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Molecular cloning and functional expression of the 5-HT$_7$ receptor in Chinese mitten crab (*Eriocheir sinensis*)

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ABSTRACT

Serotonin (5-HT) regulates numerous physiological functions and processes, such as light adaptation, food intake and ovarian maturation, and plays the role through 5-HT receptors. To our knowledge, this is the first study to isolate and characterize the serotonin receptor 7 (5-HT$_7$ receptor) cDNA encoded in *Eriocheir sinensis*, an economically important aquaculture species in China, by performing rapid-amplification of cDNA ends. The full-length of 5-HT$_7$ receptor gene cDNA is 2328 bp and encodes a polypeptide with 590 amino acids that are highly homologous with other crustaceans 5-HT$_7$ receptor genes. Analysis of the deduced amino acid sequence of the 5-HT$_7$, including 7 transmembrane domains and some common features of G protein-coupled receptors (GPCRs), indicated that 5-HT$_7$ receptor was a member of GPCRs family. A gene expression analysis of the 5-HT$_7$ receptor by RT-PCR revealed that the 5-HT$_7$ receptor transcripts were widely distributed in various tissues, in which high expression levels were observed in the cranial ganglia, thoracic ganglia and intestines. Further study about the effects of photoperiods on the 5-HT$_7$ expression in the tissues showed that a significantly increasing expression of the 5-HT$_7$ receptor was observed in the thoracic ganglia induced by constant light. In addition, in the eyestalks, the expression levels of 5-HT$_7$ mRNA in constant darkness and constant light were lower than control treatment. Then, the expression levels of the 5-HT$_7$ receptor in three feeding statuses displayed that there were significantly increasing expressions in the hepatopancreas and intestines after feeding, compared with before feeding and during the feeding period. Finally, the 5-HT$_7$ mRNA expression levels in stage III and stage IV were higher than the levels in stage I of ovarian development. Our experimental results showed that the 5-HT$_7$ receptor structurally belongs to GPCRs, and the thoracic ganglia and eyestalks are the important tissues of the 5-HT$_7$ receptor for light adaptation. The 5-HT$_7$ receptor may also be involved in the physiological regulation of the hepatopancreas and intestines after ingestion in *E. sinensis*. In addition, the 5-HT$_7$ receptor is involved in the process of ovarian maturation. The study provided a foundation for further research of light adaptation, digestive functions and ovarian maturation of the 5-HT$_7$ receptor in Decapoda.

1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is a biogenic amine neurotransmitter and found in the animal kingdom, and this neurotransmitter affects a great diversity of neuronal and physiological processes, including hormone synthesis and release (Inohara et al., 2015), locomotion (M.Cabaj et al., 2017), feeding behavior (Pratt et al., 2017), reproduction (Tomy et al., 2016), and circadian rhythm (Rodriguez-Sosa et al., 2007). 5-HT mediates these processes by binding with specific receptor subtypes. Learning the molecular and functional characteristics of these receptors is a prerequisite for understanding 5-HT's function. At present, fourteen 5-HT receptor subtypes have been found in mammals (Barnes and Sharp, 1999). Based on the pharmacological binding properties, amino acid sequence homology, and coupling to second messengers, these receptors can be classified into seven families (5-HT$_1$–5-HT$_7$). In crustaceans, many similar effects of 5-HT also have been found including behavior, feeding and reproduction (Fossat et al., 2015; Ongvarrasopone et al., 2006; Sun et al., 2015). However, crustaceans have been largely overlooked in rush to clarify physiological and pharmacological characterizations of 5-HT receptors.

