High Affinity Dopamine D2 Receptor Radioligands. 1. Regional Rat Brain Distribution of Iodinated Benzamides

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Five ^{125}I-labeled substituted benzamides, which are close structural analogues of (S)-sulpiride, eticlopride, and isoremoxipride, were evaluated for their selective in vivo uptake into dopamine D2 receptor rich tissue of the rat brain. "Iodopride" (K_D 0.88 nM), an iodine substituted benzamide structurally related to sulpiride, displayed a maximal striatum:cerebellar uptake ratio of 7.6. Demonstration of saturation of the receptor with [^{125}I]iodopride in striatum required uptake in frontal cortex to be used, rather than cerebellar uptake, to define nonspecific binding. Two other ligands structurally related to eticlopride, "iclopride" (K_D 0.23 nM) and "itopride" (K_D 0.16 nM), displayed maximal striatal:cerebellar uptake ratios of 9.8 and 3.3, respectively. The most potent ligands, "epidepride" (K_D 0.057 nM) and "ioxipride" (K_D 0.070 nM) showed striatal:cerebellar uptake ratios of 234 and 65, respectively. The observed uptake ratios correlated poorly with the affinity constants for the dopamine D2 receptor alone, but were highly correlated (r = 0.92) with the product of the receptor dissociation constant (K_D) and the apparent lipophilicity (k_w), as determined by reverse-phase HPLC at pH 7.5. Total striatal uptake also appeared dependent on lipophilicity, with maximal uptake occurring for ligands having log k_w 2.4–2.8.

C erebral dopaminergic neurotransmission has been extensively studied and has been shown to be involved in the pathogenesis of Parkinson's disease (*1*) and progressive supranuclear palsy (*2*). It has been implicated in the etiology of a number of other disorders including schizophrenia (*3*), affective disorders (*4*) and tardive dyskinesia (*5,6*). Because of the widespread interest in cerebral dopaminergic function and the availability of radiolabeled, high affinity dopaminergic ligands, in vivo studies of dopamine D2 receptors in man have been performed utilizing emission tomography. The majority of these studies of D2 receptors have utilized either a derivative of spiperone or a substituted benzamide. Spiperone and its derivatives, e.g., [^{11}C]-N-methylspiperone, have very high affinities for the D2 receptor but also are potent serotonergic ligands and have nonreversible binding in vivo (*7–10*). The substituted benzamides used in man, [^{11}C]raclopride (*11,12*) and [^{123}I]IBZM (*13,14*), are selective for the D2 receptor, have reversible binding in vivo, and have moderate affinity for the D2 receptor. Both Seeman (*15*) and Ross (*16*) have recently demonstrated that [^{3}H]raclopride binding in vivo is inhibited by endogenous free synaptic dopamine, while binding of a high affinity ligand to the D2 receptor was not significantly inhibited by dopamine (*15*). Thus, ligands with very high affinity may be needed for accurate in vivo measurements of receptor number.

A dopamine D2 ligand for single photon emission tomography (SPECT) imaging would ideally have great selectivity and high affinity for the D2 receptor, low nonspecific binding, and brain uptake adequate for imaging. The in vivo striatal:cerebellar ratios for [^{11}C]raclopride and [^{123}I]IBZM have been reported as 4:1 (*11*) and 2:1 (*14*), respectively, in man; these ratios are sufficient for imaging but not optimal. Many substituted benzamides are relatively selective for the D2 receptor (*17*); substituted benzamides with high affinity for the D2 receptor such as eticlopride (*18*) and isoremoxipride (*19*) have been synthesized. Kessler and colleagues have recently reported a series of iodinated benzamides with high affinity for the D2 receptor (*20–22*), which are structurally related to sulpiride and to these high affinity benzamides. The present study was undertaken to determine if these compounds might be suitable as potential D2 ligands for SPECT and to evaluate the relationship of receptor affinity and lipophilicity to the regional brain uptake of these tracers. The compounds evaluated include iodopride, iclopride, ioxipride, and epidepride. A preliminary evaluation of itopride, a substituted benzamide structurally related to eticlopride, was also performed. The structures of these and related compounds are shown in Figure 1.

