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Conservation of structure, signaling and pharmacology between two serotonin receptor subtypes from decapod crustaceans, *Panulirus interruptus* and *Procambarus clarkii*

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SUMMARY

Serotonin (5-HT) plays important roles in the maintenance and modulation of neural systems throughout the animal kingdom. The actions of 5-HT have been well characterized for several crustacean model circuits; however, a dissection of the serotonergic transduction cascades operating in these models has been hampered by the lack of pharmacological tools for invertebrate receptors. Here we provide pharmacological profiles for two 5-HT receptors from the swamp crayfish, *Procambarus clarkii*: 5-HT$_{2\beta}$ and 5-HT$_{1\alpha}$. In so doing, we also report the first functional expression of a crustacean 5-HT$_1$ receptor, and show that it inhibits accumulation of cAMP. The drugs mCPP and quipazine are 5-HT$_{1\alpha}$ agonists and are ineffective at 5-HT$_{2\beta}$. Conversely, methiothepin and cinanserin are antagonists of 5-HT$_{2\beta}$ but do not block 5-HT$_{1\alpha}$. A comparison of these two receptors with their orthologs from the California spiny lobster, *Panulirus interruptus*, indicates conservation of protein structure, signaling and pharmacology. This conservation extends beyond crustacean infraorders. The signature residues that form the ligand-binding pocket in mammalian 5-HT receptors are found in the crustacean receptors. Similarly, the protein domains involved in G protein coupling are conserved between the two crustacean receptors and other characterized arthropod and mammalian 5-HT receptors. Considering the apparent conservation of pharmacological properties between crustacean 5-HT receptors, these tools could be applicable to related crustacean physiological preparations.

Key words: agonist, antagonist, neuromodulation, G protein-coupled receptor, amine, cloning.

INTRODUCTION

Serotonin (5-HT) is an important neurotransmitter and neurohormone in most animal species. Diverse processes, including social behaviors and a variety of systemic physiological functions are regulated by this biogenic amine. Animals have evolved a suite of 5-HT receptor types; thus, a single neurotransmitter can have multiple and complex effects on different tissues. To date, seven classes of serotonin receptors have been identified in mammals. These receptor classes are categorized with respect to their signal transduction mechanisms and pharmacological properties (Gerhardt and van Heerikhuizen, 1997; Hoyer et al., 2002; Kroeze et al., 2002).

In invertebrate nervous systems 5-HT can modulate motor pattern generation (Hooper and DiCaprio, 2004), escape and social status (Edwards et al., 1999), aggression (Kravitz, 2000) and learning (Barbas et al., 2003; Bicker, 1999; Kandel and Schwartz, 1982). Serotonin has multiple and complex roles in crustacean models. 5-HT application to the stomatogastric nervous system of decapod crustaceans elicits distinct responses from individual identified neurons (Flamm and Harris-Warrick, 1986), and these responses can differ for the same identified neuron in different species (Katz and Tazaki, 1992). In the crayfish, the social status of an individual determines the directionality of 5-HT modulation of the lateral giant escape circuit response to sensory stimulation (Yeh et al., 1997; Yeh et al., 1996). The rate and concentration of 5-HT application to the circuit also affect this response (Teshiba et al., 2001). These studies suggest that crustaceans express several different 5-HT receptor types and that the expression or signaling of these receptors might change in response to social or environmental stimuli.

Crustacean hormonal circuits with small numbers of identifiable cells are ideal preparations for investigating the mechanistic basis of modulation and plasticity. Such studies have, however, been limited by the lack of pharmacological tools. Although the protein sequences and second-messenger couplings of 5-HT receptors are relatively well conserved across all species, their pharmacological profiles can vary significantly between vertebrate and invertebrate preparations, and even within invertebrate preparations (Tierney, 2001). This is not surprising as one would not expect selection for or against amino acids involved in binding synthetic ligands. We have recently identified and cloned two crustacean 5-HT receptors from the spiny lobster, *Panulirus interruptus*, infraorder Achelata: 5-HT$_{1\alpha Pan}$ and 5-HT$_{2\beta Pan}$ (Clark et al., 2004; Sosa et al., 2004). We were interested to know whether the actions of pharmacological agents were conserved across crustacean species. We therefore obtained full-length clones for the same two receptors from a second distantly related decapod crustacean, the swamp crayfish, *Procambarus clarkii* (infraorder Astacidea), and expressed these receptors in a heterologous system in order to determine their second messenger couplings and pharmacological profiles. These parameters were then compared across the two species.

MATERIALS AND METHODS

Animals

Crayfish, *Procambarus clarkii* Girard 1852, were obtained from Atchafalaya Biological Supply (Raceland, LA, USA) and kept

communally at 20°C in 40 l tanks with continual filtration and aeration.

Chemicals and cell lines

HEK293, NIH3T3, COS-7 and MDCK cells, Earle's minimal essential medium (EMEM), horse serum, trypsin, penicillin and streptomycin were obtained from the American Type Culture Collection (Mannassas, VA, USA). Dulbecco's modified Eagle's medium (DMEM) was from Mediatech Inc. (Herndon, VA, USA). Dialyzed fetal bovine serum (FBS), TRex cell line (293-TR), pDNA4/TO plasmid, blasticidin and zeocin were from Invitrogen (Carlsbad, CA, USA). Cinanserin was obtained from Tocris (Ballwin, MO, USA). All other chemicals were from Sigma (St Louis, MO, USA). For pharmacology experiments, amine and agonist stock solutions (10^{-1} mol l^{-1}) were made fresh for every experiment in medium or 50% ethanol, respectively. Two exceptions were tyramine (Tyr), which was made fresh as a 10^{-2} mol l^{-1} stock in medium, and methysergide, which was made as a 10^{-2} mol l^{-1} stock in DMSO and stored at –20°C. Antagonist drugs were made as 10^{-2} mol l^{-1} stock solutions in DMSO and stored at –20°C.

Cloning of full-length 5-$HT_{1\alpha}$ and 5-$HT_{2\beta}$ from *Procambarus clarkii* and generation of expression constructs

Complete cloning and sequencing of 5-$HT_{2\beta Pan}$ and 5-$HT_{1\alpha Pan}$ from *Panulirus interruptus* have been previously described (Clark et al., 2004; Sosa et al., 2004).

We also previously cloned a large segment of 5-$HT_{1\alpha Pro}$ spanning transmembrane domains III–VII from *Procambarus clarkii* (Sosa et al., 2004). We have now completed the sequencing of the 5-$HT_{1\alpha Pro}$ cDNA using rapid amplification of DNA ends (SMART RACE cDNA Amplification kit; BD Biosciences, Clontech, Cambridge, UK) as previously described (Clark et al., 2004). Constructs containing the complete ORF were assembled using standard procedures (Ausubel et al., 1990). Both strands of the construct were sequenced and errors that had been introduced in the cloning process were corrected using QuikChange site directed mutagenesis (Stratagene, La Jolla, CA, USA). The construct was then cloned into the pDNA4/TO (Invitrogen) expression plasmid.

5-$HT_{2\beta Pro}$ was cloned from crayfish cDNA using degenerate RT-PCR and RACE. Previously, 5-$HT_{2\beta Pan}$ had been identified in the *Drosophila* genome database and the ortholog from *Panulirus* was fully cloned and characterized for signal transduction properties (Clark et al., 2004). Degenerate primers were designed based on conserved regions of these *Panulirus* and *Drosophila* orthologs of 5-$HT_{2\beta Pro}$ (written 5′-3′): 5-5-1, GAYGTIYTITTYTGY-ACIGCIWSIATHATG; 5-5-2, ATGCAYYTITGYACIYTIWSI-GTIGAYMGI TT; 5-3, CATDATDATIARIGGDATRTARA-ARCA; 3-5-1, CAYGGIMGIAAYATHMGIATGGARCA; 3-5-2, WUIGARCARAARGCNACNAARGU; 3-3, YUURUURAAIA-WIGURUARRA.

Multiple cDNA preparations, each from a separate crayfish nervous tissue mRNA preparation, were used as templates for nested PCR experiments with these degenerate primers to amplify fragments of the crayfish ortholog, as described previously (Baro et al., 1994; Sosa et al., 2004). Primers specific to 5-$HT_{2\beta Pro}$ were designed to generate a large clone of 5-$HT_{2\beta Pro}$. The N- and C-terminals of 5-$HT_{2\beta Pro}$ were then cloned using SMART RACE as described above. A construct containing the complete ORF was assembled, sequenced and inserted into the pIRESneo (Clontech, Mountain View, CA, USA) expression plasmid as previously described for 5-$HT_{2\beta Pan}$ (Clark et al., 2004).

Sequence data were analyzed using Sequencher 4.1 (Gene Codes Corp., Ann Arbor, MI, USA). Sequences for other arthropod species were obtained from GenBank and alignments were created to determine sequence identities using the ClustalW algorithm with default settings in Lasergene MegAlign (DNASTAR Inc., Madison, WI, USA). Full *Procambarus* sequences have been deposited in GenBank under accession numbers EU131666 (5-$HT_{2\beta Pro}$) and EU131667 (5-$HT_{1\alpha Pro}$).

Generation of cell cultures expressing crustacean 5-HT receptors

We previously characterized the signaling and pharmacological properties of the 5-$HT_{2\beta Pan}$ receptor transiently expressed in HEK293 cells (Clark et al., 2004). We used the same techniques to characterize the *Procambarus* ortholog, 5-$HT_{2\beta Pro}$. Briefly, cells were maintained in EMEM supplemented with 10% FBS, 50 i.u. ml^{-1} penicillin and 50 μg ml^{-1} streptomycin (normal medium). Cells were plated on 60 mm dishes in EMEM without antibiotics, allowed to grow to 95–100% confluency and then transfected with 2 μg of DNA using lipofectamine (Invitrogen). The plates were supplemented to a final concentration of 10% FBS 6 h after transfection, and the medium was replaced with normal medium 24 h after transfection.

In order to functionally characterize 5-$HT_{1\alpha Pan}$ and 5-$HT_{1\alpha Pro}$, we first generated full-length constructs using standard recombinant techniques, as described above. The 5-$HT_{1\alpha}$ receptors were first cloned into pIRESneo and stably transfected into several cell lines using lipofectamine as detailed in the Results. Immunoblotting experiments coupled with the lack of growth after several weeks of selection suggested that none of these cell lines could stably express either the 5-$HT_{1\alpha Pan}$ or the 5-$HT_{1\alpha Pro}$ receptor. This did not appear to be a general phenomenon associated with crustacean receptors, as we successfully expressed 5$HT_{2\beta}$ receptors and crustacean dopamine receptors in these cell lines. The most reasonable explanation for our finding was that the cell lines could not tolerate high levels of the 5-$HT_{1\alpha}$ receptor, and for reasons unknown, cells expressing 5-$HT_{1\alpha}$ receptors were selected against. Inducible expression systems have been developed to deal with this type of problem.

