0026-895X/93/030412-07$03.00/0

MOLECULAR PHARMACOLOGY, 43:412–418

Human S31 Serotonin Receptor Clone Encodes a 5-Hydroxytryptamine$_{1E}$-like Serotonin Receptor

THOMAS GUDERMANN, FINN OLAV LEVY, MARIEL BIRNBAUMER, LUTZ BIRNBAUMER, and ALBERTO J. KAUMANN[1]

Department of Cell Biology (T.G., F.O.L., M.B., L.B., A.J.K.), Department of Molecular Physiology and Biophysics (L.B.), and the Division of Neuroscience, Baylor College of Medicine (L.B.), Houston, Texas 77030

Received June 29, 1992; Accepted November 20, 1992

SUMMARY

We reported recently the molecular cloning of a genomic fragment, designated S31, that has an open reading frame of 1095 nucleotides, encoding a protein of 365 amino acids. Amino acid similarity analysis suggested that the S31 protein could be a guanine nucleotide-binding protein-coupled receptor pertaining to the serotonin receptor subfamily. Expression of the S31 open reading frame in murine L cells confirmed this, because it led to the appearance of serotonin-mediated inhibition of adenylyl cyclase activity, which was absent in the recipient L cells. We now report some aspects of the pharmacological profile of this receptor. We found that the relative potencies with which 5-hydroxytryptamine, 5-carboxamidotryptamine, methysergide, ergotamine, 8-hydroxydipropylaminotetralin, and trifluoromethylphenylpiperazine promote inhibition of adenylyl cyclase are as follows: 5-hydroxytryptamine ≫ methysergide ≫ ergotamine ≫ trifluoromethylphenylpiperazine ≥ 8-hydroxydipropylaminotetralin > 5-carboxyamidotryptamine. This corresponds to the rank order of potencies assigned for these drugs for the 1E subtype of serotonin receptors discovered by Leonhardt and collaborators in human brain [*J. Neurochem.* **53**:465–471 (1989)].

Serotonin has a panoply of actions ranging from smooth muscle contraction to smooth muscle relaxation, from positive regulation of neuronal activity to inhibitory regulation of neuronal activity, and from regulation of nonexcitable cells such as vascular endothelial cells to regulation of excitable cells other than neurons and muscle cells, such as endocrine cells. Serotonin is found in the central nervous system, in peripheral nerve terminals including terminals of the myenteric plexus and of several organ vasculatures, in enteric chromaffin cells, and in platelets. It is thus delivered to its sites of action from local storage places, such as nerve terminals, as well as from cells arriving through the bloodstream. An elucidation of its role in normal physiology and in pathophysiological states requires as a first step the elucidation of its mechanism of action, and this in turn requires the characterization of all of its receptors, both in terms of molecular diversity and in terms of the cells in which they are expressed.

A screen of a human genomic library for sequences homologous to either 5-HT$_{1A}$ or 5-HT$_{1C}$ yielded several clones, of which clones S1, S4, S9, S12, and S8 encoded G protein-coupled receptors. S1, S4, S9, and S12 were identified as, respectively, the genes encoding the α_{2B}-adrenergic receptor, the 5-HT$_{1A}$ receptor, a 5-HT$_{1D}$-like receptor that is the structural homologue of the rodent 5-HT$_{1B}$ receptor, and a portion of the 5-HT$_2$ receptor (1). The 5-HT$_{1D}$-like receptor was cloned independently by Jin *et al.* (2) and Weinshank *et al.* (3) and has been designated 5-HT$_{1D\beta}$ (4). S8 proved to encode an incomplete open reading frame that coded for what also appeared to be a G protein-coupled receptor. S8 was used as a probe to clone another human genomic fragment, designated S31, that had a complete open reading frame of 1095 nucleotides, encoding a protein of 365 amino acids. Computer analysis of the protein predicted from the deduced amino acid sequence predicted a protein, with the seven-transmembrane domain structure typical of G protein-coupled receptors, that is evolutionarily related to the serotonin receptor subfamily. The gene was expressed in L cells and shown to confer to these cells serotonin-mediated inhibition of adenylyl cyclase. No stimulatory or inhibitory effect on adenylyl cyclase of cells expressing S31 was seen with isoproterenol, epinephrine, dopamine, melatonin, or histamine (1).

This work was supported in part by grants from the National Institutes of Health (RO1-HD-45198, R01-HD-09581, and R37-DK-19318 to L.B., R01-DK-41244 to M.B., and P30-HD-07485 and P30-DK-27685 to Baylor College of Medicine) and fellowships to T.G. from the German Research Council (Deutsche Forschungsgemeinschaft) and to F.O.L. from the Fogarty International Center, The Unger-Vetlesen Medical Fund (Jersey, Channel Islands), the Norwegian Research Council for Science and the Humanities, and the American-Scandinavian Foundation.

[1] Permanent address: Clinical Pharmacology Unit, University of Cambridge Clinical School, Addenbrook's Hospital, Cambridge CB2 2QQ, UK, and SmithKline Beecham Pharmaceuticals, The Frythe, Welwyn, Herts AL6 9AR, UK.

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; 5-CT, 5-carboxyamidotryptamine; 5-MeOT, 5-methoxytryptamine: 8-OH-DPAT, (±)-8-hydroxydipropylaminotetralin; EC$_{50}$, concentration giving a half-maximal effect; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; TFMPP, trifluoromethylphenylpiperazine; G protein, guanine nucleotide-binding protein.