METHODS

Drugs

Iodopride, (S)-N-[(1-ethyl-2-pyrrolidinyl)methyl]-5-iodo-2-methoxybenzamide (*21*), IBZM (*13,23*), eticlopride (*24*), and
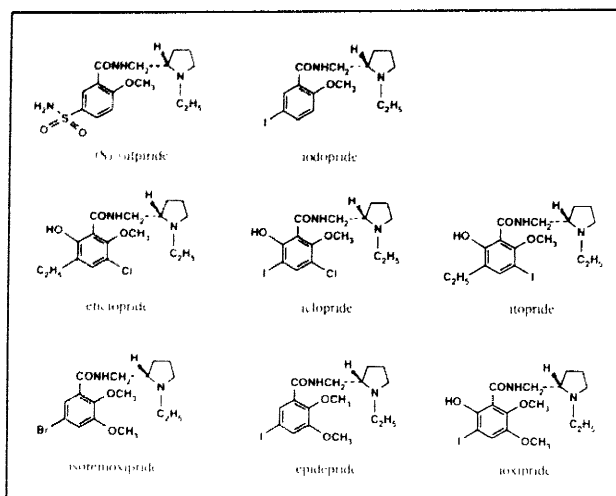
FIGURE 1. Structures of new iodinated substituted benzamides and parent compounds. The parent compounds are shown in the left column and the corresponding iodinated derivatives on the right.

ioxipride, (S)-N-[(1-ethyl-2-pyrrolidinyl)methyl]-3-iodo-5,6-dimethoxysalicylamide (*25*) were prepared as described in the literature. Iclopride, (S)-5-chloro-N-[(1-ethyl-2-pyrrolidinyl)methyl]-3-iodo-6-methoxysalicylamide (*20*), itopride, (S)-3-ethyl-N-[(1-ethyl-2-pyrrolidinyl)methyl]-5-iodo-6-methoxysalicylamide, and epidepride, (S)-N-[(1-ethyl-2-pyrrolidinyl)methyl]-5-iodo-2,3-dimethoxybenzamide (*26*), were prepared from the appropriate iodine substituted benzoic acid derivatives as described for the corresponding bromo analogues (*27*). Raclopride (*27*) and IBF (*28*) were gifts from Dr. H. Hall (Astra) and Dr. H. F. Kung (University of Pennsylvania), respectively.

Radiolabeling

Preparation of [125I]iodobenzamides at high specific activity was achieved by iododestannylation of the corresponding tributyltinbenzamide (iodopride, epidepride) (*21,26*) or by electrophilic iodination of the corresponding desiodobenzamide (ioxipride, iclopride, and itopride) (*23,29*). No-carrier-added sodium [125I]iodide (2000 Ci/mmol) was oxidized in situ by chloramine-T in dilute hydrochloric acid. Its subsequent reaction with the appropriate substituted benzamide gave the [125I]iodobenzamide with radiochemical yields of 50–60% after purification by reverse-phase liquid chromatography (Waters 10 cm × 8 mm NOVA Pak-CN, ethanol:100 m*M* phosphate buffer (30:70) at pH 7.0, flow rate 1.5 ml/min, and ambient temperature).

In Vitro Receptor Binding Studies

Male Harlan Sprague Dawley rats (200–300 g) were sacrificed, the brain removed, dissected and striatum stored at −80°C if not used on the day of sacrifice. On the day of assay, the striatum was homogenized using a Brinkman Polytron (15 sec at half-maximum speed) in a 100-fold (w:v) dilution of a 50-m*M* Tris HCl buffer, pH 7.4, containing 120 m*M* NaCl, 5 m*M* KCl, 2 m*M* CaCl₂, 1 m*M* MgSO₄, 1 m*M* NaEDTA, and 100 μ*M* Na ascorbate. The homogenate was centrifuged at 10,000 × g for 15 min at 4°C, the pellet resuspended in the same volume of buffer, centrifuged a second time, and resuspended in fresh buffer at 500 w:v.

The IC₅₀ for inhibition of [³H]spiperone binding to striatal homogenate was determined as follows. Prior to incubation, the assay tubes were placed on ice and the assay started by addition of 0.5 ml of the tissue homogenate to each tube containing [³H] spiperone (at a final concentration of 300 p*M*), 10 n*M* ketanserin, 10 μ*M* pargyline, and the displacing ligand at a final concentration of from 0.001 to 100,000 n*M* with a final volume of 2 ml. Nonspecific binding was determined using a concentration of 10 μ*M* spiperone. Each tube was removed from ice, vortexed for 3 sec, and incubated at 25°C for 50 min. Incubation was terminated by filtration through Whatman GF/B filters presoaked in 0.3% polyethylenimine, using a Brandel model M-24R cell harvester. The filters were rinsed for 10 sec with ice-cold Tris HCl buffer, the filter placed in a 20-ml plastic scintillation vial and 10 ml of scintillation fluid was added. Liquid scintillation counting was performed using a Beckman LS3801 counter; efficiency was typically 45%.

