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The Rat 5-Hydroxytryptamine$_{1B}$ Receptor Is the Species Homologue of the Human 5-Hydroxytryptamine$_{1D\beta}$ Receptor

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SUMMARY

The relationship between the serotonin 5-hydroxytryptamine$_{1B}$ (5-HT$_{1B}$) and 5-HT$_{1D}$ receptors has been the topic of much investigation and speculation since their complementary species distribution was first appreciated. The cloning of genes encoding 5-HT$_{1D}$ receptors has provided tools to investigate this relationship directly. In this study, a rat gene has been cloned that encodes the rat 5-HT$_{1B}$ receptor. Evaluation of the structure of this gene shows that it is a member of the guanine nucleotide-binding protein-coupled receptor superfamily. Comparison of the amino acid sequence of the rat gene with the human 5-HT$_{1D\beta}$ gene showed a 93% overall identity and a 96% identity in the transmembrane regions. Comparison of the two sequences revealed zero to two amino acid changes in each of these transmembrane regions, as well as a striking conservation in the connecting loops, indicative of the relationship expected for species homologues of the same gene. The rat gene was expressed transiently in COS-7 cells, and membranes derived from these cells were shown to bind [^{125}I]iodocyanopindolol. The pharmacological profile of this binding site closely matched that of the native rat 5-HT$_{1B}$ receptor (r = 0.95) but not the 5-HT$_{1D}$ receptor (r = 0.07). The cloned rat 5-HT$_{1B}$ receptor was found to couple to the inhibition of adenylate cyclase activity, as expected for a 5-HT$_{1B}$ receptor. These data indicate that, although the 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors are pharmacologically distinct, they are species variants of the same receptor gene, the 5-HT$_{1D\beta}$ gene.

The 5HT$_{1B}$ receptor was identified a decade ago as one component of 5-HT$_1$ binding, using [^{3}H]5-HT as a radioligand (1, 2). Subsequently, a number of additional serotonin receptor subtypes with high affinity for this radioligand have been described, including 5-HT$_{1C}$, 5-HT$_{1D}$, and 5-HT$_{1E}$ (3–5). A puzzling relationship between the 5-HT$_{1B}$ and 5HT$_{1D}$ receptor sites has been noted. These subtypes, although pharmacologically distinct, share several common properties. Both receptor subtypes are similarly distributed in select brain regions of reciprocal species and are particularly enriched in the substantia nigra and the basal ganglia. These receptors also couple to the same second messenger pathway, inhibition of forskolin-stimulated adenylate cyclase activity (6, 7), and function as terminal autoreceptors on serotonergic neurons originating in the raphe (8, 9). These characteristics have led to the hypothesis that the 5HT$_{1B}$ and 5HT$_{1D}$ receptors are species homologues of a single gene product, whereby the 5HT$_{1B}$ is expressed in rat, mouse, hamster, and opossum, whereas other species, such as human, dog, cow, and guinea pig, contain almost exclusively 5HT$_{1D}$ receptors (4, 7, 10). However, several lines of evidence, such as differential affinities of certain classes of compounds (notably β-adrenergic receptor blockers and some ergots) for these two receptors and the observation that both subtypes may exist in certain species (11), have suggested that the 5HT$_{1B}$ and 5HT$_{1D}$ receptors are encoded by two distinct genes that have very little homology to each other. The application of molecular cloning techniques has enabled us to assess directly the relationship between these two receptors. We have isolated a rat 5HT$_{1B}$ receptor gene by homology to a human 5HT$_{1D}$ receptor clone. We have shown that this rat gene encodes a receptor with pharmacological properties closely matching those of the 5HT$_{1B}$ receptor reported in native rat brain membranes. Therefore, the 5-HT$_{1B}$ and 5-HT$_{1D\beta}$ receptors are species homologues. A preliminary report of these findings has been presented (12, 13).

Experimental Procedures

Cloning and sequencing. A rat genomic spleen library was screened with a 1.4-kb DNA fragment derived from the human 5-HT$_{1D\beta}$

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ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; 5-CT, 5-carboxyamidotryptamine; DP-5-CT, *N,N*-dipropyl-5-carboxyamidotryptamine; 5-methoxy-DMT, 5-methoxy-dimethyltryptamine; 2-CH$_3$-5-HT, 2-methyl-5-hydroxytryptamine; 1-NP, 1-naphthylpiperazine; TFMPP, *m*-trifluromethylphenylpiperazine; TM, transmembrane; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Kb, kilobase(s).

receptor.[1] Hybridization and Southern blot analysis of phage DNA derived from plaque-purified clones were performed as previously described (14).[1] For subcloning and further Southern blot analysis, DNA was inserted into pUC18 (Pharmacia, Piscataway, NJ). Nucleotide sequence analysis was done by the dideoxy nucleotide chain-termination method of Sanger (15) on denatured double-stranded plasmid templates, using Sequenase (US Biochemical Corp., Cleveland, OH).

