Molecular analyses of crustacean hyperglycemic hormone (CHH) and CHH-like peptides and gene expression patterns in response to pathogens

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Abstract

Six transcripts, each encoding a distinct CHH (crustacean hyperglycemic hormone) or CHH-like (CHH-L) peptides, were identified in the crayfish, *Procambarus clarkia-CHH1*, *CHH1-L*, *truncated-CHH1* (*t-CHH1*), *CHH2*, *CHH2-L*, and *t-CHH2*. These transcripts were generated by alternative spicing of RNA precursors of two CHH genes (*chh1* and *chh2*).In the present study, animals were challenged with lipopolysaccharide (LPS) or white spot syndrome virus (WSSV), and tissue levels of the various CHH/CHH-L peptide transcripts were quantified thereafter. Transcripts whose levels were significantly elevated after LPS treatment include *CHH1* in the cerebral ganglia (CG), *CHH2-L* in eyestalk ganglia (EG) and CG, and *t-CHH2* in EGv. Those significantly elevated after WSSV treatment are *CHH1, CHH1-L, and CHH2-L* in TG. These results indicate that a host of CHH/CHH-L peptide transcripts are expressed in various nervous tissues, and that these neural-derived peptides are possibly involved in the processes of immune defense against invading pathogens. For future functional and structural studies, recombinant CHH1 and CHH1-L were produced, refolded, and purified by liquid chromatography; identity of the recombinant protein was confirmed using mass spectrometric (MS) analyses.

Results

Deduced amino acid sequences of preproCHHs identifiend in the crayfish *Procambarus clarkii*

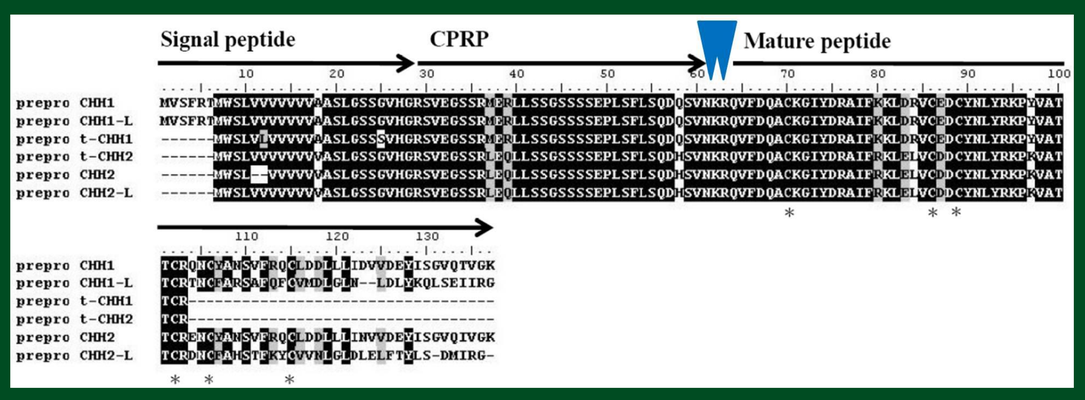

Fig. 1. sequences of preproCHHs identified in *P.clarkii*

Alignment of deduced amino acid sequences of 6 preproCHHs, eachof which consists of signal peptide, CPRP(CHH precursor related peptide), and a mature peptide (CHH, CHH-L, or t-CHH). Blue arrows indicate the cleavage sites between CPRP and mature peptide. The locations of conserved cysteyl residues are indicated by ∗ . Number at the ends of each sequence is the residue number of each mature peptide. *t-CHH1* and *t-CHH2* have shorter mature peptides than other CHH or CHH-L, and lack two conserved cysteyl residues

CHH,CHH-L(CHH-like), and truncate CHH(t-CHH) transcripts are generated via *cis*- and *trans*-splicing mechanism

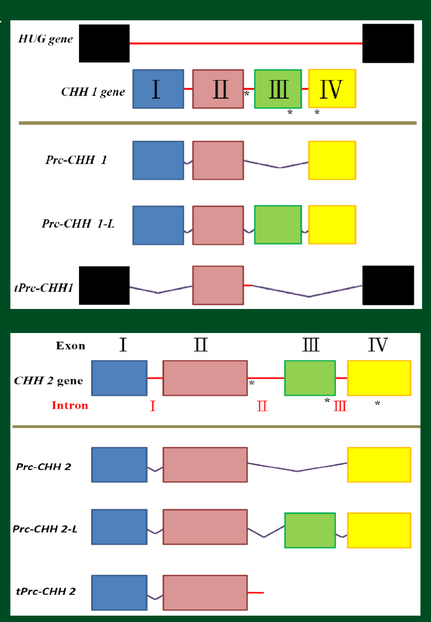

Fig. 2. CHH,CHH-L(CHH-like), and truncate CHH(*t-CHH*) transcripts are generated via *cis*- and *trans*-splicing mechanism

