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Pharmacological Characteristics of the Newly Cloned Rat 5-Hydroxytryptamine$_{2F}$ Receptor

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SUMMARY

The rat 5-hydroxytryptamine (5-HT)$_{2F}$ (serotonin$_{2F}$) receptor is a newly cloned member of the 5-HT$_{2/1C}$ receptor family. The pharmacology of the 5-HT$_{2F}$ receptor was explored using a variety of structurally different compounds in a radioligand binding assay. In addition, the 5-HT$_{2F}$ receptor was shown to stimulate production of inositol 1,4,5-trisphosphate in the transformed cells. Based on the affinities of the compounds tested, their known affinities for certain of the other 5-HT receptors, and the fact that activation of the cloned 5-HT$_{2F}$ receptor stimulates inositol 1,4,5-trisphosphate production, the 5-HT$_{2F}$ receptor was determined to be a novel receptor and a member of the 5-HT$_{2/1C}$ receptor family. In addition, several agonists and partial agonists were evaluated for contractile activity in the rat stomach fundus, and these activities were correlated with their binding affinities at the 5-HT$_{2F}$ receptor. A highly significant correlation was found, providing additional evidence that is consistent with the 5-HT$_{2F}$ receptor being the stomach fundal contractile receptor. [^{3}H]5-HT had high affinity for this receptor both at 37° and at 0° (K_d = 7.87 ± 0.55 and 0.12 ± 0.02 nM, respectively). The difference in affinity for [^{3}H]5-HT at the two temperatures prompted an investigation of potential temperature-dependent differences in the binding affinities of agonists versus antagonists. Agonists such as 5-HT, 5-methoxytryptamine, etc., showed higher affinity for the 5-HT$_{2F}$ receptor at 0° than at 37°, whereas antagonists such as methysergide, 1-naphthylpiperazine, etc., showed no difference in affinity for this receptor at the two different temperatures. Therefore, the affinity of a compound for the 5-HT$_{2F}$ receptor at 37° versus 0° was shown to be useful for predicting agonist or antagonist activity. Additionally, information is provided about some of the structural requirements for the affinity of certain tryptamines at the 5-HT$_{2F}$ receptor.

It has been known for many years that serotonin (5-HT) potently stimulates contractions of the isolated rat stomach fundus (1). Although much work has been done to characterize the receptor responsible for this effect, its identity has remained unknown. This fundal contractile receptor does not correspond to the 5-HT$_2$ receptor (2–4), the 5-HT$_{1A}$ or the 5-HT$_{1B}$ receptor (5), or the 5-HT$_3$ receptor (6). Although the receptor has some pharmacology similar to that of the 5-HT$_{1C}$ receptor, molecular biological studies have shown that it is not identical to the 5-HT$_{1C}$ receptor (7). These observations suggested that the serotonergic contractile receptor in the rat stomach fundus is indeed a unique 5-HT receptor. Recently, a novel receptor, the 5-HT$_{2F}$ receptor, has been cloned from the rat stomach fundus, and its preliminary pharmacological profile is consistent with that of the receptor that mediates contractions in the rat stomach fundus (8). The 5-HT$_{2F}$ receptor is 96% homologous at the amino acid level to a partial amino acid sequence recently reported to have been cloned from the mouse stomach fundus (9) and is identical (except for two amino acids) to the newly described rat *SRL* receptor (10). The present study was conducted to characterize further the pharmacology of the 5-HT$_{2F}$ receptor and to compare the receptor with the serotonergic contractile receptor in the rat stomach fundus. The ultimate goals were to develop reliable radioligand binding and functional assays for this cloned receptor.

Materials and Methods

Chemicals

5-Cl-T was purchased from Lancaster Synthesis (Windham, NH). TFMPP, DOI, *m*-CPP, 2-Me-5-HT, 5-CT, α-Me-5-HT, 5-MeO-T, MDL 72222, mianserin, ketanserin, ritanserin, spiperone, (*R*)-(+)-SCH-23390, (*S*)-(−)-SCH-23388, 8-OH-DPAT, rauwolscine, yohim-

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; 5-F-T, 5-fluorotryptamine; 5-Cl-T, 5-chlorotryptamine; 5-Br-T, 5-bromotryptamine; 5-Me-T, 5-methyltryptamine; 5-MeO-T, 5-methoxytryptamine; 5-CT, 5-carboxamidotryptamine; α-Me-5-HT, α-methyl-5-hydroxytryptamine; 2-Me-5-HT, 2-methyl-5-hydroxytryptamine; 5-OH-*N*′,*N*′-DiPr-T, 5-hydroxy-*N*′, *N*′-dipropyltryptamine; 5-MeO-*N*′,*N*′-DiPr-T, 5-methoxy-*N*′,*N*′-dipropyltryptamine; 5-Br-*N*′-Pr-T, 5-bromo-*N*′-propyltryptamine; 5-Br-*N*1-IsoPr-T, 1-isopropyl-5-bromotryptamine; 1-NP, 1-naphthylpiperazine; *m*-CPP, 1-(3-chlorophenyl)piperazine; TFMPP, 1-(*m*-trifluoromethylphenyl)piperazine; 2-Br-LSD, 2-bromo-lysergic acid diethylamide; LY86057, *N*1-desisopropyl homologue of LY53857; 8-OH-DPAT, (±)-8-hydroxy-2-(di-*n*-propylamino)tetralin; DOI, (±)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; IP$_3$, D-*myo*-inositol 1,4,5-trisphosphate.

