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The pharmacology and distribution of human 5-hydroxytryptamine$_{2B}$ (5-HT$_{2B}$) receptor gene products: comparison with 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors

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1 Full length clones of the human 5-HT$_{2B}$ receptor were isolated from human liver, kidney and pancreas. The cloned human 5-HT$_{2B}$ receptors had a high degree of homology (~80%) with the rat and mouse 5-HT$_{2B}$ receptors.

2 PCR amplification was used to determine the tissue distribution of human 5-HT$_{2B}$ receptor mRNA. mRNA encoding the 5-HT$_{2B}$ receptor was expressed with greatest abundance in human liver and kidney. Lower levels of expression were detected in cerebral cortex, whole brain, pancreas and spleen. Expression was not detected in heart.

3 Northern blot analysis confirmed the presence of 5-HT$_{2B}$ receptor mRNA (a 2.4 kB sized band) in pancreas, liver and kidney. An additional 3.2 kB sized band of hybridization was detected in liver and kidney. This raises the possibility of a splice variant of the receptor or the presence of an additional homologous receptor.

4 The human 5-HT$_{2B}$ receptor was expressed in Cos-7 cells and its ligand binding characteristics were compared to similarly expressed human 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors. The ligand specificity of the human 5-HT$_{2B}$ receptor (5-HT > ritanserin > SB 204741 > spiperone) was distinct from that of the human 5-HT$_{2A}$ (ritanserin > spiperone > 5-HT > SB 204741) and 5-HT$_{2C}$ (ritanserin > 5-HT > spiperone = SB 204741) receptors. On the basis of a higher affinity for ketanserin and a lower affinity for yohimbine the human 5-HT$_{2B}$ receptor also appeared to differ from the rat 5-HT$_{2B}$ receptor.

5 These findings confirm the sequence of the human 5-HT$_{2B}$ receptor and they demonstrate that the receptor has a widespread tissue distribution. In addition, these data suggest that there are differences in ligand affinities between different species homologues of the receptor. Finally, the finding of two distinct bands on the Northern blots of liver and kidney raises the possibility of splice variants or subtypes of 5-HT$_{2B}$ receptors, within these tissues.

Keywords: 5-Hydroxytryptamine; 5-HT$_{2A}$, 5-HT$_{2B}$, 5-HT$_{2C}$ receptors; human 5-HT receptors

Introduction

5-HT$_{2B}$ receptors are one of three members of the 5-hydroxytryptamine$_2$ (5-HT$_2$) family of 5-HT receptors. 5-HT$_{2B}$ receptors, like 5-HT$_{2A}$ (previously denoted as 5-HT$_2$) and 5-HT$_{2C}$ (previously denoted as 5-HT$_{1C}$) receptors are G-protein linked receptors, positively coupled to phosphoinositide metabolism. This family of 5-HT receptors share sequence homology and have the same pattern of introns and exons. Similarities in the specificity of 5-HT$_{2B}$, 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors for ligands further indicates the commonality of receptors in this family. Recent reviews of this area have been written by Martin & Humphrey (1994) and Hoyer *et al.* (1994).

5-HT$_{2B}$ receptors, initially termed 5-HT$_{2F}$ or serotonin-like receptor, were characterized in rat isolated stomach fundus (Vane, 1959; Clineschmidt *et al.*, 1985; Cohen & Wittenauer, 1987; Cohen, 1989). 5-HT-evoked contractions were found to be mediated by a receptor pharmacologically similar to the 5-HT$_{2C}$ receptor. However, relatively high affinities for 5-HT and yohimbine and relatively low affinity for ketanserin distinguished this receptor from 5-HT$_{2C}$ and 5-HT$_{2A}$ receptors (Baxter *et al.*, 1994a). Recently, the pharmacological specificity of 5-HT$_{2B}$ receptors has been further defined with the high affinity (pK_i = 7.1) 5-HT$_{2B}$ selective, antagonist, SB 204741 (Baxter *et al.*, 1994b).

Little is known about the function of 5-HT$_{2B}$ receptors in tissues other than the stomach fundus but, based on Northern blot analysis or PCR amplification of tissue-specific cDNAs, the rat (Foguet *et al.*, 1992a; Kursar *et al.*, 1992) and mouse (Foguet *et al.*, 1992b) 5-HT$_{2B}$ receptors are expressed in a several peripheral and central tissues including stomach fundus, liver, kidney, muscle, intestine and brain. This distribution, together with the discovery of a 5-HT receptor in vascular tissue with pharmacology similar to that of the 5-HT$_{2B}$ receptor (Glusa & Richter, 1993), suggests a wide ranging role for this receptor in mediating the actions of 5-HT.

Expression of the cloned 5-HT$_{2B}$ receptor has allowed its characterization by radioligand binding. The cloned receptor can be labelled with high affinity (K_i~1 nM) using [^{3}H]-5-HT and it has affinities for 5-HT$_2$ receptor ligands that are similar to those found in the stomach fundus assay. However, several small differences in the affinities of ligands for rat (Wainscott *et al.*, 1993) and mouse (Loric *et al.*, 1992) cloned 5-HT$_{2B}$ receptors suggest that there may be inter-species differences in ligand binding properties of 5-HT$_{2B}$ receptors.

