In Vitro and In Vivo Characterization of R(+)-FIDA2: A Dopamine D2-Like Imaging Agent

Janet M. Vessotskie, Mei-Ping Kung, Sumalee Chumpradit, Gemma Romaniello, William McElgin, Dana Frederick, Robert T. Malison and Hank F. Kung

Departments of Pharmacology, Psychiatry and Radiology, University of Pennsylvania, Philadelphia, Pennsylvania

R(+)-FIDA2, (R)-(+)-2,3-dimethoxy-5-iodo-N-[(1-(4'-fluorobenzyl)-2-pyrrolidinyl)-methyl]benzamide, is a new dopamine D2-like receptor imaging agent that can be labeled with either ^{123}I or ^{18}F for SPECT or PET imaging. The purpose of this study was to characterize its in vitro and in vivo binding properties. **Methods:** In vitro binding studies using [^{125}I]R(+)-FIDA2 were performed in Sf9 cells expressing dopamine D2 or D3 receptors and in rat basal forebrain homogenates, which contain a high density of dopamine D2-like receptors. A series of in vivo SPECT imaging studies in nonhuman primates (cynomologous monkeys) were performed by intravenously injecting 7.1 ± 1.0 mCi of [^{123}I]R(+)-FIDA2. At least one control study and one displacement experiment, in which a cold compound was injected intravenously 90 min after tracer injection, was performed in each monkey. Data were acquired in 10-min frames for 180 min, and the activity in regions of interest (basal ganglia and cerebellum) were plotted versus time. **Results:** Iodine-125-R(+)-FIDA2 displayed K_d values for D2 and D3 receptor subtypes expressed in Sf9 cells of 0.11 and 0.04 *nM*, respectively. As expected, SPECT images of monkey brain (transaxial sections, 2 mm) showed that the radioactivity was localized in the area of the basal ganglia and reached peak concentrations in 11.5 ± 5.8 min postinjection. An injection of R(+)7-OH-PIPAT, a new ligand that is selective for dopamine D3 receptors and the high affinity state of dopamine D2 receptors, did not show significant displacement of [^{123}I]R(+)-FIDA2 binding in the basal ganglia. **Conclusion:** These studies indicate that R(+)-FIDA2 may be a useful ligand for in vitro pharmacological characterization and in vivo imaging of CNS dopamine D2-like receptors.

Key Words: R(t)-FIDA2; dopamine D2-like receptors; single-photon emission computed tomography

J Nucl Med 1995; 36:1282–1288

In recent years, the imaging of dopamine receptors in the CNS has received much attention because abnormalities in dopamine receptor function have been implicated in neuropsychiatric disorders (*1,2*). Neuroleptics, such as haloperidol, display antagonistic activity at the dopamine D2 receptor subtype, and it is believed that this activity is responsible for the therapeutic effect of these compounds. Techniques in molecular biology, however, have revealed that dopamine D3 and D4 receptor subtypes are very similar to dopamine D2 receptors (*3–5*). Neuroleptics generally have similar affinities for the D2 and D3 receptor subtypes, however, this is not the case for the D4 receptor. Thioproperiozine, for example, is a hundredfold more selective at D2 receptors versus D4 receptors (*6*), whereas clozapine, an atypical neuroleptic, displays more selective binding to the dopamine D4 receptor subtype. Therefore, occupancy of D2, D3 and D4 receptor subtypes may be responsible for the therapeutic efficacy of neuroleptics (*7*). Using in vivo imaging techniques with SPECT and PET to investigate receptor occupancy can provide a powerful tool for the evaluation of central nervous system (CNS) function.

A variety of substituted benzamide derivatives possessing antidopaminergic properties have been used as SPECT imaging agents (*8–10*). Three agents that are well characterized are (S)-N-[(1-ethyl-2-pyrrolidinyl)]methyl-2-hydroxy-3-iodo-6-methoxybenzamide (IBZM) (*11*), (S)-5-iodo-7-N-[(1-ethyl-2-pyrrolidinyl)methyl]carboxamido-2,3, dihyrobenzofuran (IBF) (*12,13*) and (S)-(−)-N-[(1-ethyl-2-pyrrolidinyl)methyl]-5-iodo-2,3,dimethoxybenzamide (epidepride) (*14,15*). These benzamides are dopamine D2/D3 receptor antagonists and display similar high affinity (K_d in subnanomolar range) for each receptor subtype. In vivo imaging studies in monkeys indicated that these compounds accumulated in the basal ganglia and thus resulted in basal ganglia/cerebellum ratios of 1.5, 2.1 (*16*) and 4 (*17–19*), respectively, at 1 hr postinjection. The target-to-nontarget ratios for IBZM and IBF are adequate but could be improved. Epidepride displays a good target-to-nontarget ratio, but has a long half-life in the basal ganglia. Thus, the kinetic modeling of epidepride is complicated (*20*) because several hours of constant tracer infusion are required for this compound to reach an equilibrium state for data acquisition. It has been shown that each of these agents can be displaced by selective dopamine D2-like receptor

Received Jul. 13, 1994; revision accepted Oct. 3, 1994.

For correspondence or reprints contact: Janet M. Vessotskie, MS, University of Pennsylvania, Department of Pharmacology, 3700 Market Street, Room 305, Philadelphia, PA 19104.

antagonists, such as haloperidol, therefore demonstrating in vivo reversible binding of these compounds to D2-like receptors.

In PET imaging, the most widely used agents are ^{11}C-raclopride (*21*) and ^{11}C-N-methyl-spiperone (NMSP) (*22–24*). Both of these ligands display good affinities for dopamine D2/D3 receptors, but NMSP also binds to 5-HT$_2$ and α_1 adrenergic receptors (*25,26*). Carbon-11-raclopride binds reversibly, but NMSP displays irreversible binding during the time frame of a PET study (*27*).

