# The Characterization of IBF as a New Selective Dopamine D-2 Receptor Imaging Agent

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The in vivo and in vitro studies of a new iodinated benzamide analog, [$^{125}$I]IBF,5-iodo-7-N-[(1-ethyl-2-pyrrolidinyl)methyl]carboxamido-2,3-dihydrobenzofuran as a potential central nervous system (CNS) D-2 dopamine receptor imaging agent were investigated. In vivo biodistribution of IBF in rat indicated that this agent concentrated in the striatum region and displayed a remarkably high target-to-nontarget ratio (striatum/cerebellum = 48 at 120 min post-injection). The in vitro binding studies suggested that IBF binds selectively to D-2 dopamine receptors with high affinity and low nonspecific binding (Kd = 0.106 ± 0.015 nM, Bmax = 448 ± 18.2 fmole/mg protein). Ex vivo autoradiography results in rats further confirmed the high uptake and retention of this agent in the basal ganglia region. The planar images of monkey brains (lateral view of the head) after i.v. injection of [$^{123}$I]IBF clearly demonstrated that D-2 dopamine receptors can be visualized. With the excellent in vivo stability to deiodination and high target-to-nontarget ratio, [$^{123}$I]IBF may be useful as a CNS D-2 dopamine receptor imaging agent for single photon emission computed tomography (SPECT) in humans.

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A variety of substituted benzamide derivatives possessing antipsychotic properties have been reported (*1–5*). The pharmacologic effects of these agents are assumed to be induced by blocking the central nervous system (CNS) D-2 dopamine receptor. In this series of benzamide derivatives, agents with an (S)-(−)N-ethyl-2-(aminomethyl)pyrrolidinyl group appear to be the most attractive antagonists—showing the best selectivity and the highest affinity for the CNS D-2 dopamine receptor. Raclopride (*6*) and eticlopride (*7,8*) are two excellent examples which show specific D-2 antagonistic activity, with high affinity in rat striatum tissue preparations and low nonspecific binding. Radioactive iodinated benzamides are not only potentially useful as imaging agents (labeled with iodine-123 [$^{123}$I], $T_{1/2}$ = 13 hr, gamma ray energy = 159 keV), but also are very valuable as pharmacologic tools for probing the D-2 dopamine receptor under in vitro and in vivo conditions (labeled with $^{125}$I, $T_{1/2}$ = 60 days, gamma energy = 30–65 keV). Several iodinated benzamide derivatives: iodosulpiride (*9*), iodoazido-clebopride (*10*), iodopride (*11,12*), and iodinated benzamide (IBZM) (*13–15*) have been reported to show very high affinity and selectivity to the D-2 dopamine receptor in the same striatal membrane preparation. More recently, two new iodinated benzamide analogs, ioxipride and epidepride, have been shown to display remarkably high in vivo target- (striatum) to-nontarget (cerebellum) ratio in rats (*16*).

Our efforts in developing new iodinated D-2 dopamine receptor imaging agents have led us to consider bicyclic benzamide series. Several patents describing the synthesis and pharmacologic studies of bicyclic benzamide analogs have been reported (*17,18*). Of particular interest to the design of new IBZM analogs is the dihydrobenzofuran series in which the bromo and chloro derivatives displayed high pharmacologic potential and good receptor affinity in the in vitro binding assay (*18*). In our laboratory, the structural versatility of the benzamides for CNS D-2 dopamine receptor bindings have been studied in a series of new iodinated benzamides with fused ring systems, naphthlene and benzofuran (*19*). An iodinated benzofuran benzamide derivative, IBF, as the structure shown in Figure 1, was found to be the best compound among the two series tested. In this paper, the in vivo and in vitro characterizations of IBF as a selective CNS D-2 dopamine receptor imaging agent are described.