Schematic representation of alternative processing of *CHH1,CHH2 HUG genes. CHH1* and *CHH2* each consists of 4 exons (I,II, III, IV). *CHH1* and *CHH1-L* transcripts are products of a *cis*-splicing of *CHH1 gene*(A), similar to the case where *CHH2* and *CHH2-L* transcripts are products of *cis*-splicicng of *CHH2 gene*(B). Whereas *t-CHH2* transcript, also a product of *cis*-splicing of *CHH2 gene*, with a retention of a stretch of intron (B, red line) that contains a stop codon, hence causing an premature termination of the peptide sequence(c. Fig. 1), *t-CHH1* transcript is a trans-splicing product of *CHH1 gene* and *Hug gene*(A); *HUG gene* contributes sequences that constitute the 5'- and 3'-untranslate regions of t-CHH1 transcript(A, blank rectangles); retention of stretch of intron (A, red line) in *t-CHH1* transcript that also contains a stop codon cause an premature termination of the peptide sequence(cf. Fig. 1).The location of stop codon are indicated by ∗.

CHH, CHH-L, and t-CHH transcript levels in neurosecretory tissues were differentially affected by a lipopolysaccharide challenge

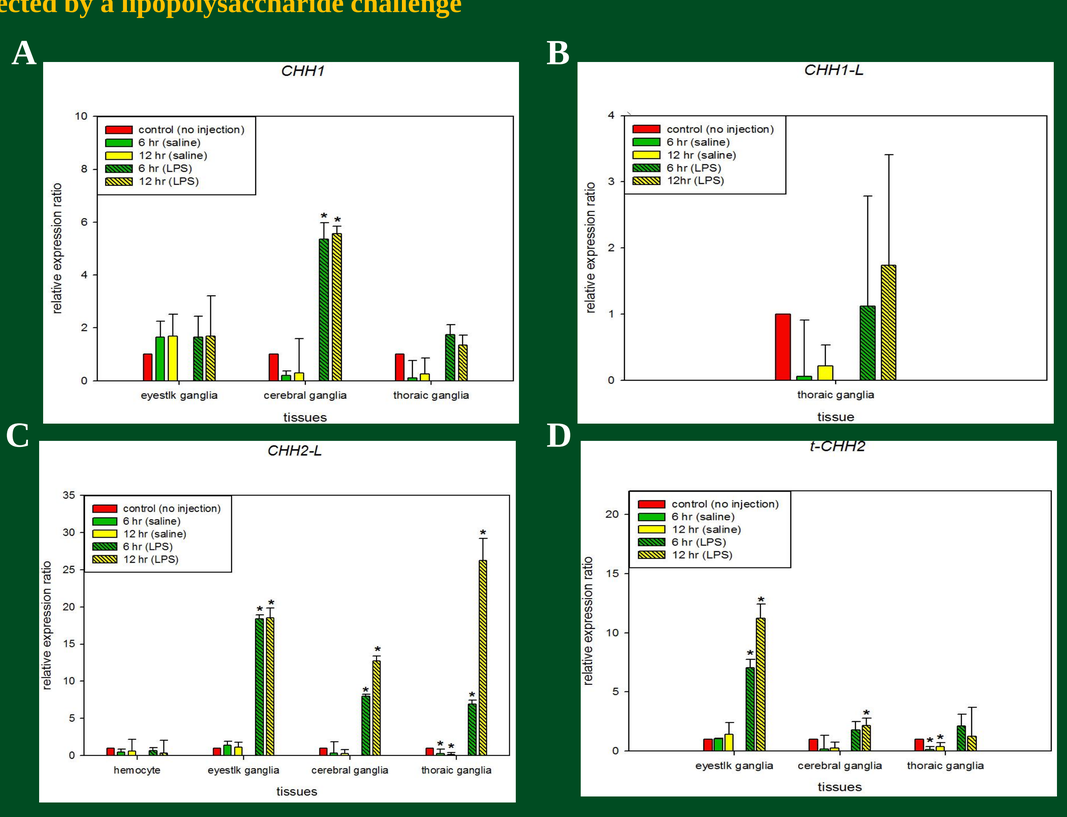

Fig. 3. Semi-quantitative real time RT-PCR of transcript levels in *P. clarkii* after a LPS challenge.