The K_D for ligand binding to striatal homogenates was determined as follows. If frozen, the tissue was thawed, homogenized at 100-fold dilution (w:v) in a Tris-HCl buffer for 15 sec using a Brinkman Polytron at half-maximum speed. The homogenate was centrifuged at 4°C at 10,000 × g for 15 min, the supernatant discarded, the pellet resuspended in the initial volume of fresh buffer, and centrifuged a second time. The supernatant was again discarded and the pellet resuspended in a Tris-HCl buffer containing 50 m*M* Tris pH 7.4, 120 m*M* NaCl, 2 m*M* CaCl₂, 1 m*M* MgSO₄, 1 m*M* NaEDTA, and 100 μ*M* Na ascorbate at a final dilution of 500 w:v. Incubation was performed in duplicate in a total of 2.0 ml Tris-HCl-Na buffer containing 0.5 ml tissue and 20 μl radioactive ligand (final concentration 0.001 n*M* to 500 n*M*). The tissue was incubated for 45 min at 25°C and the binding assay terminated by filtration as described above. The filter was placed in a gamma counting tube and gamma spectrometry performed using a Searle Analytic Inc model 1185, with 86% efficiency. Nonspecific binding was determined by adding 10 μ*M* sulpiride to the incubation mixture. Data analysis was performed using the EBDA program.

In Vivo Studies

Groups of four 200–250 g male Harlan-Sprague-Dawley rats were injected with 25 or 30 μCi of ¹²⁵I labeled iodobenzamide via tail vein. The animals were killed at 5, 20, 40, 80, 160, and when necessary, 320, 640, and 1,280 min after injection. The brains were rapidly removed, washed in iced saline, regional brain dissection was performed, and the cerebellum and striatum were weighed and counted using gamma spectrometry.

To assess the effect of haloperidol, 2 mg/kg haloperidol was administered intraperitoneally 60 min prior to the tail vein administration of 25 or 30 μCi ¹²⁵I-labeled iodobenzamide. Groups of four rats were killed at 60 min after [125I]iodopride or [125I] iclopride injection, or 80 min after injection of [125I]epidepride or [125I]ioxipride.

To assess in vivo displacement of epidepride, groups of four 200–250 g Sprague Dawley male rats were injected with 25 μCi [125I]epidepride via tail vein injection and 40 min later received either 5 mg/kg haloperidol or saline also via tail vein injection. Groups of four rats were killed at 40, 80, 160, and 320 minutes post-haloperidol injection, brain regions dissected, and tissues counted as described above.

To demonstrate receptor saturation, groups of three or four rats were injected with 25 μCi of [125I]epidepride or of 30 μCi of

[^{125}I]iodopride. Increasing doses of unlabeled ligand were used to attain specific activities of 100, 50, 20, 10, 5, 2, 1, and 0.2 Ci/mmol. The animals were killed at either 60 min or 80 min postinjection, depending upon whether iodopride or epidepride was utilized. After sacrifice, the brains were dissected and counted as described above.

Lipophilicity Measurement

The method of El Tayar et al. (30) was utilized for estimating the lipophilicity of substituted benzamides. The compounds were analyzed by C-18 reverse phase chromatography using a 3-N-(morpholino)propanesulfonic acid (MOPS) buffer (20 mM, pH 7.5) containing 2 ml per liter of n-decylamine and methanol concentrations between 30% and 60%. The capacity factor (k_x) at various methanol concentrations was calculated using the following equation: $k_x = (t_x - t_o)/t_o$ where t_x = retention time of ligand and t_o = retention time of non-retained peak.

The logarithm of the capacity factors (log k_x) were plotted versus methanol concentration and the log k_w, obtained by linear extrapolation to 0% methanol concentration. This value represents the apparent lipophilicity at pH 7.50. The HPLC system consisted of a Kontron 420 pump, a Rheodyne 4125 injection valve, a 25 cm × 4.6 mm Lichrosorb RP-18 10 μ (Alltech/Applied Sci) HPLC column protected by a Waters Resolve C-18 Guard-Pak guard column, and a Kontron 430 scanning UV detector. Data recording, reduction and analysis were carried out using the Kontron 450 software system. The column was operated at ambient temperature (21°C–23°C) at a flow rate of 1.5 ml/min.

RESULTS

In Vitro Binding

The IC_{50}s for inhibition of [^{3}H]spiperone binding to striatal membranes, the log k_w at pH 7.5, and the K_Ds for

TABLE 1
Dopamine D2 Receptor Affinities and Lipophilicities of Substituted Benzamides

Compound	[^{3}H]Spiperone binding IC_{50} (nM)	Receptor affinity K_D (nM)	Lipophilicity* log k_w (pH 7.5)
Iodopride	10.2	0.88	2.33
Iclopride	8.1	0.23	3.25
Itopride	1.5	0.16	3.55
Ioxipride	0.94	0.070	2.48
Epidepride	1.02	0.057	2.05
IBZM	6.3	0.43[†]	2.75
IBF	—	0.106[‡]	2.32
Raclopride	25.0	1.2[§]	2.66
Eticlopride	0.92	0.09[¶]	3.27
Emonapride	—	0.057**	3.53[††]