Like the ligands used for in vivo imaging, both SPECT and PET imaging techniques have advantages and disadvantages. In general, it is well recognized that PET has higher resolution, higher sensitivity and a better quantitation capability (*28*). SPECT, however, has the advantage of being more readily available, less expensive to perform, and, since it does not need an on-site cyclotron, less technically demanding. The current status in developing receptor-specific ligands is such that the data obtained from PET cannot be easily transferred to SPECT and vice versa. This incompatibility is due to the fact that different agents are used for each modality. Although in many cases these ligands are close analogs, they are not the same molecule, and therefore, pharmacokinetic and metabolic differences prevent their cross comparison.

In the evolving field of SPECT and PET imaging of dopamine D2-like receptors, there are no clearly superior ligands. An ideal agent must have a high target-to-nontarget ratio and display a kinetic profile that is suitable for quantitation of receptor density. Also, a ligand that can be used in both PET and SPECT is desirable. By using a single molecule which can be labeled with either ^{18}F or ^{123}I for both PET or SPECT, one can expect the same pharmacological profile and the same toxicology. It is highly likely, however, that ^{123}I and ^{18}F have different metabolic patterns therefore, careful analysis of the in vivo radiometabolites is necessary. In an attempt to meet these criteria, a dual-labeling dopamine D2-like agent, (R)-(+)-2,3,dimethoxy-5-iodo-N-[(1-(4′-fluorobenzyl)-2-pyrrolidinyl)-methyl]benzamide, R(+)-FIDA2, was prepared (*29*). Preliminary studies indicated that R(+)-FIDA2 displays high binding affinity toward dopamine D2-like receptors using rat basal forebrain homogenates. Further investigation of in vitro binding properties of [^{125}I]R(+)-FIDA2 indicated that R(+)-FIDA2 has a high affinity for dopamine D2, D3 and D4 receptors. Ex vivo autoradiographic studies in rats showed that [^{125}I]R(+)-FIDA2 is localized in D2-like rich brain regions. In addition, the results of a series of in vivo monkey SPECT imaging studies with [^{123}I]R(+)-FIDA2 determining the uptake and washout rates, as well as the pharmacological selectivity of [^{123}I]R(+)-FIDA2, are reported herein.

MATERIALS AND METHODS

Radiolabeling

No-carrier-added [^{123}I] or [^{125}I]R(+)-FIDA2 was prepared as previously reported (*29*). Briefly, aqueous hydrogen peroxide (50 μl, 3% w/v) was added to a mixture of 50 μl of R(+)-2,3-dimethoxy-N-[(1-(4′-fluorobenzyl)-2-pyrrolidinyl)methyl]-5-tri-n-butyltin benzamide (1 mg/ml of EtOH), 50 μl of 0.1 N HCl, and 5 μl of [^{125}I] or [^{123}I]sodium iodide (2–30 mCi, no-carrier-added) in a sealed vial. The reaction was allowed to proceed at 23°C for 30 min, after which it was terminated by the addition of 0.1 ml of sodium bisulfite (300 mg/ml). The reaction mixture was neutralized via the addition of saturated $NaHCO_3$ solution and then extracted with ethyl acetate (3 × 1 ml). The combined organic layers were passed through an anhydrous sodium sulfate column (0.2 cm × 5 cm) and evaporated to dryness by a stream of nitrogen. The residue was dissolved in 100% ethanol (50–100 μl). The desired product, [^{123}I] or [^{125}I]R(+)-FIDA2, was isolated from the unreacted compound and a small amount of unknown radioactive impurities by HPLC, using a reverse-phase column (PRP-1, Hamilton, Inc., Reno, NV) and an isocratic solvent 90% acetonitrile-10% pH 7.0 buffer (5 m*M*, 3,3-dimethylglutaric acid). The appropriate fractions were collected, condensed and extracted with ethyl acetate (1 × 3 ml). The solution containing the no-carrier-added product was evaporated to dryness and the residue was dissolved in 100% ethanol (purity >97%, overall yield 75%). Specific activity of [^{123}I]R(+)-FIDA2 was determined by injecting a known amount of the compound into the HPLC. Based on a UV standard curve the sensitivity of this system was determined to be >50,000 Ci/mmole. Based on experience with similar iodostannylation reactions where the specific activity was determined to be 2200 Ci/mmole, it is reasonable to assume that the specific activity of no-carrier-added [^{125}I]R(+)-FIDA2 is approximately 2200 Ci/mmole (*12*). The no-carrier-added products, after dilution with saline or buffer, were used for the in vivo and in vitro studies.

Membrane Preparation

Spodoptera frugiperda (Sf9) insect cell membranes were prepared as previously described (*30*). Briefly, Sf9 cells were infected with a virus containing either the rat dopamine D2$_L$ or the D3 receptor gene. The cells were centrifuged and the pellet was resuspended in 50 m*M* Tris-HCl, pH 7.4, 10 m*M* EDTA, 150 m*M* NaCl, 1 μg/ml aprotinin, 1 μg/ml leupeptin and 1 μg/ml soybean trypsin inhibitor. This homogenate was centrifuged and the pellet was resuspended in the same buffer, aliquoted, and stored at −20°C. On the day of the experiment, an aliquot was thawed, diluted with buffer and homogenized. Bovine serum albumin (1 mg/ml) was included in the final diluted homogenates for the assay.

Tissue homogenates containing dopamine D2-like receptors were prepared by dissecting the basal forebrain region from Sprague-Dawley rats (200–250 g). The tissue was homogenized in buffer (50 m*M* Tris-HCl, pH 7.4, 120 m*M* NaCl and 2 m*M* $MgCl_2$) and centrifuged at 3,000 rpm for 10 min. The pellet was discarded and the supernatant was centrifuged at 14,000 rpm for 20 min. The pellet was resuspended in the same buffer and incubated at 37°C for 20 min to remove endogenous dopamine (*31,32*). The homogenate was centrifuged at 14,000 rpm for 20 min and the pellet was resuspended in the same buffer for the following binding assay.

