Activation of neuroendocrine responses in the crayfish *Procambarus clarkii* by white spot syndrome virus

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Abstract

Crustacean hyperglycermic hormone (CHH) is a neuropeptide which participates in carbohydrate metabolism, molting inhibition, and stress-adaptive responses. In this study, we examined the effects of white spot syndrome virus (WSSV) infection on the levels of CHH peptide and its gene expression in different *Procambarus clarkii* tissues including eyestalk ganglia, cerebral ganglia, thoracic ganglia, hemolymph and hemocyte. The WSSV challenge results showed hemolymph CHH peptides were increased and significant rise in hemolymph glucose levels, meanwhile CHH peptides in the eyestalk ganglia and cerebral ganglia were significantly decreased 24 and 48 hrs after WSSV challenge but did not change significantly in the thoracic ganglia or hemocyte. Intriguingly, *chh* gene expression levels only increased in the thoracic ganglia and cerebral ganglia 24 and 48 hrs after WSSV injection. The combined data suggest WSSV activates crustacean CHH peptide release from the central (eyestalk ganglia) and peripheral (cerebral ganglia, thoracic ganglia and hemocyte) CHH-producing tissues to hemolymph and might be mediating adaptive processes (e.g., hyperglycemic response or immune responses) in response to pathogen-related stresses.

Key words: CHH, *Procambarus clarkia*, and WSSV

Results

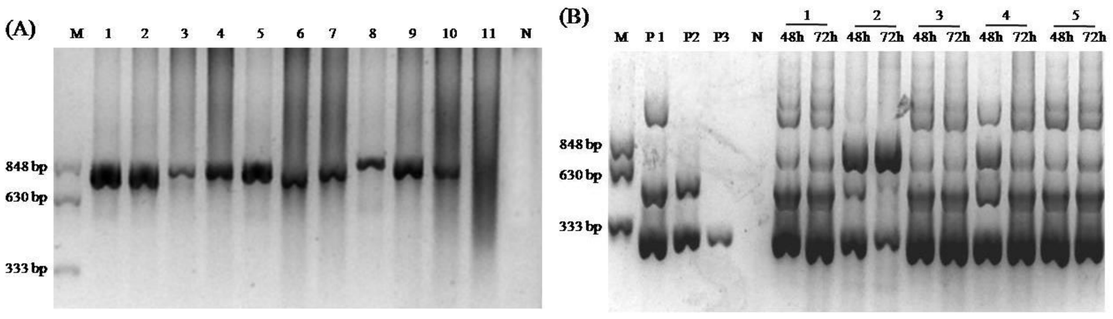

Fig 1 . Detection of WSSV in experimental animals. Tissues (pleopods) were dissected from experimental animal and processed for analysis of WSSV using a PCR-based kit IQ2000™. Separation by 1.2 % agar gel. (A): lanes M: Maker, laness 1-11: DNA samples from experimental animals, lanes N: Negative control. All animals (laness 1-11) were endogenous amplified band (~ 840 bps) which indicated WSSV negative infection. (B): lanes M Maker, lanes P1,P2 and P3 are WSSV standards at 2000 (severe infection) , 200 (moderate infection) and 20 (light infection) copies/reaction, respectively, lanes 1-5: DNA samples from experimental animals after inject WSSV at 48 or 72 hr ; lanes N: Negative control. Note that all animal (lanes 1-5) were sample of severe WSSV infection except of lanes 2 from inject WSSV at 48 or 72 hrs.