In addition to S12 (1) and S31 (5), two other cloned human serotonin receptors are known to inhibit adenylyl cyclase, i.e., the 5-HT$_{1A}$ or G21 receptor (6) and another 5-HT$_{1D}$-like receptor, M6A6 (7), designated 5-HT$_{1D\alpha}$ by Hartig *et al.* (4). When the potencies and intrinsic activities of several serotonin receptor ligands to activate S31 were compared with their effects on S12, G21, and M6A6, it became clear that the pharmacological profile of the S31 receptor is quite distinct. The profile for agonist ranking order for adenylyl cyclase inhibition strikingly resembles the binding profile described in human frontal cortex by Titeler and co-workers (8) and designated by them as the 5-HT$_{1E}$ receptor. While this manuscript was in revision McAllister *et al.* (9) reported the molecular cloning of a novel 5-HT receptor cDNA, termed AC1, which is the same as S31. Based on a study of its binding properties they concluded that this receptor is a 5-HT$_{1E}$ receptor.

Materials and Methods

All of the methods and materials used in the present work were described in our previous reports on the cloning and expression of the 5-HT$_{1D}$-like S12 serotonin receptor (1) and the cloning of the S31 serotonin receptor and its initial characterization as a receptor able to mediate inhibition of L cell adenylyl cyclase (5). For the present studies on additional pharmacological properties of the S31 receptor as an inhibitor of adenylyl cyclase, we used homogenates of the clonal cell line LS31 27.9/19, which is a secondary subclone isolated by limiting dilution from the LS31/27.9 cell line reported by Levy *et al.* (5). In addition, the S31 receptor was also transfected into LM5.36 cells that express the M5 muscarinic receptor (10), to give LM5S31/2.3 cells. Like adenylyl cyclase of LS31 cells, that of LM5S31 cells is inhibited by serotonin (data not shown). LM5S31 cells as well as LS31 cells were used to test for a potential effect of the S31 receptor to modulate basal and agonist (carbachol or ATP)-stimulated release of Ca^{2+} from intracellular stores, as assessed in fura-2-loaded cells.

Growth of cells and homogenization conditions. Approximately 1.5×10^6 cells were seeded into 150-mm plates in 10 ml of α-modified minimal essential medium supplemented with 0.4 mg/ml G418. Cells were grown for 48–60 hr, rinsed with an ice-cold balanced salt solution (126 mM NaCl, 0.64 mM KCl, 98 μM $MgCl_2$, 5 μM $CaCl_2$, 14.5 mM Tris·HCl, pH 7.5) supplemented with 0.01% glucose, scraped off with the aid of a rubber policeman in the presence of balanced salt solution/0.01% glucose, and collected by centrifugation. The cell pellet was homogenized in 1.5 ml of ice-cold 27% (w/w) sucrose, 1 mM EDTA, 20 mM Na-HEPES, pH 7.8, using a 7-ml Dounce homogenizer (eight strokes with the tight pestle), and the resulting homogenate was kept on ice for 15–30 min before assay.

Adenylyl cyclase activities were measured in 10-μl aliquots of the homogenates, in a final volume of 50 μl, in the presence of 0.1 mM [α-^{32}P]ATP ($10–15 \times 10^6$ cpm/assay), 1.5 mM $MgCl_2$, 20 μM GTP, 100 μM forskolin, 0.5% ethanol, 0.22% bovine serum albumin, 1 mM EDTA, 1 mM [^{3}H]cAMP (~10,000 cpm/assay), a nucleoside triphosphate-regenerating system (11), and drugs at the final concentrations indicated in Results and figure legends. Incubations were for 20 min at 32°. The reactions were stopped by addition of 100 μl of 40 mM ATP, 10 mM cAMP, 0.1% sodium dodecyl sulfate, and the [^{32}P]cAMP formed was isolated by the double-column chromatography method of Salomon *et al.* (12), as modified by Bockaert *et al.* (13).

Assessment of changes in intracellular Ca^{2+} by the fura-2 method was as described previously (14).

Reproducibility of results. All experiments were performed on fresh homogenates (or freshly grown cells) and repeated at least two times each. Results presented in the figures are data from individual experiments. Additional details are given in Results and the legends to the figures.

