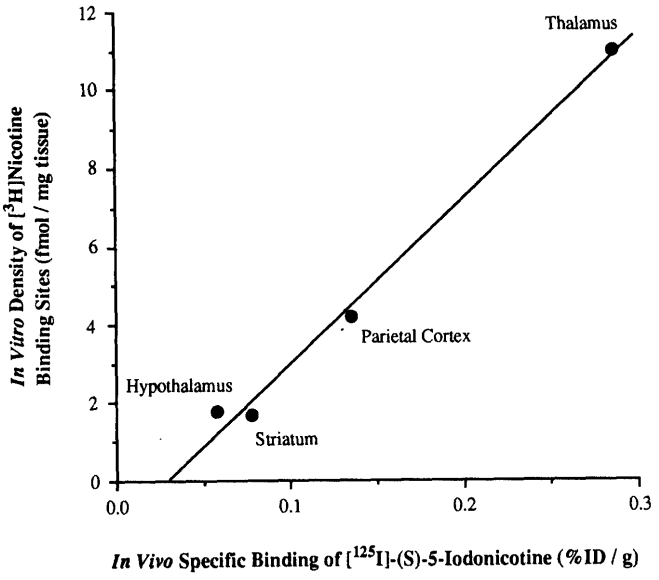

Fig. 3. Correlation of Radioactivity Levels Determined from *ex Vivo* Autoradiograms at 10 min after Injection of [^{125}I]-**4a** with Density of Nicotinic Cholinergic Receptor Sites as Determined by *in Vitro* [^{3}H]Nicotine Binding[33]

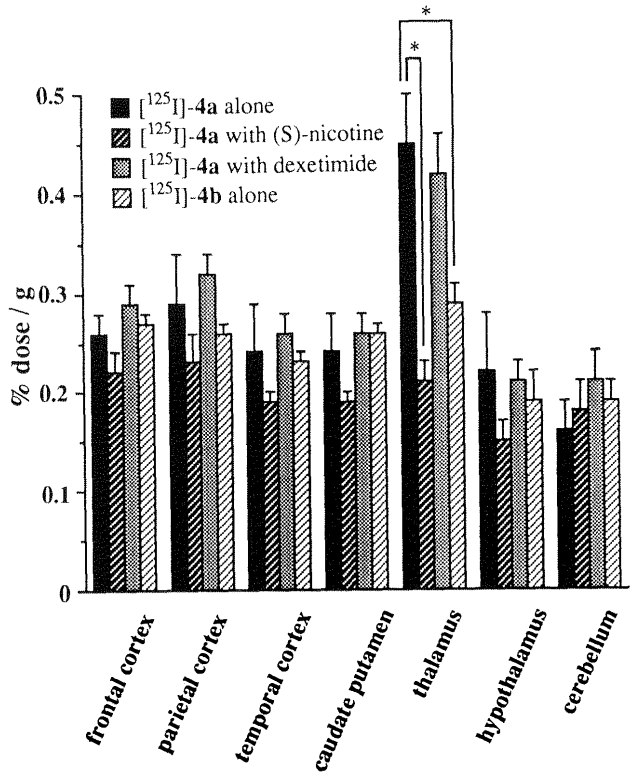

Fig. 4. Regional Cerebral Distribution of [^{125}I]-**4a** and [^{125}I]-**4b** and Effect of Various Drugs on the Regional Uptake of [^{125}I]-**4a**

Rats were injected with the radioligand alone or simultaneously with (*S*)-nicotine (60 μg/kg) and dexetimide (10 mg/kg) and were killed 10 min after injection. *$p < 0.01$ as compared with the [^{125}I]-**4a** alone (*t* test). Results are expressed in % dose/g of tissue ± S.D. for 3—4 animals.

radioactivity throughout the brain (Fig. 4). Dexetimide, a drug with high selective affinity for muscarinic cholinergic receptors,[34] caused no change in the accumulation of [^{125}I]-**4a** in any brain region examined. None of the tested compounds showed any effect on the radioactivity in the blood. These findings indicated that **4a** binds to nicotine receptors in the brain following intravenous injection.

