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Organization and expression study of the shrimp (*Metapenaeus ensis*) putative 5-HT receptor: Up-regulation in the brain by 5-HT☆

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Abstract

To study the mechanism of 5-HT action and the roles of its receptor in the control of reproduction, we have cloned and characterized the gene and the cDNA of a putative 5-HT receptor (5HT1) from the shrimp, *Metapenaeus ensis*. The *5HT1* gene is intronless in the coding region but consists of two introns in the 5′ untranslated region. 5HT1 transcript is 1.8 kb in size and the cDNA consists of an open reading frame of 1230 bp encoding for a protein of 409 amino acid residues. The deduced 5HT1 consists of the characteristic seven hydrophobic transmembrane (TM1–TM7) domains, which share a high amino acid sequence homology to those of the GPCRs. The results from phylogenetic tree analyses indicate that 5HT1 is more closely related to the octopamine/tyramine of the insect and the 5-HT receptors of the vertebrates than to the other G-protein coupled receptors. Although there is no major difference in the tissues' expression pattern of 5-HT in both sexes, the expression level of 5HT1 is much lower in the females than that in the males. 5HT1 expression in the brain and in the eyestalk is also up-regulated in the 5-HT injected shrimp. By in situ hybridization, no difference in the expression pattern of 5-HT was recorded in the eyestalk of males and females, and the pattern of 5HT1 expression in the eyestalk remained unchanged after 5-HT injection. In the eyestalk, 5HT1 transcripts can be detected in neuronal globuli cells and X-organs. The up-regulation of 5HT1 in the eyestalk and brain may be important for the sustained action in the signal transduction pathway or the regulation of other genes. This may represent an auto-regulation of 5HT1 expression in shrimp by 5-HT. To our knowledge, this is the first demonstration of 5-HT stimulation of a putative *5HT1* gene expression in shrimp.

Keywords: Shrimp; 5-Hydroxytryptamine (5-HT); G-protein coupled receptors

1. Introduction

5-Hydroxytryptamine (5-HT) is an amine-derived neurotransmitter implicated in the regulation of a wide variety of physiological and behavioral processes of vertebrates and invertebrates (Zifa and Fillion, 1992; Fingerman, 1997a,b). In vertebrates, 5-HT acts through a membrane-bound G-protein coupled receptor that stimulates the signal transduction pathway. For example, the hyperglycemia caused by 5-HT-releasing drug in rats is mediated by the 5HT1 and 5HT2 receptors. To date, more than 16 mammalian 5-HT receptor subtypes have been cloned and characterized (Hoyer and Martin, 1996; Gerhardt and Van Heerikhuizen, 1997). These 5-HT receptors were classified into seven subfamilies. Except for the 5-HT-3 receptor, which is a ligand-gated ion channel, all other 5-HT receptor subfamily members belong to the superfamily G-protein coupled receptors. These six G-protein coupled 5-HT receptor subfamilies are distinguished on the basis of their amino acid sequence homology and their coupling to particular signaling pathways. Since different 5-

Abbreviations: 5-HT, serotonin (or 5-hydroxytryptamine); 5-HTI, 5-HT receptor; CHH, crustacean hyperglycemic hormone; CNS, central nervous system; GIH, gonad inhibiting hormone; GPCR, G-protein coupled receptor; MIH, molt inhibiting hormone; RT-PCR, reverse transcriptase polymerase chain reaction; TM, trans-membrane domain.

☆ The nucleotide sequence for the shrimp *5HT1* gene and cDNA has been submitted to the GenBank database with accession numbers AY462417 and AY462418, respectively.

