In Vitro and In Vivo Evaluation of [^{123}I]IBZM: A Potential CNS D-2 Dopamine Receptor Imaging Agent

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In vitro binding characteristics of a CNS dopamine D-2 receptor imaging agent, (S)-N-[(1-ethyl-2-pyrrolidinyl)] methyl-2-hydroxy-3-iodo-6-methoxybenzamide ([^{125}I]IBZM), was carried out in rats. Also brain images, as well as organ biodistribution were determined in a monkey following the administration of ^{123}I-labeled compound. The S-(-)-I[^{125}I]IBZM showed high specific dopamine D-2 receptor binding in rat striatum (K_d = 0.426 ± 0.082 n*M*, Bmax = 480 ± 22 fmol/mg of protein). Competition of various ligands for the IBZM binding displayed the following rank order of potency: spiperone > S(-)IBZM ≫ R(+)IBZM ≥ S(-)BZM > dopamine > ketanserin > SCH-23390 ≫ propranolol, norepinephrine, serotonin. In vivo planar images of a monkey injected with [^{123}I]IBZM demonstrated a high concentration in basal ganglia of brain. The ratios of activity in the basal ganglia to cerebellum and the cortex to cerebellum in monkey brain were 4.93 and 1.44, respectively, at 120 min postinjection. These preliminary results indicate that [^{123}I]IBZM is a potentially promising imaging agent for the investigation of dopamine D-2 receptors in humans.

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Recently, positron emission tomography (PET) has attracted attention as a useful tool for studying the biochemical properties of the living human brain (*1–3*). Several agents for the imaging of CNS dopamine receptors by PET have been reported: [^{11}C]-23390 for D-1 receptors (*4*), [^{11}C]-raclopride (*5–7*), N-[^{11}C] methylspiperone (NMSP)(*8,9*) and 4′-[^{18}F]-N-methylspiperone (*10*) for D-2 receptors. They have been demonstrated to be useful in the evaluation of CNS dopamine receptors, which are essential for normal brain functions.

Because of the complexity and the cost associated with radiopharmaceuticals used for PET imaging there is a need for comparable imaging agents labeled with single photon emitting radionuclides, which may be more suitable for a widespread clinical application with single photo emission computed tomography (SPECT) instrumentation. In developing the new CNS D-2 dopamine receptor imaging agents, an iodinated benzamide, IBZM, (S)-N-[[(1-ethyl-2-pyrrolidinyl)] methyl-2-hydroxy-3-iodo-6-methoxybenzamide, has been reported (*11–13*).

IBZM (Fig. 1) belongs to a group of structurally related benzamides which display significant antidopaminergic activity (*14–19*). These benzamides show a high specificity for the CNS D-2 dopamine receptor and selectively block apomorphine-induced hyperactivity in vivo. The in vitro affinity constant (Kd) for these agents in rat striatum tissue preparation was found to be: 13, 1.1, and 0.17 n*M* for sulpiride, raclopride and eticlopride, respectively (*17*).

To further investigate the potential of this agent as a SPECT imaging agent for D-2 dopamine receptors in man, in vitro binding characteristics of [^{125}I]IBZM in rats and the biodistribution of [^{123}I]IBZM in a monkey were investigated.

MATERIALS AND METHODS

General

Male Sprague-Dawley rats weighing 200–250 g were used. The rats were housed in an animal facility with 12 hr light

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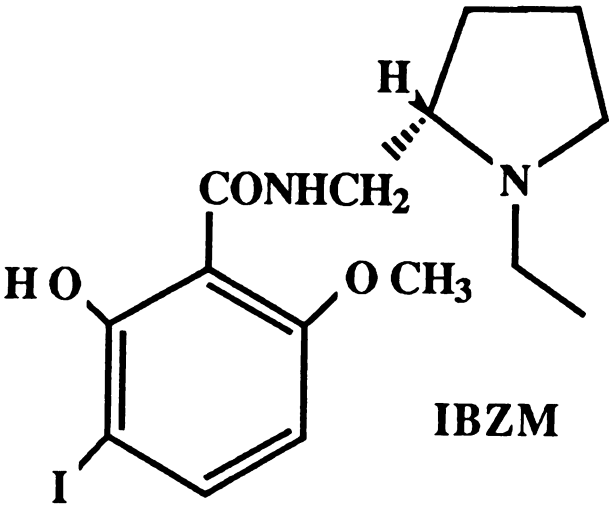