However, displacement studies of [^{125}I]-**4a** showed that a significant amount of radioactivity remained in the brain after treatment with (*S*)-nicotine. Several factors may have contributed to this phenomenon. First, since the toxicity of (*S*)-nicotine makes it technically impossible to ad-

minister high doses,[35] the dose used in this study may have been too low to inhibit completely the specific binding to the nicotine receptor. Second, (*S*)-nicotine increases cerebral blood flow and thereby would also increase the supply of radioligand to the brain.[36] This effect could result in an increase of total radioligand binding in the brain. Third, **4a** might exhibit high nonspecific binding *in vivo*.

On the other hand, the (*R*)-enantiomer, [^{125}I]-**4b** produced a significantly lower level of radioactivity in the thalamus than the (*S*)-enantiomer, [^{125}I]-**4a**, and showed less marked regional differences in brain radioactivity 10 min after injection (Fig. 2c, Fig. 4). The TLC analysis of brain homogenates at 10 min after injection of [^{125}I]-**4b** showed a single peak with the same *Rf* value as that of cold **4b** added simultaneously, indicating that the cerebral accumulation of [^{125}I]-**4b** occurred in the intact form. Since [^{125}I]-**4b** gave a blood radioactivity level and *BUI* similar to those of the [^{125}I]-**4a**, the less marked regional variation in cerebral distribution of [^{125}I]-**4b** is presumed to be due to the relative weakness of specific interaction in the brain, *i.e.*, binding to the brain nicotine receptor.

Conclusion

Routes have been developed for the syntheses of nonradioactive and ^{125}I-labeled (*S*)- and (*R*)-5-iodonicotine with high enantiomeric purity. *In vitro* competitive binding studies showed high affinity of (*S*)-5-iodonicotine for brain nicotine receptors, which was the same as that of (*S*)-nicotine and 80-fold higher than that of the (*R*)-enantiomer. *In vivo* biodistribution studies demonstrated that the brain uptake of [^{125}I]-(*S*)-5-iodonicotine was rapid and profound and that its cerebral regional distribution was consistent with the distribution of central nicotine receptors. These data suggest that [^{123}I]-(*S*)-5-iodonicotine may be potentially useful for imaging central nicotine receptors *in vivo* in conjunction with SPECT.

Experimental

Sodium [^{125}I]iodide (specific activity: 81.4 TBq/mmol) and [^{3}H]-cytisine (1.13 TBq/mmol) were purchased from Amersham International Plc and Du Pont New England Research Products, respectively. [^{99m}Tc]-HM-PAO was prepared from a freeze-dried kit (Amersham Pharmaceutical Co., Tokyo, Japan) by the addition of 370 MBq of freshly eluted [^{99m}Tc]-pertechnetate in 5 ml of saline solution just prior to injection. (*R*)-Nicotine was kindly supplied by Japan Tobacco Company (Tokyo, Japan). The other chemicals used were of reagent grade. Melting points were determined on a Yanagimoto micromelting point apparatus (Kyoto, Japan) and are uncorrected. 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 with a Hitachi M-80 model. Enantiomeric purity was determined by HPLC with a 4.0 × 100 mm Chiral-AGP column (ChromTech, Norsborg, Sweden) with a Chiral-AGP guard column (3.0 × 10 mm) at a flow rate of 0.9 ml/min. The column was eluted using the following system: solvent A = 100 mM K_2HPO_4 plus 4.5 mM decanoic acid (pH 8.2) and solvent B = methanol (0—6 min, A/B = 100/0, 6—13 min, A/B = 90/10, 13—30 min, A/B = 88/12).

