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Cloning and expression of human 5-HT_{4S} receptors. Effect of receptor density on their coupling to adenylyl cyclase

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Abstract

We have isolated a cDNA encoding the 5-HT_{4S} receptor by RT-PCR on poly (A)⁺ RNA from both human heart and brain. The sequence homology with the rat and mouse 5-HT₄ receptors was high: 93.8% of identity in the amino acid sequence. None of the 24 amino acid substitutions observed between rat and human receptors are at positions likely to modify their pharmacology. Comparing the pharmacological properties of six agonists and five antagonists on rat and human 5-HT_{4S} receptors revealed no significant differences. We have analyzed the behavior of renzapride, a full and a partial agonist on mouse colliculi neurons and human heart biological responses respectively. The coupling efficiency of renzapride was two-fold lower than that of 5-HT for the stimulation of 5-HT_{4S} receptors transfected in two different cell lines (LLC-PK1 and COS-7), but increasing the receptor density suppressed the partial agonist effect of renzapride.

Key words: Coupling efficiency; Human 5-HT₄ receptor; Receptor density

INTRODUCTION

5-HT₄ receptors were first characterized in 1988.¹ Since then they have been demonstrated in a wide variety of tissues in different species including, rodent, porcine, and human brain, guinea-pig ileum and colon, rat oesophagus, frog and human adrenocortical cells, human urinary bladder, human and porcine heart.^{2,3} The newly proposed pathophysiological properties of 5-HT₄ receptors, both in the central nervous system (increase in olfactory and spatial memory^{2,4,5}) and at the periphery (implication in arrhythmia and irritable bowel syndrome⁶), have stimulated investigations into a large series of selective and potent agonists and antagonists.² These can now be tested for their putative therapeutic use. However, due to the difficulties encountered until now in cloning 5-HT₄ receptors,^{7,8} most of these drugs have been tested on functional animal preparations. In human heart and many other preparations, benzamides have been shown to be weak partial agonists whereas they are full agonists in mouse colliculi neurons.⁹⁻¹¹ In the present study we addressed two questions: is there any species difference in 5-HT₄ receptor pharmacology; and are the differences in efficacies reported for benzamides in different species due to a difference in coupling efficiency or to receptor structure? To answer these questions we cloned the human 5-HT₄ receptor from heart and brain and analyzed their molecular pharmacological properties.

MATERIALS AND METHODS

Isolation and sequencing of a human heart and brain 5-HT₄ cDNA by RT-PCR

Total RNA was isolated from the atrium of a patient undergoing heart transplantation. Poly (A)⁺ RNA was extracted using Dynabeads Oligo (dT)₂₅ (Dynal) and reverse transcribed using Superscript II RNase H reverse transcriptase (Life Technologies) and random nonamers. RT-PCR was performed with the following oligonucleotides designed on International Patent WO 94/14957, ON1 (Sequence 7, position 5-27): 5'-TAATGGACAAACTTGATGCTAATG-3' and ON2 (Sequence 14, position 765-786): 5'-TCAGAAGCATGATTCCAGGGAT-3'. These primers are located in the 5' and 3' translated regions of the 5-HT_{4S} receptor. PCR was performed with the Taq DNA Polymerase (Eurobio) with a cycle of 94°C for 3 min followed by 30 cycles of denaturation (94°C, 30 s), annealing (55°C, 30 s) and elongation (72°C, 1 min). We used two successive PCR to have sufficient amplification. These conditions yielded a 1166 bp product which was subcloned into pGEM5zf(+) (Promega) and plasmid DNA was prepared for insert sequencing. Nucleotide sequencing was performed using the Sanger dideoxynucleotide chain-termination method on denatured double-stranded plasmid templates

using T7 DNA polymerase (Pharmacia kit). To determine the exact sequence of the human heart 5-HT₄ cDNA, we performed three independent PCRs on three independent preparations of poly(A)⁺ RNA. To confirm the sequence of the 5' part of the receptor around the ATG codon, we performed a PCR reaction using the following primers: ON3 (Patent, sequence 1, position 21-41): 5'-GGTTGGAAGGAGGAGGATGCT-3', in the 5' non-coding sequence, and ON4 (Patent, sequence 1, position 699-721): 5'-GAGGAGAAACGGGATGTAGAAGG-3', in the middle of the receptor sequence. To confirm the sequence of the 3' part of the receptor around the stop codon, we performed a PCR reaction using the following primers ON5 (Patent, sequence 1, position 1133-1150): 5'-CCCTGTTCAACCACAACC-3' in the middle of the receptor sequence and ON6 (Patent, sequence 1, position 1262-1285): 5'-AAAGGCAAGCCACGTCTTCAATCA-3' in the 3' noncoding region of the rat receptor. The PCR conditions with Taq DNA polymerase (Eurobio) were those described here above. The amplified fragments (701 bp and 153 bp, respectively) were subcloned and sequenced. cDNA encoding human brain 5-HT_{4S} receptor was cloned by RT PCR on total human brain poly(A)⁺ RNA (Clontech) by following the same procedures as described above.

