RESULTS

In order to clone 5-HT receptor cDNA of *Penaeus monodon*, total RNA from ovary undergoing stage 3 of ovarian maturation of wild broodstock prawn was reverse transcribed. The resulting cDNA was subjected to PCR amplification using the degenerate primers corresponding to the conserved amino acid sequences of the third and seventh transmembrane regions of invertebrate 5-HT receptors. RT-PCR product of an 800 bp fragment was obtained (Figure 1) and subjected to sequencing. The nucleotide and deduced amino acid sequences were blasted in GENBANK database and showed high sequence similarity to invertebrate 5-HT_1 receptors. These partial sequences were used to design gene specific primers to clone 3' and 5' end of this gene by RACE.

Following RACE, 3' RACE product obtained a 400 bp fragment (Figure 2) which was translated into 59 deduced amino acids. The 3' untranslated regions consisted of 177 nucleotide sequences. For 5' RACE, the remaining 5' end of this gene was amplified. The first 5'RACE product obtained a 850 bp fragment (Figure 3A) and the second 5'RACE product obtained a 650 bp fragment (Figure 3B).

Diagram representing the PCR products of each region is shown in Figure 4A. The complete nucleotide and deduced amino acid sequences of the full-length 5-HT_{Pm1} receptor is shown in Figure 4B. The full-length cDNA of the 5-HT_{Pm1} receptor consists of 2280-2286 bps with a 1767-1773 bps open reading frame, coding for 589-591 amino acids varying in the GGC tandem repeat in the third intracellular loop.

Sequence comparison of the 5-HT_{Pm1} with other invertebrate 5-HT receptors demonstrated that the receptor shared high sequence identity especially within the conserved seven transmembrane regions (Figure 5). This receptor displayed highest sequence identity to 5-HT_{Dro1A} (76%), followed by 75% to 5-HT_{Hel}, 71% to 5-HT_{Dro1B}, 68% to 5-HT_{Bom}, 59% to 5-HT_{Lym1}, 58% to 5-HT_{Ap2}, 47% to 5-HT_{Mou1A}, 47% to 5-HT_{Hum1A}, 44% to 5-HT_{Dro7} and 33% to 5-HT_{Lym2}. Dendrogram analysis revealed that the 5-HT_{Pm1} was grouped and clustered primarily with 5-HT_1-like receptors cloned from insect species such as 5-HT_{Dro1A}, 5-HT_{Dro1B}, 5-HT_{Hel}, and 5-HT_{Bom} (Figure 6). Sequence analysis revealed 8 potential N-linked glycosylation sites in the extracellular N-terminal domain. One potential protein kinase A phosphorylation site was found in the second intracellular loop. Two protein kinase C phosphorylation consensus sequences were found within the third intracellular loop. No palmitoylation

site was observed in the C-terminal end. Amino acids that are conserved in all G-protein coupled receptor were found in this receptor as shown in Figure 4B.