Racemic 5-Bromonicotine (1) The method of Leete *et al.* was employed.[25] bp 137 °C (9 mm Hg). ^{1}H-NMR ($CDCl_3$): 1.62—2.01 (m, 3H), 2.19 (s, 3H), 2.21—2.38 (m, 2H), 3.07 (t, 1H), 3.20 (t, 1H), 7.87 (s, 1H), 8.44 (s, 1H), 8.55 (s, 1H). HRMS *m/z*: 240.0270 (Calcd for $C_{10}H_{13}BrN_2$: 240.0262). *Anal.* Calcd for $C_{10}H_{13}BrN_2$: C, 49.81; H, 5.43; N, 11.62. Found: C, 49.93; H, 5.33; N, 11.60.

(*S*)-(−)-5-Bromonicotine (−)-α-Methoxy-α-(trifluoromethyl)phenylacetate Compound **1** (2.65 g, 11 mmol) was dissolved in 15 ml of ethyl acetate, and 1.29 g (5.5 mmol) of (−)-MTPA in 1.2 ml of ethyl acetate was added with stirring. The mixture was allowed to stand at 2 °C, after which the crystalline product was collected by filtration and washed with ethyl acetate. Three recrystallizations from acetonitrile afforded 1.15 g (44%) of colorless needles. mp 129.5—131.5 °C. *Anal.* Calcd for $C_{20}H_{22}BrF_3N_3O_3$: C, 50.54; H, 4.67; Br, 16.81; N, 5.89. Found: C, 50.41; H, 4.69; Br, 16.75; N, 5.90.

(*R*)-5-Bromonicotine (+)-α-Methoxy-α-(trifluoromethyl)phenylacetate The filtrate from the initial crystallization of the (−)-MTPA salt above was extracted with 1 N sulfuric acid (2 × 4 ml). The acid extracts were combined, washed with 10 ml of ether, made basic with NaOH, and extracted with methylene chloride. After evaporation of the solvent, the residue was dissolved in 8 ml of ethyl acetate and treated with a solution of (+)-MTPA (1.29 g, 5.5 mmol) in 2 ml of ethyl acetate. The mixture was allowed to stand at 2 °C, and the crystallized product was collected by filtration. Three recrystallizations from acetonitrile afforded 1.05 g (40%) of colorless needles, mp 129—132 °C. *Anal.* Calcd for $C_{20}H_{22}BrF_3N_3O_3$: C, 50.54; H, 4.67; Br, 16.81; N, 5.89. Found: C, 50.36; H, 4.62; Br, 16.60; N, 5.88.

(*S*)-(−)-5-Bromonicotine (2a) (*S*)-(−)-5-Bromonicotine (**2a**) (−)-MTPA salt (1.12 g, 2.35 mmol) was dissolved in 20 ml of 15% HCl and the resulting solution was washed with 20 ml of ethyl acetate. The aqueous layer was made basic with 15% NaOH solution and extracted with 30 ml of methylene chloride. The extract was dried over sodium sulfate and evaporated *in vacuo* to yield **2a** as an oil (0.46 g, 81%). Analysis by chiral HPLC indicated an enantiomeric purity of >98% (t_R = 20.6 min). ^{1}H-NMR ($CDCl_3$): 1.62—2.01 (m, 3H), 2.19 (s, 3H), 2.21—2.38 (m, 2H), 3.07 (t, 1H), 3.20 (t, 1H), 7.87 (s, 1H), 8.44 (s, 1H), 8.55 (s, 1H). $[\alpha]_D^{20}$ −139.6 (*c* = 1.0, methylene chloride). HRMS *m/z*: 240.0258 (Calcd for $C_{10}H_{13}BrN_2$: 240.0262). *Anal.* Calcd for $C_{10}H_{13}BrN_2$: C, 49.81; H, 5.43; N, 11.62. Found: C, 49.82; H, 5.52; N, 11.49.