Results

S31 receptor-mediated inhibition of adenylyl cyclase was measured in the presence of 100 μM forskolin, 20 μM GTP, and 1 mM EDTA, using 0.1 mM ATP as substrate. Tests for S31 receptor activity at varying concentrations of Mg^{2+} showed that agonist-induced inhibition of adenylyl cyclase activity increased monotonically in inverse proportion to free Mg^{2+}. Inhibition was essentially absent at concentrations of Mg^{2+} above 2 mM, 15–20% at 0.8–0.9 mM Mg^{2+} (total $MgCl_2$ added, 1.9–2.0 mM), 40–45% at 0.4 mM Mg^{2+} (total $MgCl_2$ added, 1.5 mM), and approximately 60% at 0.2 mM Mg^{2+} (total $MgCl_2$ added, 1.3 mM). However, whereas the effect of the receptor increased with decreasing Mg^{2+}, absolute adenylyl cyclase activity decreased linearly with free Mg^{2+}, making it difficult to measure at 0.2 Mg^{2+}. For the experiments reported here we compromised between increasing the efficacy of the receptor and decreasing the measured activity by assaying at 0.4 mM Mg^{2+}. Under these conditions inhibition of adenylyl cyclase by 5-HT still required the addition of forskolin, because in the absence of forskolin 5-HT failed to inhibit adenylyl cyclase activity. Standard assays were, therefore, as described in Materials and Methods and summarized above, in the presence of 1.5 mM total added $MgCl_2$. With several of the drugs, especially 5-MeOT and 5-CT, we noted nonspecific inhibition of adenylyl cyclase activity (tested at 5 mM $MgCl_2$ at concentrations above 10^{-5} M). For this reason, tests for effects of serotonin receptor ligands spanned the concentration range between 10^{-10} and 10^{-5} M. Fig. 1 illustrates typical adenylyl cyclase-inhibitory responses (or lack thereof) obtained upon exposure of S31 receptor-expressing L cells to known serotonin receptor ligands. The inhibitory effect of 5-HT was half-maximal at approximately 50 nM; full agonistic activity was obtained at 10^{-6} M. In addition to 5-HT, the following drugs acted as full agonists: ergotamine, dihydroergotamine, oxymetazoline, methysergide (Fig. 1A), and mianserine (Fig. 1B). Based on the parallelism between the dose-response curve for 5-HT and the segments of those obtained for the actions of TFMPP and 8-OH-DPAT, these compounds also appeared to be full agonists, albeit with an approximately 100–200-fold lower potency than 5-HT (Fig. 1C). In contrast, 5-MeOT, sumatriptan, and RU 24969 (Fig. 1B) appeared to act as partial agonists. We estimated the intrinsic activity of sumatriptan and RU 24969 to be about 60–70% of that of 5-HT. The intrinsic affinity of 5-MeOT appeared to be somewhat less and for the purpose of assessing an approximate potency we assumed it to be about 50% of that of 5-HT. Ketanserin had no effect on this receptor. Table 1 summarizes means of potency estimates obtained from the experiments of the type depicted in Fig. 1.

Fig. 2 presents the results of an experiment in which the blocking potential of methiothepin was tested. It can be seen that, in contrast to all other drugs, methiothepin added at concentrations above 3–5 μM had a nonspecific stimulatory effect on GTP- plus forskolin-stimulated adenylyl cyclase activity of the L cells in which the S31 receptor was being expressed. This caused an increase in the base-line activity for 5-HT dose-response curves. Under these circumstances, increasing concentrations of methiothepin had the effect of displacing the dose-response curves for 5-HT to the right, consistent with competitive blockade. Schild plots, calculated on the basis of ratios of EC_{50} values for 5-HT, gave x-axis intercepts that varied between 1.4 and 2.5 μM (mean $pK_B = 5.7 \pm$