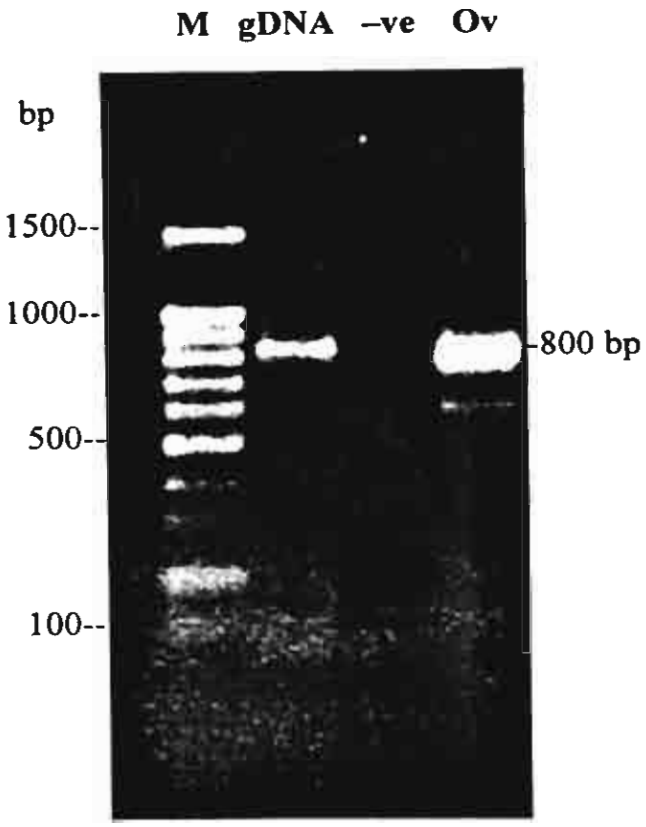


Figure 1. Nested RT-PCR product of the transmembrane region (TM III-VII)

PCR was performed in the presence of two degenerate primers, 5HTTM-III and 5HTTM-VII. cDNAs generated from total RNA of ovary (Ov) of wild broodstock was used as a template. Genomic DNA (gDNA) was used as a positive control. Negative control (-ve) was performed with distilled water. PCR products were size fractionated on 1.5% agarose gel electrophoresis. PCR product of size about 800 bp from ovary was gel purified, cloned and sequenced.

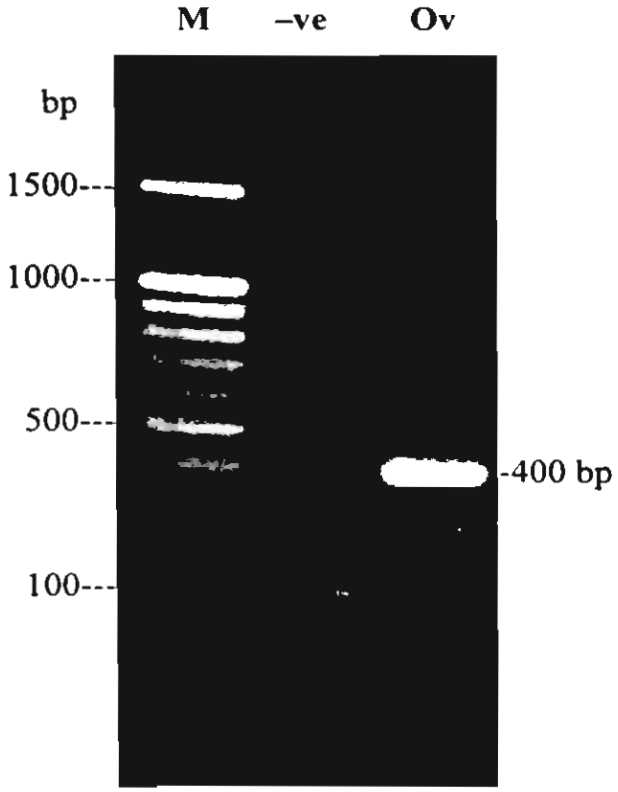


Figure 2. The nested 3'-RACE product of the 5-HT_{Pm1} receptor

The nested 3'-RACE was performed with N3RACE, a gene specific primer and PM-1, an adapter primer. cDNAs generated from total RNA of ovary (Ov) was used as a template. Negative control (-ve) was performed by adding distilled water. PCR product was size-fractionated on 1.5% agarose gel electrophoresis. Lane M is 100 bp ladder DNA marker. The cDNA fragment of size about 400 bp from ovary was gel purified, cloned and sequenced.

