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Comparison of the In-vitro Receptor Selectivity of Substituted Benzamide Drugs for Brain Neurotransmitter Receptors

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The in-vitro selectivity of a group of substituted benzamide drugs for brain neurotransmitter receptors was determined to assess the most appropriate drugs for use in human PET studies. All substituted benzamide drugs studied inhibited [^{3}H]haloperidol and [^{3}H]spiperone binding to rat striatal membranes. The most potent compounds were YM 09151-2, clebopride and raclopride. However, these substances also interacted in differing degrees with α-1, α-2, β-adrenergic, 5-HT-1, 5-HT-2, and opiate sites. Sulpiride, alizapride, SL 74205, TER 1546 and tiapride were specific for D-2 receptors, but these drugs were active only in the 10^{-7}–10^{-6} M range. Raclopride, amisulpiride and sultopride showed a 100–1000 differentiation between action on dopamine sites compared with other neurotransmitter receptors. No such selectivity was observed for clebopride or YM 09151-2. Specific substituted benzamides such as alizapride, may be appropriate in high concentrations for defining the interaction of PET ligands with brain dopamine receptors. More potent, but selective, drugs such as raclopride and amisulpiride, may be effective in low concentrations as ligands for labelling dopamine receptor sites. However, the ability of these various substituted benzamide drugs to penetrate into brain and in-vivo to identify dopamine receptors in all brain areas must be assessed.

The in-vivo identification of striatal dopamine receptors in man using positron emission tomography has successfully employed neuroleptic ligands derived from spiperone, such as [^{18}F]methylspiperone (Arnett et al 1985), [^{11}C]methylspiperone (Wagner et al 1984), [^{76}Br] (Maziere et al 1985) [^{18}F]fluoroethylspiperone (Coenen et al 1987). However, these ligands do not act only on brain dopamine receptors; they also label 5-HT and noradrenaline sites. This has not proved a difficulty in imaging dopamine receptors in striatum, since the density of noradrenaline and 5-HT sites in that brain area is low compared with that of dopamine receptors. With the advent of a new generation of PET systems with far greater anatomical resolution, visualization of dopamine receptors in other brain areas may prove possible. In these regions the interaction of dopamine receptor ligands with other receptor systems may become a limiting factor. In a recent study characterizing those receptors involved in the in-vivo accumulation of [^{3}H]spiperone in rat brain, we showed this ligand to identify dopamine receptors alone only in the substantia nigra, striatum and tuberculum olfactorium (Chivers et al 1987).

A further, but similar, difficulty lies in the use of unlabelled drugs to define the specific interaction of radioactive ligands with dopamine receptors. Most neuroleptic compounds are not specific for brain dopamine sites, but interact with a range of different neurotransmitter receptors in brain (see for example, Leysen 1981). These may include dopamine, noradrenaline, 5-HT, histamine and acetylcholine sites. So it is necessary to define those which are most selective for brain dopamine receptors in a variety of brain areas.

For these reasons interest has focussed on the selectivity of sulpiride, a substituted benzamide drug which acts on brain dopamine receptors but does not interact with other neurotransmitter receptor sites (Jenner & Marsden 1981). Sulpiride has been routinely employed to define specific binding of ligands, such as [^{3}H]spiperone, to dopamine receptors in in-vitro tissue populations (Zahniser & Dubocovich 1983; Seeman et al 1984). Use has also been made of [^{3}H](±)- or [^{3}H](−)-sulpiride to identify dopamine receptors in-vitro (Theodorou et al 1979; Woodruff & Freedman 1981).

However, sulpiride is not ideal either as a ligand for PET studies, or for defining the specificity of other ligands for brain dopamine receptors. It exhibits low lipophilicity and does not penetrate readily into brain (Benakis & Rey 1976; Honda et al 1977). As a radioactive ligand the drug has a low affinity for brain dopamine receptors in in-vitro tissue preparations (Theodorou et al 1979). Also, sulpiride may not interact equally with all dopamine receptors in brain. Thus, in a recent study of the characterization of in-vivo [^{3}H]spiperone binding in rats we showed sulpiride to prevent the accumulation of the ligand in substantia nigra, but to have inconsistent effects in the striatum, tuberculum olfactorium and nucleus accumbens (Chivers et al 1988). Attention therefore has turned to using other members of the substituted benzamide drug series in PET studies. For example [^{3}H]remoxipride and [^{3}H]raclopride have been demonstrated to identify dopamine receptors in rat brain in-vitro and in-vivo (Ogren et al 1984; Kohler et al 1985).