## MATERIALS AND METHODS

### General

Male Sprague-Dawley rats weighing 250–300 g were used. The rats were housed in an animal facility with 12 hr light and dark cycles with access to food and water ad lib. Spiperone, (+) butaclamol, (−) butaclamol, WB4101, ketanserin, dopamine, and SCH-23390 were obtained from Research Biochemicals, Inc., Wayland, MA. Propranolol was purchased from Aldrich Chemical Company (Milwaukee, WI). Naloxone and sulpiride were obtained from Sigma Chemical Company (St. Louis, MO). IBZM and IBF were prepared by the methods

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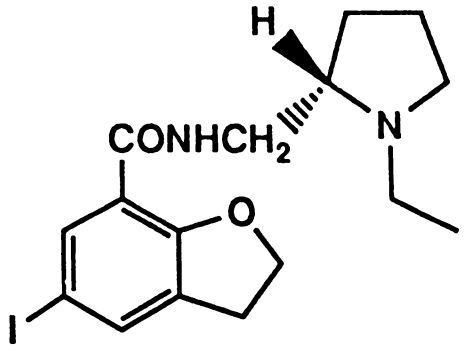


S(-)-IBF

**FIGURE 1**
Chemical structure of (S)-5-iodo-7-N-[(1-ethyl-2-pyrrolidinyl)methyl]carboxamido-2,3-dihydrobenzofuran (IBF).

reported previously (*15,19*). All other chemicals used were of chemical grade.

### Radiolabeling

The $^{125}$I- and $^{123}$I-labeled IBF were prepared by an iododestannylation reaction as reported previously (radiochemical purity >96%, overall yield ~60%, no UV detectable impurities (*19*). The specific activity of preparation synthesized in this way was determined by the receptor binding assay, determining the Ki value for "cold" IBF, with rat striatal membrane preparation (*20*). The Ki value, 0.08 ± 0.01 n*M* (n = 8), was consistently the same as that of Kd, 0.010 ± 0.015 n*M*; therefore, the [$^{125}$I]IBF is carrier-free with a theoretical specific activity value: 2,200 Ci/mmole.

### Biodistribution in Rats

Rats were administered with [$^{125}$I]IBF (0.1–5 μCi in 0.2 ml saline solution) intravenously through the femoral vein under ether anesthesia. Rats were killed at various time points after injection by cardiac excision. The organs of interest were then removed and weighed and the radioactivity concentration was determined with a Beckman gamma counter (Model 5500, San Ramon, CA). The percent dose per organ was calculated by a comparison of the tissue counts to suitably diluted aliquots of the injected material. Total activities of blood and muscle were calculated assuming that they were 7% and 40% of total body weight, respectively.

Regional brain uptake of [$^{125}$I]IBF in rats was obtained by dissecting samples from different brain regions (cortex, hippocampus, striatum, and cerebellum). The uptake ratio of each region was obtained by dividing percent dose/g of each region with that of the cerebellum. To determine the effect of spiperone, a specific D-2 antagonist, on regional brain uptake, different concentrations (0.01–100 nmole/kg of body weight) of spiperone were co-injected (intravenously) with [$^{125}$I]IBF into rats. At 60 min postinjection, the rats were killed and the brains were removed and dissected as described above.

### Re-extraction of [$^{125}$I]IBF from Rat Striatum

Thirty minutes after the i.v. injection of [$^{125}$I]IBF into three rats, the striatal tissues were removed from each rat and homogenized individually in 1.5 ml of 10 m*M* phosphate buffer (pH 7.0) in the presence of cold IBF carrier (~100 μg). The homogenates were then extracted separately with ethyl acetate (3 × 1.5 ml). The purity of [$^{125}$I]IBF in the condensed extracts was analyzed with high pressure liquid chromatography (HPLC), using a PRP-1 column, and a mixed solvent system of 90% acetonitrile/10% pH 7.0 buffer (5 m*M* dimethylglutaric acid) with a flow rate of 1 ml/min. The retention time of authentic IBF under these conditions was 6–7 min.

### Ex Vivo Autoradiography of Rat Brain

Rats were injected with 0.2 ml saline solution (intravenously) containing 1 mCi of carrier-free [$^{125}$I]IBF. The brain was rapidly removed 30 min after the i.v. injection and sectioned at low temperature (−15°C) as previously described (*13*). For blocking studies, rats were pretreated with spiperone (10 μmole/kg of body weight, i.p.) 30 min before i.v. injection, then killed 30 min after i.v. injection. The same sectioning procedure was then carried out as described for the normal rats. The autoradiograms were digitized with an image analysis system (Imagitronics S-1000, Denver, CO) developed by Lear (*21*).