Transfection of LLC-PK1 and COS-7 cells

Human 5-HT₄ cDNA was subcloned into pRK5, a CMV-based expression vector¹² and introduced into LLC-PK1 cells (a gift from Dr Falk Fahrenholz, Germany) or COS-7 cells by electroporation as described in Ref.⁸

cAMP assays

Six hours after transfection, cells were incubated overnight in DMEM without FBSd (foetal bovine serum dialysed) with 2 µCi [³H]-adenine/ml to label the ATP pool and cAMP accumulation was measured as described in Ref.¹

Membrane preparations and radioligand binding assays

Membranes were prepared from transiently transfected cells plated on 15 cm dishes and grown in DMEM with 10% FBSd for 6 h and 20 h in DMEM without FBSd. Radioligand binding studies with [³H]GR 113808 were performed as previously described.¹³ Membranes were also prepared from human atrial tissue (2 g) which was reduced to a powder under nitrogen and homogenized in 20 ml of a buffer containing 50 mM HEPES (pH 7.4), 5 mM EGTA, 1 mM EDTA, 0.32 M sucrose and antiproteases, and homogenized with an Ultra Turrax T25 at 24 000 T/min for 30 s at 4°C. The homogenate was filtered through gauze and centrifuged for 15 min

at 14 000 x g at 4°C. The pellet was resuspended in the same buffer and homogenized 10 times with a glass-Teflon pestle at 4°C. The homogenate was centrifuged at 40 000 x g for 20 min, the membrane pellet was resuspended in 50 mM HEPES (pH 7.4) and stored at -80°C until used. Radioligand binding studies with [³H]GR 113808 were performed as for membranes prepared from transiently transfected cells at 20°C for 30 min in a total volume of 250 µl with about 200 µg membrane protein.

Data analysis

Competition and saturation experiments were analyzed by non-linear regression using the computer program LIGAND.¹⁴ Saturation experiments were also analyzed according to Scatchard.¹⁵

RESULTS

Cloning and primary amino acid sequence of a human 5-HT₄ receptor

We performed RT-PCR experiments with poly(A)⁺ RNA purified from human atrium using the primer designed to clone the short form of the receptor 5-HT_{4S}. The transcript of the 5-HT₄ receptor expressed in heart has been reported to be a short form.⁷ The resulting fragment (1166 bp) ligated into the plasmid pGEM was completely sequenced on both strands. We isolated three full-length cDNA fragments, obtained from three independent PCRs, with identical sequences. Sequence analysis revealed one long open reading frame of 387 amino acids. A similar strategy was used to isolate from human brain poly (A)⁺ RNA, cDNA fragments coding for human 5-HT₄ receptor. Identical nucleotide sequences were found in clones obtained from the human heart and brain. The amino acid sequence of this human 5-HT₄ receptor was markedly homologous to that of the rat 5-HT_{4S} (Fig. 1). A difference of 111 nucleotides was found between human and rat cDNA leading to 24 differences in the amino acid sequence (Fig. 1). Compared with rat 5-HT₄ receptor, the N-glycosylated sites and most of the consensus amino acids and sequences of the biogenic amine receptors were conserved,¹⁶ including the aspartates in transmembrane domains [(TMD)-II (D66) and TMD-III (D100)] engaged in coupling to G proteins and ligand binding respectively, the cysteines of the extracellular loops e1 and e2 likely engaged in a disulfide bridge, the DRY sequence at the N-terminus of the second intracellular loop i2, which is important for activation of G proteins,¹⁷ the prolines in TMD-V, TMD-VI and TMD-VII, and a cysteine (C328) which is a potential palmitoylation site.¹⁸ As for rat 5-HT_{4S} receptor, human 5-HT_{4S} receptor has three potential

phosphorylation sites for protein kinase C (PKC) but no putative site for protein kinase A (PKA). The carboxy-terminus of the human 5-HT_{4S} receptor (h5-HT_{4S}) has five serine and six threonine residues located after a pair of acidic amino acids in a 57 amino acid peptide of the extreme carboxy-terminus. These residues are putative phosphorylation sites¹⁹ for the family of cAMP-independent G-protein-coupled receptor kinases (GRKs).²⁰

Comparison between pharmacological properties of human and rat 5-HT₄ receptors

Transient transfection of LLC-PK1 cells or COS-7 cells with cDNA encoding the h5-HT_{4S} receptor or the short form of rat 5-HT₄ (r5-HT_{4S}) resulted in the appearance of a single class of high-affinity, saturable binding sites for [³H]GR 113808, giving a Hill coefficient not different from 1 ($K_d = 0.082 \pm 0.02$ nM and 0.089 ± 0.016 nM ($n = 6$), respectively, at 20°C; Fig. 2A). No detectable binding was found in mock transfected LLC-PK1 or COS-7 cells. In order to compare the pharmacological properties of the rat and human receptors, we measured the affinities of 5-HT₄ receptor agonists and 5-HT₄ receptor antagonists by displacement experiments. All the displacement curves were monophasic, giving a Hill coefficient not different from 1. A significant correlation ($r = 0.99$) was found between the affinities of the six agonists and five antagonists tested on the rat and human 5-HT_{4S} receptors (Fig. 2B).