Cerebral gaglia

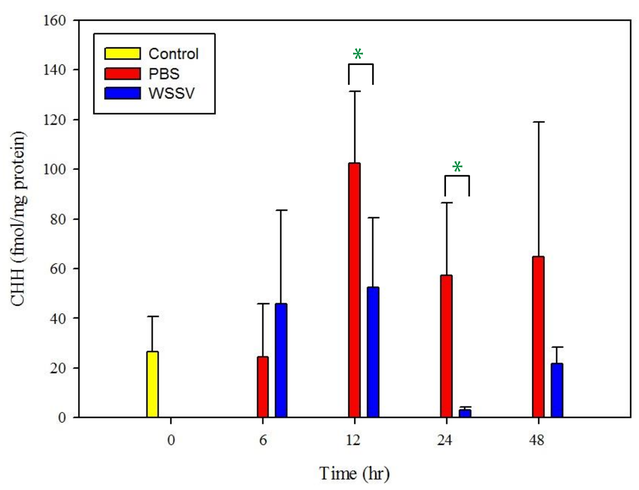

Fig 6. Effect of WSSV on CHH levels in the cerebral ganglia. Cerebral ganglia from non-injected (yellow bar), PBS- (red bar) or WSSV- (blue bar) injected crayfishes were harvested at designated time after injection and processed for ELISA. Experimental animal N=5. * represent significant difference between PBS- and WSSV-treatments at the levels of 0.05.

Thoraic ganglia

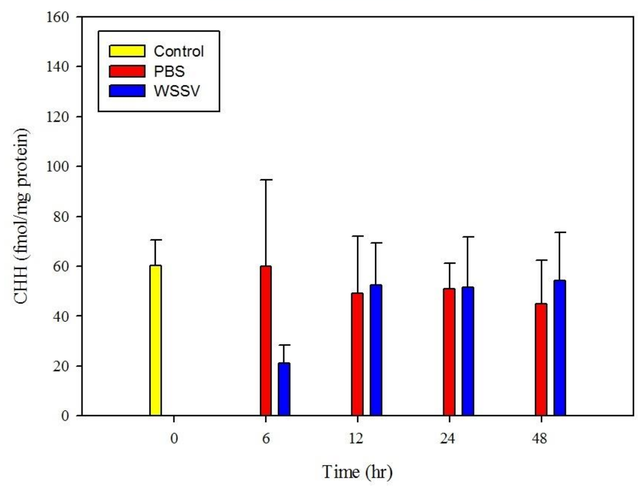

Fig 7. Effect of WSSV on CHH levels in the thoracic ganglia. Thoracic ganglia from non-injected (yellow bar), PBS- (red bar) and WSSV- (blue bar) injected crayfishes were harvested at designated time after injection and processed for ELISA. Experimental animal N=5.

Hemolymph glucose levels

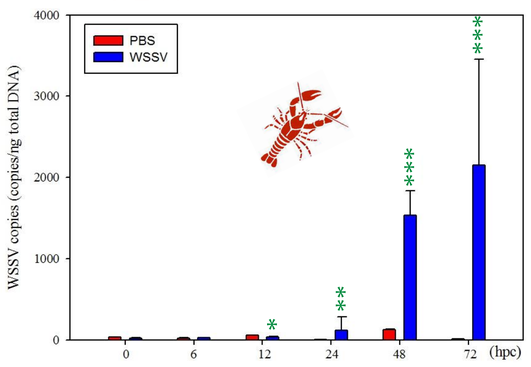

Fig 2. Real-time PCR quantification of WSSV from *Procambarus clarkii* challenged with 0.01 M PBS (red bar) and challenged with 2.125 × 10^6 WSSV viral particles for (blue bar) in hours of post-challenge (hpc). Experimental animal N=5. *, ** and * represent significant difference between WSSV-treatments 0 h and each WSSV-treatments 6, 12, 24 and 48 h at the levels of 0.05, 0.01 and 0.005, respectively.**

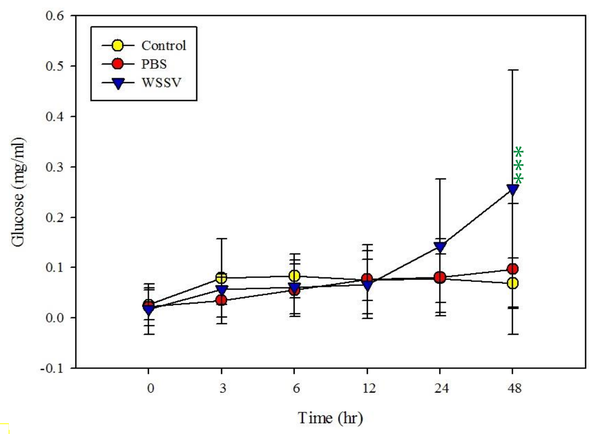

Fig 3. Effect of WSSV infection on hemolymph glucose levels. Hemolymph was withdrawn from non-injected control (●), PBS (●), and WSSV (▼) injected crayfishes at designated time after injection and processed for glucose assay. Experimental animal N=9. * represent significant difference from zero-time values at the level of 0.001.**

