

# New mouse 5-HT<sub>2</sub>-like receptor

## Expression in brain, heart and intestine

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A novel member of the family of G protein-coupled receptors has been isolated from a mouse brain cDNA library by screening with polymerase chain reaction (PCR) generated fragment of mouse genomic DNA amplified using degenerated primers. Sequence comparison demonstrates that the encoded protein sequence shows the highest homology to the 5-HT<sub>2</sub> family of receptors. The pharmacological profile of membranes from COS cells transfected with this cDNA, corresponds to a new 5-HT<sub>2</sub>-like receptor that we propose to call 5-HT<sub>2C</sub>. Its major sites of expression are in the mouse intestine and heart, also with detectable expression in brain and kidney. We speculate that it could account at least in part for the 'atypical' functions attributed to the 5-HT<sub>1C</sub>/5-HT<sub>2</sub> receptors.

G protein-coupled receptor; cDNA cloning; Polymerase chain reaction; Smooth muscle; Ritanserin

## 1. INTRODUCTION

The aminergic neurotransmitter serotonin is believed to play an important role in a multitude of cognitive and behavioral functions and dysfunctions including motor control, feeding, anxiety, depression, and sexual activity [1]. This large diversity of functions is paralleled by the pharmacological complexity of serotonin receptors. At least four classes have been distinguished pharmacologically: 5-HT<sub>1</sub>, 5-HT<sub>2</sub>, 5-HT<sub>3</sub>, and 5-HT<sub>4</sub>. These categories are defined by both binding and effector coupling properties of the receptors [2,3]. More recently, molecular biological data have confirmed the existence of multiple serotonin receptors, including both ligand-gated receptors (5-HT<sub>3</sub>) and G protein-coupled receptors (GPRs) [4]. This latter class can be split into two classes [5], reflecting the second messenger system to which the receptor is coupled: the 5-HT<sub>1C</sub> and the 5-HT<sub>2</sub> receptors which are coupled to the activation of the phospholipase C, and the 5-HT<sub>1A</sub>, B and D family, which interacts with the adenylyl cyclase; it is possible that 5-HT<sub>4</sub> belongs to this class, although it has not yet been characterized molecularly. To date the adenylyl cyclase 5-HT<sub>1</sub> subclass has been the most extensively studied, with more than five subtypes already described

[6]. By contrast only two members of the 5-HT<sub>2</sub> subfamily have been characterized, 5-HT<sub>1C</sub> and 5-HT<sub>2</sub>.

5-HT<sub>2</sub> receptors mediate many of central and peripheral physiological functions of serotonin. Cardiovascular effects include contraction of blood vessels and shape change in platelets; central nervous system effects include neuronal sensitization to tactile stimuli and mediation of hallucinogenic effects of lysergic acid diethylamide and related phenylisopropylamine hallucinogens. Many investigators have observed that 5-HT<sub>1C</sub> and 5-HT<sub>2</sub> receptors do not reflect all the properties attributed to them. For example some 5-HT<sub>2</sub>-like effects of serotonin on peripheral smooth muscles are classified as 'atypical' [7], leading to the hypothesis that other 5-HT<sub>2</sub> receptor subtypes might exist.

Therefore, we have undertaken the cloning and recombinant expression of new 5-HT<sub>2</sub> receptors in the mouse in order to study their functional properties. Previous molecular cloning experiments have demonstrated that the 5-HT<sub>1</sub> and 5-HT<sub>2</sub> receptor subclasses are encoded by members of the seven transmembrane domain containing gene superfamily. Taking advantage of the sequence conservation between related members of this family we have screened for new members of the 5-HT<sub>2</sub> class. We describe here the cloning and functional characterization of a new 5-HT<sub>2</sub>-like receptor.

