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Pharmacologic Characterization of the Human 5-Hydroxytryptamine$_{2B}$ Receptor: Evidence for Species Differences

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ABSTRACT

The 5-Hydroxytryptamine$_{2B}$ (5-HT$_{2B}$) receptor was cloned originally from rat stomach fundus and its pharmacology was determined to be consistent with that of the receptor responsible for contraction of rat fundal tissue in response to 5-HT. Recently, the cloning of the human homolog of the 5-HT$_{2B}$ receptor has been reported and, in this study, we report a detailed pharmacological characterization of this human receptor. The cloned human 5-HT$_{2B}$ receptor has high affinity for [^{3}H]5-HT (K_d = 10.6 ± 1.5 nM), and the pharmacology of this receptor matches closely the rat 5-HT$_{2B}$ receptor, consistent with the structural relatedness of these two proteins. Most compounds tested show no difference in affinity for the human or rat receptors. There were, however, groups of compounds that discriminated between the human and rat 5-HT$_{2B}$ receptors. Examples include certain ergolines such as methysergide and mesulergine, which have higher affinity for the human than for the rat receptor. Similarly, certain benzoylpiperidines, *e.g.*, ketanserin, pirenperone and pipamperone, and the antipsychotics clozapine and olanzapine have higher affinity for the human 5-HT$_{2B}$ receptor. These pharmacological findings reinforce the desirability of having the human forms of receptors when considering drug actions.

It has been almost 40 years since Vane (1957) first reported that 5-HT potently contracts the rat stomach fundus. For many years the identity of the receptor responsible for this effect had remained elusive. It had been determined previously that the fundal contractile receptor did not correspond to any of the formally classified 5-HT receptor subtypes (van Nueten *et al.*, 1982; Clineschmidt *et al.*, 1985; Cohen, 1989). Although it was known to resemble very closely the 5-HT$_{2C}$ receptor, the fundal contractile receptor was determined not to be the 5-HT$_{2C}$ receptor (Baez *et al.*, 1990) [formerly classified as the 5-HT$_{1C}$ receptor (Hoyer *et al.*, 1994)]. Subsequently, two different groups reported the cloning and pharmacological characterization of the rat stomach fundal contractile receptor called the *SRL* (Foguet *et al.*, 1992) or the 5-HT$_{2F}$ receptor (Kursar *et al.*, 1992), [now classified as the 5-HT$_{2B}$ receptor (Hoyer *et al.*, 1994)]. The mouse 5-HT$_{2B}$ receptor has also been cloned and characterized pharmacologically (Loric *et al.*, 1992). Recently, the cloning of the human 5-HT$_{2B}$ receptor was reported (Choi *et al.*, 1994; Kursar *et al.*, 1994; Schmuck *et al.*, 1994; Bonhaus *et al.*, 1995). In this study, we report a detailed pharmacological characterization of the human 5-HT$_{2B}$ receptor and describe a number of different compounds that discriminate between the human and rat 5-HT$_{2B}$ receptors.

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Methods

Chemicals. All chemicals were obtained from the sources previously described (Wainscott *et al.*, 1993). In addition, spiroxatrine, pirenperone and clozapine were obtained from Research Biochemicals, Inc. (Natick, MA). Sodium ascorbate, EGTA, melatonin and N-acetyl-5-HT were purchased from Sigma Chemical Co. (St. Louis, MO). Magnesium chloride hexahydrate was purchased from EM Science (Gibbstown, NJ). PMSF was purchased from Boehringer Mannheim Corporation (Indianapolis, IN). The following compounds were donated by the respective companies: pipamperone and risperidone from Janssen (Beerse, Belgium) and MDL 11,939 from Marion Merrell Dow, (Cincinatti OH). Olanzapine was synthesized at the Lilly Research Laboratories (Indianapolis, IN). [^{3}H]5-HT was purchased from DuPont-New England Nuclear (Boston, MA) or Amersham Corporation (Arlington Heights, IL) at 22.8 to 26.7 or 81 to 91 Ci/mmol, respectively. [^{125}I]DOI also was purchased from DuPont-New England Nuclear.

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine (serotonin); EGTA, ethylene glycol-bis(β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid; N-acetyl-5-HT, N-acetyl-5-hydroxytryptamine, N-acetylserotonin; PMSF, phenylmethanesulfonyl fluoride; DOI, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; 1-NP, 1-naphthylpiperazine; LY86057, N$_1$-desisopropyl homolog of LY53857; LY193525, N$_1$-desisopropyl homolog of amesergide; 2-Br-LSD, D-2-bromo-lysergic acid diethylamide.