Transfection. The entire coding region of clone rs38b was cloned into the eukaryotic expression vector pMO5 (16) as a 3.8-kb *Eco*RI-*Bgl*II fragment. In addition to the coding region, there was approximately 2.0 kb of 5′ sequence and 0.6 kb of 3′ sequence included in the expression vector construct. COS-7 cells were transiently transfected with the plasmid pMO5-rs38b (containing the 5-HT_{1B} receptor gene), using the DEAE dextran method, as previously described (17). These cells were used for all binding assays. To evaluate functional responses of the transfected cells, a stable cell line was produced in Y-1 cells, using the co-transfection method employing calcium phosphate precipitation (16). The selectable marker was introduced by co-transfection of a second plasmid, pGCcos3neo (18). Stable clones were then selected for their resistance to the antibiotic G-418 and their ability to bind 0.02 nM [^{125}I]iodocyanopindolol. Six cell lines, with estimated B_{max} values ranging from 1 to 14 pmol/mg of protein, were obtained. (The growth characteristics of the Y-1 cells make them unsuited to growth in large quantities for routine binding assays.) Clone Y-1-11 yielded a B_{max} of 13 pmol/mg of protein and was selected for functional characterization.

Membrane preparation. Membranes used in radioligand binding assays were prepared from transiently transfected cells, using a procedure described previously (16). Freshly prepared membranes were assayed within 1 hr of preparation.

[^{125}I]Iodocyanopindolol binding assays. [^{125}I]Iodocyanopindolol was used as a radioligand to detect the expression of 5-HT gene product in membrane fractions isolated from transiently transfected COS-7 cells. Radioligand binding assays were performed as described (19), with slight modification. Briefly, binding assays were conducted in 96-well microtiter plates, in a total volume of 250 μl of buffer (50 mM Tris·HCl, 10 mM $MgSO_4$, 0.2 mM EDTA, 0.1% ascorbic acid, 10 μM pargyline, pH 7.4 at 22°). Untransfected COS-7 cells gave a specific binding value of ~60 fmol/mg when 10 μM propranolol was used to determine nonspecific binding. Therefore, when transfected COS-7 membranes were used, isoproterenol (3 μM final concentration) was also included in the buffer to reduce binding of [^{125}I]iodocyanopindolol to endogenous β-adrenoceptors. In contrast, untransfected Y-1 cells failed to show any specific binding of [^{125}I]iodocyanopindolol when assayed under conditions that produced high levels of binding in the transfected cells. Consequently, isoproterenol was omitted from the buffer when transfected Y-1 membranes were used for selection of stable clones. All further binding characterization was performed on transiently transfected COS-7 cell membranes containing 3 μM (final concentration) isoproterenol. Saturation studies were conducted using [^{125}I]iodocyanopindolol concentrations ranging from 0.005 to 5 nM, and competition experiments were performed using a final concentration of [^{125}I]iodocyanopindolol ranging between 0.02 and 0.04 nM. 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 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, Gaithersville, 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 ReadiSafe 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).

Measurement of cAMP formation. The stably transfected cells were incubated in Ham's F10 with 5 mM theophylline, 10 mM HEPES, 10 μM pargyline, and/or appropriate concentrations of methiothepin, for 20 min at 37° in 5% CO_2. Serotonin was then added to a final concentration of 0.001 nM to 1 μM and incubated for an additional 10 min at 37° in 5% CO_2. The medium was aspirated and the reaction was stopped by the addition of 100 mM HCl. To demonstrate competitive antagonism, a dose-response curve for 5-HT was measured in parallel, using a fixed dose of methiothepin (0.32 μM). The plates were stored at 4° for 15 min and centrifuged for 5 min at 500 × *g* to pellet cellular debris, and the supernatant was aliquoted and stored at −20° before assessment of cAMP formation by radioimmunoassay (cAMP radioimmunoassay kit; Advanced Magnetics, Cambridge, MA). Radioactivity was quantified using a Packard COBRA Auto Gamma counter, equipped with data reduction software.

Drugs. Drugs were obtained from the following companies: (−)-[^{125}I]iodocyanopindolol (specific activity, 2200 Ci/mmol), New England Nuclear (Boston, MA); dihydroergotamine tartrate, pargyline hydrochloride, (±)-pindolol, serotonin creatinine sulfate, and theophylline, Sigma (St. Louis, MO); 5-CT maleate, DP-5-CT maleate, 5-methoxy-DMT oxalate, 2-CH_3-5-HT maleate, CGS-12066B dimaleate, (−)-isoproterenol bitartrate, methysergide maleate, and (−)-propranolol hydrochloride, Research Biochemical Inc. (Natick, MA); rauwolscine hydrochloride, Accurate Chemicals (Westbury, NY); and methiothepin maleate, 1-NP hydrochloride, and TFMPP hydrochloride, Biomol Research Laboratories (Plymouth Meeting, PA). Forskolin was purchased from Calbiochem (La Jolla, CA). All other chemicals were the highest purity available commercially.