We next expressed the 5-$HT_{1\alpha}$ constructs in an inducible expression system. In this system the 5-$HT_{1\alpha}$ cDNA is stably integrated into the genome of the parental HEK cells, but it is not transcribed unless tetracycline is present in the medium. Using this system we could express 5-$HT_{1\alpha}$ receptors for a defined time interval, and then perform the assays before the cells were selected against. 5-$HT_{1\alpha}$ cDNA was cloned into the inducible expression plasmid, pDNA4/TO, behind the tetracycline operator. The new constructs were then transfected into HEK293-TR cells stably expressing the tetracycline repressor protein. Stably transfected cells (i.e. those in which the plasmid carrying the receptor was incorporated into the HEK cell genome) were selected for >4 weeks in DMEM supplemented with 10% dialyzed fetal bovine serum, 5 μg ml^{-1} blasticidin and 300 μg ml^{-1} zeocin (complete medium; TRex regulated expression system, Invitrogen). Confluent plates were easily obtained after 4 weeks, suggesting that the 5-$HT_{1\alpha}$ cDNA could be maintained in tissue culture cells as long as it was not transcribed and translated. Next, western blots were used to confirm tet-repressor-regulated 5-$HT_{1\alpha Pan}$ expression as follows. Cells were plated in 60 mm dishes and induced with 1 μmol l^{-1} tetracycline 0, 6, 8, 12 and 24 h before collection and isolation of protein by previously described methods (Clark et al., 2004; Sosa et al., 2004). Protein preparations were run on a 10%

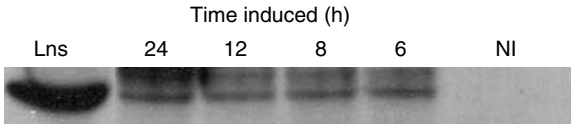

Fig. 1. 5-HT$_{1\alpha Pan}$ can be stably expressed in an inducible tissue culture system. Western blot with anti-5-HT$_{1\alpha Crust}$ showing expression of 5-HT$_{1\alpha Pan}$ in the lobster nervous system (Lns) and in stably transfected 293-TR-5-HT$_{1\alpha Pan}$ cells after induction with tetracycline for the indicated times (24, 12, 8, 6 h) and in non-induced cells (NI).

SDS-PAGE gel, transferred to PVDF membranes and probed with a custom-made rabbit anti-5-HT$_{1\alpha Crust}$ antibody (Sosa et al., 2004). Bands were visualized using chemiluminescence (Immun-Star, BioRad, Hercules, CA, USA). 5-HT$_{1\alpha Pan}$ production commences within 6 h after induction and lasts through 24 h of induction (Fig. 1). Crude protein prepared from lobster nervous system was run as a positive control. Similar results were found for induction of 5-HT$_{1\alpha Pro}$ in stably transfected 293-TR-5-HT$_{1\alpha Pro}$ lines. Based on these findings we induced cells for functional assays for 18–20 h (below).

Assay of IP release in cells expressing 5-HT$_{2\beta Pan}$ and 5-HT$_{2\beta Pro}$

Inositol phosphate (IP) release was assayed as previously described (Clark et al., 2004). Briefly, transiently transfected cells were divided among wells on a 24-well plate with 1 μCi ml^{-1} of [^{3}H]myoinositol (Amersham, Piscataway, NJ, USA) and allowed to grow to 95–100% confluency over 48 h. The cells were washed with fresh EMEM and then exposed to 10 mmol l^{-1} LiCl in EMEM for 20 min at 37°C. As applicable, antagonists were added to individual wells and allowed to incubate for an additional 10 min. 5-HT or agonist drugs were added to test well contents and cells were returned to 37°C for 60 min. The medium was removed and replaced with ice-cold 20 mmol l^{-1} formic acid. Plates were then placed on ice for 30 min. The cell lysate was collected and applied to AG1-X8 columns (BioRad, Hercules, CA, USA) equilibrated with 20 mmol l^{-1} formic acid. The columns were washed with 50 mmol l^{-1} ammonium hydroxide followed by elution of inositol phosphates (IP) with 10 ml of 1 mol l^{-1} ammonium formate–0.1 mol l^{-1} formic acid. The IP fraction was scintillation counted. Membranes attached to the wells were dissolved in 1 mol l^{-1} NaOH and scintillation counted as total phosphatidyl inositols (PI). Activation results are expressed as the fraction of radioactivity incorporated in IP over that in IP+PI and normalized

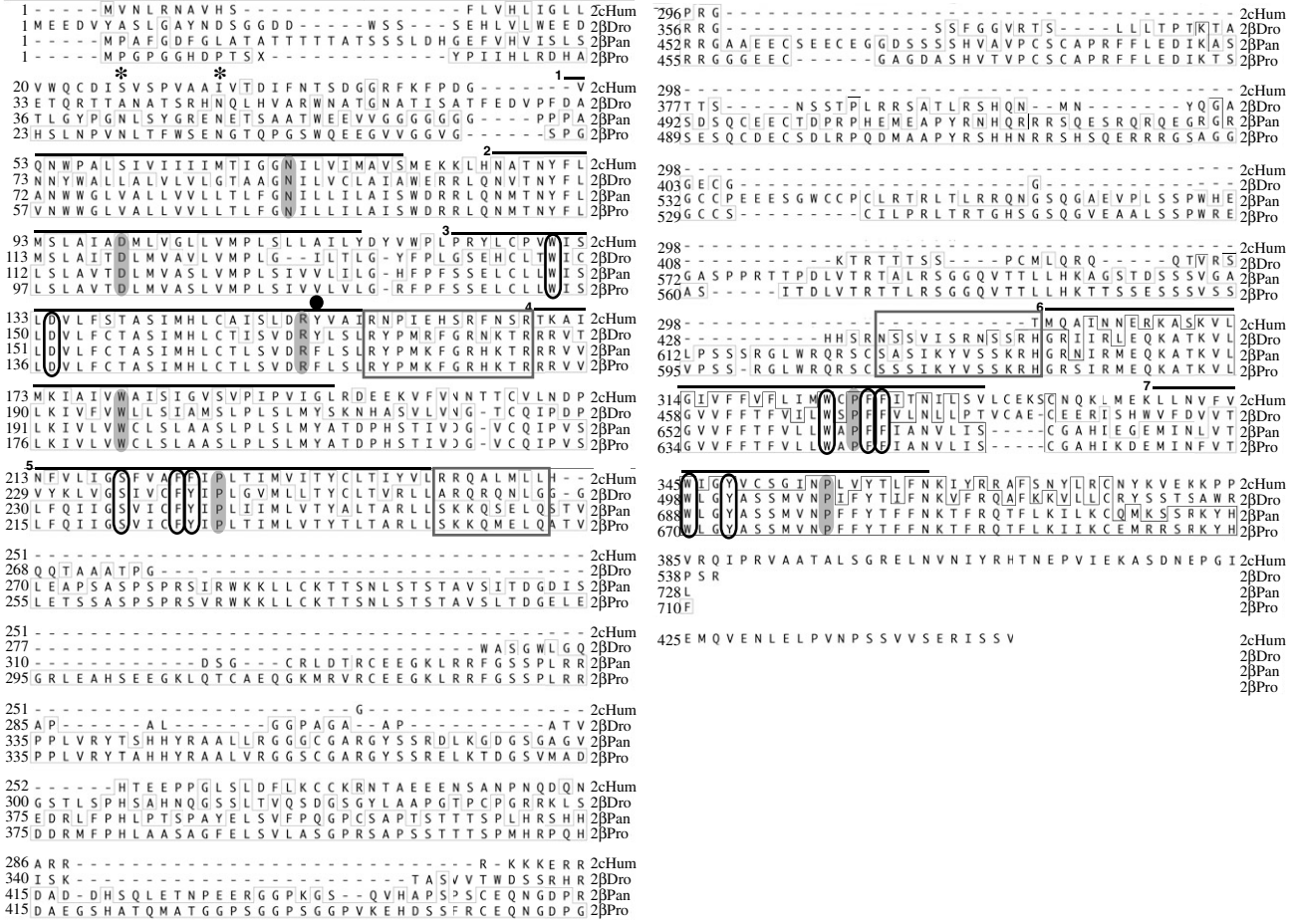

Fig. 2. 5-HT$_{2\beta}$ receptors contain key structural elements typical of the 5-HT receptor superfamily and are conserved among arthropods. Predicted protein sequences of 5-HT$_{2\beta}$ from *Panulirus* and *Procambarus* are aligned with their ortholog from *Drosophila* and the human 5-HT$_{2C}$ receptor. Residues identical to the 5-HT$_{2\beta Pro}$ sequence are indicated with thin lined boxes. Transmembrane domains are indicated with black bars above the sequence and the reference residue for numbering in each is shaded (see Results). Black ovals surround amino acids important for 5-HT ligand binding. Grey boxes surround areas important for G protein coupling or activation. Asterisks above the sequences indicate consensus sites for *N*-linked glycosylations in crustacean sequences. A black dot indicates the evolutionary change from DRY to DRF in crustacean sequences (see Results).

to activity observed in negative control wells (no drug) for every experiment.

[cAMP] determinations in cells expressing 5-HT$_{1\alpha Pan}$ or 5-HT$_{1\alpha Pro}$

Cyclic AMP levels in HEK293-TR cells stably expressing 5-HT$_{1\alpha Pan}$ or 5-HT$_{1\alpha Pro}$ were determined using a Direct cAMP kit (Assay Designs, Ann Arbor, MI, USA) as previously described (Clark et al., 2004). For receptor activation assays, stably transfected cells were plated in 24-well plates and allowed to grow to 100% confluency. The medium was replaced with 1 ml of complete medium containing 1 μg ml^{-1} tetracycline to induce expression of receptor protein. After 18–20 h the medium was replaced with 1 ml of fresh DMEM containing 2.5 mmol l^{-1} 3-isobutyl-1-methylxanthine to block phosphodiesterase activity and plates were incubated for 10 min. Antagonists were added to individual wells (if applicable) and allowed to incubate for an additional 10 min. 5-HT or agonists and forskolin (250 nmol l^{-1}), a nonspecific activator of adenylyl cyclase, were then added to individual wells and left at 37°C for 30 min. The medium was removed and replaced with 0.5 ml of 0.1 mol l^{-1} HCl containing 0.8% Triton X-100. Plates were shaken for 30 min at room temperature, the lysate collected, centrifuged for 5 min at 600 ***g*** and the supernatant assayed for [cAMP] using the Direct cAMP kit and [protein] using a BCA Protein Assay Kit (Pierce, Rockford, IL, USA). Data are presented as pmoles of cAMP per milligram of protein.

Heterologous expression system data analysis

Data for all pharmacology assays involving the heterologous expression systems were plotted and analyzed in GraphPad Prism v.4. Statistics for bar graphs were calculated using a two-way ANOVA with a Bonferroni post-test. Dose–response curves were fitted with a standard slope top–bottom or bottom–top dose–response curve to calculate EC/IC$_{50}$ and efficacy values.

RESULTS

Molecular structure of crustacean 5-HT receptors

The crustacean 5-HT$_{2\beta}$ receptor was recently cloned from *Panulirus interruptus* and characterized with respect to its signaling properties (Clark et al., 2004) and pharmacological profile (N.S. and D.J.B., unpublished). We have used degenerate RT-PCR and RACE to clone the *Procambarus clarkii* ortholog, 5-HT$_{2\beta Pro}$, from crayfish nervous system cDNA. The full-length 5-HT$_{1\alpha}$ cDNA was previously cloned from *Panulirus* and the partial sequence was reported for *Macrobrachium rosenbergii* and *Procambarus clarkii* (Sosa et al., 2004). In this study we completed the cloning and sequencing of the *Procambarus* 5-HT$_{1\alpha}$ ortholog using RACE. The predicted amino acid sequences of 5-HT$_{2\beta}$ and 5-HT$_{1\alpha}$ orthologs were very well conserved between *Panulirus* and *Procambarus*; 72 and 90%, respectively, over the entire protein (Figs 2 and 3, Table 1).

