The Journal Of The International Federation Of Clinical Chemistry And Laboratory Medicine

eJIFCC

IDENTIFICATION OF A NOVEL 5-HT2 RECEPTOR cDNA IN THE OVARY TISSUES OF BLACK TIGER SHRIMP (PANEAUS MONODON)

Nguyen Thi Phuong Mai*, Haiphong Medical University, Vietnam; Chalermporn Ongvarrasopone,

Institute of Molecular Biology and Genetics, Mahidol University, Thailand and Nguyen Thi Phuong Mai Department of Biochemistry, Haiphong Medical University, Vietnam Email: mai091273@yahoo.com.au

2.1 Abstract

The purpose of this experimental study is to isolate a 5-HT receptor from Penaeus monodon. The mRNA were isolated from ovary (stage III) of the wild broad stock then reserve transcribed to cDNA by using Oligo (dT) primer and superscript III enzyme. The template was amplified by PCR technique, used Taq DNA polymerase and two degenerate primers 5-HT-TM2 and 5-HT-TM6, corresponding to the conserved amino acid sequences of invertebrate 5-HT receptors. After cloning, checking positive PCR product, and sequencing analysis revealed an opened frame of 404 acid nucleotides, which was high identity of acid amine coding, and coded for 133 acid amines of protein G. Those result and the 5-HT-TM2 primer expressed in that opened frame were evident presentation of 5-HT2 receptor in P. monodon.

2.2 Introduction

Serotonin (5-HT, 5 hydroxytryptamin) is a neurotransmitter that plays an important role in behaviors as sleeping, memories, and reproduction. Serotonin modulates its various physiological functions by interaction with different 5-HT receptors. There are 7 families of 5-HT receptors in invertebrate, from 5-HT1 to 5-HT7, which are different in acid amine sequence and protein transmembrance. Except 5-HT3 receptor, all of others are protein G (1).

2.3 Methods

After extracting from ovary tissue of black tiger shrimp with TRI reagent, mRNA were reserved transcribed to single DNA by using enzyme superscript III and Oligo (dT) primer. Then the cDNA was used as template to amplify by using PCR with 2 rounds. The first PCR was performed by using 5-HT-TM2 as forward primer and Oligo (dT) as revered primer. Taq DNA polymerase was added at 94oC for 5 min, followed by 30 cycles of denaturizing at 94oC for 30 sec; annealing at 45oC for 30 sec, extending at 74oC for 2 min and final extending at 74oC for 7 min. Then the first PCR product was diluted into 1:50 and used to perform the second PCR, semi-nest PCR, which 5-HT-TM6 was used as revered primer. Followed by cloning PCR in pGEM-T easy vector and then the plasmid carried DNA circle would be sent to check sequence.

2.4 Results

For the amplifying DNA by PCR technique, there was a good band in size of about 900 base pairs (Figure1). After cloning in pGEM-T easy vector, there were seven out of thirteen colonies carried DNA circle (Figure 2). The results of checking sequence in figure 3 were shown an opened frame of 404 acid nucleotides, which was high identity of acid amines coding and coded for 133 acid amines. That protein was protein G but there was only 5-HT-TM2 primer expressed.

2.5 Discussion

The good band in size of 900 base pairs was shown the result of amplifying DNA by PCR technique in good condition and a sufficient amount of DNA to clone in vector. More over, the opened frame with high identity of acid amine coding and belong to protein G were evident to confirm that there was 5-HT2 **receptor in P. monodon . The absence of 5-HT-TM6 primer was explained by the lower melting temperature of the revered primer (42o C) than annealing temperature (45˚ C) – the lowest one for annealing (2).**

2.6 Acknowledgement

This work was supported by International Federation of Clinical Chemists.

References

1 Gaddum JH and Picarelli ZP. Two kinds of tryptamine receptor. Br. J Phar. Chemother.1957;12:323.

2 ambrook and Russel. Molecular cloning. Vol 2. 1992; 8.8

Figure 1: Amplifying Figure 2: cloning DNA in pGEM-T-easy vector

DNA by PCR technique M: Maker, ë Pluss ladder, 20 ng/ul

M: Maker, ë Pluss ladder, 20 ng/ul V: Vector without insert

1 ?? 3 GAA CGT GAC AAA CTA TTT CCT CGC CTC TTT TCG ATA GCA CTT CCC 47

2 ?? 0 E R D K L F P R L F S I A L P 14

3 ?? 48 TCT CCC TTG TCC TCT CGT GCT CCC CGC TCC TTG CTT TGC ATC TCT 92

4 ?? 15 S P L S S R A P R S L L C I S 29

5 ?? 93 CCT TTC CTT TCC CCT TCC CTT CGA TTC CTT TTC CTT TTC CTT TTC 137

6 ?? 30 P F L S P S L R F L F L F L F 44

7 ?? 138 CTT TCC CTT TCT TTC TAT TCC CCT TCC ACT CCA TTC CAT TCC ATT 182

8 ?? 45 L S L S F Y S P S T P F H S I 59

9 ?? 183 CCT CTT CAA TTC CCT TCC ACT TCC GTT CTG ACC TTT TCA TTT CCC 227

10 ?? 60 P L Q F P S T S V L T F S F P 74

11 ?? 228 ATT TTC TTC CTC TCT CTC AAG CTT CAG TTT TTT CCT CCT TCC CTC 272

12 ?? 75 I F F L S L K L Q F F P P S L 89

13 ?? 273 TTC CCC GTG TCC CAT ACT TCC TCT CCC CTT ATC CCT TTT CCC CTT 317

14 ?? 90 F P V S H T S S P L I P F P L 104

15 ?? 318 CCT CTC CCC TTA TCC CTT TTC CCC TTC CTC TCC CCT TAT CCC TTT 362

16 ?? 105 P L P L S L F P F L S P Y P F 119

17 ?? 363 TCC CCT TCC TCT CCA GTT ATC CCT TTT CCC CTT CCT CTC CCC 404

18 ?? 120 S P S S P V I P F P L P L P 133

Figure 3: DNA sequence and its acid amine coding