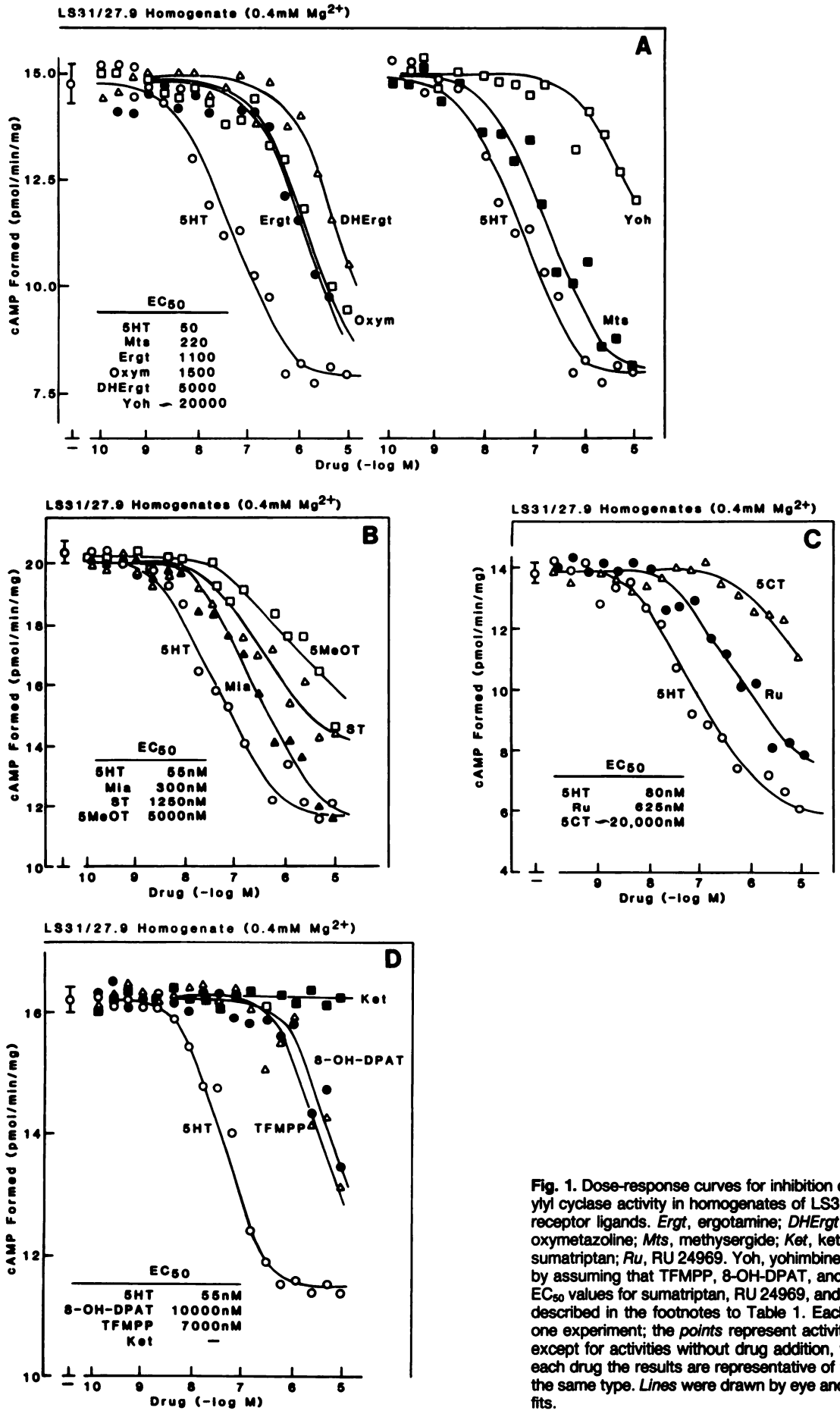

Fig. 1. Dose-response curves for inhibition of forskolin-stimulated adenylyl cyclase activity in homogenates of LS31/27.9 cells by serotonergic receptor ligands. *Ergt*, ergotamine; *DHErgt*, dihydroergotamine; *Oxym*, oxymetazoline; *Mts*, methysergide; *Ket*, ketanserin; *Mia*, mianserin; *ST*, sumatriptan; *Ru*, RU 24969. Yoh, yohimbine. EC_{50} estimates were made by assuming that TFMPP, 8-OH-DPAT, and mianserin are full agonists. EC_{50} values for sumatriptan, RU 24969, and 5-MeOT were calculated as described in the footnotes to Table 1. Each panel shows the result of one experiment; the *points* represent activities from single incubations, except for activities without drug addition, which were in triplicate. For each drug the results are representative of two or three experiments of the same type. *Lines* were drawn by eye and do not represent computer fits.

TABLE 1

Potency estimates of S31 receptor ligands as seen in adenylyl cyclase assays and comparison with affinities reported for the interaction of the same ligands with the AC1 receptor and the human frontal cortex 5-HT$_{1E}$ receptor

Values are the mean ± ½ the range for $n = 2$ and mean ± SD for $n > 3$. All compounds are full agonists except those noted as PA, which appear to be partial agonists. EC_{50} values for presumed partial agonists (as well as the maximal agonist a_∞) were derived using the curve-fitting routines of Sigmaplot 4.1 and fitting the data with a noncooperative inhibition curve of the form $\Delta_x/\Delta_{max} = (a_o - a_x)/(a_o - a_\infty) = 1/(1 + EC_{50}/X)$, where a is activity at the concentration of the drug denoted by the subscript and x is the concentration of the drug.

A. Agonists	Human S31 receptor in L cells			Human AC1 receptor in 293 cells, pK_D[b]	Human frontal cortex 5-HT$_{1E}$ receptor	
	EC_{50}	n[a]	pEC_{50}		pK_D[c]	pK_D[b]
	nM					
5-HT	58 ± 12	5	7.29	8.21	8.45	8.21
5-CT	≥20,000[d]	3	~4.70	5.48	6.04	5.67
Sumatriptan	1,475 ± 225	2	5.83 (PA)[e]	5.68		5.89
Methysergide	306 ± 90	3	6.51	6.66	7.23	6.76
Mianserine	285 ± 100	3	6.55			
5-MeOT	5,100 ± 2,300	2	5.32 (PA)[e]			
Ergotamine	1,425 ± 325	2	5.85	6.27	6.81	6.10
Dihydroergotamine	5,350 ± 350	2	5.27			
Oxymtazoline	1,375 ± 125	2	5.86			
RU 24969	840 ± 130	3	6.08 (PA)[e]			
Yohimbine	>20,000[d]	2	~4.70			
TFMPP	7,850 ± 850	2	5.10		6.24	
8-OH-DPAT	10,000	2	5.00		6.08	

B. Blocker	Human S31 receptor in L cells			AC1 receptor in 293 cells, pK_D[b]	5-HT$_{1E}$ receptor in cortex, pK_D[b]
	IC_{50}	n	pK_B		
	nM				
Methiothepin	2,280 ± 790	3	5.70	6.92	5.81

[a] n, number of experiments.
[b] Data from McAllister *et al.* (9).
[c] Data from Leonhardt *et al.* (8).
[d] Estimates are based on EC_{20} values from partial dose-response curves that appeared parallel to those of full agonists and that were assumed to reach completion according to a simple saturation isotherm.
[e] Estimates are based on partial dose-response curves that were not parallel of those of full agonists and that were assumed to reach completion according to a simple saturation isotherm.

0.1, three experiments). This is in sharp contrast to the high potency with which methiothepin blocked effects of serotonin at the S12 (1) and M6A6 (7) receptors.

For purposes of comparison, Fig. 3 presents the effects of serotonin receptor ligands not previously studied by us on adenylyl cyclase activity in cells expressing the S12 (human 5-HT$_{1D\beta}$) receptor rather than the S31 receptor. These data, together with those reported previously showing high potency for the actions of sumatriptan, 5-CT, and methiothepin (1) provide a clear pharmacological differentiation between the S31 (5-HT$_{1E}$) and S12 (^{3}HT$_{1D\beta}$) clones.