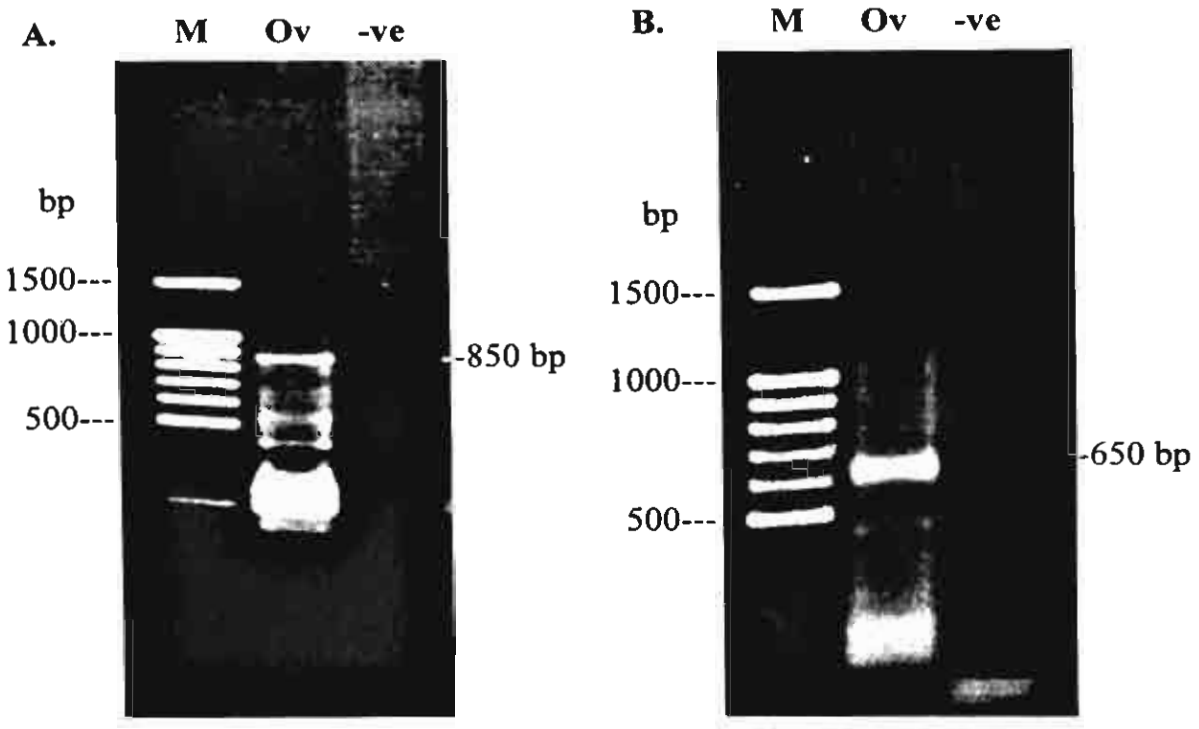


Figure 3. The nested 5'-RACE products of the 5-HT_{Pm1} receptor obtained from the first (A) and the second (B) 5'-end cDNA synthesis

The nested 5'-RACE was performed with N5RACE, a gene specific primer and PM-1, an adapter primer. cDNAs generated from total RNA of ovary (Ov) by standard protocol (A) and by partial heat denaturation (B) of reverse transcription were used as templates. Negative control (-ve) was performed by adding distilled water. PCR products were size-fractionated on a 1.5% agarose gel electrophoresis. Lane M is 100 bp ladder DNA marker. The cDNA fragment of size about 850 bp (A) and 650 bp (B) were gel purified, cloned and sequenced.