## 2. MATERIALS AND METHODS

### 2.1. Drugs and chemicals

Restriction endonucleases, AMV Reverse transcriptase, DNA polymerase I, T4 polynucleotide kinase, T4 DNA ligase, T3 or T7

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RNA polymerase, were purchased from Bethesda Research Laboratories, New England Biolabs, Boehringer-Mannheim, and Stratagene. Sequenase and sequencing reagents were purchased from United States Biochemical Corporation. Taq polymerase for PCR was purchased from Perkin-Elmer-Cetus and reactions performed in a Perkin-Elmer-Cetus cyclor. Ketanserin, ritanserin and setoperone were kindly provided by Janssen (Beerse, Belgium). ICS 205-930 and MDL 72 222 were gifts from Sandoz (Basel, Switzerland) and Merrell-Dow (Sirasbourg, France), respectively. Other neurochemicals were from the RBI, or Sigma.  $^{32}\text{P}$ -, and  $^{35}\text{S}$ -labeled nucleotides as well as [ $^{125}\text{I}$ ]DOI (2200 Ci/mmol), were from New England Nuclear.

## 2.2. Standard molecular biology techniques

Classical published procedures were used for library screening plasmid subcloning, RNA extraction, and COS cell transfections [8].

## 2.3. PCR experiments

We synthesized degenerated oligonucleotides coding for the conserved VIIth (a) and VIIIth (b and c) transmembrane domains sequences [9].

(a) TACCTCGAGGTCGACGGTATGTGGTG{C,T}CCITT-  
{C,T}TT{C,T}AT  
(b) AGAAGTAGTGGTACCCA{G,A}IGT{G,A}TAIACIA{G,A}-  
IGG{G,A}TT  
(c) AGAAGTAGTGGTACCC{G,C}{A,T}{G,A}CAIAC{G,A}TA-  
ICC{G,A,T}ATCCA

A polylinker has been added at the 5' end for subcloning. 1  $\mu\text{g}$  of mouse genomic DNA was denatured 1 min at 94°C, annealed 2 min at 55°C, and amplified 3 min at 72°C in the presence of 3 mM  $\text{MgCl}_2$  for 20 cycles for the first two sets of primers (a and b, 1  $\mu\text{g}$  each). 1/10th of the reaction was reamplified with the first and third set of primers (a and c, 1  $\mu\text{g}$  each) for 20 more cycles under the same conditions. Products were subcloned in pBluescript and sequenced. Oligonucleotides corresponding to the characterized products were synthesized and then used as probes.

For quantitative RT-PCR experiments, standard PCR reaction buffer was used in the presence of 10  $\mu\text{g}$  of total RNA. After denaturation, AMV reverse transcriptase (13 U) and Taq polymerase (5 U) were added, and extension was done at 50°C for 15 min and then a standard PCR amplification protocol [8] was used. Samples were taken after 20, 25, and 30 cycles to ensure that the reaction was in the exponential phase of synthesis. We used, as an internal standard, primers corresponding to the mRNA of the ribosomal elongation factor, EF1A, amplified in the same reaction as the NP75 primers.

## 2.4. [ $^{125}\text{I}$ ]DOI binding assays

[ $^{125}\text{I}$ ]DOI [10] was used as the radioligand to detect expression of the NP75 gene product in membrane fractions isolated from COS-7 cells 48 h after DNA transfection. Briefly, the incubation medium (200  $\mu\text{l}$ ) contained 50  $\mu\text{l}$  of radioligand, 50  $\mu\text{l}$  of buffer or of competing drug, and 100  $\mu\text{l}$  of membrane suspension (protein concentration, 50  $\mu\text{g}/\text{ml}$ ). The mixture was incubated at 30°C for 30 min. The assay was terminated by addition of ice-cold iso-osmotic solution, and rapid filtration through GF/B filters, followed by 4 washes of 5 ml of ice-cold buffer. Filters were dried rapidly and their radioactivity determined by liquid scintillation counting. Non-specific binding, determined in the presence of 10  $\mu\text{M}$  unlabeled DOI, represented about 30% of total binding.