* Determined from reverse-phase HPLC capacity factors according to El Tayar (30).
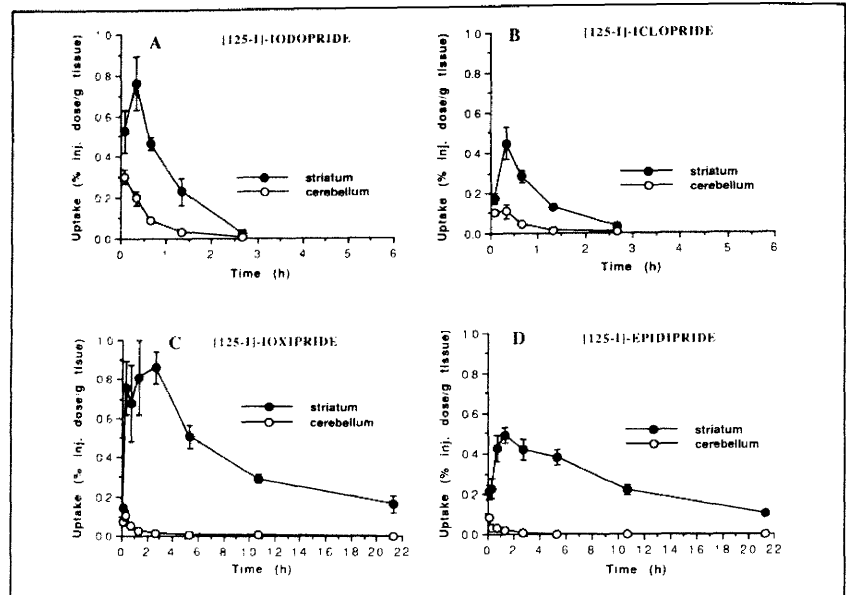[†] Data taken from reference 13.
[‡] Data taken from reference 28.
[§] Data taken from reference 48.
[¶] Data taken from reference 49.
** Data taken from reference 43.
[††] Data taken from reference 34.

binding to striatal membranes for the iodine substituted benzamides and reference compounds are shown in Table 1. Iodopride is a structural analogue of sulpiride, having an iodine atom substituted for the aminosulfonyl group in the five position of the aromatic ring. It has an IC_{50} of 10.2 nM and a K_D of 0.88 nM. Iclopride and itopride are structural analogues of eticlopride. Iclopride has an iodine atom substituted for the ethyl group in the five position of the aromatic ring, while itopride has an iodine atom substituted for the chloro atom in the three position of the aromatic ring. The IC_{50}s for these compounds are 8.1 nM and 1.5 nM, respectively; the K_Ds are 0.23 nM and 0.16 nM, respectively. Epidepride and ioxipride are structural analogues of isoremoxipride (19). Epidepride has an iodine atom substituted for the bromine atom in the five position of the aromatic ring of isoremoxipride. In addition, ioxipride has both an iodine in the five position and a hydroxy group in the six position of the aromatic ring. The IC_{50}s for displacing [^{3}H]spiperone are 1.02 nM for epidepride and 0.94 nM for ioxipride. Epidepride has an apparent K_D for striatal membranes of 57 pM, while ioxipride has an apparent K_D of 70 pM. The Hill coefficients for all five ligands ranged between 0.89 and 1.0, suggesting that all bind to a single site in striatal membranes.

In Vivo Regional Brain Uptake

Plots of time-dependent in vivo regional brain uptake for ^{125}I-labeled iodopride, iclopride, ioxipride, and epidepride are shown in Figure 2. Iodopride and iclopride have peak striatal:cerebellar ratios of 7.6:1 and 9.8:1 at 80 min after injection, respectively. Both show rapid washout from the striatum so that little radioactivity is seen by 160 min. Peak uptake is seen at 20 min after injection, at which time uptake of iodopride and iclopride are 0.76 and 0.44%/g of striatum, respectively. Itopride has a peak striatal:cerebellar ratio 3.3:1 at 320 min postinjection and a peak striatal uptake of 0.35%/g. Of note are the high peak striatal:cerebellar ratios obtained for ioxipride and epidepride, 65:1 and 234:1, respectively, at 640 min after injection and the relatively stable striatal levels of these two compounds. Striatal levels of ioxipride vary less than 22% from 20 min to 160 min after injection, at which time a striatal:cerebellar ratio of 55:1 is seen. Striatal uptake of epidepride varies only 22% from 40 to 320 min following injection, at which time a striatal:cerebellar ratio of 188:1 is seen. The percent of administered dose seen in both striatum and cerebellum was considerably higher for ioxipride than for epidepride; peak striatal uptake was 0.86%/g tissue for ioxipride and 0.49%/g tissue for epidepride.