However, it is not known which of the substituted benzamide drugs available would be the most appropriate for use as a PET ligand or for defining in-vivo the specific binding of PET ligands to brain dopamine receptors. In this study we compare the in-vitro receptor binding profiles of a group of substituted benzamide drugs to identify those compounds which, like sulpiride, show a marked selectivity for brain dopamine receptors.

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Materials and Methods

Female Wistar rats (150 g; Janssen Pharmaceutica) were used in most experiments. Animals were decapitated and the brain removed into ice. The frontal cortex, nucleus accumbens plus tuberculum olfactorium (mesolimbic tissue), striatum and hippocampus were dissected out. These areas plus the remaining cortical shell or whole forebrain were then used for individual neurotransmitter receptor ligand binding assays. In experiments designed to investigate histamine receptors female Pirbright guinea-pigs (250 g; Janssen Pharmaceutica) were used. Animals were decapitated and the brain removed onto ice. The cerebellum was dissected out and used for the preparation of tissue membranes.

[^{3}H]Haloperidol binding to striatal and mesolimbic membrane preparations

Pooled striatal or mesolimbic tissue from 50 animals was weighed and homogenized in 40 volumes of 50 mM Tris-HCl buffer (pH 7·7) for 10 s using an Ultra Turrax homogenizer. The homogenate was centrifuged for 10 min at 40 000 *g* using a Sorvall RC5B centrifuge (cooled at 4°C) and the resulting supernatant discarded. The tissue pellet was resuspended in 40 volumes of Tris-HCl buffer and homogenized using a Duall homogenizer. The tissue was again centrifuged and the procedure repeated. The final pellet was homogenized in 100 volumes of 50 mM Tris-HCl buffer containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0·1% ascorbic acid, 10 μM pargyline (pH 7·7). Aliquots (2 mL) of the membrane preparations were incubated with 0·1 mL of a solution of [^{3}H]haloperidol (2·0 nM 22·0 Ci mmol^{-1}; Janssen Pharmaceutica) and 0·1 mL of a solution of displacing drug or vehicle. Specific binding of [^{3}H]haloperidol was defined by the incorporation of 0·1 mL of a solution of 2 μM (±)-butaclamol (Ayerst Laboratories). In a typical experiment the total binding in striatal tissue would be 35 000 d min^{-1} and non-specific 10 000 d min^{-1} (≃72% specific binding), and for mesolimbic tissue total binding would be 25 000 d min^{-1} and non-specific 10 000 d min^{-1} (≃60% specific binding). The samples were incubated for 15 min at 37°C in a shaking water bath. The reaction was stopped by rapid filtration through Whatman GF/B glass fibre filters under vacuum (15–20 psi) using a 40 well filtration manifold (Multividor, Janssen Scientific Instruments Division). Filters were washed twice with 5·0 mL ice-cold Tris-HCl buffer and transferred to counting vials. Instagel (10 mL; Packard) was added and the vials shaken for 10 min. Radioactivity was estimated using Packard Tri-Carb 4530 liquid scintillation spectrometer, (counting efficiency 43–48%).

[^{3}H]Spiperone binding to striatal membrane preparations

Pooled striatal tissue from 15 animals was weighed and homogenized in 50 volumes of 50 mM Tris-HCl buffer (pH 7·4) for 10 s using a Polytron homogenizer, setting 5. The homogenate was centrifuged for 10 min at 40 000 *g* using a Sorvall RC5B centrifuge (cooled at 4°C) and the resulting supernatant discarded. The tissue pellet was resuspended in 50 volumes of Tris-HCl buffer and homogenized. The tissue was again centrifuged and the supernatant discarded. The final pellet was suspended in 500 volumes of 50 mM Tris-HCl buffer containing 120 mM NaCl. Aliquots (1 mL) of the membrane preparations were incubated with 0·05 mL of a solution of [^{3}H]spiperone (0·1 nM, 16·4–21·0 Ci mmol^{-1}; Amersham International) and 0·05 mL of a solution of displacing drug or vehicle. Specific binding of [^{3}H]spiperone was defined by the incorporation of 0·05 mL of a solution of 10 μM (±)-sulpiride (Delagrange Laboratories). In a typical experiment the total binding in striatal tissue would be 1250 d min^{-1} and non-specific 250 d min^{-1} (≃80% specific binding). The samples were incubated for 10 min at 37°C in a shaking water bath. The reaction was stopped by rapid filtration through Whatman GFC glass fibre filters under vacuum (15–20 psi) using a 24 well filtration manifold (Millipore). Filters were washed twice with 5·0 mL ice-cold Tris-HCl buffer and transferred to scintillation vial inserts. Scintillator ES-299 (5 mL; Packard) was added. Radioactivity was estimated using Packard Tri-Carb liquid scintillation spectrometer (counting efficiency 40–45%).