bine, and quipazine were purchased from Research Biochemicals, Inc. (Natick, MA). Serotonin creatinine sulfate complex, tryptamine, 5-F-T, polyethylenimine, IP_3, 1,1,2-trichlorotrifluoroethane, and tri-*n*-octylamine were purchased from Sigma Chemical Co. (St. Louis, MO). EDTA was purchased from EM Science (Gibbstown, NJ). L-Ascorbic acid was purchased from Aldrich Chemical Company (Milwaukee, WI). 2-Br-LSD was obtained from the National Institute on Drug Abuse (Rockville, MD). The following chemicals were donated by the respective companies: mesulergine and methysergide from Sandoz Pharmaceuticals (East Hanover, NJ), metergoline from Farmitalia Carlo Erba (Milan, Italy), and MK212 from Merck Sharp & Dohme (West Point, PA). [^{3}H]5-HT (27.4–38.1 Ci/mmol) and [^{3}H]IP_3 (17–21 Ci/mmol) were purchased from DuPont-NEN (Wilmington, DE). Selected ergolines and tryptamines were synthesized at the Lilly Research Laboratories (Indianapolis, IN).

Radioligand Binding Studies

Membrane preparation from transformed cells. Suspensions of cells expressing the cloned rat 5-HT_{2F} receptor (8) were harvested by centrifugation at 2200 × *g* for 15 min at 4°. Membranes for the binding assays were prepared by vortexing the pellet in 50 mM Tris·HCl, pH 7.4 (0.5×10^9 cells/30 ml). The tissue suspension was then centrifuged at 39,800 × *g* for 10 min at 4°. This procedure was repeated for a total of three washes, with a 10-min incubation at 37° between the first and second washes. The final pellet was homogenized in 67 mM Tris·HCl, pH 7.4 (at 20–40 and 12.5×10^6 cells/ml, original cell number, for cells expressing low and relatively high levels of the 5-HT_{2F} receptor, respectively), using a Tissumizer (Tekmar, Cincinnati, OH) at setting 65 for 15 sec.

[^{3}H]5-HT binding studies. Binding assays were automated using a Biomek 1000 (Beckman Instruments, Fullerton, CA) and were performed in triplicate in a 0.8-ml total volume. Membrane suspension (200 μl; 0.04–0.27 mg of protein) and 200 μl of drug dilution in water were added to 400 μl of 67 mM Tris·HCl, pH 7.4, containing [^{3}H]5-HT, pargyline, $CaCl_2$, and L-ascorbic acid. Final concentrations of pargyline, $CaCl_2$, and L-ascorbic acid were 10 μM, 3 mM, and 0.1%, respectively. Tubes were incubated at 37° for 15 min or at 0° for 2 hr (binding equilibria were verified for both of these conditions) and then rapidly filtered, using a Brandel cell harvester (model MB-48R; Brandel, Gaithersburg, MD), through Whatman GF/B filters that had been presoaked in 0.5% polyethylenimine and precooled with ice-cold 50 mM Tris·HCl, pH 7.4. The filters were then washed rapidly four times with 1 ml of ice-cold 50 mM Tris·HCl, pH 7.4. The amount of [^{3}H]5-HT trapped on the filters was determined by liquid scintillation counting (Ready Protein and Beckman LS 6000IC; Beckman Instruments). For the saturation experiments, actual free radioligand concentrations were determined by sampling the supernatant of parallel saturation experiments in which bound radioactivity had been separated by centrifugation. The concentration of [^{3}H]5-HT ranged from 0.02 to 5 nM and from 0.6 to 63 nM for saturation experiments incubated at 0° and 37°, respectively. 5-HT (10 μM) or 1-NP (10 μM) defined nonspecific binding. For competition experiments, six to 12 concentrations of displacing drugs were used, spanning 6 log units, and the final concentration of [^{3}H]5-HT was 2 nM. Protein was determined by the method of Bradford (11), using bovine serum albumin as the standard.

IP_3 Formation in 5-HT_{2F}-Transformed Cells

Formation and extraction of IP_3. The A600K-2-3-MTX cells (see Results), grown in suspension, were harvested by centrifugation at 200 × *g* and were resuspended in protein-free cell culture medium. After incubation of the cells ($2.5–3 \times 10^6$ cells/tube in 125 μl) at 37° for 10 min, 125 μl of the compound of interest, diluted in protein-free medium, were added. All incubations were performed in triplicate. When antagonists were used to inhibit the effect of 5-HT, the cells were incubated with the antagonist for 10 min (at 37°) before the addition of 5-HT. After addition of agonist, the cell suspension was vortexed and incubated for an additional 10 sec at 37° (the 10 sec includes the time for vortexing). Then, 250 μl of ice-cold 10% perchloric acid were added to terminate the reaction. The tubes were incubated for 10 min on ice and then centrifuged at 1500 × *g* for 10 min. After centrifugation, 400 μl of the supernatant were sampled. The following IP_3 extraction procedure was modified from published procedures (12, 13). The 400-μl sample was added to a 1.5-ml microfuge tube containing 100 μl of 10 mM EDTA, pH 9.0. This was followed by the addition of 500 μl of 1,1,2-trichlorotrifluoroethane/tri-*n*-octylamine (1:1, v/v). The tubes were vortexed vigorously for 5–7 min and then centrifuged at 1500 × *g* for 2 min to aid in separation of the three layers. From the top aqueous layer 100 μl were sampled for the determination of IP_3 content by the assay described below.