Competition studies for [ $^{125}\text{I}$ ]DOI-binding were performed by adding increasing concentrations of test drug to the reaction.

Data were analyzed using the iterative non-linear regression fitting program LIGAND (Version 3.0, McPershon 1985) and RS1 (Release 4.0).

## 3. RESULTS AND DISCUSSION

G protein-coupled receptors share extensive homology at the amino acid level, especially within the transmembrane region. The sixth and seventh transmem-

brane domains present the highest score of homology between several members of this family of receptors [9]. We therefore designed degenerated oligonucleotides, to perform PCR reactions on mouse genomic DNA. Several genomic fragments whose sequences were similar to known GPRs were obtained, and subcloned. Some of these products were identical to the already cloned mouse serotonin receptors [11]. Among these products a novel sequence, NP75, was identified as the most similar to the 5-HT<sub>2</sub> sequence. This we used to screen a mouse brain cDNA library.

The complete NP75 cDNA contains an open reading frame of 1510 bp encoding a protein of 504 amino acids with a predicted molecular weight of 56,508 Da (Fig. 1). On the basis of the hydropathy plot (not shown), this protein displays seven hydrophobic domains, and has extensive homology with other members of the GPR family, including one potential *N*-glycosylation site at its amino terminus (Fig. 1), and consensus sequences for phosphorylation by different protein kinases in the cytoplasmic regions. In addition the presence of 19 Ser or Thr residues in the 121 carboxyterminal residues (Fig. 1) may indicate that this region is involved in the receptor desensitization by protein kinases as has been demonstrated for the  $\beta$ -adrenergic receptor [12].

When compared with other GPRs, the NP75 transmembrane region exhibits high degree of homology with the 5-HT<sub>2</sub> and 5-HT<sub>1C</sub> receptors, especially within domains II and III where 12 and 20 amino acids, respectively, are identical. Alignment of the transmembrane region of various aminergic receptors suggests that the NP75 receptor belongs to the 5-HT<sub>2</sub> subfamily [5], being slightly more divergent from 5-HT<sub>1C</sub> than from 5-HT<sub>2</sub>; homologies reach 60% with the mouse 5-HT<sub>2</sub> receptor [11] and 57% with the mouse 5-HT<sub>1C</sub> [13]. Compared to the rat 5-HT<sub>1A</sub> receptor [14], or mouse 5-HT<sub>1B</sub> receptor [8] the homology is less than 30%. By comparison, the homologies between 5-HT<sub>2</sub> and 5-HT<sub>1C</sub> receptor of the same species are around 70%, and the 5-HT<sub>2</sub> receptors from different species have homologies over 90%. In addition, the NP75 gene includes two introns (not shown), which are present at equivalent positions to the introns in the 5-HT<sub>2</sub> gene [11]. This confirms a probable common evolutionary origin, different from the other 5-HT<sub>1</sub> genes which are devoid of introns. In order to confirm that this clone represents a new member of the 5-HT<sub>2</sub> family we analysed it further by expression studies.

To determine the pharmacological properties of the predicted NP75 protein, the coding sequence of the cDNA was introduced into the eucaryotic vector pSG5 [15] and transfected into COS-7 cells, whose membranes were then tested for their binding properties. Steady-state binding assays with [ $^{125}\text{I}$ ]DOI demonstrated that this ligand interacts specifically with the recombinant-transfected membrane preparation (and not to mock transfected cells) with high affinity and in a saturable