[^{3}H]WB4101 binding to forebrain membrane preparations

Pooled forebrain tissue from 5 animals was weighed and prepared using the same method as for [^{3}H]haloperidol binding except a 50 mM Tris-HCl buffer (pH 7·7) was used throughout. The final pellet was resuspended in 80 volumes 50 mM Tris-HCl buffer (pH 7·7). Aliquots (2 mL) of membrane preparations were incubated with 0·1 mL of a solution of [^{3}H]WB4101 (0·5 nM, 25·7 Ci mmol^{-1}; New England Nuclear) and 0·1 mL of a solution of drugs or vehicle. Specific binding of [^{3}H]WB4101 was defined by the incorporation of 0·1 mL of a solution of 100 μM noradrenaline (Janssen Chimica). In a typical experiment the total binding would be 8500 d min^{-1} and non-specific binding would be 2000 d min^{-1} (≃76% specific binding). Samples were incubated for 20 min at 25°C. Samples were filtered and measured for radioactivity.

[^{3}H]Clonidine binding to cortical membrane preparations

Pooled cortical tissue from 10 animals was weighed and prepared using the same method employed for [^{3}H]WB4101 binding. The final pellet was resuspended in 100 volumes of 50 mM Tris-HCl buffer (pH 7·7). Aliquots (2 mL) of membrane preparations were incubated with 0·1 mL of a solution of [^{3}H]clonidine (3 nM, 23·8 Ci mmol^{-1}; New England Nuclear) and 0·1 mL of a solution of drugs or vehicle. Specific binding of [^{3}H]clonidine was defined by the incorporation of 0·1 mL of a solution of 2 μM noradrenaline (Janssen Chimica). In a typical experiment the total binding would be 5500 d min^{-1} and the non-specific binding would be 1300 d min^{-1} (≃76% specific binding). Samples were incubated for 30 min at 25°C. Samples were filtered and measured for radioactivity.

[^{3}H]Dihydroalprenolol binding to cortical membrane preparations

Pooled cortical tissue from 7 animals was weighed and prepared using the same method as [^{3}H]clonidine binding except a 50 mM Tris-HCl buffer (pH 8·0) was used. The final pellet was resuspended in 80 volumes of 50 nM Tric-HCl buffer (pH 8·0). Aliquots (1 mL) of homogenate were incubated with 0·05 mL of a solution of [^{3}H](−)-dihydroalprenolol (1·0 nM, 49·0 Ci mmol^{-1}; New England Nuclear)

and 0·05 mL of a solution of drugs or vehicle. Specific binding of [^{3}H]dihydroalprenolol was defined by the incorporation of 0·05 mL of a solution of 2 μM propranolol (ICI). In a typical experiment the total binding would be 5000 d min^{-1} and non-specific binding would be 1200 d min^{-1} (≏76% specific binding). Samples were incubated for 10 min at 37°C. Samples were filtered and measured for radioactivity.

[^{3}H]5-HT binding to hippocampal membrane preparations

Pooled hippocampal tissue from 40 animals was weighed and homogenized in 40 volumes of 50 mM Tris-HCL buffer (pH 7·7) and centrifuged at 40 000 *g* for 10 min. The pellet was resuspended in 40 volumes of 50 mM Tris-HCl buffer (pH 7·7) and incubated at 37°C for 10 min to remove endogenous 5-HT. The homogenate was then centrifuged and the resulting pellet was washed once more. The final pellet was then homogenized in 80 volumes of 50 mM Tris HCl (pH 7·6) containing 4·0 mM $CaCl_2$ and 1·0 μM pargyline. Aliquots (2 mL) of membrane preparations were incubated with 0·1 mL of a solution of [^{3}H]5-HT (3 nM, 11·1 Ci mmol^{-1}; Amersham International) and 0·1 mL of a solution of drugs or vehicle. Specific binding of [^{3}H]5-HT was defined by the incorporation of 0·1 mL of a solution of 2 μM lysergic acid diethylamide. In a typical experiment the total binding would be 10 000 d min^{-1} and non-specific binding would be 2200 d min^{-1} (≏78% specific binding). Samples were incubated for 15 min at 37°C. Samples were filtered and measured for radioactivity.

