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ACCELERATED COMMUNICATION

Molecular Cloning of a Mammalian Serotonin Receptor that Activates Adenylate Cyclase

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SUMMARY

Serotonin modulates a wide range of physiological functions by activating multiple receptors, which are coupled to various effector systems. Using a strategy based on amino acid sequence homology between 5-hydroxytryptamine (5-HT) receptors, we have isolated from a mouse brain library a cDNA encoding a new 5-HT receptor, 5-HT_x, that activates adenylate cyclase. Amino acid sequence comparisons revealed that the 5-HT_x receptor was a distant relative of previously cloned 5-HT receptors, with the highest percentage of homology (42%) being with the 5-HT_{dro1} receptor, a *Drosophila* 5-HT receptor positively coupled to adenylate cyclase. In COS-7 cells transiently expressing the 5-HT_x receptor, 5-HT induced an increase in cAMP levels that was dose dependent and saturable (EC_{50} = 45 nM). Agonists displayed the following rank order of potencies: 5-carboxamidotryptamine > 5-methoxytryptamine > 5-HT > RU 24969 > 8-hydroxy-2-(di-*n*-propylamino)tetralin. The most efficient antagonists in inhibiting the stimulatory effect of 5-HT were methysergide, methiothepin, mesulergine, metergoline, clozapine, ergotamine, and (+)-butaclamol. Membranes of COS-7 cells expressing the 5-HT_x receptor displayed a single saturable binding site for [^{3}H]5-HT. The order of potencies of various drugs in displacing [^{3}H]5-HT binding was similar to the order obtained in cAMP experiments. The pharmacological profile of this receptor does not correspond to the profile of any of the classic 5-HT receptor subtypes. Expression of 5-HT_x mRNA was highest in brainstem and lower in forebrain, cerebellum, intestine, and heart. The 5-HT_x receptor might therefore correspond to 5-HT_1-like receptors that have been shown to induce relaxation in porcine vena cava and guinea pig ileum as well as tachycardia in cat heart. The high affinity of the 5-HT_x receptor for neuroleptic agents such as (+)-butaclamol and clozapine suggests also that this receptor might play a role in certain neuropsychiatric disorders.

Serotonin is involved in a wide range of behaviors (1), and serotonergic drugs are used in the treatment of a number of pathological states, such as depression, appetite disorders, and migraines (2). Pharmacological studies and molecular cloning identified several subtypes of receptors with distinct pharmacological properties, signaling systems, and tissue distributions (for review, see Refs. 3 and 4). The 5-HT receptors can be classified into two groups, i.e., G protein-coupled receptors, including the 5-HT_1, 5-HT_2, 5-HT_4, and 5-HT_5 receptors, and ligand-gated ion channels, such as the 5-HT_3 receptors. The 5-HT_1 receptors can be further subdivided in 5-HT_{1A} (5), 5-HT_{1B} (6, 7), 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ (8), 5-HT_{1E} (9, 10), and 5-$HT_{1E\beta}$ (also called 5-HT_{1F}) (11, 12) subtypes. The 5-HT_2 family contains the 5-HT_2 (13), 5-HT_{1C} (14), and 5-HT_{2F} (15) subtypes. When expressed in various mammalian cell lines the 5-HT_1 receptors inhibit adenylate cyclase activity, whereas the 5-HT_2 receptors activate phospholipase C. The 5-HT_{5A} and 5-HT_{5B} receptors define a new family of receptors that do not interact with adenylate cyclase or phospholipase C and do not correspond to classical pharmacological subtypes (16, 17). Concerning 5-HT receptors that stimulate adenylate cyclase, there are a number of reports describing a serotonin-sensitive cyclase in membranes from various organs (for review, see Ref. 18). In particular, a receptor termed 5-HT_4 has been found in colliculi and hippocampal neurons as well as in peripheral organs (for review, see Ref. 19). Two 5-HT receptors that activate adenylate cyclase have been cloned so far, the *Drosophila* 5-HT_{dro1} receptor (20) and a recently cloned receptor tentatively called 5-HT_6 (21). However, none of these receptors displays a pharmacological profile that resembles the profile of the 5-HT_4 receptor.

To isolate additional 5-HT receptors, including possibly the 5-HT_4 receptor, we took advantage of the amino acid sequence

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ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; 5-CT, 5-carboxamidotryptamine; 5-MeOT, 5-methoxytryptamine; RU 24969, 5-methoxy-3-(1,2,3,6-tetrahydropyridin-4-yl)-1*H*-indole; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin; LSD, lysergic acid diethylamide; TFMPP, 1-(3-trifluoromethylphenyl)piperazine; PCR, polymerase chain reaction; bp, base pair(s); Gpp(NH)p, guanosine-5′-($\beta\gamma$-imido)triphosphate.