IP_3 binding assay. Rat cerebellar membranes were used as the source for the IP_3-binding protein in a binding assay modified from published procedures (14, 15). Membranes were prepared by homogenizing rat cerebella in 30 volumes of homogenization buffer (1 mM EDTA and 1 mM 2-mercaptoethanol in 50 mM Tris·HCl, pH 7.7), using a Tissumizer (Tekmar) at setting 65, for 15 sec. The homogenate was centrifuged at 39,800 × *g* for 10 min at 4°. This procedure was repeated three more times, for a total of four washes. The final pellet was suspended in 30 volumes of IP_3 binding buffer (1 mM EDTA and 1 mM 2-mercaptoethanol in 64.3 mM Tris·HCl, pH 9.0) and frozen at −70° until needed.

Binding buffer (350 μl, containing [^{3}H]IP_3) and 50 μl of binding protein homogenate were added to 100 μl of the extracted IP_3 samples or known IP_3 standards that had been subjected to the extraction procedure as described above. The final concentration of [^{3}H]IP_3 was 1 nM. The tubes were incubated at 0° for 15 min and then filtered through Whatman GF/B filters [pre-wet with water and precooled with 2 ml of ice-cold IP_3 wash buffer (1 mM EDTA in 50 mM Tris·HCl, pH 9.0)] by using a Brandel cell harvester. The filters were then rapidly washed two times with 1 ml of ice-cold IP_3 wash buffer. The amount of [^{3}H]IP_3 trapped on the filters was determined by liquid scintillation counting. The amount of IP_3 in the samples was determined by comparison with the standard curve.

Smooth Muscle Contractility Studies

Isolation of stomach fundus. Male Wistar rats (150–375 g; Laboratory Supply, Indianapolis, IN) were sacrificed by cervical dislocation, and longitudinal sections of the stomach fundus were prepared for *in vitro* examination. Two to four preparations were obtained from each rat fundus. Tissues were mounted in organ baths containing 10 ml of modified Krebs' solution of the following composition (mM concentrations): NaCl, 118.2; KCl, 4.6; $CaCl_2 \cdot 2H_2O$, 1.6; KH_2PO_4, 1.2; $MgSO_4$, 1.2; dextrose, 10.0; and $NaHCO_3$, 24.8. Tissue bath solutions were maintained at 37° and equilibrated with 95% O_2/5% CO_2. Tissues were placed under optimum resting force (4 g) and were allowed to equilibrate for approximately 1 hr before exposure to drugs. Isometric contractions were recorded as changes in grams of force, using a Beckman Dynograph with Statham UC-3 transducers.

Effect of agonists. Noncumulative contractile concentration-response curves for agonists were obtained by stepwise increases in concentration, after the preceding concentrations had been washed out, every 15–20 min. Each agonist concentration remained in contact with the tissue for approximately 2–3 min before washout, and maximum response to each agonist concentration was measured. EC_{50} values were taken as the concentrations of agonist that produced half-maximal contraction.

Statistical Analysis

The K_d and B_{max} values from the saturation assays were determined for best fits to a one-site or two-site binding model, using a partial *F* test (16). The following equation was used for a one-site binding model:

$$\text{Bound} = \frac{B_{max} \times [L]}{K_d + [L]}$$

where Bound = amount of [^{3}H]5-HT specifically bound, B_{max} = maximum number of binding sites, K_d = equilibrium dissociation constant, and [L] = free concentration of [^{3}H]5-HT. The following equation was used for a two-site binding model:

$$\text{Bound} = \frac{B_{max1} \times [L]}{K_{d_1} + [L]} + \frac{B_{max2} \times [L]}{K_{d_2} + [L]}$$

where Bound = amount of [^{3}H]5-HT specifically bound, B_{max_1} = maximum number of high affinity binding sites, B_{max_2} = maximum number of low affinity binding sites, K_{d_1} = equilibrium dissociation constant for the high affinity site, K_{d_2} = equilibrium dissociation constant for the low affinity site, and [L] = free concentration of [^{3}H]5-HT. The IC_{50} values from the competition assays, the binding parameters for the IP_3 standard curve, and the EC_{50} and E_{max} values from the IP_3 assays were determined by nonlinear regression analysis of four parameter logistic equations (16) (Systat, Systat Inc., Evanston, IL). The IC_{50} values were converted to K_i values using the Cheng-Prusoff equation (17). One-way analysis of variance was performed on the pK_i values (i.e., $-\log K_i$, in M) or the pEC_{50} values (i.e., $-\log EC_{50}$, in M), followed by the Tukey-Kramer Honestly Significant Difference test (JMP; SAS Institute, Inc., Cary, NC). Student's t test was performed for the effect of incubation temperature on the K_d of [^{3}H]5-HT (Systat; Systat Inc.). A t test was performed to determine whether the ratio of the B_{max} at 37°/0° was greater than 1 or to determine whether the E_{max} values were less than 100% (JMP; SAS Institute, Inc.).

Results

Radioligand binding studies. Initially, [^{3}H]5-HT was selected as a ligand to label the 5-HT$_{2F}$ receptor, because 5-HT potently contracts the rat stomach fundus. As reported previously (8), [^{3}H]5-HT bound with high affinity (K_d = 7.87 ± 0.55 nM) and the binding was saturable for a single class of binding sites. The B_{max} values for the more highly expressing A600K-2-3-MTX cells ranged from 910 to 2357 fmol/mg of protein (Table 1). One factor that determined the number of binding sites was the method of selection used to generate the clonal isolates (8). A600K-2-3S cells expressed moderate amounts of the 5-HT$_{2F}$ receptor, whereas the A600K-2-3-MTX cell line expressed roughly 10-fold more receptors. Another factor affecting the number of binding sites appeared to be the density to which the cells were grown (Table 1). Thus, for the A600K-2-3-MTX cell line there was an inverse correlation (r = 0.93, p < 0.025) between the growth density of cells and the maximum amount of [^{3}H]5-HT bound per mg of protein.