Results

Cloning of the 5-HT_{1B} receptor. A rat genomic spleen library was screened with a 1.4-kb DNA fragment derived from the human 5-$HT_{1D\beta}$ receptor.[1] Several strongly hybridizing clones were isolated and one clone, designated rs38b, was characterized by DNA sequence analysis. Fig. 1 shows the amino acid sequence of the rat 5-HT_{1B} receptor (rs38b), indicating those positions that differ from the deduced amino acid sequence of the human 5-$HT_{1D\beta}$ receptor.[1] Overall, 93% amino acid sequence conservation was observed between the rat 5-HT_{1B} receptor and the human receptor over 386 amino acids. The greatest divergence between the rat 5-$HT_{1D\beta}$ and human 5-$HT_{1D\beta}$ receptor protein sequences was seen at the extracellular amino terminus rather than the large cytoplasmic loop found between TM region V and TM region VI. The homology between these two receptors within the TM regions alone was 96%, with only seven amino acid changes found within the

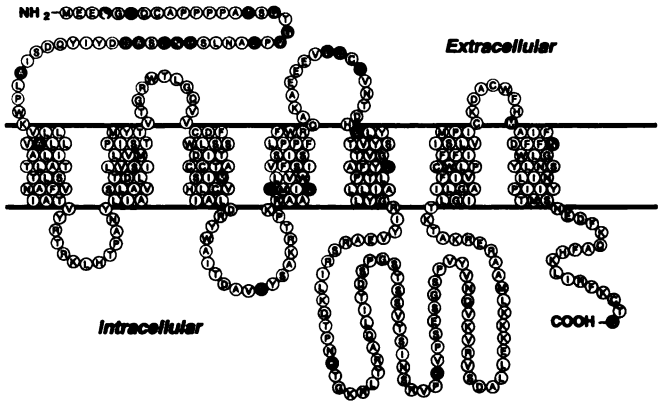

Fig. 1. Seven-TM segment model of the deduced amino acid sequence of the rat 5-HT_{1B} receptor. *Solid circles*, amino acids that differ from corresponding positions in the 5-$HT_{1D\beta}$ receptor.

[1] R. L. Weinshank, J. M. Zgombick, M. Macchi, T. A. Branchek, and P. R. Hartig. The human serotonin 1D receptor is encoded by a subfamily of two distinct genes: 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$. *Proc. Natl. Acad. Sci. U.S.A.* In press.

membrane. The close relationship between the rat 5-HT$_{1D\beta}$ and human 5-HT$_{1D\beta}$ receptor protein sequences is consistent with their classification as species homologues. A comparison of the rat 5-HT$_{1B}$ receptor with other cloned serotonin receptors is shown in Fig. 2.

Characterization of [^{125}I]iodocyanopindolol binding to the 5HT$_{1B}$ receptor. The identity of the receptor subtype encoded by the rat rs38b gene was determined from analysis of the binding properties of membranes derived from COS-7 cells transiently transfected with DNA from that clone. Preliminary experiments indicated that, at the lowest radioligand concentration used (0.005 nM), [^{125}I]iodocyanopindolol binding reached equilibrium by 60 min at 22° and remained unchanged for at least an additional 120 min. Consequently, a 90-min incubation was used in all subsequent experiments.

Saturation experiments. Saturable high affinity [^{125}I]iodocyanopindolol binding was observed with membranes prepared from COS-7 cells transiently transfected with the rs38b gene (Fig. 3). The specific [^{125}I]iodocyanopindolol 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 0.16 ± 0.03 nM (three experiments). Evaluation of the Scatchard transformation also yielded a single binding site (Fig. 3, *inset*). The binding density (B_{max}) was variable between separate transfections and ranged from 30 to 90 pmol/mg. This variability is typical of transient transfection systems. The reported K_d value for [^{125}I]iodocyanopindolol binding to the 5HT$_{1B}$ recognition site ranges between 0.16 and 0.23 nM; these values are at least 1 order of magnitude higher than those (0.01 nM) observed for β-adrenoceptors (20, 21). Saturation experiments performed using [^{3}H]5HT as radioligand yielded a K_d value of 23 nM (data not shown), a value very similar to the reported K_i value for 5-HT displacement of [^{125}I]iodocyanopindolol binding to the 5HT$_{1B}$ receptor in native rat tissue (8, 22).