### In Vitro Binding

Rat tissue homogenates were prepared as described previously (*19*). The binding assays were performed by incubating 50 μl of the tissue preparation containing a variable amount of protein (striatum, 40–60 μg; hippocampus, cortex, and cerebellum, 300–400 μg) with increasing amounts of [$^{125}$I]IBF (for saturation analysis) or appropriate amounts of ligand and competitors (for competition study) in a total volume of 0.2 ml of the assay buffer (50 m*M*, Tris buffer, pH 7.4, 120 m*M* NaCl, 5 m*M* KCl, 2 m*M* $CaCl_2$ and 1 m*M* $MgCl_2$). Incubation was performed for 20 min at 37°C and the samples were rapidly filtered and washed with cold assay buffer. The filters containing the bound radioactivity were then counted in a gamma counter with 70% efficiency. The nonspecific binding was determined in the presence of 10 μ*M* of spiperone. The data points were analyzed using the iterative, nonlinear least squares curve-fitting program, LIGAND (*22*).

### Dynamic Planar Imaging Study with Monkeys

A monkey (cynomologous, 5 lb) was sedated with ketamine (50 mg, i.m.) and an i.v. infusion of pentobarbital (0.2 ml, 65 mg/ml), followed 30 min later by an i.v. injection of [$^{123}$I]IBF (1.5 mCi/1.5 ml). Immediately after the i.v. injection, lateral images of the head (1 min per frame for 120 min) were obtained using a Picker Digital Duna Camera (Bedford, OH) equipped with an all-purpose collimator on line to a GE Star II computer system (Milwaukee, WI) 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 the whole brain were defined. The net counts in the basal ganglia region were obtained by subtracting the counts in the cortex area from that of the same number of pixels in the basal ganglia area. The net counts in the different brain regions versus time were plotted.

## RESULTS

### Radiolabeling

The carrier-free [$^{125}$I]IBF and [$^{123}$I]IBF were prepared by an iododestannylation reaction as reported previously (*19*). The desired labeled product, IBF, can be

**TABLE 1**
Biodistribution of [$^{125}$I]IBF in Rats—Percent Dose/Organ (Average of Range of 3)

| Organ | 2 min | 15 min | 30 min | 60 min | 120 min | 240 min | 23 hr |
|---|---|---|---|---|---|---|---|
| Blood | 3.50 (3.25–3.74) | 1.80 (1.48–2.17) | 1.26 (1.13–1.33) | 0.98 (0.86–1.11) | 0.53 (0.50–0.55) | 0.40 (0.36–0.42) | 0.17 (0.15–0.18) |
| Heart | 0.81 (0.54–1.04) | 0.20 (0.17–0.23) | 0.11 (0.10–0.12) | 0.05 (0.047–0.052) | 0.02 (0.020–0.021) | 0.011 (0.009–0.014) | † |
| Muscle | 11.25 (9.40–13.10) | 14.36 (13.59–15.85) | 9.00 (7.94–10.91) | 4.97 (4.71–5.61) | 2.30 (2.07–2.51) | 3.83 (1.41–4.72) | 0.648 (0.42–0.78) |
| Lung | 6.14 (4.77–6.69) | 1.85 (1.58–2.21) | 0.82 (0.72–0.87) | 0.37 (0.29–0.49) | 0.12 (0.10–0.14) | 0.06 (0.04–0.09) | 0.025 (0.014–0.038) |
| Kidney | 7.58 (6.87–8.18) | 2.92 (2.62–3.22) | 1.58 (1.11–2.03) | 1.01 (1.00–1.02) | 0.68 (0.34–0.97) | 0.33 (0.26–0.40) | 0.079 (0.050–0.095) |
| Spleen | 0.96 (0.87–1.04) | 0.58 (0.51–0.66) | 0.31 (0.27–0.34) | 0.09 (0.087–0.104) | 0.03 (0.024–0.038) | † | † |
| Liver | 16.32 (11.20–19.50) | 16.16 (11.10–19.50) | 11.59 (10.81–12.71) | 9.84 (8.65–11.80) | 3.95 (3.55–4.18) | 2.93 (2.68–3.19) | 0.817 (0.805–0.822) |
| Skin | 8.54 (7.47–9.86) | 8.46 (8.07–8.85) | 5.34 (5.15–5.45) | 2.38 (2.20–2.54) | 1.18 (1.00–1.36) | 2.93 (2.31–3.85) | 1.13 (0.99–1.67) |
| Thyroid | 0.06 (0.05–0.07) | 0.03 (0.02–0.03) | 0.02 (0.01–0.02) | 0.02 (0.01–0.03) | 0.08 (0.04–0.11) | 0.01 (0.003–0.02) | 0.358 (0.259–0.422) |
| Brain | 0.98 (0.86–1.15) | 0.54 (0.47–0.59) | 0.30 (0.24–0.34) | 0.17 (0.15–0.19) | 0.07 (0.066–0.079) | 0.01 (0.008–0.01) | † |
| Brain* Blood | 2.64 | 3.01 | 2.26 | 1.90 | 1.53 | 0.399 | 0.041 |

