CLONING AND CHARACTERIZATION OF DOPAMINE RECEPTOR FROM BLACK TIGER SHRIMP

SUCHITRAPORN SUKTHAWORN 5137034 MBMG/M

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THESIS ADVISORY COMMITTEE: APINUNT UDOMKIT, Ph.D.,
SAKOL PANYIM, Ph.D., CHALERMPORN ONGVARRASOPONE, Ph.D.,
PONGSOPEE ATTASART, Ph.D.

ABSTRACT

Reproduction of black tiger shrimp, *Penaeus monodon* is regulated by various neurohormones. Dopamine is one of the neurotransmitters that play important roles in ovarian maturation, presumably by the inhibition of a gonad-stimulating hormone (GSH). Like other neurotransmitters, dopamine modulates its physiological function through interacting with specific receptors. Therefore, the objectives of this study were to clone and characterize dopamine receptors in *P. monodon* (DAR_Pem). A cDNA encoding DAR_Pem was cloned by Rapid Amplification of cDNA Ends (RACE). The results showed that DAR_Pem cDNA was 1,990 nucleotides long composing of 1,359 nucleotides of an open reading frame (ORF) that encodes a putative protein of 452 amino acids. The deduced amino acid of DAR_Pem sequence was highly homologous to type 1 of dopamine receptors of invertebrates in phylum arthropoda and was highly conserved among other G-protein coupled receptors. Determination of DAR_Pem mRNA expression in female broodstock shrimp at each ovarian developmental stage by RT-PCR showed that dopamine receptors were expressed in various tissues such as eyestalks, brain, hepatopancrease, abdominal nerve cord, ovary, and thoracic ganglia. The high expression levels were found in nervous tissues such as brain, thoracic ganglia, and eyestalk ganglia whereas the ovary exhibited low level of DAR_Pem mRNA expression. The increased expression of DAR_Pem in the brain of shrimp from ovarian developmental stage I through stage III before starting to decline in stage IV suggested that dopamine may be involved in ovarian maturation by acting through DAR_Pem to inhibit the release of GSH from the brain and indirectly affecting the ovarian maturation. The study of DAR_Pem protein expression by western blot analysis using polyclonal antibody raised against the C-tail region of DAR_Pem in various tissues of *P. monodon* was not successful, probably because the amount of DAR_Pem presented in shrimp tissues was below the sensitivity (10 ng) of the polyclonal antibody. In addition cross-reactivity of the rabbit pre-immunized serum to shrimp proteins was also found in ovarian tissue. Recombinant DAR_Pem was transiently expressed as a membrane protein in COS-1 cells. Investigation of functional activity of DAR_Pem showed that an induction by DA increased intracellular cAMP levels in COS-1 cells confirming that DAR_Pem was a member of dopamine receptor type1. Further investigation of specific ligand binding using agonist or antagonist will help to verify the pharmacological property of DAR_Pem.

KEY WORDS: *PENAEUS MONODON*/ DOPAMINE RECEPTOR/ GONAD-STIMULATING HORMONE (GSH)

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