Competition experiments. The pharmacological profile of ligand binding to COS-7 cell membranes transfected with clone rs38b was determined by analysis of competition binding experiments. A collection of β-adrenergic blockers and serotonergic agonists and antagonists totally displaced specific [^{125}I] iodocyanopindolol binding. Representative curves are shown in Fig. 4, and the affinities of these drugs for the inhibition of [^{125}I]iodocyanopindolol binding are summarized in Table 1. The rank order of potencies for serotonergic agonists was RU 24969 > 5-CT > 5-HT > CGS12066B > sumatriptan > 5-methoxy-DMT > DP-5-CT ≥ 2-CH$_3$-5-HT. The rank order of affinities for antagonists was methiothepin > propranolol > metergoline > pindolol > methysergide > rauwolscine. Competition curves for high affinity agonists were shallow. For these cases, a two-site fit was obtained, as follows: RU 24969, K_H = 0.82 ± 0.2, K_L = 45 ± 10; 5-CT, K_H = 3.9 ± 1.38, K_L = 134 ± 58; 5-HT, K_H = 2.5 ± 1.8, K_L = 52 ± 6.5; TFMPP, K_H = 34 ± 2.5, K_L = 642 ± 142; and sumatriptan, K_H = 131 ± 37, K_L = 1668 ± 736 (all in nM). The proportion of sites in the high affinity state was variable and ranged from 50 to 80%. In order to determine whether the 5-HT$_{1B}$ receptor encoded by the rs38b gene is similar to the 5-HT$_{1B}$ receptor previously identified in rat cortex or whether its pharmacological profile resembles that of the 5-HT$_{1D}$ receptor identified in calf caudate, we compared our binding data with those of other laboratories (19, 22–24), using regression analysis of pK_i values (Fig. 5). No significant correlation (r = 0.07) was found between the affinities of the drugs for rs38b and the bovine caudate 5-HT$_{1D}$ receptors. In contrast, there was a significant correlation (r = 0.95) between affinity values for rs38b and rat 5-HT$_{1B}$ receptors.

Functional assays. 5-HT (10 μM) had no effect on either basal or forskolin-stimulated adenylate cyclase activity in untransfected 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 untransfected cells. To examine the ability of the cloned 5-HT$_{1B}$ receptor to couple to the inhibition of forskolin-stimulated cAMP levels, concentration-response curves for 5-HT-mediated inhibition of forskolin-stimulated adenylate cyclase activity (10 μM forskolin) were determined in stable transfectants. Addition of 10 μM forskolin increased the basal cAMP release (0.062 ± 0.008 pmol/ml/10 min) by 10-fold. Addition of 5-HT to this system caused a smooth monophasic (Hill slope of 1.0) inhibition of the response (Fig. 6), with an EC$_{50}$ value of 0.32 ± 0.11 nM (three experiments) and a maximum inhibition (E_{max} value) of 98 ± 2%. Further, the 5-HT$_{1B}$ response was completely blocked by the addition of the serotonergic antagonist methiothepin at a concentration of 10 μM. The dose shift produced by methiothepin yielded an apparent K_B of 21 ± 3 nM. No direct effect of methiothepin was observed. In addition, no evidence for coupling of this receptor to phosphatidylinositol turnover was detected at a dose of 10 μM 5-HT (data not shown).

Discussion

The 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors have recently been thought to be species homologues of the same receptor subtype, because they display similarities in their pharmacology, second messenger coupling, and anatomical distribution (7) and show a complementary species distribution, i.e., the 5-HT$_{1B}$ subtype appears to be confined to rat, mouse, and opossum, whereas 5-HT$_{1D}$ sites have been demonstrated in human, pig, guinea pig, and calf. In order to provide further insight into this interesting relationship, we have recently cloned a 5-HT$_{1B}$ receptor gene (rs38b) from a rat genomic library. This approach was designed to take into account our recent finding that there are at least two human genes encoding pharmacologically defined 5-HT$_{1D}$ receptors.[1] We have referred to these as 5-HT$_{1D\alpha}$ and 5-HT$_{1D\beta}$, reflecting the order in which we isolated them. These two human receptors display extremely similar pharmacological properties when expressed in mammalian cells and can both be defined as subtypes of 5-HT$_{1D}$ receptor. Each has been shown to couple to the inhibition of adenylate cyclase activity. Neither binds [^{125}I]iodocyanopindolol.