Eyestalk ganglia

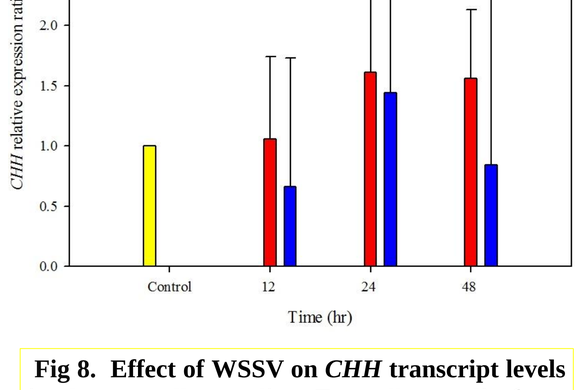

Fig 8. Effect of WSSV on *CHH* transcript levels in the eyestalk ganglia. Eyestalk ganglia from PBS- (red bar) or WSSV- (blue bar) injected crayfishes were harvested at designated time after injection and processed for quantitative real time PCR. *CHH* transcript levels are normalized to a reference gene (*GAPDH*) and expressed relative to that of zero-time control (*i.e.*, its relative expression is taken as 1, yellow bar). Experimental animal N= 5.

Cerebral gaglia

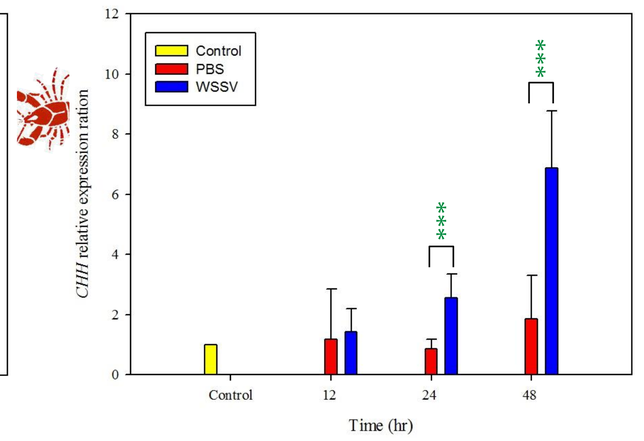

Fig 9. Effect of WSSV on *CHH* transcript levels in the thoracic ganglia. Thoracic ganglia from PBS- (red bar) or WSSV- (blue bar) injected crayfishes were harvested at designated time after injection and processed for quantitative real time PCR. *CHH* transcript levels are normalized to a reference gene (*GAPDH*) and expressed relative to that of zero-time control (*i.e.*, its relative expression is taken as 1, yellow bar). Experimental animal N=5. * represent significant difference between PBS- and WSSV- treatments at the levels of 0.001.**