D2 Receptor Blockade In Vivo

To estimate the proportion of striatal uptake due to D2 receptor binding, haloperidol (2 mg/kg i.p.) was given one hour prior to tail vein injection of the radioiodinated tracer. For iodopride and iclopride, rats were killed 40 min postinjection of tracer, while for ioxipride and epidepride

FIGURE 2. Time course of in vivo rat brain uptake of ^{125}I radioiodinated benzamides in rat striatum and cerebellum. Each point is the mean of four animals ± s.d. Rats were injected in the tail vein at t = 0 and killed at the indicated time points.

rats were sacrificed at 80 min postinjection. The percent of blockade of striatal uptake was calculated as follows:

percent blockade

$$= \frac{[(\text{striatum} - \text{cerebellum}) \text{ control} - (\text{striatum} - \text{cerebellum}) \text{ haloperidol}]}{(\text{striatum} - \text{cerebellum}) \text{ controlled}} \times 100,$$
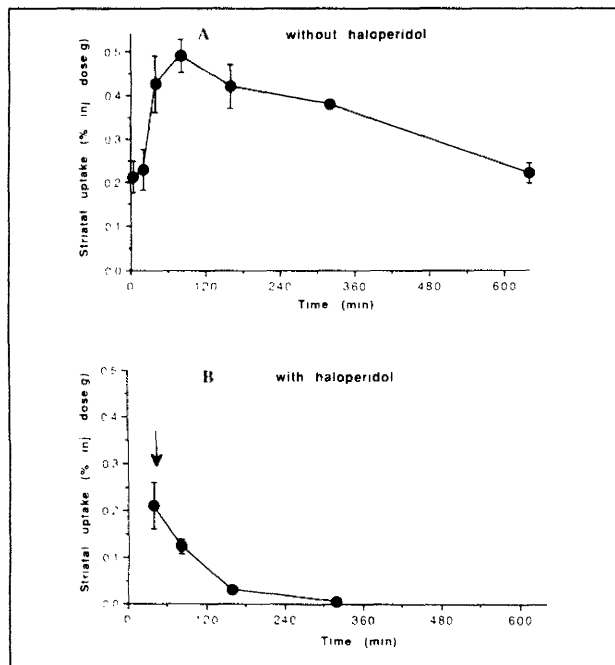

FIGURE 3. In vivo displacement of [125I]epidepride by haloperidol. (A) Time course of [125I]epidepride without haloperidol. Each point is the mean of four animals ± s.d. (B) Haloperidol displacement. Forty minutes after [125I]epidepride injection, haloperidol (5 mg/kg, i.v.) was administered and groups of animals killed at times up to 320 min. A monoexponential washout ($t_{1/2}$ = 40 min) was seen.

where striatum and cerebellum refer to the concentrations of radioactivity in each region. The blockade of uptake was 92% for iodopride, 93% for iclopride, 98% for epidepride, and ioxipride. Thus, nearly all the radioactivity in the striatum with these tracers can be blocked by pretreatment with haloperidol.

The relatively rapid washout of iodopride and iclopride from the striatum demonstrates that these ligands are reversibly bound in vivo. The slower washout of activity seen with ioxipride and epidepride leaves some question as to the ready reversibility of their binding. To ascertain the reversibility of epidepride binding, 25 μCi of [125I] epidepride was injected via tail vein, and 40 min later either saline or haloperidol was administered intravenously. Figure 3A shows the uptake of [125I]epidepride in the striatum from the injection to 320 min postepidepride injection. Figure 3B shows the washout of [125I]epidepride by haloperidol from the striatum for the period from 40 min postepidepride injection to 320 min postepidepride injection. There is a rapid and to a first approximation monoexponential washout of radioactivity from the striatum with a half-life of 40 min. The in vivo binding of [125I] epidepride appears to be completely reversible.

