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Receptor Reserve Masks Partial Agonist Activity of Drugs in a Cloned Rat 5-Hydroxytryptamine$_{1B}$ Receptor Expression System

NIKA ADHAM, BRENDA ELLERBROCK, PAUL HARTIG, RICHARD L. WEINSHANK, and THERESA BRANCHEK
Synaptic Pharmaceutical Corporation, Paramus, New Jersey 07652
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SUMMARY

The present study was undertaken to evaluate the behavior of the rat 5-hydroxytryptamine (5-HT)$_{1B}$ receptor stably expressed in a Y-1 cell line, using both radioligand binding ([^{125}I]iodocyanopindolol) and functional assays (inhibition of forskolin-stimulated cAMP release). The measured EC_{50} values for agonists were lower than expected from the measured K_i values (e.g., 5-HT, EC_{50} = 0.49 ± 0.043, nine experiments; K_i = 5.3 ± 0.82, four experiments). Furthermore, β-adrenergic antagonists such as propranolol and pindolol, which have been reported to be partial agonists or antagonists at the 5-HT$_{1B}$ receptors in other systems, were found to be full agonists. To investigate the relationship between receptor occupancy and inhibition of cAMP release (and hence the degree of receptor reserve), we used the irreversible receptor antagonist *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ). EEDQ treatment shifted the dose-response curve for 5-HT to the right by 6–7-fold, accompanied by a reduction (30–50%) in maximal response. Analysis of the data by the method of Furchgott revealed a very steep hyperbolic relationship between receptor occupancy and response for 5-HT, with 92 ± 1.4% (three experiments) receptor reserve at the 50% maximal response level. In contrast to its effect on 5-HT, EEDQ treatment did not significantly shift the dose-response curves for pindolol; rather, only the maximal responses were reduced and a linear relationship was found between receptor occupancy and response for this compound. According to classical receptor theory these data indicate that pindolol is a partial agonist, relative to 5-HT, but because of the high density of 5-HT$_{1B}$ receptors in this system the difference between the intrinsic activities of the two drugs is masked. Therefore, one has to be cautious when interpreting functional data in transfected systems that often display large receptor reserve.

The use of cloned receptors in heterologous expression systems has gained wide acceptance for the study of drug-receptor interaction. Somewhat less emphasis has been placed on the examination of the properties of functional responses of these same systems. β-Adrenergic receptor-blocking agents such as pindolol and propranolol have been shown to be antagonists, partial agonists, or full agonists at the 5-HT$_{1B}$ receptor (1, 2). The present study was undertaken to further characterize the cloned 5-HT$_{1B}$ receptor stably expressed in the Y-1 cell line, using both radioligand binding and functional assays. For several compounds including 5-HT, a large discrepancy was found between EC_{50} values obtained from functional assays and K_i values obtained from binding assays, indicating a large receptor reserve for these compounds. To investigate the relationship between receptor occupancy and inhibition of FSK-stimulated cAMP release, we examined the degree of receptor reserve in this system before and after irreversible receptor inactivation with EEDQ (3, 4). The results indicated that a large receptor reserve exists for the 5-HT-induced response.

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Materials and Methods

Cloning and sequencing. The rat 5-HT$_{1B}$ receptor gene was isolated by homology to the human 5-HT$_{1D\beta}$ gene, using a rat spleen genomic library (Stratagene), as described previously (5).

Transfection. The entire coding region of the rat 5-HT$_{1B}$ gene was cloned into the eukaryotic expression vector pMO5 (6), and the plasmid pMO5-rs38b was stably transfected into Y-1 cells by using the co-transfection method employing calcium phosphate precipitation (7). The selectable marker was introduced by co-transfection of a second plasmid, pGCcos3neo (8). Stable clones were then selected for their resistance to the antibiotic G418 and their ability to bind 20 pM [^{125}I] ICYP, as described previously (5). Of the six positive clones obtained, clone Y-1-11, which yielded an estimated B_{max} of 8.0 pmol/mg of protein, was selected for further studies.

Measurement of cAMP formation. Receptor-mediated inhibition of FSK-stimulated cAMP release in stable transfectants (Y-1-11 cells) was assayed as described previously (5). For EEDQ experiments, intact cells were preincubated with concentrations of EEDQ ranging between 10 nM and 1 mM. The cells were then washed three times with 1 ml of Ham's F-10 medium and 5-HT responses were investigated. Intracellular cAMP levels were measured by radioimmunoassay (cAMP radioimmunoassay kit; Advanced Magnetics, Cambridge, MA). Radioactiv-

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; ICYP, iodocyanopindolol; 5-CT, 5-carboxyamidotryptamine; EEDQ, *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline; FSK, forskolin; PTX, pertussis toxin; Gpp(NH)p, 5′-guanylylimidodiphosphate; OK, opossum kidney.

ity was quantitated using a Packard COBRA Auto Gamma Counter equipped with data reduction software.