FIGURE 1
Chemical structure of IBZM (S)-N-[(1-ethyl-2-pyrrolidinyl)] methyl-2-hydroxy-3-iodo-6-methoxybenzamide.

and dark cycle with access to food and water ad lib. All chemicals used were of chemical grade. Spiperone, (+)-butaclamol, (−)-butaclamol, (±)-6,6-ADTN, serotonin, ketanserin, dopamine, eticlopride·HCL, clonidine, apomorphine, and papaverine were obtained from Research Biochemical Inc, Wayland, MA; chlorpromazine, norepinephrine and propranolol were purchased from Aldrich Chemical Company.

Radiolabeling

The iodine-125 (^{125}I) and iodine-123 (^{123}I) labeled IBZM were prepared by a procedure reported previously (radiochemical purity ≥ 94%, overall yield ~60%, no uv detectable impurities) (*11*). The theoretical specific activities for [^{125}I] IBZM and [^{123}I]IBZM are 2.2×10^3 and 2.4×10^5 Ci/mmol, respectively. After dilution with saline, these agents were used in the studies described below.

Tissue Preparation

Male Sprague-Dawley rats (200–250 g) were killed and the brains removed and placed on ice. Striatal tissues were excised, pooled, and homogenized (glass and teflon) in 100 volumes (w/v) of ice-cold Tris-HCl buffer (50 m*M*), pH 7.4. The homogenates were centrifuged at 20,000 *g* for 20 min. The resultant pellets were rehomogenized in the same buffer and centrifuged again. The final pellets were resuspended in assay buffer containing: 50 m*M* Tris-HCl buffer pH 7.4, 120 m*M* NaCl, 5 m*M* KCl, 2 m*M* $CaCl_2$ and 1 m*M* $MgCl_2$. The protein concentration of homogenate was determined by the method of Lowry (*20*).

Binding Assays

Binding assays were carried out by incubating 50 ml of tissue preparations containing a variable amount of protein (striatum, 40–60 mg; hippocampus, 300 mg; cortex, 400 mg) with appropriate amounts of [^{125}I]IBZM and competitors in a total volume of 0.2 ml of the assay buffer. After an incubation period of 15 min at 37°C, samples were rapidly filtered with a cell harvester (Brandel M-24R) under vacuum through Whatman GF/B glass fiber filters pretreated with 0.2% poly-L-lysine, and the filter paper washed with 3 × 5 ml of cold (4°C) 50 m*M* Tris-HCl buffer, pH 7.4. Nonspecific binding was obtained in the presence of 10 m*M* spiperone. Radioactivity bound on the filters was counted in a gamma counter (Beckman 5500) at an efficiency of 70%.

Data Analysis

Both Scatchard and competition experiments were analyzed using the iterative nonlinear least squares curve-fitting program LIGAND (*21*). Each saturation analysis used 11–12 concentrations of [^{125}I]IBZM between 0.05 and 1.5 n*M*. Scatchard analysis of the binding data using tissues from striatum, hippocampus and frontal cortex always resulted in linear plots with saturability. The Hill coefficients were nearly one in all cases.

Re-extraction of [^{125}I]IBZM from Rat Striatum

Thirty minutes after the i.v. injection of [^{125}I]IBZM into a rat, the striatal tissues were removed, homogenized in 1 ml Tris buffer (50 m*M*, pH 7.4) and extracted with ethyl acetate (3 × 1 ml) in the presence of cold IBZM carrier (400 mg). The combined organic extracts were condensed and the residue injected into the high performance liquid chromatography (HPLC) with the same solvent system as described above. The retention time of the major peak (>98%) was comparable to the original [^{125}I]IBZM under the same conditions. A total of three rats was used for the experiment.

