Annals of Nuclear Medicine Vol. 8, No. 1, 17–22, 1994

^{11}C-Labeled 2′-iododiazepam for PET studies of benzodiazepine receptors: Synthesis and comparison of biodistribution with its radioiodinated compound

Yasuhiko IIDA,* Hideo SAJI,* Yasuhiro MAGATA,** Junji KONISHI,** Iwao NAKATSUKA,***
Akira YOSHITAKE*** and Akira YOKOYAMA*

*Faculty of Pharmaceutical Sciences, **School of Medicine, Kyoto University
***Environmental Health Sciences Laboratory, Sumitomo Chemical Co. Ltd.

For PET studies of benzodiazepine receptors, N-^{11}C-methyl-2′-iododiazepam (2′-IDZ) was synthesized by N-methylation of its desmethyl derivative with ^{11}C-methyl iodide, and was subsequently purified by HPLC. The labeling and purification procedures were completed within 45 min after ^{11}C-methyl iodide trapping, and the radiochemical yield (corrected for decay) was approximately 40% based on the initial trapped radioactivity of ^{11}C-methyl iodide. Biodistribution studies in mice demonstrated that ^{11}C-2′-IDZ was rapidly and noticeably accumulated in the brain, and subsequently decreased with time. Accumulation was greater in the cortex than in other brain regions. When compared with ^{125}I-2′-IDZ, the distribution was almost the same until 5 min after injection, but levels were low after 20 min. Metabolic studies indicated that the difference between these two compounds in the time course of brain radioactivity distribution may be due to N-demethylation *in vivo*.

Key words: diazepam derivative, ^{11}C, ^{125}I, benzodiazepine receptor, PET

INTRODUCTION

ALTERATIONS in the biochemical integrity of the benzodiazepine receptor system have been related to the development and evolution of various neurological and psychiatric disorders, including epilepsy,[1,2] Huntington's disease,[3,4] and Alzheimer's disease.[5] The *in vivo* imaging of benzodiazepine receptors by positron emission tomography (PET) and single photon emission tomography (SPECT) has therefore been a growing field of interest.[6–9] For such studies, a ligand capable of being labeled with both positron- and single photon-emitting radionuclides would be useful, since it would then become possible to investigate benzodiazepine receptors by PET and SPECT with a single radioligand and to compare both imaging methods directly.[10–12]

The basic requirements of a radioligand for *in vivo* studies of benzodiazepine receptors include rapid and quantitatively significant brain uptake following peripheral administration and a high affinity for the target receptors.[13–15] 1,4-Benzodiazepine compounds such as diazepam show signs of high extraction by the brain.[6–9,16] In addition, structure-activity studies have indicated that the introduction of electron-withdrawing groups such as halogens at position 2′ of the 5-phenyl-1,4-benzodiazepine molecule increases its receptor affinity.[17–20]

Accordingly, we recently developed a diazepam derivative, 2′-iododiazepam (2′-IDZ), which has both a methyl group at the N-l position as a site for introducing ^{11}C and an iodine atom at the 2′ position as the site for introducing ^{123}I (Fig. 1).[21,22] This compound was found to have a high affinity for cerebral benzodiazepine receptors, approximately 9 times greater than that of diazepam (K_d = 0.66 nM).[21,22] In addition, radioiodinated 2′-IDZ was synthesized and was found to bind to benzodiazepine receptors *in vivo*.[22] In the present study, ^{11}C-labeled 2′-IDZ was synthesized, and its *in vivo* behavior was compared with that of its ^{125}I-labeled analog. In addition, its usefulness for imaging cerebral benzodiazepine receptors was assessed.

Received May 26, 1993, revision accepted September 3, 1993.

For reprint contact: Hideo Saji, Ph.D., Department of Radiopharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto 606–01, JAPAN.

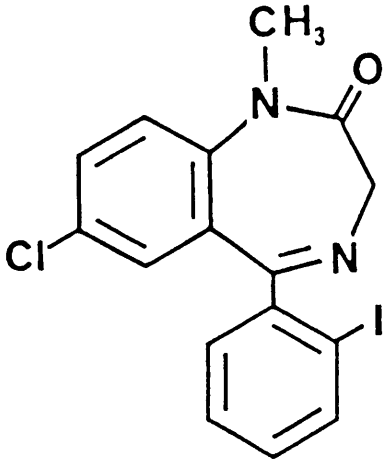

Fig. 1 Chemical structure of 2′-iododiazepam (2′-IDZ).