When comparing 5-HT receptors from various species, the overall sequence identity can fall very quickly. This is mainly due to the N- and C-terminal domains and the majority of the third intracellular loop, all of which are highly variable in 5-HT receptors. These regions are not thought to be critical to signaling or pharmacology (Kroeze et al., 2002; Saudou et al., 1992; Witz et al., 1990). When these variable regions were excluded from the alignment, a core region representing 34–59% of the protein remained. This core consisted of transmembrane domains and short linker regions important for maintenance of protein structure, ligand binding and signaling. The identity between *Panulirus* and *Procambarus* orthologs in the core protein was very high at 97% for 5-HT$_{2\beta}$ and 98% for 5-HT$_{1\alpha}$ (Table 1). The complete 5-HT$_{2\beta}$ sequence from each crustacean was 45% identical to the predicted protein sequence of their ortholog from the fruit fly, with an increase to 68% for the core protein. Similarly, crustacean 5-HT$_{1\alpha}$ receptors showed 29–53% identity to orthologs from fly, budworm and butterfly with the core protein sharing at least 76% identity between any of these five arthropod species. In addition, the cores of both crustacean receptors had greater than 40% identity to their human homologs.

5-HT receptors from all species share key conserved residues in their transmembrane domains that are responsible for forming the ligand binding pocket. Because the N-terminal and extra-membrane loops of diverse GPCRs are of variable lengths, referring to residues by their absolute position within these proteins does not allow for comparisons of structural domains between proteins. The Ballesteros-Weinstein numbering scheme used here identified a crucial and conserved characteristic residue common to all G protein coupled receptors (GPCRs) within each transmembrane domain (TM) that was arbitrarily assigned the number 50 (grey, Figs 2 and 3). Other residues within that TM domain were then numbered with the TM number followed by the residue number in relation to this identified amino acid [i.e. Phe (6.51) immediately follows the reference residue in TM6, Pro (6.50)] (Ballesteros and Weinstein, 1995). In biogenic amine receptors the charged residue Asp (3.32) is thought to act as a counterion for the protonated amine moiety of amine ligands, agonists and antagonists and is required for ligand binding but not receptor activation (Kristiansen et al., 2000). The presence of Asp (3.32) in combination with Trp (7.40) is considered a unique fingerprint for biogenic and trace amine GPCRs. These amino acids are involved in ligand binding and receptor activation. In addition to these residues, 5-HT receptors typically have a conserved group of hydrophobic amino acids [Trp (3.28), Phe (5.47), Phe (5.48), Trp (6.48), Phe (6.51), Phe (6.52), Trp (7.40), Tyr (7.43)] that form the hydrophobic ligand-binding pocket within the tertiary structure of the receptor [Kristiansen (Kristiansen, 2004) and references therein; (Roth et al., 1997)]. The hydroxyl group of 5-HT is thought to be stabilized by the Ser (5.43) residue in transmembrane helix 5. Finally, a disulfide bridge formed between Cys (3.25) and a Cys in extracellular loop 2 (EL 2) is important in maintaining tertiary structure and stabilizing the ligand binding pocket. All of these key amino acids (first defined in mammalian receptors) were conserved in 5-HT$_{2\beta}$ and 5-HT$_{1\alpha}$ from crayfish and spiny lobster (Figs 2 and 3).

Crucial residues for Gα subunit binding specificity are located in cytoplasmic amphipathic α-helical extensions of TM5 and 6 in intracellular loop 3 (IL3) (reviewed by Blenau and Baumann, 2001; Gether, 2000; Kristiansen, 2004; Strader et al., 1995). The C-terminal region of IL3 near the membrane interface with TM6 interacts with the C-terminal end of the Gα protein, helping to confer the receptor's specificity for a specific Gα subtype. Again, these regions were very well conserved when comparing the crustacean receptor orthologs to each other, to orthologs from other arthropods and to a human homolog (Figs 2 and 3). Although IL2 does not appear to be involved in determining Gα specificity, this loop contains an α-helix that is thought to act in conjunction with the adjacent DRY motif as a switch between active and inactive states making this loop important for efficient G protein activation. Ligand binding results in protonation of the

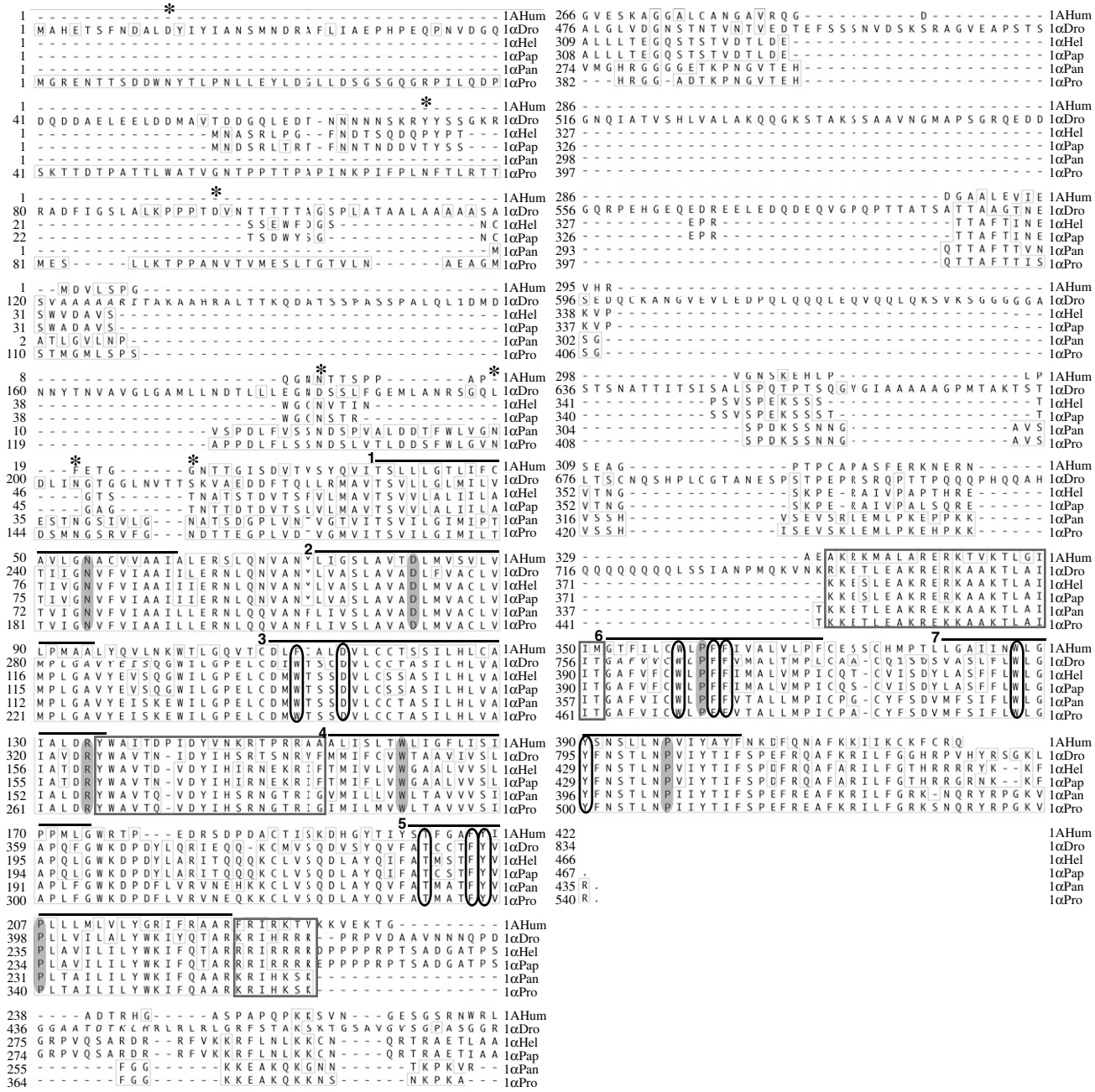

Fig. 3. 5-HT$_{1\alpha}$ receptors contain key structural elements typical of the 5-HT receptor superfamily and are conserved among arthropods. Predicted protein sequences of 5-HT$_{1\alpha}$ from *Panulirus* and *Procambarus* are aligned with their orthologs from *Drosophila*, *Heliothis* and *Papilio* and the human 5-HT$_{1A}$ receptor. Residues identical to the 5-HT$_{1\alpha Pro}$ sequence are boxed with thin lines. Transmembrane domains are indicated with black bars above the sequence and the reference residue for numbering in each is shaded (see Results). Black ovals surround amino acids important for 5-HT ligand binding. Grey boxes surround areas important for G protein coupling or activation. Asterisks above the sequences indicate consensus sites for *N*-linked glycosylations in crustacean sequences.

Asp (3.49) in the DRY motif and in significant rotation of TM3 and TM6 and transition to the active state of the receptor. In addition, the DRY motif and the residues surrounding it are important for constitutive activation in 5-HT receptors (Gether, 2000; Shapiro et al., 2002). Interestingly, the 5-HT$_{2\beta}$ receptor cloned from *Panulirus* has evolved a DRF sequence in place of the DRY that confers increased basal activity of the receptor when stably expressed in cell culture (Clark et al., 2004); this sequence alteration was conserved in 5-HT$_{2\beta Pro}$ from crayfish (Fig. 2).

Serotonin receptors are extensively post-translationally modified by several mechanisms. Most known GPCRs, including 5-HT$_{2\beta Pro}$ and 5-HT$_{1\alpha Pro}$ have several consensus sites for N-linked glycosylation (Asn–X–Ser/Thr) in the N-terminal tail (Figs 2 and 3) and sometimes in other extracellular regions such as EL2. Proper glycosylation of at least some of these sites is required to obtain appropriate levels of receptor expression on the cell surface (Lanctot et al., 2006). Efficiency of ligand binding and functional activity of receptors are not known to be affected by the glycosylation state in receptors that are expressed in the membrane.

Table 1. Predicted protein sequences of arthropod 5-HT$_{2\beta}$ and 5-HT$_{1\alpha}$ receptors are well conserved

	2BHum	2βDro	2βPan	2βPro	1αHel	1αDro	1AHum	1αPan	1αPro
2BHum	X	X	X	X	X	X	X	X	X
2βDro	31 (47)	X	X	X	X	X	X	X	X
2βPan	29 (43)	46 (68)	X	X	X	X	X	X	X
2βPro	28 (43)	45 (68)	**72 (97)**	X	X	X	X	X	X
1αHel	21 (33)	21 (35)	16 (34)	16 (34)	X	X	X	X	X
1αDro	22 (33)	25 (37)	17 (33)	18 (33)	60 (81)	X	X	X	X
1AHum	21 (33)	21 (36)	15 (33)	15 (33)	33 (50)	18 (49)	X	X	X
1αPan	22 (34)	21 (36)	14 (31)	15 (32)	53 (77)	29 (76)	36 (50)	X	X
1αPro	22 (34)	22 (36)	14 (31)	14 (32)	53 (77)	30 (77)	37 (49)	**90 (98)**	X
1αPap	21 (33)	21 (34)	16 (33)	16 (34)	92 (98)	35 (82)	37 (50)	56 (76)	53 (77)

Percentage identities between 5-HT$_2$ and 5-HT$_1$ receptors from Human (Hum), *Drosophila* (Dro), *Panulirus* (Pan) and *Procambarus* (Pro). 5-HT$_{1\alpha}$ receptors from two other arthropods, the tobacco budworm *Heliothis virescens* (Hel) and the swallowtail butterfly *Papilio xuthus* (Pap), are also included. Identity was determined for the entire protein and the core region (parentheses) using the ClustalW algorithm in MegAlign (Lasergene). Comparisons amongst 5-HT$_2$ and 5-HT$_1$ receptors are shaded and crustacean ortholog comparisons are in bold. Accession numbers are human (5-HT$_{2BHum}$, NM_000868; 5-HT$_{1A}$, AAH69159), *Drosophila* (5-HT$_{2\beta Dro}$, NP_731257 plus NP_649805; 5-HT$_{1\alpha Dro}$ P28285), *Panulirus* (5-HT$_{2\beta Pan}$, AY550910; 5-HT$_{1\alpha Pan}$, AY528823), *Heliothis* (5-HT$_{1Hel}$, CAA64863), *Papilio* (5-HT$_{1Pap}$, BAD72868), *Procambarus* (5-HT$_{2\beta Pro}$, EU131666; 5-HT$_{1\alpha Pro}$, EU131667).