Dose-response curves for the drug-induced inhibition of the FSK-stimulated cAMP response were analyzed using six to 10 concentrations of the agonist in triplicate. EC_{50} values were obtained by computer-assisted nonlinear regression using InPlot (GraphPad Inc., San Diego, CA).

Pseudo-dissociation constants (K_a) for drug-induced inhibition of FSK-stimulated cAMP release were obtained by the method of Furchgott and Bursztyn (9), using the equation:

$$\frac{1}{[A]} = \frac{1}{q[A']} + \frac{1-q}{qK_a}$$

where $[A]$ is the concentration of agonist necessary to produce a specific level of response before receptor alkylation, $[A']$ is the concentration needed to produce the same response after inactivation, and q is the fraction of remaining functional receptors. The equiactive doses were resolved at several levels of response (between 30 and 70% of the maximum response after EEDQ treatment) from best-fit dose-response curves derived by using InPlot. Equiactive concentrations of 5-HT from before and after inactivation were then plotted in a double-reciprocal plot and the slope and y-intercept of the resulting straight line were determined. The K_a value was calculated from the following equation:

$$K_a = \frac{\text{slope} - 1}{y\text{-intercept}}$$

The fractional receptor occupancy (f) at a particular dose, $[A]$, was calculated from the K_a values by the following relationship:

$$f = [RA]/[R_T] = [A]/K_a + [A]$$

where $[RA]$ is the concentration of receptor-agonist complex and $[R_T]$ is the total concentration of functional receptors. Fractional receptor occupancy at a given dose was then plotted against fractional response at that dose.

Membrane preparation. Membranes used in radioligand binding assays were prepared from stably transfected Y-1 cells (Y-1–11 cells) as described previously (6). Freshly prepared membranes were assayed within 1 hr of preparation. To investigate the effect of EEDQ on [^{125}I] ICYP binding, intact cells were preincubated with 320 μM EEDQ for 60 min at 37°. The cells were then washed and membranes were harvested as described above.

[^{125}I]ICYP binding assays. [^{125}I]ICYP was used as a radioligand to detect the expression of 5-HT_{1B} gene products in membrane fractions isolated from Y-1–11 cells. Radioligand binding assays were performed according to a modification of the method of Offord *et al.* (10). Isoproterenol was omitted as a masking drug from the buffer because nontransfected Y-1 cells are devoid of β-adrenergic receptors (5). Saturation studies were conducted using [^{125}I]ICYP concentrations ranging from 1 pM to 5 nM and competition experiments were performed using a final concentration of [^{125}I]ICYP ranging between 20 and 40 pM. Unlabeled 5-HT (10 μM) was used to define nonspecific binding. Assays were initiated by the addition of 50 μl of membrane homogenate (~0.1 μg of protein/well). After a 90-min incubation at 22° (in the dark), the assay was terminated by rapid filtration using a Brandel cell harvester (model 48R; Brandel, Gaithersburg, MD). Specific binding represented 95% of total binding at the K_d value. Radioactivity trapped on GF/B filter strips was quantitated by liquid scintillation counting in a Beckman LS5000 TA scintillation counter, using Ready Safe liquid scintillation cocktail (Beckman Instruments, Fullerton, CA), at an efficiency of 80%. Data were analyzed by computer-assisted nonlinear analysis (Accufit and Accucomp; Lundon Software, Chagrin Falls, OH). Protein was determined by the method of Bradford (11).

Drugs. Drugs were obtained from the following companies: (−)-[^{125}I]ICYP (specific activity, 2200 Ci/mmol), New England Nuclear (Boston, MA); dihydroergotamine tartrate, pargyline hydrochloride, (±)-pindolol, 5-HT creatinine sulfate, EEDQ, PTX, and theophylline, Sigma Chemical Co. (St. Louis, MO); 5-CT maleate, 5-methoxytryptamine oxalate, CGS-12066B dimaleate, and (−)-propranolol hydrochloride, Research Biochemical Inc. (Natick, MA); and methiothepin maleate and *m*-trifluoromethylphenylpiperazine hydrochloride, Biomol Research Laboratories (Plymouth Meeting, PA). FSK was purchased from Calbiochem (La Jolla, CA). All other chemicals were of the highest purity available commercially.

Results

Functional assays for cAMP production. 5-HT (10 μM) had no effect on either basal or FSK-stimulated adenylate cyclase activity in nontransfected or mock-transfected Y-1 cells (data not shown), indicating that endogenous cyclase-coupled serotonin receptors (including the 5-HT_{1B} receptor) are not present in nontransfected cells. Addition of 10 μM FSK increased the basal cAMP release (0.062 pmol/ml/10 min) by ~10-fold in Y-1–11 cells. Addition of 5-HT to this system caused a smooth monophasic (n_H of ~1.0) inhibition of the response (Fig. 1), with an EC_{50} value of 0.49 ± 0.043 nM (nine experiments) and a maximum inhibition (E_{max} value) of 99 ± 0.58%. The affinity and efficacy of various other compounds for the 5-HT_{1B} receptor were determined by their inhibition of 10 μM FSK-stimulated cAMP production. All compounds tested that elicited this response acted as full agonists (Fig. 1). A summary of the EC_{50} values for drugs that inhibit FSK-stimulated cAMP production in Y-1–11 cells is presented in Table 1. 5-CT was the most potent agonist tested, displaying an EC_{50} of 0.10 nM. It was previously reported (5) that methiothepin antagonizes the 5-HT-mediated inhibition of FSK-stimulated cAMP response with an apparent K_b of 21 ± 2 nM (three experiments). Interestingly, the β-adrenergic antagonists pindolol and propranolol acted as full agonists, with EC_{50} values of 34 and 42 nM, respectively (Table 1).