Radioligand Binding Studies

Membrane preparation from transformed cell lines. Membranes were prepared as described previously using AV12 cells (Syrian hamster fibroblast, ATCC no. CRL 9595) stably transformed with the human 5-HT$_{2A, 2B, 2C}$ or rat 5-HT$_{2B}$ receptor (Wainscott *et al.*, 1993). Briefly, cells expressing the receptor of interest were grown in suspension and harvested by centrifugation. The cells were resuspended in a minimal volume of a hypotonic buffer, 50 mM Tris-HCl, pH 7.4, and frozen at −70°C until needed. On the day of the assay, the suspension was thawed and diluted to 35 ml/0.5 × 10^9 cells, original cell number, with 50 mM Tris-HCl, pH 7.4, and centrifuged at 39,800 × *g*, 4°C. The resulting pellet was resuspended by vortexing and incubated at 37°C for 10 min, then centrifuged at 39,800 × *g*, 4°C. This pellet was resuspended and centrifuged one more time. To achieve a homogenous membrane suspension, the final pellet was resuspended using a Tissumizer (Tekmar, Cincinnati, OH) at setting 75 for 10 to 15 sec in 67 mM Tris-HCl, pH 7.4, for cells expressing the human or rat 5-HT$_{2B}$ receptor or 67 mM Tris-HCl, pH 7.4, containing 13 mM $MgCl_2$ and 0.67 mM EDTA for cells expressing the human 5-HT$_{2A}$ or 5-HT$_{2C}$ receptors.

5-HT$_{2B}$ [^{3}H]5-HT binding studies. Rat or human 5-HT$_{2B}$ binding assays using [^{3}H]5-HT were performed using a previously described method and AV12 cells transformed with the human or rat 5-HT$_{2B}$ receptor (Wainscott *et al.*, 1993). The assay was automated using a Biomek 1000 (Beckman Instruments, Fullerton, CA). [^{3}H]-5-HT in Tris-HCl containing $CaCl_2$, pargyline and L-ascorbic acid, adjusted to pH 7.4, was added to drug dilutions, spanning 6 log units, in water. Then 200 μl of membrane resuspension (approximately 100–150 μg of protein) was added with mixing and incubated for 15 min at 37°C. The total incubation volume was 800 μl and all incubations were performed in triplicate. The assay was terminated by vacuum filtration through Whatman GF/B filters which had been presoaked with 0.5% polyethylenimine (w/v) and precooled with 4 ml of ice-cold wash buffer (50 mM Tris-HCl, pH 7.4), using a Brandel cell harvester (model MB-48R; Brandel, Gaithersburg, MD). The filters were then washed rapidly 4 times with 1 ml of ice-cold wash buffer. The amount of [^{3}H]5-HT trapped on the filters was determined by liquid scintillation spectrometry (Ready Protein, LS 6000IC, Beckman Instruments, Fullerton, CA). The final concentration S of $CaCl_2$, pargyline, Tris and L-ascorbic acid were 3 mM, 10 μM, 50 mM and 0.1%, respectively. The final [^{3}H]5-HT concentration for competition studies was approximately 2 nM (range = 1.7–2.5 nM). For saturation studies, the [^{3}H]5-HT concentrations ranged from approximately 0.7 to 35 or 0.7 to 65 nM. The actual free radioligand concentration was determined by sampling the supernatant of identical tubes in which the incubation had been terminated by centrifugation instead of filtration. Nonspecific binding was defined with 10 μM 5-HT or 10 μM 1-NP. The amount of protein was determined by the method of Bradford (1976), with bovine serum albumin as the standard.

5-HT$_{2B}$ [^{125}I]DOI binding studies. The method for [^{125}I]DOI binding to the human 5-HT$_{2B}$ receptor was adapted from that of Choi *et al.* (1994). Briefly, membranes from cells expressing the human 5-HT$_{2B}$ receptor were prepared as described above, except that after thawing they were diluted in 4 mM EDTA, 1 mM EGTA, 0.1 mM PMSF and 10 mM imidazole, adjusted to pH 7.3 with NaOH. All of the intermittent resuspensions were in 50 mM Tris-HCl, pH 7.4, as described above, and the final resuspension was in 75 mM KCl, 5 mM $MgCl_2$, 1 mM EGTA and 10 mM imidazole, pH 7.3. [^{125}I]DOI (50 μl) in Tris-HCl, pH 7.4, was added to 50 μl of drug dilutions, spanning 6 log units, in water (two experiments) or in Tris-HCl, pH 7.4 (one experiment). Then 100 μl of membrane resuspension (approximately 30–40 μg of protein) was added with mixing and incubated for 30 min at 30°C. The total incubation volume was 200 μl and all incubations were performed in triplicate. The assay was terminated by filtration through Whatman GF/B filters which had been presoaked with 0.5% polyethylenimine and precooled with 4 ml of ice-cold wash buffer (50 mM Tris-HCl, pH 7.4), using a Brandel cell harvester. The filters were then washed rapidly 4 times with 1 ml of ice-cold wash buffer. The amount of [^{125}I]DOI trapped on the filters was determined by gamma counting. The final concentrations of KCl, $MgCl_2$, EGTA, imidazole and Tris were 37.5, 2.5, 0.5, 5 and 25 mM, respectively. The final [^{125}I]DOI concentration for competition studies was approximately 0.17 nM. Nonspecific binding was defined with 10 μM 5-HT or 10 μM 1-NP.