As expected, stimulation of human 5-HT_{4S} receptors transfected into LLC-PK1 or COS-7 cells resulted in a dose-dependent accumulation of cAMP. EC₅₀ and PKi values were measured in transiently transfected LLC-PK1 cells expressing human 5-HT_{4S} or rat 5-HT_{4S} receptors as described above. As reported in Table 1, none of the substitutions observed between rat and human 5-HT₄ receptors modify the pharmacology or the transduction properties of one receptor compared to the other.

Are benzamides full or partial agonists because of a species or coupling difference?

One of the main pharmacological differences between the 5-HT₄ receptors described in different tissues or species is that benzamides are either full (in mouse colliculi neurons)¹¹ or partial agonists (in mouse hippocampal membranes or human heart).^{3,9} Figure 3A is an illustration of our previous results showing the partial agonism of renzapride on human heart 5-HT₄ receptors, compared with the full agonist behavior of this drug in mouse colliculi neurons.^{1,10,11} It was of interest, therefore, to examine why renzapride had such a poor efficacy on human heart 5-HT₄ receptors. Saturation binding with [³H]GR 113808 identified 8 ± 2 fmol/mg 5-HT₄ receptors in human right atrium with a K_d of 0.1 ± 0.03 nM, close to that of the cloned human 5-HT_{4S} receptors (not shown). In COS-7 cells expressing a low density of

receptors, renzapride acted indeed as a partial agonist. However, when tested on cells expressing an increasing number of receptors, the partial agonism of renzapride was progressively suppressed (Fig. 3B, C). According to the classical pharmacological²¹ and operational models,²² if one supposes that the agonist (H) interacts with the receptors (R) following a simple mass action kinetic:

$$RH = RT \frac{H}{Kd + H} \quad (\text{Equation 1})$$

and that a chain of hyperbolic coupling steps (including the interaction of RH with G proteins),



occurs between the formation of RH and the final response. Then, the final response is expected to be a hyperbolic function of the ligand concentration, as generally observed. Therefore the final response (E) (cAMP production) is also a hyperbolic function of RH:

$$E = ET \frac{RH}{C_t + RH} \quad (\text{Equation 3})$$

It is possible to determine the coupling constant (C_t) for 5-HT and renzapride as well as maximal cAMP which can be produced (ET) when RH is very high compared to C_t . Plotting E as a function of E/RH (Fig. 4) gives a straight line, the slope of which is equal to C_t . ET is equal to E when E/RH = 0. In COS-7 cells, the coupling constant for 5-HT was twice as high as the coupling constant for renzapride (135 +/- 9 and 70 +/- 3 fmol/mg, respectively). As indicated in Fig. 4A, ET values were identical for 5-HT and renzapride. This implied that at high receptor density, renzapride was a full agonist. Interestingly in LLC-PK1, a similar analysis also gave a 2-fold difference between C_t values for 5-HT and renzapride (46 +/- 4 and 26 +/- 2 fmol/mg, respectively) and again similar values of ET for the two drugs.

DISCUSSION

We have isolated a cDNA encoding 5-HT_{4S} receptor by RT-PCR on poly (A)⁺ RNA from both human heart and brain using specific primers from the rat 5-HT_{4S} cDNA sequence.^{7,7} The nucleotide sequences of the clones were identical, indicating that the same 5-HT₄ receptor is expressed in both heart and brain. The human 5-HT₄ receptor that we cloned was also highly homologous to those previously cloned from rat and mouse brain.^{7,8} Although not formal proof, this suggests that only one gene coding for 5-HT₄ receptors exists in different species.

None of the 24 amino acid substitutions observed between rat and human 5-HT₄ receptors are at a position likely to modify the pharmacology or the transduction properties of

