Evaluation of N-Alkyl Derivatives of Radioiodinated Spiperone as Radioligands for *in Vivo* Dopamine D_2 Receptor Studies: Effects of Lipophilicity and Receptor Affinity on the *in Vivo* Biodistribution

Hideo SAJI,[a] Taro TOKUI,[a] Iwao NAKATSUKA,[b] Akihiko SAIGA,[a] Yasuhiro MAGATA,[a] Kunio SHIBA,[b] Akira YOSHITAKE,[b] and Akira YOKOYAMA*,[a]

Department of Radiopharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Kyoto University,[a] Yoshida Shimoadachi, Sakyo-ku, Kyoto 606, Japan and Environmental Health Science Laboratory, Sumitomo Chemical Co., Ltd.,[b] Kasugade-naka, Konohana-ku, Osaka 554, Japan.
Received May 21, 1991

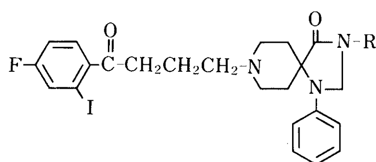
A series of radioiodinated spiperone (2′-ISP) derivatives bearing amide N-alkyl substituents (*N*-methyl-2′-ISP, *N*-ethyl-2′-ISP, and *N*-propyl-2′-ISP) were synthesized and evaluated as potential singlet photon emission computed tomographic radiopharmaceuticals for visualizing dopaminergic receptors. The lipophilicity of these ligands (*i.e.*, the partition coefficient for octanol–phosphate buffer) increased as the chain length increased. Investigation of blood–brain barrier permeability in rats showed a parabolic relationship between the brain uptake index and the partition coefficient. *In vitro* competitive binding studies showed that the relative affinity for the dopamine D_2 receptor was in the order of *N*-propyl-2′-ISP〉2′-ISP〉*N*-methyl-2′-ISP≈*N*-ethyl-2′-ISP. *In vivo* biodistribution studies showed that the initial brain uptake correlated fairly well with the brain uptake index and that the kinetics of the radioactivity specifically bound to the striatum were strongly influenced by the dopamine receptor binding affinity of the compounds. Thus, the *in vivo* behavior of these N-alkylated 2′-ISP derivatives involved a complex interplay between receptor affinity, lipophilicity, and blood–brain barrier permeability.

Keywords radioiodinated spiperone derivative; N-alkylation; dopamine D_2 receptor affinity; lipophilicity; blood–brain barrier permeability; biodistribution; mouse brain region

Introduction

The *in vivo* demonstration of central dopamine D_2 receptors should be helpful for the investigation, diagnosis, and evaluation of the results of treatment of several neurological and psychiatric disorders, including Parkinson's disease and schizophrenia.[1] Various compounds labeled with positron-emitting radionuclides have already been synthesized and evaluated to determine their usefulness for dopamine D_2 receptor imaging by positron emission tomography (PET).[2] However, since the requirement for an on-site cyclotron limits their application, the development of a compound labeled with a more widely usable single photon-emitting radionuclide would be desirable.[3]

Single photon-emitting ^{123}I nuclides have become available, and radioiodinated compounds would seem to have a great potential for application to this field.[3] Accordingly, we recently developed 2′-iodospiperone (2′-ISP), a spiperone derivative iodinated at the *ortho* position of the *p*-fluorobutyrophenone moiety, as a radioligand for single photon emission computed tomography (SPECT) studies of the dopamine D_2 receptor.[4] This compound has a high and selective affinity for dopamine D_2 receptors *in vivo* as well as *in vitro*. However, its brain accumulation is lower than that of [^{11}C]-*N*-methylspiperone, the radioligand most commonly used for PET studies of dopamine D_2 receptors.



