

A novel inhibitory gonadotropin-releasing hormone-related neuropeptide in the ascidian, *Ciona intestinalis*

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The gonadotropin-releasing hormone (GnRH) family peptides are most widely distributed neuropeptides and/or neurophysiological hormones. GnRH is involved in diverse neuroendocrine, paracrine, autocrine, and neurotransmitter/neuromodulatory functions in the central and peripheral nervous system as well as peripheral tissues. In the present study, we show the identification of a novel GnRH-related peptide, Ci-GnRH-X, in the ascidian, *Ciona intestinalis*. Intriguingly, Ci-GnRH-X possesses a unique primary sequence consisting of 16 amino acids, although typical GnRH family peptides are composed of 10 amino acids. On the other hand, Ci-GnRH-X shares the GnRH consensus motifs, including the N-terminal pQHWS ('pQ' indicates a pyro-glutamic acid) and C-terminal Gly-amide. Reverse transcription (RT)-PCR analysis shows that the Ci-GnRH-X gene is expressed exclusively in the central nervous system. Moreover, in situ hybridization demonstrated that the *Ciona* GnRH-1 gene encoding *Ciona* GnRHs (t-GnRH-3, -5 and -6) was co-expressed with the Ci-GnRH-X gene in neurons of the cerebral ganglion. Of particular interest is that Ci-GnRH-X exhibited moderate (10-50%) inhibitory activity against t-GnRHs at their cognate receptors. Ci-GnRH-X repressed the elevation of the intracellular calcium and cAMP production by t-GnRH-6 at Ci-GnRHR1, and cAMP production by t-GnRH-3, and t-GnRH-5 via Ci-GnRHR-3 was also inhibited by Ci-GnRH-X. In contrast, no inhibitory effect of Ci-GnRH-X at Ci-GnRHR2 was observed. The localization and biochemical assays revealed that Ci-GnRH-X acts as an endogenous antagonist for the *Ciona* GnRHergic system. This is the first molecular and functional characterization of an endogenous inhibitor of GnRHs in an animal species.

Table. 1

Peptide	Sequence	Species
Ci-GnRH-X	pQHWSNWWIPGAPGYNG-amide	Ascidian (<i>C. intestinalis</i>)
t-GnRH-3	pQHWSYEFMPG-amide	Ascidian (<i>C. intestinalis</i>)
t-GnRH-5	pQHWSYEYMPG-amide	Ascidian (<i>C. intestinalis</i>)
t-GnRH-6	pQHWSKGYSYPG-amide	Ascidian (<i>C. intestinalis</i>)
GnRH-I	pQHWSYGLRPG-amide	Human
GnRH-II	pQHWSHGWYPG-amide	Human
GnRH-III	pQHWSYGWLPG-amide	Fish

Fig. 1

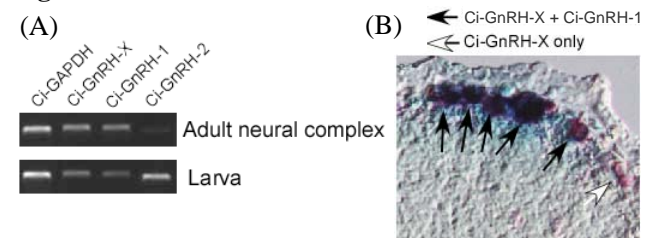


Fig. 2

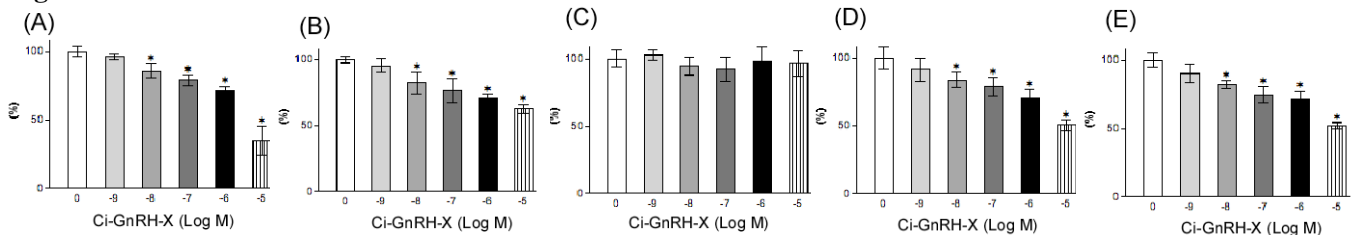


Table.1: Sequence alignments of Ci-GnRH-X and typical chordate GnRHs. The N-terminal and C-terminal GnRH consensus motifs are indicated by the red characters. **Fig.1:** Gene expression of the Ci-GnRH-1, 2 and X in the adult neural complex and larva (A). Localization of the Ci-GnRH-X and Ci-GnRH-1 mRNAs in the neural ganglion. The cells co-expressing Ci-GnRH-X and -1 genes were stained with dark purple (black arrows), while red cells indicated by a white arrow are the cells expressing only Ci-GnRH-X gene (B). **Fig.2:** Inhibitory activity of Ci-GnRH-X against t-GnRH-6 for intracellular calcium mobilization (A) and cAMP production (B) at Ci-GnRHR-1, against t-GnRH-6 for cAMP production at Ci-GnRHR-2 (C), against t-GnRH-3 (D) and t-GnRH-5 (E) for cAMP production at Ci-GnRHR-3. The activity of each 10^{-8} -M t-GnRH in the absence of Ci-GnRH-X was taken as 100%. The error bars denote S.E. (n=5, *P<0.05).