Hemolymph

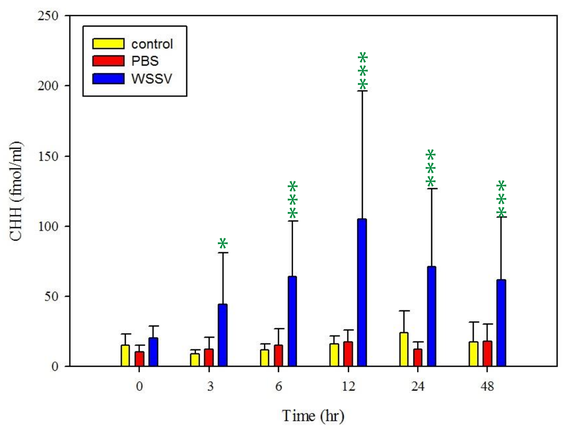

Fig 4. Effect of WSSV on CHH levels in the hemolymph. Hemolymph from non-injected (yellow bar), PBS- (red bar) and WSSV- (blue bar) injected crayfishes were harvested at designated time after injection and processed for enzyme-linked immunosorbent assay (ELISA). Experimental animal N≧8 . *,* represent significant difference between WSSV- treatments 0 h and each WSSV- treatments 3, 6, 12, 24 and 48 h at the levels of 0.05, 0.005, respectively.**

Eyestalk ganglia

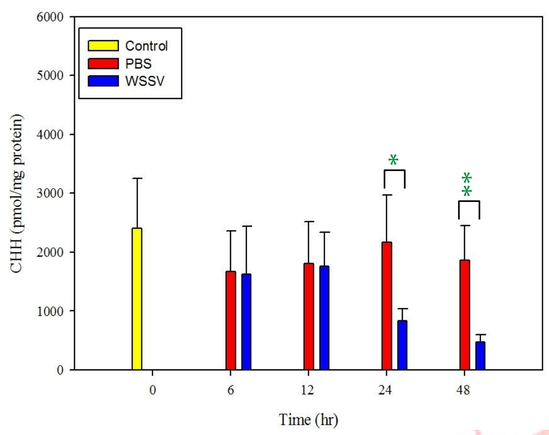

Fig 5. Effect of WSSV on CHH levels in the eyestalk ganglia. Eyestalk ganglia from non-injected (yellow bar), PBS- (red bar) or WSSV- (blue bar) injected crayfishes were harvested at designated time after injection and processed for ELISA. Experimental animal N=5. *, ** represent significant difference between PBS- and WSSV-treatments at the levels of 0.05, 0.01, respectively.

Thoraic ganglia

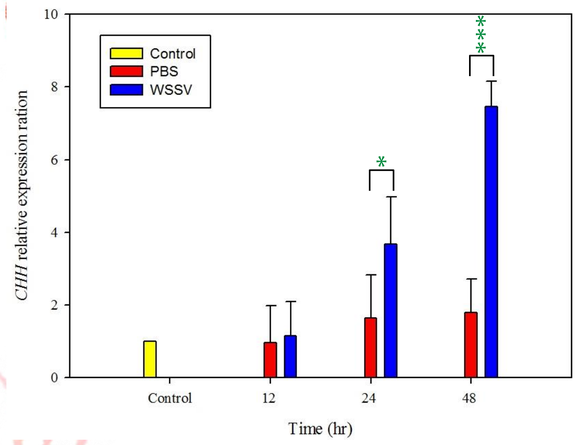

Fig 10. Effect of WSSV on *CHH* transcript levels in the cerebral ganglia. Cerebral ganglia from PBS- (red bar) or WSSV- (blue bar) injected crayfisheswere harvested at designated time after injection and processed for quantitative real time PCR. *CHH* transcript levels are normalized to a reference gene (*GAPDH*) and expressed relative to that of zero-time control (*i.e.*, its relative expression is taken as 1, yellow bar). Experimental animal N=5. * represent significant difference between PBS- and WSSV- treatments at the levels of 0.001.**

Conclusions

1. **White spot syndrome virus (WSSV) significantly proliferated in *Procambarus clarkii* 12 h after injection into animals and afterwards .**
2. **WSSV injection also increased significantly hemolymph CHH and glucose levels in animals.**
3. **The increased hemolymph CHH levels resulted mainly from WSSV-induced massive releases of the hormone from the eyestalk ganglia.**
4. **Transcription of *CHH* gene after WSSV infection was enhanced in the extra-eyestalk ganglia (cerebral ganglia and thoracic ganglia), but not in the central site of CHH biosynthesis (i.e., eyestalk ganglia).**
5. **Possible immune regulatory function of the WSSV-induced production and release of CHH merits investigation**