5-HT$_{2A,2C}$ [^{125}I]DOI binding studies. Human 5-HT$_{2A}$ or 5-HT$_{2C}$ binding studies were performed essentially as described for [^{3}H]5-HT binding to the 5-HT$_{2B}$ receptor with the following exceptions. The assay buffer contained, in final concentration, 10 μM pargyline, 9.75 mM $MgCl_2$, 0.5 mM EDTA, 0.1% sodium ascorbate and 50 mM Tris-HCl, pH 7.4. Incubations were performed at 37°C for 30 min with approximately 40 and 30 μg of protein for the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors, respectively, then filtered through Whatman GF/C filters which had been presoaked in 0.5% (w/v) polyethylenimine and precooled with 4 ml of ice-cold wash buffer. The filters were then washed rapidly 4 times with 1 ml of ice-cold wash buffer. The amount of [^{125}I]DOI trapped on the filters was determined using a gamma counter. Nonspecific binding was determined with 10 μM mianserin for 5-HT$_{2C}$ and 1 μM ketanserin for 5-HT$_{2A}$ receptors. The final concentration of [^{125}I]DOI was approximately 0.07 to 0.15 nM for competition experiments.

Statistical analysis. Nonlinear regression analysis for the saturation and competition curves was performed as described previously (Wainscott *et al.*, 1993). One-way analysis of variance was performed on the pK_i values (*i.e.*, $-\log K_i$, molar) followed by the Tukey-Kramer Honestly Significant Difference test (JMP; SAS Institute Inc., Cary, NC). IC_{50} values from the competition curves were converted to K_i values using the Cheng-Prusoff (1973) equation. For [^{125}I]DOI-labeled receptors, the K_d of [^{125}I]DOI for the 5-HT$_{2A}$ or 5-HT$_{2C}$ receptors was determined using a rearrangement of the Cheng-Prusoff equation giving: $K_d = IC_{50} - [L]$, where IC_{50} is the concentration of unlabeled DOI causing 50% inhibition of specific [^{125}I]DOI binding and [L] = the free concentration of [^{125}I]DOI.

Results

Radioligand binding studies. [^{3}H]5-HT saturation of the human 5-HT$_{2B}$ receptor revealed a single, saturable site with a K_d of 10.6 ± 1.5 nM and a maximum number of binding sites of 1040 ± 90 fmol/mg of protein (n = 5).

A large number of compounds with their human and rat 5-HT$_{2B}$ receptor affinities are listed in table 1. Among the compounds listed (excluding sumatriptan, melatonin and N-acetyl-5-HT), there was a strong correlation between rat and human 5-HT$_{2B}$ receptor affinities (fig. 1A, r = 0.89, $P < .001$). Although most compounds displayed similar affinities for the human and rat receptors, many compounds were able to distinguish between the two receptors. The N_1-substituted ergolines, including methysergide, mesulergine, LY53857 and amesergide, displayed significantly higher affinity (*i.e.*, lower K_i) for the human than for the rat 5-HT$_{2B}$ receptor (table 2). The N_1-unsubstituted ergolines LY86057, LY193525 and 2-Br-LSD did not discriminate between the two receptors. Similarly, none of the tryptamines showed any species differences. As reported previously (Kursar *et al.*, 1994), ketanserin and spiperone showed higher affinity for the human form of the 5-HT$_{2B}$ receptor. In addition, the related ligands pipamperone and pirenperone exhibited higher affinity for the human 5-HT$_{2B}$ receptor (table 3). The antipsychotics risperidone, clozapine and olanzapine also displayed higher human than rat 5-HT$_{2B}$ receptor affinity (tables 3 and 4). None of the tested compounds had statisti-

TABLE 1

Pharmacological profile of the human 5-HT_{2B} receptor

Drug competition for binding was performed as described under "Materials and Methods." K_i values (nanomolar) are the mean ± S.E. of the number of separate experiments given in parentheses. The rat and human 5-HT_{2B} receptors were labeled with [^{3}H]5-HT. The human 5-HT_{2A} and 5-HT_{2C} receptors were labeled with [^{125}I]DOI. ABBREVIATIONS: 5-Cl-T, 5-chlorotryptamine; 5-MeO-T, 5-methoxytryptamine; α-Me-5-HT, α-methyl-5-hydroxytryptamine; TFMPP, 1-(*m*-trifluoromethylphenyl)piperazine; *m*-CPP, 1-(3-chlorophenyl)piperazine; 5-F-T, 5-fluorotryptamine; 5-Br-T, 5-bromotryptamine; 5-CT, 5-carboxamidotryptamine; 2-Me-5-HT, 2-methyl-5-hydroxytryptamine; 8-OH-DPAT, (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin.