(*R*)-(+)-5-Bromonicotine (2b) The (*R*)-isomer (**2b**) was prepared by employing identical reaction conditions to those described for the (*S*)-isomer (**2a**) from **2b** (+)-MTPA salt (1.0 g, 2.1 mmol) in 87% yield (0.44 g). Analysis by chiral HPLC indicated an enantomeric purity of >98% (t_R = 15.9 min). ^{1}H-NMR ($CDCl_3$): 1.66—2.01 (m, 3H), 2.19 (s, 3H), 2.20—2.38 (m, 2H), 3.07 (t, 1H), 3.21 (t, 1H), 7.87 (s, 1H), 8.44 (s, 1H), 8.55 (s, 1H). $[\alpha]_D^{20}$ +133.1° (*c* = 1.0, methylene chloride). HRMS *m/z*: 240.250 (Calcd for $C_{10}H_{13}BrN_2$: 240.0262). *Anal.* Calcd for $C_{10}H_{13}BrN_2$: C, 49.81; H, 5.43; N, 11.62. Found: C, 49.94; H, 5.62; N, 11.47.

(*S*)-(−)-5-(Tri-*n*-butylstannyl)nicotine (3a) Compound **2a** (0.46 g, 1.9 mmol) and hexa-*n*-butylditin (2.76 g, 4.76 mmol) were dissolved in dry toluene (5 ml), and a catalytic amount of tetrakis(triphenylphosphine)palladium (22 mg, 0.019 mmol) was added. The mixture was refluxed with stirring for 18 h under an argon atmosphere. After cooling, the reaction mixture was filtered through Celite. The filtrate was concentrated *in vacuo*. The oily residue was then purified by silica gel flash chromatography with chloroform–methanol (20 : 1) to yield **3a** as a colorless oil (0.80 g, 94%). ^{1}H-NMR ($CDCl_3$): 0.85—1.59 (m, 27H), 1.66—2.01 (m, 3H), 2.19 (s, 3H), 2.20—2.38 (m, 2H), 3.07 (t, 1H), 3.21 (t, 1H), 7.72 (s, 1H), 8.42 (s, 1H), 8.47 (s, 1H). HRMS *m/z*: 452.2211 (Calcd for $C_{22}H_{40}N_2Sn$: 452.2213).

(*R*)-(+)-5-(Tri-*n*-butylstannyl)nicotine (3b) Compound **3b** was prepared from **2b** (400 mg, 0.83 mmol) in 45% yield (340 mg) by employing the same reaction conditions as described for the (*S*)-isomer (**3a**). ^{1}H-NMR ($CDCl_3$): 0.85—2.01 (m, 30H), 2.19 (s, 3H), 2.20—2.38 (m, 2H), 3.07 (t, 1H), 3.21 (t, 1H), 7.87 (s, 1H), 8.44 (s, 1H), 8.55 (s, 1H). HRMS *m/z*: 452.2216 (Calcd for $C_{22}H_{40}N_2Sn$: 42.2213).

(*S*)-(−)-5-Iodonicotine (4a) A solution of iodine monochloride (20 mg, 0.12 mmol) in 1 ml of chloroform was added dropwise to stirred suspension of **3a** (46 mg, 0.10 mmol) in chloroform (2 ml). The reaction mixture was stirred at ambient temperature for an additional 1 h, and then washed with 10% aqueous sodium thiosulfate (5 ml) and water (5 ml) and dried over sodium sulfate prior to removal of the solvent *in vacuo*. The oil was purified by HPLC (Cosmosil 5C18, 10 × 250 mm [Nacalai Tesque, Kyoto, Japan]; methanol : 0.01 M ammonium acetate solution = 1 : 1; flow rate 1.5 ml/min; retention time 43 min). The fractions containing **4a** were collected, and evaporated to remove the methanol. The residual solution was made basic with 15% NaOH solution and extracted with methylene chloride. The extract was dried over sodium sulfate and evaporated *in vacuo* to yield 12 mg (40%) of compound **4a** as an oil. Analysis by chiral HPLC indicated an