Biodistribution Study in Monkey

A monkey (Cynomologous, 5 lb) was sedated with ketamine (50 mg) and an intravenous infusion of pentobarbital (0.2 ml, 25 mg/ml) and followed 30 min later by an intravenous injection of the [^{123}I]IBZM (1.7 mCi/1.2 ml, radiochemical purity 94.4%). Immediately after the i.v. injection, lateral images of the head (1 min per frame for 120 min) were obtained using a Picker Digital Dyna Camera equipped with an all purpose collimator on line to a GE Star II computer system with a 20% window set at an energy peak of 159 keV. For brain washout analysis the dynamic planar images were summed (5 min × 24). Regions of interest for whole brain were defined. The net counts were obtained by subtracting the counts in the brain from that of the same number of pixels in the soft tissue near the neck. The net counts of the whole brain versus time were plotted.

At the end of 120 min the monkey was killed. The brain and other tissues of interest were removed and weighed. The brain was sliced into four sections along the cantho meatal line. Planar images of the brain sections were obtained using the same camera. The brain slices were then cut into smaller samples and counted along with standards (a diluted initial dose) and samples of other tissues: liver, lung, kidney, spleen, thyroid, and muscle. The % dose/g and % dose/organ were calculated by comparing the net counts of the tissue sample to that of the standard and using 40% and 7% of the body weight for total muscle and blood, respectively.

RESULTS AND DISCUSSION

Saturation Analysis

Results of the binding studies show specific binding of [^{125}I]IBZM in the striatum membrane preparation with a K_d value of 0.426 n*M* (Table 1). The B_{max} of [^{125}I]IBZM in the striatum of rats was 480 fmol/mg of

TABLE 1
Binding Constants of [^{125}I]IBZM in Different Regions of Rat Brain

Region	K_d (n*M*)	Bmax (fmol/mg protein)
Striatum	0.426 ± 0.082	480 ± 22
Frontal cortex	0.984 ± 0.191	47.0 ± 6.2
Hippocampus	0.914 ± 0.154	87.1 ± 7.7

protein, which greatly exceeded the values for hippocampus (87 fmol/mg) and frontal cortex (47 fmol/mg) (Table 1). The B_{max} value is comparable to that reported in the literature using a similar procedure and [^{3}H] spiperone as the ligand (*22*). The nonspecific binding in striatum was 5% of the total bound at the K_d concentration, and lower than 50% found in the hippocampus and frontal cortex. The result is consistent with the fact that the striatum has more D-2 dopamine receptors than that in any other region of the brain.

Competition Studies

D-2 Dopamine antagonists, e.g., spiperone, (+)-butaclamol and chlorpromazine inhibit [^{125}I]IBZM binding more potently than the D-2 agonist (±)-ADTN and the D-1 antagonist SCH-23390 (Table 2). Agonists or antagonists of other receptors including serotonin, ketanserin, norepinephrine, propranolol, prazosin and clonidine, do not compete with the binding of IBZM. The data demonstrate that the ligand is a very specific ligand with which to study D-2 dopamine receptors. The fact that the displacement of the S(−)-isomer of IBZM was more effective than its R(+)-isomer (by a factor of ~300), and that (−)-butaclamol did not inhibit the binding of IBZM proves that the binding of [^{125}I] IBZM is highly stereoselective.

Re-Extraction of [^{125}I]IBZM from Rat Striatum

At 30 min after an i.v. injection, the radioactivity in rat striatal tissue can be re-extracted (> 95%) by ethylacetate. The extractable material displaced the same retention time on the HPLC as that of authentic [^{125}I] IBZM. The data strongly suggests that the radioactivity in striatum is the original compound and in vivo metabolism is minimum (if any) in the target tissue containing a high density of D-2 dopamine receptors.