(A) *CHH1*, (B) *CHH1-L*, (C) *CHH2-L* and (D) *t-CHH2* transcript levels in neurosecretory tissues from *P. clarkii* at different time points before injection or after saline or LPS injection. Bars represented the mean (n=3). Significant differences between the LPS-treated and the control animals were indicated with an asterisk ($p < 0.05$).

CHH and CHH-L transcript levels in neurosecretory tissues were differentially affected by a WSSV(white spot syndrome virus) challenge

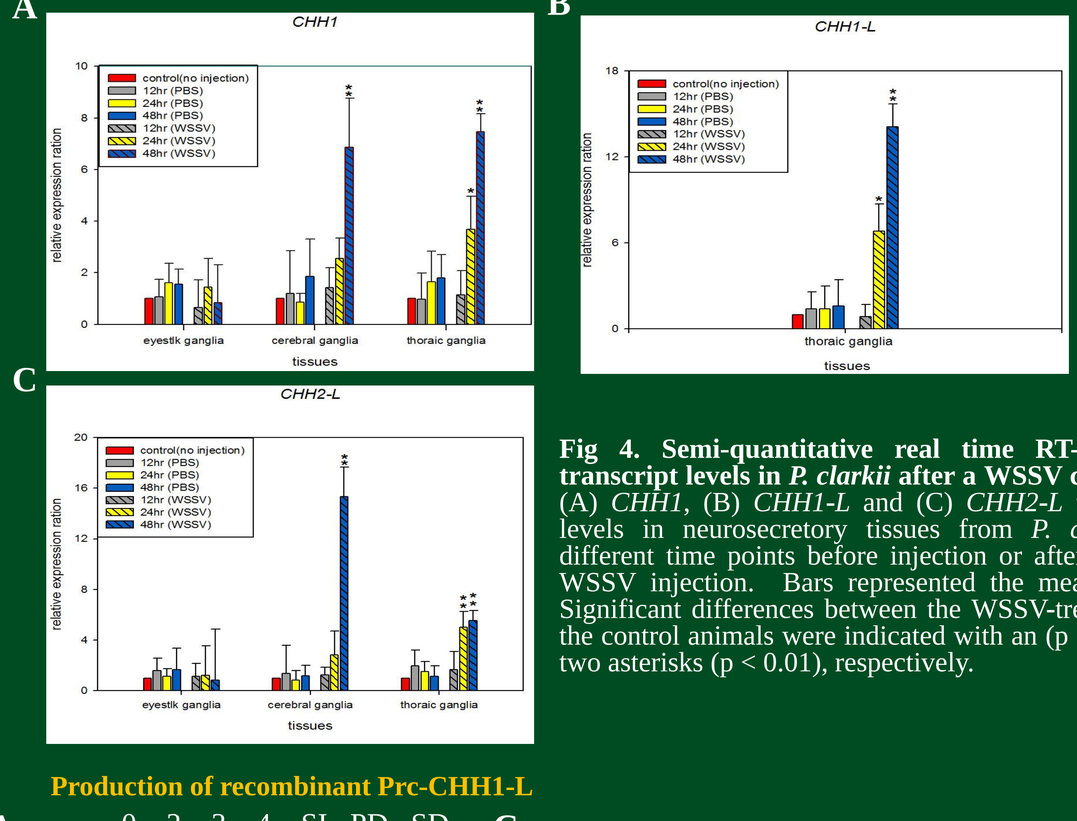

Fig 4. Semi-quantitative real time RT-PCR of transcript levels in *P. clarkii* after a WSSV challenge.

(A) *CHH1*, (B) *CHH1-L* and (C) *CHH2-L* transcript levels in neurosecretory tissues from *P. clarkii* at different time points before injection or after PBS or WSSV injection. Bars represented the mean (n=5). Significant differences between the WSSV-treated and the control animals were indicated with an ($p < 0.05$)or two asterisks ($p < 0.01$), respectively.