[^{3}H]Ketanserin binding to frontal cortex membrane preparations

Pooled frontal cortex tissue from 50 animals was weighed and homogenized in 10 volumes of 0·25 M sucrose with a Duall homogenizer. The homogenate was then centrifuged at 1000 *g* for 10 min. The supernatant was decanted and kept. The pellet was washed in 5 volumes of 0·25 M sucrose and centrifuged at 1000 *g* for 10 min. The supernatant was decanted and pooled with the first supernatant, the pellet was discarded. The resulting sample was diluted with 50 mM Tris-HCl buffer (pH 7·7) to 40 volumes then centrifuged for 20 min at 40 000 *g*. The resulting pellet was resuspended in 400 volumes of 50 mM Tris-HCl buffer (pH 7·7). Aliquots (4 mL) of membrane preparations were incubated with 0·2 mL of a solution of [^{3}H]ketanserin (1 nM, 17·9 Ci mmol^{-1}); Janssen Pharmaceutica) and 0·2 mL of a solution of drugs or vehicle. Specific binding of [^{3}H]ketanserin was defined by the incorporation of 0·2 mL of a solution of 1 μM methysergide (Sandoz). In a typical experiment the total binding would be 13 000 d min^{-1} and non-specific binding would be 3500 d min^{-1} (≏73% specific binding). Samples were incubated for 15 min at 37°C. Samples were filtered and measured for radioactivity.

[^{3}H]Dexetimide binding to striatal membrane preparations

Pooled striatal tissue from 25 animals was weighed and homogenized in 10 volumes of distilled water with a Duall homogenizer. This was then made up to 200 volumes with 50 mM phosphate buffer (pH 7·5). Aliquots (1 mL) of homogenate were incubated with 0·05 mL of a solution of [^{3}H]dexetimide (2 mM, 16·3 Ci mmol^{-1}; Janssen Pharmaceutica) and 0·05 mL of a solution of drugs or vehicle. Specific binding of [^{3}H]dexetimide was defined by the incorporation of 0·05 mL of a solution of 0·2 μM dexetimide (Janssen Pharmaceutica). In a typical experiment the total binding would be 42 000 d min^{-1} and nonspecific would be 2000 d min^{-1} (≏95% specific binding). Samples were incubated for 20 min at 37°C. Samples were filtered and measured for radioactivity.

[^{3}H]Sufentanil binding to forebrain membrane preparations

Pooled forebrain tissue from 5 animals was weighed and prepared using the same method as for [^{3}H]ketanserin. The final pellet was suspended in 200 volumes of 50 mM Tris-HCl buffer. Aliquots (5 mL) of membrane preparations were incubated with 0·25 mL of a solution of [^{3}H]sufentanil (0·5 nM, 15·0 Ci mmol^{-1}; Janssen Pharmaceutica) and 0·25 mL of a solution of drugs or vehicle. Specific binding of [^{3}H]sufentanil was defined by incorporating 0·25 mL of a solution of 0·5 μM dextramoramide (Janssen Pharmaceutica). In a typical experiment total binding would be 4200 d min^{-1} and non-specific binding would be 150 d min^{-1} (≏64% specific binding). Samples were incubated for 15 min at 37°C. Samples were filtered and measured for radioactivity.

[^{3}H]Mepyramine binding to guinea-pig cerebellar membrane preparations

Pooled guinea-pig cerebellar tissue from 10 animals was weighed and homogenized in 10 volumes of distilled water with a Duall homogenizer. This was then made up to 100 volumes with 50 mM phosphate buffer (pH 7·5). Aliquots (1 mL) of membrane preparations were incubated with 0·05 mL of a solution of [^{3}H]mepyramine (4 nM, 23·7 Ci mmol^{-1}, New England Nuclear) and 0·05 mL of a solution of drugs or vehicle. Specific binding of [^{3}H]mepyramine was defined by incorporating 0·05 mL of a solution of 1 μM astemizole (Janssen Pharmaceutica). In a typical experiment total binding would be 14 000 d min^{-1} and non-specific binding would be 2000 d min^{-1} (86% specific binding). Samples were incubated for 30 min at 25°C, then filtered and radioactive content measured.