MATERIALS AND METHODS

^{125}I-2′-IDZ was prepared by a bromine-iodine exchange reaction with sodium ^{125}I-iodide (Amersham International Plc.), as described previously.[21,22] All other chemicals used were of reagent grade. Male ddY mice were supplied by Japan SLC Co. Ltd.

Preparation of ^{11}C-2′-IDZ

^{11}C-Methyl iodide was prepared according to the method described previously.[23] ^{11}C-Carbon dioxide was produced via proton bombardment of nitrogen gas and the ^{14}N(p,α)^{11}C reaction in an ultracompact cyclotron (Sumitomo, Model 325), and was trapped in a solution of lithium aluminum hydride in tetrahydrofuran (THF). After evaporation of the THF, 54% hydroiodic acid was added, and the ^{11}C-methyl iodide thus produced was trapped in an acetone solution (350 μl) containing 0.4 mg of 2′-iodonordiazepam (IND) and 5 μl of 10 N sodium hydroxide. Then the reaction vial was sealed and heated at 90°C for 8 min. After cooling and neutralization of the solution with 1 N hydrochloric acid, the resulting mixture was purified by high-performance liquid chromatography (HPLC) on a 7.5 × 300 mm Lichrosorb RP-18 column eluted with ethanol : water (9 : 11) at 1.5 m*l*/min (R_t = 25 min for 2′-IND; R_t = 30 min for 2′-IDZ). The fraction corresponding to 2′-IDZ was collected and evaporated, after which the residue was dissolved in ethanolic saline (< 0.5% ethanol) and filtered through a 0.22 μm cellulose acetate filter (Milex, Millipore Corp.).

The radiochemical purity of the product was determined by thin-layer chromatography (TLC) and analytical HPLC. TLC was performed on a silica gel plate with a chloroform : acetone (4 : 1) solvent (R_f = 0.62 – 0.68). HPLC was performed on a 7.5 × 300 mm Lichrosorb RP-18 column eluted with methanol : water : 4 mM phosphate buffer (pH 7.4) (11 : 5 : 4) at a flow rate of 1.5 m*l*/min (R_t = 62 min). The specific activity was estimated from the U.V. absorbance at 315 nm.

2′-IND was synthesized from 2-iodobenzonitrile by the addition of 4-chloroaniline, followed by coupling with bromoacetyl chloride, amination, and cyclization. The structure of the compound was confirmed by infrared analysis (IR), proton nuclear magnetic resonance (NMR) spectrometry, and mass spectrometry. Details of the synthesis and characterization of this compound will be published elsewhere.

Biodistribution study in mice

Male ddY mice weighing about 30 g were injected via the tail vein with ^{11}C-2′-IDZ (1.11 MBq, 3.1 μg/kg) or ^{125}I-2′-IDZ (18.5 kBq, 0.03 μg/kg) in 0.1 m*l* of ethanolic saline. At designated times afterwards, the mice were killed by decapitation and their organs were removed. For *in vivo* brain distribution studies, the brains were dissected on an ice-cold plate according to the method of Glowinski and Iversen.[24] All samples were weighed, and the radioactivity was counted in an NaI well scintillation counter. Results are presented as the % injected dose/g tissue weight.

Metabolic study

Mice weighing about 30 g were injected intravenously with 18.5 MBq of ^{11}C-2′-IDZ or 55.5 kBq of ^{125}I-2′-IDZ and then decapitated at 5 or 20 min after injection. The brains were removed immediately and homogenized in 1 m*l* of methanol. After centrifugation, the precipitate was washed twice with 1 m*l* of methanol and the washings were combined with the supernatant. For both compounds, approximately 95% of the radioactivity in the homogenate was extractable by our organic solvent technique. The combined methanol extracts were evaporated, and the resultant residue was redissolved in a small volume of methanol for analysis by HPLC. HPLC was performed as described above on a Lichrosorb RP-18 column.