In Vitro Binding Assay

The membrane preparations (100 μl) were incubated with 50 μl [^{125}I]R(+)-FIDA2 and 50 μl of buffer at 37°C for 30 min. For assays with Sf9 cell membrane preparations, 2–4 μg of protein per sample were used, whereas assays involving tissue homogenates required 10–20 μg of protein per sample. Nonspecific binding was determined in the presence of 10 μ*M* of spiperone. After the incubation period, 5 ml of wash buffer (25 m*M* Tris-HCl, pH 7.4) were added to the samples to stop the reaction and a Brandel (M24-R) cell harvester (Brandel, Gaithersburg, MD) was used for

filtration through glass fiber filters (pretreated with 1.0% polyethylenimine). The filters were counted in a gamma counter (Packard 5000, Packard Instrument Co., Downers Grove, IL) with 70% efficiency.

Ex Vivo Autoradiography

Male Sprague-Dawley rats (200–225 g) received an intravenous injection containing 0.5 mCi (0.1 μg) of [^{125}I]R(+)-FIDA2 under ether anesthesia. At 60 min postinjection, the rats were killed by cardiac excision while under ether anesthesia. The brain was rapidly removed, placed in OTC embedding medium (Miles Laboratory, Elkhart, IN) and frozen with powdered dry ice. After attaining equilibration at −20°C, consecutive 20-μm coronal sections were sliced using a cryostat microtome (Hacker Instruments, Fairfield, NJ) and thaw mounted on acid washed, gelatin-coated microscope slides. These slides, along with ^{125}I standards (Amersham, Arlington Hills, IL), were exposed to x-ray film in an autoradiographic cassette for 15 days and developed using an automatic processor. For blocking studies, rats were pretreated with spiperone, 10 mg/kg, i.p. 30 min prior to [^{125}I]R(+)-FIDA2 injection, and the brain sections thus obtained were processed similarly to those of the control rats. The autoradiograms were digitized using an image analysis program (NIH Image, version 1.47).

Anesthesia Procedure

Eight male cynomolgus monkeys (~5 kg) were the subjects of 20 SPECT imaging studies. Most of the animals underwent repeat imaging with at least one control experiment per monkey. In addition, an MR image of each monkey's brain was obtained.

Fasted animals were immobilized with ketamine (10–20 mg/kg, intramuscularly) and xylazine (2–3 mg/kg, intramuscularly) and maintained on a 1.5% isoflurane/98.5% oxygen mixture via an endotracheal tube. For MR image acquisition, only ketamine and xylazine were used. Glycopyrrolate (10 μg/kg, subcutaneously), an anticholinergic drug that does not cross the blood-brain barrier, was administered at the beginning of the study to decrease digestive and respiratory secretions. Vital signs were monitored throughout the study, and core body temperature was maintained by blankets circulating warm water. An intravenous perfusion line with 0.9% NaCl was used to administer the radiotracer and nonradioactive drugs into the saphenous vein. This line was maintained throughout the study. The head was immobilized with a "bean bag" which hardens on evacuation.

SPECT Data Acquisition

Twelve control experiments and eight displacement experiments, in which nonradioactive compounds were administered 90 min postinjection of radiotracer, were performed.

Sequential dynamic SPECT scans were acquired immediately with an intravenous injection of tracer on a triple-head camera (7 mm FWHM) equipped with fanbeam collimators. Scan lengths for these experiments were 10 min over a period of 180 min. The acquisition parameters were a 20% energy window at 159 keV, 120 projection angles over 360°, a 128 × 128 matrix, and a zoom factor 1.78 in a slice thickness of 2 mm. The projection data was reconstructed with a count dependent three-dimensional Wiener filter. Chang's first order correction method was used to compensate for the ^{123}I photon attenuation.

Kinetic analysis was performed on regions of interest (ROIs) which outline the right and left basal ganglia, occipital cortex, frontal cortex, cerebellum and eye orbits. The ROIs were positioned by reference to a monkey brain atlas and the activity distribution, and were confirmed by comparison with MR images. The ROI template was applied to the images acquired throughout the study. The activity is expressed as average cpm/pixel and the rates of washout of brain activity were determined for the 60 min period following peak basal ganglia levels as % control.

TABLE 1
In Vitro Binding of Iodine-125-R(+)-FIDA2 to Dopamine D2/D3 Receptors

	Sf9 Cells			Rat basal forebrain
	$D2_S$	$D2_L$	D3	
K_d (nM)	0.11 ± 0.04	0.11 ± 0.08	0.04 ± 0.01	0.08 ± 0.04

[^{125}I]R(+)-FIDA2 was incubated with the membrane preparations for 30 min at 37°C. Nonspecific binding was determined in the presence of 10 μM of spiperone. Data is expressed as mean ± s.d.

Pharmacological Selectivity

Displacement experiments in which nonradioactive compounds were injected 90 min after [^{123}I]R(+)-FIDA2 injection (<0.1 μg) were designed to show dopamine D2/D3 receptor selectivity, as well as to establish the in vivo pharmacological profile of [^{123}I]R(+)-FIDA2 binding in the basal ganglia area. The first 90 min of the displacement experiments employed exactly the same conditions as the control experiments, therefore, these data were analyzed as control data. In order to determine that R(+)-FIDA2 is selective for dopamine D2-like receptors, doses of raclopride (1.3 mg/kg) and haloperidol (0.04 mg/kg) were used as displacers. R(+)7-OH-PIPAT (1 mg/kg) (*33*), a selective ligand for dopamine D3 receptors and the high affinity state of D2 receptors, was also used as a competing compound. SCH23390 (1.25 mg/kg), a dopamine D1 receptor antagonist, ketanserin (0.9 mg/kg), a 5-HT_2/5-HT_{1C} antagonist, and scopolamine (0.007 mg/kg), a muscarinic receptor antagonist, were used to show the specificity of R(+)-FIDA2.

Magnetic Resonance Imaging

Magnetic resonance images were acquired on anesthetized monkeys with the same headholders used in the SPECT studies. An image of each animal's brain was obtained. Spoil gradient images of 1-mm thick sections were acquired using a TR of 35 and a TE of 5 msec with a 1.5 T instrument.