enantomeric purity of >98% (t_R=23.4 min). ^{1}H-NMR ($CDCl_3$): 1.66—2.01 (m, 3H), 2.19 (s, 3H), 2.20—2.38 (m, 2H), 3.07 (t, 1H), 3.21 (t, 1H), 7.87 (s, 1H), 8.44 (s, 1H), 8.55 (s, 1H). $[\alpha]_D^{20}$ −111.4° (c=1.0, dichloromethane). HRMS m/z: 288.0126 (Calcd for $C_{10}H_{13}IN_2$: 288.0124). *Anal.* Calcd for $C_{10}H_{13}IN_2$: C, 41.69; H, 4.55; N, 9.72. Found: C, 41.57; H, 4.53; N, 9.48.

(*R*)-(+)-5-Iodonicotine (4b) Compound **4b** was prepared from **3b** (300 mg, 0.66 mmol) in 9% yield (15 mg) by employing the same reaction conditions as described for compound **4a**. Analysis by chiral HPLC indicated an enantomeric purity of >98% (t_R=21.0 min). ^{1}H-NMR ($CDCl_3$): 1.66— 2.01 (m, 3H), 2.19 (s, 3H), 2.20—2.38 (m, 2H), 3.07 (t, 1H), 3.21 (t, 1H), 7.87 (s, 1H), 8.44 (s, 1H), 8.55 (s, 1H). $[\alpha]_D^{20}$ +111.0° (c=1.0, dichloromethane). HRMS m/z: 288.0128 (Calcd for $C_{10}H_{13}IN_2$: 288.0124). *Anal.* Calcd for $C_{10}H_{13}IN_2$: C, 41.69; H, 4.55; N, 9.72. Found: C, 41.76; H, 4.58; N, 9.48.

Radiolabeling Ten microliters of 30% aqueous hydrogen peroxide was added to a mixture of sodium [^{125}I]iodide (10 μl, 37 MBq) and compound **3a** or **3b** (0.1 mg in 50 μl of 0.1 N HCl). The vial was sealed and the reaction mixture was stirred for 30 min at room temperature. The resulting solution was made basic with 1 N sodium hydroxide, the product was extracted with diethyl ether (3×0.5 ml), and the combined organic layers were evaporated under a stream of nitrogen. The residue was dissolved in 100—150 μl of methanol, applied to a reverse-phase HPLC column (Cosmosil 5C_{18}-300, 10×250 mm, Nacalai Tesque, Kyoto, Japan), and eluted with methanol : 10 mM ammonium acetate (1 : 1) at a flow rate of 1.5 ml/min (t_R=40 min for (*S*)- and (*R*)-5-[^{125}I]-iodonicotine). The fraction corresponding to compound **4a** or **4b** was collected, evaporated to remove the residual organic solvent, and sterilized by filtration through a 0.22 μm cellulose acetate filter (Milex filter, Millipore Corp.).

The radiochemical purities of [^{125}I]-**4a** and [^{125}I]-**4b** determined by TLC and HPLC were greater than 98%. TLC was performed on a silica gel plate with chloroform : methanol (20 : 1) (t_R=0.37—0.45 for [^{125}I]-**4a** or [^{125}I]-**4b**). HPLC was performed using a chiral analytical column under the conditions described (t_R=23.4 min for [^{125}I]-**4a** and 21.0 min for [^{125}I]-**4b**).

***In Vitro* Brain Nicotine Receptor Binding Studies** The affinity of (*S*)- and (*R*)-5-iodonicotine (**4a** and **4b**) for brain nicotinic receptors was measured in terms of displacement of [^{3}H]cytisine from a preparation of synaptosomal membranes, according to the method of Pabreza *et al.*[37] In brief, the cerebral cortex of a male rat (150—200 g) was homogenized in 50 mM Tris–HCl buffer (pH 7.0) containing 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, and 2.5 mM $CaCl_2$ using a Teflon-glass homogenizer. The homogenate was centrifuged at 40000×g for 10 min at 4 °C, and the pellet was resuspended in fresh buffer to yield a synaptosomal membrane suspension with a protein concentration of 10 mg protein/ml.

