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Myocardial accumulation of a dopamine D_2 receptor-binding radioligand, 2′-iodospiperone

Hideo SAJI,* Yoshiharu YONEKURA,** Kiyoko TANAHASHI,* Yasuhiko IIDA,* Yasushi IWASAKI,** Yasuhiro MAGATA,** Junji KONISHI,** and Akira YOKOYAMA*

**Faculty of Pharmaceutical Sciences and **School of Medicine, Kyoto University, Kyoto 606–01, Japan*

^{125}I-2′-iodospiperone (2′-ISP), which has a high and selective affinity for dopamine D_2 receptors, produced a high myocardial accumulation of radioactivity in the early phase after intravenous injection into mice. A human scintigraphic study also showed that the myocardium was clearly visualized soon after intravenous injection of the tracer. Analysis of the myocardial homogenate obtained from mice showed that ^{125}I-2′-ISP was metabolically stable and was taken up the myocardium in its intact form. Administration of spiperone significantly reduced the myocardial uptake of ^{125}I-2′-ISP in mice. Treatment with haloperidol and (+) butaclamol, which have a high affinity for dopamine D_2 receptors, also tended to reduce the myocardial uptake of radioactivity, while (−)-butaclamol, which has no affinity for dopamine D_2 receptors, caused no change in uptake. These findings suggest that the myocardial accumulation of 2′-ISP occurred in association with dopamine D_2 (DA_2) receptors.

Key words: radioiodinated 2′-iodospiperone, myocardium, dopamine receptor, SPECT.

INTRODUCTION

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[1–3] (Fig. 1). *In vitro* and *in vivo* studies in mice and rats have shown that this compound binds to dopamine D_2 receptors in the central nervous system with a high affinity and stereospecificity.[1,2] Furthermore, a preliminary human imaging study with ^{123}I-2′-ISP showed its specific uptake by the basal ganglia, a region of the brain known to have a high density of dopamine D_2 receptors.[3]

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Address for correspondence: Hideo Saji, Department of Radiopharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto 606–01, JAPAN

In the myocardium, dopamine D_2 (DA_2) receptors are located on postsynaptic sympathetic nerves and inhibit the release of norepinephrine from sympathetic nerve storage sites.[4,5] In this study, the myocardial accumulation of radioiodinated 2′-ISP was investigated in mice and humans, and its usefulness for imaging of the myocardium was assessed.

MATERIALS AND METHODS

^{123}I-sodium iodide produced by the indirect method using the ^{127}I (p, 5n) ^{123}Xe reaction was obtained from Nihon Medi-Physics Co. Ltd. (specific activity: 8.88 TBq/μmol), and ^{125}I-sodium iodide (specific activity: 81.4 GBq/μmol) was purchased from Amersham International, Plc. All other chemicals used were of reagent grade. Male ddY mice were supplied by Japan SLC Co. Ltd. (Hamamatsu, Japan).

Synthesis of ^{123}I- and ^{125}I-2′-ISP

^{123}I- and ^{125}I-2′-ISP were synthesized by a bromine-

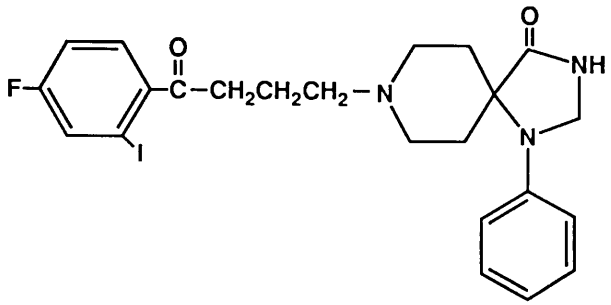

Fig. 1 Chemical structure of 2'-iodospiperone (2'-ISP).

radioiodine exchange reaction. A solution of ^{123}I- or ^{125}I-sodium iodide was evaporated to dryness. To the residue was added 37.5 μl of an 80% aqueous solution of dimethylformamide (DMF) containing 200 μg of 2'-bromospiperone, 13 μg of sodium iodine, 200 μg of copper sulfate pentahydrate, and 180 μg of 1-naphthalenesulfonic acid dihydrate. After heating at 95°C for 1 hr, the product was purified by high-performance liquid chromatography (HPLC) (Lichrosorb RP-18, $H_2O/CH_3OH/(C_2H_5)_3N$=50/75/1; flow rate: 2 ml/min).