Drugs

All drugs were dissolved in pure ethanol and diluted just before the assay in 10% ethanol in water. The displacing drugs used were (±)-sulpiride (Delagrange Laboratories), (±)-sultopride (Delagrange Laboratories), metoclopramide (Beecham Pharmaceuticals), tiapride (Delagrange Laboratories), clebopride (Almirall), amisulpiride (Delagrange Laboratories), TER 1546 (*N*-(1-methyl-3-pyrrolidyl)-2-methoxy-5-sulphamoyl benzamide; Delagrange Laboratories), prosulpride (Delagrange Laboratories), alizapride (Delagrange Laboratories), YM 019151-2 (*N*-(1-benzyl-2-methyl-3-pyrrolidinyl)-5-chloro-2-methoxy-4-methylaminobenzamide; Yamanouchi, Japan), SL-74205 (5-(aminosulphonyl)-*N*-(1-(4-fluorophenyl)methyl)-2-pyrrolidinymethyl)-2-methoxy) benzamide; Synthelabo), haloperidol (Janssen Pharmaceutica), raclopride tartrate and remoxipride hydrochloride (Astra Lakemedel AB).

Analysis of samples

Radioactivity was assessed and the data expressed as disintegrations per minute (d min^{-1}). In each experiment a

number of concentrations of displacing drugs were used. In initial experiments drugs were screened using concentrations in the range 10^{-4}–10^{-9} M. Experiments were repeated using at least six drug concentrations which were found to be on the linear portion of the displacement curve. All experiments were performed in duplicate and each experiment was performed three times if the drug showed any activity and twice if the drug showed no activity at a concentration greater than 10^{-5} M. Each IC50 value is therefore the mean of three independent experiments. IC50 values were calculated using semi-log probit analysis, as the concentration of drug needed to inhibit the specific binding by 50%. Specific binding was calculated as the difference between the total binding and the binding in the presence of the displacing agent used to determine specific binding.

Results

Interaction with D-2 dopamine sites (Table 1)

All the substituted benzamide drugs were able to inhibit [^{3}H]haloperidol binding to D-2 sites in striatal tissue preparations. All the substituted benzamides were less potent than haloperidol in inhibiting [^{3}H]haloperidol binding, except YM 09151-2 which was approximately 5 times more potent. Clebopride, amisulpiride and sultopride were approximately 5–10 times less potent than haloperidol; all the other benzamides were less potent than clebopride, amisulpiride and sultopride.

All the substituted benzamides were able to inhibit [^{3}H]spiperone binding to D-2 sites in striatal tissue. YM 09151-2 was the most potent, followed by raclopride and then haloperidol and clebopride which were equipotent. All other substituted benzamides were less potent than haloperidol and clebopride.

Table 1. IC50 values obtained from in-vitro ligand binding assays of [^{3}H]haloperidol and [^{3}H]spiperone to striatal membrane preparations.

	IC50 (nM)	
Drug	[^{3}H]Haloperidol	[^{3}H]Spiperone
YM 09151-2	0·56	4·8
Raclopride	ND	5·6
Haloperidol	3·2	7·1
Clebopride	17	7·4
Amisulpiride	30	14
Sultopride	32	48
SL 74205	130	ND
Sulpiride	160	130
Prosulpride	190	210
Metoclopramide	300	270
Alizapride	340	66
TER 1546	740	380
Tiapride	1000	890
Remoxipride	ND	1400

IC50 values (nM) were calculated using semi-log probit analysis and represent the concentrations of drug needed to inhibit specific binding by 50%. Specific binding of [^{3}H]haloperidol (2·0 nM) was defined using 2 μM (±)-butaclamol. Specific binding of [^{3}H]spiperone (0·1 nM) was defined using 10 μM (±)-sulpiride. IC50 values are the mean of three experiments performed in triplicate.
ND = not done.

Table 2. IC50 values obtained from in-vitro ligand binding assays of [^{3}H]-WB 4101 to forebrain membrane preparations and [^{3}H]clonidine and [^{3}H]dihydroalprenolol to cortical membrane preparations.