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Although, only a few studies on 5-HT receptors have been reported in crustaceans, such as 5-HT_{1Mac} and 5-HT_{2Mac} of the freshwater prawn *Macrobrachium rosenbergii* (Vazquez-Acevedo et al., 2009), 5-HT_{1Pem} of *Penaeus monodon* (Ongvarrasopone et al., 2006), 5-HT_{1Pan} and 5-HT_{2Pan} of *Panulirus interruptus* (Clark et al., 2004; Sosa et al., 2004), and 5-HT_{1Pro} and 5-HT_{2Pro} of *Procambarus clarkii* (Spitzer et al., 2008). No cloned of 5-HT receptors has to date been identified from economically important decapods crustaceans, such as Chinese mitten crab, *Eriocheir sinensis*, which is an economically important freshwater species in China. Its production had increased to approximately 812,103 tons in 2016. In order to improve the culture performance of *E. sinensis*, planting aquatic plants providing shelter and changing the photoperiods, promoting the feeding efficiency, and induction of oocyte maturation and spawning, are effective methods to be used.

The 5-HT receptors are commonly considered to regulate light reactions in invertebrates. For example, in mollusks and crustaceans, 5-HT enhances the sensitivity of light receptors to light (Aréchiga et al., 1990; Benzid et al., 2006). There is evidence that exogenous supply of 5-HT in vivo can change the phototaxis of several amphipod crustaceans (Guler and Ford, 2010; Perrot-Minnot et al., 2013). In addition, studies have shown that in *Drosophila* larva, honeybee and *P. clarkii*, the neuromodulation of light behavior is carried out by 5-HT neurons and may be mediated by 5-HT_{1A} receptors (Moncalvo and Campos, 2009; Rodriguez-Sosa et al., 2008; Rodriguez-Sosa et al., 2007; Thamm et al., 2010). Despite, light regime has been adjusted to prevent cannibalism in crustaceans, the effects of 5-HT on *E. sinensis* in different photoperiods are still unclear.

The 5-HT can regulate gastrointestinal motility in mammals (Grundy, 2008), and it can bind to 5-HT_2 receptors in the stomatogastric ganglion, which participates in the modulation of stomatogastric motor output (Clark et al., 2004). Stimulation of 5-HT_{1A} receptors and 5-HT_4 receptors in the nucleus accumbens inhibited ingestion, accompanied by changes in locomotor activity (Clissold et al., 2013; Jean et al., 2007; Pratt et al., 2009). In contrast, stimulation of 5-HT_6 receptors can promote ingestion (Pratt et al., 2009). In addition, stimulation of 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2B} receptors in the ventral tegmental area of the rat's midbrain were able to change the food intake (Pratt et al., 2016). Many studies have published that L-tryptophan, a precursor of 5-HT, which was beneficial to improve growth performance by mediating 5-HT in juvenile mud crab, *Scylla serrata*, and freshwater crayfish, *Astacus leptodactylus* (Harlıoğlu et al., 2014; Laranja et al., 2010). The effective mechanism of L-tryptophan or 5-HT in this process has not been reported by far. Our previous study has proved that 5-HT can promote the food transit in digestive tract, 5-HT_2 receptor distributes in intestine and hepatopancreas by immunohistochemical techniques (Li, 2016; Yang et al., 2015). But it is unknown that which receptor involve in gastrointestinal motility of function.

In addition to regulating light reactions and feeding behavior, 5-HT can also participate in the gonad maturation process. Studies have shown that 5-HT promotes release of gonad stimulating hormone (GSH) in *P. clarkii*, and promotes testicular maturation in the red swamp crayfish, *P. clarkii* and the fiddler crab, *Uca pugilator* (Prasad et al., 2014). The ovarian stimulating factor is released through the thoracic ganglia, 5-HT indirectly promotes oocytes maturation and ovarian development in *M. rosenbergii* (Meeratana et al., 2006). In addition, it was reported that the 5-HT_{1Pem} receptor plays an indispensable role in promoting ovarian maturation and spawning in *P. monodon* (Ongvarrasopone et al., 2006).