The radiochemical purity of the products was more than 98% as determined by thin-layer chroatography (TLC) (CH_2Cl_2/C_2H_2OH=6/1, Rf=0.6–0.7) and HPLC (Lichrosorb RP-18, $H_2O/CH_3OH/(C_2H_5)_3N$=50/75/1; flow rate: 2 ml/min; Rt=39 min). The specific radioactivity of ^{123}I- and ^{125}I-2'-ISP was 9.25 GBq/μmol and 1.1 GBq/μmol respectively, as estimated by the ultraviolet absorbance at 249 nm.

Biodistribution in mice

Male ddY mice weighing an average of 30 g were injected intravenously with ^{125}I-2'-ISP (18.5 kBq in 0.1 ml of ethanolic saline solution, 0.29 μg/kg). At designated times afterward, the mice were killed by decapitation, blood samples were collected by cardiac puncture, and the organs of interest were removed. All samples were weighed, and the radioactivity was counted in a well-type NaI scintillation counter.

Metabolic studies in mouse myocardium

Mice were injected intravenously with 18.5 kBq of ^{125}I-2'-ISP, and were killed at 10 min after injection. The hearts were removed immediately and homogenized in a mixture of 340 μl of methanol, 120 μl of water, 30 μl of dimethyl sulfoxide (DMSO) and 30 μl of 2N HCl. After centrifugation, the precipitate was washed with a mixture of 340 μl of methanol, 120 μl of water, 30 μl of DMSO and 30 μl of 2N HCl, and the washings were combined with the supernatant. The solution was then analyzed by TLC (CH_2Cl_2/C_2H_5OH=6/1).

Effect of various drugs on myocardial uptake in mice

Several dopaminergic drugs were injected intravenously into mice along with 18.5 kBq of ^{125}I-2'-ISP. Spiperone (1 mg/kg), haloperidol (1 mg/kg), or unlabeled 2'-ISP (1, 5, 10 mg/kg) in a mixture of ethanol and 2% acetic acid (2: 100) was injected simultaneouly with the radioligand, and (+) or (−)-butaclamol (5 mg/kg) in ethanolic saline solution was injected intraperitoneally 30 min before radioligand administration. The animals were killed 10 min after radioligand administration, their hearts were removed, and the radioactivity was counted as described above.

Human scintigraphic study

Imaging was performed with a scintillation camera with a low-energy collimator (Gamma View-E RC150E, Hitachi).

An ^{123}I-2'-ISP solution (59 MBq, 48 ng/kg) was injected into a healthy male volunteer and serial planar images of the chest and abdomen were obtained for 60 min afterwards.

RESULTS AND DISCUSSION

One strategy for imaging the myocardium and evaluating its function is to utilize a radiolabled ligand that is known to bind to specific myocardial receptors.[6,7] Accordingly, this study examined the localization of 2'-ISP, a ligand for dopamine D_2 receptors,[1–3] which are located on postsynaptic sympathetic nerves in the myocardium.[4,5]

Table 1 shows the biodistribution of ^{125}I-2'-ISP in mice. Radioactivity was cleared rapidly from the blood. In the heart, a high uptake was observed during the early phase after injection, following which it declined with time. Therefore, a high heart-to-blood ratio of 4.4–10.8 was obtained during the first 10 min of the study. The lungs showed a high initial uptake, but they cleared rapidly. The liver and kidneys showed a steep increase in uptake until 5 min, and thereafter their radioactivity remained nearly constant.

The scintigraphic study in the human volunteer was in agreement with the results of the biodistribution study in mice, and a high myocardial accumulation of radioactivity was observed in the early phase after injection. Fig. 2 presents an image obtained at 15 min after injection of ^{123}I-2'-ISP, a time when the myocardium was clearly visualized. The high accumulation in the myocardium and the success of myocardial imaging in the human volunteer suggested that further *in vivo* evaluation of this tracer was justified.

At 10 min after the intravenous injection of ^{125}I-2'-ISP into mice, the radioactivity in the extract

Table 1 Biodistribution of ^{125}I-2′-ISP in mice

Organ	Time (min)				
	2	5	20	20	30
Blood	1.43 (0.59)	1.41 (0.48)	1.39 (0.76)	1.43 (0.21)	1.41 (0.22)
Heart	14.11 (2.31)	7.57 (1.89)	5.48 (0.98)	3.20 (0.27)	2.45 (0.27)
Lung	31.23 (3.79)	23.02 (5.52)	17.19 (2.43)	11.50 (1.88)	10.43 (2.37)
Liver	5.37 (0.57)	8.61 (1.82)	7.89 (0.99)	9.07 (0.97)	10.22 (1.23)
Kidney	14.60 (1.09)	22.25 (5.76)	20.80 (2.39)	20.96 (1.68)	17.73 (4.01)