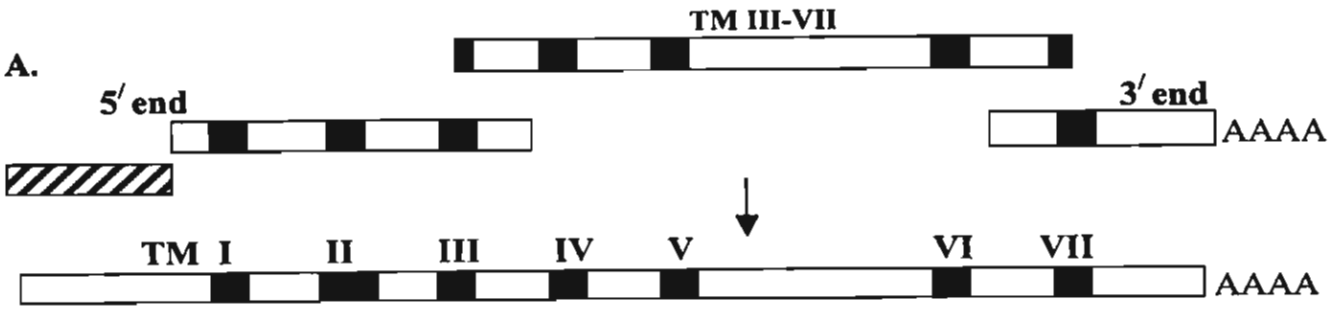


Figure 4. (A) Diagram showing PCR products of the central region (from TM-III to TM-VII), 3' and 5' end. (B) The complete nucleotide and amino acid sequences of 5-HT_{Pm1} receptor of *Penaeus monodon*. The deduced amino acid sequence of the 5-HT_{Pm1} is shown in single letter under the respective codon. Asterisks represent the terminator codons. Seven putative transmembrane domains are boxed and numbered (I-VII). Amino acid letters with underline represent residues that are conserved in all G-protein coupled receptors. Arrows represent potential N-linked glycosylation sites. Triangles represent putative protein kinase C phosphorylation sites. Circle represents putative protein kinase A phosphorylation site.