Compound	K_i Values (*n*)			
	Human 5-HT_{2B}	Rat 5-HT_{2B}	Human 5-HT_{2A}	Human 5-HT_{2C}
	nM	*nM*	*nM*	*nM*
5-HT	9.46 ± 1.97 (6)	11.3 ± 1.8 (8)	7.77 ± 0.59 (3)	15.5 ± 2.8 (3)
Tryptamine	73.2 ± 4.0 (4)	113 ± 6[a] (3)		
5-F-T	11.0 ± 1.0 (4)	5.65 ± 0.55[a] (3)		
5-Cl-T	13.0 ± 1.5 (4)	6.21 ± 0.55 (6)		
5-Br-T	13.9 ± 1.0 (4)	39.3 ± 14.0[a] (4)		
5-MeO-T	16.4 ± 2.2 (3)	9.15 ± 1.48[a] (3)		
5-CT	116 ± 10 (6)	147 ± 13 (7)		
α-Me-5-HT	11.5 ± 0.9 (4)	10.5 ± 1.5[a] (4)		
2-Me-5-HT	197 ± 33 (3)	284 ± 13[a] (3)		
N-Acetyl-5-HT	7,220 ± 830 (3)		89,100 ± 800[b] (3)	28,800 ± 700[c] (3)
Melatonin	12,900 ± 1,900 (3)		62,300 ± 2,100[c] (3)	39,500 ± 2,700 (3)
Sumatriptan	18,200 ± 5,400 (3)	>10,000[a] (3)		
RU 24969	31.8 ± 4.0 (4)	17.1 ± 1.7[a] (3)		
1-NP	3.72 ± 0.72 (9)	4.50 ± 0.47 (12)		
m-CPP	28.7 ± 0.8 (3)	26.8 ± 2.5[a] (3)		
TFMPP	39.9 ± 2.6 (3)	84.8 ± 15.2[a] (5)		
Quipazine	48.7 ± 4.0 (3)	131 ± 16 (7)		
Methysergide	1.59 ± 0.05 (4)	6.28 ± 0.91[a,c] (4)		
Mesulergine	3.62 ± 0.17 (3)	35.9 ± 4.1[a,b] (3)		
2-Br-LSD	8.56 ± 2.33 (3)	12.6 ± 0.8[a] (4)	0.48 ± 0.13[b] (3)	7.14 ± 0.19 (3)
LY53857	1.63 ± 0.11 (4)	6.87 ± 0.55[a,c] (3)		
LY86057	12.3 ± 0.4 (4)	12.2 ± 1.5[a] (3)		
Amesergide	1.96 ± 0.52 (4)	12.0 ± 2.7[b] (8)	15.1 ± 2.3[b] (5)	6.27 ± 1.44 (3)
LY193525	3.87 ± 1.10 (4)	9.55 ± 2.16 (7)	0.90 ± 0.32[b] (5)	10.8 ± 2.6 (3)
Clozapine	7.57 ± 1.03 (5)	31.3 ± 11.9[c] (3)	6.51 ± 0.39 (3)	36.0 ± 1.3[b] (3)
Olanzapine	11.8 ± 1.8 (8)	53.6 ± 7.2[b] (5)	2.53 ± 0.35[b] (3)	28.6 ± 3.6 (3)
Ritanserin	1.73 ± 0.77 (3)	5.18 ± 0.52[a] (3)		
Ketanserin	395 ± 45 (4)	3,240 ± 340[b] (4)	2.01 ± 0.16[b] (5)	161 ± 47 (3)
Mianserin	9.57 ± 0.33 (3)	52.5 ± 0.6[a,b] (4)		6.52 ± 0.76 (3)
Spiroxatrine	505 ± 74 (4)	193 ± 24 (4)		
Risperidone	29.2 ± 3.5 (9)	203 ± 20[b] (6)	0.42 ± 0.08[b] (3)	64.0 ± 6.2 (3)
Spiperone	704 ± 39 (4)	3,000 ± 290[b] (4)		
Pirenperone	61.0 ± 7.6 (3)	598 ± 64[b] (3)	1.08 ± 0.14[b] (3)	77.0 ± 2.5 (3)
Pipamperone	47.4 ± 8.0 (10)	569 ± 54[b] (7)	7.21 ± 0.07[b] (3)	227 ± 23[b] (3)
Rauwolscine	15.1 ± 1.3 (7)	35.8 ± 3.8[a] (3)	252 ± 8[b] (3)	1,690 ± 310[b] (3)
Yohimbine	44.4 ± 4.8 (4)	53.1 ± 4.6[a] (4)		
Cisapride	62.3 ± 10.3 (4)	135 ± 37 (3)	10.2 ± 1.8[b] (3)	508 ± 26[b] (3)
8-OH-DPAT	2,200 ± 230 (3)	4,120 ± 310[a] (3)		
DOI	20.2 ± 2.8 (5)	26.6 ± 1.6 (5)	0.65 ± 0.07[b] (9)	2.36 ± 0.29[b] (3)
MDL 11,939	3,020 ± 380 (4)	4,700 ± 360 (3)	6.06 ± 0.19[b] (3)	1,020 ± 170 (3)

[a] Denotes values taken from Wainscott *et al.* (1993).
[c] Denotes significant difference ($P < .05$) from the K_i value for the same compound at the human 5-HT_{2B} receptor.
[b] Denotes significant difference ($P < .001$) from the K_i value for the same compound at the human 5-HT_{2B} receptor.

cally significantly higher affinity for the rat than for the human 5-HT_{2B} receptor.

A number of compounds were compared for their affinities at the human 5-HT_{2A} and 5-HT_{2C} receptors *vs.* the human 5-HT_{2B} receptor. In comparison of the 5-HT_{2A} with the human 5-HT_{2B} receptor, there was a significant correlation with respect to the compounds tested (fig. 1B, $r = 0.67$, $P < .01$). The compounds 2-Br-LSD, LY193525, olanzapine, ketanserin, risperidone, pirenperone, pipamperone, cisapride, (±)-DOI and MDL 11,939 all exhibited significantly higher affinity for the human 5-HT_{2A} than for the human 5-HT_{2B} receptor (table 1). Four of the compounds tested, rauwolscine, amesergide, melatonin and N-acetyl-5-HT, had higher affinity for the human 5-HT_{2B} receptor. It should be noted that when melatonin and N-acetyl-5-HT were omitted from the correlational analysis, the remaining compounds did not give a significant correlation between the human 5-HT_{2A} and 5-HT_{2B} receptors ($r = 0.001$, $P = .997$). The K_d of [^{125}I]DOI for the human 5-HT_{2A} receptor was determined to be 0.65 ± 0.07 nM ($n = 9$).