Toxin sensitivity. To investigate whether the cloned 5-HT_{1B} functional responses are mediated via the inhibitory guanine nucleotide-binding regulatory protein G_i, we examined the effect of PTX. Preincubation of transfected cells with 100 ng/ml PTX for 18 hr resulted in attenuation of the 5-HT-

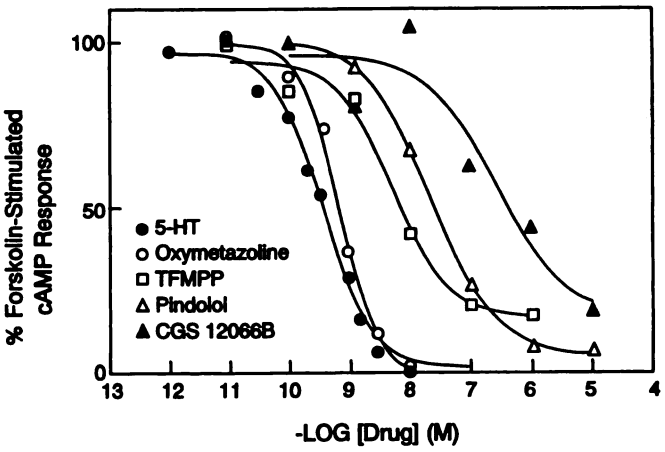

Fig. 1. Inhibition of FSK-stimulated cAMP production by agonists in stable transfectants (Y-1–11 cells) expressing the cloned rat 5-HT_{1B} receptor. cAMP measurements on intact cells were as described in Materials and Methods. Data were analyzed by computer-assisted nonlinear regression analysis (InPlot; GraphPad Inc.). Each *curve* represents the mean of triplicates from a single experiment representative of at least two others. *TFMPP*, *m*-trifluoromethylphenylpiperazine.

TABLE 1

Drug affinities for the inhibition of FSK-stimulated cAMP release and the displacement of [^{125}I]ICYP binding to the cloned rat 5-HT$_{1B}$ receptor

cAMP measurements on intact cells were as described in Materials and Methods. In binding assays, membranes harvested from cells were incubated with 20–40 pM [^{125}I]ICYP in the presence of 10–14 concentrations of unlabeled competitors for 90 min at 22°. Nonspecific binding was defined with 10 μM unlabeled 5-HT. IC_{50} values were converted to K_i values using the Cheng-Prusoff equation (12). EC_{50} and K_i values are expressed as mean values ± standard errors from at least three determinations. K_i values of compounds at the 5-HT$_{1B}$ receptor in transiently transfected COS-7 cells were obtained from previous studies (5).

Chemical class	Drug[a]	Y-1-11, EC_{50}	K_i	
			Y-1-11	COS-7
		nM	nM	
Tryptamines	5-CT	0.10 ± 0.014	19 ± 6.5	7.3 ± 1.3
	RU 24969	0.33 ± 0.010	2.6 ± 0.73	1.6 ± 0.17
	5-HT	0.49 ± 0.043	5.3 ± 0.82	16 ± 0.58
	5-MeOT[a]	1.8 ± 0.030	31 ± 10	46 ± 2.1
	Sumatriptan	44 ± 12	465 ± 187	465 ± 85
Alkoloids	DHE	0.28 ± 0.07	3.7 ± 0.65	4.2 ± 1.7
Piperazines	TFMPP	6.7 ± 0.20	60 ± 7.0	55 ± 8.5
	CGS 12066B	104 ± 29	147 ± 12	110 ± 11
Others	Oxymetazoline	0.91 ± 0.17	2.5 ± 0.41	ND[b]
	(±)-Pindolol	34 ± 11	62 ± 8.9	153 ± 62
	(−)-Propranolol	42 ± 0.50	ND	57 ± 4.0

[a] 5-MeOT, 5-methoxytryptamine; DHE, dihydroergotamine; TFMPP, *m*-trifluoromethylphenylpiperazine.
[b] ND, not determined.