LM5S31 cells, in which expression of S31 causes serotonin to inhibit forskolin-stimulated adenylyl cyclase activity as it does in LS31 cells, were used to explore whether S31 would affect intracellular Ca^{2+} levels by itself or affect intracellular Ca^{2+} previously raised by carbachol through the M5 receptor or by ATP through the purinergic receptor resident in these cells. Serotonin did not affect intracellular Ca^{2+} in cells that express S31 (not shown).

Discussion

In previous articles we reported the molecular cloning of two new members of the serotonin receptor family, S12 and S31, both of which inhibit adenylyl cyclase. Pharmacologically the S12 and S31 receptors proved to belong to the 5-HT$_1$ class. One, S12, is a human receptor with the pharmacological profile ascribed by Hartig *et al.* (4) to the 5-HT$_{1D\beta}$ subtype but a structure orthologous to that of the receptor referred to in the rat as 5-HT$_{1B}$ (2), which has pharmacological properties that are quite distinct from those of 5-HT$_{1D}$ receptors. The other receptor reported on here has a phamacological profile that resembles quite closely that described by Leonhardt *et al.* (8) for a serotonin receptor they designated as 5-HT$_{1E}$. This conclusion is based on comparison of potency estimates obtained by us for the action of receptor ligands on adenylyl cyclase and affinities of sites labeled by [^{3}H]5-HT in human cerebral cortex (8) (Table 1). The low affinity for 5-CT, TFMPP, and 8-OH-DPAT distinguishes the receptor encoded by S31 from the 5-HT$_{1D\alpha}$ (M6A6) (7), 5-HT$_{1D\beta}$ (S12) (1, 2), and 5-HT$_{1A}$ (G21) (6) receptors.

This conclusion, which is based on an analysis of receptor function, is in complete agreement with that of McAllister *et al.* (9), who also concluded that this receptor, designated by them AC1 and analyzed by competitive displacement studies, is a 5-HT$_{1E}$ receptor (Table 1). The two studies complement each other, in that, whereas one evaluated the interaction with the receptor through direct binding and provided K_d values (9), we evaluated the drug-receptor interaction through the consequence of receptor activation (Figs. 1 and 2). Of the 15 drugs studied (including 5-HT), all but two had agonistic activities, and none was more potent than 5-HT. One, methiothepin, proved to be a weak competitive inhibitor with the complicating property of having at high concentrations a stimulating effect on forskolin-activated adenylyl cyclase activity that was present in cells without serotonin receptors (Fig. 2). Schild analysis performed using dose ratios calculated on the basis of concentrations of 5-HT giving 50% of maximal effects, rather than equal effects, gave a pK_b value in the adenylyl cyclase assay of

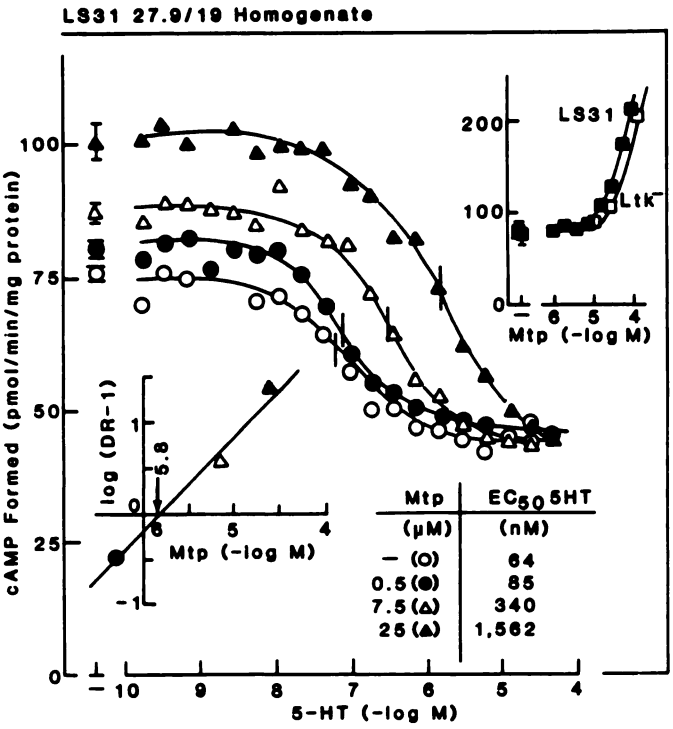

Fig. 2. Low potency of methiothepin to interfere with serotonin-induced activation of the human S31 receptor. Dose-response curves for serotonin were obtained in the absence and presence of 0.5, 7.5, and 25 μM methiothepin (*Mtp*). Lower left *Inset*, Schild transformation using dose ratios calculated with 5-HT concentrations that give 50% of maximal inhibition at each of the concentrations of methiothepin (*vertical bars* and *table*); Upper right *Inset*, results from a separate experiment on the nonspecific action of methiotepin to increase cAMP formation under the assay conditions used (■, forskolin- plus GTP-stimulated activities of LS31 27.9/19 cell homogenates; □, forskolin- plus GTP-stimulated activities of parent Ltk⁻ cell homogenates). LS31 27.9/19 is a clonal cell line derived by limiting dilution from the clonal cell line LS31/27.9, which was used in the remainder of the adenylyl cyclase experiments of this report.