* % dose/gram ratio.
† Below 0.01% dose/organ.

effectively separated from the starting material, the tributyltin compound, by HPLC. The radiolabeled compound ([$^{125}$I]IBF or [$^{123}$I]IBF) and the nonradioactive authentic sample showed a consistent HPLC retention profile with a retention time of 7 min in PRP-1 column (Hamilton, Reno, NV), an isocratic eluent of 90% acetonitrile/10% pH 7.0 buffer (5 m*M* 3,3-dimethylglutaric acid), and a flow rate of 1 ml/min.

### Biodistribution in Rats

Iodine-125-IBF showed good brain uptake in rats (Table 1). The initial uptake (0.98% dose/organ) at 2 min postinjection was lower than that of [$^{125}$I]IBZM (2.87% dose/organ). At later time points, the brain uptake decreased; at 1 hr postinjection a large portion of the radioactivity had washed out from the brain (0.17% dose/organ). The brain washout pattern in rats is similar to that of [$^{125}$I]IBZM. High initial uptake in the lungs (6.14% dose/organ) was also observed, but it was rapidly cleared. At 30 and 120 min, the lung uptake dropped to 0.82% and 0.37%, respectively. Liver uptake remained high throughout the first hour and gradually decreased at 2 hr postinjection. The thyroid uptake appeared to be fairly low ranging from 0.02% to 0.08% of the injected dose up to 4 hr postinjection, suggesting minimal in vivo deiodination.

### Regional Distribution of [$^{125}$I]IBF in Rat Brain

Utilizing a brain regional dissection technique, the regional uptake of [$^{125}$I]IBF in rats was determined (Fig. 2). The striatum, a region rich in D-2 dopamine receptors, showed a higher uptake than other regions, i.e., cortex, hippocampus, and cerebellum, after the initial 5 min postinjection. The striatal uptake was retained whereas the uptake of the other regions showed decrement (Fig. 2). The striatum/cerebellum (ST/CB) ratio displayed a dramatic increase with time: 2.0, 3.8, 6.2, 18, 48, and 54 at 2, 15, 30, 60, 120, and 240 min, respectively (Fig. 3). This remarkable increase in the target-to-nontarget ratio versus time was not observed

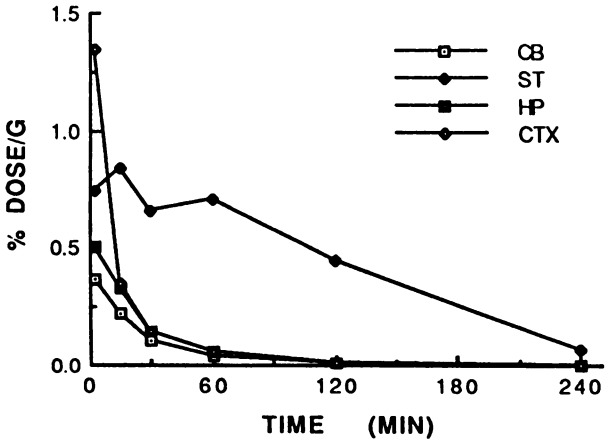


**FIGURE 2**
Regional brain uptake of [$^{125}$I]IBF in rats. CTX: cortex; HP: hippocampus; ST: striatum; CB: cerebellum. Each point represents the mean of three animals.