R=H : 2′-ISP
$R=CH_3$: *N*-methyl-2′-ISP
$R=C_2H_5$: *N*-ethyl-2′-ISP
$R=n\text{-}C_3H_7$: *N*-propyl-2′-ISP

Fig. 1. Chemical Structures of the Compounds Studied

It has been reported that N-alkylation at the amide nitrogen of the spirodecane moiety of spiperone can increase its lipophilicity and thus strongly influence the *in vivo* pharmacokinetics of this compound.[5] Therefore, further improvement of 2′-ISP as a SPECT radiopharmaceutical would seem to require the systematic study of a series of N-alkylated derivatives containing methyl, ethyl, and *n*-propyl groups of diverse chain lengths (Fig. 1). The present study was performed to investigate the effects of N-alkylation on the dopamine D_2 receptor affinity and biodistribution of various 2′-ISP derivatives.

Materials and Methods

[^{125}I]Sodium iodide [specific activity 81.4 TBq (2200 Ci)/mmol] and [^{3}H]spiperone [592 GBq (16 Ci)/mmol] were purchased from Amersham International Plc. The other drugs and reagents used were obtained from commercial sources. Male Wistar rats and male ddY mice were supplied by Japan SLC Co., Ltd.

Synthesis of 3-*N*-Alkyl-2′-[^{125}I]iodospiperone Compounds ^{125}I-Labeled N-alkylated 2′-ISP was synthesized by the method previously reported for the N-alkylation of spiperone and brominated spiperone derivatives.[5] Firstly, [^{125}I]-2′-ISP was synthesized as described previously,[4] and then reacted with the corresponding alkyl iodide in the presence of NaH or tetrabutylammonium hydroxide. The final products were purified by high performance liquid chromatography (HPLC, Lichrosorb RP-18, $H_2O/CH_3OH/CH_3CN/(C_2H_5)_3N=80/250/33/0.2$, flow rate: 1.0 ml/min).

The radiochemical purity of the products was more than 98% as determined by HPLC and thin-layer chromatogaphy (TLC, $CH_2Cl_2/C_2H_5OH=9/1$), and the specific activity estimated by the ultraviolet (UV) absorbance at 254 nm was 1.1 TBq (30 Ci)/mmol.

Nonradioactive standard compounds were prepared similarly and their structures were confirmed by infrared (IR), nuclear magnetic resonance (NMR), and mass spectrometry analysis. The details of the synthesis and characterization of these compounds will be published elsewhere.

Inhibition of [^{3}H]Spiperone Binding to Rat Striatal Membranes Rat striatal membranes were prepared as described previously.[5a)] An aliquot (0.8 ml, 0.4 mg protein) of the striatal membrane preparation was incubated at 23°C for 30 min with different concentrations of the unlabeled competing drugs (10^{-10} to 10^{-5} M), ketanserine (10^{-7} M), and [^{3}H]spiperone (2×10^{-10} M). Incubation was terminated by adding 5 ml of ice-cold TEAN buffer (15 mM Tris–HCl, pH 7.4, 5 mM ethylenediaminetetraacetic acid disodium (Na_2EDTA), 1.1 mM ascorbate, and 12.5 μM nialamide) followed

© 1992 Pharmaceutical Society of Japan

NII-Electronic Library Service

by rapid filtration through a Whatman GF/B filter. Each filter was immediately washed twice with ice-cold TEAN buffer (5 ml), and the bound [^{3}H]spiperone retained on the filter was extracted with 10 ml of ACS II (Amersham) and counted. All incubations were done in triplicate. Nonspecific binding was determined using tubes containing (+)butaclamol at a final concentration of 10^{-6} M, and specific binding was calculated by subtracting the nonspecific binding from the total binding. The IC_{50} values, the concentrations of the tested compounds that produced 50% inhibition of specific [^{3}H]spiperone binding, were assessed by triplicate assays of six to eight samples with different concentrations of the inhibitors.

Measurement of the Octanol/Water Partition Coefficient Partition coefficients for the radioiodinated compounds were determined by a slight modification of the method previously reported.[6]

A 20-μl aliquot of each radioiodinated sample was mixed with 3 ml each of 1-octanol and 0.1 M phosphate buffer (pH 7.4) in a test tube. This tube was vortexed (3 × 1 min), incubated for 1 h at room temperature, and then centrifuged for 5 min. Then 500-μl aliquots of each phase were removed and counted for ^{125}I activity in a well-type NaI scintillation counter. The partition coefficient (P) was determined by calculating the ratio of the cpm/ml for octanol to that for buffer, and the natural logarithm of this ratio (log P) was used for assessing correlations with physiological parameters.