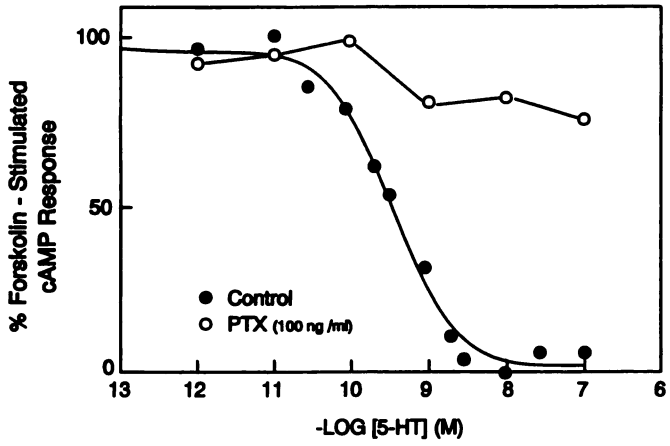

Fig. 2. Attenuation by PTX (100 ng/ml) of 5-HT-induced inhibition of FSK-stimulated cAMP production in stable transfectants (Y-1–11 cells) expressing the cloned rat 5-HT$_{1B}$ receptor. cAMP measurements on intact cells were as described under Materials and Methods. Basal activity was not altered by PTX. Data were analyzed by computer-assisted nonlinear regression analysis (InPlot; GraphPad Inc.). Each *curve* represents the mean of triplicates from a single experiment representative of at least two others.

mediated response to about 20% of the response seen in untreated cells (Fig. 2).

Characterization of [^{125}I]ICYP binding to the 5-HT$_{1B}$ receptor in Y-1–11 cells. The properties of the 5-HT$_{1B}$ receptor subtype were determined from analysis of the binding studies using membranes derived from Y-1–11 cells. Preliminary experiments indicated that, at the lowest radioligand concentration used (5.0 pM), [^{125}I]ICYP binding reached equilibrium by 60 min at 22° and remained unchanged for at least an additional 120 min. Consequently, a 90-min incubation time point was used in all subsequent experiments.

Saturation experiments. Saturable, high affinity, [^{125}I]ICYP binding was observed with membranes prepared from Y-1–11 cells (Fig. 3). The specific [^{125}I]ICYP binding represented >95% of the total binding at a ligand concentration equal to its equilibrium dissociation constant. Nonlinear analysis of saturation data indicated only a single class of binding sites, with an equilibrium dissociation constant (K_d) of 0.12 ± 0.013 nM and a binding density (B_{max}) of 7.4 ± 1.5 pmol/mg of protein (nine experiments). The transformation of data according to Scatchard also yielded a single binding site (Fig. 3, *inset*). Addition of 100 μM Gpp(NH)p had no significant effect on the saturation binding parameters [with Gpp(NH)p, K_d = 0.14 ± 0.005 nM; B_{max} = 12 ± 6.2 pmol/mg of protein; two experiments).

Competition experiments. The pharmacological profile of ligand binding to Y-1–11 cell membranes was determined by analysis of competition binding experiments. The affinities of these drugs (K_i values) for inhibiting [^{125}I]ICYP binding are summarized in Table 1. The rank order of potencies for the compounds listed is very similar to that previously obtained with transiently transfected COS-7 cells (5) (Table 1) and is consistent with 5-HT$_{1B}$ pharmacology reported for the native rat cortical membranes (10, 13, 14) and for OK cells (2). Most of the agonist competition binding curves were shallow in the absence of Gpp(NH)p. For 5-HT, the binding was resolved into high and low affinity components with a ~50-fold difference in affinity for the agonist (K_H = 0.46 ± 0.13 nM; K_L = 22 ± 6.7 nM; n_H = 0.55 ± 0.03; three experiments), with 50 ± 4.1% of the sites being of high affinity. In the presence of 100 μM Gpp(NH)p, competition binding curves for 5-HT were shifted to the right (Fig. 4) and were best fit by a one-site model (n_H = 0.83 ± 0.09); 5-HT competed for [^{125}I]ICYP binding sites with a K_i value of 17 ± 3.0 nM (five experiments), approximating the K_L value measured in the absence of Gpp(NH)p. On the other hand, pindolol competition curves were best fit by a one-site model (K_i = 62 ± 8.9; n_H = 0.78 ± 0.084; three experiments), and addition of Gpp(NH)p had no significant effect on either the K_i (55 ± 12 nM; three experiments) or the Hill coefficient (n_H = 0.77 ± 0.024; three experiments).

EEDQ experiments for investigation of degree of receptor reserve for 5-HT$_{1B}$ receptor-mediated functional responses in Y-1–11 cells. Comparison of the EC_{50} values obtained for agonist-elicited inhibition of FSK-stimulated cAMP release and the corresponding K_i values derived from binding experiments (Table 1) indicated a significant amount of receptor reserve in these transfected cells. The ratio of K_i/EC_{50} ranged from ~1.5 in the case of CGS 12066B to ~200 for 5-CT (Table 1). To investigate the degree of receptor reserve, cells were pretreated with EEDQ as described above. Preliminary results indicated that the dose of EEDQ necessary to produce 30% or greater reduction in the maximum response to 5-HT, without significantly affecting either the basal or the FSK-stimulated levels of cAMP release, was 320 μM. Consequently, this dose of EEDQ was used for further studies. EEDQ treatment produced dose-dependent shifts to the right in the 5-HT dose-response curve for inhibition of the FSK-stimulated cAMP response and at high doses caused a reduction in the maximal response. The shift was significantly different from control at EEDQ concentrations between 10 and 320 μM, whereas the reduction in E_{max} was significant only at 100 and 320 μM EEDQ (Table 2). At the maximum dose of EEDQ used (320 μM), the EC_{50} for 5-HT was shifted rightward by 6–7-fold and the maximum response was attenuated by 30–50% (Table