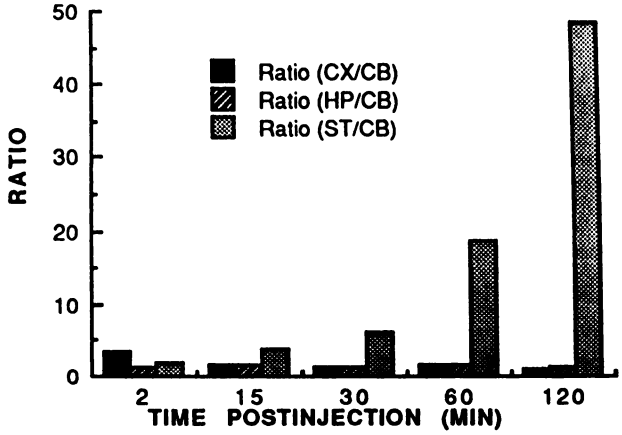


**FIGURE 3**
Ratios of regional brain uptake of [$^{125}$I]IBF (CX: cortex; HP: hippocampus; ST: striatum; CB: cerebellum). The dramatic increase of ST/CB ratio with time suggests that the agent is specifically concentrated in the target tissue, in which the D-2 dopamine receptor density is high.

for the other two regions (hippocampus and cortex). The competitiveness of [$^{125}$I]IBF binding in the regional brain uptake with spiperone is shown in Figure 4. The uptake of radioactivity in the hippocampus, cortex, and cerebellum was not significantly different at any of the doses of spiperone given. By contrast, the uptake of radioactivity in the striatum was saturable and dose-dependent. The data suggests that the binding of IBF to striatum is a specific process related to D-2 dopamine receptors.

### Metabolic Stability of [$^{125}$I]IBF in Rat Striatum

At 30 min after i.v. injection, the radioactivity in rat striatal tissue can be extracted (>92%) by ethyl acetate. The extractable material displayed a single peak (>98% pure) on HPLC with the same retention time as that of authentic IBF.

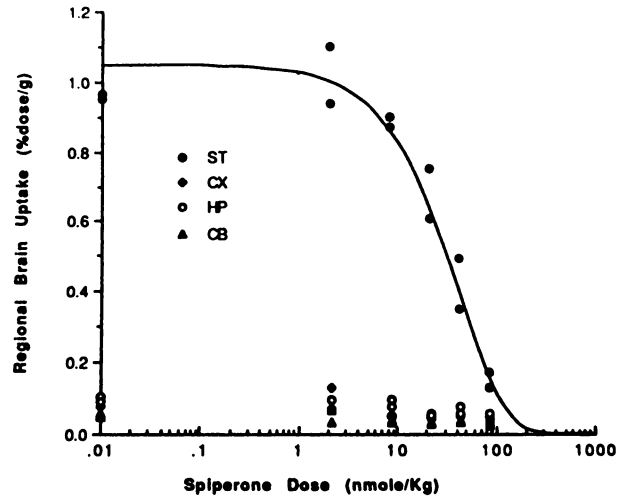


**FIGURE 4**
Competition binding of spiperone with [$^{125}$I]IBF in rat striatum, cortex, hippocampus, and cerebellum. Animals were killed 60 min after injection. ST: striatum, CB: cerebellum, HP: hippocampus, CX: cortex.