Determination of the Brain Uptake Index (BUI) The BUI values of [^{125}I]-2′-ISP and the N-alkylated derivatives were determined by the Oldendorf method.[7] A mixture of 200 μl of ethanolic saline (<2% ethanol) containing the labeled compounds [37 kBq (1 μCi)] and tritiated water [^3H_2O; 37 kBq (1 μCi)] was injected into the right common carotid artery of male Wistar rats (280—320 g), which were killed by decapitation at 15 s after injection. Part of the midbrain was removed from each rat and the ^{125}I radioactivity (A) was counted in a well-type NaI scintillation counter. The same sample was then treated with NCS tissue solubilizer (Amersham) and the radioactivity was counted in a liquid scintillation counter (B). The radioactivity of suitably diluted aliquots of the [^{125}I]-2′-ISP sample was also counted in both a well-type NaI scintillation counter (C) and a liquid scintillation counter (D). Then, the ^{3}H radioactivity of the BUI sample was determined as follows: ^{3}H radioactivity $= B - [A \times D/C]$. Finally, the BUI was calculated with the following formula:

$$\mathrm{BUI} = \frac{(^{125}\mathrm{I\ in\ brain\ tissue}/^{3}\mathrm{H\ in\ brain\ tissue})}{(^{125}\mathrm{I\ in\ injectate}/^{3}\mathrm{H\ in\ injectate})} \times 100$$

Biodistribution in Mice A dose of 18.5 kBq (0.5 μCi) of each ^{125}I-labeled compound was injected intravenously into male ddY mice weighing about 30 g. At appropriate time intervals, the mice were killed by decapitation, their organs were excised and samples of blood were collected by cardiac puncture. All samples were weighed and the radioactivity was counted in a well-type NaI scintillation counter. Results were presented as the % dose/g organ weight.

Regional Cerebral Localization Studies Male ddY mice weighing 30 g were intravenously injected with 0.1 ml of an ethanolic saline solution (<2% ethanol) of each radioiodinated compound [55.5 kBq (1.5 μCi)/mouse] (2′-ISP, 0.87 μg/kg; N-methyl-2′-ISP, 0.89 μg/kg; N-ethyl-2′-ISP, 0.92 μg/kg; and N-propyl-2′-ISP, 0.94 μg/kg). At various time intervals after the injection, the mice were sacrificed by decapitation and their brain regions were dissected out on an ice-cold plate according to the method of Glowinski and Iversen.[8] The wet tissue samples were then weighed and the radioactivity was determined using a well-type NaI scintillation counter. Results were calculated in terms of the percent injected dose per gram of tissue by comparison of tissue radioactivity with suitably diluted aliquots of the injectate.

Metabolic Stability in the Brain Four mice were given intravenous injections of 55.5 kBq (1.5 μCi) of each ^{125}I-labeled compound. At 60 min after injection, the animals were killed and pooled striata were homogenized with a mixture of 500 μl of methanol and 20 μl of 10% trichloroacetic acid (TCA) solution. After centrifugation, the precipitate was washed with 200 μl of methanol and the washings were combined with the supernatant. The solution was analyzed by TLC on Merck silica gel ($CH_2Cl_2/C_2H_5OH = 9/1$).

Drug Displacement Studies Several neuroleptic drugs were intravenously injected into mice along with 55.5 kBq (1.5 μCi) of each ^{125}I-labeled compound. Spiperone (1.0 mg/kg) was injected simultaneously with the radioligand, and (+) or (−)butaclamol (0.5 mg/kg) was injected intraperitoneally 30 min before radioligand administration. The animals were killed 60 min after radioligand administration and the uptake of radioactivity by various brain regions was determined as described above.

Results

Dopamine D_2 Receptor Binding Affinity Measurement of the binding affinities of 2′-ISP and its N-alkyl derivatives for the dopamine D_2 receptor was carried out by a competitive binding assay with [^{3}H]spiperone using rat striatal membranes. The results are given in Table I as IC_{50} values, including that of spiperone. All the compounds tested competed with [^{3}H]spiperone for binding to rat striatal membranes, and the binding affinity was in the order of N-propyl-2′-ISP ≈ spiperone > 2′-ISP > N-methyl-2′-ISP ≈ N-ethyl-2′-ISP. Thus, the propyl derivative of 2′-ISP showed the highest affinity, which was approximately 4.5 times that of 2′-ISP itself and slightly higher than that of spiperone. The methyl and ethyl derivatives had about 5 times less affinity than 2′-ISP.