The rat 5-HT$_{1B}$ receptor, based upon a much stronger amino acid sequence identity to the 5-HT$_{1D\beta}$ receptor than the 5-HT$_{1D\alpha}$ receptor, appears to be the species homologue of the 5-HT$_{1D\beta}$ receptor. It exhibits an overall amino acid sequence identity of 93% to the human 5-HT$_{1D\beta}$ receptor sequence and a 96% identity in the TM region, indicative of the relationship expected for species homologues. Further, there is a high degree of conservation in the connecting loops, including the third intracellular loop. Other examples of species homologues include the human (25) and rat (26) 5-HT$_{1A}$ receptors, the human (27, 28) and rat (29) 5-HT$_2$ receptors, and the human (30) and rat (31) α$_{2B}$-adrenergic receptors. In all of these cited examples, the human and rat receptors exhibit very similar pharmacolog-

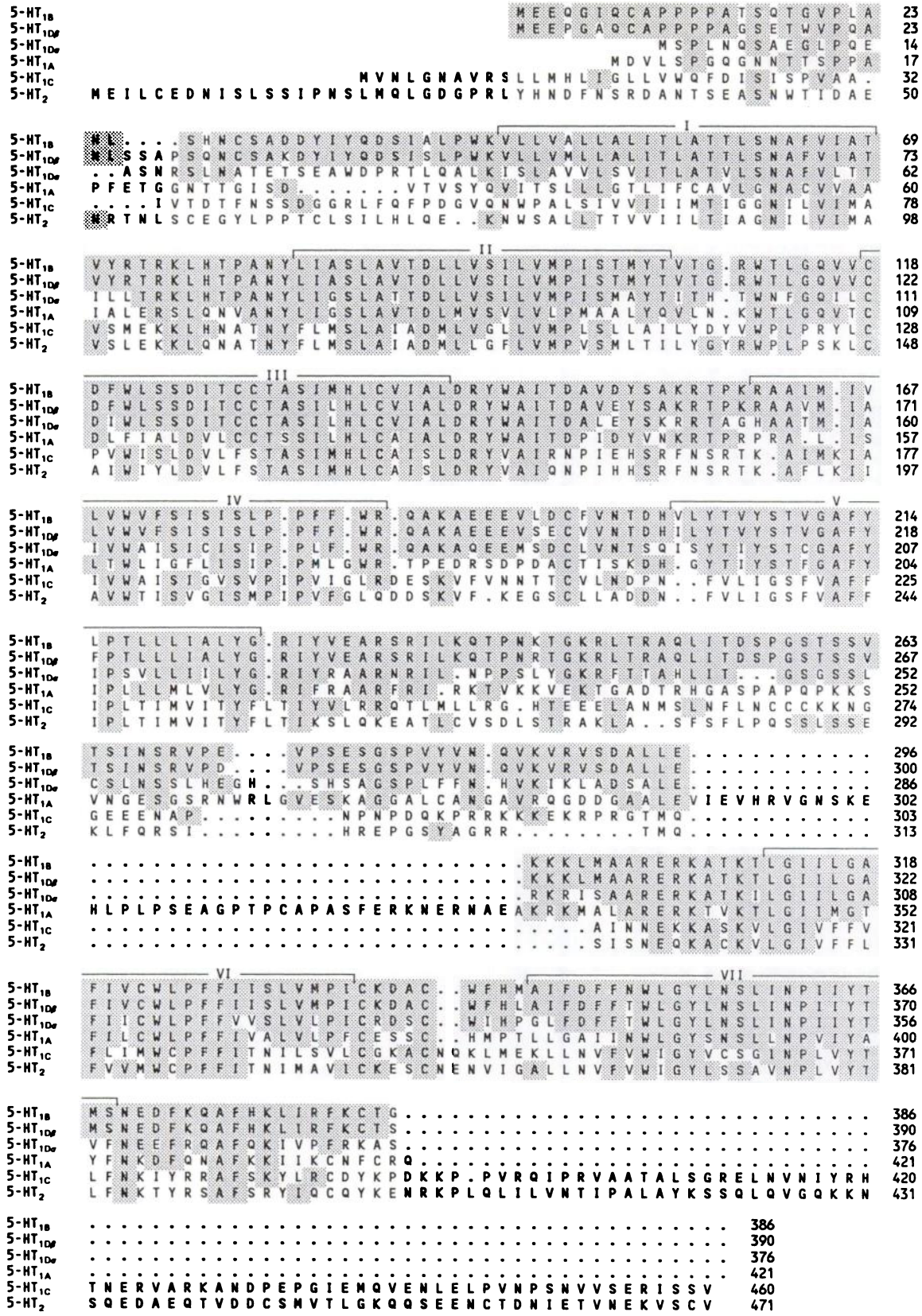

Fig. 2. Comparison of the 5-HT$_{1B}$ receptor deduced amino acid sequence with other serotonin receptors. *Solid bars*, the seven putative membrane-spanning domains (TM I–VII); *shading*, homologies between the 5-HT$_{1B}$ receptor and other receptors.