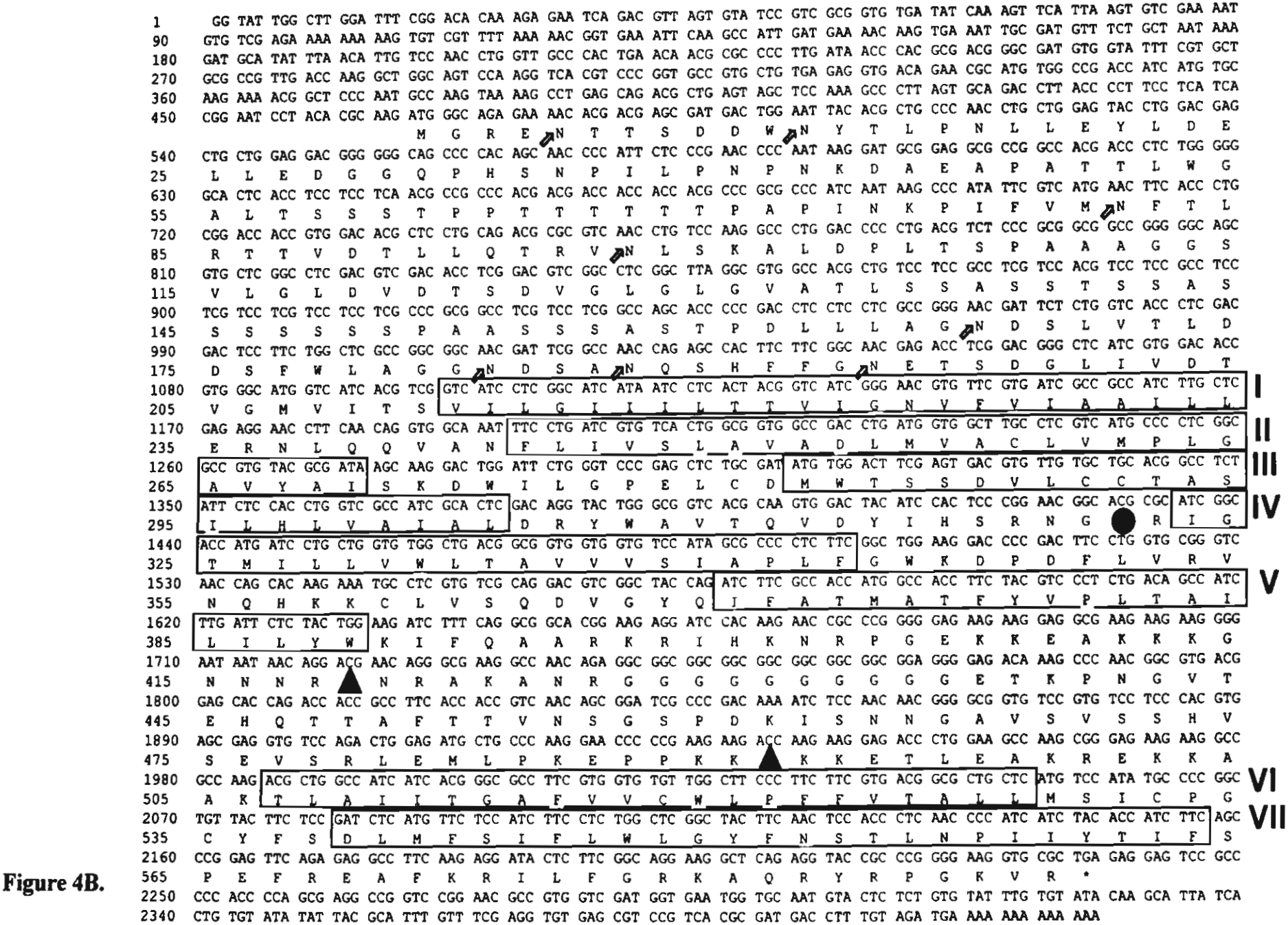


Figure 4B.