Lipophilicity The lipophilicity of 2′-ISP and its N-alkyl derivatives was assessed by octanol–phosphate buffer extraction. As shown in Table I, the partition coefficient (log P) increased from 2.3 to 2.9 as the chain length increased. This correlation of lipophilicity with the chain length was also supported by HPLC studies, since the elution time of a substrate is directly related to its lipophilicity in reversed-phase HPLC (column 3 in Table I).[9]

Blood–Brain Barrier Permeability The permeability of 2′-ISP and the N-alkyl derivatives to the blood–brain barrier was assessed by determining the BUI for each compounds. Following Oldendorf's method,[7] the BUI was determined at 15 s after the injection of a mixture of the radioiodinated compounds and tritiated water. The BUI was 26.3 ± 4.4 for 2′-ISP, 59.8 ± 6.6 for N-methyl-2′-ISP, 64.8 ± 6.0 for N-ethyl-2′-ISP, and 39.2 ± 1.6 for N-propyl-2′-ISP. Thus, the methyl and ethyl derivatives had a large BUI, and the propyl derivative and 2′-ISP showed a smaller BUI value.

Biodistribution in Mice Table II shows the results of the biodistribution study of radioiodinated 2′-ISP and its N-alkyl derivatives. Data are expressed as a percentage of the dose administered per gram of wet tissue at 5—120 min following intravenous injection. These four compounds generally showed a similar biodistribution, with radioactivity clearing rapidly from the blood and the kidneys and liver showing a high uptake. However, their brain uptake differed. N-Methyl-2′-ISP showed the highest initial brain uptake followed by N-ethyl-2′-ISP, 2′-ISP, and N-propyl-2′-ISP in that order. For N-methyl-2′-ISP, ethyl-2′-ISP, and 2′-ISP, the radioactivity in the brain remained unchanged from 5—15 min after injection and thereafter

Table I. Dopamine D_2 Receptor Affinity, Partition Coefficient, and Retention Time on Reversed-Phase HPLC of Spiperone and N-Alkylated Analogues of 2′-ISP

Compound	IC_{50}[a] ($\times 10^{-8}$ M)	log P[b]	t_R[c] (min)
Spiperone	0.26	2.11	19.4
2′-ISP	0.93	2.30	25.6
N-Methyl-2′-ISP	5.28	2.41	29.4
N-Ethyl-2′-ISP	5.55	2.81	33.1
N-Propyl-2′-ISP	0.20	2.90	44.1

a) Binding to dopamine D_2 receptors was assessed using rat striatal membrane preparations (*vs.* [^{3}H]spiperone, see Methods). b) Logarithm of the partition coefficient between n-octanol and phosphate buffer (pH 7.4). c) Retention time on a Lichrosorb RP-18 HPLC column (7.5 × 300 mm, $H_2O/CH_3OH/CH_3CN/(CH_2H_5)_3N = 80/250/33/0.2$, flow rate; 1.0 ml/min).

NII-Electronic Library Service

TABLE II. Biodistribution of ^{125}I-Labeled 2′-ISP and Its N-Alkylated Derivatives in Mice