### In Vitro Saturation Binding and Displacement Studies

As reported previously for [$^{125}$I]IBZM, the new iodinated benzamide analog, [$^{125}$I]IBF, displayed a high affinity and a saturable binding to the rat striatal membrane preparation (Fig. 5). The saturation curve also indicated a very low nonspecific binding (<5% at Kd) for [$^{125}$I]IBF, which is superior to the other iodinated benzamide ligands reported, including IBZM. The Kd and Bmax values for [$^{125}$I]IBF, generated from the scatchard plot (Fig. 5 insert), are 0.106 ± 0.015 n*M* and 448 ± 18.2 fmole/mg protein, respectively. These values were comparable to those for [$^{125}$I]IBZM (Kd = 0.426 n*M*, Bmax = 480 fmole/mg protein) measured under similar conditions (*15*). The binding constants (Kd and Bmax) were also measured for the other regions of rat brain as well as striatal tissue. The Kd values were 0.102 n*M*, 0.242 n*M* and 0.397 n*M* for cortex, hippocampus, and cerebellum regions, respectively. The binding of [$^{125}$I]IBF in all the regions examined can be totally displaced with 10 $\mu M$ sulpiride, a selective dopamine D-2 ligand. Furthermore, the Bmax of [$^{125}$I]IBF in the striatum (448 fmole/mg protein) greatly exceeds the values for hippocampus (95 fmole/mg protein), cortex (34 fmole/mg protein), and cerebellum (57 fmole/mg protein). The result is consistent with the fact that the striatum has more D-2 dopamine receptors than any other region of the brain.

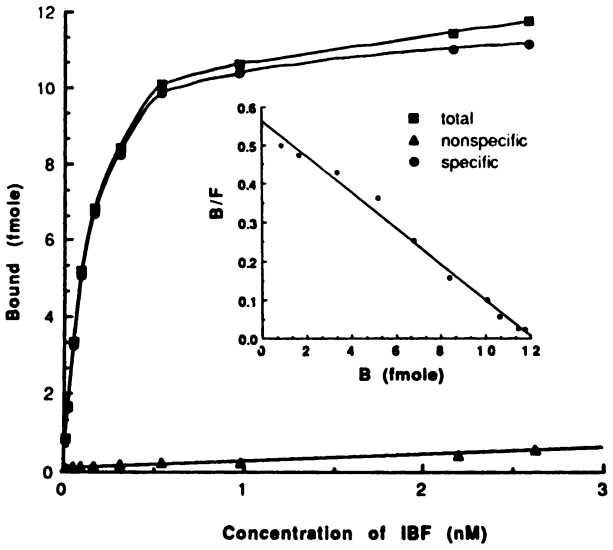


**FIGURE 5**
Binding of [$^{125}$I]IBF to membranes prepared from rat striatum. Nonspecific binding was determined in the presence of 10 $\mu M$ of spiperone. The amount of [$^{125}$I]IBF specifically bound (●) was calculated as the difference between total (■) and nonspecific (▲) binding. Inset: scatchard plot of the saturation isotherm. The Kd and Bmax values from this experiment are 0.106 n*M* and 448 fmole/mg protein, respectively.

**TABLE 2**
Inhibition Constants of Various Compounds on the Binding of [$^{125}$I]IBF to Receptors on Membranes from Rat Striatum*

| Compound | Ki (n*M*, mean ± s.e.m.) |
|---|---|
| Spiperone | 0.015 ± 0.002 |
| S(−)IBZM | 0.261 ± 0.018 |
| S(−)IBF | 0.085 ± 0.010 |
| (+)Butaclamol | 1.19 ± 0.14 |
| WB4101 | 20.2 ± 1.8 |
| Ketaserin | 491 ± 49 |
| Dopamine | 843 ± 150 |
| SCH23390 | 820 ± 164 |
| (−)Butaclamol | >5,000 |
| Naloxone | >2,000 |
| Propranolol | >10,000 |

* 0.05–0.15 n*M* [$^{125}$I]IBF was incubated in the presence of the indicated compounds in 7–11 concentrations and of membrane preparation from rat striatum. Each value represents the mean ± s.e.m. of three to five determinations.