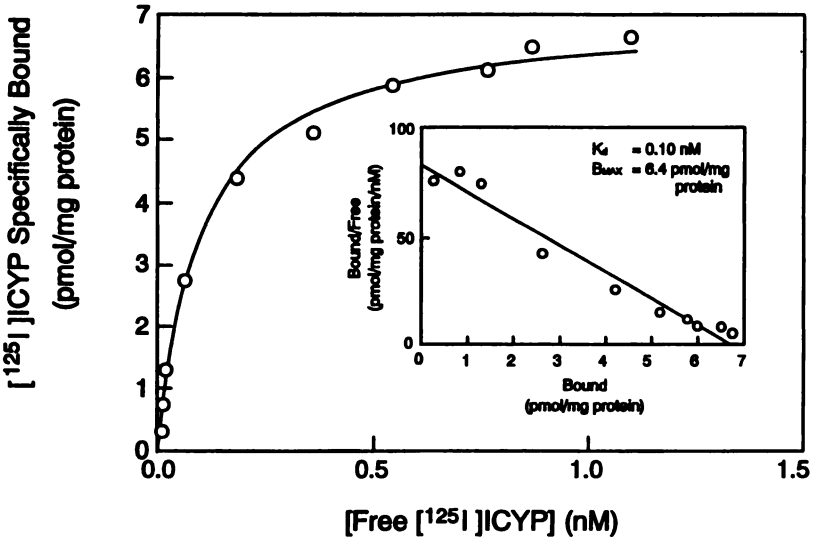

Fig. 3. Determination of the equilibrium dissociation constant (K_d) of [^{125}I]ICYP for the cloned rat 5-HT$_{1B}$ receptor. Membranes harvested from stable transfectants (Y-1–11 cells) were incubated with 10–12 concentrations of [^{125}I]ICYP (0.005–1.5 nM) for 90 min at 22°. Nonspecific binding was defined with 10 μM unlabeled 5-HT. Each *data point* is the mean of triplicate determinations from a single experiment representative of at least two others, and standard deviations averaged <5%. K_d and B_{max} values were determined by computer-assisted nonlinear regression analysis (Accufit; Lundon Software) and these values are illustrated in the form of a Scatchard plot (*inset*).

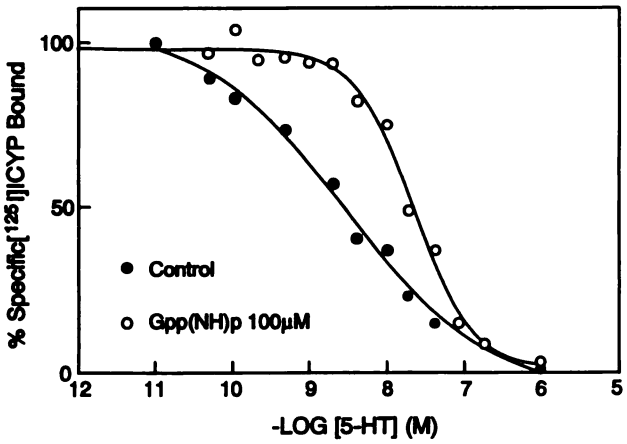

Fig. 4. Inhibition by 5-HT of [^{125}I]ICYP binding to the cloned rat 5-HT$_{1B}$ receptor, in the absence and presence of Gpp(NH)p (100 μM). Membranes harvested from stable transfectants (Y-1–11 cells) were incubated with [^{125}I]ICYP (20–40 pM) for 90 min at 22°. Nonspecific binding was defined with 10 μM unlabeled 5-HT. Each *data point* is the mean of triplicate determinations from a single experiment representative of at least two others, and standard deviations averaged <5%. Data were analyzed by computer-assisted nonlinear regression analysis (Accufit; Lundon Software).

TABLE 2

Effect of different doses of EEDQ on the EC_{50} and E_{max} of 5-HT- and pindolol-induced inhibition of FSK-stimulated cAMP production in Y-1-11 cells expressing the cloned rat 5-HT$_{1B}$ receptor

cAMP measurements on intact cells were as described in Materials and Methods. EC_{50} and E_{max} values were obtained from dose-response curves by computer-assisted nonlinear regression analysis (InPlot; GraphPad Inc.). Results are means ± standard errors of at least three separate experiments.