RESULTS

In Vitro Binding of Iodine-125-R(+)-FIDA2 to Dopamine D2 and D3 Receptors

Iodine-125-R(+)-FIDA2, a benzamide derivative, showed similar binding properties for dopamine D2 and D3 receptors expressed in Sf9 cells (Table 1). The D2 receptor has been found to exist in two forms, $D2_{(short)}$ and $D2_{(long)}$, which are splice variants of a common gene (*34*) that differ by a 29 amino acid sequence. Iodine-125-R(+)-FIDA2 displayed a similar affinity for both forms, having a K_d of 0.11 n*M* at the $D2_{(long)}$ receptor and a K_d of 0.11 n*M* at the $D2_{(short)}$ receptor. In insect cells expressing the dopamine D3 receptor, a K_d value of 0.04 n*M* was obtained (Table 1). Another benzamide derivative, NCQ298, which is a selective D2/D3 antagonist, has comparable affinities in the in-

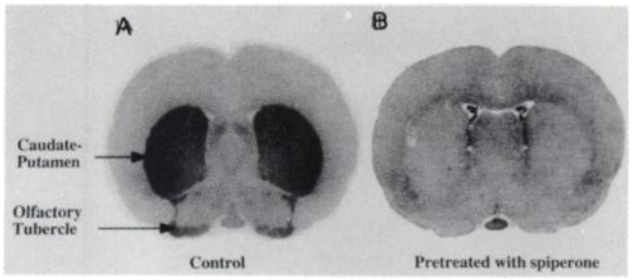

FIGURE 1. Ex vivo autoradiography of rat brain coronal sections 60 min postinjection of 0.5 mCi of [^{125}I]R(+)-FIDA2. (A) Control. (B) Pretreated: animals received 10 mg/kg intraperitoneally spiperone 30 min prior to [^{125}I]R(+)-FIDA2 injection.

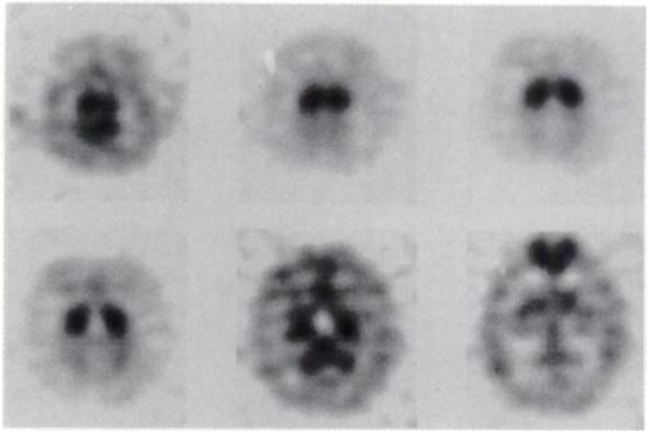

FIGURE 2. Transaxial SPECT images of monkey brain acquired 90–120 min following the injection of 7.3 mCi [^{123}I]R(+)-FIDA2. All images are normalized to maximum pixel.

fected cell lines. The number of receptor sites labeled with R(+)-FIDA2, however, is lower than that labeled with NCQ298 under similar assay conditions (data not shown). Further experiments must be performed to provide a possible explanation for this phenomena.

In the basal forebrain (a region containing the caudate-putamen, nucleus accumbens and olfactory tubercle), the affinity of [^{125}I]R(+)-FIDA2 is 0.08 n*M*, which is consistent with the value (K_d = 0.02 n*M*) previously reported in striatal homogenates (*29*). Similar to other benzamide derivatives, R(+)-FIDA2 requires the presence of NaCl in order to bind to dopamine D2-like receptors; however, R(+)-FIDA2 will retain its binding properties in much lower NaCl concentrations than other benzamides (data not shown).

Ex Vivo Autoradiography

Ex vivo autoradiography was used to evaluate the cerebral regional distribution of [^{125}I]R(+)-FIDA2 in rats. At 60 min postinjection, the autoradiograms showed the compound to be localized in the caudate-putamen, nucleus accumbens, olfactory tubercle and substantia nigra (Fig. 1). These areas are known to have a high density of dopamine D2-like receptors. Very low levels of nonspecific binding were observed in brain regions lacking dopamine receptors. Pretreatment with spiperone (10 mg/kg intraperitoneally), a potent dopamine D2-like (and 5-HT_2) receptor antagonist, showed blocking of specific uptake of [^{125}I]R(+)-FIDA2, indicating that these compounds are competing for the same binding sites.

In Vivo SPECT Imaging Studies Using Iodine-123-R(+)-FIDA2

SPECT images showed that activity was localized in the basal ganglia. The frontal pole, occipital pole, cerebellum, eye orbits and midbrain, including the hypothalamus, were also examined, but activity did not localize in these areas (Fig. 2). Reference to MRI scans and the monkey brain atlas confirmed the anatomical regions. The camera resolution prevented identification of individual structures in the basal ganglia and in the midbrain.

The activity reached peak levels in the basal ganglia in 11.5 ± 5.8 min (n = 19) postinjection. The target-to-non-target ratio reached 8.1 (n = 9) at 3 hr postinjection (Table 2). Iodine-123-R(+)-FIDA2 displayed an average washout rate of 27.9% ± 6.3%/hr (n = 19) from the basal ganglia and 67.7% ± 14.4%/hr (n = 19) from the cerebellum when the activity was measured from peak levels for 60 min postinjection. The average washout rates from the basal ganglia and the cerebellum were 11.4% ± 3.9%/hr (n = 9) and 4.32% ± 2.2%/hr (n = 9), respectively, when measured from 90–150 min following tracer injection.

A series of displacement studies were conducted to determine the reversibility and selectivity of R(+)-FIDA2 binding. Because of intersubject variability, the washout rates of activity from the basal ganglia starting at 90 min postinjection of tracer for a control study and a displacement study in one animal were compared (Fig. 3). The injection of raclopride, haloperidol and cold R(+)-FIDA2, all selective D2-like antagonists, produced washout rates of 34.1%/hr, 38.8%/hr and 33.1%/hr, respectively, from the basal ganglia. These rates are much higher than the washout rates of 6.7%/hr, 10.7%/hr and 10.6%/hr, respectively, produced in control studies. The change in washout rate indicates that [^{123}I]R(+)-FIDA2 was displaced because of competition between the tracer and cold compound at the binding sites (Fig 4). R(+)7-OH-PIPAT binds to D2 receptors in the high-affinity state and D3 receptors (*35*). The introduction of R(+)7-OH-PIPAT or ketanserin, a 5-HT_{1C}/5-HT_2 antagonist, at 90 min postinjection of

TABLE 2
Basal Ganglia/Cerebellum of Iodine-123-R(+)-FIDA2 SPECT Monkey Imaging Studies

Time	Basal Ganglia/Cerebellum	n
Peak	1.15 ± 0.37	20
1 hr	3.41 ± 0.86	19
2 hr	6.32 ± 2.9	9
3 hr	8.14 ± 4.8	9

Data is expressed as mean ± s.d.