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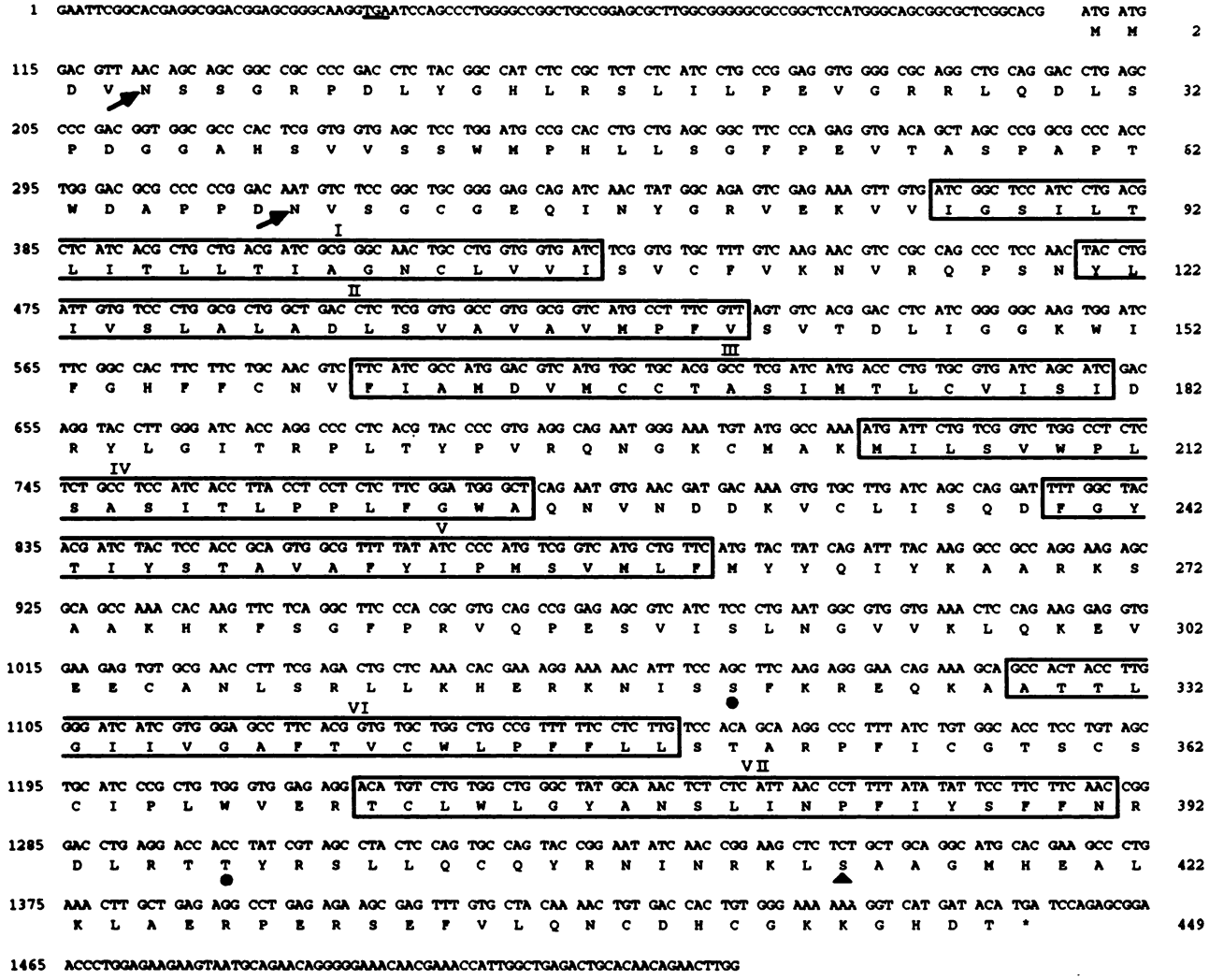

Fig. 1. Nucleotide sequence of the 5-HT$_x$ cDNA. The 3.2-kilobase *Eco*RI-*Xho*I cDNA fragment was sequenced on both strands from the *Eco*RI site to position 1539. The remaining 1700 nucleotides were not sequenced. The seven putative transmembrane domains are *boxed* and *numbered* (*I* to *VII*). *Arrows*, sites of potential *N*-linked glycosylation. *Circles* and *triangles*, consensus sites for phosphorylation by protein kinase C and protein kinase A, respectively. An in-frame stop codon upstream of the ATG is *underlined*. *, Terminal stop codon. EMBL/GenBank accession number: Z23107.

homologies found in transmembrane domains 3, 6, and 7 of 5-HT receptors. A reverse PCR was performed on RNA prepared from mouse colliculi neurons and the PCR fragments were used to screen a mouse brain cDNA library. One of the resulting cDNAs was shown to encode a functional 5-HT receptor. Sequence comparisons revealed that this receptor is a new member of the G protein-coupled receptor family that is most homologous (42% amino acid identity) to the 5-HT$_{dro1}$ receptor. When expressed in COS-7 cells, like the 5-HT$_{dro1}$ receptor our new receptor stimulated adenylate cyclase activity. We therefore named it 5-HT$_x$, while awaiting a final nomenclature. In addition, the 5-HT$_x$ receptor displayed a high affinity for [^{3}H] 5-HT, and its pharmacological profile did not correspond to that of any known serotonin receptor subtype, including the 5-HT$_4$ receptor and the recently cloned 5-HT$_5$ and 5-HT$_6$ receptors. Our results therefore reveal an unexpected heterogeneity among 5-HT receptors that are positively coupled to adenylate cyclase. Interestingly, the 5-HT$_x$ receptor had a high affinity for psychotropic drugs such as clozapine, raising the possibility that this receptor plays a role in neuropsychiatric disorders involving the serotonergic system.