In the initial attempts to define a radioligand binding assay for the 5-HT$_{2F}$ receptor, it was noted that the incubation temperature of the assay affected binding. [^{3}H]5-HT had higher affinity (p < 0.001) for the 5-HT$_{2F}$ receptor at 0° (K_d = 0.12 ± 0.02 nM) than at 37° (K_d = 7.87 ± 0.55 nM). In addition, the B_{max} was greater (p < 0.025) at 37° than at 0°.

Table 2 shows the pharmacological profile for the [^{3}H]5-HT-labeled 5-HT$_{2F}$ receptor. When a number of known agonists that contract the rat stomach fundus (α-Me-5-HT, 5-MeO-T, 5-CT, and 2-Me-5-HT) (18), antagonists [1-NP (3), rauwolscine, methysergide (4), ritanserin (7), yohimbine (4, 18), and metergoline (3, 4)], and partial agonists [quipazine (4) and TFMPP (4, 5)] were examined, it was noted that agonists displayed significantly higher affinity (p < 0.001) for the 5-HT$_{2F}$ receptor at 0° than at 37°, whereas antagonists showed no difference in affinity for this receptor at the two temperatures. It is interesting to note that the partial agonists quipazine and TFMPP also had significantly greater affinity for the 5-HT$_{2F}$ receptor at 0° than at 37° (p < 0.001), although the difference was not as great as that seen with full agonists.

Stimulation of IP_3 formation. We had previously shown that 5-HT could stimulate [^{3}H]inositol phosphate accumulation via the 5-HT$_{2F}$ receptor in cells prelabeled with *myo*-[^{3}H]inositol (8). The present work was conducted to extend the functional evaluation to include additional compounds and to determine specifically whether agonists stimulate IP_3 formation. Maximal IP_3 levels occurred at approximately 5 sec after the addition of 5-HT and returned to near-basal values by 30 sec (data not shown). Ten seconds was chosen for the standard length of the agonist incubations, because shorter times were not practical for routine assays. A group of compounds was selected that was expected to cover a spectrum of full agonists, partial agonists, and antagonists.

IP_3 formation in 5-HT$_{2F}$-expressing cells showed that 5-HT, 5-Br-T, 5-MeO-T, and 5-MeO-*N*′,*N*′-DiPr-T were full agonists (Fig. 1A; Table 3). TFMPP, quipazine, and 5-OH-*N*′,*N*′-DiPr-T displayed partial agonist activity. For the seven compounds listed in Table 3, there was a significant correlation between the pEC_{50} values for stimulation of IP_3 production and the pK_i for inhibition of [^{3}H]5-HT binding (see data obtained at 37° in Table 2) (r = 0.85, p < 0.02).

Mianserin, methysergide, rauwolscine, and 1-NP displayed no agonist activity in the IP_3 assay (Fig. 1B). When cells

TABLE 1

Saturation summary of B_{max} versus cell density and K_d versus temperature

K_d and B_{max} values for [^{3}H]5-HT saturation of the 5-HT$_{2F}$ receptor are shown. Each row represents the K_d and B_{max} values for an individual experiment. Saturation curves were best fit by a one-site model. B_{max} values are given as fmol/mg of protein. Density refers to the concentration to which the cells were grown in culture.

Cells	Density	[^{3}H]Serotonin binding				
		37°		0°		Ratio of B_{max} at 37°/B_{max} at 0°
		K_d	B_{max}	K_d	B_{max}	
	10^6/ml	nM	fmol/mg	nM	fmol/mg	
A600K-2-3S	4.2	7.25	99	0.16	66	1.49
A600K-2-3S	2.25	7.27	229	0.12	127	1.81
A600K-2-3-MTX	2.3	6.83	2357	0.09	1616	1.46
A600K-2-3-MTX	1.7	7.03	2053			
A600K-2-3-MTX	3.7	6.97	1380			
A600K-2-3-MTX	4.6	8.99	910			
A600K-2-3-MTX	2.4	10.73	2101			
Mean ± standard error		7.87 ± 0.55[a]		0.12 ± 0.02		1.59 ± 0.11[b]

[a] Significant difference from the K_d determined at 0° (p < 0.001).
[b] Ratio significantly greater than 1.0 (p < 0.025).

TABLE 2

Pharmacological profile

Drug competition for binding was performed as described in Materials and Methods. K_i values are the mean ± standard error of the number of separate experiments given in parentheses.