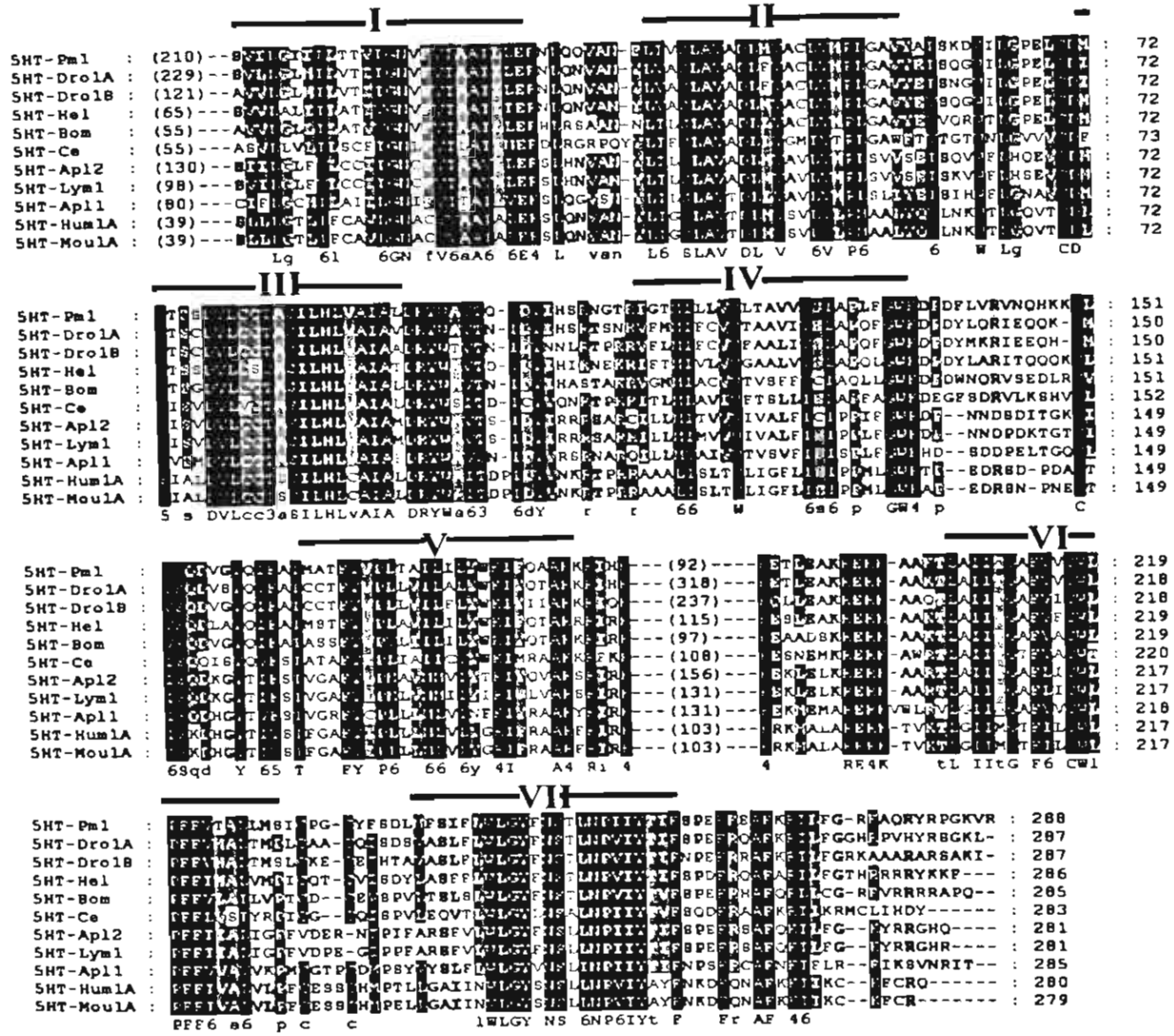
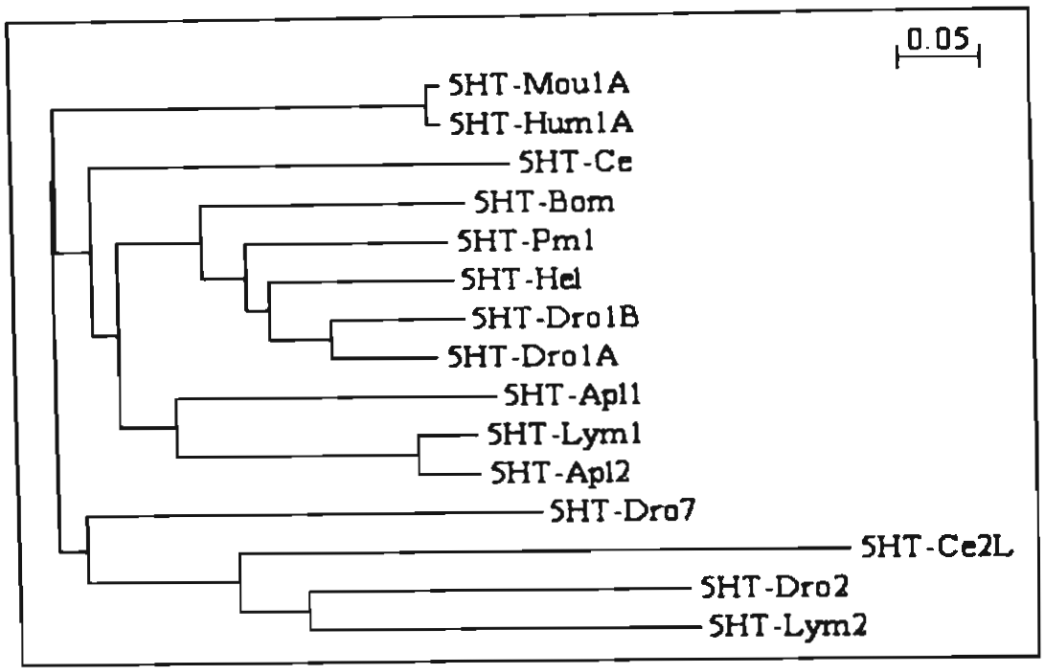


Figure 5. Amino acid sequence similarity between the 5-HT$_{Pml}$ receptor and other member of 5-HT$_1$ receptor subfamily

The amino acid sequences of the seven transmembrane domains and the adjacent regions of 5-HT$_1$ receptors from *Drosophila melanogaster* (5HT –Dro1A and 5HT-Dro1B), *Heliothis virescens* (5HT-Hel), *Bombyx mori* (5HT-Bom), *C. elegans* (5HT-Ce), *Aplysia californica* (5HT-Apl1 and 5HT-Apl2), *Lymnaea stagnalis* (5HT-Lym1), human (5HT-Hum1A), and mouse (5HT-mou1A) were aligned by using clustal X program. Predicted transmembrane domain I-VII are overlined. Black boxes represent amino acids which are identical in all of the receptors. Numbers in parentheses correspond to the number of amino acids at the N-terminal end and the third intracellular loop that are not presented in the alignment.