In addition to the human 5-HT_{2A} receptor, there was a good correlation between the affinities of the compounds at the 5-HT_{2B} receptor *vs.* affinity at the 5-HT_{2C} receptor (fig. 1C, $r = 0.85$, $P < .001$). There were, however, several compounds that gave higher affinity for the 5-HT_{2B} receptor than for the 5-HT_{2C} receptor. N-acetyl-5-HT, rauwolscine, clozapine, pipamperone and cisapride had significantly higher 5-HT_{2B} receptor affinity (table 1). DOI was the only compound tested that had higher 5-HT_{2C} than 5-HT_{2B} receptor

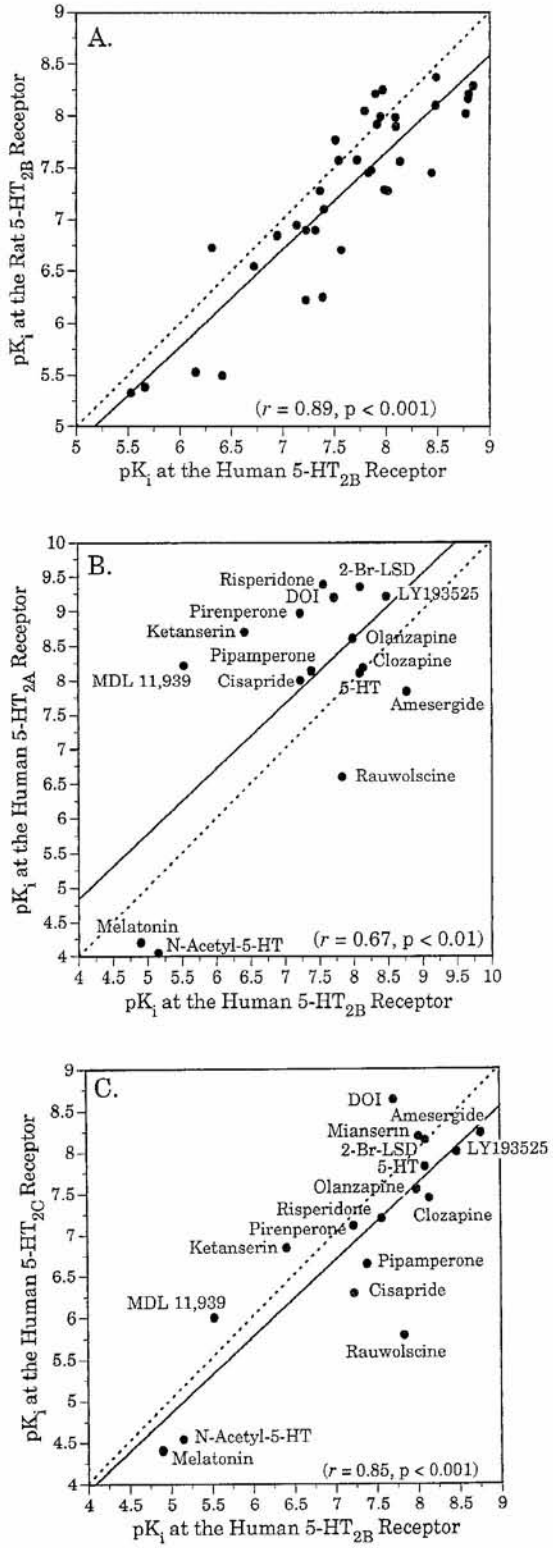


Fig. 1. A, correlation between the pK$_i$ for inhibition of [^{3}H]5-HT binding to the human and the rat 5-HT$_{2B}$ receptor. B, correlation between the pK$_i$ for inhibition of [^{3}H]5-HT binding to the human 5-HT$_{2B}$ receptor and the pK$_i$ for inhibition of [^{125}I]DOI binding to the human 5-HT$_{2A}$ receptor. C, correlation between the pK$_i$ for inhibition of [^{3}H]5-HT binding to the human 5-HT$_{2B}$ receptor and the pK$_i$ for inhibition of [^{125}I]DOI binding to the human 5-HT$_{2C}$ receptor. For all three graphs, the solid line is the fitted regression line and the dashed line is the line of identity.

affinity. The K_d of [^{125}I]DOI for the human 5-HT$_{2C}$ receptor was found to be 2.36 ± 0.29 nM (n = 3).