1	TGA GTC ACC AAA AGG CGA ATG GCT TCA TCT TAT AAA ATG TCT GAA CAA AGC ACA ACT TCT GAG CAC ATT TTA CAG	19
	* M A S S Y K M S E Q S T T S E H I L Q	
76	AAG ACA TGT GAT CAC CTG ATC CTG ACT AAC CGT TCT GGA TTA GAG ACA GAC TCA GTA GCA GAG GAA ATG AAG CAG	44
	K T C D H L I L T N R S G L E T D S V A E E M K Q	
151	ACT GTG GAG GGA CAG GGG GAT ACA GTG CAC TGG GCA GCT CTC CTG ATA CTC GCG GTG ATA ATA CCG ACC ATT GGT	69
	T V E G Q G H T V H W A A L L I L A V I I P T I G	
226	GGG AAC ATC CTT GTG ATT CTG GCT GTT GCA CTG GAG AAA AGG CTG CAG TAC GCT ACC AAC TAC TTT TTA ATG TCC	94
	G N I L V I L A V A L E R R L Q Y A T N Y F L M S	
301	TTG GCG ATA GCA GAT TTG CTG GTT GGA TTG TTT GTG ATG CCG ATT GCC CTC TTG ACA ATC ATG TTT GAG GGT ATA	119
	L A I A D L L V G L F V M P I A L L T I M F E A I	
376	TGG CCC CTC CCA CTG GCC CTG TGT CCT GCC TGG TTA TTC CTC GAT GTT CTC TTT TCA ACT GCC TCC ATC ATG CAT	144
	W F L P L A L C P A W L F L D V L F S T A S I M H	
451	CTC TGT GCC ATT TCC CTG GAC CUC TAT ATA GCC ATC AAA AAG CCA ATT CAG GCC AAT CAG TGC AAC ACC CGG GCT	169
	L C A I S L D R Y I A I K K P I Q A N Q C N T R A	
526	ACT GCA TTC ATC AAG ATT ACA GTG GTA TGG TTA ATT TCA ATA GGC ATC GCC ATC CCA GTC CCT ATT AAA GGA ATC	194
	T A F I K I T V V W L I S I G I A I P V P I K G I	
601	GAG ACT GAT GTG ATT AAT CCA CAC AAT GTC ACC TGT GAG CTG ACA AAG GAC CGC TTT GGC AGT TTT ATG GTC TTT	219
	E T D V I N P H V N V T C R L T K D R F G S F M V F	
676	GGG TCA CTG GCT GCT TTC TTC GTA CCT CTC ACC ATC ATG GTA GTC ACT TAC TTT CTC ACC ATT CAC ACT TTA CAG	244
	G S L A A F F V P L T I M V V T Y F L T I H T L Q	
751	AAG AAA GCT TAC TTG GTC AAA AAT AAG CCA CCT CAA CGC CTA ACA CGG TGG ACT GTG CCC ACA GTT TTC CTA AGG	269
	K K A Y L V K N K P P Q R L T R W T V P T V F L R	
826	GAA GAC TCA TCC TTT TCA TCA CCA GAA AAG GTG GCA ATG CTG GAT GGG TCT CAC AGG GAT AAA ATT CTA CCT AAC	294
	E D S S F S P E K V A M L D G S H R D K I L P N	
901	TCA AGT GAT GAG ACA CTT ATG CGA AGA ATG TCC TCA GTT GGA AAA AGA TCA GCC CAA ACC ATT TCT AAT GAG CAG	319
	S S D S T L M R R M S S V G K R S A Q T I S N E Q	
976	AGA GCC TCG AAG GCC CTT GGA GTC GTG TTT TTC CTT TTT CTG CTT ATG TGG TGC CCC TTT TTT ATT ACA AAT CTA	344
	R A S K A L G V V F F L F L L M W C P F F I T N L	
1051	ACT TTA GCT CTG TGT GAT TCC TGC AAT CAG ACC ACT CTC AAA ACA CTC CTG GAG ATA TTT GTG TGG ATA GGC TAC	369
	T L A L C D S C N Q T T L K T L L E I F V W I G Y	
1126	GTT TCC TCG GGG GTG AAT CCT CTG ATC TAT ACA CTC TTC AAT AAG ACA TTT CCG GAA GCA TTT GGC AGG TAC ATC	394
	V S S G V N P L I Y T L F N K T F R E A F G R Y I	
1201	ACC TGC AAT TAC CGA GCC ACA AAG TCA GTA AAA GCA CTT AGG AAG TTT TCC AGT ACA CTT TGT TTT GCG AAT TCA	419
	T C N Y R A T K V K A L R K F S T L C F G N S	
1276	ATG GTA GAA AAC TCT AAA TTT TTC ACA AAA CAT GGA ATT CGA AAT GGG ATC AAC CCT GCC ATG TAC CAG AGC CCA	444
	M V E N S K F F T K H G I R N G I N P A M Y Q S P	
1351	ATG AGG CTC CGA TGT TCA ACC ATT CAG TCC TCA TCA ATC ATC CTC CTC GAT ACC CTT CTC ACT GAA AAC GAT GGC	469
	M R L R C S T I Q S S S I I L L D T L L T E N D G	
1426	GAC AAA GCG GAA GAG CAG GTC AGC TAC ATA TTG CAG GAA CGG GCC GGC CTC ATC TTG AGA GAG GGT GAT GAG CAG	494
	D K A E E Q V S Y I L Q E R A G L I L R E G D E Q	
1501	GAC GCA CGC GCA CCA TGG CAG GTT CAA GAG TGA	504
	D A R A P W Q V Q E *	