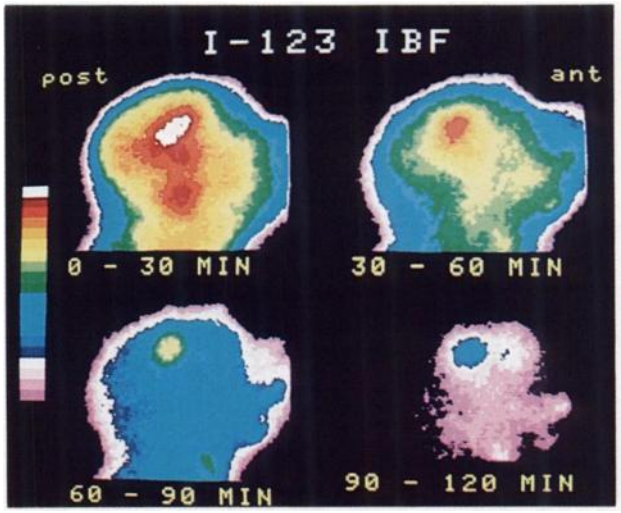


**FIGURE 7**
Brain images (lateral view) of a monkey after injection of 2 mCi of [$^{123}$I]IBF. The radioactivity is concentrated in the basal ganglia of the brain.

D-2 dopamine antagonists, e.g., spiperone, IBZM, and (+)butaclamol, inhibit [$^{125}$I]IBF binding more potently (lower Ki) than the D-2 agonist dopamine or the D-1 antagonist SCH-23390 and the alpha-adrenergic ligand WB4101 (Table 2). However, (−)butaclamol and the ligands for other receptors, including ketanserin, naloxone and propranolol, do not compete with the binding of IBF.

### Ex Vivo Autoradiography in Rats

The regional cerebral distribution of [$^{125}$I]IBF in brain sections at 30 min postinjection showed distinct uptake in the caudate putamen and accumbens nucleus, regions known to have high concentrations of D-2 receptors relative to the rest of the brain (Fig. 6). This pattern of distribution is similar to that observed for [$^{125}$I]IBZM. Furthermore, autoradiograms of brain sections from a rat pretreated with spiperone, a known D-2 dopamine antagonist, showed specific and total blockage of the uptake in the areas of caudate putamen (Fig. 6).

### Imaging Study of [$^{123}$I]IBF in a Monkey

Results of an imaging study of [$^{123}$I]IBF in a cynomologous monkey is shown in Figure 7. The summed images demonstrate that the agent is concentrated in the basal ganglia region, where the dopamine receptors are located. The time-radioactivity curve of the images in the brain regions of interest is presented in Figure 8.

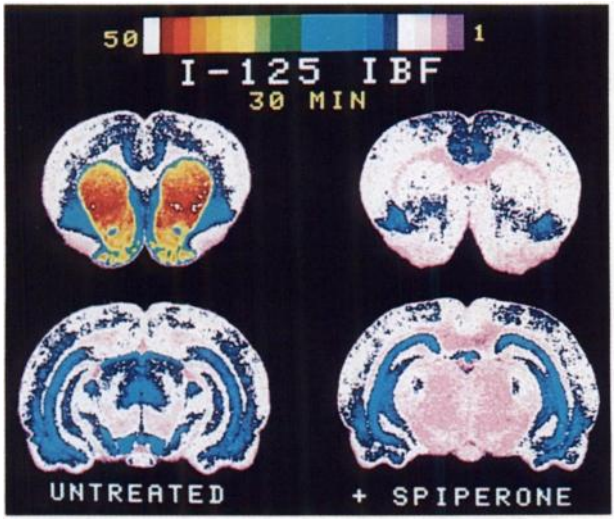


**FIGURE 6**
In vivo autoradiography of rat coronal brain sections, 30 min uptake of [$^{125}$I]IBF. The two sections on the right are from a rat pretreated with spiperone (10 μmole/kg, i.p.) 30 min before the i.v. injection and killed at 30 min postinjection.