As we all know, most crustaceans 5-HT receptors are 5-HT_1, 5-HT_2, and 5-HT_7 (Northcutt et al., 2016; Tierney, 2001). Although 5-HT_7 receptor in mammals and insects have been described, and is associated with a number of physiological and pathological responses, including regulation of photoperiods behavior, antidepressant-like behavior and modulating intestinal inflammation, no study on the cloning of this receptor was found in crustaceans (Guseva et al., 2014; Kim and Khan, 2014; Thamm et al., 2010; Vleugels et al., 2014). Based on the sequencing of transcriptomes information of 5-HT_7 receptor from our research groups (Wei et al., 2017; Zhang et al., 2017), the present study aims to clone the 5-HT7 full-length cDNA and analyze its expression in *E. sinensis*' various tissues. In the literature, no such studies have been published. Moreover, we investigated the changes expression of 5-HT_7 in different photoperiods, feeding status and ovarian stages. The aim of this study is to verify the hypothesis that 5-HT involve in the regulation in above physiological process in *E. sinensis*. This study will provide a foundation for further studying the physiological function of 5-HT receptor and in practical applications in Decapoda.

2. Materials and methods

2.1. Animals and sampling

In our study, all crabs were well-limbed and vigorous and provided from the Shuxin crab base (Shanghai, China). The crabs were sheltered in clear glass aquaria with water temperature of 26 ± 1 °C and light of L:D = 12 h:12 h for 7 days, and they were fed from 9:00 to 10:00 every day.

A total of 30 crabs (13.43 ± 1.81 g) were acclimatized to 26 ± 1 °C and a photoperiod of L:D = 12 h:12 h (control), L:D = 0 h:24 h (constant darkness) or L:D = 24 h:0 h (constant light) for 14 days (Tilden et al., 2001). Then, crabs were frozen on ice, dissecting the tissues including cranial ganglia, thoracic ganglia, intestine, gill, heart, muscle, eyestalks, hepatopancreas and hemolymph from control group; cranial ganglia, thoracic ganglia and eyestalks were obtained from the constant darkness group and the constant light group.

After 7 days' temporarily rearing, another 18 crabs were dissected at 8:00 (before feeding), 10:00 (feeding period, the feeding time was 9:00 to 10:00), and 16:00 (after feeding, the feces were mostly in the hindgut and the crabs began to evacuate after feeding 6 h) (Li, 2016), hepatopancreas and intestines were collected and stored at −80 °C for RNA isolation.

With the development and maturation of the ovary, we collected the ovaries from different developmental stages (stage I–stage V) (Wu et al., 2017).

2.2. Nucleic acid extraction

According to the manufacturer's protocol, total RNA was extracted from the different tissues using RNAiso™ plus reagent (RNA Extraction Kit, TaKaRa, Japan). Using micro-volume ultraviolet-visible spectrophotometer (Quawell Q5000; Thmorgan, China) and agarose-gel electrophoresis to estimate the concentration and quality of the total RNA, respectively.

2.3. Cloning of full-length E. sinensis 5-HT_7 cDNA

Transcriptomes sequences of 5-HT_1, 5-HT_2, and 5-HT_7 were obtained from the Y-organ of *E. sinensis*. BlastX analysis showed that the amino acid of the 5-HT_7 EST (length: 661 bp) was certainly homologous to the *Homarus americanus* serotonin receptor 7 (AOG12998.1), so on the basis of the EST, to clone the full-length 5-HT_7 cDNA from *E. sinensis*.

Two gene-specific primers (GSP), 5-HT_7 3′ and 5-HT_7 5′ (Table 1), were designed based on the EST to clone the full-length cDNA of 5-HT_7 by rapid amplification of cDNA ends (RACE) using the SMARTer® RACE 5′/3′ Kit (Clontech, USA). The 3′- and 5′-end cDNA templates were synthesized according to the user's manual. The RACE PCR reaction was carried out in a total volume of 25 μL that contained 12.5 μL of SeqAmp Buffer, 2.5 μL of Universal Primer A Mix (UPM), 1.25 μL of the first-strand cDNA, 0.5 μL of GSP, 0.5 μL of SeqAmp DNA Polymerase, and 7.75 μL of PCR-Grade Water. The PCR amplification conditions were as follows: 94 °C for 5 min; 5 cycles at 94 °C for 30 s, 72 °C for 3 min;