Receptor Saturation

Receptor binding should be saturable in vivo. To demonstrate saturability, increasing doses of unlabeled ligand were added to 25 μCi of ^{125}I-labeled ligand. Saturation curves were obtained for one of the lower affinity ligands, iodopride, at 60 min postinjection and one of the higher affinity ligands, epidepride, at 80 min postinjection (see Figs. 4A–B). Cerebellar uptake was initially used as a measure of nonspecific binding and free ligand in striatum. Saturation could not be demonstrated for iodopride and the uptake curve (Fig. 4A) suggests that the cerebellum underestimates nonspecific binding in the striatum. When
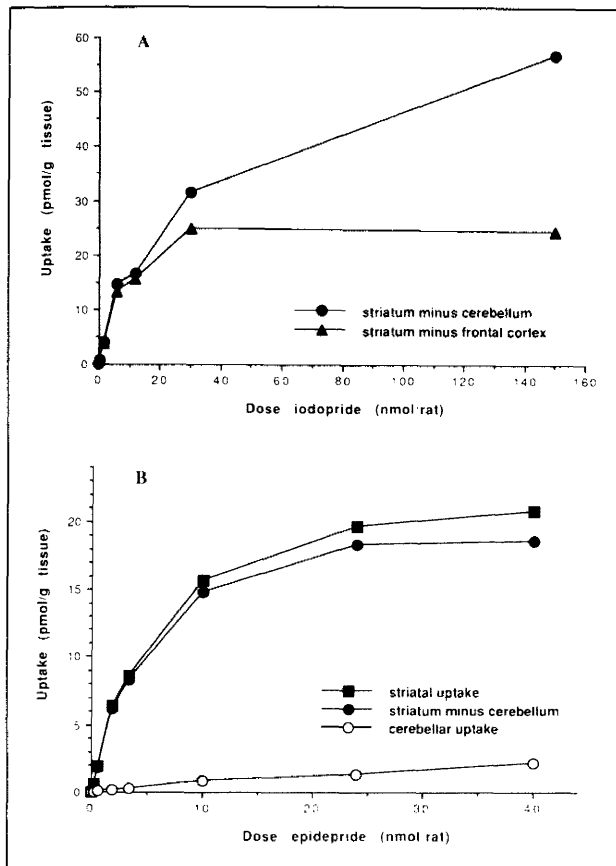
FIGURE 4. In vivo saturation of the dopamine D2 receptor in rat brain. (A) [^{125}I]iodopride saturation. Groups of four rats were injected intravenously with 30 μCi [^{125}I]iodopride with specific activities ranging from 20 to 0.2 Ci/mmol. Rats were killed at 60 min after injection. No saturation was demonstrated using cerebellum as a measure of nonspecific binding and free ligand; saturation was shown when frontal cortex was used to estimate nonspecific binding plus free ligand. (B) Iodine-125-epidepride saturation. Groups of four rats were intravenously administered 25 μCi [^{125}I]epidepride with specific activities ranging from 100 to 0.2 Ci/mmol and killed at 80 min after injection. Cerebellar uptake was used as a measure of nonspecific binding plus free ligand.

FIGURE 5. Correlation of peak striatal:cerebellar ratios in rat brain with the affinity for the dopamine D2 receptor (K_A). The association constants (1/K_D) are from Table 1. The correlation coefficient was 0.35.

Relationship of In Vivo Regional Brain Uptake to Receptor Affinity and Lipophilicity

With a given receptor concentration in a target tissue, it has been demonstrated that the affinity of a ligand places a limit on the contrast between receptor rich and receptor poor tissues (31,32). To study the relationship of affinity for the D2 receptor to the striatal:cerebellar ratios for various benzamides, a plot of K_A versus reported striatal:cerebellar was performed (Fig. 5). This plot demonstrates only a weak correlation (r = 0.35) between K_A and this ratio. Of some note is the observation that the ligands with the highest ratios also had extremely high affinity for the D2 receptor.

Previous work on optimization of ligands for the estrogen receptors (33) has indicated that contrast between receptor rich and receptor poor tissues is a function of both receptor affinity and nonspecific binding. To examine this hypothesis, in vivo affinity was represented by the in

the frontal cortex was used as a measure of nonspecific binding and free ligand, saturation could be demonstrated, with B_{max} = 26 pmole/g tissue in the striatum. This is in reasonable agreement with in vitro determinations, which found a B_{max} of 35 pmole/g in striatum. In support of using the frontal cortex as opposed to the cerebellum is the observation that with haloperidol blockade, the striatal uptake fell to the level seen in the frontal cortex (within experimental error) but remained 50% higher than seen in the cerebellum. The same findings were seen with iclopride following haloperidol pretreatment. With epidepride, saturation can be demonstrated using cerebellum as measure of nonspecific binding since the levels of nonspecific binding are very low (Fig. 4B).
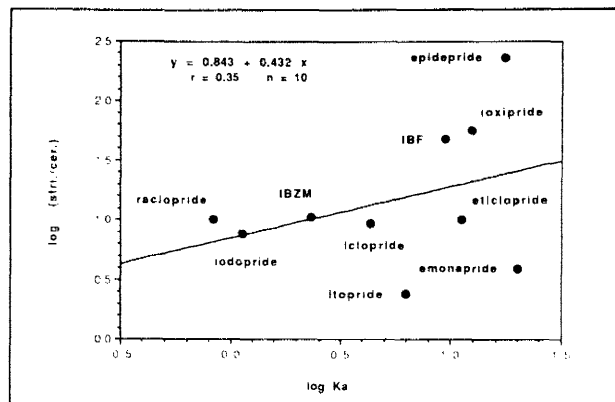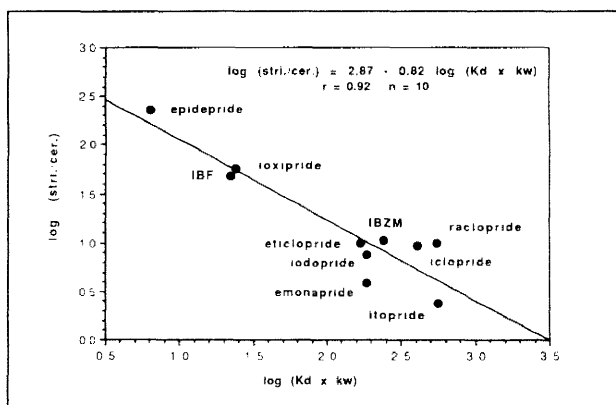