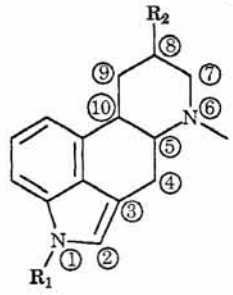
Discussion

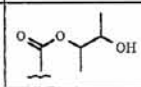
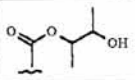
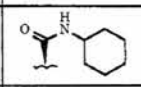
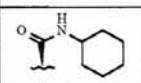
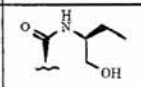
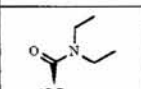
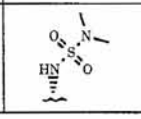
This work describes the rat and human 5-HT$_{2B}$ receptor affinities of a large number of structurally diverse compounds. As reported previously (Kursar *et al.*, 1994), the human 5-HT$_{2B}$ receptor has high affinity for [^{3}H]5-HT (K_d = 10.6 ± 1.5 nM), which allowed for the use of filtration for the separation of bound from free radioligand, thus providing a high throughput assay. The high affinity for the native ligand, 5-HT, was very close to that reported previously for the rat 5-HT$_{2B}$ receptor [7.87 nM (Wainscott *et al.*, 1993)] and, in fact, all of the tryptamines examined showed similar affinities between the 5-HT$_{2B}$ receptors from the two species. Overall, comparison of a large variety of compounds revealed a strong correlation between rat and human 5-HT$_{2B}$ receptor affinities (fig. 1A), a finding that is consistent with the degree of amino acid homology between these two species homologs, *i.e.*, 82% homology overall and 91.5% within the putative transmembrane regions (Kursar *et al.*, 1994).

Although most compounds displayed similar affinities for the human and rat receptors, certain groups of compounds were able to distinguish between the two receptors. These fall roughly into three classes as follows: 1) ergolines (table 2). Ergolines that were alkylated (either methyl or isopropyl) in position N$_1$ had higher affinity for the human 5-HT$_{2B}$ receptor than the rat, whereas the N$_1$ unsubstituted ergolines showed no species difference; 2) *p*-fluorobenzoyl-containing structures (table 3). We noted previously (Kursar *et al.*, 1994) that ketanserin and spiperone exhibited higher affinity for the human 5-HT$_{2B}$ receptor than for the rat. In the present work it was seen that two other *p*-fluorobenzoyl-containing compounds, pipamperone and pirenperone, also have higher affinity for the human form of the receptor. In addition, risperidone, which contains a fluorobenzisoxazole that could serve as an isostere for the *p*-fluorobenzoyl group, also showed selectivity for the human form of the receptor; and 3) polycyclic ring systems (table 4). A third structural type that appeared to show a trend toward species selectivity was found in mianserin, clozapine and olanzapine. These compounds, which also displayed significantly higher affinity for the human than rat 5-HT$_{2B}$ receptor, all contain, at least superficially, a degree of similarity in the ring systems that they contain.

Although the analyses of structural features that allow compounds to discriminate between the species homologs of the 5-HT$_{2B}$ receptor are at a very preliminary stage, the patterns that have emerged suggest that these may ultimately be useful in determining the ligand recognition topology of the 5-HT$_{2B}$ receptor. The species differences between the rat and human receptors can be viewed as natural mutations that provide shortcuts to determining the parts of the receptor involved in ligand recognition. Thus, within the transmembrane spanning domains of the human and rat 5-HT$_{2B}$ receptors there are only about 13 to 15 amino acid differences (depending upon where one draws the transmembrane boundaries) (Kursar *et al.*, 1994). Some of these amino acid differences are quite conservative, but others are significantly different and will serve as initial points for future mutational studies to define the domains within the 5-HT$_{2B}$

Table 2. **5-HT2B species differences of selected ergolines.**



Ergolines showing higher affinity at the human than the rat 5-HT2B receptor				Ergolines showing no significant species differences			
Compound	R_1	R_2	$\frac{K_i \text{ Rat}}{K_i \text{ Human}}$	Compound	R_1	R_2	$\frac{K_i \text{ Rat}}{K_i \text{ Human}}$
LY53857	isopropyl		4.2	LY86057	H		0.99
Amesergide	isopropyl		6.1	LY193525	H		2.5
Methysergide [a]	methyl		3.9	2-Br-LSD [a, b]	H		1.5
Mesulergine	methyl		9.9				

[a] Denotes 9 - 10 unsaturated

[b] Denotes Br at position 2

receptor that are responsible for the binding of agonists and antagonists.

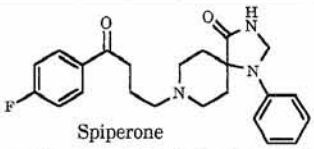
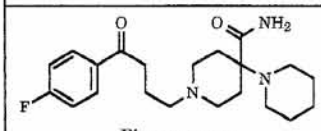
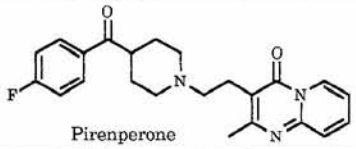
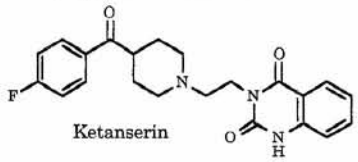
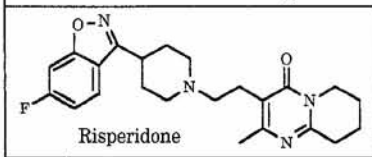
The finding of pharmacological differences in species homologs of the same receptor is not unusual, and several other examples exist within the 5-HT family of receptors. In some cases the differences can be quite small as occurs for the 5-HT_{2A} receptor, in which a single amino acid difference in transmembrane region five results in affinity differences that are restricted to certain ergolines and tryptamines (Johnson *et al.*, 1993, 1994; Nelson *et al.*, 1993). In other cases, the affinity differences can be very striking. For example, for many years it was assumed that the rat $5HT_{1B}$ and human 5-HT_{1D} receptors were two different receptors, based on the strikingly different affinities of selected compounds for these two receptors. Since the cloning of the rat 5-HT_{1B} and the human 5-$HT_{1D\beta}$ receptors, it has been determined that these receptors are species homologs of each other (Adham *et al.*, 1992; Hamblin *et al.*, 1992; Jin *et al.*, 1992). Furthermore, it has been demonstrated that a single amino acid difference between these two receptors in transmembrane region seven accounts for the difference in pharmacology (Metcalf *et al.*, 1992; Oksenberg *et al.*, 1992; Parker *et al.*, 1993).