the receptor. However, a single amino acid substitution may drastically modify the binding properties or the coupling properties of G protein-coupled receptors. The now classical example of the rodent 5-HT_{1B} and human 5-HT_{1DB} receptors which differ by only 32 amino acids, but which recognized β -adrenergic antagonists with high and low affinity, respectively, should encourage us not to make rapid conclusions. In this the latter an asparagine in the TMD-VII of rodent which is necessary for high affinity for β_2 -adrenergic antagonists is substituted by threonine in the human 5-HT_{1DB}.²³ Human as well as rat and mouse 5-HT₄ contained no potential PKA phosphorylation site, consistent with the absence of cAMP-mediated desensitization of 5-HT₄ receptors. In colliculi neurons and rat oesophagus, 5-HT₄ receptors are rapidly and potently desensitized by homologous desensitization.²⁴ In contrast, in human heart, 5-HT₄-mediated responses do not desensitize in a notable manner.²⁵ Comparison of the primary sequences of 5-HT_{4S} receptors expressed in colliculi neurons and human heart reveal no differences in the putative phosphorylated sites for GRK. It has been proposed that GRK are acidotropic kinases preferring acidic amino acids in proximity of putative phosphorylation sites. The β_2 -adrenergic receptor rhodopsin, an N-formyl peptide receptor, as well as the α_2 -adrenergic receptor have a pair of acidic residues, at the N-terminal side of the most amino-terminal phosphorylated residue, such as ED, DE or EE.¹⁹ In rat, mouse and human 5-HT_{4S} receptors the acidic doublet DE is present at position 331 and 332 followed by 11 serine or threonine residues, which are susceptible to phosphorylation by a family of GRK proteins.²⁰ In the long form of mouse and rat 5-HT₄ receptors (5-HT_{4L}), another acidic doublet (ED) is present at positions 396 and 397 followed by three and four serine residues respectively, including the C-terminal residue. This could explain preliminary results indicating that 5-HT_{4L} is more susceptible to desensitization than is 5-HT_{4S}.²⁶ Both the long and the short forms of 5-HT₄ receptors are present in neurons,⁸ whereas only the short form seems to be present in human heart [7](#) (we were unable to amplify the long form by RT-PCR using poly (A)⁺ RNA prepared from human atrium). This may explain why homologous desensitization is more efficient in neurons. We have studied the question of the partial agonism of benzamides on different preparations expressing 5-HT₄ receptors.³ When transfected into LLC-PK1 cells and COS-7 cells the coupling constant (C_i) showed that in both cell lines the coupling capacity of the renzapride/5-HT₄ receptor complex was two-fold lower than that of the 5-HT/5-HT₄ receptor complex. However, in both cell lines it is clear that at high receptor density renzapride and 5-HT produce the same maximal cAMP accumulation (ET). It is thus likely that the partial agonism of renzapride in some tissues, and especially in heart, is due to a low receptor

expression level. We measured the level of 5-HT₄ receptors in human atrium as 8 +/- 2 fmol/mg protein.

The fact that a partial agonist behaves as a full agonist when receptor density is increased is consistent with the classical models of pharmacology but has not always been found. We previously reported that when 5-HT_{1A} receptors were transfected into NIH 3T3 cells, a partial agonist remained a partial agonist whatever the receptor density.²⁷ Further studies are required to explain the nature of this difference.

CONCLUSION

We have cloned the human 5-HT_{4S} receptor from brain and heart. The same nucleotidic sequences were found in both tissues. None of the 24 residue substitutions observed between rat and human 5-HT_{4S} receptors induced any differences in the pharmacological profile, established by testing the characteristics of classical 5-HT₄ agonists and antagonists. The present study clearly shows that the efficacy of renzapride relative to that of 5-HT was dependent on the receptor density and not on receptor structure. The coupling efficiency of renzapride was two-fold lower than that of 5-HT in two cell lines, but increasing the receptor density suppressed the partial agonist effect of renzapride.

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Compound	Binding		Adenylyl cyclase stimulation		
	h5-HT _{4S}	r5-HT _{4S}	h5-HT _{4S}	r5-HT _{4S}	
	n°	pK _d	pEC ₅₀	pEC ₅₀	
Agonists					
5-HT	1	7.25 ± 0.12	7.41 ± 0.14	8.05 ± 0.28	8.58 ± 0.15
Bimu 8	2	7.34 ± 0.16	7.72 ± 0.18	7.88 ± 0.15	7.63 ± 0.22
Renzapride	3	6.76 ± 0.14	6.95 ± 0.20	7.55 ± 0.23	7.35 ± 0.19
Zacopride	4	6.55 ± 0.09	6.73 ± 0.12	6.72 ± 0.17	6.87 ± 0.28
5-MeOt	5	6.81 ± 0.18	6.91 ± 0.10	7.84 ± (0.12)	8.61 ± 0.34
5-CT	6	4.62 ± 0.25	5.04 ± 0.21	5.56 ± 0.23	5.67 ± 0.25
		pK _d	pK _d	pK _i	pK _i
Antagonists					
DAU 6285	7	7.98 ± 0.15	8.27 ± 0.17	7.73 ± 0.20	7.65 ± 0.26
GR 113808	8	10.03 ± 0.22	10.51 ± 0.28	10.56 ± 0.36	10.68 ± 0.19
GR 125487	9	10.66 ± 0.26	11.05 ± 0.33	12.06 ± 0.27	12.04 ± 0.16
SB 207266	10	9.52 ± 0.28	9.32 ± 0.22	10.85 ± 0.35	11.25 ± 0.30
RS 100235	11	12.24 ± 0.24	12.40 ± 0.21	12.20 ± 0.21	12.32 ± 0.12

Table 1

Pharmacological profile of the human 5-HT_{4S} receptor. Comparison with the rat 5-HT_{4S} receptor previously cloned by Gerald and co-workers.⁷

cAMP production was measured as described in Materials and Methods in transiently transfected LLC-PK1 cells expressing human 5-HT_{4S} or rat 5-HT_{4S} receptors. EC₅₀ values were determined graphically and corresponded to the concentrations of agonists required to obtain a half-maximal stimulation of adenylyl cyclase. Data are expressed as means ± s.e.m. in duplicate determinations performed at least in four separate transfected LLC-PK1 cell cultures. For the antagonists, K_i values were calculated from the concentration of the drugs that reversed 50% the stimulation induced by 5-HT (0.1 μM). Data are expressed as means ± s.e.m. of 3–5 separate transfected LLC-PK1 cell cultures performed in duplicate.