Materials and Methods

Isolation and sequencing of the 5-HT$_x$ cDNA. A doubly nested PCR experiment was performed with the following oligonucleotides: (i) (C/G)(T/A)(A/G)TTG(A/G)C(A/G)TAGCC(C/A)A(A/G/T)CCA, (ii) CTTGATATCGAATTCGA(T/C)(A/G)T(A/G/C/T)CT(A/G/C/T)TG(C/T)TG(C/T)AC, (iii) GGTATCGATAAGCTTAT(C/T/A)GC(C/T)CT(A/G/C/T)GA(C/T)(C/A)G(A/G/C/T)TA, and (iv) AGAACTAGTGGATCCAA(A/G)AA(A/G/C/T)GG(A/G/C/T)A(A/G)CCA(A/G)CA. Five micrograms of total RNA from mouse embryonal colliculi neurons were reverse transcribed in the presence of 1 μg of oligonucleotide i and 12 units of avian myeloblastosis virus reverse transcriptase (Pharmacia). One half of that reaction was then amplified for 30 cycles in the presence of *Thermus aquaticus* polymerase (5 units; Cetus) and oligonucleotides i and ii (1 μg of each). One twentieth of that reaction was amplified for 30 cycles with oligonucleotides i and iii. The resulting products were size selected between 300 bp and 700 bp on a 2% agarose gel. Finally, one fourth of the material extracted from the gel was amplified for 30 more cycles with oligonucleotides iii and iiii. Two discrete fragments were obtained, 500 bp and 700 bp long. These fragments were digested with *Bam*HI and *Hin*dIII, inserted in the Bluescript plasmid, and sequenced. The 500-bp fragment exhibited homology with 5-HT receptors. It was labeled by random priming and

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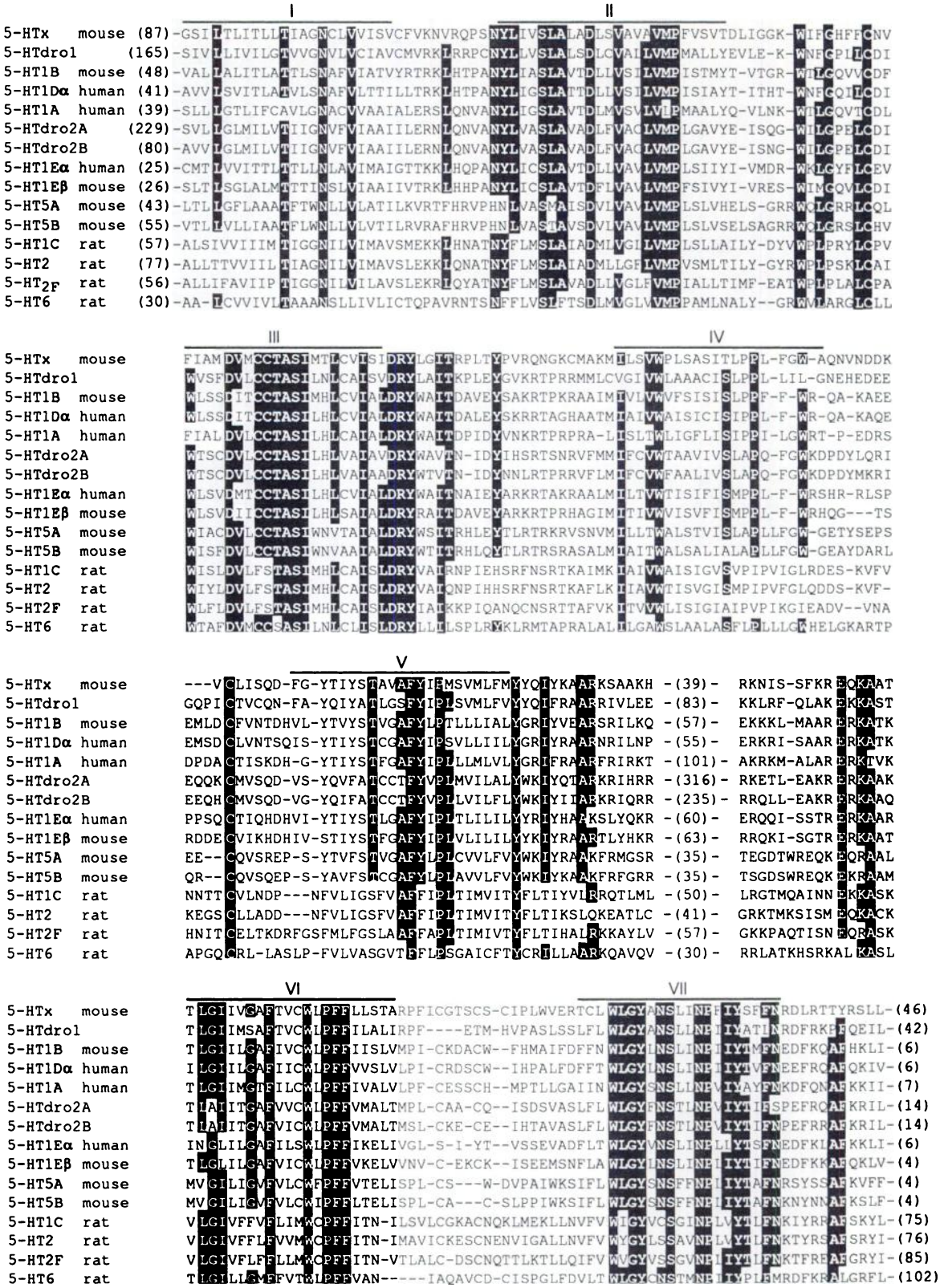