5.7. This agrees with the pK_d of 5.8 obtained by McAllister *et al.* (9) by competitive binding in human frontal cortex membranes with 170 fmol of 5-HT_{1E} receptors/mg of membrane protein but differs from the value of 6.9 those same authors found in cells expressing the cloned receptor at 8400 fmol/mg of membrane protein, using the same procedure. It may be of interest in future studies to assess whether affinities for antagonists may be influenced by membrane factors other than the receptor itself. The other drug that had no agonistic property was ketanserin (Fig. 1B), indicatng either that it does not bind to this receptor or that, if it does, it might be a blocker. Although we did not test for its potential blocking activity, McAllister *et al.* (9) failed to see an inhibitory effect of 10 μM ketanserin on 5-HT binding. This indicates that ketanserin is not a 5-HT_{1E} receptor ligand. In contrast to ketanserin, mianserin, which is also a potent 5-HT_{1C} and 5-HT_2 antagonist, had an agonistic activity at the S31 5-HT_{1E}-like receptor.

Little if anything is known about the physiological role of the 5-HT_{1E} receptor. In addition to characterizing distinctive aspects of its pharmacological profile, Leonhardt *et al.* (8) demonstrated that this receptor is under guanine nucleotide regulation. This was a strong functional indication that this receptor belongs to the family of G protein-coupled receptors. Their studies could not, however, define signaling modes for this receptor, e.g., ion channel regulation, adenylyl cyclase stimulation or inhibition, or phospholipase stimulation, nor did the binding studies define pre- or postsynaptic locations of the receptor sites.

Likewise, neither the physiological role nor the primary signaling mechanism are known for the S31 5-HT_{1E}-like receptor characterized here. Our studies indicate that its signaling characteristics differ from those of the 5-HT_{1A} receptor, which, unlike the 5-HT_{1E}-like S31 receptor, mediates not only inhibition of adenylyl cyclase but also stimulation of phospholipase C and mobilization of intracellular Ca^{2+} (15). Our studies indicate further that in L cell membranes the 5-HT_{1E}-like S31 receptor is a "weak" inhibitor of adenylyl cyclase, compared with S12 (5-$HT_{1D\beta}$). Thus, the free Mg^{2+} concentration in the assays had to be lowered from 0.8–1.0 mM to 0.3–0.4 mM for observation of agonistic effects of 5-HT with S31 similar to those seen with S12. Whether this also applies in the natural environment of the receptor is not known. As occurs with all G protein-coupled receptors, the effects of the 5-HT_{1E}-like S31 receptor depend on both the type of G_i to which it can couple, e.g., abundance of G_{i1} relative to G_{i3}, and the subtype of adenylyl cyclase that is expressed in the cells in which it is acting.

It needs to be pointed out that the signaling potential of the receptor cloned here has by no means been fully explored. For example, the primary function of an 5-HT_{1E}-like receptor, S31 in this case, may not be reduction of cAMP but attenuation of Ca^{2+} currents or stimulation of phospholipase C and/or a K^+ channel. In cells where inhibition of Ca^{2+} currents has been studied, this type of effect is mediated by G_o and not G_i forms, and L cells lack both a G_o-type G protein[2] and a voltage-gated Ca^{2+} channel (16). If for the sake of argument we assume that S31 and the frontal cortex 5-HT_{1E} sites are the same, then the fact that potencies for cyclase inhibition are shifted rightward with respect to the binding affinities of agonists (Table 1, Fig. 4) could be construed as evidence that a signaling function other than inhibition of adenylyl cyclase is the primary *raison d'être* of the S31/5-HT_{1E} receptor. On the other hand, G protein-coupled receptors are known to exist in both a high affinity form and at least one form of lower affinity. Binding studies carried out in the absence of GTP with agonist as the binding probe measure mostly, if not exclusively, the high affinity form of the receptor. Functional studies are carried out in the presence of GTP and it could be that the EC_{50} values of agonists correlate with the K_d values of the low affinity state(s) of the receptor, rather than those of its high affinity state. Although intrinsic activities of β-adrenergic receptor ligands correlate with their high affinity binding properties (17, 18), activation of phospholipase C by the chemoattractant (formyl-methionyl-leucyl-phenylalanine) receptor has been shown to be mediated by both its high and low affinity states (19). Translated to the present situation, it may be that the low rather the high affintiy form of the receptor mediates inhibition of adenylyl cyclase, as has been proposed also for the 5-HT_{1A} receptor [see the report of Frazer *et al.* (20) for futher discussion of this issue].

Definitive studies on the physiological actions of these receptors and their molecular signaling mechanisms will require that the cell types in which the receptors are expressed be determined and that additional model cell systems be developed for mechanistic studies. The availability of nucleotide and deduced amino acid sequences should help in the development of hy-

[2] J. Codina and L. Birnbaumer, unpublished observations.