The putative glycosylation sites of 5-HT$_{2\beta}$ and 5-HT$_{1\alpha}$ were conserved between orthologs from *Panulirus* and *Procambarus* (Figs 2 and 3). Many GPCRs also have a Cys residue in the proximal region of the C-terminal tail that is a putative palmitoylation site; this creates a membrane anchor, generating an additional cytoplasmic loop. This site was present in crustacean 5-HT$_{2\beta}$ receptors but not in 5-HT$_{1\alpha}$.

The high degree of conservation of the key structures within the crustacean receptors led us to predict that their signaling pathways will also be the same as those of their vertebrate and invertebrate homologs. Because of the high level of overall conservation, we also might expect the spiny lobster and crayfish orthologs of 5-HT$_{2\beta}$ and 5-HT$_{1\alpha}$ to exhibit similar pharmacological profiles. In order to compare the functional properties of *Panulirus* and *Procambarus* 5-HT receptors, we heterologously expressed the proteins in cell culture and used second messenger assays to determine their ligand specificity, signaling and pharmacological properties.

Amine specificity of 5-HT$_{2\beta Pan}$ and 5-HT$_{2\beta Pro}$

The arthropod 5-HT$_{2\beta}$ receptor was initially cloned from *Panulirus* and shown to be specific for 5-HT over other biogenic amines (Clark et al., 2004). When stably expressed in HEK cells, activation of this receptor resulted in increased intracellular levels of inositol phosphates (IP), activation of protein kinase C (PKC) and no change in cAMP levels. In addition, stably expressed 5-HT$_{2\beta Pan}$ demonstrated constitutive activity conferred by the DRY motif (Clark et al., 2004). In this study we transiently expressed 5-HT$_{2\beta Pan}$ in HEK cells and measured IP release in response to amines and putative pharmacological agents. Interestingly, unlike stably transfected cultures, we found no constitutive activity of 5-HT$_{2\beta Pan}$ when the receptor was transiently expressed (see Discussion).

Non-transfected parental HEK cells did not respond to 1 mmol l^{-1} concentrations of any of the monoamines in the IP assay (Fig. 4A). 5-HT$_{2\beta Pan}$ responded to 5-HT, dopamine and tyramine with IP release (Fig. 4B). The EC_{50} for 5-HT was 52 nmol l^{-1} whereas greater than 50 μmol l^{-1} dopamine (DA) and tyramine (Tyr) were required to activate 5-HT$_{2\beta Pan}$ (Fig. 4C). At 1 mmol l^{-1} these amines also had an efficacy less than 55% that of 5-HT (Table 2), indicating that 5-HT is the preferred functional ligand for the 5-HT$_{2\beta Pan}$ receptor. As observed for transiently expressed 5-HT$_{2\beta Pan}$, we found no constitutive activity of transiently expressed 5-HT$_{2\beta Pro}$. 5-HT$_{2\beta Pro}$ responded strongly to 5-HT with a smaller response to DA (Fig. 4D). The EC_{50} for 5-HT$_{2\beta Pro}$ was 270 nmol l^{-1} whereas 1 mmol l^{-1} DA elicited only a minimal response (Fig. 4E, Table 2).

Amine specificity of 5-HT$_{1\alpha Pan}$ and 5-HT$_{1\alpha Pro}$

We were not able to express 5-HT$_{1\alpha Pan}$ or 5-HT$_{1\alpha Pro}$ in traditional systems including HEK293, NIH3T3, MDCK or COS-7 cells; all cells that produced the receptor protein, as determined by western blot analysis, were unhealthy and did not grow beyond 3 weeks. In the few cases where a stable cell line was generated, 5-HT$_{1\alpha}$ protein could not be detected by western blot analysis, suggesting rearrangements in the DNA construct. We do not understand why mammalian cell lines were unable to stably express 5-HT$_{1\alpha}$ using traditional methods. 5-HT$_{1\alpha Pan}$ and 5-HT$_{1\alpha Pro}$ appeared to be constitutively active (below) so high levels of expression of the receptors in standard expression systems may have resulted in toxicity. Alternatively, the protein synthesis, export or turnover machineries of the cells may have been overly taxed by high levels of 5-HT$_{1\alpha}$ expression such that they were not able to maintain normal functions.

In order to functionally characterize 5-HT$_{1\alpha Pan}$ and 5-HT$_{1\alpha Pro}$, we therefore employed an inducible expression system. 5-HT$_{1\alpha Pan}$ or 5-HT$_{1\alpha Pro}$ constructs were stably transfected into 293-TR cells expressing the tetracycline repressor protein. In this system the 5-HT$_{1\alpha}$ construct is under control of the Tet operator sequence, which binds the repressor protein in the absence of tetracycline, thereby preventing expression of 5-HT$_{1\alpha}$. Upon addition of tetracycline to the media, the repressor protein dissociates and 5-HT$_{1\alpha}$ is transcribed and translated into protein. The western blot in Fig. 1 indicates that non-induced cells (tetracycline absent) did not express detectable levels of 5-HT$_{1\alpha Pan}$. After induction (tetracycline present) we were able to obtain high levels of 5-HT$_{1\alpha Pan}$ expression within 6 h that lasted for at least 24 h (Fig. 1). Similar results were obtained for cells induced to express 5-HT$_{1\alpha Pro}$ (not shown).

When compared with non-induced cells, induced cells expressing either 5-HT$_{1\alpha Pan}$ or 5-HT$_{1\alpha Pro}$ showed an increased sensitivity to forskolin, a non-specific activator of adenylyl cyclase (Fig. 5A,C). This supersensitization of adenylyl cyclase is typical of cells expressing a constitutively active Gi/o-coupled receptor

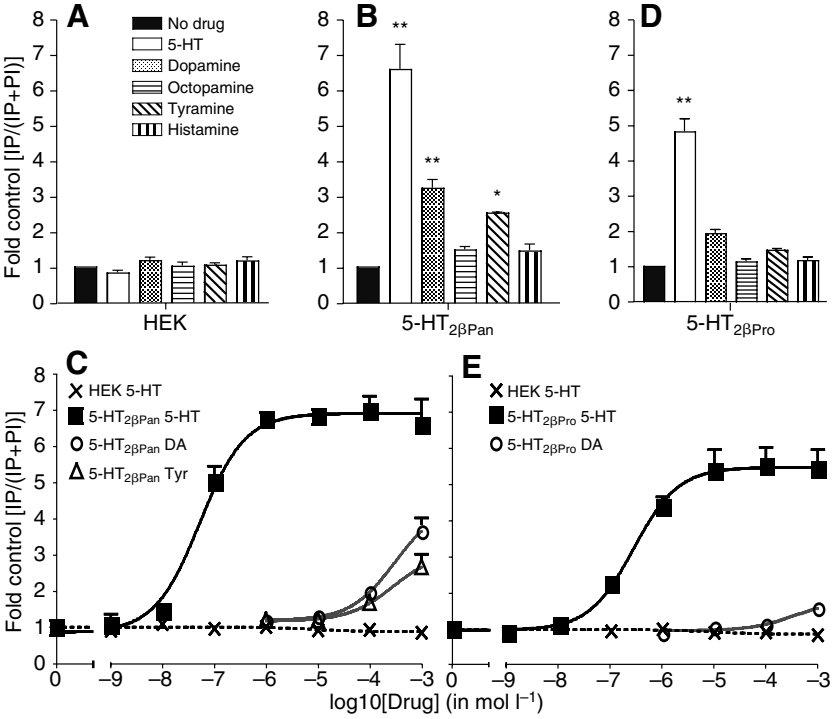

Fig. 4. 5-HT is the only biogenic amine that acts as a potent agonist at 5-$HT_{2\beta Pan}$ and 5-$HT_{2\beta Pro}$. (A) Non-transfected parental HEK cells do not show significant IP responses to any of the amines tested. (B) IP release in response to biogenic amines (10^{-3} mol l^{-1}) in cells transiently expressing 5-$HT_{2\beta Pan}$. Cells expressing 5-$HT_{2\beta Pan}$ demonstrate a greater than sixfold increase in IP release in response to 5-HT and a smaller but significant increase in response to dopamine and tyramine. Values are means ± s.e.m., N=3, $^{**}P$<0.001 and $^{*}P$<0.05 *versus* no drug control. (C) Dose–response curves of 5-$HT_{2\beta Pan}$ to biogenic amines in IP assay. 5-$HT_{2\beta Pan}$ responds to 5-HT (squares) with an EC_{50} of 52 nmol l^{-1}. Dopamine (DA; circles) and tyramine (Tyr; triangles) activate the receptors only at very high concentrations. Non-transfected HEK cells do not respond to 5-HT (crosses). Values are means ± s.e.m., N=3. (D) IP release in response to biogenic amines (10^{-3} mol l^{-1}) in cells transiently expressing 5-$HT_{2\beta Pro}$. IP release is increased more than fourfold in response to 1 mmol l^{-1} 5-HT in cells expressing 5-$HT_{2\beta Pro}$. A smaller but significant increase in response to dopamine is observed. Values are means ± s.e.m., N=3, $^{**}P$<0.001 *vs* no drug control. (E) Dose–response curves of 5-$HT_{2\beta Pro}$ to biogenic amines in IP assay. 5-$HT_{2\beta Pro}$ responds to 5-HT (squares) with an EC_{50} of 270 nmol l^{-1}. Dopamine (circles) activates the receptor only at very high concentrations. Non-transfected HEK cells do not respond to 5-HT (crosses). Values are means ± s.e.m., N=3.

(Johnston and Watts, 2003). Constitutive activity has been observed in mammalian 5-HT_1 receptors as well as other G protein-coupled receptors (Berg et al., 2005; Cosi and Koek, 2000; Johnston and Watts, 2003; Liu et al., 1999).

All known vertebrate and invertebrate 5-HT_1 receptors inhibit adenylyl cyclase, resulting in decreased cAMP levels after stimulation with forskolin (Hoyer et al., 2002; Tierney, 2001). As expected for a 5-HT_1 receptor expressed in a HEK293 cell line, 5-$HT_{1\alpha Pan}$ activation with 5-HT inhibited forskolin-stimulated cAMP accumulation, presumably *via* Gi/o inhibition of adenylyl cyclase. At 1 mmol l^{-1} concentrations, 5-HT was the only monoamine to significantly activate 5-$HT_{1\alpha Pan}$ (Fig. 5A). 5-HT is a highly effective ligand at 5-$HT_{1\alpha Pan}$ with an EC_{50} of 8.4 nmol l^{-1} (Fig. 5B, Table 2). No significant change in cAMP levels was observed with any biogenic amine in non-induced 293-TR-5-$HT_{1\alpha Pan}$ cells (Fig. 5A).