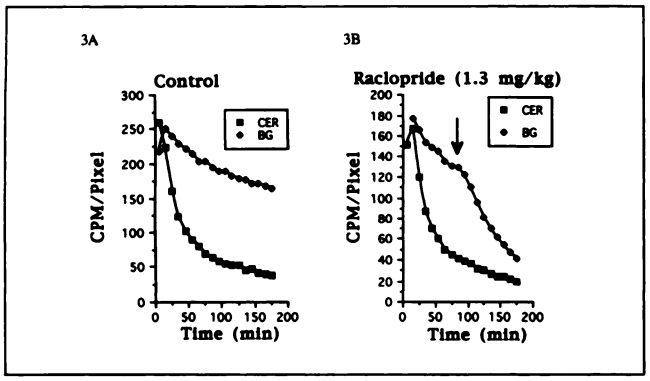

FIGURE 3. (A) Control SPECT imaging study in monkey brain following an [^{123}I]R(+)-FIDA2 injection (7.3 mCi, i.v.). (B) A displacement SPECT imaging study in which raclopride (1.3 mg/kg, intravenously), a dopamine D2/D3 receptor antagonist, was injected 90 min postinjection of [^{123}I]R(+)-FIDA2 (7.0 mCi, i.v.). The same monkey was used for both studies. A relative radioactivity measurement was obtained for the basal ganglia and the cerebellum.

[^{123}I]R(+)-FIDA2 did not dramatically alter the tracer washout rate from the basal ganglia. In addition, SCH23390, a dopamine D1 antagonist, or scopolamine, a muscarinic antagonist, did not affect tracer clearance. Basal ganglia washout rates of 20.5%/hr (control) versus 20.1%/hr following the addition of SCH23390 and 13.0%/hr prior to scopolamine administration compared to 12.7%/hr following treatment were observed. Iodine-123-R(+)-FIDA2 is displaced by compounds that are selective for D2-like receptors, indicating reversible and selective binding at these sites.

DISCUSSION

The use of in vivo techniques to image dopamine D2-like receptors is advantageous because it is a noninvasive procedure that can be used as a diagnostic tool as well as a way to evaluate receptor occupancy and the efficacy of treatment. Neuroleptic drugs are dopamine antagonists that are believed to be therapeutically active because of dopamine D2 receptor blockade. Recently, with the cloning of the dopamine D3 and D4 receptors, it has been proposed that neuroleptics may be efficacious because of activity at dopamine D3 or D4 receptors (*5*). Both PET (*36*) and SPECT (*37*) techniques can be used in schizophrenic patients to monitor receptor occupancy and subsequently titrate the levels of neuroleptics or to change to an alternative medication.

R(+)-FIDA2, which contains a N-p-fluorobenzyl substitution group on the pyrrolidine ring, has a high affinity for dopamine D2-like receptors. Although the high affinity for dopamine D2/D3 receptors is consistent with other benzamide derivatives, which have N-ethyl groups on the same pyrrolidine ring, R(+)-FIDA2 displays several unique characteristics. Normally, benzamides with N-ethyl substitutions display higher affinity to dopamine D2 and D3 receptors with the S(−) isomer (*38*). In contrast, the R(+) isomer is the preferred configuration for FIDA2, displaying