Given the possibility of inter-species differences in 5-HT$_{2B}$ receptor pharmacology and given the paucity of pharmacological information on human 5-HT$_{2B}$ receptors, the goals of the current study were to clone a human 5-HT$_{2B}$ receptor, to assess its expression in a variety of tissues, and to characterize its pharmacological properties. Consequently, the distribution and ligand binding properties of the human cloned 5-HT$_{2B}$ receptor were compared to those of the human 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors. The ligand binding properties of these cloned

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receptors were also compared to their rat homologues and, where possible, to native 5-HT$_2$ receptors in human post-mortem tissue.

Methods

Cloning and expression of human 5-HT$_2$ receptors

The full length human 5-HT$_{2A}$ receptor gene was amplified from human brain cDNA using the PCR primers CTACAAGTTCTGGCTTAGACATG (sense) and CACGGCAACTAGCCTATCACACAC (anti-sense). The receptor was cloned into the PCRII (TA) vector and then sub-cloned into the eukaryotic expression vector pSW104. The clone was then sequenced on both strands.

To clone the human 5-HT$_{2B}$ receptor, PCR primers based on the third exon of the mouse 5-HT$_{2B}$ receptor were used to isolate a 200 base-pair probe from human genomic DNA. This probe was extended in the 3' direction using a two step, nested PCR, procedure and human liver cDNA as a template. The PCR primers, CTATGTCCTGCCTGGTTATTTCT (sense) and ACGAAIACIATGAAGAAIGGGCA (degenerate anti-sense primer) were used for the first amplification step while the PCR primers TATGTCCTGCCTGGTTATTTC (sense nested primer) and ACGAAGCAGATGAAGAAGGGGCACCACAT (anti-sense nested primer) were used for the second amplification step. The resulting 650 base-pair product was used as a probe to screen 1×10^6 plaques of a human liver library. One cDNA clone was identified. This clone encompassed the entire 5' coding region but lacked 50 amino acids from the C terminus. The 3' end of the human 5-HT$_{2B}$ receptor was generated using λgt10 flanking primer and the specific sense primer TGCCATGTACCAGAGTCCAATGAG. Based on the above data, two primers were designed to amplify the complete human 5-HT$_{2B}$ receptor gene. The full length 5-HT$_{2B}$ receptor was then amplified from human liver, kidney and pancreas cDNA using CTCGAGCTCAGCAAATGGCTCTCTCTTACAGAG (sense) and CTTCGCTAGCTATACATAACTAACTTGCTCTTC (anti-sense) primers. These PCR products were sub-cloned into SAC I and Nhe I ends of the poly-linker region of the mammalian expression vector pSW104, a derivative of pCD-SRα (Takebe *et al.*, 1988).

The full length human 5-HT$_{2C}$ receptor gene was amplified from human brain cDNA using the PCR primers TAACACTGAAGCAATCATGG (sense) and GACTGTGCTGTTCTTTCTCACAC (anti-sense). The 1.5 kB amplified product was cloned into PCRII (TA) cloning vector and then sub-cloned into the eukaryotic expression vector pSW104. The clone was sequenced and confirmed to be that of the 5-HT$_{2C}$ receptor.

5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors were transfected into Cos-7 cells using the lipofection method (Flegner *et al.*, 1987). Cells were harvested 65 h after transfection in phosphate buffered saline and centrifuged at 3000 *g* for 15 min. Cell pellets were frozen until required for radioligand binding studies.

Expression of rat 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors

NIH-3T3 mouse fibroblast cells expressing rat 5-HT$_{2C}$ or 5-HT$_{2A}$ receptors were grown in Dulbecco's Modified Essential Media with 10% foetal calf serum and 250 μg ml^{-1} Geneticin in 5% CO_2 until confluent. Cells were then harvested as described above.

Preparation of membranes from cells and human tissues

Tissues were either homogenized in 0.32 M sucrose using a motor driven (550 rpm. 10 strokes), glass teflon homogenizer (human cortex) or were disrupted with a polytron (human choroid plexus). Tissue homogenates were centrifuged at 900 *g* for 10 min and the pellets were washed once in 0.32 M sucrose. The supernatants were combined and then centrifuged at 12,000 *g* (cortex) or 45,000 *g* (choroid plexus) for 15 min. The resulting pellets were resuspended, incubated at 37°C for 15 min, and then centrifuged at 48000 *g* for 15 min. The final pellet was resuspended in a small volume of 50 mM Tris-HCl, divided into aliquots of 1 ml and stored at −70°C until required.

Cells containing expressed receptors were homogenized in 50 mM Tris-HCl with 5 mM Na_2EDTA (pH 7.4 at 4°C) using a Polytron tissue disrupter. The homogenates were centrifuged at 48,000 *g* for 15 min. The pellets were washed by re-suspension and centrifugation in homogenizing buffer and incubated at 37°C for 15 min. After an additional wash, the membranes were stored in 1 ml aliquots at −70°C.