Production of recombinant Prc-CHH1-L

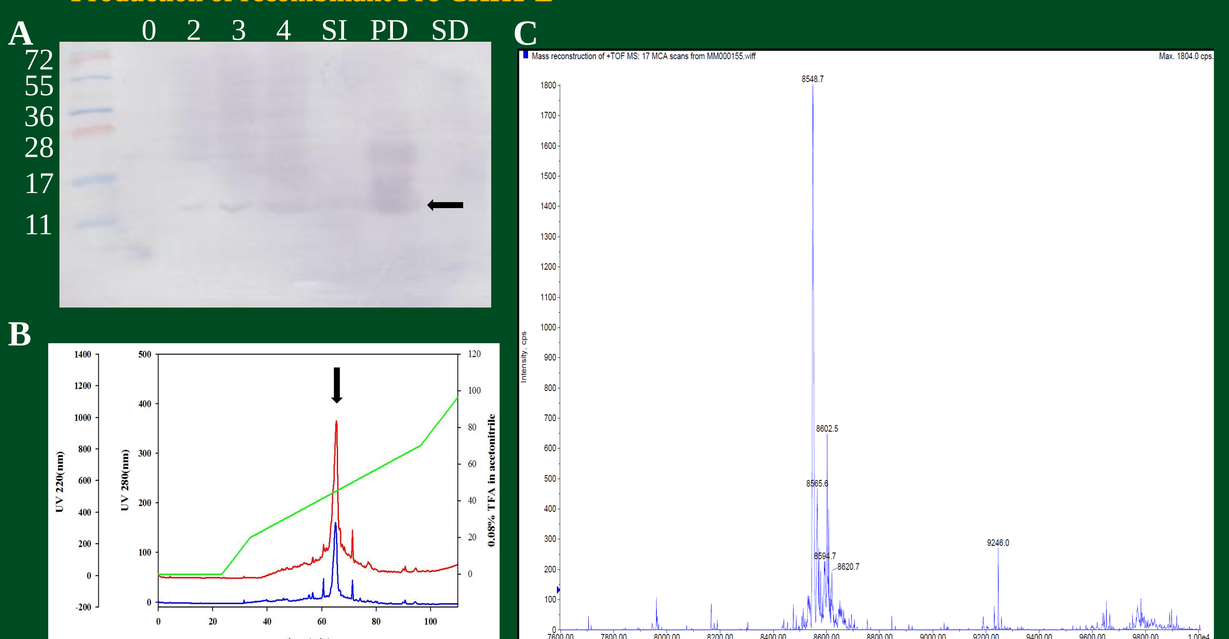

Fig. 5. Recombinant CHH1-L was produced, refolded, and purified by liquid chromatography; identity of the recombinant protein was confirmed using mass spectrometric (MS) analyses

(A) Using anti-L-phe3-CHH (α-LA) to detect aliquots of total cell homogenate, supernatant and pellet protein. The arrow indicated that an immunoreactive band was detected with an apparent molecular mass of ~9 kDa in pellet by induced 2,3 and 4 h and Cell disrupted(A, 0、2、3 and 4: Incubation time after IPTG added; SI: Supernatant of 4 hour after IPTG treatment; PD: The pellets of cell disrupted at 30 KPSI of 4 hour after IPTG treatment, and centrifuged; SD: The supernatant of cell disrupted at 30 KPSI of 4 hour after IPTG treatment)

(B)Reversed phase high performance liquid chromatograph purification of rPrc-CHH1-L.The insoluble proteins, derived from cells transformed with recombinant plasmids Prc-CHH1-L/pET-22b(+) were dissolved and eluted on a Sep-Pak cartridge. The 60% acetonitrile-eluted fractions were refolded and fractionated by RP-HPLC. Fractions (B, arrow) of major UV peaks eluated at 63.5-66.0 min.

(C) The resulting chromatogram revealed a major UV peak eluated at at 63.5-66.0 min (arrow, B), which contains rPrc-CHH1-L as identified by MS analyses. Result in accordance with rPrc-CHH1-L theoretical molecular weight 8549.9 Da..

Conclusion

(1) In *P. clarkii*, at least 6 CHH or CHH-L transcripts are present, which are generated post-transcriptionally by *cis*- or *trans*-splicing mechanisms.

(2) CHH and CHH-L peptides are differentially expressed in neurosecretory tissues.

(3) Immune challenges - LPS or WSSV treatments - significantly increase CHH or CHH-L transcript levels in neurosecretory tissues, suggesting the encoded peptides play roles in modulating immune activity -a scenario that manifest a concerted response between the immune and neuroendocrine systems when eminent immune challenges are present.

(4) Recombinant protein CHH1-L were successfully produced using an E. coil expression system. For future functional and structural studies