Fig. 2. Amino acid similarity between the 5-HT$_x$ receptor and other 5-HT receptors. The amino acid sequences of the mouse 5-HT$_x$ receptor and the *Drosophila* 5-HT$_{dro1}$ (20), mouse 5-HT$_{1B}$ (7), human 5-HT$_{1D\alpha}$ (8), human 5-HT$_{1A}$ (5), *Drosophila* 5-HT$_{dro2A}$ and 5-HT$_{dro2B}$ (38), human 5-HT$_{1E\alpha}$ (9), mouse 5-HT$_{1E\beta}$ (recently named 5-HT$_{1F}$) (11), mouse 5-HT$_{5A}$ (16) and 5-HT$_{5B}$ (17), rat 5-HT$_{1C}$ (recently named 5-HT$_{2C}$) (14) and 5-HT$_2$ (recently named 5-HT$_{2A}$) (13), rat stomach fundus 5-HT$_{2F}$ (recently named 5-HT$_{2B}$) (15), and rat 5-HT$_6$ (21) receptors were aligned. *Numbers in parentheses*, number of amino acids that are not represented. Putative transmembrane domains are indicated (*I* to *VII*).

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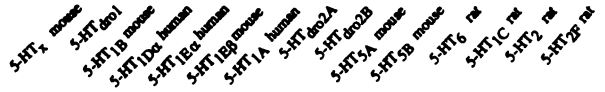
A

5-HT$_x$	mouse	100													
5-HT$_{dro1}$		42	100												
5-HT$_{1B}$	mouse	36	38	100											
5-HT$_{1D\alpha}$	human	36	41	69	100										
5-HT$_{1E\alpha}$	human	36	37	52	52	100									
5-HT$_{1E\beta}$	mouse	36	40	51	53	59	100								
5-HT$_{1A}$	human	34	43	47	46	42	44	100							
5-HT$_{dro2A}$		34	41	41	40	37	42	42	100						
5-HT$_{dro2B}$		34	40	40	41	38	42	42	82	100					
5-HT$_{5A}$	mouse	32	35	34	35	35	36	34	37	37	100				
5-HT$_{5B}$	mouse	32	35	34	34	34	33	34	38	38	73	100			
5-HT$_6$	rat	30	33	27	29	29	27	27	28	29	29	28	100		
5-HT$_{1C}$	rat	27	30	29	31	33	31	28	28	29	27	28	28	100	
5-HT$_2$	rat	29	27	30	29	30	28	28	28	28	27	26	30	67	100
5-HT$_{2F}$	rat	24	29	27	27	27	26	29	28	28	26	26	28	55	58	100



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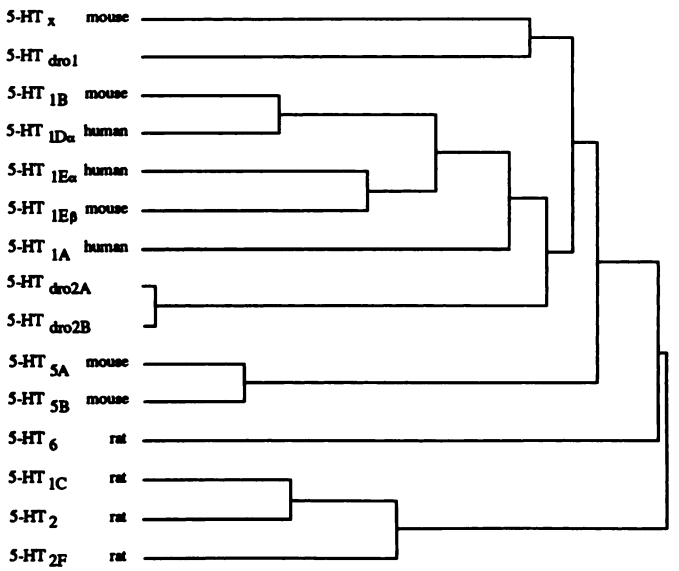

Fig. 3. A, Percentages of amino acid homology between the 5-HT$_x$ receptor and other 5-HT receptors. These percentages of homology were calculated over the sequences that are represented in Fig. 2. B, Dendrogram. The sequences of the 5-HT receptors were compared and clustered with the program CLUSTAL (39). The lengths of the *horizontal lines* are inversely proportional to the percentages of homology between receptors or groups of receptors.

used to screen a mouse brain cDNA library constructed in the Uni-Zap phage (Stratagene). Positive phages were isolated, and the cDNA inserts were recovered in the Bluescript plasmid and sequenced on both strands by the dideoxynucleotide technique, using successive synthetic oligonucleotides.

Expression of the 5-HT$_x$ receptor in COS-7 cells. The *Eco*RI-*Xho*I cDNA fragment (Fig. 1) was inserted between the *Eco*RI and *Xho*I sites of expression vector p513, which is a derivative of pSG5 (22) containing a multiple cloning site. The resulting recombinant was introduced into COS-7 cells by calcium phosphate-mediated transfection (20 μg/10-cm dish). Cells were exposed to the precipitate for 24 hr, after which the medium was replaced with fresh medium. Twenty-four hours later the cells were harvested or the cAMP assay was performed.

cAMP assays. COS-7 cells were seeded into 12-well plates at a density of 10^5 cells/well. Cells were transfected as described above. Forty-eight hours later cells were washed once with phosphate-buffered saline and incubated for 15 min at 37° with 100 μM isobutylmethylxanthine and test agents in phosphate-buffered saline. The reaction was stopped by aspiration of the medium, followed by the addition of 500 μl of ice-cold ethanol. After 2 hr at room temperature, the ethanol fraction was collected and lyophilized. The pellet was reconstituted and cAMP was quantified using a radioimmunoassay (Immunotech radioimmunoassay kit 1117).