In Vivo Biodistribution of [^{123}I]IBZM in a Monkey

Immediately after the i.v. injection of [^{123}I]IBZM significant uptake was observed in the monkey brain. The uptake appeared to reach a maximum by ~10 min postinjection (Fig. 2). Since the agent is a lipid-soluble material (partition coefficient: 1-octanol/buffer = 111 and 211 at pH 7.0 and 7.4, respectively), it is likely that IBZM penetrates the blood-brain barrier by simple diffusion (*23*). The summed images (Fig. 3) demonstrates that the agent is concentrated in the basal ganglia. The total brain washout curve (Fig. 4) shows a single component with a $T_{½}$ of 100 min.

TABLE 2
Inhibition Constants of Compounds on [^{125}I]IBZM Binding to Rat Striatal Membranes*

Compounds	Ki (n*M*)
S(−)IBZM	0.633 ± 0.049
R(+)IBZM	30.3 ± 0.84
S(−)BZM	31.1 ± 5.78
(+)Butaclamol	0.851 ± 0.174
Chloropromazine	4.01 ± 0.80
(±)ADTN	52.6 ± 6.60
Dopamine	296 ± 59
SCH23390	600 ± 15
Ketanserin	359 ± 79
Apomorphine	262 ± 38

*Each value represents the mean ± s.e.m. of four to six determinations. Kd = 0.426 ± 0.082 n*M*, Bmax = 480 ± 22 fmol/mg of protein. Norepinephrine, propranolol, serotonin, (−)-butaclamol displayed Ki > 1,000 n*M*.

The ex vivo image of the sectioned brain shows a clear cut concentration of the agent in the basal ganglia, where the D-2 dopamine receptors are located (Fig. 4). This observation is confirmed by the in vitro experiments. The ratios between the basal ganglia and cerebellum, and the cortex and cerebellum were 4.93 and 1.44, respectively at 120 min postinjection (Table 3). These findings are consistent with those observed in rats (*11*). Using the same tissue counting technique the total brain uptake was 3.71% dose/organ (total brain = 55 g), which suggests that there is high residual uptake in the brain at 2 hr postinjection. Based on the fact that

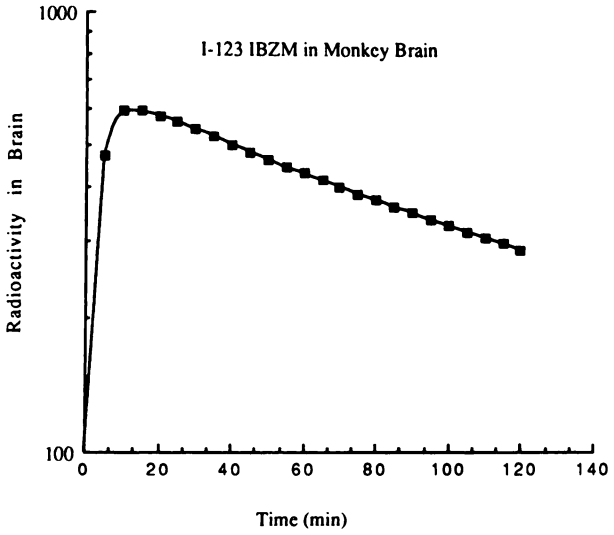

FIGURE 2
Time activity curve for the whole brain of a monkey after an i.v. injection of [^{123}I]IBZM. The total brain curve appears to show a single component wash out with a $T_{½}$ of ~100 min.

