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Identification and expression analyses of a novel serotonin receptor gene, *5-HT$_{2\beta}$*, in the field cricket *Gryllus bimaculatus*

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Abstract

Biogenic amine serotonin (5-HT) modulates various aspects of behaviors such as aggressive behavior and circadian behavior in the cricket. In our previous report, in order to elucidate the molecular basis of the cricket 5-HT system, we identified three genes involved in 5-HT biosynthesis, as well as four 5-HT receptor genes (*5-HT$_{1A}$*, *5-HT$_{1B}$*, *5-HT$_{2\alpha}$*, and *5-HT$_{7}$*) expressed in the brain of the field cricket *Gryllus bimaculatus* DeGeer [7]. In the present study, we identified *Gryllus 5-HT$_{2\beta}$* gene, an additional 5-HT receptor gene expressed in the cricket brain, and examined its tissue-specific distribution and embryonic stage-dependent expression. *Gryllus 5-HT$_{2\beta}$* gene was ubiquitously expressed in the all examined adult tissues, and was expressed during early embryonic development, as well as during later stages. This study suggests functional differences between two 5-HT$_2$ receptors in the cricket.

Introduction

The biogenic monoamine serotonin (5-HT) is an ancient intracellular signaling molecule found in all phyla possessing nervous systems. 5-HT functions as a neurotransmitter/modulator, or as a neurohormone, and in the insect nervous system, 5-HT modulates various principal behaviors, such as feeding, circadian behavior, sleep, sexual behavior, and social behavior. In our previous study [7], we identified genes involved in biosynthesis and transduction of 5-HT, including four 5-HT receptor genes (*5-HT$_{1A}$*, *5-HT$_{1B}$*, *5-HT$_{2\alpha}$*, and *5-HT$_{7}$*), in the field cricket *Gryllus bimaculatus*, and examined their tissue-specific distribution. In addition to these four 5-HT receptor genes, another type 2 5-HT receptor gene, *5-HT$_{2\beta}$*, is present in the insect genomes [1]. However, its cDNA cloning, pharmacological characterization, and expression analysis have not been carried out in any insect species. Here, we report the molecular cloning and expression analysis of *Gryllus 5-HT$_{2\beta}$* gene, and discuss about functional differences between two *Gryllus* type 2 5-HT receptors (5-HT$_{2\alpha}$ and 5-HT$_{2\beta}$) based on their differential expression patterns in the adult tissues and during embryonic development.

Materials and methods

Molecular cloning of *Gryllus 5-HT$_{2\beta}$* gene was performed according to the experimental procedure described previously [7]. Briefly, at first, partial cDNA of *5-HT$_{2\beta}$* was amplified using degenerate primers. Degenerate primers were designed on the basis of conserved amino acid sequences (EQKATKV and WAPFFV) among the insect 5-HT$_{2\beta}$ receptor proteins (forward degenerate primer, GGGARCARAARGCNACNAARGT; reverse degenerate primer, CGAARAANGGNGCCCA). Then, rapid amplification of cDNA ends

were performed to extend partial cDNA clones.

RT-PCR analyses of *Gryllus* 5-HT receptor genes were performed according to the experimental procedure described previously [7]. The developmental stages of the cricket embryo were determined according to [4]. Following gene specific primers were used to amplify cDNA fragment of *Gryllus* *5-HT$_{2\beta}$* gene: forward primer, 5'-GCCCTTCTTCGTGCTCAACC-3'; reverse primer; 5'-GCCGCCACACCTTCTTGC-3'.