Radioligand binding assay. Membranes were prepared as described (23). [^{3}H]5-HT saturation and competition binding experiments were performed with 10–20 μg of protein/sample, in a final volume of 500 μl of 50 mM Tris·HCl, pH 7.4, 4 mM $CaCl_2$, at 37° for 20 min. Reactions were terminated by vacuum filtration over Whatman GF/B glass fiber filters, which were then rinsed four times with 4 ml of 50 mM Tris·HCl, pH 7.4. Nonspecific binding was defined with 10 μM 5-HT.

PCR analysis. To perform PCR analysis we used the following oligonucleotides: (i) CCGACAGAATCATTTTGGCCATAC and (ii) GAGCAGATCAACTATGGCAGAGTC, corresponding to positions 733 and 331, respectively (Fig. 1). One microgram of total RNA from various organs was reverse transcribed with 5 units of avian myeloblastosis virus reverse transcriptase (Pharmacia) and 300 ng of oligonucleotide i for 45 min at 42°. One fifth of that reaction was amplified in the presence of 5 units of *T. aquaticus* polymerase (Cetus) and 500 ng of oligonucleotides i and ii for 20 cycles. These PCR products were transferred to filters and hybridized with the *Eco*RI-*Xho*I cDNA probe. This fragment was ^{32}P-labeled by random priming and was hybridized to the filter at high stringency (42°, 50% formamide, 5× saline sodium citrate (0.75 M NaCl 175 mM sodium citrate), 1× Denhardt's solution, 20 mM sodium phosphate buffer, pH 6.5, 0.1% sodium dodecyl sulfate, 100 μg/ml tRNA). Washings were performed at high stringency (60°, 0.1× saline sodium citrate, 0.1% sodium dodecyl sulfate).

Results

The isolated mouse cDNA clone encodes a new member of the G protein-coupled receptor family. Sequence comparisons of serotonin receptors have revealed a striking amino acid sequence conservation, particularly in certain putative transmembrane domains such as domains III, VI, and VII. We therefore decided to use degenerate oligonucleotides corresponding to these regions to perform a series of reverse PCR experiments on RNA extracted from mouse colliculi neurons. The resulting fragments were subcloned and sequenced. One of these fragments was used to screen a mouse brain cDNA library. We obtained a phage recombinant that contained a 3.2-kilobase cDNA insert. Sequence analysis revealed one long open reading frame (448 amino acids) (Fig. 1). Hydropathy analysis of the predicted protein revealed seven hydrophobic domains (numbered I to VII in Fig. 1), a feature shared by all other cloned members of the G protein-coupled receptor family. The amino-terminal end displayed two putative sites for *N*-linked glycosylation and the presumed cytoplasmic domains contained consensus sites for phosphorylation by protein kinases C and A (Fig. 1), features that are found in most members of that family.

Amino acid sequence comparisons revealed homologies with G protein-coupled receptors in the putative transmembrane domains and short connecting loops but not in the amino- and carboxyl-terminal ends or in the third cytoplasmic loop, which are very variable in sequence and in length within this gene

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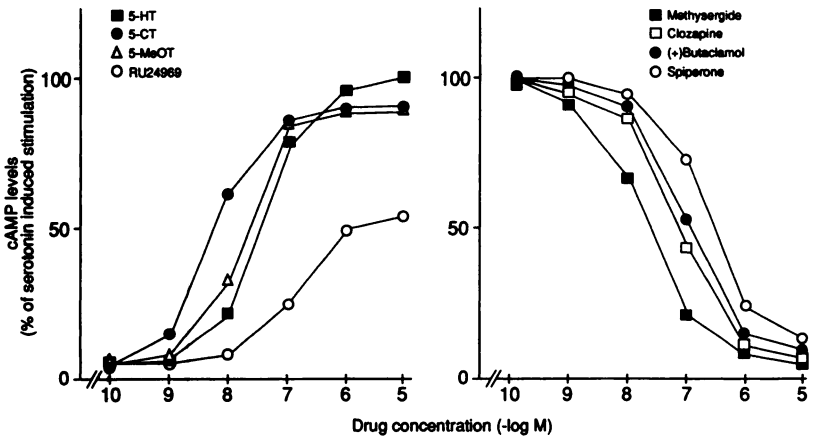

Fig. 4. 5-HT-induced increase in cAMP levels in COS-7 cells expressing the 5-HT$_x$ receptor and the effect of agonists and antagonists. COS-7 cells were transfected and cAMP levels were determined as described in Materials and Methods. *Left*, effect of various concentrations of 5-HT, 5-CT, 5-MeOT, and RU 24969. cAMP levels were expressed as a percentage of the value obtained with 10 μM 5-HT. Typically, 10 μM 5-HT yielded an 8-fold increase in cAMP levels (from 0.25 to 2.0 pmol/10^5 cells). *Right*, antagonist effect of methysergide, clozapine, (+)-butaclamol, and spiperone on cAMP levels induced by 100 nM 5-HT. cAMP levels were expressed as a percentage of the values obtained with 100 nM 5-HT. Data are representative of at least two independent experiments, with each *point* being measured in triplicate.