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

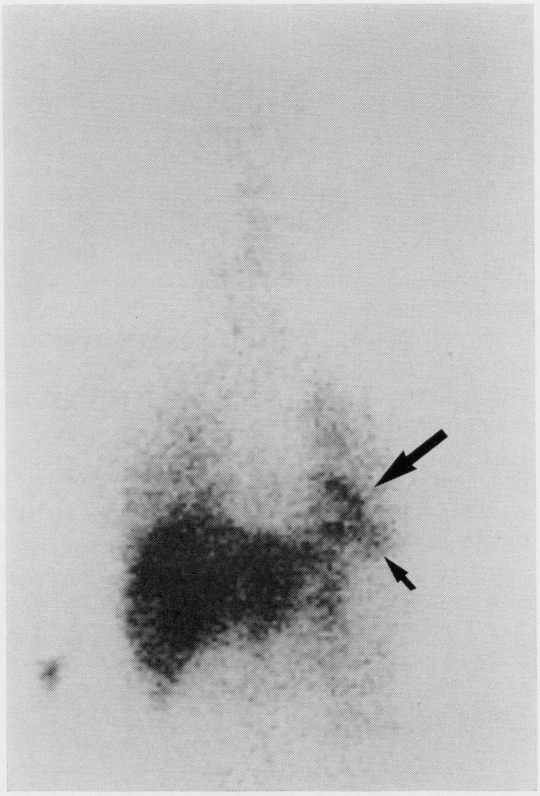

Fig. 2 Scintigram obtained with ^{123}I-2'-ISP in a healthy human volunteer. The image was obtained 15 min after intravenous injection of the radiotracer. Arrows indicate the myocardium.

Table 2 Effect of various drugs on the myocardial uptake of ^{125}I-2'-ISP at 10 min after injection into mice

Drug	% uptake/g	% of control
None (control)	5.48 (0.98)	100
Spiperone	3.86 (0.72)*	70
Haloperidol	5.04 (0.27)	92
(+) Butaclamol	4.97 (1.02)	91
(−) Butaclamol	5.67 (1.02)	103

Each value is the mean (S.D.) of 4 animals.
* $P<0.05$ compared with the control by Student's t-test.

of myocardial homogenate was analyzed by TLC. Approximately 80% of the radioactivity in the homogenate was extractable by our organic solvent technique, and the extractable material displayed a single peak which co-migrated with authentic 2′-ISP. These results showed that most of the myocardial uptake and distribution of the tracer occurred in the intact form and indicated its metabolic stability in the myocardium.

To assess the dopaminergic nature of the ^{125}I-2′-ISP binding sites, the effects of several drugs on the myocardial uptake of this tracer were studied at 10 min after injection. As shown in Table 2, the administration of spiperone significantly reduced the myocardial uptake of radioactivity. Treatment with haloperidol and (+) butaclamol also tended to reduce the myocardial uptake. We also tested (−) butaclamol, which has no affinity for dopamine D_2 receptors, and found that it caused no change in the myocardial uptake of radioactivity. Furthermore, the effect of the carrier itself on the myocardial uptake of ^{125}I-2′-ISP at 10 min after injection was investigated with various doses of unlabeled 2′-ISP (1–10 mg/kg). As shown in Fig. 3, the myocardial uptake of radioactivity was decreased in a dose-dependent manner by unlabeled 2′-ISP injection. Since 2′-ISP is highly selective for dopamine D_2 receptors,[1,2] these displacement studies indicated that ^{125}I-2′-ISP binds to dopamine D_2 receptors (DA_2 receptors) in the myocardium. However, a significant amount of radioactivity remained in the myocardium after displacement. Quantitative studies of neuroreceptors *in vivo* require the use of a radioligand that displays a fairly high specific to non-specific binding ratio (ideally$>$10).[8,5] Thus, if the fraction displaced by dopaminergic drugs in this study represents the true level of specific binding, the specific binding of 2′-ISP may be considerably less than ideal for quantitatively evaluating myocardial dopamine DA_2 receptors, although the imaging of such receptors would be possible. However, it is not clear at present that this displaced fraction represents the true specific binding, i.e., that the