RESULTS AND DISCUSSION

^{11}C-2′-IDZ was prepared by N-methylation of 2′-iodonordiazepam (2′-IND) with ^{11}C-methyl iodide, and was well separated from the starting material (2′-IND) and some radioactive by-products by reverse-phase HPLC. Radioactivity due to compounds other than ^{11}C-2′-IDZ (including $^{11}CH_3I$) was detected in the early fractions. The labeling and purification procedures were completed within 45 min after the finish of ^{11}C-methyl iodide trapping and the radiochemical yield (corrected for decay) was approximately 40% based on the initial radioactivity of ^{11}C-methyl iodide. Radiochemical purity of the product was greater than 98% as determined by HPLC and TLC. The specific activity was 3.7–4.8 TBq/mmol at the end of synthesis when 2.6 MBq of ^{11}C-methyl iodide was utilized in the procedure.

Table 1 Biodistribution of ^{11}C-2′-IDZ in mice

Organ	Time (min)			
	1	5	20	60
Blood	1.97 (0.25)*	1.24 (0.18)	1.06 (0.06)	0.84 (0.18)
Liver	3.62 (0.36)	8.53 (1.72)	7.54 (0.45)	5.59 (1.15)
Kidney	7.30 (0.66)	6.22 (1.21)	3.99 (0.23)	2.70 (0.69)
Lung	7.48 (1.28)	3.11 (1.26)	2.50 (0.31)	1.45 (0.41)
Heart	10.26 (2.07)	3.75 (0.74)	2.61 (0.07)	1.63 (0.41)
Brain	6.37 (2.26)	4.22 (0.98)	1.98 (0.17)	0.90 (0.10)
Br/Bl**	3.14 (0.87)	3.27 (0.62)	1.88 (0.17)	1.02 (0.10)

* Each value is the mean (S.D.) for 4 animals (% dose/g organ).
** Brain-to-blood ratio.

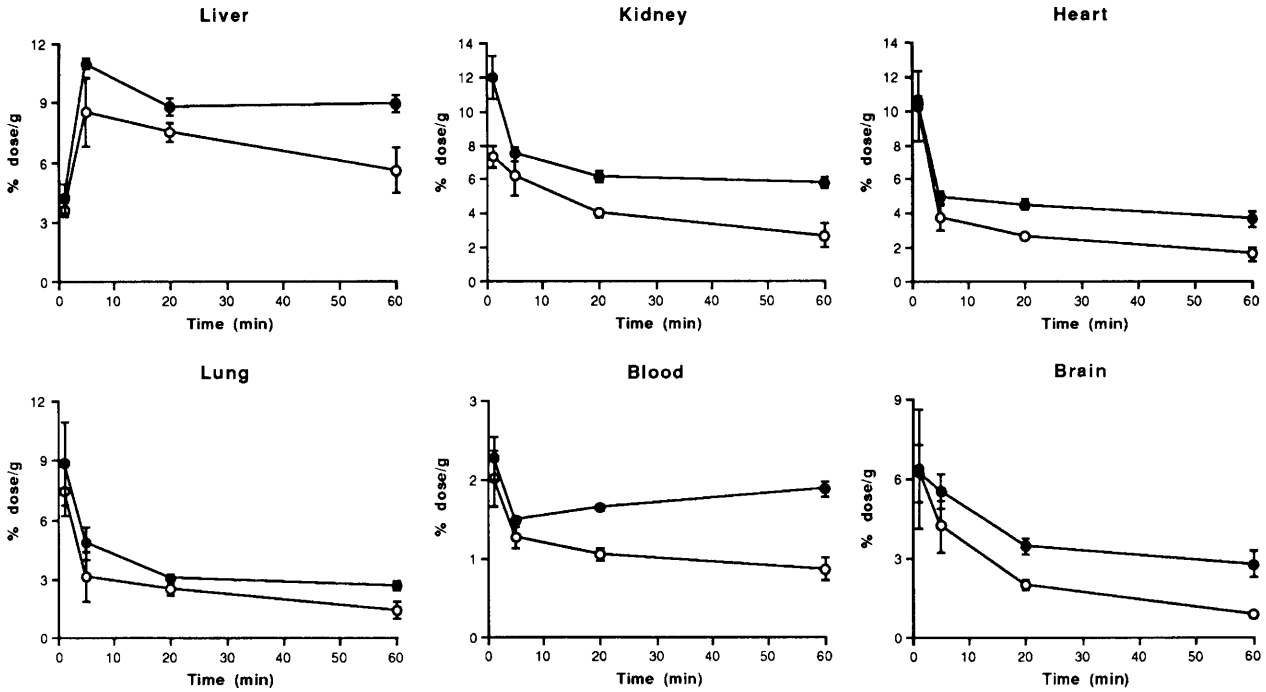

Fig. 2 Comparison of the biodistribution of ^{11}C-labeled and ^{125}I-labeled 2′-IDZ in mice after intravenous injection. Each point is the mean of 4 animals. ○: ^{11}C-2′-IDZ, ●: ^{125}I-2′-IDZ.