	IC50 (nM)		
Drug	[^{3}H]-WB 4101	[^{3}H]-Clonidine	[^{3}H]-Dihydroalprenolol
YM 09151-2	630	> 10 000	6300
Raclopride	32900	> 10 000	ND
Haloperidol	22	> 10 000	> 10 000
Clebopride	7100	> 10 000	> 10 000
Amisulpiride	> 10 000	1600	> 10 000
Sultopride	> 10 000	2200	< 10 000
SL 74205	> 10 000	> 10 000	> 10 000
Sulpiride	> 10 000	> 10 000	> 10 000
Prosulpiride	> 10 000	> 10 000	> 10 000
Metoclopramide	> 10 000	2200	> 10 000
Alizapride	> 10 000	> 10 000	> 10 000
TER 1546	> 10 000	> 10 000	> 10 000
Tiapride	> 10 000	> 10 000	> 10 000
Remoxipride	26 100	23 700	ND

IC50 values (nM) were calculated using semilog probit analysis and represent the concentration of drug needed to inhibit specific binding by 50%. Specific binding of [^{3}H]clonidine (3·0 nM) was defined using 2·0 μM noradrenaline. Specific binding of [^{3}H]dihydroalprenolol. IC50 values are the mean of three experiments performed in triplicate. An IC50 value of greater than 10 000 indicates there was no displacement of ligands at 10^{-5} M. If some displacement occurred at this concentration, higher concentrations were employed to determine IC50 values. Where IC50 values were not reached, a value of > 10 000 nM is recorded.
ND = not done.

Most drugs had IC50 values of the same order of magnitude for inhibiting [^{3}H]haloperidol and [^{3}H]spiperone binding with the exception of YM 09151-2 and alizapride. Alizapride was found to be 5 times more potent at displacing [^{3}H]spiperone binding than [^{3}H]haloperidol binding. YM 09151-2 however was found to be approximately 10 times more potent at displacing [^{3}H]haloperidol than [^{3}H]spiperone.

Interaction with α- and β-adrenergic sites (Table 2)

Haloperidol was approximately 10 times less potent in inhibiting [^{3}H]WB4101 binding to α-1 sites compared to its activity at D-2 sites. Amongst the substituted benzamides, YM 09151-2, (in the high nanomolar range) clebopride, remoxipride and raclopride (in the micromolar range) inhibited [^{3}H]WB4101 binding. YM 09151-2 was approximately 25 times less potent than haloperidol, and clebopride was 300 times less potent, while remoxipride and raclopride were approximately 800–1000 times less potent than haloperidol. Although haloperidol showed no activity in inhibiting [^{3}H]clonidine binding to α-2 sites, amilsupiride, sultopride, metoclopramide and remoxipride were active in the micromolar range. Only YM 09151-2 inhibited [^{3}H]dihydroalprenolol binding to β-adrenoceptor sites and this was in the micromolar range; other substituted benzamides had no effect.

Interaction with 5-HT-1 and 5-HT-2 sites (Table 3)

Haloperidol did not inhibit [^{3}H]5-HT binding to 5-HT-1 sites, but YM 09151-2, clebopride and prosulpride showed

Table 3. IC50 values obtained from in-vitro ligand binding assays of [^{3}H]5-HT to hippocampal membrane preparations and [^{3}H]ketanserin to frontal cortical membranes.

	IC50 (nM)	
Drug	[^{3}H]5-HT	[^{3}H]Ketanserin
YM 09151-2	79	79
Raclopride	ND	26 100
Haloperidol	> 10 000	74
Clebopride	6300	350
Amisulpiride	> 10 000	5600
Sultopride	> 10 000	> 10 000
SL 74205	> 10 000	> 10 000
Sulpiride	> 10 000	> 10 000
Prosulpride	4000	> 10 000
Metoclopramide	> 10 000	7100
Alizapride	> 10 000	> 10 000
TER 1546	> 10 000	> 10 000
Tiapride	> 10 000	> 10 000
Remoxipride	ND	> 10 000

IC50 values (nM) were calculated using semi-log probit analysis and represent the concentration of drug needed to inhibit specific binding by 50%. Specific binding of [^{3}H]5-HT (3 nM) was defined using 2 μM LSD. Specific binding of [^{3}H]ketanserin (1 nM) was defined using 1 μM methysergide. IC50 values are the mean of three experiments performed in triplicate. An IC50 value of greater than 10 000 indicates there was no displacement of ligands at 10^{-5} M. If some displacement occurred at this concentration, higher concentrations were employed to determine IC50 values. Where IC50 values were not reached a value of > 10 000 nM is recorded.