5-HT receptor	Accession No.	%Identity
5HT-Dro1A	Z11489	76
5HT-Hel	X95605	75
5HT-Dro1B	Z11490	71
5HT-Bom	X95604	68
5HT-Lym1	L06803	59
5HT-Apl2	AF372526	58
5HT-Apl1	AF041039	53
5HT-Cel	U15167	51
5HT-Mou1A	U39391	47
5HT-Hum1A	AB041403	47
5HT-Dro7	M55533	44
5HT-Lym2	U50080	33
5HT-Dro2	X81835	32
5HT-Ce2L	AF031414	25

Figure 6. Phylogenetic analysis and % identity of the 5-HT$_{Pm1}$ receptor of *P. monodon* and other 5-HT receptors

5-HT receptors from *Drosophila melanogaster* (5HT –Dro1A, 5HT-Dro1B, 5HT-dro2 and 5HT-Dro7), *Heliothis virescens* (5HT-Hel), *Bombyx mori* (5HT-Bom), *C. elegans* (5HT-Ce and 5-HT-ce2L), *Aplysia californica* (5HT-Apl1 and 5HT-Apl2), *Lymnaea stagnalis* (5HT-Lym1 and 5HT-Lym2), human (5HT-Hum1A), and mouse (5HT-mou1A) were aligned by using Clustal X program and constructed a dendrogram. Analysis was based on the amino acid sequences of the seven transmembrane domains and the adjacent regions.

DISCUSSION

Serotonin or 5-HT plays important roles in various biological functions in both vertebrate and invertebrates. In penaeus prawn, it involves in ovarian maturation and spawning (Vaca and Alfero, 2000). Serotonin has been proposed to stimulate the release of gonad stimulating hormone from brain and thoracic ganglion and resulted in an induction of ovarian maturation and spawning (Sarojini et al., 1995). It mediates the effects by interacting with its receptor. Most of them are G-protein coupled receptors with the exception of 5-HT_3 which is a ligand-gated ion channel receptor. Most of invertebrate 5-HT receptors are 5-HT_1 -, 5-HT_2 -, or 5-HT_7 - like family. To our knowledge, this is the first report to clone a full-length cDNA of a putative 5-HT receptor from penaeus prawn. An identification of the cDNA coding for the 5-HT_{Pm1} receptor will enable us to understand how 5-HT transduces the signal and induces its various physiological functions especially ovarian maturation and spawning in *Penaeus monodon*. In this study, the full-length cDNA encoding a putative 5-HT_{Pm1} receptor was cloned by RT-PCR technique using the degenerate primers designed from the most conserved amino acid sequences in the third and the seventh transmembrane domains of invertebrate 5-HT_1 –like receptors. The 3' and 5' end of this gene were cloned by RACE method using the derived gene specific sequences as primers. The combined full-length cDNA of 5-HT_{Pm1} receptor of various clones varied from 2280-2286 nucleotides encoding 589-591 amino acids varying in the GGC tandem repeat. The coding region of the 5-HT_{Pm1} receptor consists of seven predicted transmembrane domains which is a major characteristic of the G-protein coupled receptor, variable regions in the third intracellular loop and a short carboxy tail. In addition, it has high amino acid sequence similarity to other invertebrate 5-HT_1 receptors. Therefore, sequence analysis shows that 5-HT_{Pm1} receptor is possibly a 5-HT_1 –like receptor.