In the biodistribution study, ^{11}C-2′-IDZ was found to rapidly enter the brain, and high uptake was observed during the early phase, after which the brain levels declined with time (Table 1). The radioactivity was cleared rapidly from the blood, and the highest brain-to-blood ratio of 3.3 was obtained at 5 min after injection. At the initial sampling point of 1 min, there was a higher uptake in the heart and lungs than in the brain, but the radioactivity in these organs fell below the brain level at 5 min after injection. The kidneys also showed a high initial uptake, but the clearance of radioactivity was slower than in the heart and lungs. If the distribution of a compound is only dependent on blood perfusion, the organs with a high uptake show more rapid washout.[25] Thus, the results obtained in this study suggest that the distribution of ^{11}C-2′-IDZ was not only dependent on perfusion. In addition, the liver showed a rapid increase in radioactivity, which reached a maximum at 5 min after injection, and then gradually decreased with time.

The biodistribution of ^{11}C-2′-IDZ was almost the same as that of ^{125}I-2′-IDZ within 5 min after injection. However, the levels of ^{11}C-2′-IDZ in all organs tested were lower than those of ^{125}I-2′-IDZ in the late phase (Fig. 2).

In the liver, diazepam and its analogs are known to be rapidly metabolized to 3-hydroxylated and N-demethylated compounds, which are still pharmacologically active and can redistribute in other organs.[16,19,20,26–29] In contrast, it has been reported that diazepam is not metabolized in the brain.[29] If 2′-IDZ was metabolized in a manner similar to diazepam, it would be transformed to 3-hydroxylated and N-demethylated analogs in the liver (Fig. 3). 3-Hydroxylation is unlikely to cause the loss of radioactivity from either ^{11}C-2′-IDZ or ^{125}I-2′-IDZ. On the other hand, it can be predicted that,

after N-demethylation, ^{11}C-N-methylated 2′-IDZ would lose its radioactivity by excretion through the lungs as $^{11}CO_2$, while ^{125}I-2′-IDZ would be transformed in the liver to an N-demethylated metabolite that traps its radioactivity and is distributed to some other organs including the brain (Fig. 3).[25,29,30] Thus, the difference in the biodistribution of these two compounds may be due to their metabolism by N-demethylation. In fact, radio-HPLC analysis (corrected for decay) of brain homogenates obtained at 20 min after radioligand injection showed that, apart from the hydrophilic metabolite (R_t = 8 min), the number of major metabolites was one (R_t = 42 min) and two (R_t = 42 min and 44 min) for ^{11}C-2′-IDZ and ^{125}I-2′-IDZ, respectively (Fig. 4). The metabolites eluted at 42 min and 44 min would correspond to the 3-hydroxy and N-demethyl analogs, respectively, as judged from their retention times on HPLC. On the other hand, both compounds showed the same HPLC profiles at 5 min after injection, at which time most of the radioactivity (> 94%) in the brain homogenates remained as 2′-IDZ. These results indicate that this compound enters the brain in its unchanged form soon after administration.