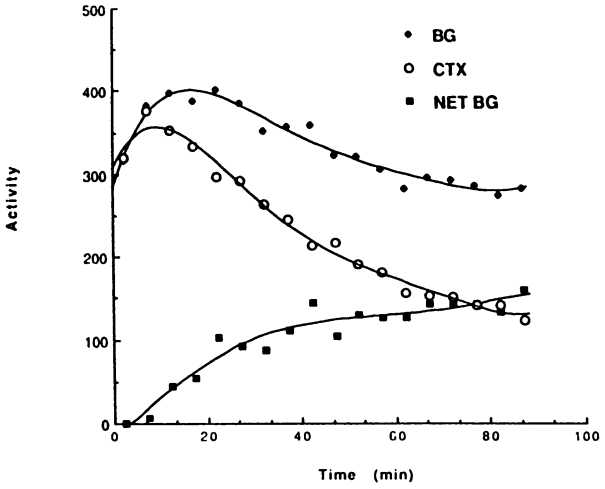


**FIGURE 8**
Time-activity curve after administration of [$^{123}$I]IBF in different brain regions based on lateral view of monkey head. Net activity in basal ganglia region was obtained by subtracting counts in cortex from that in basal ganglia.

The uptake in basal ganglia showed a plateau from 10–20 min after the injection and basal ganglia were clearly visualized from the surrounding tissue at later time periods because of the slower clearance profile. The net counts in basal ganglia were constant during the time period from 45–90 min, as shown in Figure 8. The basal ganglia/cortex ratio was ~2.0 at 75 min after administration.

## DISCUSSION

In this paper, we report that IBF, an iodinated dihydrobenzofuran benzamide, can be successfully radiolabeled with $^{125}I$ and $^{123}I$ by an iododestannylation reaction. The in vivo biodistribution studies in rats with [$^{125}I$]IBF showed high accumulation in the striatum, a region of high dopamine receptor density, whereas its accumulation was much less pronounced in the hippocampus, cortex, and cerebellum, regions of lower dopamine receptor density. As compared to IBZM, an iodinated benzamide ligand previously developed in our laboratory (*14,15*), IBF displayed higher target-to-nontarget (striatum versus cerebellum) ratios (48 versus 10 at 2 hr postinjection). Similar high target-to-nontarget ratios on the in vivo uptake of [$^{125}I$]ioxipride and [$^{125}I$]epidepride (two close analogs of IBZM and IBF) in rat brains have been reported (*16*). Ioxipride showed a peak striatal to cerebellum ratio of 65:1; epidepride displayed a remarkably high peak striatal to cerebellum ratio of 234:1. An iodinated 2′-iodospiperone(2′-ISP) also reported a striatum to cerebellum ratio of 13.6 at 2 hr in rats for this iodinated spiperone derivative (*23*). In addition, the in vivo striatal binding of [$^{125}I$]IBF in rats can be blocked by coinjection with spiperone, a specific D-2 antagonist, indicating that IBF is a selective dopamine D-2 ligand.

The superior in vivo selectivity of IBF was further corroborated by the in vitro displacement studies with various receptor ligands. The most potent displacement for in vitro IBF binding was seen with the D-2 antagonists, e.g., spiperone, IBZM, and (+)butaclamol. A clear-cut stereospecificity was demonstrated in the competition experiment performed with the two isomers of butaclamol. The (+)-isomer showed the specific inhibition of the IBF binding, while the (−)-isomer displayed low inhibition. The competition data demonstrate that IBF is a very selective ligand for studying D-2 receptors and the results are consistent with the pharmacologic profile of a D-2 receptor antagonist.

The analysis of organic extracts of rat striatal homogenate at 30 min postinjection of [$^{125}I$]IBF registered the presence of >98% of the original compound, a good indication of its in vivo stability. Ex vivo autoradiography results in rats and an in vivo monkey imaging study of IBF further confirm the high uptake and retention of IBF in basal ganglia region. It was observed that the nonspecific uptake of IBF in regions low in dopamine receptors is lower than that for IBZM. This is also consistent with the characteristic of very low in vitro nonspecific binding and very high in vivo target-to-nontarget ratio of IBF.

In conclusion, the in vitro and in vivo data indicate that IBF is an excellent D-2 dopamine receptor ligand with specific and selective affinity. When labeled with $^{123}I$, it is a potentially useful D-2 dopamine receptor imaging agent in humans.

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