	[^{3}H]5-HT binding		
	K_i at 37°	K_i at 0°	K_i at 37°/K_i at 0°
	nM	nM	
Tryptamines			
5-HT	10.2 ± 2.0[a] (6)	0.19 ± 0.03 (8)	53.7
Tryptamine	113 ± 6[a] (3)	3.75 ± 0.68 (3)	30.1
5-F-T	5.65 ± 0.55 (3)		
5-Cl-T	5.09 ± 0.43 (3)		
5-Br-T	39.3 ± 14.0[a] (4)	0.42 ± 0.08 (4)	93.6
5-Me-T	32.8 ± 5.0[a] (3)	0.95 ± 0.08 (3)	34.5
5-MeO-T	9.15 ± 1.47[a] (3)	0.26 ± 0.08 (4)	35.2
5-CT	150 ± 21[a] (4)	1.48 ± 0.34 (3)	101.4
α-Me-5-HT	10.5 ± 1.5[a] (4)	0.45 ± 0.05 (4)	23.3
2-Me-5-HT	284 ± 13[a] (3)	5.53 ± 0.59 (3)	51.4
5-OH-*N*′,*N*′-DiPr-T	61.0 ± 16.5[a] (5)	7.26 ± 4.20 (4)	8.4
5-MeO-*N*′,*N*′-DiPr-T	169 ± 50[a] (4)	14.1 ± 2.7 (4)	12.0
5-Br-*N*′-Pr-T	131 ± 19[a] (4)	17.2 ± 1.5 (3)	7.6
5-Br-*N*1-IsoPr-T	475 ± 81[a] (5)	15.2 ± 3.3 (4)	31.3
Sumatriptan	>10,000 (3)		
RU 24969	17.1 ± 1.7 (3)		
Arylpiperazines			
1-NP	5.29 ± 1.36 (3)	1.87 ± 0.05 (3)	2.8
m-CPP	26.8 ± 2.6 (3)		
TFMPP	84.8 ± 15.2[a] (5)	13.8 ± 1.1 (3)	6.1
Quipazine	130 ± 26 [a] (4)	12.3 ± 0.4 (3)	10.6
MK212	408 ± 56 (3)		
Ergolines			
Methysergide	6.28 ± 0.91 (4)	7.24 ± 1.30 (4)	0.9
Metergoline	6.62 ± 0.09 (4)	5.94 ± 3.09 (4)	1.1
Mesulergine	35.9 ± 4.1 (3)	15.7 ± 1.9 (3)	2.3
2-Br-LSD	12.6 ± 0.9 (4)	22.7 ± 8.9 (4)	0.6
LY53857	6.87 ± 0.55 (3)		
LY86057	12.2 ± 1.5 (3)		
Amesergide	10.7 ± 1.1 (3)		
Miscellaneous			
Ketanserin	3,559 ± 175 (3)		
Ritanserin	5.18 ± 0.52 (3)	3.65 ± 0.49 (3)	1.4
Mianserin	52.5 ± 0.6[b] (4)	15.1 ± 3.6 (4)	3.5
Spiperone	3,278 ± 92 (3)		
8-OH-DPAT	4,115 ± 312 (3)		
DOI	27.5 ± 1.7[a] (4)	1.43 ± 0.11 (4)	19.2
Rauwolscine	35.8 ± 3.8 (3)	24.0 ± 2.2 (3)	1.5
Yohimbine	53.1 ± 4.6 (4)	61.7 ± 11.7 (4)	0.9
(*S*)-(−)-SCH-23388	3,320 ± 116 (3)		
(*R*)-(+)-SCH-23390	82.9 ± 14.1 (3)		
MDL 72222	>10,000 (3)		

[a] Significant difference from K_i value determined at 0° ($p < 0.001$).
[b] Significant difference from K_i value determined at 0° ($p < 0.05$).

expressing the 5-HT$_{2F}$ receptor were preincubated with mianserin, methysergide, rauwolscine, or 1-NP before the addition of 5-HT, the 5-HT curves were shifted to the right and the E_{max} values were decreased, relative to 5-HT alone (Fig. 1C). It should be noted that, under the conditions of this assay, agonists do not have time to come to equilibrium with the receptors; thus, it is not possible to calculate antagonist affinity constants. In fact, high affinity antagonists would be expected to appear as noncompetitive agents, because in the 10-sec time period of agonist exposure insignificant amounts of the antagonist would be able to dissociate from the receptors. This appears to be the case, because all of the antagonists examined caused a decrease in the E_{max} produced by 5-HT (Fig. 1C).

Comparison of agonist potency in the rat stomach fundus with binding affinities. Fig. 2 shows the correlation for inhibition of [^{3}H]5-HT binding, at 37°, to the cloned 5-HT$_{2F}$ receptor versus contraction of the rat stomach fundus for a number of different agonists and partial agonists. There was a highly significant linear correlation between the pK_i for inhibition of [^{3}H]5-HT binding and the pEC$_{50}$ for contraction of the rat stomach fundus ($r = 0.76$, $p < 0.001$).

Discussion

This study characterized further the 5-HT$_{2F}$ receptor as a novel receptor in the 5-HT$_{2/1C}$ receptor family, as has recently been reported (8). [^{3}H]5-HT had high affinity for this receptor at both 37° and 0°. These data, along with the sequence data (8), provide clear evidence that this is a serotonergic receptor. The data obtained at 37° (Table 2) also establish the unique pharmacology of the 5-HT$_{2F}$ receptor, relative to certain of the other 5-HT receptor subtypes. For example, the 5-HT$_{2F}$ receptor is clearly different from the 5-HT$_{1A}$ receptor, because, unlike the 5-HT$_{1A}$ receptor (19), the 5-HT$_{2F}$ receptor has very low affinity for 8-OH-DPAT. It is also clearly different from the 5-