Receptor binding methods

5-HT$_{2A}$ receptors were labelled with [^{3}H]-ketanserin in human cortex, in Cos-7 cells expressing a cloned human 5-HT$_{2A}$ receptor and in NIH3T3 cells expressing the rat 5-HT$_{2A}$ receptor. For competition binding studies the ligand concentration was approximately 0.1 nM. For saturation binding studies concentrations of radioligand ranged from 0.01 nM to 2.0 nM. Assays were conducted in 0.5 ml of assay buffer containing 50 mM Tris-HCl, 4 mM $CaCl_2$ and 0.1% ascorbic acid (pH 7.4 at 4°C). Non-specific binding was defined with 10 μM unlabelled ketanserin. After a 60 min incubation at 32°C, membranes were harvested onto filters treated with 0.1% polyethylenimine and the bound radioactivity was determined.

Human 5-HT$_{2B}$ receptors were labelled in Cos-7 cells as described above except that the radioligand was [^{3}H]-5-HT and that the assay buffer contained 10 μM pargyline and 0.1% ascorbic acid. For competition binding studies the radioligand concentration was approximately 0.4 nM while for saturation binding studies the concentration of [^{3}H]-5-HT ranged from 0.05 to 8 nM. Non-specific binding was defined with 10 μM 5-HT. Incubations were for 120 min at 4°C.

5-HT$_{2C}$ receptors were labelled in choroid plexus, Cos-7 cells expressing the human 5-HT$_{2C}$ receptor and in NIH3T3 cells expressing the rat 5-HT$_{2C}$ receptor. Assays were conducted as described for the 5-HT$_{2A}$ receptor except that the radioligand was [^{3}H]-mesulergine. The radioligand concentration for competition studies was approximately 0.2 nM while for saturation binding studies the concentration ranged from 0.1 to 18 nM. Nonspecific binding was defined with 10 μM unlabelled mesulergine.

Competition radioligand binding data were analysed with a four parameter logistic equation and iterative curve-fitting techniques to obtain estimates of the IC_{50} and Hill slope. K_d values, determined from saturation binding studies, where then used to calculate inhibition dissociation constants (K_i) according to the method described by Cheng & Prusoff (1973). Data were reported as the negative logarithm of the K_i (pK_i) rather than K_i since pK_i values tend to be normally distributed thus allowing presentation of mean and standard error of the mean.

Saturation binding data were analysed with the iterative nonlinear curve fitting programmes in 'Ligand' (Munson & Rodbard, 1980). Protein concentrations were determined with the Biorad colorimetrical method with bovine gamma-globulin as the standard (Bradford, 1979). Comparison of binding affinities of specific ligands in different tissues was made using independent *t* tests with a *P* value of less than 0.05 used as the criteria for statistically significant differences (CSS statistical package, Tulsa, OK U.S.A.).

Northern and reverse transcription PCR methods for determining receptor distribution

Northern analysis was performed on multiple tissue blots using RNA probes labelled with ^{32}P by random priming. Hybridization was conducted under conditions of medium strin-

gency (42°C, 50% formamide/5 × SSC, 5 × Denhardt's solution, 2% SDS and 100 μg ml^{-1} single strand DNA).

Reverse transcription - PCR was conducted using primers containing specific sequences appropriate for each receptor subtype. cDNA from human cerebral cortex, pancreas, kidney, heart, liver, spleen and whole brain were screened using a two step nested PCR amplification procedure. For the first cycle of PCR the samples were denatured at 94°C for 1 min, annealed at 50°C for 2 min and extended at 72°C for 3 min for 30 cycles. The second step of the PCR reaction was performed using 10 μl from the first step as the template and primers that were internal to those used in the first step to amplification. PCR conditions were identical except that the annealing temperature was increased to 55°C. The PCR products were run on a 1% agarose gel and bands were detected by staining with ethidium bromide. The identities of the PCR products were confirmed by sequencing.

Materials

[^{3}H]-mesulergine (75 Ci mmol^{-1}, 99% pure) and [^{3}H]-5-HT (91 Ci mmol^{-1}, 99% pure) were obtained from Amersham Corporation (Arlington Heights, IL, U.S.A.). [^{3}H]-ketanserin, (62 Ci mmol^{-1}, 99% pure) was obtained from Dupont-NEN (Boston, MA, U.S.A.) Methysergide was a gift from Sandoz Pharma Ltd (Basel, Switzerland). Paroxetine was a gift from SmithKline Beecham Pharmaceuticals (Essex, England). SB 200646A, (N-(1-methyl-5-indolyl)-*N'*-(3-pyridyl)urea hydrochloride (Forbes *et al.*, 1993)), its analogue SB 204741 (Baxter *et al.*, 1994b) and 5-carboxamidotryptamine (5-CT) were synthesized in the Institute of Organic Chemistry, Syntex Discovery Research (Palo Alto, CA U.S.A.). Other chemicals and reagents were purchased from Sigma Chemicals (St. Louis MO U.S.A.) or Research Biochemicals Incorporated (Natick, MA U.S.A.). NIH3T3 cells transfected with rat 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors were a generous gift from Dr David Julius, Department of Pharmacology, University of California, San Francisco, U.S.A. Post mortem brain tissues were obtained from the Cooperative Human Tissue Network (CHTN) and the National Disease Research Interchange (NDRI) or Dr Gavin Reynolds (University of Sheffield, United Kingdom). Human cDNA libraries and human poly-A RNA were obtained from Clontech, Palo Alto, CA U.S.A.. The TA cloning vector was obtained from Invitrogen, San Diego, CA U.S.A.