TABLE 1

Pharmacological profile of the 5-HT$_x$ receptor

Binding data correspond to competition for [^{3}H]5-HT binding to membranes of COS-7 cells transiently expressing the 5-HT$_x$ receptor. IC_{50} values required to displace 50% of [^{3}H]5-HT binding were determined experimentally and converted to pK_d values according to the equation $K_d = IC_{50}/(1 + C/K_d)$, where C is the [^{3}H]5-HT concentration (2 nM) and K_d is the equilibrium dissociation constant of [^{3}H]5-HT. cAMP experiments were performed as described in Materials and Methods and in the legend to Fig. 4. 2-Bromo-LSD, bromocriptine, dihydroergocryptine (DHC), buspirone, and TFMPP behaved as antagonists but not as agonists at 10 μM. EC_{50} values were determined experimentally and correspond to the concentrations of agonists required to obtain a half-maximal stimulation of adenylate cyclase. The efficacy of agonists corresponds to the maximal stimulation of adenylate cyclase, and values are expressed as a percentage of the value obtained with 10 μM 5-HT. The concentrations of antagonists required to obtain a half-maximal inhibition of 5-HT-induced cAMP levels (IC_{50}) were determined experimentally and converted to pK_i values according to the equation $K_i = IC_{50}/(1 + C/K_d)$, where C is the 5-HT concentration (100 nM) and K_d is the EC_{50} value for 5-HT (45 nM). The presented data are representative of at least two independent experiments with each point being measured in triplicate.

Agonist	Binding, pK_d	Cyclase stimulation, pEC_{50}	Efficacy	Antagonists	Binding, pK_d	Cyclase stimulation, pK_i
			%			
5-CT	9.0	8.3	91	Methiothepin	8.2	7.7
5-HT	8.3	7.3	100	2-Bromo-LSD	8.0	
5-MeOT	8.2	7.7	89	Methysergide	7.9	8.2
Bufotenine	7.0	6.3	92	Mesulergine	7.6	7.6
RU 24969	6.9	6.7	54	Metergoline	7.5	7.6
8-OH-DPAT	6.6	6.1	95	(+)-Butaclamol	7.5	7.5
Cisapride	5.8	<5		Clozapine	7.4	7.7
Sumatriptan	5.7	<5		Ergotamine	7.3	7.7
Dopamine	4.7	<5		Spiperone	7.2	7.2
DOI[a]	4.6	<5		DHC	7.0	
(−) Norepinephrine	3.8			Mianserin	7.0	
				Bromocriptins	6.9	
				Ketanserin	6.4	6.4
				Amitriptyline	6.4	
				Buspirone	6.4	
				Haloperidol	6.3	5.3
				TFMPP	6.3	
				Chlorpromazine	5.3	
				(−)-Butaclamol	4.8	
				(−)-Pindolol	4.7	<5
				(−)-Propranolol	4.7	
				Remoxipride	<4	

[a] DOI, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane.

family. Percentages of homology were therefore calculated over the conserved regions (Fig. 2) and were used to establish a dendrogram (Fig. 3B). The percentages of homology with other known receptors are low (Fig. 3A), with the best score being 42% with the *Drosophila* serotonin receptor 5-HT$_{dro1}$. The next closest receptors are the 5-HT$_{1B}$, 5-HT$_{1D}$, and 5-HT$_{1E}$ serotonergic receptors (36%).

The 5-HT$_x$ receptor is positively coupled to adenylate cyclase. To determine whether our cDNA clone encoded a functional receptor, we introduced it into a eukaryotic expression vector and transfected COS-7 cells with the resulting recombinant. Serotonin induced an increase in cAMP levels that was dose dependent and saturable (EC_{50} = 45 nM) (Fig. 4). In a control experiment, serotonin had no effect on cAMP levels in nontransfected COS-7 cells. This result suggests that we have isolated a 5-HT receptor that is positively coupled to adenylate cyclase. To characterize this new receptor we analyzed cAMP levels in response to a number of serotonergic agonists and antagonists. 5-CT, a 5-HT$_1$ agonist, was the most efficient compound at increasing cAMP levels in COS-7 cells transiently expressing the 5-HT$_x$ receptor. The rank order of potencies of serotonergic agonists was as follows: 5-CT > 5-MeOT > 5-HT > RU 24969 > bufotenin > 8-OH-DPAT (Fig. 4; Table 1). Sumatriptan, TFMPP, buspirone, and (−)-pindolol were inactive. We also tested the ability of various serotonergic drugs to antagonize the stimulatory activity of 5-HT. Methio-

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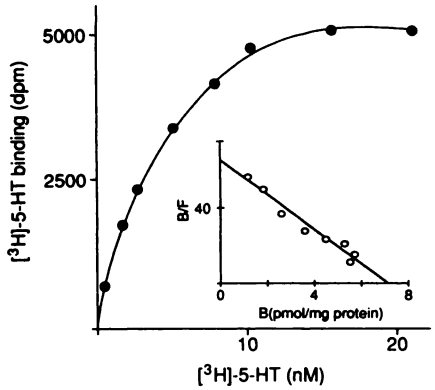

Fig. 5. Saturation isotherm of [^{3}H]5-HT binding to membranes of COS-7 cells expressing the 5-HT$_x$ receptor. Membranes were incubated with concentrations of [^{3}H]5-HT ranging from 0.1 nM to 20 nM, with or without 10 μM 5-HT. Specific binding is represented. *Inset*, Scatchard analysis of [^{3}H]5-HT binding (K_d = 3.6 nM, B_{max} = 6.9 pmol of receptor/mg of membrane protein). Data are representative of three independent experiments, with each *point* being measured in triplicate.