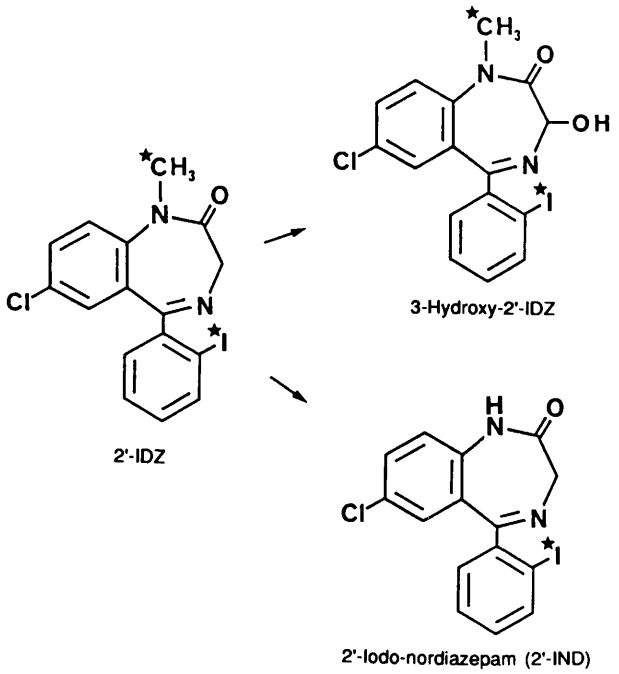

Fig. 3 Possible metabolic pathway of 2′-IDZ in mice. *C: ^{11}C, *I: ^{125}I

The regional distribution of radioactivity in the mouse brain is shown in Table 2. The cortex tended to show a higher uptake of radioactivity than the other brain regions until 5 min after injection, although the regional variation in distribution became small after 20 min. This differential regional distribution during the early period paralleled the distribution of benzodiazepine receptors shown by previous *in vitro* and PET studies,[6,8,9,31,32] suggesting that

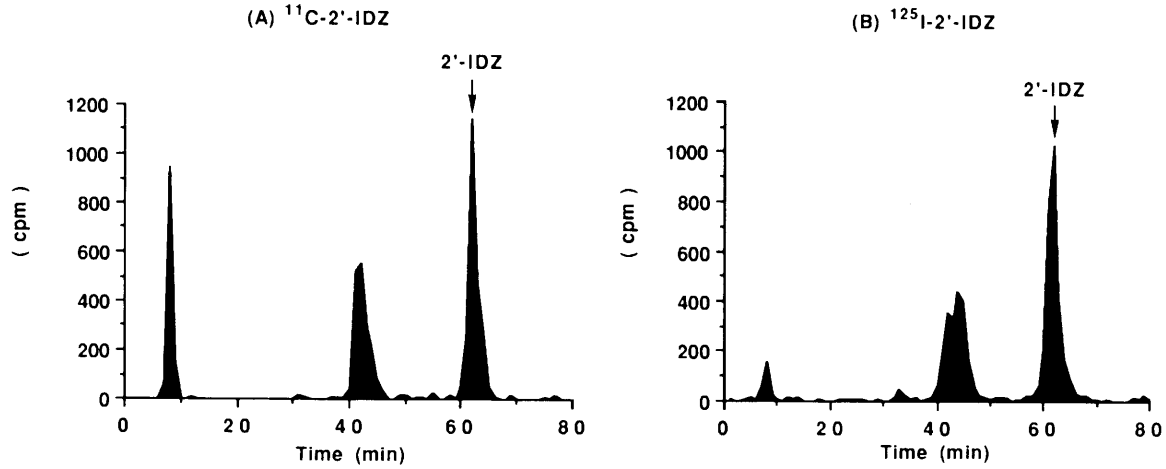

Fig. 4 HPLC profiles of the methanol-extractable fraction of brain homogenates at 20 min after intravenous injection of ^{11}C-2′-IDZ (A) and ^{125}I-2′-IDZ (B). Eluates were collected at 1-min intervals, and the radioactivity of each fraction was determined and corrected for decay.

Table 2 Regional cerebral distribution of ^{11}C-2′-IDZ in mice

Region	Time (min)			
	1	5	20	60
Cortex	8.04 (1.96)	5.18 (0.69)	2.14 (0.27)	0.98 (0.07)
Striatum	7.38 (1.79)	4.18 (0.69)	1.74 (0.31)	0.79 (0.11)
Hippocampus	6.22 (1.64)	4.46 (0.74)	1.90 (0.25)	0.89 (0.03)
Cerebellum	7.38 (2.07)	4.50 (0.61)	1.90 (0.14)	0.85 (0.07)

Each value is the mean (S.D.) for 4 animals (% dose/g tissue).

^{11}C-2′-IDZ bound to benzodiazepine receptors in the brain.

In conclusion, ^{11}C-2′-IDZ showed prominent uptake by the brain regions containing benzodiazepine receptors soon after injection, and therefore appears to be a potentially useful radiopharmaceutical for PET studies of cerebral benzodiazepine receptors. In addition, our results suggested that 2′-IDZ had the potential for use in direct comparative PET and SPECT imaging for the localization and quantification of benzodiazepine receptors with a single tracer with different radioactive moieties (i.e., ^{11}C-2′-IDZ and ^{123}I-2′-IDZ).