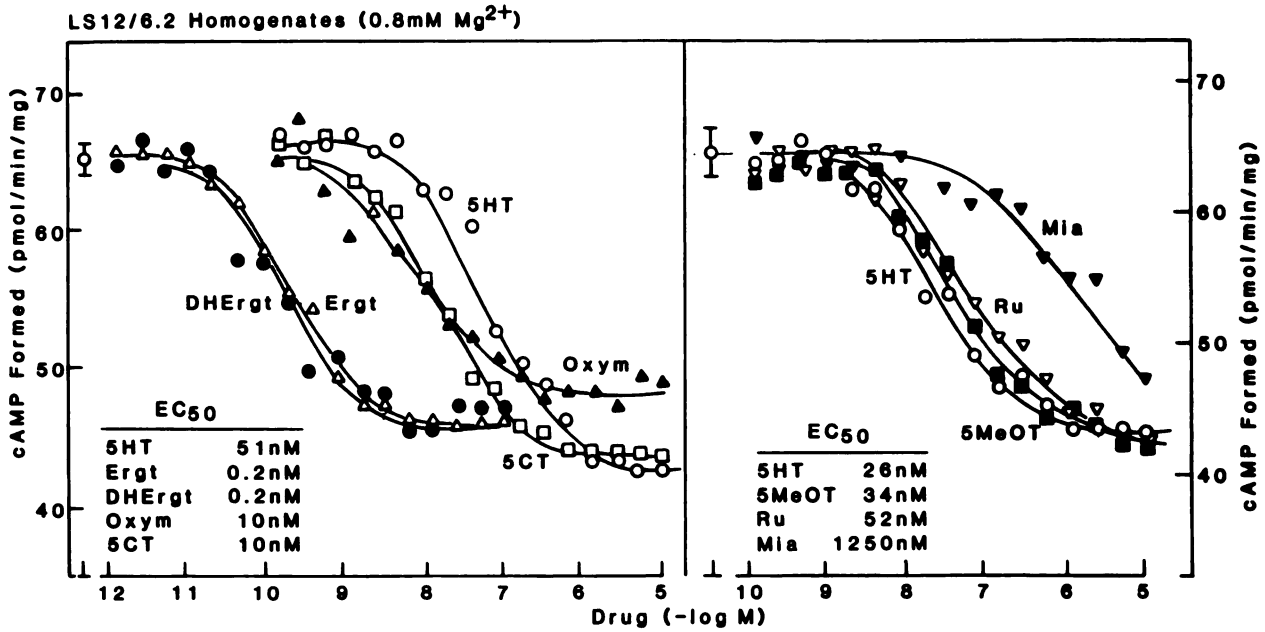

Fig. 3. Dose-response curves for inhibition by serotonergic receptor ligands of the forskolin-stimulated adenylyl cyclase activity in homogenates of cells expressing the human S12 (5-HT$_{1D\beta}$) receptor. *Ergt*, ergotamine; *DHErgt*, dihydroergotamine; *Oxym*, oxymetazoline; *Mia*, mianserin; *Ru*, RU 24969. The results are from a single experiment and are represented in two panels for clarity. *Points* represent activities from single incubations, except for activities without drug addition, which were in triplicate. The results are representative of two experiments of the same type. Note that, in contrast to experiments on S31 receptor-mediated inhibition of adenylyl cyclase, incubations for measurement of S12 receptor-mediated inhibition of adenylyl cyclase were with 0.8 mM Mg^{2+}.

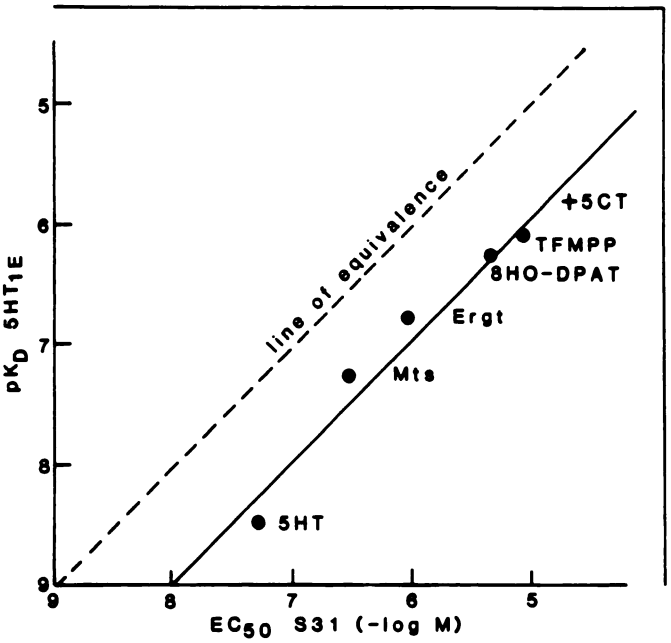

Fig. 4. Comparison of potencies determined for serotonergic agonists at the S31 receptor and affinities determined for the same agonists for the human cerebral cortex 5-HT$_{1E}$ receptor. Data for the *x*-axis are from Table 1 and *y*-axis values are from Ref. 8. +, EC$_{50}$ was estimated from its EC$_{20}$ value (e.g., Fig. 1B), assuming full agonistic activity. *Ergt*, ergotamine; *Mts*, methysergide.

bridization and antibody probes to locate the S31 receptors in their normal environment. The capacity to transfect the genes that encode these receptors may allow expression in biochemically and eletrophysiologically more well equipped model cells, for study of the mechanism of action of these receptors. To these ends, the cloning of these genes and their fundamental pharmacological subclassification have been an essential first step.