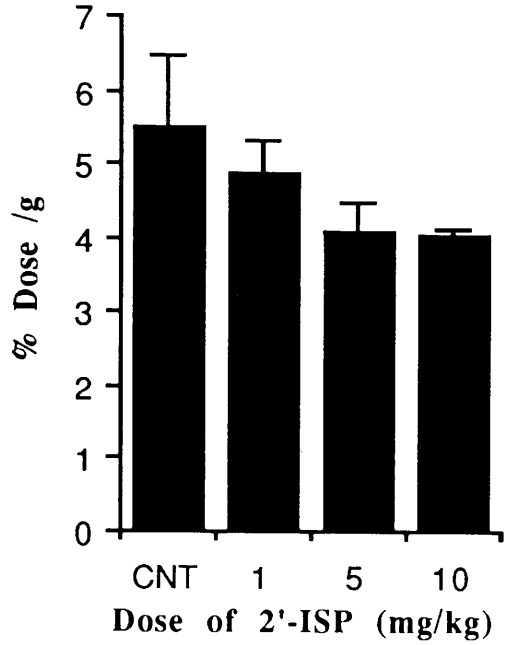

Fig. 3 Effect of unlabeled 2'-ISP on the myocardial uptake of ^{125}I-2'-ISP at 10 min after injection into mice. CNT: control.

fraction remaining after displacement represents true nonspecific binding. As has been found for other receptor-binding radiotracers,[10–13] the retention of radioactivity after displacement might have been due to the presence of high-capacity binding sites or the so-called "occultation" phenomenon in which the receptor becomes refractory to competing ligands following initial binding. However, this high retention was not caused by metabolism of the compound, since the myocardial homogenate study showed that 2'-ISP remained intact in the myocardium. Further studies are required to evaluate the *in vivo* behavior of this ligand.

In conclusion, the results obtained in this study indicate that 2'-ISP showed rapid and high myocardial uptake and that its distribution was partly associated with dopamine DA_2 receptors. Although further investigations are still required, 2'-ISP seems to hold some promise for use in functional studies of the myocardium.

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REFERENCES

1. Nakatsuka I, Saji H, Shiba K, et al: In vitro evaluation of radioiodinated butyrophenones as radiotracer for dopamine receptor study. *Life Sci* 41: 1989–1997, 1987
2. Saji H, Nakatsuka I, Shiba K, et al: Radioiodinated 2'-iodospiperone: a new radioligand for in vivo dopamine receptor study. *Life Sci* 41: 1999–2006, 1987
3. Saji H, Iida Y, Magata Y, et al: Preparation of ^{123}I-labeled 2'-iodospiperone and imaging of D_2 dopamine receptors in the human brain using SPECT. *Nucl Med Biol* 19: 523–529, 1992
4. Murphy MB, Bass AS, Goldberg LI: Modulation of the dopaminergic system in cardiovascular therapeutics. In Enzymes as Targets for Drug Design, Palfreyman MG, McCann PP, Lovenberg W, Temple JG, Sjoerdsma A, (eds), San Diego, *Academic Press*, pp 17–29, 1990
5. Terai M, Usuda S, Maeno H: Dopamine receptors. *Protein Nucleic Acid Enzyme* 29: 1328–1337, 1984
6. Eckelman WC, Grissom M, Conklin J, et al: In vivo competition studies with analogues of 3-quinuclidinyl benzilate. *J Pharm Sci* 73: 529–534, 1984
7. Syrota A, Comar D, Paillotin G, et al: Muscarinic cholinergic receptor in the human heart evidenced under physiological conditions by positron emission tomography. *Proc Natl Acad Sci USA*: 82: 584–588, 1985
8. Eckelman WC, Reba RC, Gibson RE, et al: Receptor-binding radiotracers: a class of potential radiopharmaceuticals. *J Nucl Med* 20: 350–357, 1979
9. Kung HF: Radiopharmaceuticals for CNS receptor imaging with SPECT. *Nucl Med Biol* 17: 85–92, 1990
10. Beer HF, Blauenstein PA, Hasler PH, 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
11. Pappata S, Cornu P, Samson Y, et al: PET study of carbon-11-PK 11195 binding to peripheral type benzodiazepine sites in glioblastoma: a case report. *J Nucl Med* 32: 1608–1610, 1991
12. Rogers GA, Parsons SM: Very high-affinity analogs of vesamicol, and an acetylcholine storage inhibitor. *Neuroreport* 1: 11–14, 1990
13. Efange SMN, Dutta AK, Michelson RH, et al: Radioiodinated 2-hydroxy-3-4(-iodophenyl)-1-(4-phenylpiperidinyl)propane: poteitial radiotracer for mapping central cholinergic innervation in vivo. *Nucl Med Biol* 19: 337–348, 1992