Fig. 1. Sequence of the NP75 cDNA. The NP75 cDNA and deduced protein sequences are displayed. Roman numbers over the boxes localize the transmembrane domains. The left numbering is for the DNA sequence, the right is for the protein. In frame stop codons are shown by \*. The circled N indicates putative *N*-glycosylation sites. Circled serines or threonines represent consensus for phosphorylation by protein kinase A (A), protein kinase C (C), and casein kinase (K).

fashion (Fig. 2). The resulting saturation data exhibits a best-fit to a single site mode with an apparent  $K_d$  of  $25.8 \pm 0.54$  nM ( $n = 3$ ). The apparent  $B_{max}$  varied between 14.8 and 21.0 pmol/mg protein depending on the transfection efficiency. The  $K_d$  values of DOI for classical 5-HT<sub>2</sub> receptor is 2.2 nM [10].

Competitive inhibition studies were performed in

order to refine the pharmacological profile of this receptor. Table I shows the following rank order of potencies for selected drugs: Ritanerine > *N*-acetyl 5-HT > Methysergide > Setoperone = Cyproheptadine > Spiperone > Ketanserin > Tryptamine > 5-HT > 8-CH-DPAT. This rank order as well as the apparent  $K_d$  values for these compounds correlate with those of 5-

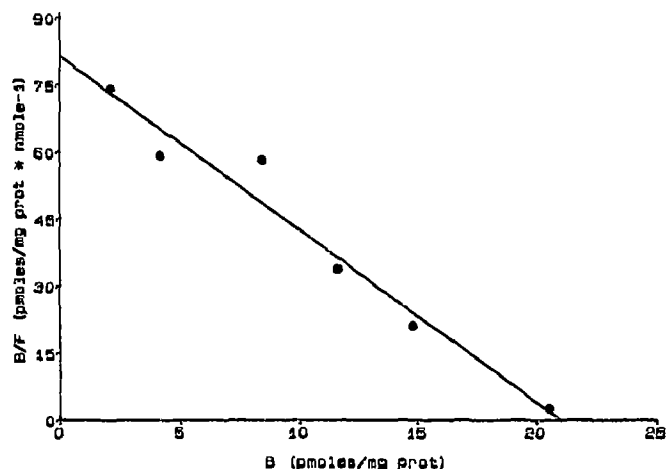


Fig. 2. Saturation binding of NP75 COS transfected membranes. The scatchard plot of the dose binding curves with [ $^{125}$ I]DOI as radioligand is represented with each value as the mean of at least 3 independent trials run in triplicate. The mean  $K_d$  value and the maximal binding capacity value are respectively 25.8 nM and 18.4 pmol/mg of protein.