Compound	Time (min)	% dose/g tissue[a]					
		Blood	Intestine	Liver	Kidney	Heart	Brain
2′-ISP	5	1.27 (0.13)	3.53 (0.23)	8.84 (0.52)	20.78 (1.50)	7.43 (1.42)	0.78 (0.02)
	15	1.29 (0.10)	4.47 (0.43)	9.25 (0.62)	18.79 (1.36)	3.85 (0.72)	0.77 (0.11)
	30	1.24 (0.13)	5.28 (0.26)	9.66 (1.02)	15.17 (3.70)	2.27 (0.21)	0.60 (0.03)
	60	1.04 (0.08)	6.26 (0.77)	10.06 (1.84)	8.96 (1.72)	1.28 (0.27)	0.36 (0.08)
	120	0.97 (0.28)	6.67 (3.78)	8.29 (4.61)	5.40 (1.92)	0.39 (0.20)	0.16 (0.03)
N-Methyl-2′-ISP	5	1.05 (0.08)	2.24 (0.17)	6.81 (1.04)	12.38 (0.84)	4.15 (0.46)	1.43 (0.09)
	15	1.16 (0.15)	3.69 (0.36)	8.21 (0.78)	12.39 (1.37)	2.68 (0.22)	1.38 (0.11)
	30	1.28 (0.08)	5.58 (0.86)	8.04 (1.28)	9.78 (0.48)	2.04 (0.04)	1.09 (0.10)
	60	1.17 (0.25)	6.52 (0.62)	6.64 (0.24)	7.40 (0.55)	1.39 (0.20)	0.64 (0.11)
	120	0.98 (0.18)	6.34 (0.47)	5.68 (0.86)	6.16 (0.83)	0.73 (0.06)	0.28 (0.03)
N-Ethyl-2′-ISP	5	1.67 (0.05)	3.18 (0.67)	9.09 (1.57)	14.13 (1.34)	4.20 (0.39)	1.03 (0.05)
	15	1.50 (0.04)	5.84 (0.47)	9.27 (0.84)	13.10 (1.04)	2.81 (0.09)	1.02 (0.05)
	30	1.49 (0.09)	7.60 (0.65)	9.07 (0.63)	10.36 (0.56)	2.07 (0.19)	0.80 (0.14)
	60	1.31 (0.16)	8.59 (1.41)	8.51 (1.41)	8.15 (1.30)	1.44 (0.22)	0.48 (0.07)
	120	1.34 (0.13)	10.52 (1.50)	7.20 (0.19)	6.46 (0.40)	1.05 (0.07)	0.32 (0.02)
N-Propyl-2′-ISP	5	1.07 (0.10)	2.50 (0.30)	10.78 (1.79)	12.74 (2.19)	3.61 (0.44)	0.52 (0.09)
	15	0.96 (0.15)	4.67 (0.57)	11.00 (1.54)	12.62 (2.25)	2.44 (0.26)	0.61 (0.15)
	30	0.92 (0.06)	7.48 (1.24)	9.85 (1.19)	10.69 (1.51)	1.61 (0.30)	0.44 (0.13)
	60	1.02 (0.06)	9.77 (2.26)	8.41 (0.77)	8.40 (0.91)	1.19 (0.13)	0.34 (0.04)
	120	1.02 (0.22)	11.27 (1.01)	6.37 (0.47)	8.57 (2.85)	1.06 (0.12)	0.30 (0.04)

a) Each value is the mean (S.D.) of data from 4 animals.