Binding assays were performed by incubating 100 μl of the cortical membrane preparation (10 mg protein/ml) with [^{3}H]cytisine (5 nM) at 2 °C and various concentrations of competitors in 0.15 ml of 50 mM Tris–HCl buffer (pH 7.0) containing 120 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, and 2.5 mM $CaCl_2$. Incubation was performed for 75 min at 2 °C, after which the samples were rapidly filtered through polylysine-soaked Whatman GF/C filters, and the filters were washed rapidly three times with 4 ml of ice-cold assay buffer. Each filter was then placed into a 20-ml scintillation vial containing 10 ml of ACS II (Amersham) and the radioactivity bound to the filter was measured with a liquid scintillation counter (LS 500TA, Beckman). All incubations were performed in duplicate. Nonspecific binding was determined in the presence of 1 nM (*S*)-nicotine. IC_{50} values were determined from displacement curves of the percent inhibition of [^{3}H]cytisine binding *versus* the inhibitor concentration using the LIGAND curve-fitting computer program (Elsevier-Biosoft, Cambridge, U.K.). K_i values were calculated by the method of Cheng and Prusoff.[38] For calculation of K_i, the value of 0.96 nM obtained by Pabreza *et al.* was used as the K_d for cytisine.[37] Results are presented as the average of 3 separate experiments.

Determination of the Brain Uptake Index (*BUI*) The *BUI* values for [^{125}I]-**4a**, [^{125}I]-**4b** and [^{99m}Tc]-HM-PAO were determined by the method of Oldendorf.[39] A mixture of 200 μl of saline containing the labeled compounds (37 kBq) and tritiated water (37 kBq) was injected into the right common carotid artery of male Wistar rats (280—300 g), and the rats were killed by decapitation at 15 s after the injection. Part of the midbrain was removed from each rat and the ^{125}I or ^{99m}Tc radioactivity (A) was counted using an NaI well scintillation counter. The sample was then treated with NCS tissue solubilizer (Amersham) and the radioactivity was counted with a liquid scintillation counter (B). In the case of ^{125}I-labeled compounds, the radioactivity of suitably diluted aliquots of the injected sample was also counted using both an NaI well scintillation counter (C) and a liquid scintillation counter (D), and the ^{3}H radioactivity of the brain tissue was determined as follows: ^{3}H radioactivity = $B-(A\times D/C)$. For the ^{99m}Tc-labeled compounds, ^{3}H radioactivity was counted with a liquid scintillation counter after all of the ^{99m}Tc had decayed. Finally, the *BUI* was calculated with the following formula:

$$BUI=\frac{(^{125}\text{I or }^{99m}\text{Tc in brain tissue}/^{3}\text{H in brain tissue})}{(\text{injected }^{125}\text{I or }^{99m}\text{Tc}/\text{injected }^{3}\text{H})}\times 100$$

Biodistribution Studies in Mice Male ddY mice weighing about 30 g were injected with ^{125}I-labeled compound [^{125}I]-**4a** or [^{125}I]-**4b** (18.5 kBq in saline) *via* the tail vein. At the designated times afterwards, the mice were killed by decapitation, and their organs were removed. All samples were weighed, and the radioactivity was counted using an NaI well scintillation counter. Results are presented as the % dose/g organ weight.

***Ex Vivo* Autoradiographic Studies in Rats** Male Wistar rats weighing about 260 g were intravenously injected with 4.8 MBq of [^{125}I]-**4a** or [^{125}I]-**4b**. At the designated times afterwards, the rats were decapitated, and their brains were quickly removed, frozen, and cut into 20-μm thick sections using a cryomicrotome. The sections were thaw-mounted on precleaned gelatin-coated slides, which were then placed on autoradiography film (MARG-^{3}H type, Konica, Japan) for one week along with calibrated ^{125}I-labeled external standards ([^{125}I]Microscales, Amersham, U.K.). Densitometric analysis of the autoradiograms was performed with a videodensitometry system (Excel TVIP-4100, Nippon Avionics, Japan) coupled to a personal computer.[40] Results are presented as the % dose/g tissue weight.