[EEDQ]	5-HT		Pindolol	
	EC_{50}	E_{max}	EC_{50}	E_{max}
μM	nM	% inhibition of FSK	nM	% inhibition of FSK
0.0	0.49 ± 0.043	99 ± 0.58	34 ± 11	94 ± 2.4
1.0	0.65 ± 0.19	99 ± 1.0	34 ± 13	84 ± 1.2[a]
10	0.93 ± 0.20[a]	92 ± 6.0	31 ± 15	70 ± 5.2[b]
100	2.4 ± 0.05[d]	83 ± 4.5[d]	25 ± 11	58 ± 8.6[b]
320	3.2 ± 0.20[d]	64 ± 8.5[d]	19 ± 4.0	39 ± 8.5[c]

[a–d] Student's *t* test, significant difference from control (in the absence of EEDQ) values: [a] $p < 0.05$, [b] $p < 0.01$, [c] $p < 0.001$, [d] $p < 0.0001$.

2; Fig. 5). The mean K_a value obtained from three independent experiments was 14 ± 4.5 nM, which is close to the K_i value obtained for 5-HT inhibition of [^{125}I]ICYP binding in the presence of Gpp(NH)p [with 5-HT plus 100 μM Gpp(NH)p, K_i = 17 ± 3.0 nM]. K_a values could not be determined at any other dose of EEDQ because the maximum response to the agonist has to be reduced by at least 50% to obtain an accurate estimate (9) and, therefore, higher doses of EEDQ had to be used, which were found to be toxic to the cell. The K_a values were used to calculate fractional receptor occupancy, depicted in Fig. 7. In the Y-1–11 cells there was a very steep hyperbolic relationship for 5-HT, with half-maximal response (50%) requiring approximately 4% (mean = 3.7 ± 1.4%; three experiments) receptor occupancy, indicating a 92% (mean = 92 ± 1.4%, three experiments) receptor reserve for 5-HT-induced inhibition of FSK-stimulated cAMP response. EEDQ treatment did not shift the dose-response curve for pindolol, but the maximal response was reduced significantly between 1.0 and 320 μM EEDQ (Table 2; Fig. 6). The K_a value obtained for pindolol was 72 ± 28 nM (five experiments), which was very close to the K_i value determined from binding assays (pindolol K_i = 62 ± 8.9 nM). At the highest concentration of EEDQ the maximum response to pindolol was reduced by 50–70% (Table 2). A linear relationship was obtained between receptor occupancy and response (Fig. 7), indicating that there was no receptor reserve for this compound.

Binding results indicated that EEDQ produced a dose-dependent decrease in the maximum number of receptors (statistically significant between 10 and 320 μM EEDQ), with no significant effect on the K_d of [^{125}I]ICYP except at the highest dose of EEDQ, where the K_d value was increased ~2.5-fold (Table 3). However, under these conditions there was a large standard error for the estimation of K_d and B_{max}, because the signal to noise ratio was greatly reduced such that the specific binding was only 25% of the total binding at the K_d. An estimated total of 7% of receptors remained intact after pretreatment with the maximum dose of EEDQ (Table 3).

Discussion

Similarly to results obtained previously using transiently transfected COS-7 cells (5), the pharmacological profile of Y-1

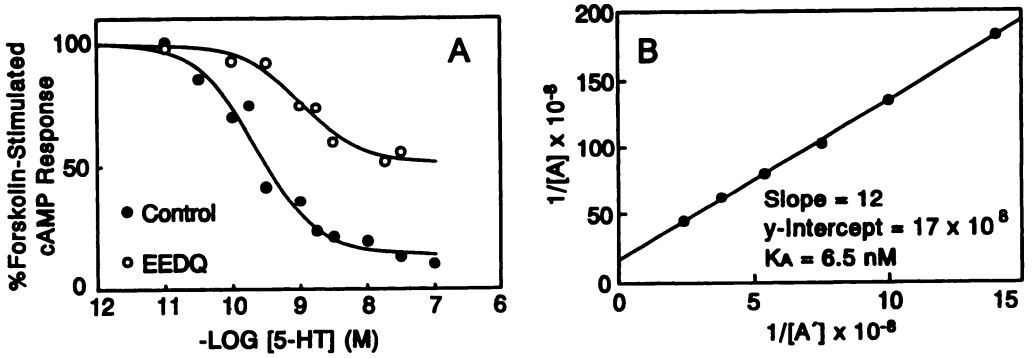

Fig. 5. A, Effect of EEDQ pretreatment (320 μM) on 5-HT-induced inhibition of FSK-stimulated cAMP production in stable transfectants (Y-1–11 cells) expressing the cloned rat 5-HT$_{1B}$ receptor. cAMP measurements on intact cells were as described in Materials and Methods. Data were analyzed by computer-assisted nonlinear regression analysis (InPlot; GraphPad Inc.). Each *curve* represents the mean of triplicates from a single experiment representative of at least two others. B, Double-reciprocal plot for the data from the two curves in A, according to the method of Furchgott, as described in Materials and Methods. K_a value indicated was derived from the slope and the *y*-intercept of the regression line, as described in Materials and Methods.