Similarly, 5-$HT_{1\alpha Pro}$ activation with 1 mmol l^{-1} 5-HT also blocks forskolin-stimulated cAMP accumulation. Tyr was the only other

Table 2. Agonist profiles of 5-$HT_{2\beta}$ and 5-$HT_{1\alpha}$ from *Panulirus* and *Procambarus* are very similar

	Potency (EC_{50}, μmol l^{-1})				Efficacy (%5-HT effect)			
Drug	5-$HT_{2\beta Pan}$	5-$HT_{2\beta Pro}$	5-$HT_{1\alpha Pan}$	5-$HT_{1\alpha Pro}$	5-$HT_{2\beta Pan}$	5-$HT_{2\beta Pro}$	5-$HT_{1\alpha Pan}$	5-$HT_{1\alpha Pro}$
5-HT	0.052	0.27	0.0084	0.031	100	100	100	100
Dopamine	310	362	IA	IA	54	19	IA	IA
Octopamine	IA	IA	IA	IA	IA	IA	IA	IA
Tyramine	283	IA	IA	30	32	IA	IA	67
Histamine	IA	IA	IA	IA	IA	IA	IA	IA
DOI	4.5	IA	Bkd	NS	32	IA	Bkd	NS
5-CT	6.1	4.6	2.2	NS	79	50	100	NS
2-Me-5-HT	0.78	5.2	Bkd	0.043	96	104	Bkd	73
MeOTryp	1.0	1.5	4.2	NS	80	29	94	NS
N-acetyl-5-HT	IA	IA	IA	NS	IA	IA	IA	NS
Quipazine	**IA**	**IA**	Bkd	**111**	**IA**	**IA**	Bkd	**92**
α-Me-HT	1.5	7.3	1.1	0.22	79	76	120	67
8-OH-DPAT	0.27	1.1	7.6	65	77	64	105	68
mCPP	**IA**	**IA**	**139**	**109**	**IA**	**IA**	**97**	**70**
Methysergide	0.11	0.11	0.089	0.42	48	19	81	109

EC_{50} values (potency) and relative efficacy were calculated from dose–response curves for each drug. Efficacy is presented as a given drug's ability to activate the receptor compared to the maximum activation obtained from 5-HT (100%). Drugs that activate one and not the other of 5-$HT_{2\beta}$ and 5-$HT_{1\alpha}$ for each species are indicated in bold.

IA, inactive; Bkd, drug has background activity on non-induced cells and was not tested; NS, curve could not be fit because of complex effects of the drug. $N \geqslant 3$ separate experiments for each drug.

DOI, 2,5-dimethoxy-4-iodoamphetamine; 5-CT, 5-carboxamidotryptamine; 2-Me-5-HT, 2-methyl-serotonin; MeOTryp, 5-methoxytryptamine; α-Me-5-HT, α-methyl-serotonin; 8-OH-DPAT, (±)-8-hydroxy-2-(di-*n*-dipropylamino) tetralin; mCPP, 1-(*m*-chlorophenyl)-piperazine.

biogenic amine that resulted in a significant decrease of cAMP in cells expressing 5-$HT_{1\alpha Pro}$ (Fig. 5C). Serotonin and tyramine are therefore the only amines that significantly inhibit adenylyl cyclase *via* activation of the 5-$HT_{1\alpha Pro}$ receptor. Histamine, dopamine and octopamine all produced an increase in cAMP levels in non-induced cells. Similar levels of cAMP were observed in the induced cells, suggesting that these amines do not act at the 5-$HT_{1\alpha Pro}$ receptor. Non-induced 293-TR-5-$HT_{1\alpha Pro}$ cells gave a positive cAMP response at high 5-HT concentrations (Fig. 5D, crosses). Unlike the cell line expressing *Panulirus* 5-$HT_{1\alpha}$, during the selection period, the 5-$HT_{1\alpha Pro}$ cell line appears to have initiated expression of an endogenous 5-HT receptor that is positively coupled to adenylyl cyclase. It is not unusual for cell cultures to change their karyotypes or expression profiles over time due to the lack of selection pressure to maintain a constant genome. Such changes can be significant and can lead to different net signaling effects for the same protein (Clark and Baro, 2007; Friedman et al., 2002). Cells that were induced to produce 5-$HT_{1\alpha Pro}$ responded to low 5-HT concentrations with a decrease in forskolin-induced cAMP mediated by 5-$HT_{1\alpha Pro}$. This effect was dampened and partially reversed at higher 5-HT concentrations through activation of the endogenous receptor (Fig. 5D, black squares). Subtraction of the 5-HT curves of non-induced cells (Fig. 5D, crosses) from induced cells (Fig. 5D, black squares) resulted in a sigmoidal dose–response for 5-HT at 5-$HT_{1\alpha Pro}$ with an EC_{50} of 31 nmol l^{-1} (Fig. 5D, grey squares). Tyramine was an inefficient agonist and slightly reduced cAMP accumulation only at high concentrations (Fig. 5D, triangles) indicating that 5-HT is the preferred functional ligand for 5-$HT_{1\alpha Pro}$.

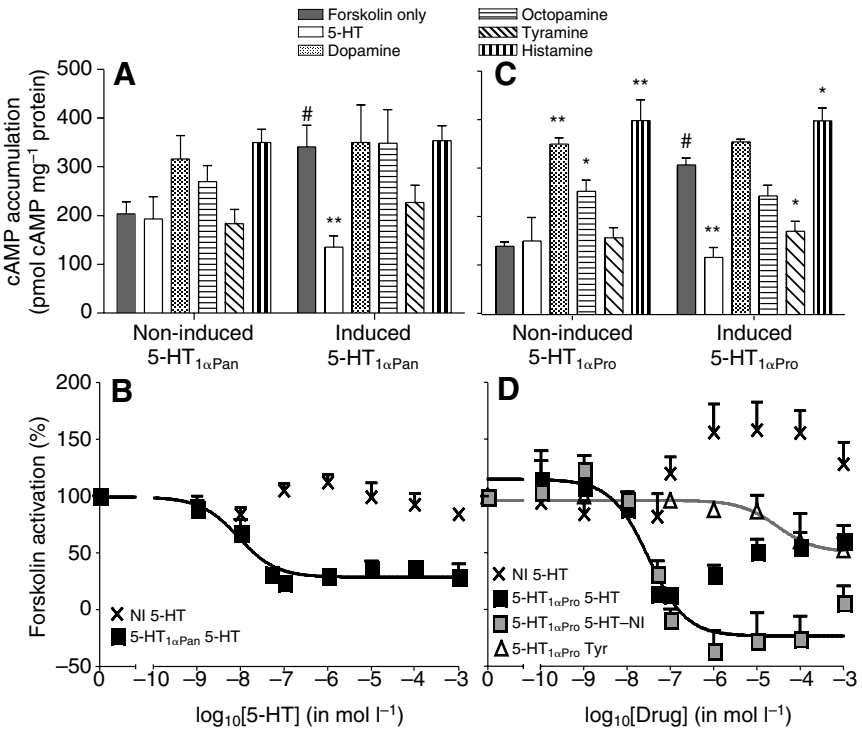

Fig. 5. 5-HT is the only biogenic amine that acts as a potent agonist at 5-$HT_{1\alpha Pan}$ and 5-$HT_{1\alpha Pro}$. (A) Inhibition of forskolin-stimulated cAMP accumulation in response to biogenic amines (10^{-3} mol l^{-1}) in cells induced to express 5-$HT_{1\alpha Pan}$. Non-induced (NI) cells do not significantly respond to biogenic amines (left). Induced cells show supersensitivity and accumulate significantly higher levels of cAMP in response to forskolin only (grey bars). In induced cells adenylyl cyclase is significantly inhibited in response to 1 mmol l^{-1} 5-HT but no other biogenic amines (right). Values are means ± s.e.m., N=3, $^{\#}P$<0.05 *vs* non-induced, $^{**}P$<0.001 *vs* forskolin only. (B) Dose–response curve of 5-$HT_{1\alpha Pan}$ (squares) to 5-HT. The EC_{50} of 5-HT at 5-$HT_{1\alpha Pan}$ is 8.4 nmol l^{-1}. Non-induced cells do not show significant changes in forskolin-stimulated cAMP accumulation in response to 5-HT (crosses). Values are means ± s.e.m., N=3. (C) Inhibition of forskolin-stimulated cAMP accumulation in response to amines (10^{-3} mol l^{-1}) in cells induced to express 5-$HT_{1\alpha Pro}$. Adenylyl cyclase in non-induced cells is activated by dopamine, octopamine and histamine (left). Induced cells accumulate significantly higher levels of cAMP in response to forskolin only (grey bars). In induced cells adenylyl cyclase is significantly inhibited in response to 1 mmol l^{-1} 5-HT and tyramine but no other biogenic amines (right). As observed in non-induced cells, histamine elicits an increase in cAMP levels in cells expressing 5-$HT_{1\alpha Pro}$. Values are means ± s.e.m. N=3, $^{\#}P$<0.001 *vs* non-induced, $^{**}P$<0.001 and $^{*}P$<0.05 *vs* forskolin only. (D) Non-induced (NI) cells show a positive response in cAMP levels at high concentrations of 5-HT (crosses). Owing to this background activity, the dose–response curve of 5-$HT_{1\alpha Pro}$ (black squares) to 5-HT is biphasic. When the activity of non-induced cells in response to 5-HT is subtracted from the response of induced cells (5-HT–NI; grey squares), a sigmoidal dose–response curve is obtained. This curve has an EC50 for 5-HT at 5-$HT_{1\alpha Pro}$ of 31 nmol l^{-1}. Even at high concentrations tyramine has a minimal effect on 5-$HT_{1\alpha Pro}$ (triangles). Values are means ± s.e.m., N=3.

Agonist drugs of crustacean 5-HT receptors

In order to determine if the conservation in sequence and signaling extends to the receptors' responses to pharmacological agents, we tested crayfish 5-$HT_{2\beta Pro}$ and 5-$HT_{1\alpha Pro}$ with a suite of agonist drugs. All drugs that showed significant activity at 10^{-3} mol l^{-1} in an initial overview (Fig. 6) were tested in dose–response curves (see Fig. 7 for examples), which were then used to determine the potency and efficacy (% of maximum 5-HT response at highest concentration) of the drugs. The EC_{50} is a measure of the potency of a drug and reflects its binding affinity at the receptor. Because the maximum effect, or efficacy, achieved by any drug is dependent on the number of receptors expressed we ran a parallel dose–response curve for 5-HT in every experiment and normalized all agonist maximum effects to the maximum 5-HT response, set at 100%. These data have been previously reported for the *Panulirus* receptor (N.S. and D.J.B., unpublished) and are summarized with the *Procambarus* receptor data in Table 2 for comparison.