Results

We identified a type 2β serotonin receptor gene, *5-HT$_{2\beta}$*, expressed in the cricket brain. We performed RT-PCR and obtained 493-bp cDNA fragment (GenBank accession number: AB667995) encoding the C-terminal region of the receptor. This clone contains an ORF spanning 1–273 bp, resulting in a protein product of 91 amino acids. The partial cDNA fragment of *Gryllus* 5-HT$_{2\beta}$ receptor encoded the two transmembrane (TM) segments corresponding to the sixth and seventh TM segments of GPCR (TM6 and TM7 in Fig. 1). Comparison of the deduced amino acid sequence of *Gryllus* 5-HT$_{2\beta}$ with those of other type 2 5HT receptors indicated that *Gryllus* 5-HT$_{2\beta}$ is structurally most similar to the other known insect 5-HT$_{2\beta}$: 80.4% identical to *Drosophila* 5-HT$_{2\beta}$ (CG42796; GenBank accession number, AAN13390), 76.9% identical to *Tribolium* 5-HT$_{2\beta}$ (GenBank accession number, EFA04642), and 80.2% identical to *Apis* 5-HT$_{2\beta}$ (GenBank accession number, NP_001191178).

Next, we examined the tissue-specific expression pattern of *Gryllus 5-HT$_{2\beta}$* by using RT-PCR analysis (Figure 2A). After 35 cycles of amplification, PCR products of *Gryllus 5-HT$_{2\beta}$* were detected in the lanes of all of the examined adult tissues. We also examined expression of two 5-HT$_2$ receptor genes (*5-HT$_{2\alpha}$* and *5-HT$_{2\beta}$*) during embryogenesis (Figure 2B). Our results indicated that gene expression of the two 5-HT$_2$ receptors appears to be differentially regulated during embryonic development: *5-HT$_{2\alpha}$* gene is not expressed during early embryonic stages, and starts to express after stage 12. On the other hand, *5-HT$_{2\beta}$* gene starts to express at stage 2 and, then the expression was decreased at stage 7. After stage 8, *5-HT$_{2\beta}$* gene is abundantly re-expressed in the embryos.

Discussion

In our previous study, we identified four *Gryllus* 5-HT receptor genes and examined their distributions in the adult tissues [7]. In the present report, we performed the molecular cloning and expression analysis of an additional 5-HT receptor gene, *5-HT$_{2\beta}$*, in *G. bimaculatous*. Although a homologue of *5-HT$_{2\beta}$* gene is predicted in most of the sequenced insect genomes, its cDNA fragment has not been actually cloned and sequenced in any insects. This study is the first report on the molecular cloning and expression analysis of the insect *5-HT$_{2\beta}$* gene.

Our expression analyses of two type 2 serotonin receptors in the various adult tissues

suggested distinct physiological roles of these receptors in the cricket. We previously reported that, in the adult cricket, *Gryllus 5-HT$_{2\alpha}$* gene is selectively expressed in several tissues including the central brain, SOG, and salivary gland. On the contrary, *Gryllus 5-HT$_{2\beta}$* is ubiquitously expressed in the all examined tissues. Although the pharmacological property and downstream signal transduction pathway of the insect *5-HT$_{2\beta}$* receptor has not been investigated yet, structural similarity among insect and vertebrate type 2 5-HT receptors suggests that these receptors have similar characteristics. Further analyses are needed to elucidate subtype-specific physiological functions of the type 2 5-HT receptors in the cricket.

5-HT plays a role in development before it acts as a neurotransmitter/neuromodulator in the nervous system. In both vertebrates and invertebrates, the type 2 serotonin receptors play crucial roles in embryonic development [6]. In *Drosophila*, the *5-HT$_{2\alpha}$* gene (*5-HT$_{2Dro}$*) starts to express at the onset of gastrulation, and is co-expressed with a pair-rule gene *fushi-tarazu* [2]. During embryogenesis, *Drosophila* 5-HT$_{2\alpha}$ receptor plays a function in ectodermal cell adhesion [3]. In the *Gryllus* embryos, not *5-HT$_{2\alpha}$* but *5-HT$_{2\beta}$* gene was expressed during early embryonic stages. Our data suggests that the type 2 5-HT receptor-mediated 5-HT signaling also plays roles during early embryonic development in the cricket, and that the molecular mechanisms underlying the embryonic 5-HT signaling differ between the basal direct-developing insects and the evolutionary advanced holometabolous insects. In addition, during late stage embryogenesis, both *5-HT$_{2\alpha}$* and *5-HT$_{2\beta}$* genes start to express after the onset of neuronal differentiation that takes place around 90 hr after egg laying (stage 7 to 8) [5]. This data suggests that expression of type 2 5-HT receptors during late embryonic stages takes place in the differentiated neurons.