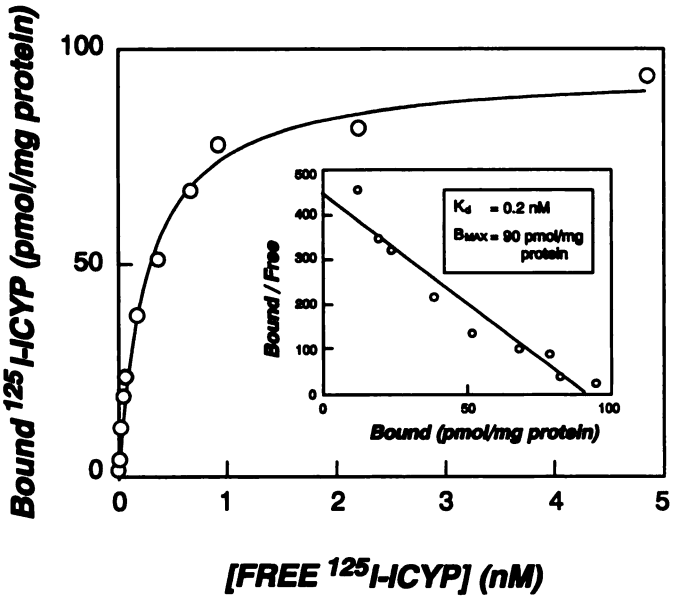

Fig. 3. Determination of the equilibrium dissociation constant (K_d) of [^{125}I] iodocyanopindolol (^{125}*I-ICYP*) for the cloned rat 5-HT$_{1B}$ receptor. Membranes harvested from transient transfectants (COS-7 cells) were incubated with 10–12 concentrations of [^{125}I]iodocyanopindolol (0.005–5 nM), in the presence of 3 μM isoproterenol to block endogenous β-adrenergic receptors, for 90 min at 22°. Nonspecific binding was defined by 10 μM unlabeled 5-HT. Each data point is the mean of triplicate determination, 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*). Calculated K_d and B_{max} values for this experiment are 0.20 nM and 90 pmol/mg of protein, respectively. Protein was determined by the method of Bradford (41).

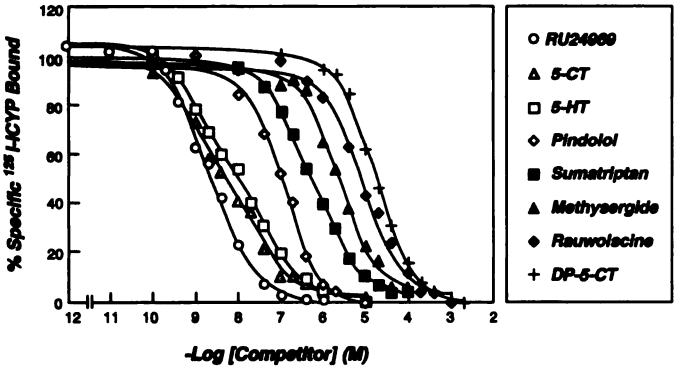

Fig. 4. Inhibition of specific [^{125}I]iodocyanopindolol (^{125}*I-ICYP*) binding to the cloned rat 5-HT$_{1B}$ receptor. Membranes from transiently transfected COS-7 cells were incubated with 0.02–0.04 nM [^{125}I]iodocyanopindolol in the presence of 3 μM isoproterenol to block endogenous β-adrenergic receptors. Nonspecific binding was defined by 10 μM unlabeled 5-HT. Data were analyzed by computer-assisted nonlinear regression analysis (ACCUCOMP; Lundon Software). Each *curve* represents the mean of triplicates from a single experiment, representative of at least three separate experiments.

ical properties, indicating that they are essentially identical receptor genes.

Evaluation of the pharmacological profile of cells transfected with the rs38b gene identifies this gene as encoding a rat 5-HT$_{1B}$ receptor. When K_i values from competition binding studies are plotted in log-log correlation plots, a very weak linear correlation coefficient of $r = 0.07$ is obtained comparing rs38b clone with the calf caudate 5-HT$_{1D}$ site, whereas a strong correlation coefficient of $r = 0.95$ is obtained in comparison with the rat 5-HT$_{1B}$ site. These data indicate that, although the amino acid identity between the rat and the human gene is very high, the 5-HT$_{1B}$ receptor is an unusual species homologue of the 5-HT$_{1D\beta}$ receptor because it displays distinct pharmacological properties. The recent suggestion by Guan *et al.* (32) that a single amino acid, Asn-385, plays an important role in the interaction between pindolol derivatives and the 5-HT$_{1A}$ receptor is relevant to this apparently large difference in pharmacology between very closely related genes. The rat 5-HT$_{1B}$ sequence contains this conserved asparagine residue at position 351 (see Fig. 2). In contrast, the human homologue (5-HT$_{1D\beta}$) does not contain this residue and neither does the human 5-HT$_{1D\alpha}$ sequence. The human receptors both have a threonine residue at this position (45).[1] Apparently, although both are uncharged but polar amino acids, the asparagine enables the binding of iodocyanopindolol. The human receptors do not bind [^{125}I]iodocyanopindolol. Therefore, the single naturally occurring "mutation" between the human and rat sequences in this TM region VII amino acid may underlie the substantial difference in pharmacology that led the identification of the 5-HT$_{1D}$ receptor as a subtype distinct from the 5-HT$_{1B}$ receptor.