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HT receptor subtypes have been identified, 5-HT is postulated to regulate different neuronal systems through the selective activation of distinct receptor subtypes. In invertebrates, 5-HT plays an important role in learning, feeding, locomotion, circadian rhythm, and defense behavior responses (Fingerman, 1997a,b). In crustaceans, 5-HT has been shown to mediate many physiological processes including glucose metabolism, circadian rhythms, behavior, feeding, and reproduction (Fingerman et al., 1994; Fingerman, 1997a,b). The levels of 5-HT and the localizations of 5-HT-immunoreactive neurons have been determined by biochemical and immunohistochemical techniques in the nervous systems of several crustaceans (Livingstone et al., 1981; Beltz et al., 1990; Elofsson, 1983; Rodriguez-Sosa, 1997). In crayfish and lobster, 5-HT modulates the escape and aggressive behaviors in laboratory conditions (Kravitz, 2000; Huber et al., 1997). It also modulates retinal sensitivity in crayfish (Arcehiga et al., 1990) and stimulates the increase of hemolymph glucose concentration in different crustaceans (Luschen et al., 1993; Lee et al., 2001, 2000). Moreover, 5-HT also enhanced the darkening of crayfish by the release of pigment-dispersing hormone (Fingerman et al., 1994), stimulated the molting event (Mattson and Spaziani, 1985), and stimulated rapid gonad maturation (Vaca and Alfaro, 2000). The effect is postulated to act via the secretion of different eyestalk neurohormones (Fingerman, 1997a,b; Lee et al., 2001; Sarojini et al., 1995). Despite the large volume of information on the effects of 5-HT no different physiological responses of the crustaceans, there is limited information for the mechanism of responses mediated by the receptors. 5-HT also induces ovary maturation both in vitro and in vivo (Vaca and Alfaro, 2000; Sarojini et al., 1995). This gonad-stimulating effect is proposed to act through the release of a gonad-stimulating hormone from the brain and thoracic ganglion. Although several 5-HT receptors have been identified in insects (Pietrantonio et al., 2001) and mollusks (Gerhardt et al., 1996; Sugamori et al., 1993; Angers et al., 1998; Barbas et al., 2002), only a few studies described the cloning of 5-HT receptors in crustaceans. As a first step to study the role of 5-HT and the involvement of its receptor in shrimp reproduction, we describe the isolation and characterization of DNA encoding the putative 5-HT receptor genes in the sand shrimp *Metapenaeus ensis*. We also study the expression of 5HT1, provide evidence for the differential expression in different sexes, and up-regulate the expression of 5HT1 in the brain and eyestalk of *M. ensis* after 5-HT injection.

2. Materials and methods

2.1. Nested PCR amplification

Poly(A)$^+$ RNA was isolated from *M. ensis* heart tissue using the PolyAT tract mRNA Isolation kit (Promega, USA). The first strand cDNA was synthesized by MMLV reverse transcriptase (USB, USA). PCR was performed (denaturation at 95 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 2 min) with the degenerate primers 5HTDegF (GAY GTI YTI TGY TGY ACI GCI WCI AT) and 5HTDegR1 (IAR ISW RTT RAA ICC IAR CAA) based on the highly conserved transmembrane domains 3 and 7 of different 5-HT receptors. The second round PCR was performed (denaturation at 95 °C for 1 min, annealing at 55 °C for 1 min, extension at 72 °C for 2 min) with primers 5HTDegF and 5HTDegR2 (corresponding to the conserved region of transmembrane domain 6). PCR products were analyzed on a 1.5% agarose gel. The targeted PCR product was gel-purified (BIO 101, USA), subcloned into a pBluescript cloning vector (Stratagene, USA), and sequenced by autosequencing (Applied Biosystems, USA).

2.2. Cloning of the shrimp 5HT1 gene

Four GenomeWalker libraries were constructed from a Genome Walker™ kit (Clontech, USA). The first round PCR was performed using the gene-specific reverse primer, 5HT1-5′Walk1 (TCC GCA ACC GCG AGA GAC ACG ATG AAA TAG), and the forward primer, AP1 (GTA ATA CGA CTC ACT ATA GGG C), for seven cycles at 94 °C for 2 s, 68 °C for 3 min, followed by 35 cycles at 94 °C for 2 s, 63 °C for 3 min, and a final cycle of 63 °C for 5 min. Nested PCR was performed using the reverse primer, 5HT1-5′Walk2 (GCC AAC GGT GAA ACA AGT GAC GAT ACT), and the adapter primer, AP2 (ACT ATA GGG CAC GCG TGGT), for five cycles of 94 °C for 2 s, 68 °C for 3 min, followed by 20 cycles of 94 °C for 2 s, 63 °C for 3 min, and a final cycle of 63 °C for 5 min. PCR products were analyzed by a 1.5% gel and subcloned, and the sequence was determined.

For genomic Southern blot analysis, DNA (15 μg) was digested with 1–2 U of *Bam*HI, *Bgl*II, and *Alu*I at 37 °C overnight. DNAs were resolved on a 0.8% agarose gel and transferred to a nylon membrane (Nytran, Schleicher, USA). The membrane was UV-crosslinked before incubating in pre-hybridization buffer (5 × SSC, 5 × Denhardt's, 0.1% SDS) at 68 °C for 2 h. Hybridization was performed in a fresh hybridization buffer with the addition of a ^{32}P-dATP-labeled probe. The membranes were washed in different stringency conditions (1 × SSC, 1% SDS at 65 °C for 15 min; 0.5 × SSC, 1% SDS at 65 °C for 15 min; 0.1 × SSC, 1% SDS at 65 °C for 15 min.) in order to remove the strong and weak hybridization signals from the other homologous neurotransmitter receptor genes. After the final wash, the membrane was exposed to X-ray film and autoradiography was performed.

2.3. Northern blot, RT-PCR, and RACE analysis of 5HT1

RNA from different tissues (the eyestalk, hepatopancreas, ovary, testis, swimming leg, heart and CNS) was extracted with standard procedures (Chomczynski and