Invertebrate 5-HT_1-like receptors have been identified from *Drosophila melanogaster* : 5-HT_{1A}dro and 5-HT_{1B}dro (Saudou, et al., 1992), *C. elegan* : 5-HT_{Ce} (Olde and McCombie, 1997), sea snail (*Aplysia californa*) : 5-HT_{Ap1} and 5-HT_{Ap2} (Anger, et al., 1998 and Barbas, et al., 2002), and fresh water snail (*Lymnaea stagnalis)* : 5-HT_{1Lym} (Sugamori, et al., 1993). Most of them encode 445-834 amino acids. Most of 5-HT_1- like receptors couple to Gαi protein and result in a reduction of cAMP level. Some receptors also couple to stimulate phospholipase C leading to an increase in inositol triphosphate. The pharmacological binding properties of the invertebrate 5-HT_1-like receptors do not follow the mammalian counterparts. Therefore, the functional characterization of the cloned 5-HT receptor of *Penaeus monodon* will be essential for understanding the signaling mechanisms of 5-HT receptors and define a specific agonist that can be potentially used to inject in black tiger prawn to induce ovarian maturation and spawning without eyestalk ablation.

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PROJECT OUTPUTS

1. Publication

Ongvarrasopone, C., Roshorm, Y., Pothiratana, C. and Panyim. S. Molecular Cloning of a Putative 5-HT Receptor From Black Tiger Prawn *(Penaeus monodon)*. Manuscript in preparation (submit to Journal of Biochemistry and Molecular Biology).

2. Oral Presentation

Chalermporn Ongvarrasopone, Yaowaluck Roshorm, Chetsada Pothiratana and Sakol Panyim. Identification of a Novel Serotonin Receptor From Black Tiger Prawns (*Penaeus Monodon*)

Oral Presentation in The Annual Scientific Meeting of the Hong Kong Society of Neurosciences on 9th and 10th December 2002 at the Hong Kong Baptist University.

IDENTIFICATION OF A NOVEL SEROTONIN RECEPTOR FROM BLACK TIGER PRAWNS *(PENAEUS MONODON)*

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Biogenic amines such as serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmiter that has various physiological functions in both vertebrates and invertebrates. In crustacean, it has been proposed to stimulate the release of gonad stimulating hormone from brain and thoracic ganglia and resulted in an induced ovarian maturation and spawning. Serotonin modulates its various physiological functions by interacting with different 5-HT receptors which most of them are members of a G-protein coupled receptor family. In this study, we isolate the partial cDNA sequences encoding a putative 5-HT receptor from a black tiger prawn *(Penaeus monodon)*. Total RNAs isolated from an ovary (stage III) of a wild broodstock prawn, and from brain and thoracic ganglia of juvenile prawns were reverse transcribed by using an oligo(dT) primer and ImProm-II™ reverse transcriptase. The double stranded cDNAs were amplified by using two degenerate primers corresponding to the conserved amino acid sequences in the third (TM III) and seventh (TM VII) transmembrane domains of 5-HT_1 receptors from invertebrates species. The 3′ and 5′ end cDNAs of this gene were amplified by 3′ and 5′ Rapid Amplification of cDNA End. The partial nucleotide sequences contained 1458 nucleotides encoding a protein of 425 amino acids. Deduced amino acid sequences were blasted in GENBANK database and showed high amino acid sequence identities with invertebrates and vertebrates 5-HT_1 receptors. The sequence analysis showed that this 5-HT receptor contained seven transmembrane domains, a characteristic of G-protein coupled receptors. Moreover, it presented a large third intracellular loop, a short carboxy tail and several conserved amino acids which are characteristics of 5-HT_1 receptor family. Sequence comparisons revealed that this receptor shared amino acid sequence identity (86 % to 53%) to the invertebrate 5-HT receptors. Characterization of the pharmacological binding properties and the signal transduction pathway of this cloned receptor will be essential to identify the type of this receptor in the future.

Oral Presention in The Annual Scientific Meeting of The Hong Kong Society of Neurosciences on 9th and 10th December 2002 at the Hong Kong Baptist University.