Functional responses were obtained from cells transfected with the rat 5-H$_{1B}$ receptor gene. The magnitude of this 5-HT$_{1B}$ receptor response signal ($E_{max} > 90\%$) was large, relative to that typically seen in native rat membranes (20%) (24). This difference might be attributed to the use of intact rather than broken cell preparations, as has been reported (33). Excellent responses with intact OK cells, which contain a native 5-HT$_{1B\text{-like}}$ receptor, have been observed (E_{max}, ~80% inhibition of PTH-stimulated cAMP production) (34). In 5-HT$_{1B}$-transfected cells, the 5-HT$_{1B}$ response was completely blocked by the addition of the serotonergic antagonist methiothepin, at a concentration (10 μM) previously shown to block 5-HT$_{1B}$-mediated adenylate cyclase inhibition in native brain membranes (24). The effect of methiothepin was receptor specific, because it was seen only in the presence of 5-HT. The apparent K_B for the nonselective antagonist methiothepin (21 ± 2 nM; calculated from single-point Schild analysis) was in good agreement with that derived from binding experiments (13 ± 4 nM). In contrast, the EC$_{50}$ for 5-HT (0.37 nM) was higher than expected from the measured K_i value (20 nM). This apparent discrepancy may be the result of a significant amount of receptor reserve in these transfected cells. This latter point may be tested experimentally using alkylation protocols. However, the EC$_{50}$ value for 5-HT more closely correlates with the high affinity component of binding from two-site analysis of competition studies ($K_H = 2.5$ nM).

In humans and other species, it has been postulated that there are multiple subtypes of 5-HT$_{1D}$ receptor (35, 36). We have provided evidence for two distinct 5-HT$_{1D\text{-like}}$ receptors by using a molecular cloning approach (5). The 5-HT receptor encoded by the gene reported here is clearly a 5-HT$_{1B}$ receptor. The close relationship between the human and rat genes indicates that the human genome does not have a 5-HT$_{1B\text{-like}}$ receptor, as defined pharmacologically. This supports the previous data of Lebel *et al.* (37), using [^{3}H]CP96,501, in which no specific binding of this 5-HT$_{1B}$-selective radioligand was detected to membranes derived from human, guinea pig, pig, or bovine brain. These data do not exclude the possibility that the rat expresses a 5-HT$_{1D}$ receptor that is the species homologue

TABLE 1

Drug affinities for cloned rat 5-HT$_{1B}$ receptor

Apparent dissociation constants (K_i values) of serotonergic ligands for the cloned rat 5-HT$_{1B}$ receptor are shown in comparison with the pharmacologically defined 5-HT$_{1B}$ and 5-HT$_{1D}$ receptors from native brain membrane preparations. Membranes were incubated with 0.02–0.04 nM [^{125}I]iodocyanopindolol in the presence of 10–14 concentrations of unlabeled competitors, for 90 min at 22°. Isoproterenol (3 μM) was included to block endogenous β-adrenergic receptors. Nonspecific binding was defined by 10 μM unlabeled 5-HT. Affinity constants were determined from IC$_{50}$ values obtained by computer-assisted nonlinear curve analysis (ACCUCOMP; Lundon Software), using the Cheng-Prussoff equation. K_i values are expressed as mean ± standard error values from at least three determinations. Affinity constants for the 5-HT$_{1B}$ receptor in rat frontal cortical membranes and for the 5-HT$_{1D}$ receptor in bovine caudate membranes were obtained from literature values.

Chemical class	Drug	K_i		
		rs38b	5-HT$_{1B}$[a]	5-HT$_{1D}$[a]
			nM	
Tryptamine derivatives	RU24969	1.6 ± 0.17	3.8[b]	151
	5-CT	7.3 ± 1.3	5.0	2.5
	5-HT	16 ± 0.58	25	4.0
	Sumatriptan	465 ± 85	500[c]	17[d]
	5-MethoxyDMT	3,594 ± 212	1,259	32
	DP-5-CT	>10,000	>10,000	63
	2-CH$_3$-5-HT	>10,000	>10,000	398
Alkaloids	Dihydroergotamine	4.2 ± 1.7	0.79	80
	Metergoline	129 ± 33	40	0.79
	Methysergide	1,823 ± 297	1,585	4.0
	Rauwolscine	6,295 ± 1,261	5,012	20
Piperazines	Methiothepin	13 ± 3.9	50	50
	1NP	40 ± 14	250	16
	TFMPP	55 ± 8.5	26[e]	630
	CGS12066B	110 ± 11	130	2.9
Others	(−)-Propranolol	57 ± 4.0	50	3,162
	(±)-Pindolol	153 ± 62	398	6,310

[a] From Hoyer (43).
[b] From Schoeffter and Hoyer (44).
[c] From Schoeffter and Hoyer (24).
[d] From Peroutka and McCarthy (23).
[e] From Offord *et al.* (19).