In the present study, none of the compounds tested had statistically significantly higher affinity for the rat than for the human 5-HT_{2B} receptor. Spiroxatrine, which has a K_i of 193 ± 24 nM for the rat and a K_i of 505 ± 74 nM for the human 5-HT_{2B} receptor, came the closest to showing a preference for the rat receptor. This information, along with the fact that most of the compounds tested do not discriminate between the human and the rat 5-HT_{2B} receptors, provides evidence that the differences seen between these species homologs are not an artifact of the assay system.

During the course of our studies, Choi *et al.* (1994) published on the cloning of the human 5-HT_{2B} receptor and described its pharmacological profile. To our surprise, a comparison of affinities obtained from our clone, using [^{3}H]5-HT, with those of Choi *et al.* (1994) using [^{125}I]DOI, produced a plot showing no statistically significant correlation (fig. 2A). Examination of this indicated that the primary difference was for the affinity of N-acetylserotonin (N-acetyl-5-HT). Whereas Choi *et al.* (1994) reported a K_i for N-acetyl-5-HT of 7.08 nM, we found a value of 7220 nM. Likewise, we found that N-acetyl-5-HT had very low affinity for both the human 5-HT_{2A} and 5-HT_{2C} receptors (table 1). The related compound, melatonin (N-acetyl-5-methoxytryptamine) also showed low affinity at all three members of the 5-HT_2 receptor family (table 1), and has been reported to have low affinity for the 5-$HT_{1A,\ 1B \text{ and } 2A}$ receptors (Guardiola-Lemaitre, 1991).

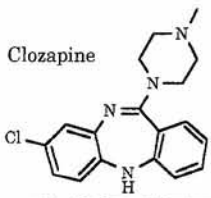
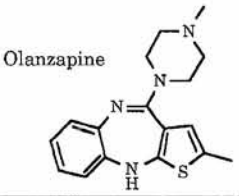
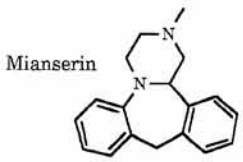
In order to rule out the possibility that the different radioligands or buffer conditions could account for the discrepancies between these results and those of Choi *et al.* (1994), the

TABLE 3
Ratio of the K_i's of selected *p*-fluorobenzoyl-containing compounds for the rat 5-HT$_{2B}$ receptor to the human 5-HT$_{2B}$ receptor*

Compound	$\frac{K_i \text{ Rat}}{K_i \text{ Human}}$
 Spiperone	4.3
 Pipamperone	12.0
 Pirenperone	9.8
 Ketanserin	8.2
 Risperidone	7.0

*All Compounds in this series showed significantly higher affinity for the human 5-HT$_{2B}$ receptor compared to the rat.

TABLE 4
Ratio of the K_i's of selected polycyclic compounds for the rat 5-HT$_{2B}$ receptor to the human 5-HT$_{2B}$ receptor*

Compound	$\frac{K_i \text{ Rat}}{K_i \text{ Human}}$
 Clozapine	4.1
 Olanzapine	4.5
 Mianserin	5.5

*All three compounds of this type showed statistically significantly higher affinity for the human 5-HT$_{2B}$ receptor compared to the rat.

conditions of Choi *et al.* (1994) were adapted to determine the affinity of selected compounds for the [^{125}I]DOI-labeled human 5-HT$_{2B}$ receptor in our laboratory. In our laboratory, when the affinities, pIC_{50} values (*i.e.*, $-\log IC_{50}$, molar), were measured against the [^{125}I]DOI-labeled human 5-HT$_{2B}$ receptor using the conditions of Choi *et al.* (1994), a strong correlation was found *vs.* the affinities, pK_i values, when measured against the [^{3}H]5-HT-labeled human 5-HT$_{2B}$ receptor (fig. 2B, $r = 0.97$, $P < .001$). Thus, the exact reason for the differences between the two laboratories remains unknown.

Low affinity of N-acetyl-5-HT for the 5-HT receptors would not be unexpected, because in N-acetyl-5-HT the amino group of 5-HT has been converted to an amide, which is not charged at physiologic pH and thus cannot form an ionic interaction with the aspartate residue in transmembrane region three of the monoamine receptors that is believed to anchor the amino portion of the monoamine neurotransmitters (Hibert *et al.*, 1991). Interestingly, however, the affinity for N-acetyl-5-HT determined by Choi *et al.* (1994) is very similar to what they found for 5-HT itself, raising the question of whether the N-acetyl-5-HT was being deacylated to form 5-HT in their binding assay. Removal of N-acetyl-5-HT from the plot of [^{3}H]-5-HT-labeled human 5-HT$_{2B}$ receptors in this laboratory *vs.* the results of Choi *et al.* (1994) resulted in a statistically significant correlation ($r = 0.67$, $P < .01$), but a number of compounds still showed as much as a 10-fold difference in the calculated K_i values (*e.g.*, spiperone, clozapine, α-methyl-5-HT, methysergide, mesulergine and ritanserin). The basis for these differences remains unknown, because in our laboratory there was very good agreement between the affinities of these compounds determined against both [^{125}I]DOI and [^{3}H]5-HT binding.