thepin, methysergide, and ergotamine, which are nonspecific 5-HT antagonists, and mesulergine, which is a 5-HT$_{1C}$/5-HT$_2$ antagonist, were the most efficient compounds at decreasing cAMP levels (Fig. 4; Table 1). Spiperone, a 5-HT$_{1A}$/5-HT$_2$ antagonist, was active and ketanserin, a 5-HT$_2$ antagonist, was weakly active. The 5-HT$_{1A}$ agonist 8-OH-DPAT was a weak agonist at the 5-HT$_x$ receptor but (−)-pindolol, a 5-HT$_{1A}$ antagonist, was inactive. The 5-HT$_4$ agonist cisapride had no effect on the 5-HT$_x$ receptor. The 5-HT$_x$ receptor differs also from the 5-HT$_4$ receptor in its high affinity for methiothepin, mesulergine, metergoline, and spiperone, which are all inactive at the 5-HT$_4$ receptor (19). The profile for these agonists and antagonists does not correspond to the profile of any classical 5-HT receptor subtype. Interestingly, two neuroleptics, (+)-butaclamol and clozapine, were efficient antagonists of the 5-HT$_x$ receptor.

The 5-HT$_x$ receptor displays a high affinity for [^{3}H]5-HT. To further characterize the 5-HT$_x$ receptor we performed binding assays on membranes of COS-7 cells transiently expressing the 5-HT$_x$ receptor. [^{3}H]5-HT displayed a single saturable binding site in membranes of transfected cells, whereas no specific binding was found in membranes of nontransfected cells (K_d = 3.6 nM and B_{max} = 6.9 pmol/mg of membrane protein) (Fig. 5). [^{3}H]5-HT binding was displaced by a number of drugs, with the following order of potencies: 5-CT > 5-HT = 5-MeOT = methiothepin > 2-bromo-LSD > methysergide > mesulergine > (+)-butaclamol = clozapine > spiperone > RU 24969 > 8-OH-DPAT (Table 1). This pharmacological profile is atypical, because the 5-HT$_x$ receptor has a high affinity both for 5-HT$_1$ ligands such as 5-CT and for 5-HT$_2$/5-HT$_{1C}$ ligands such as mesulergine. 5-CT, 5-HT, 5-MeOT, bufotenin, and 8-OH-DPAT, which are all agonists of the 5-HT$_x$ receptor, were more potent at displacing [^{3}H]5-HT than at stimulating adenylate cyclase. In contrast, in the case of antagonists the pK_i values obtained in the binding experiments were similar to those obtained in the cAMP experiments. Such a difference of behavior between agonists and antagonists has often been observed with G protein-coupled receptors and suggests that [^{3}H]5-HT might label the high affinity state of the 5-HT$_x$ receptor. However, we have not been able to confirm this hypothesis thus far, because Gpp(NH)p had little or no effect on [^{3}H]5-HT binding (data not shown).

The 5-HT$_x$ receptor is expressed in the central nervous system as well as in intestine and heart. Expression of the 5-HT$_x$ transcripts was analyzed by Northern analyses and reverse PCR experiments. The Northern analysis performed on poly(A)$^+$ RNA samples extracted from various adult mouse tissues (brain, heart, kidney, lung, liver, and intestine) did not reveal any transcripts in these organs (data not shown). Because the 5-HT$_x$ cDNA had been isolated from a mouse brain cDNA library the corresponding mRNA was likely to be found in brain. To detect this mRNA we used a more sensitive technique, reverse PCR, which was performed on total RNA from various organs and which yielded a specific 404-bp fragment (see Materials and Methods and Fig. 6). The strongest signal was detected in brainstem. A weaker signal was found in forebrain, cerebellum, and embryonal colliculi neurons. In peripheral organs, a weak signal was detected in intestine and heart. In a control experiment, no bands could be detected when reverse transcriptase was omitted, indicating that the signal we observe corresponds to mRNA and not to contaminant genomic DNA.

Discussion

We have isolated a novel 5-HT receptor that is positively coupled to adenylate cyclase. The amino acid sequence of this

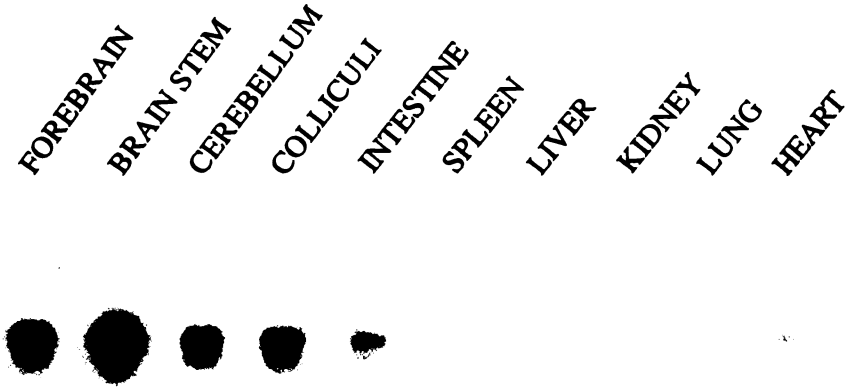

Fig. 6. Distribution of 5-HT$_x$ transcripts. PCR analysis was performed with 1 μg of total RNA from various organs, as described in Materials and Methods. The specific 404-bp PCR product corresponds to the 5-HT$_x$ mRNA.