References

1. Levy, F. O., T. Gudermann, E. Perez-Reyes, M. Birnbaumer, A. J. Kaumann, and L. Birnbaumer. Molecular cloning of a human serotonin receptor (S12) with a pharmacological profile resembling that of the 5-HT$_{1D}$ subtype. *J. Biol. Chem.* **267**:7553–7562 (1992).
2. Jin, H., D. Oksenberg, A. Ashkenazi, S. J. Peroutka, A. M. V. Duncan, R. Rozmahel, Y. Yang, G. Mengod, J. M. Palacios, and B. F. O'Dowd. Characterization of the human 5-hydroxytryptamine$_{1B}$ receptor. *J. Biol. Chem.* **267**:5735–5738 (1992).
3. Weinshank, R. L., J. M. Zgombick, M. J. Macchi, T. A. Branchek, and P. R. Hartig. Human serotonin 1D receptor is encoded by a subfamiliy of two distinct genes: 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$. *Proc. Natl. Acad. Sci. USA* **89**:3630–3634 (1992).
4. Hartig, P. R., T. S. Brancheck, and R. L. Weinshank. A subfamily of 5-HT$_{1D}$ receptor genes. *Trends Pharmacol. Sci.* **13**:152–159 (1992).
5. Levy, F. O., T. Gudermann, M. Birnbaumer, A. J. Kaumann, and L. Birnbaumer. Molecular cloning of a human gene (S31) encoding a novel serotonin receptor mediating inhibition of adenylyl cyclase. *FEBS Lett.* **296**:201–206 (1992).
6. Fargin, A., J. R. Raymond, M. J. Lohse, B. K. Kobilka, M. G. Caron, and R. J. Lefkowitz. The genomic clone G-21 which resembles a β-adrenergic receptor sequence encodes the 5-HT$_{1A}$ receptor. *Nature (Lond.)* **335**:358–360 (1988).
7. Hamblin, M. W., and M. A. Metcalf. Primary structure and functional characterization of a human 5-HT$_{1D}$-type serotonin receptor. *Mol. Pharmacol.* **40**:143–148 (1991).
8. Leonhardt, S., K. Herrick-Davis, and M. Titeler. Detection of a novel serotonin receptor subtype (5-HT$_{1E}$) in human brain: interactions with a GTP-binding protein. *J. Neurochem.* **53**:465–471 (1989).
9. McAllister, G., A. Charlesworth, C. Snodin, M. S. Beer, A. J. Noble, D. N. Middlemiss, L. L. Iversen, and P. Whiting. Molecular cloning of a serotonin receptor from human brain (5-HT$_{1E}$): a fifth 5-HT$_1$-like subtype. *Proc. Natl. Acad. Sci. USA* **89**:5517–5521 (1992).
10. Liao, C.-F., A. P. N. Themmen, R. Joho, C. Barberis, M. Birnbaumer, and L. Birnbaumer. Molecular cloning and expression of a fifth muscarinic acetylcholine receptor (M5-mAChR). *J. Biol. Chem.* **264**:7328–7337 (1989).
11. Birnbaumer, L., P. C. Yang, M. Hunzicker-Dunn, J. Bockaert, and J. M. Duran. Adenylyl cyclases in ovarian tissues. I. Homogenization and conditions of assay in Graafian follicles and corpora lutea of rabbits, rats and pigs: regulation by ATP and some comparative properties. *Endocrinology* **99**:163–184 (1976).

12. Salomon, Y., C. Londos, and M. Rodbell. A highly sensitive adenylate cyclase assay. *Anal. Biochem.* **58**:541–548 (1974).
13. Bockaert, J., M. Hunzicker-Dunn, and L. Birnbaumer. Hormone-stimulated desensitization of hormone-dependent adenylyl cyclase: dual action of luteinizing hormone on pig Graafian follicle membranes. *J. Biol. Chem.* **251**:2653–2663 (1976).
14. Liao, C. F., W. P. Schilling, M. Birnbaumer, and L. Birnbaumer. Cellular responses to stimulation of the M5 muscarinic acetylcholine receptor as seen in murine L cells. *J. Biol. Chem.* **265**:11273–11284 (1990).
15. Fargin, A., J. R. Reymond, J. W. Regan, S. Cotecchia, R. J. Lefkowitz, and M. G. Caron. Effector coupling mechanisms of the cloned 5-HT_{1A} receptor. *J. Biol. Chem.* **264**:14848–14852 (1989).
16. Perez-Reyes, E., H. S. Kim, A. E. Lacerda, W. Horne, X. Wei, D. Rampe, K. P. Campbell, A. M. Brown, and L. Birnbaumer. Induction of calcium currents by the expression of the α_1-subunit of the dihydropyridine receptor from skeletal muscle. *Nature (Lond.)* **340**:233–236 (1989).
17. DeLean, A., J. M. Stadel, and R. J. Lefkowitz. A ternary complex model explains the agonist-specific binding properties of the adenylate cyclase-coupled *beta*-adrenergic receptor. *J. Biol. Chem.* **255**:7108–7117 (1980).
18. Kent, R. S., A. DeLean, and R. J. Lefkowitz. A quantitative analysis of *beta*-adrenergic receptor interactions: resolution of high and low affinity states of the receptor by computer modeling of ligand binding data. *Mol. Pharmacol.* **17**:14–23 (1980).
19. Gierschik, P., M. Stisslinger, D. Sidiropolous, E. Herrmann, and K. H. Jakobs. Dual Mg^{2+} control of formyl peptide receptor: G protein interaction in HL-60 cells: evidence that the low agonist affinity receptor interacts with and activates the G protein. *Eur. J. Biochem.* **183**:97–105 (1989).
20. Frazer, A., S. Maayani, and B. B. Wolfe. Subtypes of receptors for serotonin. *Annu. Rev. Pharmacol. Toxicol.* **30**:307–348 (1990).

Send reprint requests to: Lutz Birnbaumer, Department of Cell Biology, Baylor College of Medicine, Houston, TX 77030.