Binding data correspond to the affinities of various compounds that compete for [³H]GR 113808 binding in transfected LLC-PK1 cell membrane (15 mg) expressing 120 ± 22 and 145 ± 28 fmol/mg protein of human 5-HT_{4S} or rat 5-HT_{4S} receptors respectively. IC₅₀ values correspond to a displacement of 50% of [³H]GR 113808 binding determined experimentally on membrane harvested from transiently transfected LLC-PK1 cells as described in Materials and Methods and converted to K_d values according to the equation of Cheng and Prusoff. Data are expressed as means ± s.e.m. of at least four separate experiments. The concentration of [³H]GR 113808 used was 0.25 nM. Non-specific binding, determined by addition of 50 μM 5-HT, represented only 10 ± 3% of total binding.

FIGURE LEGENDS

Figure 1

Nucleotide sequence of a human 5-HT_{4S} receptor: comparison with the rat 5-HT_{4S} receptor. The receptor was sequenced on both strands from ON1 (position 1) to the end of ON2 (position 1166). The seven putative transmembrane domains are boxed and numbered (I-VII). Changes in amino acids in human vs rat receptor are shaded and rat amino acids are indicated below. ●, sites of potential N-linked glycosylation. ◆, site of potential palmitoylation. ▲, consensus sites for phosphorylation by protein kinase C, *, putative sites for G-protein coupled receptor kinases (GRK), ★, terminal stop codon.

Figure 2

Pharmacological characteristics of a human 5-HT_{4S} receptor: comparison with the rat 5-HT_{4S} receptor. (A) Characteristics of [³H]GR 113808 binding in membranes (20 μg of protein in a total volume of 250 μl) prepared from transiently transfected LLC-PK1 cells, electroporated with 1 μg human or rat 5-HT_{4S} receptor cDNA/10⁶ cells and incubated with eight different concentrations of [³H]GR 113808 (0.01-1.5 nM) for 30 min at 20°C. The presented typical experiment is representative of three separate experiments. Each assay was performed in triplicate. Inset: Scatchard analysis of saturation experiments of [³H]GR 113808 binding to human and rat 5-HT_{4S} receptors. (B) Correlation between the affinities of 11 5-HT₄-active drugs (six agonists and five antagonists) for [³H]GR 113808 binding sites of cells (LLC-PK1) transfected with the cloned human 5-HT_{4S} receptor or transfected with the cloned rat 5-HT_{4S} receptor. Binding data correspond to competition of [³H]GR 113808 (0.25 nM) binding to membranes (20 μg) of LLC-PK1 cells transiently expressing human 5-HT_{4S} receptor (120 +/- 18 fmol/mg protein) or rat 5-HT_{4S} receptor (105 +/- 21 fmol/mg protein). IC₅₀ values required to displace 50% of [³H]GR 113808 binding were determined experimentally and converted to K_d values according to the Cheng-Prusoff equation:²⁸ $K_d = IC_{50}/(1 + S/K_{dS})$ where S is [³H]GR 113808 concentration (0.25 nM) and K_{dS} is the equilibrium constant of [³H]GR 113808. The x axis shows pK_d values for 11 5-HT₄-active drugs for the cloned human 5-HT_{4S} and the y axis shows the pK_d values for cloned rat 5-HT_{4S} receptors. All values are taken from Table 1. The numbers in Fig. 2B correspond to the numbers in Table 1.

Figure 3

Effect of the receptor densities on the dose-response curves for 5-HT and renzapride on endogenous 5-HT₄ receptors in human heart and on human 5-HT_{4S} receptor transiently expressed in COS-7 cells. (A) Effect of 5-HT and renzapride on increasing Ca²⁺ current (ICa) in human cardiomyocytes (data from Ref.¹⁰). (B) Effect of human 5-HT_{4S} receptor density on the dose-response curves of 5-HT in COS-7 cells. The densities of 5-HT₄ receptors were 10, 30, 36, 123, 258 and 311 fmol/mg protein in COS-cells transfected with 25, 50, 100, 500 and 1000 ng of cDNA, respectively. (C) Effect of human 5-HT_{4S} receptor density on the dose-response curves of renzapride in COS-7 cells. The expression levels of 5-HT₄ receptors were the same as in (B). cAMP productions measured at different concentrations of drugs are expressed as the percentage over basal cAMP produced in the mock-transfected cells (control). The percentage conversion of [³H]-ATP to [³H]-cAMP in control was 0.158 +/- 0.04. In (B) and (C) results are mean +/- standard error of triplicate determinations from a typical experiment.