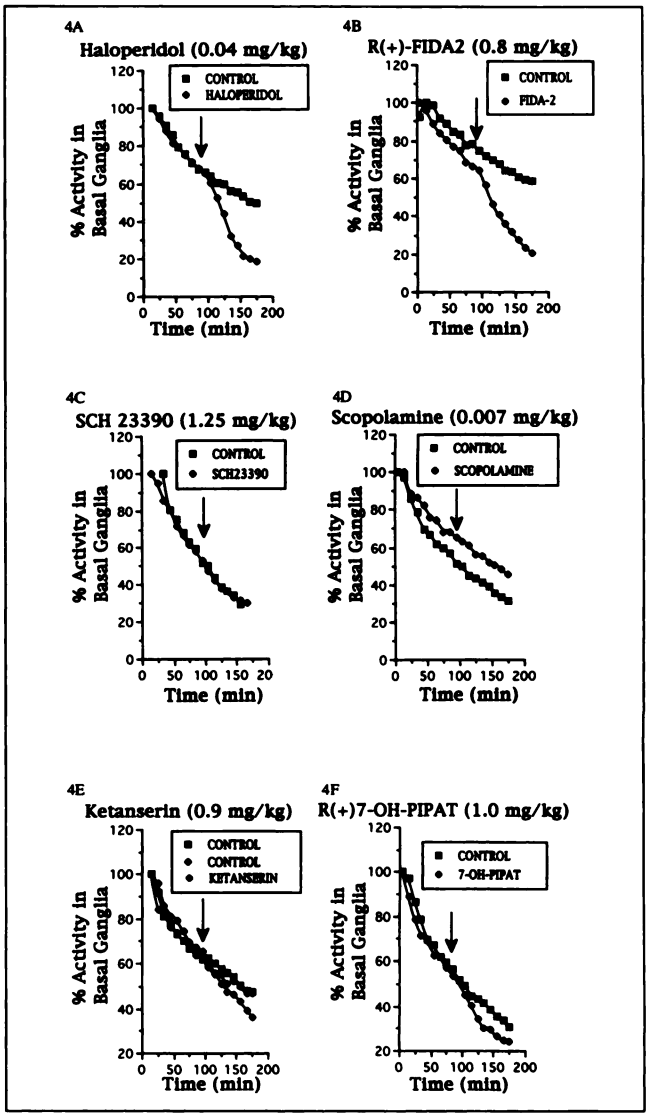

FIGURE 4. A series of SPECT monkey brain imaging displacement studies after intravenous injection of [^{123}I]R(+)-FIDA2 (5–9 mCi). At 90 min postinjection, a cold compound was intravenously injected. Each animal had at least one control study in which no displacer was injected and [^{123}I]R(+)-FIDA2 clearance from the basal ganglia in the control and displacement studies were compared. (A) Haloperidol was used to compete with [^{123}I]R(+)-FIDA2. (B) Cold R(+)-FIDA2 was injected to determine the reversibility of [^{123}I]R(+)-FIDA2 binding. The selectivity of [I-123]R(+)-FIDA2 for dopamine D2-like receptors was determined by competition with (C) SCH23390, (D) scopolamine and (E) ketanserin. (F) R(+)7-OH-PIPAT, a compound which binds to dopamine D2 receptors in the high-affinity state and D3 receptors, was used in a competition study.

a K_d value of 80 p*M* in rat basal forebrain. A characteristic of benzamide binding to dopamine D2 receptors, such as epidepride with a N-ethyl substitution, is NaCl sensitivity (*39*). The affinity of dopamine D2 receptors for agonists and substituted benzamide antagonists varies according to the conformational state of the receptor. S(−)-epidepride potency is greatly reduced in the presence of 40 m*M* NaCl (*14*). Although R(+)-FIDA2 requires NaCl for specific binding to D2 receptors, it binds with comparable potency

in the presence of either 40 m*M* NaCl or 120 m*M* NaCl. This reduced sensitivity to NaCl is consistent with other non-benzamide dopamine D2 receptor antagonists, such as spiperone (*39*). Finally, it is reported that raclopride binds poorly to dopamine D4 receptors (K_i = 240 n*M*) (*5*). Preliminary data indicated that [^{125}I]R(+)-FIDA2 binds potently to dopamine D4 receptors with a K_d value of 0.2 n*M* (unpublished data).

The N-benzyl substitution on the pyrrolidine ring appears to change the stereoselectivity from S(−) to R(+) isomer. The change of steric preference appears to be associated with higher binding affinity to dopamine D4 receptors. It is interesting to note that another N-benzylated benzamide derivative, YM-09151-2, displays high affinity to dopamine D4 receptors, as well as to the dopamine D2 and D3 receptor subtypes (*40*). It may be reasonable to predict that tropapride (*41*) and other N-benzyl-substituted benzamide derivatives (*42*) may also display equal potency to all dopamine D2-like receptor subtypes. In contrast, epidepride, IBF and IBZM, the N-ethyl-substituted benzamides, do not display a high affinity for dopamine D4 receptors (unpublished data). Whether this will detract from using [^{123}I]R(+)-FIDA2 as an imaging agent remains to be seen.

Ex vivo autoradiography showed that [^{125}I]R(+)-FIDA2 localized in areas rich in D2-like receptors. In situ hybridization studies in the rat brain have shown that mRNA for dopamine D2 receptors is heavily localized in the striatum, nucleus accumbens, and olfactory tubercle (*43*). Dopamine D3 receptor mRNA is found mainly in the islands of Calleja and the nucleus accumbens, with minimal detectable levels in the caudate-putamen (*43*). Dopamine D4 receptor mRNA is also not localized in the caudate-putamen, however, levels of D4 mRNA are highest in cortical and limbic regions (*5*). The ex vivo autoradiograms of [^{125}I]R(+)-FIDA2 show high activity in regions consistent with high mRNA levels of D2-like receptors, such as the caudate-putamen, nucleus accumbens and olfactory tubercle, with low nonspecific binding in other brain regions.

In vivo SPECT imaging studies indicated that [^{123}I]R(+)-FIDA2 localized in the basal ganglia, a brain region rich in dopamine D2-like receptors. In addition, [^{123}I]R(+)-FIDA2 has a better target-to-nontarget ratio than IBF or IBZM of 3.4 versus 2.1 or 1.6, respectively, at 1 hr postinjection and 6.3 versus 2.9 or 1.4, respectively, at 2 hr postinjection (*16*).

High affinities of R(+)-FIDA2 for dopamine D2-like receptors have been observed. R(+)-FIDA2 also displays reversible binding in vivo during the course of the imaging studies. In comparison, other ligands which have a high affinity for dopamine D2-like receptors, such as N-methylspiperone do not display reversible binding. The rapid uptake and reversible binding displayed by [^{123}I]R(+)-FIDA2 make it amenable for use in three-compartment or equilibrium models in conjunction with either SPECT or PET imaging. It is likely that R(+)-FIDA2 binding to the D2-like receptors will be less sensitive to the level of endogenous dopamine. The possibility of displacement by increased endogenous dopamine levels produced by d- amphetamine treatment remains to be investigated (*44*).

Iodine-123-R(+)-FIDA2 can be displaced in vivo by raclopride, haloperidol and R(+)-FIDA2, which are compounds having a high affinity for dopamine D2-like receptors, but SCH23390, a dopamine D1 selective antagonist, did not affect [^{123}I]R(+)-FIDA2 binding. R(+)7-OH-PIPAT (*33*), a compound which binds to dopamine D2 receptors in the high-affinity state and dopamine D3 receptors, did not significantly displace [^{123}I]R(+)-FIDA2 binding in vivo. These results are consistent with preliminary in vitro autoradiographic studies with [^{125}I]R(+)-FIDA2 and R(+)7-OH-PIPAT which suggest that competition at R(+)-FIDA2 binding sites does not appear to be significant. It may be possible that R(+)7-OH-PIPAT is not able to displace the binding of a high-affinity antagonist, such as R(+)-FIDA2. An alternative explanation may be that the number of D2 dopamine receptors in the high-affinity state and D3 dopamine receptors that are occupied by [^{123}I]R(+)-FIDA2 is very small, so competition at these sites does not appear to significantly affect [^{123}I]R(+)-FIDA2 binding. Additional experiments are needed to fully characterize the relationship of selective dopamine D2-like receptor ligands for in vivo binding and displacement.