In addition to the 5-HT$_{2B}$ receptor, a number of compounds were also tested at the agonist-labeled forms of the human 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors. As seen in figure 1, B and C, there was a significant correlation with the 5-HT$_{2A}$ receptor as well as a highly significant correlation with the 5-HT$_{2C}$ receptor. However, as noted previously, when melatonin and N-acetyl-5-HT were removed from the human 5-HT$_{2B}$ *vs.* human 5-HT$_{2A}$ correlational analysis, the remaining compounds did not give a significant correlation. There were many compounds that displayed higher affinity for the 5-HT$_{2A}$ receptor than for the 5-HT$_{2B}$. However, only four of the compounds tested, melatonin, N-acetyl-5-HT, rauwolscine and amesergide, displayed significantly higher affinity for the 5-HT$_{2B}$ receptor than the 5-HT$_{2A}$. Only one of the compounds tested, DOI, displayed higher affinity for the 5-HT$_{2C}$ than for the 5-HT$_{2B}$ receptor. N-acetyl-5-HT, clozapine, pipamperone, rauwolscine and cisapride showed higher 5-HT$_{2B}$ than 5-HT$_{2C}$ receptor affinity, although rauwolscine, with 112-fold selectivity, was the only compound with greater than 10-fold selectivity. Overall, these results demonstrated the difficulty in finding compounds having sufficient selectivity for either the 5-HT$_{2B}$ or the 5-HT$_{2C}$ receptor so that these receptors could be distinguished *in vivo*.

In conclusion, the present study demonstrates that the

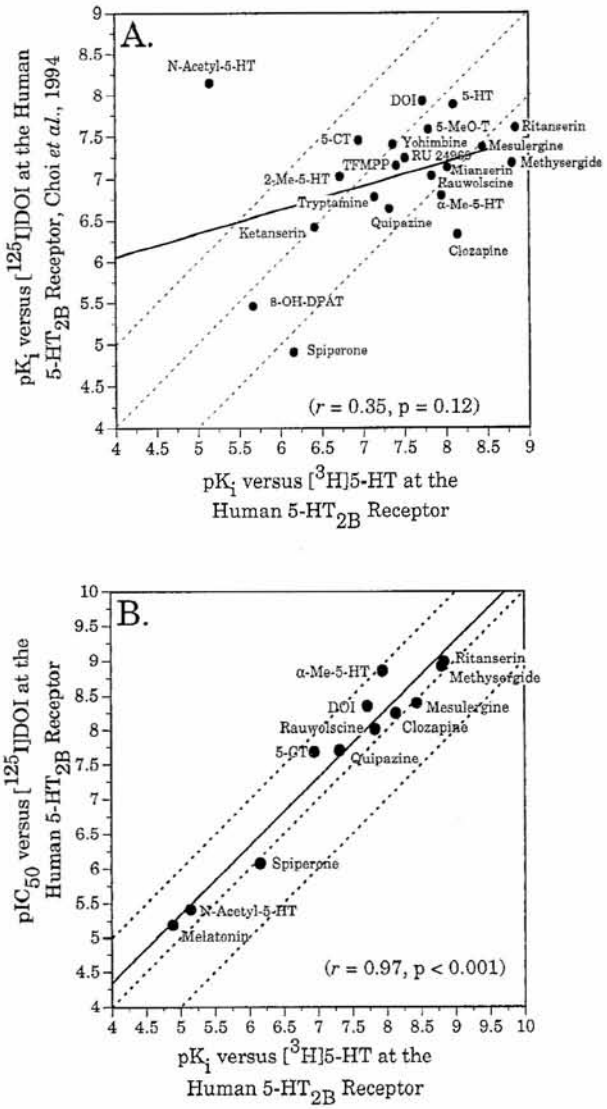


Fig. 2. A, correlation between the pK_i for inhibition of [^{125}I]DOI binding (Choi *et al.*, 1994) and the pK_i for inhibition of [^{3}H]5-HT binding in the present study to the human 5-HT_{2B} receptor. B, correlation between the pIC_{50} for inhibition of [^{125}I]DOI binding in the current study and the pK_i for inhibition of [^{3}H]5-HT binding in the current study to the human 5-HT_{2B} receptor. For both graphs, the solid line is the fitted regression line and the middle dashed line is the line of identity. The outer dashed lines are one log unit from the line of identity.

human 5-HT_{2B} receptor has an overall pharmacological profile consistent with its rat homolog. However, there are sufficient differences to emphasize the importance of defining the species when describing a receptor's pharmacology and the importance of having access to the human form of the receptor when considering drug actions. The pharmacological differences between the rat and human forms of the 5-HT_{2B} receptor should aid in determining the topology of the ligand recognition domain of this receptor and in the development of 5-HT_{2B}-selective compounds.

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