5-$HT_{2\beta}$ agonists

Most of the drugs that activated 5-$HT_{2\beta Pro}$ had an efficacy close to that of 5-HT (Fig. 6A, Table 2). Methysergide (EC_{50}=110 nmol l^{-1}) was more potent than 5-HT (EC_{50}=270 nmol l^{-1}), however, it elicited only 19% of the total response obtained with 5-HT. Thus, when both potency and efficacy are considered, 5-HT is the strongest ligand at 5-$HT_{2\beta Pro}$. The rank potency of effective agonists at 5-$HT_{2\beta Pro}$ was methysergide>5-HT>8-OH-DPAT>MeOTryp>5-CT>2-Me-5-HT>α-Me-5-HT. This was very similar to the potency ranking of these drugs at 5-$HT_{2\beta Pan}$. However, as can be seen in Table 2, the potency of drugs at the crayfish receptor was generally lower than in lobster, with 5-HT being five times less potent. The crayfish ortholog did not respond to 2,5-dimethoxy-4-

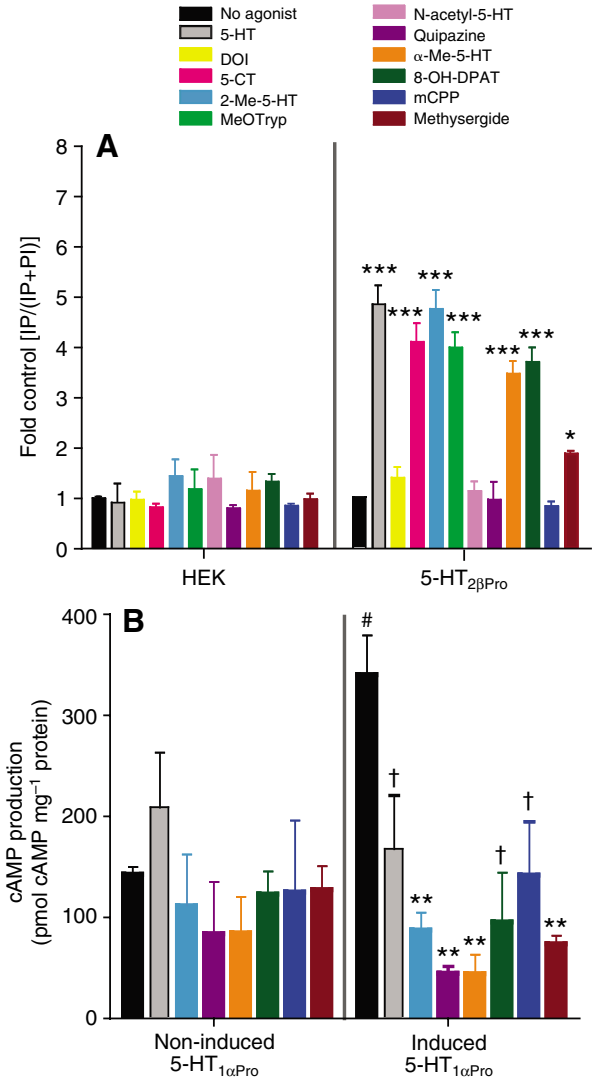

Fig. 6. Specific drugs have differential agonist activity at 5-$HT_{2\beta}$ and 5-$HT_{1\alpha}$. (A) Release of IP in cells expressing 5-$HT_{2\beta Pro}$ (right) in response to various putative agonist drugs (10^{-3} mol l^{-1}, except methysergide, 10^{-5} mol l^{-1}). Non-transfected HEK cells (left) show no IP release in response to the drugs. Values are means ± s.e.m., *N*=3, ***P<0.001 and *P<0.05 *vs* no drug. (B) Inhibition of forskolin-stimulated cAMP production in response to the same drugs in cells induced to express 5-$HT_{1\alpha Pro}$. Values are means ± s.e.m., *N*=3, #P<0.05 *vs* non-induced, **P<0.001 and †P<0.05 *vs* forskolin only.

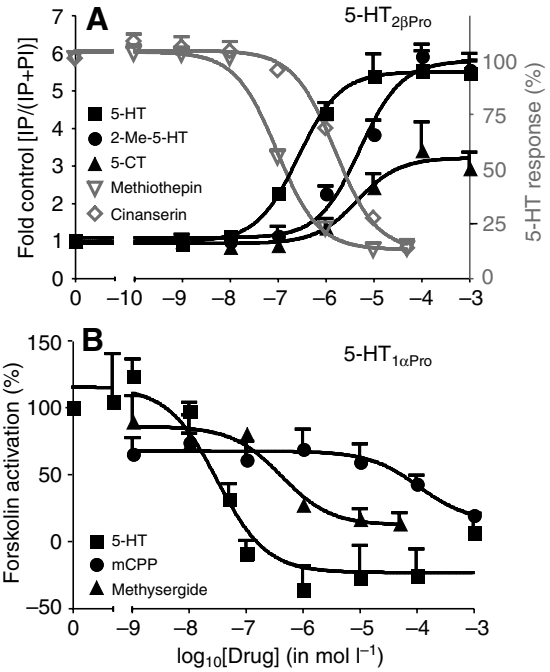

Fig. 7. Example dose–response curves for agonists and antagonists at 5-$HT_{2\beta Pro}$ and 5-$HT_{1\alpha Pro}$. (A) Cells expressing 5-$HT_{2\beta Pro}$ respond with a dose-dependent increase in IP levels in response to 5-HT (10^{-3} mol l^{-1}; black squares) and two agonists with different potencies and efficacies, 2-methyl-5-HT (black circles) and 5-XT (black triangles). The 5-HT-indexed IP response is blocked by increasing concentrations of two antagonists, methiothepin (grey inverted triangles) and cinanserin (grey diamonds). Values are means ± s.e.m., *N*=3. (B) Dose-dependent responses to 5-HT (squares) and two agonists, mCPP (circles) and methysergide (triangles) with different potencies and efficacies in cells induced to express 5-$HT1_{aPro}$. Values are means ± s.e.m., *N*=3.

iodoamphetamine (DOI), which was a relatively weak agonist of 5-$HT_{2\beta Pan}$. The potencies of methysergide, 5-carboxamido-tryptamine (5-CT) and 5-methoxytryptamine (MeOTryp) were similar for the two orthologs, but their efficacies were twofold lower at the 5-$HT_{2\beta Pro}$ ortholog (Table 2). However, 2-methyl-serotonin (2-Me-5-HT) was less potent at 5-$HT_{2\beta Pro}$, whereas ortholog efficacies were equivalent. Together these data indicate that, whereas the pharmacological profiles of 5-$HT_{2\beta Pro}$ and 5-$HT_{2\beta Pan}$ are conserved in terms of which drugs are active, 5-$HT_{2\beta Pro}$ was consistently less sensitive to agonist stimulation. As observed for 5-$HT_{2\beta Pan}$, no change in IP level was detected in cells expressing 5-$HT_{2\beta Pro}$ after application of 10^{-3} mol l^{-1} *N*-acetyl-5-HT, quipazine, or 1-(*m*-chlorophenyl)-piperazine (mCPP). None of the drugs had significant effects on non-transfected parental HEK cells (Fig. 6A).

5-$HT_{1\alpha}$ agonists

Most of the putative agonists tested (at 1 mmol l^{-1}) resulted in some activation of the 5-$HT_{1\alpha Pro}$ receptor (Fig. 6B, Table 2). The rank potency of effective agonists at 5-$HT_{1\alpha Pro}$ was 5-HT > 2-Me-5-HT > α-Me-5-HT > methysergide > tyramine > 8-OH-DPAT > mCPP > quipazine. The agonist 2-Me-5-HT was almost as potent but only 73% as efficacious as 5-HT. This pharmacological profile is very similar to that of its lobster ortholog, 5-$HT_{1\alpha Pan}$. However, as for the 5-$HT_{2\beta Pan}$ receptor, the crayfish ortholog of 5-$HT_{1\alpha Pro}$ was generally less sensitive to agonists than the lobster ortholog. ±-Methyl-serotonin (α-Me-5-HT) was more potent, and (±)-8-hydroxy-2-(di-*n*-dipropylamino) tetralin (8-OH-DPAT) was less potent at 5-$HT_{1\alpha Pro}$ relative to 5-$HT_{1\alpha Pan}$, however, both these drugs had lower efficacies at the crayfish ortholog. The potency of mCPP was comparable for *Panulirus* and *Procambarus* 5-$HT_{1\alpha}$, although it was less efficient at the crayfish ortholog. Methysergide was less potent but had a higher efficacy at 5-$HT_{1\alpha Pro}$ compared to 5-$HT_{1\alpha Pan}$. Two drugs that could not be tested at 5-$HT_{1\alpha Pan}$ because they altered cAMP levels in non-induced 293-TR-5-$HT_{1\alpha Pan}$ cells, 2-Me-5-HT and quipazine, are effective agonists of 5-$HT_{1\alpha Pro}$. Several drugs (DOI, 5-CT, MeOTryp, *N*-acetyl-5-HT) had complex effects on induced 293-TR-5-$HT_{1\alpha Pro}$ cells that could not be fitted with standard dose–response curves. These complex effects are probably due to endogenous 5-HT receptors expressed by the non-induced cell line as observed in the 5-HT dose–response curve above (Fig. 5D, crosses). We were

therefore not able to determine an EC_{50} or relative efficacy measurements for these drugs.

In summary, we identified two agonists that would activate 5-$HT_{1\alpha}$ but not 5-$HT_{2\beta}$ (Table 2). mCPP is an agonist of 5-$HT_{1\alpha}$ but not 5-$HT_{2\beta}$ in *Procambarus* and *Panulirus* whereas quipazine is also inactive at 5-$HT_{2\beta}$ from either species and activates 5-$HT_{1\alpha Pro}$ but could not be tested on 5-$HT_{1\alpha Pan}$.

Antagonist drugs of crustacean 5-HT receptors

Because pharmacological agents can be active at multiple 5-HT receptors, strategic combinations of drugs will be necessary to identify the receptors involved in physiological 5-HT effects. In addition to agonists, we therefore tested a suite of putative antagonists on 5-$HT_{2\beta}$ and 5-$HT_{1\alpha}$ from *Procambarus* and compared them to the antagonist profile for *Panulirus* receptors (N.S. and D.J.B., unpublished).

Antagonists were applied to cells 10 min before 5-HT application and second messenger assays were used to test receptor activation. Antagonists were first screened at 10^{-5} mol l^{-1} (Fig. 8) and then dose–response curves were generated for any drugs that significantly blocked 5-HT activation of second messengers at that concentration (see Fig. 7 for examples). The IC_{50} was calculated and is reported as a measure of potency for the drug. The efficacy, or maximum effect, for each drug again depends on receptor expression levels and is therefore reported as a percentage reduction from the level of receptor activation achieved by 5-HT alone in the same experiment (Table 3).

5-$HT_{2\beta}$ antagonists

Putative antagonists had no effect on parental HEK cells (Fig. 8A). Several antagonists (at 10 μmol l^{-1}) significantly reduced 5-HT-induced (1 mmol l^{-1}) IP release in HEK cells expressing 5-$HT_{2\beta Pro}$ (Fig. 8A, Table 3). The rank potency of effective antagonists at 5-$HT_{2\beta Pro}$ was (+)butaclamol > methiothepin > ritanserin > cinanserin > clozapine (Table 3). Of the antagonists tested, ketanserin, spiperone, prazosin, (–)butaclamol, gramine and atropine had no effect at 10^{-5} mol l^{-1}. The potency profile of active antagonists was nearly conserved between 5-$HT_{2\beta}$ from crayfish and lobster, but four of the five drugs had lower IC_{50}s at the crayfish ortholog. In addition, all of the drugs blocked the crayfish receptor by >80%, but the lobster receptor by only 48–84%. 5-$HT_{2\beta}$ from lobster and crayfish diverge by almost 30% in the variable regions (Table 1) and these differences could contribute to the increase in potency and efficacy of antagonists at 5-$HT_{2\beta Pro}$.

5-$HT_{1\alpha}$ antagonists

Twenty-nine putative antagonists, including those that were characterized at 5-$HT_{2\beta Pro}$, were tested on 5-$HT_{1\alpha Pro}$ (Fig. 8B, Table 3). None of 14 putative antagonists (at 10 μmol l^{-1}) were able to block 5-HT (at 10 μmol l^{-1}) activation of 5-$HT_{1\alpha Pro}$. The antagonists were ineffective even at 10 nmol l^{-1} (data not shown). These results agree with what we found for 5-$HT_{1\alpha Pan}$ from *Panulirus* where the same putative antagonists also did not block activation of the receptor (Table 3). Because of background activity, presumably at endogenous GPCRs in the non-induced cell line, we were not able to test the 15 other putative antagonists on cells induced to express 5-$HT_{1\alpha Pro}$ (Table 3). It is not surprising that the uninduced Pan and Pro cell lines showed different background activities, as it is well established that cell lines diverge with time in culture (Clark and Baro, 2007). Further, multiple copies of the plasmid DNA can be integrated into the host genome, and this has the potential to alter expression of nearby genes. It is unlikely that the number or location of plasmid insertions would be identical in the two cell lines.