Blocking muscarinic receptors with scopolamine or 5-HT_2 receptors with ketanserin did not influence the kinetics of [^{123}I]R(+)-FIDA2. Previously, Dewey et al. has shown in separate experiments in primates using ^{11}C-raclopride and PET imaging techniques that both compounds increase the basal ganglia washout of ^{11}C-raclopride (*45*). R(+)-FIDA2, however, has over a hundredfold greater affinity for dopamine D2/D3 receptors than raclopride, making it less susceptible to competition. Displacement by selective dopamine D2-like receptor compounds, in addition to the lack of an effect by nondopamine selective compounds, demonstrates the in vivo specificity of [^{123}I]R(+)-FIDA2.

CONCLUSION

In vitro binding studies demonstrate that [^{125}I]R(+)-FIDA2 has a high affinity to dopamine D2 and D3 receptors expressed in cell lines and in native tissues. Ex vivo autoradiographic studies confirmed the specific binding of [^{125}I]R(+)-FIDA2 to brain regions containing a high density of dopamine D2-like receptors. In vivo SPECT imaging studies have shown the advantages of [^{123}I]R(+)-FIDA2 over other dopamine D2-like receptor imaging agents. This agent may be a useful ligand for SPECT and PET imaging of CNS dopamine receptors.

ACKNOWLEDGMENTS

The authors thank Drs. Virginia Boundy and Perry Molinoff for supplying Sf9 cells expressing dopamine receptors, and Ms. Susan West for her assistance in preparing this manuscript. This work was supported by the Society of Nuclear Medicine Educa-

tion and Research Foundation and National Institutes of Health grant NS-24538.