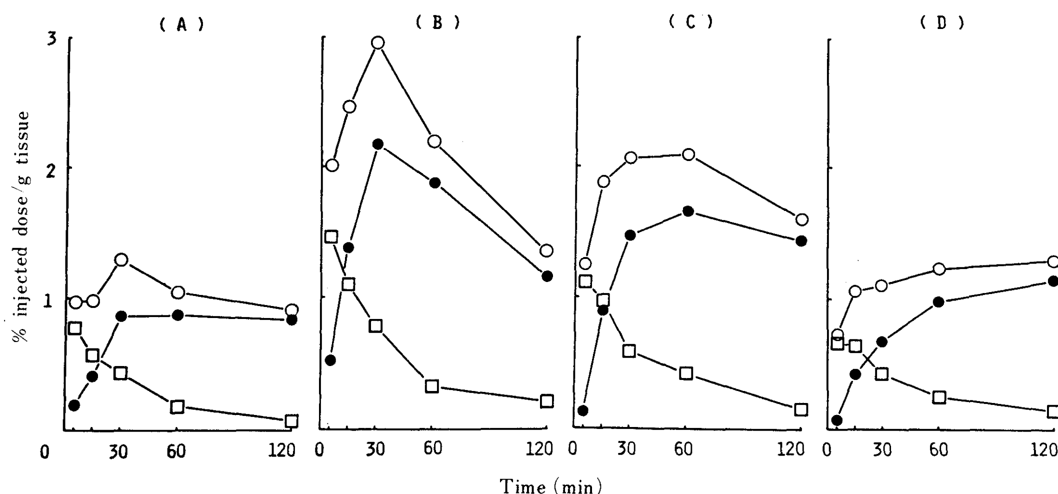


Fig. 2. Biodistribution of [^{125}I]-2′-ISP (A), [^{125}I]-*N*-Methyl-2′-ISP (B), [^{125}I]-*N*-Ethyl-2′-ISP (C), and [^{125}I]-*N*-Propyl-2′-ISP (D) in the Striatum (○) and Cerebellum (□) of Mice

The value of the differences obtained by subtracting the cerebellar values from the corresponding values for the striatum are marked as (●). Each point is the mean of data from 4 animals.

decreased with time. *N*-Propyl-2′-ISP also showed similar behavior, although the maximum uptake was observed at 15 min.

Regional Cerebral Distribution in Mice Figure 2 shows the time course of the change of radioactivity in the striatum and cerebellum. The differences between striatal and cerebellar radioactivity levels (obtained by simple subtraction) are also plotted.

The density of dopamine D_2 receptors is very high in the striatum and negligible in the cerebellum.[10] In addition, it is considered that nonspecific binding is equal in both regions,[11] so that a plot of the differences in radioactivity between them may give an indication of the kinetics of specifically bound radioactivity.

For all four compounds, the radioactivity at 5 min after injection was similar in the two regions, while later times the striatum showed higher levels of radioactivity than the cerebellum. Thus, all the tested compounds showed specific regional localization to the striatum.

However, some differences in the brain distribution kinetics of these compounds were observed. The methyl derivative exhibited the highest initial striatal uptake and then showed a decrease after 30 min. The ethyl derivative also showed a high level of accumulation, which was almost equal to that of the methyl derivative and only decreased slightly at 120 min. The striatal uptake of 2′-ISP remained constant over time, and the propyl derivative displayed a low initial striatal uptake and a continuous increase over time. Specific binding values for these compounds paralled their striatal uptake.

In addition, the effect of various drugs on the striatal uptake of the four compounds was studied. As shown in

NII-Electronic Library Service

TABLE III. Effects of Various Drugs on the Striatal to Cerebellar Ratios of ^{125}I-Labeled *N*-Alkyl-2′-ISP Analogues

Compound	Striatum/cerebellum ratio[a]			
	Control	Spiperone	(+)Butaclamol	(−)Butaclamol
2′-ISP[b]	6.00 (0.51)	1.38 (0.09)	1.46 (0.07)	6.18 (0.96)
N-Methyl-2′-ISP	6.95 (0.74)	1.20 (0.05)	1.42 (0.16)	6.15 (0.38)
N-Ethyl-2′-ISP	6.21 (0.96)	1.82 (0.17)	1.63 (0.18)	5.52 (0.69)
N-Propyl-2′-ISP	4.91 (0.41)	1.70 (0.21)	1.61 (0.27)	3.98 (0.16)

a) Each ^{125}I-labeled compound was injected intravenously 30 min after the administration of 5.0 mg/kg of butaclamol isomers or simultaneously with 1.0 mg/kg of spiperone. At 60 min after injection of the radioligand, the radioactivities of the striatum and cerebellum were measured. Values represent the mean (S.D.) of data for 4 animals (% dose/g tissue ratio). *b*) Reference 4*b*.

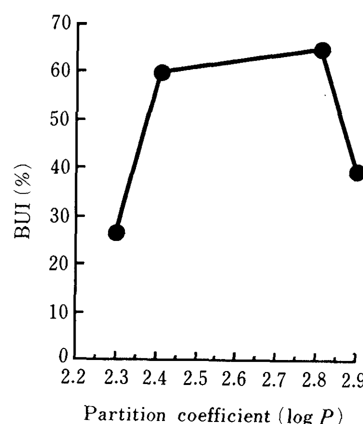


Fig. 3. The Relationship between the Octanol: Buffer (pH 7.4) Partition Coefficient (log *P*) and the BUI for [^{125}I]-2′-ISP and Its N-Alkylated Derivatives

Table III, the administration of spiperone and (+)butaclamol prevented striatal uptake, but (−)butaclamol caused no changes in binding.