REFERENCES

1. Savic I, Roland P, Sedvall G, Persson A, Pauli S, Widen L. *In-vivo* demonstration of reduced benzodiazepine receptor binding in human epileptic foci. *Lancet* II: 863–866, 1988.
2. Bartenstein P, Ludolph A, Schober O, Lottes G, Scheidhauer K, Sciuk J, et al. Benzodiazepine receptors and cerebral blood flow in partial epilepsy. *Eur J Nucl Med* 18: 111–118, 1991.
3. Whitehouse PJ, Trifiletti RR, Jones BE, Folstein S, Price DL, Snyder SH, et al. Neurotransmitter receptor alterations in Huntington's disease: autoradiographic and homogenate studies with special reference to benzodiazepine receptor complexes. *Ann Neurol* 18: 202–210, 1985.
4. Trifiletti RR, Snowman AM, Whitehouse PJ, Marcus KA, Snyder SH. Huntington's disease: increased number and altered regulation of benzodiazepine receptor complexes in frontal cerebral cortex. *Neurology* 37: 916–922, 1987.
5. Owen F, Poulter M, Waddington JL, Mashal RD, Crow TJ. [H-3]Ro 05-4864 and [H-3]flunitrazepam binding in kanate-lesioned rat striatum and in temporal cortex of brains from patients with senile dementia of Alzheimer type. *Brain Res* 278: 373–375, 1983.
6. Shinotoh H, Yamasaki T, Inoue O, Itoh T, Suzuki K, Hashimoto K, et al. Visualization of specific binding sites of benzodiazepine in human brain. *J Nucl Med* 27: 1593–1599, 1986.
7. Ishiwata K, Yanai K, Ido T, Miura-Kanno Y, Kawashima K. Synthesis and biodistribution of [^{11}C]fludiazepam for imaging benzodiazepine receptors. *Nucl Med Biol* 15: 365–371, 1988.
8. Beer H-F, Blauenstein PA, Hasler PH, Delaloye B, Riccabona G, Bangerl I, et al. *In vitro* and *in vivo* evaluation of iodine-123-Ro 16-0154: a new imaging agent for SPECT investigations of benzodiazepine receptors. *J Nucl Med* 31: 1007–1014, 1990.
9. Sadzot B, Frost JJ. Benzodiazepine receptors. *In* Quantitative Imaging: Neuroreceptors, Neurotransmitters, and Enzymes, Frost JJ, Wagner Jr HN (eds.), New York, Raven Press, pp. 109–127, 1990.
10. Wilson AA, Dannals RF, Ravert HT, Wagner Jr HN. Preparation of [^{11}C]- and [^{125}I]IMB: a dopamine D-2 receptor antagonist. *Appl Radiat Isot* 40: 369–373, 1989.
11. Van Dort ME, Kilbourn MR, Chakraborty PK, Richfield EK, Gildersleeve DL, Wieland DM. Iodine-125 and fluorine-18 labeled aryl-1,4-dialkylpiperazines: potential radiopharmaceuticals for *in vivo* study of the dopamine uptake system. *Appl Radiat Isot* 43: 671–680, 1992.
12. Wong DF, Wilson AA, Chen C, Minkin E, Dannals RF, Ravert HT, et al. *In-vivo* studies of [I-125]idodbenzamide and [C-11]iodobenzamide—a ligand suitable for positron emission tomography and single photon emission tomography imaging of cerebral D(2)-dopamine receptors. *Synapse* 12: 236–241, 1992.
13. Kilbourn MR, Zalutsky MR. Research and clinical potential of receptor based radiopharmaceuticals. *J Nucl Med* 26: 655–662, 1985.
14. Arnett CD, Shiue CY, Wolf AP, Fowler JS, Logan J, Watanabe M. Comparison of three ^{18}F-labeled butyrophenone neuroleptic drugs in the baboon using positron emission tomography. *J Neurochem* 44: 835–844, 1985.
15. Kung HF. Radiopharmaceuticals for CNS receptor imaging with SPECT. *Nucl Med Biol* 17: 85–92, 1990.
16. Van Der Kleijn E. Kinetics of distribution and metabolism of diazepam and chlordiazepoxide in mice. *Arch Int Pharmacodyn* 178: 193–215, 1969.
17. Sternbach LH, Randall LO, Banziger R, Lehr H. Structure-activity relationships in the 1,4-benzodiazepine series. *In* Drugs Affecting The Central Nervous System, Volume 2, Burger A (ed.), New York, Mercel Dekker Inc., pp. 237–264, 1968.
18. Boera PA, Gilli G, Bertolasi V. Application of the Free-Wilson model to the analysis of three different pharmacological activity tests in benzodiazepines. *Farmaco-Ed Sc* 34: 1073–1082, 1979.
19. Haefely W, Kyburz E, Gerecke M, Mohler H. Recent advances in the molecular pharmacology of benzodiazepine receptors and in the structure-activity relationships of their agonists and antagonists. *In* Advances in Drug Research, Volume 14, Testa B (ed.), London, Academic Press Inc., pp. 165–322, 1985.
20. Gupta SP, Saha RN, Muichandani V. Quantitative structure-activity relationship studies on benzodiazepine receptor binding: recognition of active sites in receptor and modelling of interaction. *J Mol Recognit* 5: 75–80, 1992.
21. Saji H, Iida Y, Ariyoshi K, Nakatsuka I, Kataoka M, Yoshitake A, et al. An approach for benzodiazepine receptor binding assay with radioiodinated ligand: ^{125}I-labeled diazepam derivative. *Biol Pharm Bull* 16: 513–515, 1993.
22. Saji H, Iida Y, Nakatsuka I, Kataoka M, Ariyoshi K, Magata Y, et al. Radioiodinated 2′-iododiazepam: a potential imaging agent for SPECT investigations of benzodiazepine receptors. *J Nucl Med* 34: 932–937, 1993.
23. Saji H, Magata Y, Yamada Y, Tajima K, Yomekura Y, Konishi J, et al. Synthesis of (S)-N-[methyl-^{11}C]nicotine and its regional distribution in the mouse brain: a potential tracer for visualization of brain nicotinic receptors by positron emission tomography. *Chem Pharm Bull* 40: 734–736, 1992.
24. Glowinski J, Iversen L. Regional studies of catecholamines in the rat brain-I: the disposition of [^{3}H]norepinephrine, [^{3}H]dopamine and [^{3}H]dopa in various regions on the brain. *J Neurochem* 13: 655–669, 1966.
25. Comar D, Maziere M, Cepeda C, Godot J-M, Menini C, Naquet R. The kinetics and displacement of [^{11}C]flunitrazepam in the brain of the living baboon. *Eur J Pharmacol* 75: 21–26, 1981.
26. Kaplan SA, Jack ML. Metabolism of the benzodiazepines:

pharmacokinetic and pharmacodynamic considerations. *In* The Bezodiazepines: From Molecular Biology to Clinical Practice, Costa E (ed.), New York, Raven Press, pp. 173–199, 1983.
27. Xie X, Steiner H, Bickel MH. Kinetics of distribution and adipose tissue storage as a function of lipophilicity and chemical structure. *Drug Metab Disposition* 19: 15–19, 1991.
28. Richelson E, Nelson A, Neeper R. Binding of benzodiazepines and some major metabolites at their sites in normal human frontal cortex *in vitro*. *J Pharmacol Exp Ther* 256: 897–901, 1991.
29. Schwartz MA, Postma E. Metabolism of diazepam *in vitro*. *Biochem Pharmacol* 17: 2443–2449, 1968.
30. Saji H, Iida Y, Nakatsuka I, Kataoka M, Yoshitake A, Yokoyama A. Evaluation of radioiodinated desmethyl diazepam as radiotracer for *in vivo* quantitative studies of benzodiazepine receptors. *J Labelled Compd Radiopharm* 32: 340–341, 1993.
31. Richard JG, Mohler H. Benzodiazepine receptors. *Neuropharmacology* 23: 233–242, 1984.
32. Samson Y. Hantraye P, Baron JC, Soussaline F, Comar D, Maziere M. Kinetics and displacement of [C-11]Ro 15-1788, a benzodiazepine antagonist, studies in human brain *in vivo* by positron tomography. *Eur J Pharmacol* 110: 247–251, 1985.