HT2 ( $P = 0.0436$ ) or 5-HT1C ( $P = 0.022$ ) (Kendall rank correlation), but not with other 5-HT2 receptor subtypes ( $P > 0.05$ ); the low affinity of this receptor for serotonin would place it in the 5-HT2 family of receptors, that is confirmed by the low affinity for the 5-HT1A selective agonist 8-OH-DPAT. In addition the

Table 1

Pharmacology of the mouse 5-HT receptor expressed in COS cells

Drug	COS cell binding	5-HT2	5-HT1C
<b>Agonists</b>			
5-HT	5.90	5.5	7.5
2-Me-5-HT	5.18	5.2	5.8
Tryptamine	6.70	6.0	7.2
1-Me-5-HT	5.60	6.3	8.4
NNdiMe-5-MeOT	5.13	6.2	7.0
8-OH-DPAT	5.19	5.0	5.2
Quipazine	5.18	6.2	6.7
Histamine	<4.0	<4.0	<4.0
<b>Antagonists</b>			
Ritanserlin	8.44	9.3	8.6
Methysergide	7.90	8.6	8.6
Setoperone	7.61	8.6	7.3
Cyproheptadine	7.65	8.5	7.9
Spiperone	7.30	8.8	5.9
Ketanserin	6.69	8.9	7.0
ICS 205-930	5.30	5.3	4.6
MDL72222	4.62	6.7	<5.0
Chlorpromazine	<4.0	<4.0	<4.0

Competition experiment for [ $^{125}$ I]DOI labeled membrane from NP75 transfected COS cells as compared with published values for native rat 5-HT1C and 5-HT2 receptor taken from [22]. Each value, expressed as  $pK_d$  ( $-\log \text{mol/l}$ ) is the mean of at least 3 independent trial runs in triplicate.

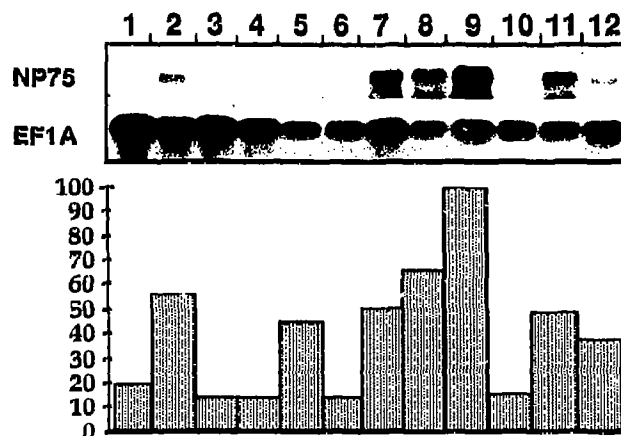


Fig. 3. RT-PCR analysis of NP75 in vivo expression. PCR experiments were done in the presence of 10  $\mu\text{g}$  of total RNA, using NP75 and EF1A specific primers. PCR products were analyzed and revealed by hybridization to  $^{32}\text{P}$ -labeled primers different from the amplimers, after 25 cycles for NP75 (row NP75) or after 20 cycles for EF1A (row EF1A). The resulting autoradiograms were scanned and the EF1A normalized intensity is displayed (lower panel). Lane 1 contains RNA from LMTK cell line; lane 2, from 10.5 days mouse embryo; lane 3, from mouse testis; lane 4, from 3T6 cell line; lane 5, from MBK cell line; lane 6, from mouse liver; lane 7, from mouse cerebellum; lane 8, from mouse heart; lane 9, from mouse intestine; lane 10, from mouse spleen; lane 11, from mouse kidney; and lane 12, from total mouse brain.

poor competition by the 5-HT3 selective compounds ICS 205-930, MDL 72222, and quipazine rule out the possibility that this belongs to the 5-HT3 family. The receptor affinity for ketanserin is too low for it to be a typical 5-HT2, although the high affinity that it has for the 5-HT2 antagonist ritanserin makes it difficult to classify NP75 within the 5-HT2/5-HT1C family. Furthermore, preliminary evidence shows that like 5-HT2 and 5-HT1C receptors, NP75 is coupled to the PLC second messenger system (not shown).