In conclusion, we identified a novel type 2 5-HT receptor gene, *5-HT$_{2\beta}$*, in the field cricket *G. bimaculatous*. RT-PCR analysis revealed ubiquitous expression of *5-HT$_{2\beta}$* gene in the adult tissues, as well as its expression during the early embryonic development. Here, we identified a complete set of insect 5-HT receptor genes in *G. bimaculatous*. As a next step of our research, we are planning to perform pharmacological manipulation of each 5-HT receptor and receptor subtype-specific RNAi, in order to reveal which subtype(s) of 5-HT receptors modulate specific aspects of behavior in the cricket brain.

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Figure and figure legends

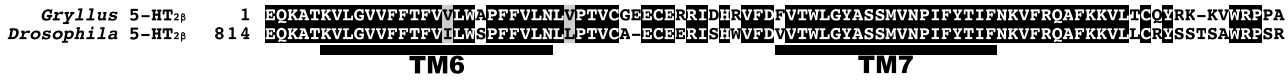

Fig. 1. Alignment of the partial deduced amino acid sequence of *Gryllus 5-HT$_{2\beta}$* with that of *Drosophila 5-HT$_{2\beta}$* (CG42796). Identical and similar amino acid residues are indicated by reverse shading and grey shading, respectively, using the BOXSHADE program. Black lines indicate the transmembrane segments (TM).

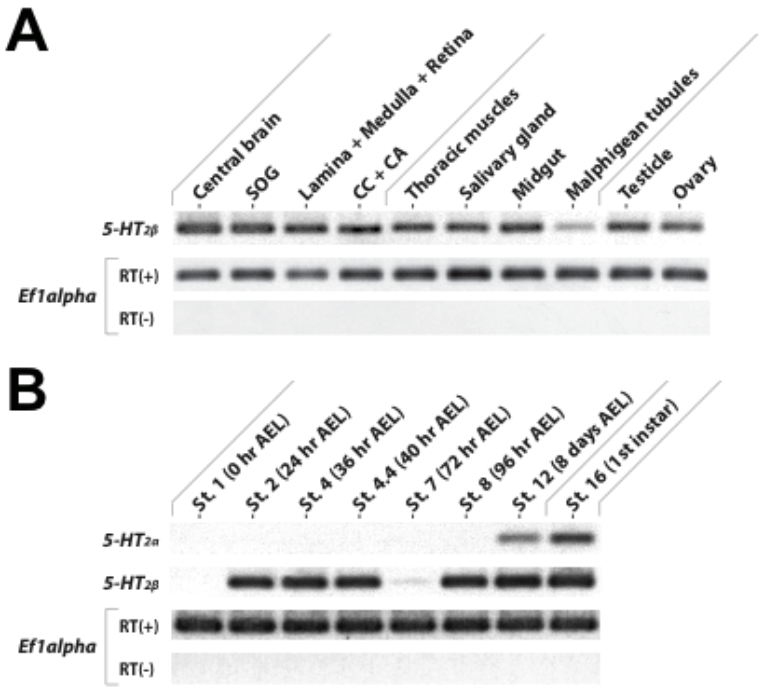

Fig. 2. RT-PCR analyses of the type 2 5-HT receptor genes in the cricket. (A) Tissue-specific expression pattern of *5-HT$_{2\beta}$* gene. (B) Stage-specific expression patterns of the type 2 5-HT receptor genes (*5-HT$_{2\alpha}$* and *5-HT$_{2\beta}$*) during embryonic development. *Ef1alpha* gene was amplified as an internal control gene.