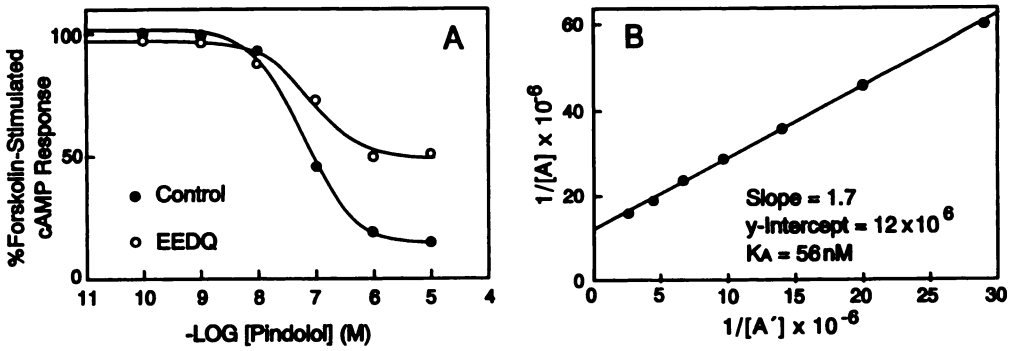

Fig. 6. A, Effect of EEDQ pretreatment (320 μM) on pindolol-induced inhibition of FSK-stimulated cAMP production in stable transfectants (Y-1–11 cells) expressing the cloned rat 5-HT$_{1B}$ receptor. cAMP measurements on intact cells were as described in Materials and Methods. Data were analyzed by computer-assisted nonlinear regression analysis (InPlot; GraphPad Inc.). Each *curve* represents the mean of triplicates from a single experiment representative of at least two others. B, Double-reciprocal plot for the data from the two curves in A, according to the method of Furchgott, as described in Materials and Methods. K_a value indicated was derived from the slope and the *y*-intercept of the regression line, as described in Materials and Methods.

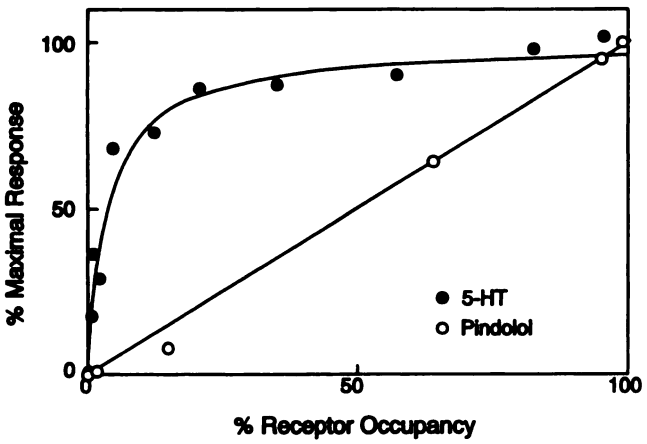

Fig. 7. Maximal inhibition by 5-HT and pindolol of FSK-stimulated cAMP production in stable transfectants (Y-1–11 cells) expressing the cloned rat 5-HT$_{1B}$ receptor, as a function of receptor occupancy. cAMP measurements on intact cells were as described in Materials and Methods. Fractional receptor occupancy for the agonists was calculated using the pseudo-K_a values (obtained by Furchgott analysis after partial receptor alkylation using EEDQ) and the corresponding control dose-response curves, as described in Materials and Methods. The pindolol data points were subjected to linear regression (r = 0.99). Each *curve* represents the mean of triplicates from a single experiment representative of at least two others.

TABLE 3

Effect of different doses of EEDQ on the binding parameters of [^{125}I]ICYP in Y-1-11 cells expressing the 5-HT$_{1B}$ receptor

Membranes harvested from stable transfectants were incubated with 10–12 concentrations of [^{125}I]ICYP (0.005–1.5 nM) for 90 min at 22°. Nonspecific binding was defined with 10 μM unlabeled 5-HT. K_d and B_{max} values were determined by computer-assisted nonlinear regression analysis (Accufit; Lundon Software). Results are means ± standard errors of at least two separate experiments.

[EEDQ]	K_d	B_{max}
μM	nM	pmol/mg of protein
0.0	0.12 ± 0.013	7.4 ± 1.5
1.0	0.14 ± 0.01	3.3 ± 0.25
10	0.19 ± 0.04	1.7 ± 0.35[a]
100	0.17 ± 0.002	0.68 ± 0.02[a]
320	0.29 ± 0.18[a]	0.55 ± 0.18[a]

[a] Student's t test, significant difference from control (in the absence of EEDQ) values, $p < 0.05$.

cells stably transfected with the rat 5-HT$_{1B}$ gene matched that of the native 5-HT$_{1B}$ receptors found in rat cerebral cortex (10, 12) and OK cells (2). In contrast to the data reported by Murphy and Bylund (2) for the 5-HT$_{1B}$ receptors in the OK cells, [^{125}I] ICYP saturation binding in Y-1–11 cells was insensitive to Gpp(NH)p and could not be resolved into two sites, although the 5-HT competition curves were shallow and were shifted to a single low affinity state by Gpp(NH)p. One explanation could be that in our system ICYP is acting either as an antagonist or as a partial agonist, relative to 5-HT, such that the difference

between the affinities of its high and low affinity states is not as great as that for 5-HT. This is supported by the observation that Gpp(NH)p had no effect on the competition curves obtained for pindolol, which is a closely related analogue of ICYP and a partial agonist in this system.