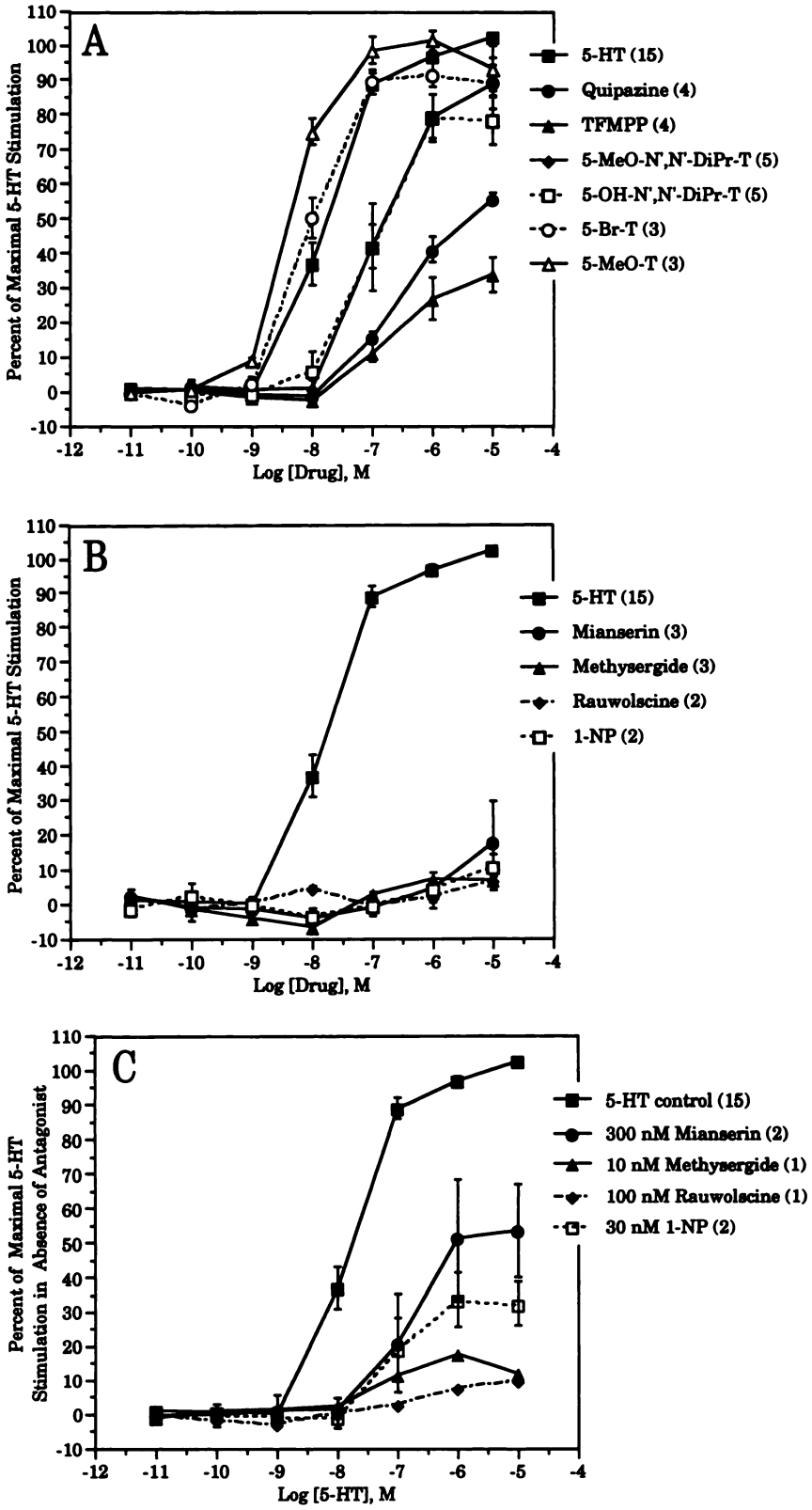

Fig. 1. IP_3 formation in cells transformed with the 5-HT_{2F} receptor. A, Agonist or partial agonist stimulation of IP_3 production. B, Stimulation by compounds with no agonist activity, compared with 5-HT. C, Effect of antagonist pretreatment on 5-HT stimulation of IP_3 production. Where more than two experiments were performed, *points* are the mean ± standard error of the number of separate experiments given in parentheses. Where two experiments were performed, *points* are the mean ± one half the range of the two separate experiments.

$HT_{1D\alpha}$, also called the 5-HT_{1D} (20), and the 5-$HT_{1D\beta}$, also called the 5-HT_{1B} (21, 22), receptors because both of these receptors have high affinity for 5-CT (23). The selective 5-HT_3 antagonist MDL 72222 (24) also has very low affinity for the 5-HT_{2F} receptor.

A number of serotonergic compounds have been tested at the 5-HT_{2F} receptor, showing that its pharmacological profile is consistent with it being a member of the 5-$HT_{2/1C}$ receptor family (see data obtained at 37° in Table 2). Although the 5-HT_{2F} receptor shares similarities with both the 5-HT_2 and 5-HT_{1C} receptors, there are several compounds that discriminate between the 5-HT_{2F} receptor and the 5-HT_2 or 5-HT_{1C} receptors. Ketanserin, mianserin, spiperone, metergoline, and methysergide have higher affinity for the 5-HT_2 receptor (K_i values

TABLE 3

Agonist-stimulated production of IP_3 in cells transformed with the 5-HT_{2F} receptor

EC_{50} and E_{max} values were calculated by nonlinear regression analysis, as described in Materials and Methods. EC_{50} and E_{max} values are the mean ± standard error of the number of separate experiments given in parentheses.