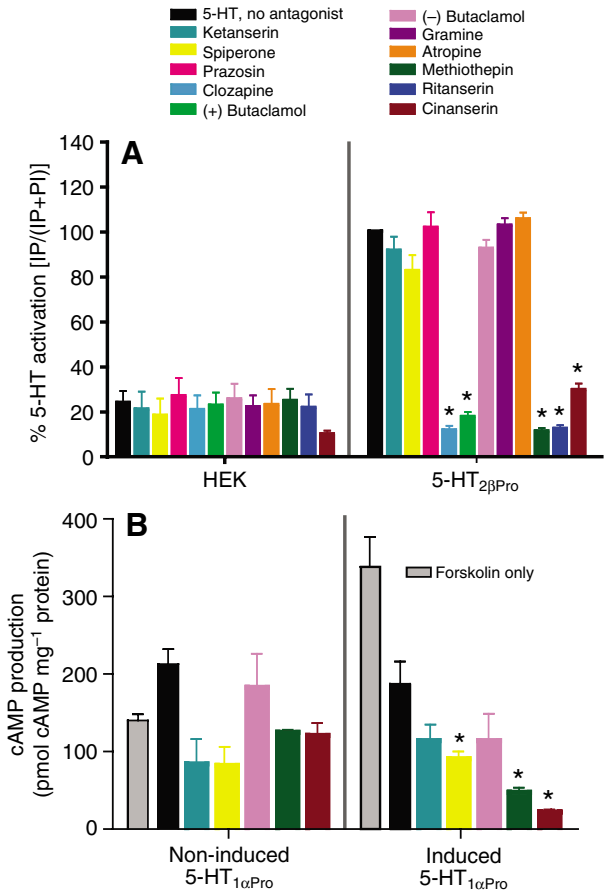

Fig. 8. Identification of antagonists of 5-$HT_{2\beta}$ and 5-$HT_{1\alpha}$. (A) Putative antagonists have no effect on the parental HEK cell line (left). Several of the antagonists (10^{-5} mol l^{-1}) block 5-HT (1 mmol l^{-1})-stimulated increases in IP levels in cells expressing 5-$HT_{2\beta Pro}$ (right). Values are means ± s.e.m., N=3, *P<0.05 *vs* 5-HT only. (B) Antagonist drugs have no significant effect on non-induced 5-$HT_{1\alpha Pro}$ cells (left). None of these drugs (10 mmol l^{-1}) blocks inhibition of adenylyl cyclase by 10^{-5} mol l^{-1} 5-HT in cells induced to express 5-$HT_{1\alpha Pro}$ (right). Some putative antagonists actually increase the efficacy of the 5-HT effect. Values are means ± s.e.m., N=3, *P<0.05 *vs* 5-HT only.

As was seen at 5-$HT_{1\alpha Pan}$, several putative antagonists appeared to increase the efficacy of the 5-HT effect on cAMP accumulation in cells expressing 5-$HT_{1\alpha Pro}$ (Fig. 8B). When tested without 5-HT, however, these drugs had no significant effect on forskolin-stimulated cAMP production (not shown), indicating that they are not acting as agonists. Further studies will be necessary to determine the relationship between antagonist drugs and the crustacean 5-$HT_{1\alpha}$ receptors.

Although we could not find effective 5-$HT_{1\alpha Pro}$ antagonists, we were able to identify several drugs that would block 5-$HT_{2\beta}$ while not affecting 5-HT activation of 5-$HT_{1\alpha}$ (Table 3). Methiothepin and cinanserin efficiently blocked 5-$HT_{2\beta Pro}$ but had no activity at 5-$HT_{1\alpha Pro}$. Similarly, cinanserin, (+)butaclamol and ritanserin all blocked 5-$HT_{2\beta Pan}$ but not 5-$HT_{1\alpha Pan}$. All four of these drugs are effective 5-$HT_{2\beta}$ antagonists but some could not be tested at the 5-$HT_{1\alpha}$ receptor from one or the other species to confirm specificity because of differences in the parental 5-$HT_{1\alpha}$ cell lines.

Table 3. Antagonist profiles of 5-$HT_{2\beta}$ and 5-$HT_{1\alpha}$ from *Panulirus* and *Procambarus* are well conserved

	Potency (IC_{50}, μmol l^{-1})				Efficacy (% reduction)	
Drug	5-$HT_{2\beta Pan}$	5-$HT_{2\beta Pro}$	5-$HT_{1\alpha Pan}$	5-$HT_{1\alpha Pro}$	5-$HT_{2\beta Pan}$	5-$HT_{2\beta Pro}$
Clozapine	6.8	2.5	Bkd	Bkd	79	86
Ritanserin	**0.57**	0.18	**IA**	Bkd	58	86
Methiothepin	0.66	**0.097**	Bkd	**IA**	**84**	**88**
(+)Butaclamol	**0.14**	0.017	**IA**	Bkd	48	82
Cinanserin	**1.2**	**1.6**	**IA**	**IA**	**73**	**88**
Gramine	IA	IA	IA	Bkd	IA	IA
(–)Butaclamol	IA	IA	IA	IA	IA	IA
Ketanserin	IA	IA	IA	IA	IA	IA
Spiperone	IA	IA	IA	IA	IA	ND
Prazosin	IA	IA	IA	Bkd	IA	IA
Atropine	IA	IA	IA	Bkd	IA	IA
Chlorpromazine	ND	ND	IA	IA	ND	ND
Flupenthixol	ND	ND	IA	Bkd	ND	ND
Domperidone	ND	ND	IA	Bkd	ND	ND
Fluphenazine	ND	ND	IA	Bkd	ND	ND
Haloperidol	ND	ND	IA	IA	ND	ND
Metoclopride	ND	ND	IA	IA	ND	ND
(–)Sulpiride	ND	ND	IA	Bkd	ND	ND
WAY100635	ND	ND	IA	IA	ND	ND
Yohimbine	ND	ND	IA	IA	ND	ND
S(–)Propanolol	ND	ND	Bkd	IA	ND	ND
SB269970	ND	ND	IA	IA	ND	ND
Metergoline	ND	ND	Bkd	Bkd	ND	ND
Cyproheptadine	ND	ND	Bkd	Bkd	ND	ND
SB224289	ND	ND	IA	Bkd	ND	ND
BRL15572	ND	ND	IA	IA	ND	ND
TFMPP	ND	ND	IA	IA	ND	ND
SCH23390	ND	ND	Bkd	Bkd	ND	ND
S(–)eticlopride	ND	ND	Bkd	Bkd	ND	ND

IC_{50} values (potency) and relative efficacy were calculated from dose–response curves for each drug. Efficacy is presented as the percentage reduction of the total effect obtained from 5-HT in the absence of antagonist. Drugs that block one and not the other of 5-$HT_{2\beta}$ and 5-$HT_{1\alpha}$ for each species are indicated in bold. $N \geqslant 3$ separate experiments for each drug.

IA, inactive; Bkd, drug has background activity on non-induced cells and was not tested on induced cells, ND, not determined.

DISCUSSION

Two 5-HT receptors from two infraorders of decapod crustaceans, *Panulirus* (Achelata) and *Procambarus* (Astacidea) are highly conserved in their sequence, signaling and pharmacological properties. A significant portion of the 5-$HT_{1\alpha}$ receptor from the giant freshwater prawn *Macrobrachium rosenbergii*, has also been cloned and is also highly conserved with its lobster and crayfish orthologs (Sosa et al., 2004). Together, these data suggest that the conservation of pharmacological function observed between the species studied here may extend beyond reptantian crustaceans, in which case the drugs identified here could perhaps be useful for dissecting 5-HT mechanisms in diverse crustacean systems. We have identified several antagonists, (+)butaclamol, cinanserin and ritanserin for *Panulirus* and methiothepin and cinanserin for *Procambarus*, that will block 5-$HT_{2\beta}$ while leaving 5-$HT_{1\alpha}$ unaffected. In addition, mCPP weakly activates 5-$HT_{1\alpha}$ while having no effect on 5-$HT_{2\beta}$ for both species whereas quipazine activates 5-$HT_{1\alpha Pro}$ but not 5-$HT_{2\beta Pro}$. Combinations of these drugs can be applied in studies of the mechanisms underlying 5-HT modulation in identified circuits and cells of the California spiny lobster, crayfish and related crustacean nervous systems.

Conservation of crustacean 5-HT receptor structure and signaling

Panulirus and *Procambarus* orthologs of 5-$HT_{2\beta}$ and 5-$HT_{1\alpha}$ have the 5-HT signature sequences required for ligand binding and G protein coupling. Most of the sequence differences between receptor orthologs were located in the amino termini and the nonconserved center of the third intracellular loop. These regions may contribute to differences in sensitivity to pharmacological agents observed between receptor orthologs; however, no specific function has been ascribed to these regions to date (Kroeze et al., 2002). Indeed, in characterizing the *Drosophila* 5-HT_1 and 5-HT_7 receptors, the amino termini were removed to increase expression levels in cell culture, with no apparent effect on receptor function (Saudou et al., 1992; Witz et al., 1990).

5-HT was the only biogenic amine to significantly activate 5-$HT_{2\beta Pro}$, however, DA and Tyr were also weak agonists of 5-$HT_{2\beta Pan}$, suggesting that these amines could activate 5-$HT_{2\beta Pan}$ in a physiological context. By contrast, Clark et al. (Clark et al., 2004) found that 5-HT was the only biogenic amine to significantly activate 5-$HT_{2\beta Pan}$. Thus, it is not clear whether or not DA and Tyr have any effect on the 5-$HT_{2\beta Pan}$ receptor in the native system. If these amines do activate native receptors, then is likely to be a concern only at synaptic sites where concentrations can reach up to 1 mmol l^{-1} [(Clements, 1996; Frerking and Wilson, 1996) and references therein] because, high levels of DA and Tyr are required to activate 5-$HT_{2\beta Pan}$. The difference between the two studies may be because we measured IP release [i.e. phospholipase Cβ (PLCβ) activity] whereas the previous study measured PKC activity. Because PKC is downstream of PLCβ and dependent on the consequent release of Ca^{2+}, low levels of PLCβ activation by DA and Tyr may not be sufficient to initiate a cascade culminating in PKC activation. Alternatively, the difference may stem from our

use of transiently transfected cells whereas Clark et al. (Clark et al., 2004) studied stable transfectants. In addition, 5-$HT_{2\beta Pan}$ was found to be constitutively active when stably expressed in HEK293 cells (Clark et al., 2004). However, in our transient transfections with 5-$HT_{2\beta Pan}$ and 5-$HT_{2\beta Pro}$ we found no constitutive activity. The reason for the difference is unclear.

Crustacean 5-$HT_{1\alpha}$ receptors are typical type 1 5-HT receptors, preferentially responding to 5-HT over other biogenic amines with EC_{50} values of 8.4 and 31 nmol l^{-1}, respectively. Other arthropod orthologs of 5-$HT_{1\alpha Pan}$ and 5-$HT_{1\alpha Pro}$ have been cloned and characterized from *Drosophila* (5-HT_{1ADro}, originally 5-HT_{dro2A}) and *Boophilus microplus* and these have comparable EC_{50} values of 30 and 83 nmol l^{-1} for 5-HT, respectively (Chen et al., 2004; Saudou et al., 1992). Whereas the lobster ortholog responded only to 5-HT, the crayfish ortholog was also activated by Tyr. Activation of 5-HT_1 receptors, including those described here, results in inhibition of forskolin-stimulated cAMP accumulation. In addition, the crustacean 5-$HT_{1\alpha}$ receptor shows agonist-independent activity when expressed in HEK cells.