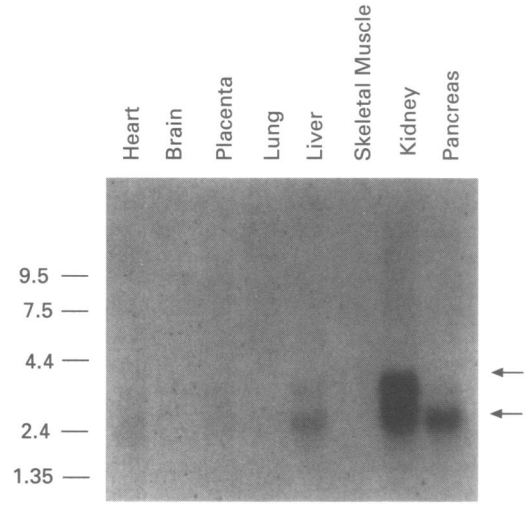

Figure 1 Northern blot of 5-HT$_{2B}$ receptor mRNA hybridization in different tissues. Note the 2.4 kB sized band which corresponds to previously detected 5-HT$_{2B}$ receptor mRNA as well as a second, 3.2 kB sized, band of hybridization in liver and kidney.

Table 1 PCR amplification of 5-HT$_2$ receptor subtypes

Human cDNA	*5-HT$_{2A}$*	*5-HT$_{2B}$*	*5-HT$_{2C}$*
Cerebral cortex	+ +	+ +	+ +
Whole brain	+ +	+ +	+ +
Spleen	+ +	+ +	+ +
Liver	+ +	+ +	+
Pancreas	+	+ +	+ +
Kidney	+	+ +	+
Heart	–	–	+ +

Human tissue cDNA was probed for the presence of 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptor cDNA using two steps of PCR with nested primers. (+ +) Indicates that a PCR product was detected after one round (30 cycles) of PCR. (+) Indicates that a PCR product was detected after two rounds of PCR amplification. (–) Indicates that a PCR product was not detected after two rounds of PCR amplification. PCR products were sequenced to confirm their identity.

Results

Cloning

The sequence of the cloned 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors were identical to those previously published except for a single nucleotide change in the human 5-HT$_{2A}$ receptor and two nucleotide changes in the human 5-HT$_{2C}$ receptor (Saltzman *et al.*, 1991). Sequencing of the appropriate genomic DNA de-

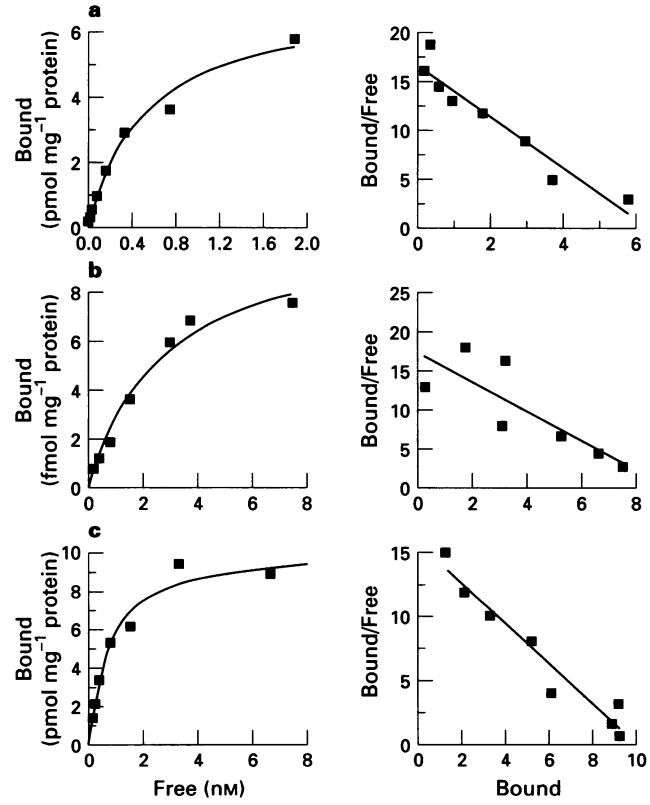

Figure 2 Saturation binding isotherms and Scatchard transformations for cloned human 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors: (a) 5-HT$_{2A}$, (b) 5-HT$_{2B}$ and (c) 5-HT$_{2C}$ receptors. The radioligand and the concentrations used in each experiment are given in the Methods section. Note the change in scale and the lower expression for the 5-HT$_{2B}$ receptor.

Table 2 Saturation binding parameters for human 5-HT$_2$ receptors

Tissue	*Radioligand*	n	K_d (nM)	B_{max} (fmol mg^{-1} protein $^{-1}$)
Human 5-HT$_{2A}$ in Cos-7	[^{3}H]-Ketanserin	3	0.45 (0.017)	4870 (1180)
Human 5-HT$_{2B}$ in Cos-7	[^{3}H]-5-HT	2	2.29 (0.04)	10.3 (1.13)
Human 5-HT$_{2C}$ in Cos-7	[^{3}H]-mesulergine	3	0.67 (0.07)	8830 (1620)

Values are the mean, with s.e.mean in parentheses, from 2–3 determinations. Fitting the data to a two site model did not give a statistically better fit than a single site model.

