Molecular aspects of the serotonergic system in the cricket CNS: implication in the adaptive modulation of behavior

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Abstract: Serotonin (5-HT) modulates various aspects of behaviors in animals. We use the field cricket *Gryllus bimaculatus* as a model animal to study the neural basis of the adaptive modulation of behavior in the insect microbrain system. In this paper, we examined genes involved in 5-HT synthesis and transduction in the cricket central nervous system (CNS). As a result, three genes involved in 5-HT synthesis and four 5-HT receptor genes were identified. Expression analysis of the 5-HT related genes revealed that the 5-HT system is widely distributed in the cricket.

Keywords: Serotonin, TRH, TPH, AADC, 5-HT receptors, Gryllus bimaculatus

1. INTRODUCTION

5-HT functions as a neurotransmitter/modulator, or as a neurohormone that modulates various principal behaviors, such as feeding, circadian behavior, sleep, sexual behavior, and social behavior. We focus on the serotonergic modulation of aggressive and escape behaviors in the field cricket *Gryllus bimaculatus* to understand the neural basis of the adaptive modulation of behavior. Although physiological and behavioral aspects of the cricket serotonergic system have been extensively studied [2, 3, 4], its molecular basis has not been investigated yet. In this paper, we identified genes involved in synthesis and transduction of 5-HT in the cricket.

2. MOLECULAR ASPECTS OF 5-HT SYSTEM IN INSECTS

2.1 5-HT synthesis pathway

5-HT is synthesized by a two step reaction process. The first step of 5-HT synthesis is catalyzed by tryptophan hydroxylase, which encoded by two distinct genes (*TRH* and *TPH*) in insects [5]. The second step of 5-HT synthesis is catalyzed by AADC (Fig. 1).



Fig. 1 5-HT synthesis pathway in the cricket.

2.2 5-HT receptors

All of the insect 5-HT receptors belong to the G-protein-coupled receptor (GPCR) superfamily, and are classified into three subtypes (5-HT₁, 5-HT₇, and 5-HT₂) which are coupled with subtype-specific signal transduction mechanisms (Fig. 2, Table 1) [6].

Table 1Insect 5-HT receptors.

Family	Subtype	Down-stream signal transduction	
5-HT ₁	$5-HT_{1A}$	G _i -coupled	Decrease in cAMP
	$5-HT_{1B}$		Increase in IP ₃
5-HT ₂	$5\text{-}\text{HT}_{2\alpha}$	G _q -coupled	Increase in IP ₃ (?)
	$5-HT_{2\beta}$?	?
5-HT ₇		G _s -coupled	Increase in cAMP

3. MATERIALS AND METHODS

In the present study, we identified *TRH*, *TPH*, *AADC*, 5- HT_{1A} , 5- HT_{1B} , 5- HT_{2a} , and 5- HT_7 in *G. bimaculatus*.

To obtain the partial cDNA fragment of the target genes, we searched EST clones corresponding to the target genes on the GenBank database, otherwise we designed degenerate primers to amplify the partial cDNA fragment of the target genes. 5' and 3' RACEs were performed to extend partial cDNA clones by amplifying the 5' and 3' sequences of the corresponding mRNAs. Tissue-specific expression patterns were examined by RT-PCR.



Fig. 2 5-HT signaling.

4. RESULTS AND DISCUSSION

4.1 *TRH*, *TPH*, *AADC* genes and their distribution in the cricket (Fig. 3).

We identified three genes involved in 5-HT synthesis in the cricket CNS. We found two transcript variants of *TRH* gene in the central brain. Two tryptophan hydroxylase genes (*TRH* and *TPH*), which are selectively expressed in the neuronal and peripheral tissues, respectively in the fruitfully [7], were co-expressed in the neuronal tissues in the cricket. This data suggests that TRH- and TPH-mediated 5-HT biosynthesis pathways are not compartmentalized into neuronal and peripheral tissues in the cricket, and the cricket CNS has two distinct mechanisms of the regulation of 5-HT synthesis.

AADC gene, which is involved not only in 5-HT biosynthesis but also in biosynthesis of dopamine and melanin, was ubiquitously expressed in the cricket. Dopamine functions as a neurotransmitter or neuromodulator, and melanin plays essential roles in cuticular tanning/sclerotization and the immune response in insects. Ubiquitous expression of *AADC* might reflect its functional importance in various biochemical pathways.



Fig. 3 Expression patterns of the 5-HT synthesis pathway-related genes.

4.2 Four 5-HT receptor genes and their distribution in the cricket (Fig. 4).

We identified four 5-HT receptor genes ($5-HT_{1A}$, $5-HT_{1B}$, $5-HT_{2a}$, and $5-HT_7$) expressed in the cricket CNS. The 5-HT system is involved in modulating various behaviors in the insect CNS, and in the peripheral tissue, 5-HT controls various physiological phenomena such as heart beat, salivary gland secretion, diuresis in the Malpighian tubules. Tissue-specific expression patterns of four 5-HT receptor genes showed that the 5-HT system is widely distributed in the cricket, and that the 5-HT system might regulate various aspects of physiological phenomena via distinct 5-HT receptor pathways.



Fig. 4 Expression patterns of four 5-HT receptors.

5. CONCLUSION

To elucidate molecular basis of the cricket 5-HT system, we identified seven genes essential for the synthesis and transduction of 5-HT in the cricket *G. bimaculatus*. Our data suggest that two distinct 5-HT synthesis pathways co-exist in the cricket CNS. The four of 5-HT receptor genes were expressed in various tissues at differential expression levels, suggesting that the 5-HT system is widely distributed in the cricket. Functional analysis of each 5-HT receptor will reveal the differential involvement of specific 5-HT receptor subtypes to behavior in the cricket.

REFERENCES

- [1] T. Watanabe, H. Sarasota, H. Aonuma, "Identification and expression analysis of the genes involved in serotonin biosynthesis and transduction in the field cricket *Gryllus bimaculatus*." *Insect Molecular Biology*. Revised.
- [2] AS. Saifullah, K. Tomioka, "Serotonin sets the day state in the neurons that control coupling between the optic lobe circadian pacemakers in the cricket *Gryllus bimaculatus*" *J Exp Biol*, Vol. 205, pp. 1305–1314, 2002.
- [3] PA. Stevenson, HA. Hofmann, K. Schoch, K. Schildberger, "The fight and flight responses of crickets depleted of biogenic amines" *J Neurobiol*, Vol. 43, pp. 107–120, 2000.
- [4] T. Nagao, T. Tanimura, T. Shimozawa, "Neurohormonal control of the mating interval in the male cricket, *Gryllus bimaculatus* DeGeer" *J Comp Physiol*, Vol. 168, pp. 159–164, 1991.
- [5] CM. Coleman, WS. Neckameyer, "Serotonin synthesis by two distinct enzymes in *Drosophila melanogaster*" *Arch Insect Biochem Physiol*, Vol. 59, pp. 12–31, 2005.
- [6] AJ. Tierney, "Structure and function of invertebrate 5-HT receptors: a review" Comp Biochem Physiol A Mol Integr Physiol, Vol. 128, pp. 791–804, 2001.
- [7] WS. Neckameyer, CM. Coleman, S. Eadie, SF. Goodwin, "Compartmentalization of neuronal and peripheral serotonin synthesis in *Drosophila melanogaster*", *Genes Brain Behav*, Vol. 6, pp. 756–769, 2007.