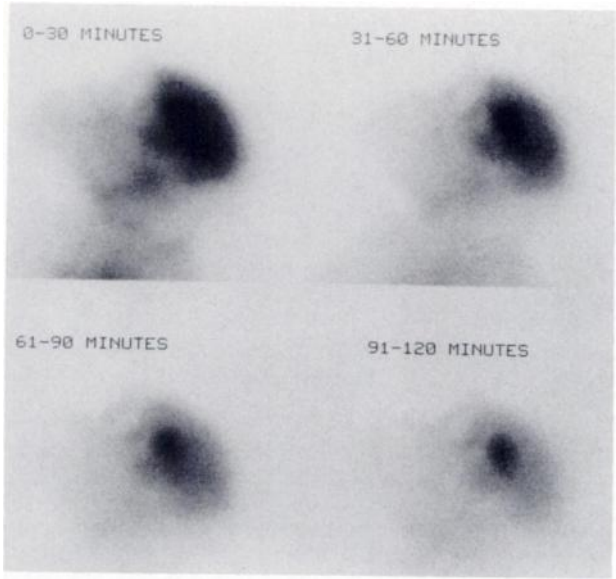

FIGURE 3
Summed images (lateral view) of a monkey head at 1–30 min, 31–60 min, 61–90 min and 91–120 min after an i.v. injection of [^{123}I]IBZM. The early images demonstrate that the agent is initially localized in the whole brain as well as in the basal ganglia. However, delayed images show washout of this agent from the cortex while basal ganglia appear to retain high concentrations of ligand.

the $T_{½}$ in the brain is ~100 min, the maximum total brain uptake is estimated to be 7% dose/organ. Other tissue and organ uptake is summarized in Table 4. Liver and kidney were the organs with higher uptake. The use of [^{123}I]FLB-981, chemical equivalent of [^{123}I]

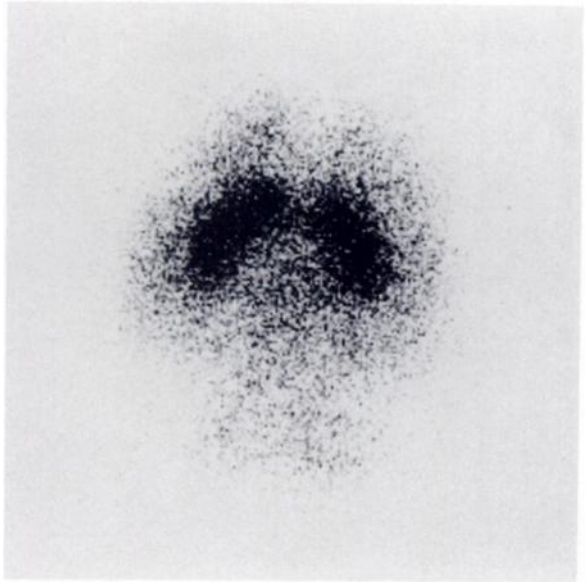

FIGURE 4
Ex vivo image of brain slice at the level of the basal ganglia along the cantho meatal line after an i.v. injection of [^{123}I] IBZM. This image showed a clear cut concentration of the agent in the basal ganglia (BG), where D-2 dopamine receptors are located, and low uptake in the cerebellum (CB) with a low concentration of these receptors.

TABLE 3
Uptake Ratios of [^{123}I]IBZM in Different Regions of Monkey Brain

Basal ganglia/cerebellum ratio	4.93*
Cortex/cerebellum ratio	1.44*

* Ratio of % dose/g for each region.

IBZM, for imaging of one human brain has recently been reported, however, no data on the chemical characterization were described (*24*). This agent showed lower brain uptake than that for [^{77}Br]bromospiperone.

In conclusion, the results of the in vitro binding studies suggest that the binding is highly selective for D-2 dopamine receptors. The in vivo images and tissue counting data described in this paper clearly demonstrate that [^{123}I]IBZM is localized in basal ganglia of a monkey. The planar images (lateral view of the head) with this agent clearly demonstrate that D-2 dopamine receptors can be visualized in a species close to human being. Based on this data it is our belief that with single photon emission computed tomography (SPECT), which improves the image quality and provides quantitative information, the use of this agent will allow the generation of images of these receptors in the human brain.

TABLE 4
Biodistribution of [^{123}I]IBZM in a Monkey*

Organ	% Dose/organ	% Dose/g
B. ganglia	—	0.212
Cerebellum	—	0.043
B. Stem	—	0.062
Hip.	—	0.160
Gray	—	0.062
White	—	0.054
Brain (total)	3.71	0.0675
Liver	4.19	0.0873
Muscle	12.64	0.0126
Lungs	1.885	0.134
Thyroid	0.146	—
Spleen	0.281	0.0385
Kidney	1.198	0.0974

* The monkey was killed at 120 min after the i.v. injection of [^{123}I]IBZM.

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