Functional responses were obtained from Y-1 cells transfected with the rat 5-HT_{1B} receptor gene. The magnitude of this 5-HT_{1B} receptor response was large (E_{max} > 99%). The 5-HT_{1B} receptor expressed in Y-1–11 cells is likely to be coupled to G_i proteins, because agonist binding is sensitive to Gpp(NH)p and PTX attenuates 5-HT-induced inhibition of FSK-stimulated cAMP release. A similar conclusion was reached by Murphy and Bylund (2) for the 5-HT_{1B} receptor in OK cells. Apart from methiothepin, which acts as an antagonist (5), all the compounds tested, including β-adrenergic antagonists such as pindolol and propranolol, which have been reported to be antagonists or partial agonists at the 5-HT_{1B} receptor in other systems, behaved as full agonists. This observation, together with the fact that the affinities for the agonists were greater than those expected from the measured K_i values, indicated a significant amount of receptor reserve for agonist-induced inhibition of FSK-stimulated cAMP release in these transfected cells. The ratio of K_i/EC_{50} varied between different compounds and the highest value (~200) was obtained for 5-CT, indicating that a larger receptor reserve may exist for this compound, compared with 5-HT (K_i/EC_{50}, ~10). Interestingly, 5-CT has been shown to be more efficacious than 5-HT in inhibiting FSK-stimulated cAMP release via 5-HT_{1B} receptors in the rat substantia nigra (1). In the present study, a low K_i/EC_{50} value was observed for pindolol (~2), suggesting a smaller receptor reserve for this drug, compared with 5-HT. This hypothesis was verified by alkylation experiments using EEDQ. There was a substantial receptor reserve for 5-HT in this system, inasmuch as blockade of approximately 80% of the receptors with the irreversible antagonist EEDQ resulted in a shift to the right of the 5-HT dose-response curve, with no apparent change in the maximal response until >90% of the receptors were alkylated, at which point the EC_{50} approximated the apparent K_i value. In the case of pindolol, on the other hand, the reduction in E_{max} caused by EEDQ pretreatment was directly proportional to the loss of available receptors, suggesting little receptor reserve for this compound. Using Furchgott analysis, an estimated 92% receptor reserve was obtained for 5-HT at the 50% response level, whereas no receptor reserve was found for pindolol, indicating that pindolol is a partial agonist, relative to 5-HT, but because of the large numbers of receptors that are expressed the difference between the intrinsic activities of the two compounds is not obvious.

The EC_{50} value and intrinsic activity for a given drug have been shown to depend on the number of receptors expressed in a given system. For example, in the case of muscarinic M_1 receptors transfected into B82 fibroblasts a linear correlation was obtained between the total number of receptors and the maximum response. Moreover, for the high expressors the K_i/EC_{50} ratio was significantly greater than unity, indicating the existence of spare receptors (15). Similarly, for 5-HT_{1A} receptors transfected into HeLa cells the intrinsic activity of a given drug was changed from 1 (full agonist) to 0 (silent antagonist) in a lower expressor (16). In a recent study (17) using 5-HT_{1A} receptors transfected into NIH-3T3 cells, the maximum responses to full and partial agonists were found to change with receptor density but the EC_{50} values of both full and partial agonists were independent of the receptor density. In a strict sense the results of the present study cannot be compared with those mentioned above, because the experimental approach used to reduce receptor number was different; nevertheless it can be concluded that, unlike 5-HT_{1A} receptors in NIH-3T3 cells, the data obtained for the coupling of 5-HT_{1B} receptors to adenylate cyclase in Y-1 cells are consistent with classical receptor theory.

Receptor number, however, does not appear to be the sole determinant of intrinsic activity. This is illustrated in the case of the β-adrenergic antagonists propranolol, pindolol, and cyanopindolol, whose intrinsic activities at the 5-HT_{1B} receptor can vary markedly in different systems expressing similar receptor densities. For instance, these compounds are potent antagonists in the rat brain (1, 14) but full agonists in OK cells, despite the presence of a similar number of 5-HT_{1B} receptors in the two systems (OK cells, ~100 fmol/mg of protein; rat brain, 150–300 fmol/mg of protein, as determined by [^{125}I]ICYP binding) (2, 14). These results and the present study indicate that the efficiency of coupling not only is dependent on receptor number and ligand efficacy but also is determined by the guanine nucleotide-binding protein and effector. These data suggest that there are substantial differences in the degree of amplification at the level of the receptor-guanine nucleotide-binding protein-effector complex among different systems.

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Send reprint requests to: Nika Adham, Department of Pharmacology, Synaptic Pharmaceutical Corp., 215 College Road, Paramus, NJ 07652.