Metabolic Stability in the Brain At 60 min after the intravenous injection of the N-alkylated analogs of 2′-ISP, the radioactivity in the TCA–methanol extract of mouse striatal homogenate was analyzed by TLC. Approximately 75% of the radioactivity in the striatal homogenate obtained after injection of each radioligand was extractable by methanol and the extractable material displayed a single peak which migrated with the same *Rf* as the authentic compound. These results showed that most of their distribution in the brain occurred as an intact entity and indicated the metabolically stable nature of the compounds in the brain.

Discussion

In the present study, N-alkylation of the amide nitrogen in 2′-ISP was shown to strongly influence the lipophilicity, dopamine D_2 receptor affinity, blood–brain barrier permeability, and *in vivo* biodistribution of all the compounds tested.

The lipophilicity (*P* value) as assessed by octanol–phosphate buffer (pH 7.4) extraction increased from 2.3 to 2.9 with an increase of chain length from C_1–C_3. The *in vivo* penetration of the blood–brain barrier assessed by the BUI was also increased by N-alkylation. However, the BUI was in the order of *N*-ethyl-2′-ISP ≈ *N*-methyl-2′-ISP > *N*-propyl-2′-ISP > 2′-ISP, and showed a parabolic relationship with the log *P* values (Fig. 3). Similar parabolic relationships between brain uptake and lipophilicity have been noted for some other compounds including radiolabeled spiperone analogs, and have been explained by the opposing effects of increased blood–brain barrier penetration and increased plasme protein binding and/or precipitation as the lipophilicity of a compound is enhanced.[5a,c,12] These considerations may also apply to this series of 2′-ISP analogs. In addition, since the molecular weight and size of a compound was increased by N-alkylation, the blood–brain barrier permeability might also be affected by these factors.

In vitro assay of the relative affinity of these compounds for the dopamine D_2 receptor showed a decrease in the order of *N*-propyl-2′-ISP > 2′-ISP > *N*-methyl-2′-ISP ≈ *N*-ethyl-2′-ISP, and this order was contrary to the BUI data. Welch has also reported that the *in vitro* binding affinity of spiperone analogs to D_2 receptors is influenced by N-alkylation.[5e)]

These interesting results called for an *in vivo* evaluation of the compounds. All four compounds showed specific regional uptake with a high concentration of radioactivity in the striatum, a region of high dopamine receptor density,[10] and a low concentration in the cerebellum, a region of negligible dopamine receptor density.[10] However, the level of striatal uptake was influenced strongly N-alkylation, *i.e.*, striatal uptake was initally increased and then decreased as the alkyl chain length increased, as shown in Fig. 2. These *in vivo* biodistribution findings roughly correlated with the BUI data, although *N*-ethyl-2′-ISP showed a slightly lower uptake than would have been expected from its BUI value. On the other hand, washout of the specifically bound radioactivity from the striatum increased in the order of *N*-methyl-2′-ISP > *N*-ethyl-2′-ISP > 2′-ISP > *N*-propyl-2′-ISP, and this order showed an approximately inverse correlation with the dopamine D_2 receptor affinity of these compounds. Thus, our data indicate that the *in vivo* behavior of N-alkylated 2′-ISP derivatives involves a complex interplay between receptor affinity, lipophilicity and blood–brain barrier permeability.

When *in vivo* SPECT studies are performed, a radioligand that exhibits high and specific uptake by brain regions with a high dopamine D_2 receptor density and subsequent long-term retention will provide good images of dopamine D_2 receptor localization, and will allow estimation of degree of *in vivo* specific receptor binding from such images.[13] On the other hand, a radioligand that accumulates specifically in the regions with high dopamine D_2 receptor density and is then cleared rapidly can provide information on the dissociation of the ligand from the receptor, a potentially important kinetic parameter.[14] The results obtained in this study suggest that 2′-ISP and its N-propyl derivative belong to the former class of radioligands, while the N-methyl and ethyl derivatives belong to the latter class. Further investigation is necessary to clarify the usefulness of these radioligands for *in vivo* functional studies of dopamine D_2 receptors.