The heterogeneity of 5-HT2 receptors has already been detected on preparations of cortical membranes, where the agonist competition curves for [ $^3\text{H}$ ]ketanserin are shallow, indicating the presence of two types of binding site. The observation that the high affinity sites represented only a small subfraction of the ketanserin sites in addition to the existence of tissues containing only low affinity binding sites for the amphetamine derivative 4-bromo-2,5-dimethoxyphenylisopropylamine (DOB) led Peroutka and coworkers to propose the hypothesis that the high affinity agonist DOB binding must represent a unique receptor subtype 5-HT2A, whereas the low-affinity site (labeled by ketanserin but not by DOB) 5-HT2B represents the classical 5-HT2 receptor [16]. The low affinity of the NP75 receptor for ketanserin makes it unlikely to correspond to any one of these subtypes. Therefore this new receptor appears distinct from the 5-HT1C, 5-HT2A, and 5-HT2B, so we propose to call it 5-HT2C.

In order to have more complete information concern-

ing the properties of this new serotonin receptor, we analyzed for its *in vivo* expression. NP75 transcripts were analyzed by Northern blot (not shown) and by quantitative RT-PCR experiments. Fig. 3 displays the pattern of expression of NP75 seen after 25 cycles of amplification using 10 µg of total RNA. The highest expression is detected in the mouse intestine and in the mouse heart as well as at a lower level in the brain and kidney. In contrast, no expression is seen in the liver and spleen. We used the ribosomal elongation factor EF1A as an internal standard and primers located in two different exons in order to distinguish the amplification of RNA from DNA templates. The NP75 mRNA is also detected in the mouse embryo at day 10.5, and in the kidney derived cell line BHK. *In situ* experiments in the mouse brain as well as in the heart and intestine are in progress in order to refine these results.

The localization of this receptor to peripheral as well as to the central nervous system is puzzling. However, several reports have provided evidence that the peripheral action of serotonin cannot be mediated by the classical 5-HT<sub>1C</sub> or 5-HT<sub>2</sub> receptors. For example, in the rat, administration of 5-HT causes tachycardia. This effect is antagonized by ketanserin, cyproheptadine, methysergide and metiothepine. Since DOI which exhibits a partial agonist action with 5-HT<sub>2</sub> receptors, neither mimics nor blocks this tachycardiac action, the 5-HT<sub>2</sub> receptor involved has been classified as 'atypical' [7]. In addition coronary vasoconstriction has been shown in several species to be mediated by a 5-HT<sub>2</sub>-like receptor [17], effects which are potentiated in coronary artery disease [7]. Similarly, the contractions of the rat stomach are mediated by an unknown serotonin receptor [18]. Furthermore, the activation of the peripheral serotonin receptor in platelets and in vascular smooth muscle has been shown to stimulate PI turnover. Nevertheless, mRNA analysis from several peripheral tissues, including heart and intestine, has failed to detect specific transcripts for either the 5-HT<sub>2C</sub> or the 5-HT<sub>2</sub> receptor [19]. Therefore, we propose that some of the peripheral actions of 5-HT, specifically on smooth muscles, are mediated by the NP75 receptor.

Development of specific drugs to NP75/5-HT<sub>2C</sub> receptor could be used therapeutically in some cases of human cardiac pathology. For example, the recent finding that serotonin, known to have a vasodilating effect on normal human coronary arteries, has a direct vasoconstricting effect via 5-HT<sub>2</sub>-like receptors when the endothelium is damaged, (as in coronary artery disease) [20], makes it urgent to characterize in more detail the receptors involved.

#### *Note added in proof*

During the reviewing process of this paper, a similar receptor has been described in rat [21]. These authors described the restricted expression of this receptor in the rat stomach. Although both protein sequences are very similar, we don't know if the difference in expression is species specific.