ND = not done

Table 4. IC50 values obtained from in-vitro ligand binding assays of [^{3}H]dexetimide to striatal membrane preparations, [^{3}H]sufentanil to forebrain membrane preparations and [^{3}H]mepyramine to guinea-pig cerebellar membrane preparations.

	IC50 (nM)		
Drug	[^{3}H]Dexetimide	[^{3}H]Sufentanil	[^{3}H]Mepyramine
YM 09151-2	> 10 000	3200	> 10 000
Raclopride	> 10 000	ND	> 10 000
Haloperidol	> 10 000	3200	8000
Clebopride	> 10 000	> 10 000	> 10 000
Amisulpiride	> 10 000	> 10 000	> 10 000
Sultopride	> 10 000	> 10 000	> 10 000
SL 74205	> 10 000	> 10 000	> 10 000
Sulpiride	> 10 000	> 10 000	> 10 000
Prosulpride	> 10 000	> 10 000	> 10 000
Metoclopramide	> 10 000	> 10 000	> 10 000
Alizapride	> 10 000	> 10 000	> 10 000
TER 1546	> 10 000	> 10 000	> 10 000
Tiapride	> 10 000	> 10 000	> 10 000
Remoxipride	> 10 000	ND	> 10 000

IC50 values (nM) were calculated using semi-log probit analysis and represent the concentration of drug needed to inhibit specific binding by 50%. Specific binding of [^{3}H]dexetimide (2 nM) was defined with 0·02 μM dexetimide. Specific binding of [^{3}H]sufentanil (0·5 nM) was defined with 0·5 μM dextramoramide. Specific binding of [^{3}H]-mepyramine (4 nM) was defined with 1 μM astemizole. IC50 values are the mean of three experiments performed in triplicate. An IC50 value of greater than 10 000 indicates there was no displacement of ligands at 10^{-5} M. If some displacement occurred at this concentration, higher concentrations were employed to determine IC50 values. Where IC50 values were not reached a value of > 10 000 nM is recorded.

ND = not done.

activity. YM 09151-2 showed reasonable potency, but clebopride and prosulpride were only active in the micromolar range. No other substituted benzamides inhibited specific [^{3}H]5-HT binding.

Haloperidol inhibited [^{3}H]ketanserin binding to 5-HT-2 sites, but was approximately 30 times less potent compared with its ability to inhibit [^{3}H]haloperidol binding to D-2 sites. YM 09151-2 was equipotent with haloperidol at 5-HT-2 sites and, interestingly, was equipotent at both 5-HT-1 and 5-HT-2 sites. Clebopride was approximately 5 times less potent than haloperidol and YM 09151-2. Metoclopramide, amisulpiride and raclopride only inhibited [^{3}H]ketanserin specific binding in micromolar concentrations.

Interaction with muscarinic, opiate and histamine sites (Table 4)

None of the drugs, including haloperidol, was active in inhibiting [^{3}H]dexetimide binding to muscarinic sites. Only YM 09151-2 and haloperidol showed any activity at inhibiting [^{3}H]sufentanil binding to opiate sites, in the micromolar range. Haloperidol also inhibited [^{3}H]mepyramine binding to H-1 sites, in high concentrations. No other substituted benzamide drug or haloperidol showed activity at opiate or H-1 sites.

Discussion

The in-vivo identification of brain dopamine receptors using neuroleptic drugs requires some appropriate characteristics in candidate molecules. Drugs to be used as ligands for PET investigations should, in the low concentrations used, show high affinity and selectivity for the receptor population to be identified. Compounds to be used in an unlabelled form to define the specific binding of PET ligands should show specificity for a particular receptor population in the high concentrations to be employed. In addition, all compounds, whether used as ligands or as defining drugs should penetrate readily into brain following systemic administration. The substituted benzamide drugs may be one class that can fulfil these criteria.

Substituted benzamide drugs have been developed for their ability to interact with brain dopamine receptors as antagonists. Not surprisingly all the compounds examined were able, in varying degrees, to inhibit both [^{3}H]haloperidol and [^{3}H]spiperone binding to dopamine receptors in striatal preparations. The most potent compounds examined were YM 09151-2 and raclopride. Clebopride, amisulpiride and sultopride also were active in the high nanomolar range. YM 09151-2 was more active than haloperidol in inhibiting both [^{3}H]haloperidol and [^{3}H]spiperone to their specific binding sites. Raclopride and clebopride were equiactive with haloperidol in inhibiting [^{3}H]spiperone to specific binding sites. The values obtained are in good agreement with previous data on these compounds (Usuda et al 1981; Terai et al 1983; Kohler et al 1985; Niznik et al 1985). Other substituted benzamides were active in the range 10^{-6}–10^{-8} M against both ligands, with the exception of remoxipride which showed some activity in-vitro in inhibiting [^{3}H]spiperone binding to specific binding sites, but only at micromolar concentrations, which is in agreement with the findings of Hall et al (1986). However, remoxipride has been shown to

displace specific [^{3}H]spiperone binding in-vivo, being more active than tiapride but less active than haloperidol (Ogren et al 1984).