Compound	EC_{50}	E_{max}
	nM	% of 5-HT maximum
5-HT	23.3 ± 5.4	100.0 (15)
5-Br-T	9.2 ± 1.1	91.4 ± 3.1 (3)
5-MeO-T	4.7 ± 0.6	97.1 ± 3.9 (3)
5-OH-*N',N'*-DiPr-T	106 ± 30[a]	77.8 ± 6.7[b] (5)
5-MeO-*N',N'*-DiPr-T	201 ± 68[c]	94.5 ± 5.1 (5)
TFMPP	295 ± 58[c]	34.5 ± 5.0[d] (4)
Quipazine	465 ± 134[c]	60.8 ± 1.9[d] (4)

[a] Significant difference ($p < 0.01$) from the EC_{50} value for 5-HT.
[b] Significant difference ($p < 0.025$) from 100% for the E_{max} value.
[c] Significant difference ($p < 0.001$) from the EC_{50} value for 5-HT.
[d] Significant difference ($p < 0.001$) from 100% for the E_{max} value.

of 0.39, 1.4, 0.53, 0.28, and 0.94 nM, respectively) (25) than for the 5-HT_{2F} receptor (Table 2). However, yohimbine has a 10-fold higher affinity for the 5-HT_{2F} receptor (Table 2) than for the 5-HT_2 receptor (K_i = 660 nM) (25). Mesulergine, ketanserin, and metergoline have higher 5-HT_{1C} receptor affinity (K_i values of 1.6, 97.7, and 0.51 nM) (26) than 5-HT_{2F} receptor affinity (Table 2). Conversely, RU 24969 has higher 5-HT_{2F} receptor affinity (K_i = 17.1 ± 1.7 nM) than 5-HT_{1C} receptor affinity (K_i = 398 nM) (26). Therefore, the 5-HT_{2F} receptor is clearly pharmacologically distinct from the 5-HT_2 or the 5-HT_{1C} receptor.

Further evidence is provided here that is consistent with the 5-HT_{2F} receptor being the fundal contractile receptor. Comparison of 19 agonists and partial agonists revealed a significant correlation between the pK_i for inhibition of [^{3}H]5-HT binding (at 37°) to the 5-HT_{2F} receptor and the pEC_{50} for agonist contraction of the rat stomach fundus ($r = 0.76$, $p < 0.001$; Fig. 2). This information, along with the fact that the receptor was cloned from rat stomach fundus cDNA (8) and the fact that this receptor, like the 5-HT receptor in the rat stomach fundus, does not correspond to any of the known serotonergic receptors, provides additional evidence suggesting that the 5-HT_{2F} receptor is indeed the fundal contractile receptor.

Temperature was an important factor in determining agonist affinity at the 5-HT_{2F} receptor. It was first noticed that the 5-HT_{2F} receptor had higher affinity for [^{3}H]5-HT at 0° than it did at 37°. It was then found that other compounds previously reported to be full contractile agonists in the rat stomach fundus had 20–100-fold higher affinity for the 5-HT_{2F} receptor at 0° than at 37°. Conversely, antagonists displayed similar affinity for the 5-HT_{2F} receptor at the two temperatures. Known partial agonists had a smaller 37°/0° ratio than full agonists but a larger ratio than antagonists. A similar phenomenon has been reported for the β-adrenergic receptor, where agonist binding was enthalpy driven and antagonist binding was entropy driven (27).

Based on the 37°/0° binding ratio of 93.6 for 5-Br-T, 5-Br-T was predicted to behave as an agonist and did indeed maximally contract the rat stomach fundus (data not shown). However, because 5-MeO-*N',N'*-DiPr-T and 5-OH-*N',N'*-DiPr-T had 37°/0° binding ratios of 12.0 and 8.4, respectively, they were expected to behave as partial agonists like TFMPP or quipazine, which had 37°/0° binding ratios of 6.1 and 10.6, respectively. However, 5-MeO-*N',N'*-DiPr-T was a full agonist and 5-OH-*N',N'*-DiPr-T was a partial agonist in the rat stomach fundus (data not shown).

IP_3 formation in 5-HT_{2F} receptor-containing cells confirmed that 5-HT, 5-Br-T, and 5-MeO-T were full agonists (Fig. 1A), in agreement with the high 37°/0° binding ratio and the functional data in the rat stomach fundus. In addition, methysergide, rauwolscine, and 1-NP displayed no agonist activity for IP_3 formation (Fig. 1B), as expected from the binding studies. TFMPP and quipazine were partial agonists for IP_3 formation,

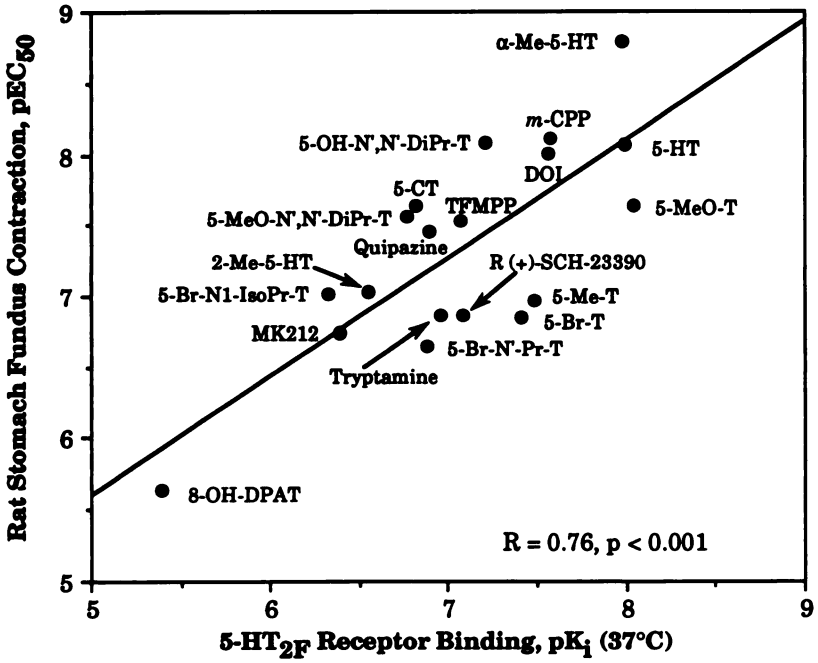

Fig. 2. Correlation between the pK_i for inhibition of [^{3}H]5-HT binding to the 5-HT_{2F} receptor at 37° and the pEC_{50} for contraction of the rat stomach fundus.