FIGURE 6. Correlation of peak striatal:cerebellar ratios in rat brain with the product of the dissociation constant (K_D) and the apparent lipophilicity (k_w, pH 7.5). The dissociation constants, log k_w's and striatal:cerebellar ratios are taken from Table 1 and the literature (28,38-42).

vitro K_D; nonspecific binding was assumed to be related to lipophilicity, and this was represented by the log k_w at pH 7.5. The log K_D (nM) + log k_w (pH 7.5) was plotted against log striatal:cerebellar ratios (Fig. 6) for the same compounds as in Figure 5. The apparent lipophilicities, log k_ws, were measured in our laboratory for all ligands except emonapride (YM 09151-2) where the value reported by El Tayar (*34*) was utilized. There was an excellent linear correlation (r = 0.92) demonstrating that contrast between striatum and cerebellum depends on both the affinity of a ligand for the dopamine D2 receptor and its apparent lipophilicity (log k_w) at pH 7.5.

The image contrast, i.e., striatal:cerebellar ratio, is an important variable in imaging of D2 receptors, but total uptake in the tissue of interest, i.e., striatum is an important concern. If it is assumed that there is a passive transfer across the blood-brain barrier, then one would expect a relationship between brain uptake and lipophilicity. A plot of striatal uptake versus log k_w at pH 7.5 for the compounds studied is shown in Figure 7. Peak uptake occurred at a log k_w of about 2.4–2.8 and declined above and below that value, suggesting an inverted parabolic relationship.

DISCUSSION

The present study has evaluated the affinity for the dopamine D2 receptor, lipophilicity, and in vivo rat brain distribution of four new iodinated benzamides and preliminarily evaluated a fifth. Previous studies of in vitro binding have demonstrated that epidepride (*35,36*), ioxipride (*37*), and iodopride (*22*) are potent and specific ligands for the dopamine D2 receptor. Several observations suggest that iclopride and itopride are selective ligands for the in vivo study of striatal D2 receptors. These observations include the following: (a) high affinity for the D2 receptor; (b) Hill coefficients of 1.0 and 0.97, respectively, indicating a single striatal binding site; (c) inhibition of in vitro striatal binding by sulpiride; (d) blockade of specific
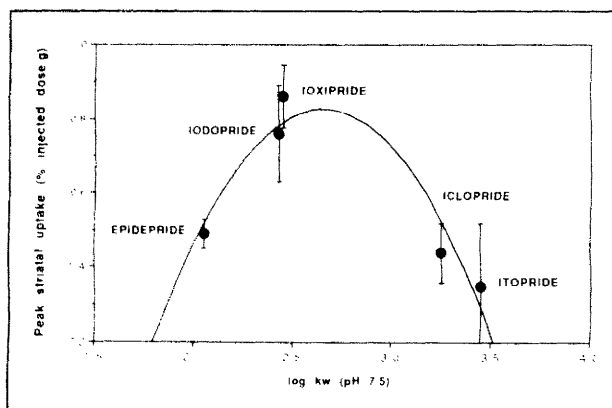

FIGURE 7. Relationship between apparent lipophilicity (log k_w) at pH 7.5 and peak striatal uptake (% administered dose/gram tissue in the rat brain). Peak uptake occurred between log k_w of 2.4 to 2.8. Each point is the mean of four animals ± s.d.

in vivo uptake by haloperidol; (e) the low levels of in vivo uptake in the frontal cortex and hippocampus (data not shown) as well as cerebellum; and (f) the close structural similarity of itopride and iclopride to eticlopride and raclopride, both specific D2 ligands.