REFERENCES

1. Seeman P. Brain dopamine receptors. *Pharmacol Rev* 1980;32:229–269.
2. Seeman P. Dopamine receptors and the dopamine hypothesis of schizophrenia. *Synapse* 1987;1:133–152.
3. Bunzow JR, Van Tol HHM, Grandy DK, et al. Cloning and expression of a rat D2 dopamine receptor cDNA. *Nature* 1988;336:783–787.
4. Sokoloff P, Giros B, Martres M-P, Bouthenet M-L, Schwartz J-C. Molecular cloning and characterization of a novel dopamine receptor (D3) as a target of neuroleptics. *Nature* 1990;347:146–151.
5. Van Tol HHM, Bunzow JR, Guan HC, Sunahara RK, Niznik HB, Civelli O. Cloning of the gene for the human D4 receptor with high affinity for the antipsychotic clozapine. *Nature* 1991;350:610–614.
6. Schwartz J-C, Giros B, Martres M-P, Sokoloff P. Multiple dopamine receptors as molecular targets for antipsychotics. In: Brunella N, Mendlewicz J, eds. *New generation of antipsychotic drugs. Novel mechanisms of action*. Basal: Karger; 1993:1–14.
7. Seeman P. Dopamine receptor sequences. Therapeutic levels of neuroleptics occupy D2 receptors, clozapine occupies D4. *Neuropsychopharmacology* 1992;7:261–284.
8. Högberg T, Rämsby S, Ögren S-O, Norinder U. New selective dopamine D-2 antagonists as antipsychotic agents. Pharmacological, chemical, structural and theoretical considerations. *Acta Pharm Suec* 1987;24:289–328.
9. Högberg T, Strom P, Hall H, Kohler C, Halldin C, Farde L. Synthesis of [^{125}I], [^{123}I] and unlabelled (S)3-iodo-5,6-dimethoxy-N-[(1-ethyl-2-pyrrolidinyl)methyl] salicylamide (NCQ298), a selective ligand for the study of dopamine D-2 receptors. *Acta Pharm Nord* 1990;2:53–60.
10. Högberg T, Ström P, de Paulis T, et al. Potential antipsychotic agents. 9. Synthesis and stereoselective dopamine D-2 receptor blockade of a potent class of substituted (R)-N-[(1-Benzyl-2-pyrrolidinyl)methyl]benzamides. Relations to other side chain congeners. *J Med Chem* 1991;34:948–955.
11. Kung HF, Guo Y-Z, Billings JJ, et al. Preparation and biodistribution of [^{125}I]IBZM: a potential CNS D2 dopamine receptor imaging agent. *Nucl Med Biol* 1988;15:195–201.
12. Kung M-P, Kung HF, Billings JJ, Yang Y, Murphy RA, Alavi A. The characterization of IBF as a new selective dopamine D-2 receptor imaging agent. *J Nucl Med* 1990;31:648–654.
13. Murphy RA, Kung HF, Kung M-P, Billings JJ. Synthesis and characterization of iodobenzamide analogues: potential D-2 dopamine receptor imaging agents. *J Med Chem* 1990;33:171–178.
14. Kessler RM, Ansari MS, Schmidt DE, et al. High affinity dopamine D2 receptor radioligands. II. [^{125}I]Epidepride, a potent and specific radioligand for the characterization of striatal and extrastriatal dopamine D2 receptors. *Life Sci* 1991;49:617–628.
15. Kessler RM, Ansari MS, de Paulis T, et al. High affinity dopamine D2 receptor radioligands. I. Regional rat brain distribution of iodinated benzamides. *J Nucl Med* 1991;32:1593–1600.
16. Kung HF. Iodinated D1 and D2 dopamine receptor imaging agents for SPECT. In: Burns HD, Gibson RE, Dannals RF, Siegal PKS, eds. *Nuclear imaging in drug discovery, development and approval*. Boston: Birkhäuser; 1993:227–244.
17. Kessler RM, Votaw JR, Schmidt DE, et al. High affinity dopamine D2 receptor radioligands. III. [^{123}I] and [^{125}I]epidepride: in vivo studies in rhesus monkey brain and comparison with in vitro pharmacokinetics in rat brain. *Life Sci* 1993;53:241–250.
18. Kessler RM, Whetsell WO, Ansari MS, et al. Identification of extrastriatal dopamine D2 receptors in postmortem human brain with [^{125}I]epidipride. *Brain Res* 1993;609:237–343.
19. Kessler RM, Mason NS, Votaw JR, et al. Visualization of extrastriatal dopamine D2 receptors in the human brain. *Eur J Pharmacol* 1992;223:105–107.
20. Votaw JR, Kessler RM, de-Paulis T. Failure of the three compartment model to describe the pharmacokinetics in brain of a high affinity substituted benzamide. *Synapse* 1993;15:177–190.
21. Farde L, Ehrin E, Eriksson L, et al. Substituted benzamides as ligands for visualization of dopamine receptor binding in the human brain by positron emission tomography. *Proc Natl Acad Sci USA* 1985;82:3863–3867.
22. Wong DF, Wanger HN Jr., Pearlson G, et al. Dopamine receptor binding of C-11-3-N-methylspiperone in the caudate in schizophrenia and bipolar disorder: a preliminary report. *Psychopharmacol Bull* 1985;21:595–598.
23. Wong DF, Broussolle EP, Wand G, et al. In vivo measurement of dopamine receptors in human brain by positron emission tomography. Age and sex differences. *Ann NY Acad Sci* 1988;515:203–214.
24. Kohler C, Hall H, Ogren SO, Gawell L. In vitro and in vivo binding of [^{3}H]raclopride: a potent substituted benzamide drug with high affinity for dopamine D2 receptors in rat brain. *Biochem Pharmacol* 1985;34:2251–2259.
25. Frost JJ, Smith AC, Kuhar MJ, Dannals RF, Wagner HN Jr. In vivo binding of ^{3}H-N-methylspiperone to dopamine and serotonin receptors. *Life Sci* 1987;40:987–995.
26. Wong DF, Wagner HN Jr., Dannals RF, et al. Effects of age on dopamine and serotonin receptors measured by positron emission tomography in the living human brain. *Science* 1984;226:1393–1396.
27. Farde L, Eriksson L, Blomquist G, Halldin C. Kinetic analysis of central [^{11}C]raclopride binding to D2-dopamine receptors studied by PET—a comparison to the equilibrium analysis. *J Cereb Blood Flow Metab* 1989;9:696–708.
28. Budinger TF. Advances in emission tomography: quo vadis? *J Nucl Med* 1990;31:628–631.
29. Chumpradit S, Kung M-P, Billings JJ, Mach R, Kung HF. Fluorinated and iodinated dopamine agents (FIDA): D2 imaging agents for PET and SPECT. *J Med Chem* 1993;36:221–227.
30. Boundy VA, Luedtke RR, Gallitano AL, et al. Expression and characterization of the rat D3 dopamine receptor: pharmacologic properties and development of antibodies. *J Pharmacol Exp Ther* 1993;264:1002–1011.
31. Seeman P, Guan H-C, Niznik HB. Endogenous dopamine lowers the dopamine D2 receptor density as measured by [^{3}H]raclopride: implications for positron emission tomography of the human brain. *Synapse* 1989;3:96–97.
32. Seeman P, Niznik HB. Dopamine receptors and transporters in Parkinson's disease and schizophrenia. *Faseb J* 1990;4:2737–2744.
33. Burris KD, Filtz T, Chumpradit S, et al. Characterization of [^{125}I](R)-2'-*trans*-7-hydroxy-2-[N-n-propyl-N-(3'-iodo-2"-propenyl)amino]tetralin ([^{125}I](R)trans-7-OH-PIPAT) binding to dopamine D3 receptors in rat olfactory tubercle. *J Pharmacol Exp Ther* 1994;268:935–942.
34. Sibley DR, Monsna FJJ. Molecular biology of dopamine receptors. *Trends Pharmacol Sci Rev* 1992;13:61–69.
35. Kung M-P, Kung HF, Chumpradit S, Foulon C. In vitro binding of a novel dopamine D3 receptor ligand: [^{125}I]*trans*-7-OH-PIPAT-A. *Eur J Pharmacol* 1993;235:165–166.
36. Farde L, Wiesel FA, Halldin C, Sedvall G. Central D2-dopamine receptor occupancy in schizophrenic patients treated with antipsychotic drugs. *Arch Gen Psychiatry* 1988;45:71–76.
37. Pilowsky LS, Costa DC, Ell PJ, Murray RM, Verhoeff NP, Kerwin RW. Clozapine, single photon emission tomography, and the D2 dopamine receptor blockade hypothesis of schizophrenia. *Lancet* 1992;340:199–202.
38. Baldessarini RJ, Kual NS, McGrath CR, Bakthavachalam V, Kebabian JW, Neumeyer JL. Isomeric selectivity at dopamine D3 receptors. *Eur J Pharmacol* 1993;269–270.
39. Neve KA. Regulation of dopamine D2 receptors by sodium and pH. *Mol Pharmacol* 1991;39:570–578.
40. Tang L, Todd RD, Heller A, O'Malley KL. Pharmacological and functional characterization of D2, D3 and D4 dopamine receptors in fibroblast and dopaminergic cell lines. *J Pharmacol Exp Ther* 1994;268:495–502.
41. Cantineau R, Guillaume M, Damhaut P, et al. Synthesis and biodistribution of [5-^{131}I]iodotropapride: a potential D2 dopamine receptor imaging agent. *Nucl Med Biol* 1994;21:255–262.
42. Mach RH, Luedtke RR, Unsworth CD, et al. Fluorine-18-labeled benzamides for studying the dopamine D2 receptor with positron emission tomography. *J Med Chem* 1993;36:3707–3720.
43. Bouthenet M-L, Souil E, Martes MP, Sokoloff P, Giros B, Schwarz JC. Localization of dopamine D3 receptor mRNA in rat brain using in situ hybridization histochemistry: comparison with D2 receptor mRNA. *Brain Res* 1991;564:203–219.
44. Al-Tikriti MS, Baldwin RM, Zea-Ponce Y, et al. Comparison of three high affinity SPECT radiotracers for the dopamine D2 receptors. *Nucl Med Biol* 1993;21:179–188.
45. Dewey SL, Smith GS, Logan J, et al. Effects of central blockade on striatal dopamine release measured with positron emission tomography in normal human subjects. *Proc Natl Acad Sci USA* 1993;90:11816–11820.