Figure 4

Determination of the coupling constant (C_t) of 5-HT and renzapride and their maximal efficacy (E_t). (A) cAMP production (E) for maximal concentrations of 5-HT and renzapride ($> 1 \mu\text{M}$) was measured at different concentrations of human 5-HT_{4S} receptor (RH) expressed in COS-7 cells. E was plotted as a function of (E/RH). For COS-7 cells, data are taken from Fig. 3. (B) cAMP production for maximal concentrations of 5-HT and renzapride ($> 1 \mu\text{M}$) was measured at different concentrations of human 5-HT_{4S} receptor (HR) expressed in LLC-PK1 cells. The expression level of human 5-HT_{4S} receptors was 11, 18, 25, 55, 39, 56 and 77 fmol/mg protein in LLC-PK1 cells transfected with 50, 100, 200, 400, 500, 800 and 1000 ng of human 5-HT_{4S} receptor cDNA, respectively. The presented typical experiment is representative of three separate experiments. E plotted as a function of E/RH gives a straight line, the slope of which is C_t . E_t is equal to E when $E/\text{RH} = 0$. The coupling constant for 5-HT in COS-7 cells was 2-fold higher than that for renzapride (135 +/- 9 and 70 +/- 3 fmol/mg, respectively). As indicated in Fig. 4, E_t was identical for 5-HT and renzapride. This means that at high receptor density, renzapride is a full agonist. Interestingly, in LLC-PK1 cells, a similar analysis gave also a 2-fold difference between the values of C_t for 5-HT and renzapride (46 +/- 4 and 26 +/- 2 fmol/mg protein, respectively) and again a similar E_t for the two drugs.

1 ta ATG GAC AAA CTT GAT GCT AAT GTG AGT TCT GAG GAG GGT TTC GGG TCA GTG GAG AAG 59
 1 M D R L D A N V S S N E G F G S V E K 19

60 GTG GTG CTG CTC ACG TTT CTC TCG ACG GTT ATC CTG ATG GCC ATC TTG GGG AAC CTG CTG 119
 20 V V L L T F F S M V I L M A I L G N L L 39

120 GTG ATG GTG GCT GTG TGC TGG GAC AGG CAG CTC AGG AAA ATA AAA ACA AAT TAT TTC ATT 179
 40 V M V A V C W D R Q L R K I K T N Y F I 59

180 GTA TCT CTT GCT TTT GCG GAT CTG CTG GTT TCG GTG CTG GTG ATG CCC TTT GGT GCC ATT 239
 60 V S L A F A D L L V S V L V M P F G A I 79

240 GAG CTG GTT CAA GAC ATC TGG ATT TAT GGG GAG GTG TTT TGT CTT GTT CGG ACA TCT CTG 299
 80 E L V Q D I W F Y G E M F C L V R T S L 99

300 GAC GTC CTG CTC ACA ACG GCA TCG AAT TTT CAC CTG TGC TGC AAT TCT CTG GAT AGG TAT 359
 100 D V L L T T A S I F H L C C I S L D R Y 119

360 TAC GCC ATC TGC TGC CAG CCT TTG GTC TAT AGG AAC AAG ATG ACC CCT CTG CGC ATC GCA 419
 120 Y A I C C Q P L V R N K M T P L R I A 139

420 TTA ATG CTG GGA GGC TGC TGG GTC ATC CCC ACG TTT ATT TCT TTT CTC CCT ATA ATG CAA 479
 140 L M L G G C W V I P F I S F L P I M Q 159

480 GGC TGG AAT AAC ATT GGC ATA ATT GAT TTG ATA GAA AAG AGG AAG TTC AAC CAG AAC TCT 539
 160 G W N N I G I V D V I E K R K F N Q N S 179

540 AAC TCT ACG TAC TGT GTC TTC ATG GTC AAC AAG CCC TAC GCC ATC ACC TGC TCT GTG GTG 599
 180 N S T F C V F M V N K P Y A I T C S V V 199

600 GCC TTC TAC ATC CCA TTT CTC CTC ATG GTG CTG GCC TAT TAC CGC ATC TAT GTC ACA GCT 659
 200 A F Y I P F L L M V L A Y Y R I Y V T A 219

660 AAG GAG CAT GCC CAT CAG ATC CAG ATG TTA CAA CGG GCA GGA GCC TCC TCC GAG AGC AGG 719
 220 K E H A Q Q I Q M L Q R A G A S S E S R 239

720 CCT CAG TCG GCA GAC CAG CAT AGC ACT CAT CGC ATG AGG ACA GAG ACC AAA GCA GCC AAG 779
 240 P Q S A D Q H S T H R M R T E T K A A K 259

780 ACC CTG TGC ATC ATC ATG GGT TGC TTC TGC CTC TGC TGG GCA CCA TTC TTT GTC ACC AAT 839
 260 T L C I M G C F C C W A P F F V T N 279