Table 3 Affinities (pK$_i$) of ligands for human 5-HT$_2$ receptors in Cos-7 cells

	5-HT$_{2A}$	*5-HT$_{2B}$*	*5-HT$_{2C}$*
Yohimbine	< 6.0	6.4 (0.11)	< 5.0
mCPP	7.3 (0.17)	7.5 (0.20)	6.6 (0.11)
Mianserin	8.4 (0.07)	7.7 (0.14)	8.3 (0.03)
Spiperone	9.0 (0.15)	5.8 (0.05)	< 6.0
Ketanserin	8.5 (0.11)	6.2 (0.14)	6.7 (0.07)
Methysergide	8.4 (0.04)	8.1 (0.15)	8.8 (0.03)
Ritanserin	10.8 (0.07)	8.3 (0.29)	9.6 (0.02)
SB 200646a	< 5.0	6.2 (0.08)	6.4 (0.06)
SB 204741	< 5.0	7.1 (0.08)	5.7 (0.04)
5-HT	7.2 (0.09)	9.1 (0.16)	6.8 (0.09)

Values are the mean, with s.e.mean in parentheses, from at least three determinations.

monstrated these mutations to be PCR artifacts. These artifacts were corrected by site directed mutagenesis prior to expression of the receptors.

There was no difference between the sequences of the 5-HT$_{2B}$ receptors isolated from liver, kidney or pancreas. The sequences were identical to those previously found for human 5-HT$_{2B}$ receptors (Kursar *et al.*, 1994; Schmuck *et al.*, 1994).

Distribution

The distribution of 5-HT$_{2B}$ receptor mRNA was examined by Northern analysis. The 5-HT$_{2B}$ probe hybridized to a 2.4 kB band in pancreas, liver and kidney. The probe also hybridized to a 3.2 kB band in liver and kidney. In kidney the 3.2 kB band was the major product. 5-HT$_{2B}$ mRNA was not detected in heart, brain, placenta, lung or skeletal muscle (Figure 1).

The distribution of 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors was also examined by PCR amplification of tissue-specific cDNAs. A PCR product corresponding to the 5-HT$_{2A}$ receptor was found after one step of amplification in cerebral cortex, liver, spleen and whole brain. PCR products corresponding to this receptor were also detected in pancreas and kidney following two steps of amplification. PCR products corresponding to the 5-HT$_{2B}$ receptor were detected following one step of PCR amplification in cDNA from cerebral cortex, whole brain, pancreas, liver, kidney and spleen but not heart. PCR products corresponding to the 5-HT$_{2C}$ receptor were detected following one step of amplification in cerebral cortex, whole brain, spleen, pancreas and heart. PCR products corresponding to the 5-HT$_{2C}$ receptor were further detected in kidney and liver following two steps of amplification (Table 1).

Receptor characterization by radioligand binding

Specific, high affinity, saturable binding was detected in Cos-7 cells expressing each of the transiently human 5-HT$_2$ receptors,

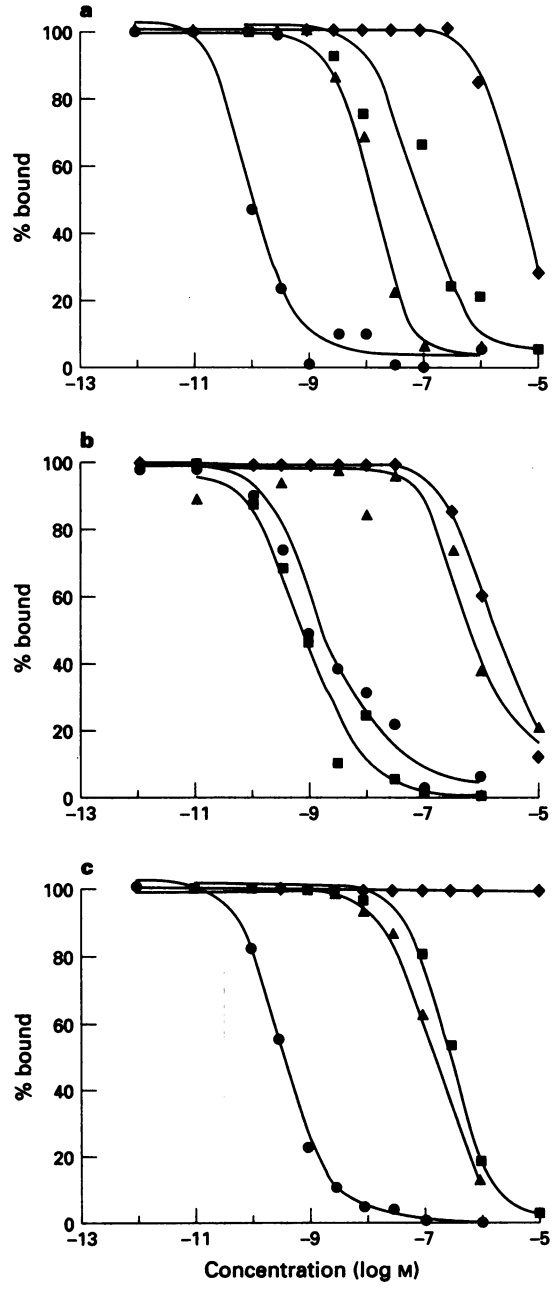

Figure 3 Competition binding curves for cloned human 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors: (a) 5-HT$_{2A}$, (b) 5-HT$_{2B}$ and (c) 5-HT$_{2C}$ receptors. In each case, (●) ritanserin, (▲) ketanserin, (■) 5-HT and (◆) yohimbine. In each assay, in the absence of displacing ligand, specific binding accounted for greater than 85% of total binding.