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#### REFERENCES

- [1] Wilkinson, L.O. and Dourish, C.T. in: *Serotonin Receptor Subtypes: Basic and Clinical Aspects* (S.J. Peroutka, Ed.) Receptor Biochemistry and Methodology, Vol. 15, Wiley-Liss, New York, 1991, pp. 147-210.
- [2] Schmidt, A.W. and Peroutka, S.J. (1989) *FASEB J.* 3, 2242-2249.
- [3] Frazer, A., Maayani, S. and Wolfe, B.B. (1990) *Annu. Rev. Pharmacol. Toxicol.* 30, 307-348.
- [4] Hartig, P.R. (1989) *Trends Pharmacol. Sci.* 10, 64-69.
- [5] Peroutka, S.J. (1992) *Neuropharmacology* 31, 609-613.
- [6] McAllister, G., Charlesworth, A., Snodin, C., Beer, M.S., Noble, A.J., Middlemiss, D.N., Iversen, L.L. and Whiting, P. (1992) *Proc. Natl. Acad. Sci. USA* 89, 5517-5521.
- [7] Saxena, P.R. and Villalon, C. (1991) *Trends Pharmacol. Sci.* 12, 223-227.
- [8] Maroteaux, L., Saudou, F., Amlaiky, N., Boshert, U., Plassat, J.L. and Hen, R. (1992) *Proc. Natl. Acad. Sci. USA* 89, 3020-3024.
- [9] Strosberg, A.D. (1991) *Eur. J. Biochem.* 196, 1-10.
- [10] Glennon, R.A., Seggel, M.R., Soine, W.H., Herrick-Davis, K., Lyon, R.A. and Titeler, M. (1988) *J. Med. Chem.* 31, 5-7.
- [11] Chen, K., Yang, W., Grimsby, J. and Shih, J.C. (1992) *Mol. Brain Res.* 14, 20-26.
- [12] Hausdorff, W.P., Caron, M.G. and Lefkowitz, R.J. (1990) *FASEB J.* 4, 2881-2889.
- [13] Yu, L., Nguyen, H., Le, H., Bloem, L.J., Kosak, C.A., Hoffman, B.J., Snutch, T.P., Lester, H.A., Davidson, N. and Lubbert, H. (1991) *Mol. Brain Res.* 11, 143-149.
- [14] Albert, P.R., Zhou, Q.-Y., Tol, V.M.H.H., Bunzow, J.R. and Civelli, O. (1990) *J. Biol. Chem.* 265, 5825-5832.
- [15] Green, S., Issemann, I. and Sheer, E. (1988) *Nucleic Acids Res.* 16, 396.
- [16] Pierce, P.A. and Peroutka, S.J. (1989) *J. Neurochem.* 52, 656-658.
- [17] Cushing, D.J. and Cohen, M.L. (1992) *Pharmacol. Exp. Ther.* 261, 856-862.
- [18] Baez, M. and Cohen, M.L. (1990) *Mol. Pharmacol.* 38, 31-37.
- [19] Huang, K.N. and Julius, D. in: *Serotonin Receptor Subtypes: Basic and Clinical aspects* (S.J. Peroutka, Ed.) Receptor Biochemistry and Methodology, Vol. 15, Wiley-Liss, New York, 1991, pp. 1-17.
- [20] Golino, P., Piscione, F., Willerson, J.T., Capelli-Bigazzi, M., Focaccio, A., Villari, B., Indolfi, C., Russolillo, E., Condorelli, M. and Chiariello, M. (1991) *New Engl. J. Med.* 324, 641-648.
- [21] Foguet, M., Hoyer, D., Pardo, L.A., Parekh, A., Kluxen, F.W., Kalkman, H.O., Stuhmer, W. and Lubbert, H. (1992) *EMBO J.* 11, 3481-3487.
- [22] Goyer, D. and Schoeffter, P. (1991) *J. Receptor Res.* 11, 197-214.