Most compounds studied were equally active in inhibiting [^{3}H]haloperidol and [^{3}H]spiperone binding to their specific binding sites. Surprisingly, YM 09151-2 was found to be approximately 10 times more active in inhibiting [^{3}H]haloperidol binding compared with [^{3}H]spiperone binding to striatal homogenates. Conversely, tiapride was found to be more active in displacing [^{3}H]spiperone than [^{3}H]haloperidol binding from specific binding sites.

Analysis of the activity of these substituted benzamide drugs at the D-2 receptor site suggests YM 09151-2, raclopride, clebopride, amisulpiride and sultopride would be good candidates for PET scanning studies due to their high activity in displacing [^{3}H]haloperidol and [^{3}H]spiperone specific binding. However, analysis of other receptor systems gives information about their selectivity. Both YM 09151-2 and clebopride showed activity in displacing ligands from α-1, 5HT-1, and 5HT-2 sites in the 10^{-6}–10^{-8} M range and YM 09151-2 also showed some activity at β-adrenergic and opiate sites. This lack of selectivity was also shown by Terai et al (1983). In their study YM 09151-2 was found to have activity at D-2, α-1, α-2, β-adrenergic, H-1, opiate, 5-HT-1, 5-HT-2 and muscarinic sites. However, unlike in our study, the affinity of YM 09151-2 for other receptor sites was at least three orders of magnitude smaller than for the D-2 binding site. Raclopride also showed some weak activity in inhibiting [^{3}H]ligand binding to α-1 and 5-HT-2 sites in the 10^{-4}–10^{-6} M range. These results are similar to those found by Kohler et al (1985) although they also observed some inhibition at α-2 and H-1 sites. Amisulpiride and sultopride in the micromolar range showed activity in inhibiting [^{3}H]clonidine binding to α-2 sites, and amisulpiride showed activity in inhibiting [^{3}H]ketanserin binding to 5-HT-2 sites.

Only sulpiride, TER 1546, alizapride, tiapride and SL 74205 (all with activity in the 10^{-6}–10^{-7} M range) showed activity at D-2 sites but no activity on the other receptor systems studied.

So, which of the substituted benzamide drugs investigated would be the most appropriate for use in PET studies in man? Clearly compounds such as YM 09151-2 or clebopride, which show interactions with a number of neurotransmitter receptors and where there is little differentiation between the effective concentrations, would not be suitable as PET ligands or for the definition of specific binding of other PET ligands. Ideally, the most suitable compounds as ligands would be those substituted benzamide drugs which are specific for D-2 receptors. However, these compounds had a relatively low affinity for these sites and so would not be appropriate. Rather, drugs such as alizapride may be useful in defining the binding of other PET ligands since in the high concentration employed they would only interact with D-2 sites. So it would appear that the most appropriate substituted benzamide drugs for use as PET ligands would be those compounds showing a reasonable affinity for D-2 sites but with the greatest differentiation between D-2 activity and effects on other neuronal systems. In this respect, raclopride only displaces ligands from α-1 and 5-HT-2 sites of very high concentrations at least three orders of magnitude higher than for the D-2 binding sites. Similarly, amisulpiride and sultopride only show activity on other receptor sites in the micromolar range, with two orders of magnitude greater concentrations required than for the D-2 binding site. Indeed [^{11}C]raclopride has recently been reported as an effective ligand for use in human PET scanning studies (Farde et al 1986).

Whether any of the compounds examined are appropriate for PET scanning studies requires further investigation since in-vitro binding assays do not take into account drug distribution, metabolism, lipophilicity, penetration and regional accumulation. In-vivo studies in animals must be carried out on these compounds before a true evaluation of their potential use in PET studies can be made. Finally, there is the problem of whether such compounds are chemically suitable for labelling for PET investigations.

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