Conservation of crustacean 5-HT receptor pharmacological profiles

The pharmacological profiles of 5-HT receptors are very similar for *Procambarus* and *Panulirus*, but show more variability when compared with homologs from other invertebrates. Agonist activity at crustacean 5-$HT_{2\beta}$ and 5-$HT_{1\alpha}$ orthologs is quite conserved. Two agonists that would differentiate between 5-$HT_{2\beta}$ and 5-$HT_{1\alpha}$ were identified. mCPP activates 5-$HT_{1\alpha}$ but not 5-$HT_{2\beta}$ in both *Procambarus* and *Panulirus*. Quipazine is also specific to 5-$HT_{1\alpha Pro}$ over 5-$HT_{2\beta Pro}$ and, similarly, is inactive at 5-$HT_{2\beta Pan}$ but could not be tested on 5-$HT_{1\alpha Pan}$. Quipazine binds weakly to a molluskan 5-HT_1 receptor (Sugamori et al., 1993) and to 5-$HT_{2\alpha Dro}$ (Colas et al., 1995), indicating that its specificity may not be highly conserved among invertebrates. 8-OH-DPAT was an effective agonist of both crustacean 5-$HT_{2\beta}$ and 5-$HT_{1\alpha}$, and is also the only agonist reported to bind 5-$HT_{1\alpha Dro}$ (Saudou et al., 1992). 8-OH-DPAT was considered a specific mammalian 5-HT_1 agonist but was later found to also activate 5-HT_7 receptors (Bard et al., 1993; Sprouse et al., 2004) and it activates *Drosophila* 5-HT_{7Dro} (Witz et al., 1990). Interestingly, methysergide is an agonist at crustacean 5-$HT_{2\beta}$ and 5-$HT_{1\alpha}$. This drug is a functional antagonist of 5-HT_{7Dro} (Witz et al., 1990) and of vertebrate 5-HT_2 receptors but has agonist activity at some vertebrate 5-HT_1 receptors (Silberstein, 1998).

The antagonist profiles of 5-$HT_{2\beta}$ receptors from *Panulirus* and *Procambarus* are also very well conserved. Cinanserin, (+)butaclamol, ritanserin and methiothepin were identified as antagonists that would block crustacean 5-$HT_{2\beta}$ but not 5-$HT_{1\alpha}$ receptors, however not all of these could be tested at 5-$HT_{1\alpha}$ from both species. The antagonist profiles of the crustacean receptors could not be compared to other arthropod orthologs, as 5-$HT_{2\beta}$ receptors have only been cloned from crustaceans, though they have been found in the fruit fly, honey bee and mosquito sequence databases. A *Drosophila* paralog of the 5-$HT_{2\beta}$ receptor, 5-$HT_{2\alpha Dro}$ [originally 5-HT_{2Dro} (see Tierney, 2001; Clark et al., 2004)] binds strongly to α-Me-5-HT, 2-Me-5-HT, ritanserin and methysergide (Colas et al., 1995), all of which we found to be functionally active at 5-$HT_{2\beta Pan}$ and 5-$HT_{2\beta Pro}$. These drugs may therefore, be 5-HT_2-specific antagonists in arthropods. N-acetyl-5-HT and ketanserin were completely inactive at crustacean 5-$HT_{2\beta}$ but have very high binding constants at 5-$HT_{2\alpha Dro}$. Effective binding of a drug to a receptor may not directly reflect that drug's functional properties at the receptor. 5-$HT_{2\beta}$ and 5-$HT_{2\alpha}$ may therefore be more or less similar, functionally, than suggested by these comparisons. Looking beyond arthropods, several 5-HT_1 and 5-HT_2 type receptors from mollusks and nematodes, also bind methiothepin or ritanserin (Angers et al., 1998; Gerhardt et al., 1996; Hamdan et al., 1999; Olde and McCombie, 1997; Sugamori et al., 1993). Similarly, (+)butaclamol binds a variety of 5-HT receptor subtypes from diverse species (Hamdan et al., 1999; Olde and McCombie, 1997; Saudou et al., 1992). In sum, the antagonist profile of 5-HT receptors appears to be well conserved among crustaceans but this may not extend between phyla.

Crustacean 5-$HT_{1\alpha}$ receptors are highly resistant to antagonists. Even the potent and selective mammalian 5-HT_{1A} blocker WAY100635 (Hoyer et al., 2002) was ineffective at 5-$HT_{1\alpha Pan}$. Prazosin, which functionally blocks 5-HT_{1Dro} (Saudou et al., 1992), also had no effect on crustacean 5-$HT_{1\alpha}$. Many of these putative antagonists block functional activation of mammalian (Hoyer et al., 2002) and invertebrate (Barbas et al., 2002; Hobson et al., 2003; Li et al., 1995; Saudou et al., 1992; Witz et al., 1990) 5-HT receptors. Although some of these may act as allosteric modulators and therefore be sensitive to the high variability of the N-terminal tail amongst 5-HT receptors (May et al., 2004), several of the drugs do efficiently displace radioligands at arthropod receptors (Tierney, 2001) and therefore presumably bind at or near the ligand binding pocket. For none of these to be capable of blocking 5-HT activation of crustacean 5-$HT_{1\alpha}$ is unexpected. 5-$HT_{1\alpha}$ may have an extraordinarily high affinity and selectivity for 5-HT such that the antagonists are not able to overcome the binding of and/or conformational changes elicited by 5-HT. Radioligand studies done on other arthropod 5-$HT_{1\alpha}$ receptors, however, do not fully support this hypothesis as some putative antagonist drugs bind more strongly than 5-HT (Colas et al., 1995; Saudou et al., 1992). Alternatively, the exogenously expressed crustacean 5-$HT_{1\alpha}$ may be in a hyperactive state that overcomes any antagonist effects. This might explain the high response to almost all the putative agonists we tested.

Putative function of characterized 5-HT receptors in crustacean physiological systems

We previously showed that 5-$HT_{1\alpha}$ was extensively expressed in thoracic ganglia of crayfish and *Macrobrachium rosenbergii*, the giant freshwater prawn, in similar patterns (Sosa et al., 2004; Spitzer et al., 2005). Crustacean 5-HT receptors may therefore be conserved in their expression patterns as well as in their molecular structure and function. In crayfish, 5-$HT_{1\alpha}$ is observed in somata and the neuropil throughout the central nerve cord. It is also localized to processes surrounding the nerve roots, to superficial flexor muscles of the abdomen and to processes on the vasculature (Spitzer et al., 2005). The 5-$HT_{1\alpha}$ receptor therefore provides numerous targets for 5-HT modulation in the crayfish.

Several of the drugs characterized here have been used to investigate physiological mechanisms of 5-HT neuromodulation in crustaceans. Based on the pharmacological characterization of crustacean 5-$HT_{2\beta}$ and 5-$HT_{1\alpha}$ we can now hypothesize which receptors might be involved in mediating specific effects and behaviors. In the lateral giant neuron of crayfish, mCPP elicited the inhibitory component of the 5-HT response (Yeh et al., 1997) indicating that 5-$HT_{1\alpha Pro}$ may be responsible. The facilitatory component of the 5-HT response was elicited by α-Me-5-HT (Yeh et al., 1997), an effective agonist of both 5-$HT_{2\beta Pro}$ and 5-$HT_{1\alpha Pro}$. One or both of these receptors could therefore contribute to facilitation of the circuit. In freely behaving crayfish, mCPP injection mimicked the effects of 5-HT on posture (Tierney et al.,

2004; Tierney and Mangiamele, 2001) indicating that 5-$HT_{1\alpha Pro}$ may mediate 5-HT effects on posture. Crayfish abdominal muscles, essential to posture, are modulated by 5-HT at the motor neurons and the neuromuscular junction (Strawn et al., 2000). 5-$HT_{1\alpha Pro}$ receptors are localized on abdominal superficial flexor motorneurons (Spitzer et al., 2005) and could mediate this effect. In addition, 5-CT enhances agonistic behavior in *Procambarus* (Tierney and Mangiamele, 2001), indicating that 5-$HT_{2\beta Pro}$ and/or 5-$HT_{1\alpha Pro}$ could mediate 5-HT signals eliciting these behaviors. Because posture is an essential component of agonistic behavior, these receptors may therefore contribute to different aspects of the agonistic behavioral program (Edwards and Kravitz, 1997; Huber et al., 1997; Kravitz, 2000). Although 5-$HT_{1\alpha Pro}$ is expressed by neurons throughout the crayfish eyestalk (Spitzer et al., 2005), it may not mediate 5-HT-elicited hyperglycemia as this was not mimicked by mCPP (Lee et al., 2000). 5-$HT_{2\beta Pro}$ may, however, be involved as the response was evoked by both 5-CT and 8-OH-DPAT. In the stomatogastric nervous system of the crab, *Cancer borealis*, cinanserin blocked 5-HT-mediated acceleration of the pyloric rhythm (Zhang and Harris-Warrick, 1994) suggesting that the crab 5-$HT_{2\beta}$ ortholog may play a role in modulation of pyloric frequency. Indeed, we have identified roles for 5-$HT_{2\beta Pan}$, 5-$HT_{1\alpha Pan}$ and an unidentified 5-HT receptor in modulation of the pyloric rhythm in *Panulirus* using the drugs identified here (N.S. and D.J.B., unpublished).

Considering uncharacterized 5-HT receptors

At least three crustacean 5-HT receptors (5-$HT_{1\beta}$, 5-$HT_{2\alpha}$, 5-HT_{7}) remain uncharacterized (Clark et al., 2004; Tierney, 2001) and, as we demonstrate in comparing crustacean 5-$HT_{2\beta}$ and 5-$HT_{1\alpha}$ receptor pharmacology, many drugs are active at multiple 5-HT receptor subtypes. Uncharacterized 5-HT receptors could therefore be contributing to observed effects. Especially when using a single agonist drug to elicit a '5-HT effect', the possibility of activating multiple 5-HT or other aminergic receptors must be considered. In addition, radioligand displacement assays of *Drosophila* 5-$HT_{1\alpha}$ and 5-$HT_{1\beta}$, indicated that arthropod 5-HT receptor paralogs can have similar pharmacological properties (Saudou et al., 1992); their effects may therefore be difficult to tease apart even using several drugs. Combinations of drugs were, however, used to assign specific modulatory roles to 5-$HT_{2\beta}$, 5-$HT_{1\alpha}$ and an uncharacterized receptor in the stomatogastric nervous system of *Panulirus* (N.S. and D.J.B., unpublished). Profiling of additional receptor subtypes, the use of multiple characterized drugs and expression studies should allow greater confidence in assigning specific receptor types to behavioral effects.

Conclusion

In conclusion, we have shown that crustacean 5-$HT_{1\alpha}$ receptors are functionally similar to homologous receptors across multiple vertebrate and invertebrate species; furthermore, 5-HT receptors are conserved in protein structure and signaling properties. For 15 drugs that we were able to test at both receptors in both species, the effects on *Panulirus* and *Procambarus* receptors were very comparable. Furthermore, the effects of 19 additional drugs that could only be compared for one receptor type were also similar between the two species. These comparisons suggest that the pharmacological profiles of 5-HT receptor orthologs between these two infraorders are relatively well conserved. Finally, we have identified drugs specific to 5-HT receptor subtypes and thus developed a pharmacological toolset for use in crustacean physiological preparations.

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