Table 4 Affinities (pK_i) of ligands at native and cloned 5-HT$_{2A}$ receptors

	Human 5-HT$_{2A}$ clone		*Human cortex*		*Rat 5-HT$_{2A}$ clone*	
	pK_i	n_H	pK_i	n_H	pK_i	n_H
Yohimbine	< 6.0		< 5.0		< 5.0	
mCPP	7.3 (0.17)	0.90 (0.16)	6.4 (0.13)	0.84 (0.18)	6.4 (0.08)	0.93 (0.09)
Mianserin	8.4 (0.07)	0.84 (0.22)	8.2 (0.23)	0.49 (0.04)*	8.4 (0.06)	1.04 (0.09)
Spiperone	9.0 (0.15)	1.25 (0.18)	9.0 (0.32)	0.79 (0.06)	9.5 (0.06)	0.91 (0.03)
Ketanserin	8.5 (0.11)	1.10 (0.17)	9.4 (0.30)	0.79 (0.01)	8.9 (0.07)	1.05 (0.08)
Methysergide	8.4 (0.04)	1.22 (0.18)	8.4 (0.01)	0.90 (0.11)	8.8 (0.01)	1.04 (0.12)
Ritanserin	10.8 (0.07)*	1.32 (0.19)	9.0 (0.18)	0.94 (0.17)	9.5 (0.09)	1.21 (0.05)
SB 200646a	< 5.0		< 5.0		< 5.0	
5-HT	7.2 (0.09)	1.23 (0.24)	6.7 (0.04)	0.72 (0.09)	6.9 (0.27)	0.84 (0.07)

Values are the mean pK_i (s.e.mean) and mean Hill (n_H) slope (s.e.mean) of at least three determinations.

Table 5 Affinities (pK_i) of ligands at human and rat 5-HT$_{2B}$ receptors

	Human 5-HT$_{2B}$ clone		*Rat 5-HT$_{2B}$ clone*
	pK_i	n_H	pK_i
Yohimbine	6.4 (0.11)	1.21 (0.17)	7.3
mCPP	7.5 (0.20)	1.19 (0.22)	7.6
Mianserin	7.7 (0.14)	0.95 (0.16)	7.3
Spiperone	5.8 (0.05)	1.13 (0.09)	5.5
Ketanserin	6.2 (0.14)	0.87 (0.14)	5.4
Methysergide	8.1 (0.15)	1.21 (0.31)	8.2
Ritanserin	8.3 (0.29)	1.10 (0.44)	8.3
SB 200646a	6.2 (0.08)	1.17 (0.16)	—
5-HT	9.1 (0.16)	0.90 (0.02)	8.0

Data for binding to the rat 5-HT$_{2B}$ receptor were taken from Wainscott *et al.* (1993).

Table 6 Affinities (pK_i) of ligands at 5-HT$_{2C}$ receptors

	Human 5-HT$_{2C}$ clone		*Human choroid plexus*		*Rat 5-HT$_{2C}$ clone*	
	pK_i	n_H	pK_i	n_H	pK_i	n_H
Yohimbine	< 5.0		< 5.0		< 5.0	
Spiperone	< 6.0		6.3 (0.27)	0.47 (0.06)	6.1 (0.08)	0.91 (0.10)
Ketanserin	6.7 (0.07)	1.03 (0.02)	7.3 (0.01)	0.85 (0.11)	7.2 (0.06)	0.96 (0.14)
Methysergide	8.8 (0.03)	0.97 (0.06)	ND	ND	8.7 (0.10)	1.14 (0.03)
Ritanserin	9.6 (0.02)*	1.03 (0.14)	ND	ND	8.8 (0.11)	1.19 (0.07)
SB 200646a	6.3 (0.06)	1.02 (0.04)	ND	ND	6.6 (0.10)	0.83 (0.04)
Mianserin	8.3 (0.03)	1.04 (0.03)	ND	ND	8.7 (0.02)	0.95 (0.04)
mCPP	6.6 (0.11)	0.94 (0.09)	ND	ND	6.9 (0.12)	0.95 (0.05)

Values are the mean, with s.e.mean in parentheses, of at least three determinations. *The affinity of ritanserin was statistically ($P < 0.05$) greater at the human receptor than at the rat receptor. ND, not determined.

in NIH3T3 cells expressing rat 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors, in human cortex and in human choroid plexus membranes. The saturation binding isotherms for [^{3}H]-ketanserin binding to Cos-7 cells expressing the human 5-HT$_{2A}$ receptors [^{3}H]-5-HT binding to Cos-7 cells expressing the human 5-HT$_{2B}$ receptor and for [^{3}H]-mesulergine binding to Cos-7 cells expressing the human 5-HT$_{2C}$ receptor were best fitted by a single site model (Figure 2). The affinities of [^{3}H]-ketanserin, [^{3}H]-mesulergine were appropriate for the labelling of 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors respectively (Table 2).