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receptor is not closely related to that of any other mammalian serotonin receptor. The nearest relative of the 5-HT_x receptor is the *Drosophila* 5-HT_{dro1} receptor, which is also coupled positively to adenylate cyclase. The resemblance between these receptors might therefore be related to their common coupling to second messengers. A similar situation was found in the 5-HT_1 and 5-HT_2 families, which can be distinguished by their interactions with second messengers. All of the members of the 5-HT_1 family, including the mammalian 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-$HT_{1E\alpha}$, and 5-$HT_{1E\beta}$ receptors, as well as two *Drosophila* receptors, 5-HT_{dro2A} and 5-HT_{dro2B}, are negatively coupled to adenylate cyclase. Similarly, all members of the 5-HT_2 family, such as the 5-HT_2, 5-HT_{1C}, and recently cloned stomach fundus 5-HT_{2F} receptors, activate phospholipase C. The existence in both vertebrates and invertebrates of families of 5-HT receptors with distinct intracellular signaling properties suggests that these families appeared early in evolution and that they might play distinct fundamental roles in all nervous systems.

To obtain insights into the possible physiological roles of the 5-HT_x receptor, we have tried to relate it to receptors with similar properties that have already been reported. Unfortunately, few 5-HT receptors positively coupled to adenylate cyclase and expressed in the central nervous system have been characterized in sufficient detail to allow a meaningful comparison with the 5-HT_x receptor. These receptors include the 5-HT_4 receptor expressed in embryonal colliculi neurons and in hippocampus (19) and a 5-HT_{1A}-like receptor expressed in hippocampus (24, 25). Compared with the 5-HT_{1A} receptor, the 5-HT_x receptor has a lower affinity for 8-OH-DPAT and spiperone and a higher affinity for mesulergine and clozapine. Concerning the 5-HT_4 receptor, there is even less resemblance. The 5-HT_x receptor has a much higher affinity for 5-CT, 8-OH-DPAT, RU 24969, spiperone, methiothepin, and mesulergine than does the 5-HT_4 receptor. The 5-HT_x receptor also does not correspond to a number of other stimulatory 5-HT receptors such as those found in NCB20 cells (26) and in pulmonary artery smooth muscle cells (27). In addition, the 5-HT_x receptor differs markedly from a recently cloned 5-HT receptor that is also positively coupled to adenylate cyclase and that has been tentatively named 5-HT_6 (21). Although the amino acid sequence of the 5-HT_x receptor is related most closely to that of the 5-HT_{dro1} receptor and to a lesser extent to those of members of the 5-HT_1 family, the 5-HT_6 receptor does not resemble any other 5-HT receptor (Fig. 3). Unlike the 5-HT_x receptor, the 5-HT_6 receptor has a low affinity for 5-CT and mesulergine (21). There are a number of additional cases where 5-HT-sensitive adenylate cyclases have been reported, such as in cortex (28), hypothalamus, and spinal cord (29, 30), but the pharmacological profiles of these activities have not been established with enough compounds to allow a comparison with the 5-HT_x receptor. In the cardiovascular and gastrointestinal systems, there are also reports of 5-HT_1-like receptors stimulating adenylate cyclase (31). In particular, 5-CT has been shown to mediate relaxation and elevation of cAMP in the porcine vena cava (32). In addition, a 5-HT_1-like receptor that induces tachycardia in isolated cat hearts displays a pharmacological profile that is similar to the profile of the 5-HT_x receptor (33–35). In both cases, agonists and antagonists display the same rank order of potencies; 5-CT is the most efficient agonist, whereas methiothepin is the strongest antagonist. This receptor might correspond to the 5-HT_x receptor, because we could detect 5-HT_x mRNA in heart. Another receptor that might be related to the 5-HT_x receptor induces relaxation in guinea pig ileum and is labeled by ^{125}I-LSD (36). The order of potency of agonists is 5-CT > 5-HT > 8-OH-DPAT = RU 24969, whereas the order of potencies of antagonists is metergoline = mesulergine > spiperone > haloperidol. Such a profile, where metergoline, mesulergine, and spiperone display similar high potencies ($8 > pK_i > 7$), does not correspond to that of any of the classic 5-HT subtypes. Again, the fact that we found 5-HT_x mRNA in intestine makes it a good candidate for the 5-HT receptor that relaxes the guinea pig ileum.

Interestingly, a number of therapeutically important drugs, such as the typical antipsychotic drugs butaclamol and spiperone and the atypical antipsychotic agent clozapine, have a high affinity for the 5-HT_x receptor. These drugs are believed to exert their therapeutic effects by interacting mainly with D_2 dopaminergic receptors. However, atypical effects of clozapine such as a reduced incidence of extrapyramidal side effects have been suggested to be mediated by D_4 receptors or 5-HT_2 receptors, both of which have a higher affinity for clozapine than do D_2 receptors (37). The high affinity of clozapine for the 5-HT_x receptor and the fact that this receptor is expressed in the brain makes this receptor an additional candidate to explain some of the atypical effects of clozapine. A more precise localization of the sites of expression of the 5-HT_x receptor might allow us to further document this idea. In addition, the availability of the 5-HT_x gene should allow us to alter the expression of the 5-HT_x receptor *in vivo* and to study the consequences of such alterations for physiology and behavior.

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