In regard to regional brain distribution, the high striatal:cerebellar ratio seen with [^{125}I]epidepride in the rat (234:1) is the highest that has been reported to date. The relatively high and stable in vivo uptake of [^{125}I]epidepride, together with the high contrast found between dopamine-rich and dopamine-poor brain regions, make epidepride an excellent candidate radioligand for SPECT imaging. Iodine-125-ioxipride administration also results in a high striatal:cerebellar ratio (65:1) with stable striatal levels, but with 70% higher peak striatal uptake than seen with epidepride. In a recent report, a striatal:cerebellar ratio of 15:1 3 hr postinjection of [^{123}I]ioxipride (NCQ 298) was obtained in cynomologous monkeys (*37*).

In comparison to currently available SPECT ligands for the D2 receptor, e.g., IBZM (*38*), IBF (*28*), 2-iodospiperone (*39,40*), and spectramide (*41,42*), the new iodobenzamides, epidepride and ioxipride, appear to be superior candidate ligands. The previously reported ligands have in vivo rodent striatal:cerebellar uptake ratios of 10:1, 48:1, 14:1, and 4:1, respectively, in comparison to 234:1 and 65:1 for epidepride and ioxipride. Ioxipride and epidepride have relatively stable striatal uptake in the rat, with the half maximum striatal level during washout attained at 7 and 10 hr postinjection, respectively; in contrast, IBF (*28*) has a more rapid washout, with the half-maximum level being reached at approximately 2 hr. The K_Ds reported here for ioxipride and epidepride are lower than those reported for the above ligands, except spectramide. Spectramide (*42*), with a reported K_D of 25 pM, is clearly a very potent D2 ligand. However, like emonapride (YM 09151-2) (*43,44*), to which it is structurally related, spectramide shows only modest in vivo contrast. The K_Ds reported here must be regarded as preliminary, as careful optimization of binding conditions for each ligand was not performed.

The relationship between affinity, lipophilicity, and in vivo striatal:cerebellar ratios suggests that in addition to high affinity, relatively low apparent lipophilicity is crucial to achieve high tissue contrast. Eticlopride (*15,45*), as well as its structural analogue itopride, and emonapride (*43, 44*) have high affinity for the D2 receptor. However, the relatively high log k_w ($\geq$3.27) of each compound results in relatively high levels of nonspecific binding; therefore, tissue contrast is relatively moderate (10:1, 3.3:1, and 4:1, respectively). Spectramide, being structurally related to emonapride, is presumably highly lipophilic as well, which explains its relatively low in vivo striatal:cerebellar contrast despite its high affinity for the dopamine D2 receptor. In contrast, epidepride and ioxipride both have very high affinity and relatively low lipophilicity, a combination that results in the very high in vivo contrast seen with these

compounds. These compounds differ from iodopride and IBZM only by the presence of a 3-methoxy substituent. This substituent has the unexpected property of raising affinity by an order of magnitude while lowering apparent lipophilicity.

As discussed above, an inverted parabolic relationship between striatal uptake of tracer and log k_w is seen. These findings are similar to those of Dischino (46), who examined the influence of lipophilicity on first pass extraction. The presumed explanation for this behavior is that molecules with relatively low lipophilicity do not cross the blood-brain barrier efficiently whereas highly lipophilic molecules strongly bind to plasma proteins and cell membranes. Consequently, only a small fraction of the ligand in plasma is free and able to cross the blood-brain barrier. In concordance with this explanation is the observation that N-fluoroethyl substituted benzamides (47), which are very lipophilic (log k_w greater than 3.6, D. Schmidt, unpublished observations), have high plasma-protein binding.

The use of the cerebellar uptake for estimating nonspecific binding and free ligand bears some discussion. Saturation of [^{125}I]iodopride binding in vivo could not be demonstrated using cerebellar uptake, while saturation could be seen using uptake in the frontal cortex. These findings suggest that for iodopride cerebellar uptake underestimates nonspecific binding in the striatum. Similarly, [^{11}C]-FLB 472 (the inactive enantiomer of raclopride) has a lower distribution ratio in cerebellum (11.4) than in striatum (15.4) (12). For epidepride, these effects were not significant. Thus, for less potent benzamides, the cerebellum is probably not an appropriate "blank" for binding studies in vivo.

The following conclusions may be drawn from this study. First, the new iodobenzamide derivatives studied, i.e., epidepride and ioxipride, appear to be superior candidates as SPECT ligands for study of the D2 receptor. Second, not only high affinity but also relatively low lipophilicity are needed to achieve high contrast between receptor-rich and receptor-poor brain regions. Third, a lipophilicity range, corresponding to low k_w = 1.7 to 3.3, is required for reasonable brain uptake. Thus, ligands with a log k_w 1.7 to 2.5 and a K_D of 0.1 nM or less, should have optimal affinity and lipophilicity for imaging. An understanding of these relationships will help rational design of future radioligands for brain imaging.

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