Competition binding curves were consistent with homogeneous populations of receptors in the Cos-7 cells expressing the human 5-HT$_2$ receptors in that Hill slopes did not differ from unity and in that the affinities of displacing ligands were consistent with the labelling of 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors in the appropriate tissues (Figure 3). The 5-HT$_{2A}$ receptor could be distinguished from 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors on the basis of a relatively high affinity for spiperone and ketanserin. The 5-HT$_{2B}$ receptor could be distinguished from the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors on the basis of a lower affinity for ritanserin and higher affinities for 5-HT and yohimbine. The compound with the highest selectivity for the 5-HT$_{2B}$ receptor was SB 204741 (Table 3). The 5-HT$_{2C}$ receptor could be distinguished from the 5-HT$_{2A}$ receptor on the basis of a low affinity for spiperone and ketanserin, and from the 5-HT$_{2B}$ receptor on the basis of a low affinity for yohimbine.

The affinities of ligands at the cloned human 5-HT$_{2A}$ receptor were compared to those at the cloned rat 5-HT$_{2A}$ receptor and to a binding site in human cortex labelled with [^{3}H]-ketanserin (Table 4). The affinities of most ligands at the human and rat receptors were similar. However, ritanserin and mCPP exhibited 3–10 fold higher affinity for the human receptor than for the rat receptor. Similar affinities were found for a site labelled with [^{3}H]-ketanserin in human cortex. However, ketanserin had a 10 fold higher affinity for the site in cortex than for the human cloned 5-HT$_{2A}$ receptor while ri-

tanserin had ~60 fold lower affinity in human cortex. Hill slopes of displacement curves generated with mianserin, in the cortical preparation, were less than unity.

The affinities of ligands at the human 5-HT$_{2B}$ receptor in Cos-7 cells were compared to those values previously reported for the rat 5-HT$_{2B}$ receptor (Table 5). The affinities of most ligands for the human receptor were similar to those at the rat binding site. However, the human 5-HT$_{2B}$ receptor had about a 10 fold lower affinity for yohimbine and about a 10 fold higher affinity for ketanserin and 5-HT.

The affinities of ligands at the human 5-HT$_{2C}$ receptor in the Cos-7 cells were compared to those at the cloned rat 5-HT$_{2C}$ receptor and to those at a [^{3}H]-mesulergine binding site in human choroid plexus (Table 6). The affinities of ligands at the human and rat receptor were similar. However, ritanserin had about 10 fold higher affinity for the human receptor. Values in the human choroid plexus were similar to those at the cloned receptor.

Discussion

In the present study, human 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors were cloned, expressed in Cos-7 cells, and characterized. The distribution of the 5-HT$_2$ receptor gene products in a variety of tissues was then examined by both Northern blot hybridization and reverse transcription PCR.

The human 5-HT$_{2B}$ receptors cloned in the current study had nucleotide sequences identical to those previously reported (Saltzman *et al.*, 1991; Kursar *et al.*, 1994; Schmuck *et al.*, 1994). There is a high degree of homology between human 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors and their counterparts in rats and mice (Cohen, 1989; Yang *et al.*, 1992; Foguet *et al.*, 1992a,b; Kursar *et al.*, 1992; Martin & Boess, 1994). However, the homology between subtypes of 5-HT$_2$ receptors is only about 40%, a somewhat surprising finding, given the similarities in the ligand binding and signal transduction properties of these receptors.

The expression of 5-HT$_{2B}$ receptor mRNA in different tissues was examined by both Northern analysis and the more sensitive reverse transcription PCR amplification method. Northern analysis resulted in the detection of 5-HT$_{2B}$ receptor mRNA (a 2.4 kB band) in pancreas, liver and kidney but not heart, brain or placenta. PCR amplification of 5-HT$_{2B}$ receptor cDNA confirmed the expression of this receptor in these tissues. PCR amplification also indicated the presence of 5-HT$_{2B}$ receptor mRNA in human cerebral cortex, whole brain and spleen but not heart. While estimating the level of mRNA expression by PCR is qualitative, these findings suggest relatively higher levels of expression of 5-HT$_{2B}$ receptor mRNA in liver, kidney and pancreas than in cortex and spleen with little or no expression in heart. This pattern of receptor expression is similar to that previously reported by Schmuck *et al.* (1994) or Kursar *et al.* (1994) with the exception that we failed to detect PCR product for this receptor in heart cDNA. Taken together, these findings suggest that 5-HT$_{2B}$ receptors are expressed in a wide range of tissues including brain.