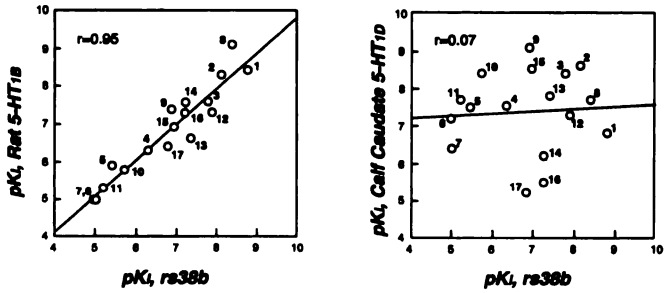

Fig. 5. Correlation between affinity constants (pK_i values) of serotonergic ligands for the cloned rat 5-HT$_{1B}$ receptor and the pharmacologically defined 5-HT$_{1B}$ receptor in rat cortex (A) and 5-HT$_{1D}$ receptor in bovine caudate (B). The correlation coefficient (r) is listed in each panel. *1*, RU24969; *2*, 5-CT; *3*, 5-HT; *4*, sumatriptan; *5*, 5-methoxy-DMT; *6*, DP-5-CT; *7*, 2-CH$_3$-5-HT; *8*, dihydroergotamine; *9*, metergoline; *10*, methysergide; *11*, rauwolscine; *12*, methiothepin; *13*, 1NP; *14*, TFMPP; *15*, CGS12066B; *16*, propranolol; *17*, pindolol.

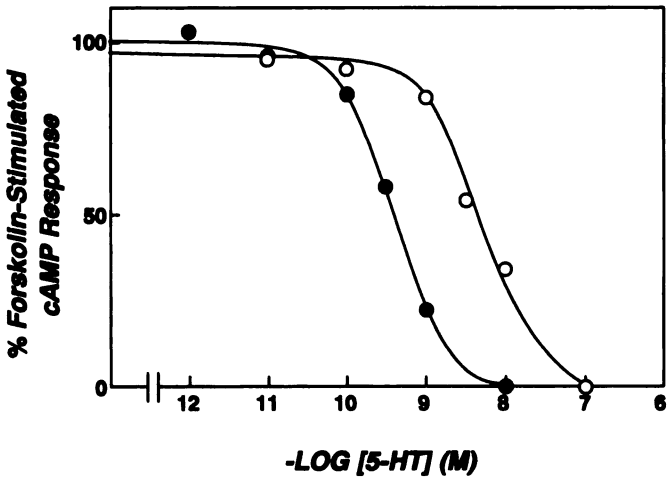

Fig. 6. Inhibition of forskolin-stimulated cAMP production by 5-HT and antagonism by methiothepin in stable transfectants (Y-1-11 cells) expressing the cloned rat 5-HT$_{1B}$ receptor. cAMP measurements on intact cells were as described in Experimental Procedures. 5-HT concentration-effect curves are represented in the absence (●) and in the presence (○) of methiothepin (0.32 μM). 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 three separate experiments. Values of E_{max} (maximal effect) and EC$_{50}$ (concentration producing the half-maximal effect) were derived from this analysis and are indicated in the figure. The apparent dissociation constant of antagonist (K_B) was calculated according to the formula: $K_B = [B]/(A'/A) - 1]$, where $[B]$ is the concentration of antagonist and A' and A are the EC$_{50}$ values of agonist measured, respectively, in the presence and in the absence of antagonist (42).

of the 5-HT$_{1D\alpha}$ gene. Reports of 5-HT$_{1D\text{-like}}$ receptors in the rat have appeared (11, 38, 39). Although we did not find the rat homologue of the 5-HT$_{1D\alpha}$ in any of our libraries, a preliminary report has appeared indicating the cloning of a 5-HT$_{1D\text{-like}}$ receptor, with a 5-HT$_{1D\text{-like}}$ pharmacology, isolated from the rat. The amino acid sequence of this rat clone is nearly identical to that of the human 5-HT$_{1D\alpha}$ (40). No functional responses were obtained in their study.

The analysis of the relationship between pharmacologically distinct and genetically distinct receptor subtypes is of key importance for both basic science and pharmaceutical development. Analysis of differences in the structure of the proteins encoded by receptor genes can be coupled with the ability to study the detailed binding properties and functional responses of the receptors in heterologous expression systems. These

approaches can be used to develop selective agonists and antagonists of individual receptor subtypes, which will contribute to our understanding of the roles of these receptors in physiology and pathophysiology.

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