For *in vivo* inhibition studies, (*S*)-nicotine (60 μg/kg, 0.37 μmol/kg) and (*S*)-(+)-dexetimide hydrochloride (10 mg/kg, 25 μmol/kg) in 0.1 ml of saline were injected simultaneously with 4.8 MBq of [^{125}I]-**4a**. The rats were killed 10 min after radioligand administration, their brains were quickly removed, and the regional cerebral distribution of the radioactivity was determined by quantitative autoradiography as described above.

Metabolic Studies Mice (about 30 g) and rats (about 260 g) were injected intravenously with 111 kBq of [^{125}I]-**4a** or [^{125}I]-**4b** and then decapitated at the designated times afterwards. The brains were removed immediately and homogenized in 1 ml of methanol. After centrifugation, the precipitate was washed twice with 1 ml of methanol and the washings were combined with the supernatant. Then the combined methanol extracts were evaporated under a stream of nitrogen, and the resulting residue was re-dissolved in a small volume of methanol and analyzed by TLC on a silica gel plate with a solvent of chloroform–methanol–25% aqueous ammonia (100 : 5 : 0.15). The blood samples were collected in heparinized tubes and centrifuged at 1500 rpm for 10 min to collect plasma samples. These plasma samples were extracted three times with 1 ml of methanol, and the combined methanol extracts were analyzed by TLC as described above.

Statistical Analysis Data are expressed as the mean ± standard deviation when appropriate. Each result was statistically analyzed using the unpaired t test.

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References

1) Nordberg A., Winblad B., *Neurosci. Lett.*, **72**, 115—119 (1986).
2) Whitehouse P. J., Martino A. M., Antuono P. G., Lowenstein P. R., Coyle J. T., Price D. L., Kellar K. J., *Brain Res.*, **371**, 146—151 (1986).
3) London E. D., Ball M. J., Waller S. B., *Neurochem. Res.*, **14**, 745—750 (1989).
4) Aquilonius S-M., "Nicotinic Receptors in the CNS Their Role in Synaptic Transmission," ed. by Nordberg A., Fuxe K., Holmstedt B., Elsevier Science Publishers, Amsterdam, 1989, pp. 329—333.