The pattern of tissue distribution of the 5-HT$_{2B}$ receptor was different from that of the 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors since 5-HT$_{2C}$ receptor cDNA was detected in heart cDNA and since 5-HT$_{2A}$ receptor cDNA was only detected following two cycles of PCR amplification in either the pancreas or the kidney. These findings suggest that human heart tissue may be enriched in 5-HT$_{2C}$ receptors while human kidney may be enriched in 5-HT$_{2B}$ receptors (assuming some correspondence between mRNA levels and receptor expression). The reason for the apparent differences in 5-HT$_{2B}$ receptor expression in heart found here, as compared to prior studies (Kursar *et al.*, 1994; Schmuck *et al.*, 1994), will need to be examined further.

In addition to the expected 2.4 kB band of hybridization found on Northern blots from liver and kidney, a 3.2 kB band was also detected in these tissues. Indeed, in the kidney this larger band was the most prominent band of hybridization. The identity of the 3.2 kB band was not determined although, given the stringency of conditions used in the hybridization assays, it probably had at least moderate homology with the 2.4 kB band. One possibility is that there are splice variants of the 5-HT$_{2B}$ receptor. Alternatively, these findings raise the possibility that human liver and kidney contain a novel receptor having homology with the 5-HT$_{2B}$ receptor.

Comparison of the receptor binding properties of the human 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors confirmed the identities of these receptors and further defined their pharmacological characteristics. The human 5-HT$_{2B}$ receptor was distinguished from the human 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors on the basis of a low affinity for ritanserin and a higher affinity for yohimbine. The receptor also had a higher affinity for 5-HT, although agonist-mediated conformational changes may have accounted for this higher affinity. The rank order of affinities of ligands binding to the human 5-HT$_{2B}$ receptor, as expressed in Cos-7 cells, was similar to that previously reported by Kursar *et al.* (1994); however, in the current study a 10 fold higher affinity of the receptor for 5-HT was detected. The reason for this difference was not determined but differences in the cell lines used to express the receptor may be one explanation. The most selective 5-HT$_{2B}$ receptor ligand to be tested was SB 204741 with approximately 20 fold selectivity for the human 5-HT$_{2B}$ receptor as compared to the human 5-HT$_{2C}$ receptor. These data confirm preliminary reports by Baxter *et al.* (1994b) and further define the binding pharmacology of human 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors.

Comparison of the affinities of ligands at the cloned human and rat 5-HT$_2$ receptors extend previous indications of interspecies heterogeneity in 5-HT$_2$ receptor pharmacology (Pazos *et al.*, 1985; Kao *et al.*, 1992; Nelson *et al.*, 1993; Choi *et al.*, 1994). Thus for 5-HT$_{2A}$ receptors, mCPP and ritanserin were shown to have 10–20 fold higher affinity for the cloned human receptor in comparison to the cloned rat receptor. Whereas, for 5-HT$_{2C}$ receptors, ritanserin was nearly 10 fold selective for the human receptor, in general, comparison of affinities of ligands at rat and human 5-HT$_{2B}$ receptors revealed no striking differences in the affinities of antagonists, although yohimbine tended to have a higher affinity for the rat receptor while ketanserin had a higher affinity at the human receptor. 5-HT also had about 10 fold higher affinity for the human 5-HT$_{2B}$ receptor but this may be a consequence of the effect of different temperatures on agonist binding affinity (Wainscott *et al.*, 1993).

To validate data obtained with the cloned human receptors, binding assays were established in human cortex tissue using [^{3}H]-ketanserin and human choroid plexus using [^{3}H]-mesulergine (Pazos *et al.*, 1984). The affinity of displacing ligands for human choroid plexus were consistent with the labelling of a 5-HT$_{2C}$ receptor but the difficulty in obtaining sufficient quantities of this tissue precluded a more extensive evaluation. The affinity values for ligands in human cortex membranes, in general, matched those obtained with the cloned human 5-HT$_{2A}$ receptor. However, one striking difference was found with ritanserin in that this compound was nearly 100 fold more potent for the cloned receptor than for the receptor labelled in human membranes. Conversely, ketanserin was found to have approximately 10 fold higher affinity for the cloned receptor. Whether these differences reflect an artifact of the cloned receptor or the binding of the ligand to heterogeneous binding sites in cerebral cortical membranes remains to be determined. The observation that Hill slopes of the competition curves, obtained in human cortex, tended to be less than unity supports the latter idea. Nonetheless, these discrepancies between affinity estimates in human cortical membranes and human receptors expressed in cell lines raise a cautionary note to the reliance on only cloned receptors for predicting ligand affinity at endogenous receptors.

In summary, these studies further characterize the distribution and receptor binding properties of human 5-HT$_{2A}$, 5-HT$_{2B}$ and 5-HT$_{2C}$ receptors. Based on the expression of mRNA, these receptors have unique and heterogeneous pat-

terns of distribution in peripheral and central tissues. The 5-HT$_{2B}$ receptor was found to be widely distributed in numerous tissues with a distribution pattern distinct from that of 5-HT$_{2A}$ or 5-HT$_{2C}$ receptors. The ligand binding specificity of these receptors generally matched that of their counterparts cloned from rat tissues although differences in the affinities of several ligands support previous indications of pharmacologically distinct species homologues. Finally, the finding of two distinct bands of hybridization on the Northern blots raises the possibility of splice variants or subtypes of 5-HT$_{2B}$ receptors.

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