840 AAT GTG GAT CCA TTC ATA GAC TAC ACT GTC CCT GGG CAG GTG TGG ACT GCT TTC CTC TGG 899
 280 I V D P F I D Y T V P E Q V W T A F L W 299

900 CTC GGC TAT ATC AAT TCC GGG TTG AAC CCT TTT CTC TAC GCC TTC TTG AAT AAG TCT TTT 959
 300 L G Y I N S G L N P F L Y A F L N K S F 319

960 AGA CGT GCC TTC CTC ATC ATC CTC TGC TGT GAT GAT GAG CGC TAC CGA AGA CCT TCC ATT 1019
 320 R R A F L I I L C C D D E R Y R P S I 339

1020 CTG GGC CAG ACT GTC CCT TGT TCA ACC ACA ACC APT AAT GGA TCC ACA CAT GTA CTA AGG 1079
 340 L G Q T V P C S T T T I N G S T H V L R 359

1080 TAC ACC GTT CTG CAC AGG GGA CAT CAT CAG GAA CTC GAG AAA CTG CCC ATA CAC AAT GAC 1139
 360 Y T V L H S G H Q E L E K L P I H N D 379

1140 CCA GAA TCC CTG GAA TCA TGC TTC TGA 1166
 380 P E S L E S C F ★ 388

* * * * *

Figure 1

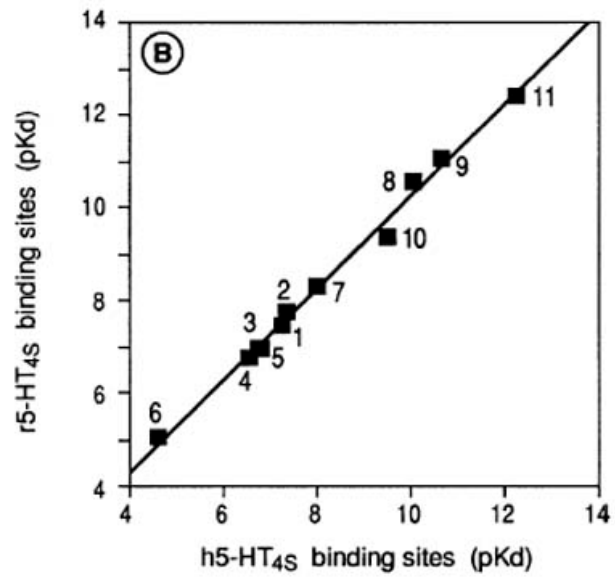
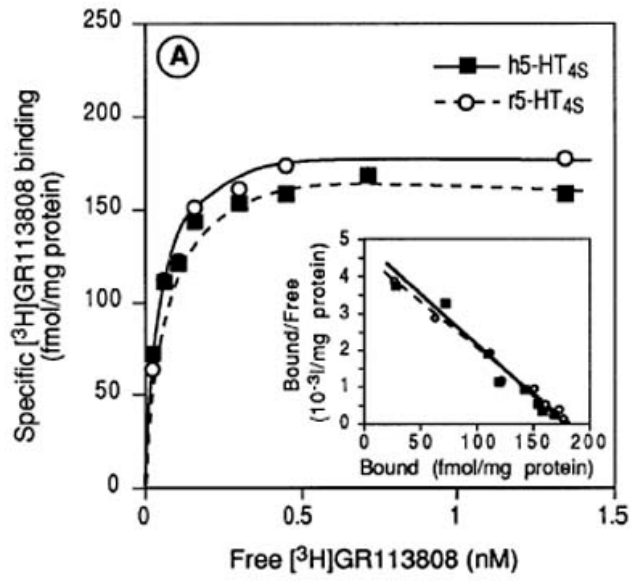