consistent with their lower 37°/0° binding ratios. However, mianserin, whose 37°/0° binding ratio was significantly greater than 1, had no agonist activity (Fig. 1B). The activity of 5-MeO-*N*′,*N*′-DiPr-T and 5-OH-*N*′,*N*′-DiPr-T in IP_3 formation was consistent with their activity in the rat stomach fundus, showing agonist and partial agonist activity, respectively. Therefore, at the 5-HT$_{2F}$ receptor, although the 37°/0° binding ratio may provide some clues about agonist versus antagonist activity, the ratio does not always predict the absolute intrinsic activity of compounds.

Examination of the saturation data revealed differences in the apparent B_{max} values for [^{3}H]5-HT binding at the 5-HT$_{2F}$ receptor, depending on the incubation temperature of the assay, i.e., the B_{max} was consistently higher at 37° than at 0° ($p < 0.025$). The reason for this difference was not readily apparent. However, one possible explanation relates to the fact that most guanine nucleotide-binding protein-coupled receptors exist in two affinity states, agonist high and low affinity, and to the fact that the difference in affinity at 0° versus 37° resulted in the need to use different concentration ranges of [^{3}H]5-HT to generate appropriate saturation curves. Because the 5-HT$_{2F}$ receptor had much higher affinity for [^{3}H]5-HT at 0°, the maximum concentration of [^{3}H]5-HT used in the saturation assays varied from 1.2 to 5 nM, whereas at 37° the maximum concentration varied from 32 to 63 nM. At 37°, the maximum concentrations of [^{3}H]5-HT may have been high enough to cause some labeling of the agonist low affinity state of the 5-HT$_{2F}$ receptor, yielding a higher apparent B_{max} value. The magnitude of labeling of such a site, relative to the agonist high affinity state, would have to be low, because nonlinear regression analysis of the saturation curves at 37° showed that a two-site fit was not statistically significantly better than a one-site fit. Two affinity states of this receptor were not detected in these saturation isotherms; this suggests that a filtration assay such as the one used might not be appropriate for reliably determining the K_d of a putative agonist low affinity state for the 5-HT$_{2F}$ receptor. Future studies using centrifugation assays or antagonist ligands will address the nature of the putative agonist low affinity state.

The present study has also yielded preliminary information about the structural requirements for affinity of the tryptamines at the 5-HT$_{2F}$ receptor (see the data obtained at 37° in Table 2). At the C5 position of the indole nucleus, fluoro ($p < 0.001$), chloro ($p < 0.001$), hydroxy ($p < 0.001$), and methoxy ($p < 0.001$) substitutions resulted in increased affinity of the compound for the 5-HT$_{2F}$ receptor, relative to tryptamine, whereas methyl, carboxamido, or bromo substituents resulted in no statistically significant change in affinity, compared with tryptamine. The rank order for the effect of the C5 substituent on the affinity of the tryptamines for the 5-HT$_{2F}$ receptor was 5-F-T = 5-Cl-T > 5-HT = 5-MeO-T > 5-Me-T = 5-Br-T > tryptamine = 5-CT. Substitution on other parts of the molecule produced varying effects on the affinity of the compound for the 5-HT$_{2F}$ receptor. α-Me-5-HT had an affinity for the 5-HT$_{2F}$ receptor that was equal to that of 5-HT, but a methyl substituent at the C2 position of the indole nucleus, as in 2-Me-5-HT, greatly decreased affinity of the compound, relative to that of 5-HT ($p < 0.001$).

Although smaller substitutions were not examined, the 5-HT$_{2F}$ receptor did not tolerate substitutions at the terminal nitrogen that were as large as a propyl group. 5-HT and 5-MeO-T had high affinity; however, their *N*′,*N*′-dipropyl homologues each had significantly decreased affinity for the 5-HT$_{2F}$ receptor ($p < 0.001$). Even a single propyl substitution at the terminal nitrogen decreased affinity for the 5-HT$_{2F}$ receptor, as evidenced by 5-Br-T versus 5-Br-*N*′-Pr-T ($p < 0.05$). In addition, 5-Br-*N*1-IsoPr-T had decreased affinity, relative to 5-Br-T ($p < 0.001$), demonstrating that the 5-HT$_{2F}$ receptor did not tolerate an isopropyl substitution at the N1 position of the indole nucleus.

In summary, the 5-HT$_{2F}$ receptor is a novel serotonergic receptor of the 5-HT$_2$ receptor family whose pharmacology is consistent with this receptor being the same as the receptor that mediates 5-HT-induced contractions in the rat stomach fundus. The availability of the cloned receptor will facilitate the development of compounds that are selective for this receptor. Such compounds should permit the study of the 5-HT$_{2F}$ receptor in tissues other than the rat stomach fundus.

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