5) Giacobini E., *J. Neurosci. Res.*, **27**, 548—560 (1990).
6) Benwell M. E., Balfour D. J. K., Anderson J. M., *J. Neurochem.*, **50**, 1243—1247 (1988).
7) Maziere M., Comar D., Marazano C., Berger G., *Eur. J. Nucl. Med.*, **1**, 255—258 (1976).
8) Nyback H., Nordberg A., Langstrom B., Halldin C., Hartvig P., Ahlin A., Swahn C-G., Sedvall G., "Nicotinic Receptors in the CNS Their Role in Synaptic Transmission" ed. by Nordberg A., Fuxe K., Holmstedt B., Elsevier Science Publishers, Amsterdam, 1989, pp. 313—319.
9) Nordberg A., Hartvig P., Lundqvist H., Antoni G., Ulin J., Langstrom B., *J. Neural Transm.* [P-D Sect]., **1**, 195—205 (1989).
10) Nordberg A., Hartvig P., Lilja A., Viitanen M., Amberla K., Lundqvist H., Andersson Y., Ulin J., Winblad B., Langstrom B., *J. Neural Transm.* [P-D Sect]., **2**, 215—224 (1990).
11) Nordberg A., Lilja A., Lundqvist H., Hartvig P., Amberla K., Viitanen M., Warpman U., Johansson M., Hellastrom-lindahl E., Bjurling P., Fasth K-J., Langstrom B., Winblad B., *Neurobiol. Aging*, **13**, 747—758 (1992).
12) Saji H., Magata Y., Yamada Y., Tajima K., Yonekura Y., Konishi J., Ohmomo Y., Yokoyama A., *Chem. Pharm. Bull.*, **40**, 734—736 (1992).
13) Sheridan R. P., Nilakantan R., Dixon J. S., Venkataraghavan R., *J. Med. Chem.*, **29**, 899—906 (1986).
14) Barlow R. B., Johnson O., *Br. J. Pharmacol.*, **98**, 799—808 (1989).
15) Glennon R. A., Maarouf A., Fahmy S., Martin B., Fan F., Yousif M., Shafik R. M., Dukat M., *Med. Chem. Res.*, **2**, 2, 546—551 (1993).
16) Roano C., Goldstein A., *Science*, **210**, 647—650 (1980).
17) Ikushima S., Muramatsu I., Sakakibara Y., Yokotani K., Fujiwara M., *J. Pharmacol. Exp. Ther.*, **22**, 22, 463—470 (1982).
18) Reavill C., Jenner P., Kumar R., Stolerman I. P., *Neuropharmacology*, **27**, 235—241 (1988).
19) Counsell R. E., Ice R. D., "Drug Design," ed. by Ariens E., Academic Press, New York, 1975, pp. 172—259.
20) Magata Y., Saji H., Arano Y., Horiuchi K., Torizuka K., Yokoyama A., *Nucl. Med. Biol.*, **14**, 7—13 (1987).
21) Eckelman W. C., "Radiopharmaceuticals," ed. by Nunn A. D., Marcel Dekker, New York, 1992, pp. 167—219.
22) Roe A., Hawkins G. F., *J. Am. Chem. Soc.*, **69**, 2443—2444 (1947).
23) Rondahl L., *Acta Pharm. Suec.*, **14**, 113—118 (1977).
24) Chan S. M., Basmadjian G. P., Marten D. F., Sadek S. A., Magarian R. A., Grunder J. R., Ice R. D., *J. Label. Comp. Radiopharm.*, **20**, 1017—1025 (1983).
25) Leete E., Bodem G. B., Manuel M. F., *Photochemistry*, **10**, 2687—2692 (1971).
26) Jacob P., III *J. Org. Chem.*, **47**, 4165—4167 (1982).
27) Oldendorf W. H., *Proc. Soc. Exp. Biol. Med.*, **147**, 813—816 (1974).
28) Pardridge W. M., Landaw E., *J. Clin. Invest.*, **74**, 745—752 (1984).
29) Shimohara S., Taniguchi T., Fujiwara M., Kameyama M., *J. Neurochem.*, **45**, 604—610 (1985).
30) Wonnacott S., *Human Toxicol.*, **6**, 343—353 (1987).
31) Broussolle E. P., Wong D. F., Fanelli R. J., London E. D., *Life Sci.*, **44**, 1123—1132 (1989).
32) Flesher J. E., Scheffel U., London E. D., Frost J. J., *Life Sci.*, **54**, 1883—1890 (1994).
33) London E. D., Waller S. B., Wamsley J. K., *Neurosci. Lett.*, **53**, 179—184 (1985).
34) Laduron P. M., Verwimp M., Leysen J. E., *J. Neurochem.*, **32**, 421—427 (1979).
35) Barlow R. B., McLeod L. J., *Br. J. Pharmac.*, **35**, 161—174 (1969).
36) Bedran de Castro M. T., Crystal G. J., Downey H. F., Bashour F. A., *Stroke*, **15**, 690—694 (1984).
37) Pabreza L. A., Dhawan S., Kellar K., *Mol. Pharmacol.*, **39**, 9—12 (1991).
38) Cheng Y. C., Prusoff W. H., *Biochem. Pharmacol.*, **22**, 3099—3108 (1973).
39) Oldendorf W. H., *Brain Res.*, **24**, 372—376 (1970).
40) Saji H., Iida Y., Takahashi M., Sasa M., Serikawa T., Yamada J., Yokoyama A., *Brain Res.*, **601**, 76—79 (1993).