References

1) H. N. Wagner and D. F. Wong, "Quantitative Imaging; Neuroreceptors, Neurotransmitters and Enzymes," ed. by J. J. Frost and

NII-Electronic Library Service

H. N. Wagner, Revan Press, New York, 1990, pp. 97—108; G. Sedvall, *Trends Neurosci.*, **13,** 302 (1990).

2) B. Maziere and M. Maziere, "Wenner-Gren International Symposium Series Vol. 53, Visualization of Brain Functions," ed. by D. Ottoson and W. Rostene, Stockton Press, New York, 1989, pp. 167—181.

3) H. F. Kung, *Nucl. Med. Biol.*, **17**, 85 (1990); H. F. Kung, Y. Ohmomo, and M.-P. Kung, *Sem. Nucl. Med.*, **20**, 290 (1990).

4) *a*) I. Nakatsuka, H. Saji, K. Shiba, H. Shimizu, M. Okuno, A. Yoshitake, and A. Yokoyama, *Life Sci.*, **41**, 1989 (1987); *b*) H. Saji, I. Nakatsuka, K. Shiba, T. Tokui, K. Horiuchi, A. Yoshitake, K. Torizuka, and A. Yokoyama, *ibid.*, **41**, 1999 (1987).

5) *a*) S. M. Moerlein, P. Laufer, and G. Stocklin, *Int. J. Nucl. Med. Biol.*, **12**, 353 (1985); *b*) C. D. Arnett, C.-Y. Shiue, A. P. Wolf, J. S. Fowler, J. Logan, and M. Watanabe, *J. Neurochem.*, **44**, 835 (1985); *c*) M. J. Welch, D. Y. Chi, C. J. Mathias, M. R. Kilbourn, J. W. Brodack, and J. A. Katzenellenbogen, *Nucl. Med. Biol.*, **13**, 523 (1986); *d*) H. H. Coenen, P. Laufer, G. Stocklin, K. Wienhard, G. Pawlik, H. G. Bocher-Schwarz, and W.-D. Heiss, *Life Sci.*, **40**, 81 (1987); *e*) M. J. Welch, J. A. Katzenellenborgen, C. J. Mathias, J. W. Brodack, K. E. Carlson, D. Y. Chi, C. S. Dense, M. R. Kilbourn, J. S. Pelmutter, M. E. Raichle, and M. M. Ter-pogossian, *Nucl. Med. Biol.*, **15**, 83 (1988).

6) T. Hosotani, A. Yokoyama, Y. Arano, K. Horiuchi, H. Saji, and K. Torizuka, *Appl. Radiat. Isot.*, **37**, 505 (1986).

7) W. H. Oldendorf, *Brain Res.*, **24**, 372 (1970).

8) J. Glowinski and L. L. Iverson, *J. Neurochem.*, **13**, 655 (1966).

9) D. J. Minick, J. H. Frenz, M. A. Patrick, and D. A. Brent, *J. Med. Chem.*, **31**, 1923 (1988).

10) D. R. Burt, I. Creese, and S. H. Snyder, *Mol. Pharmacol.*, **12**, 800 (1976).

11) C. D. Arnett, J. S. Fowler, A. P. Wolf, and R. P. MacGregor, *J. Neurochem.*, **40**, 455 (1983); M. A. Mintun, M. E. Raichle, M. R. Kilbourn, G. F. Wooten, and M. J. Welch, *Ann. Neurol.*, **15**, 217 (1984).

12) D. D. Dischino, M. J. Welch, M. R. Kilbourn, and M. E. Raichle, *J. Nucl. Med.*, **24**, 1030 (1983).

13) D. F. Wong, A. Gjedde, and H. N. Wagner, Jr., *J. Cereb. Blood Flow Metab.*, **6**, 137 (1986).

14) C. D. Arnett, C.-Y. Shiue, A. P. Wolf, J. S. Fowler, J. Logan, and M. Watanabe, *J. Neurochem.*, **44**, 835 (1985).

NII-Electronic Library Service