Figure 2

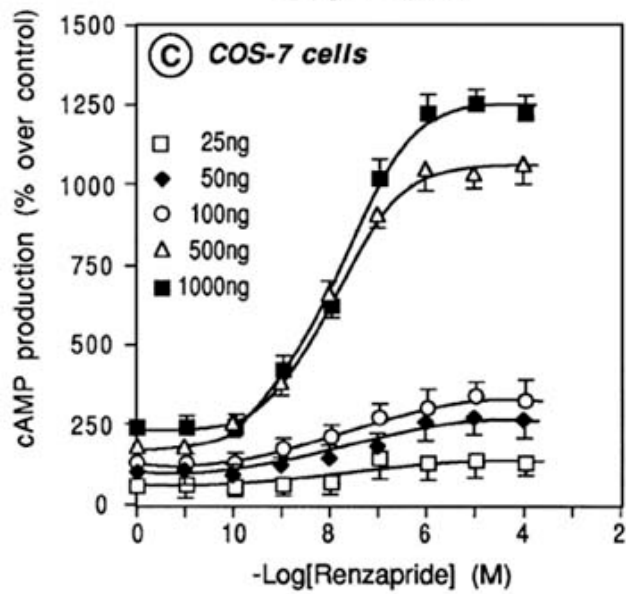
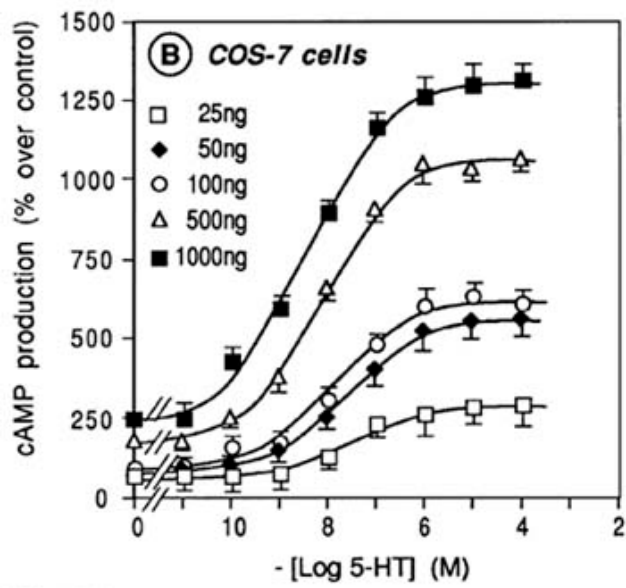
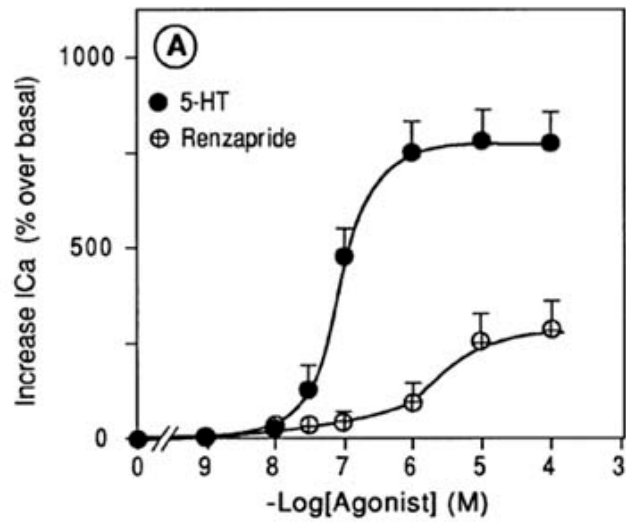


Figure 3

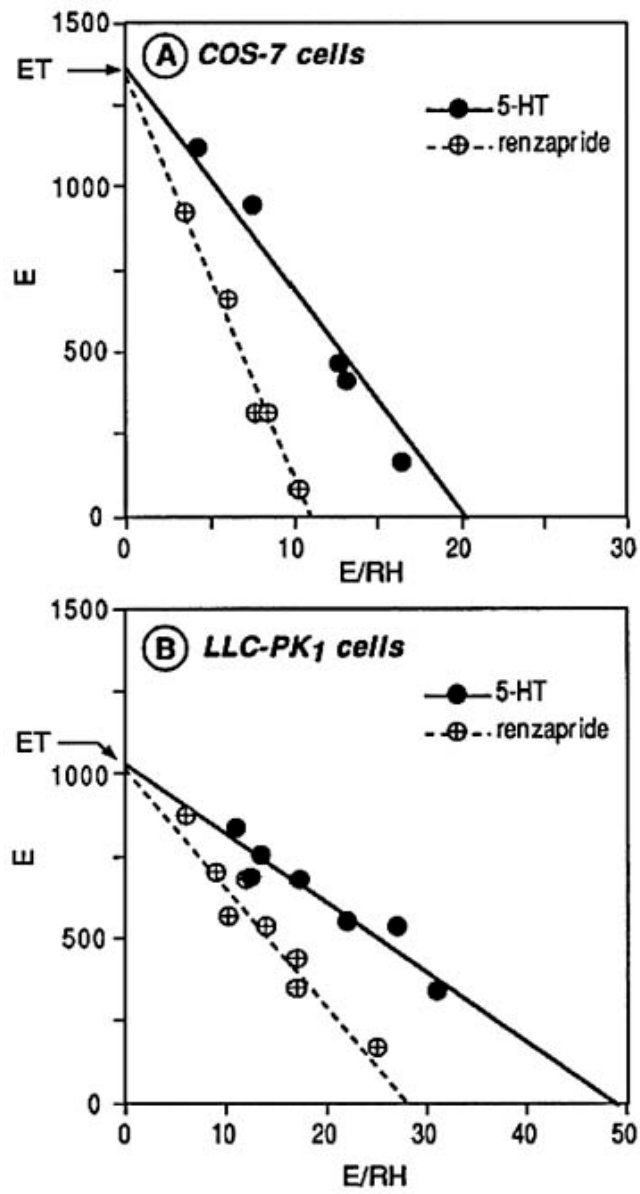


Figure 4