## Updated research on hormonal regulation on crustacean health: Bursicons and their role in immune modulation

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## Abstract:

Neuropeptides are small proteins or polypeptides generally synthesized and secreted by neurons or neuroendocrine cells to regulate the physiological functions of animals through activation of specific receptors. In crustaceans, eyestalk and the central nervous system (CNS) are the primary sites from which most crustacean neuropeptides are synthesized and released into the hemolymph. So far, more than one hundred neuropeptides have been identified in crustaceans, which play central roles in the control of various physiological processes, including reproduction, growth, molting, metabolism, and immunity. Transcriptome analysis and in silico mining of neuropeptides in the freshwater prawn Macrobrachium rosenbergii eyestalk and central nervous system could predict various preproneuropeptide transcripts, including bursicons (Burs), crustacean cardioactive peptide (CCAP), crustacean hyperglycemic hormones (CHH), eclosion hormone (EH), pigment-dispersing hormones (PDH), diuretic hormones (DH), neuropeptide F (NPF), neuroparsins (NPs), SIFamide, and sulfakinin (SK). These transcripts are most prominent within the eyestalk and CNS. Bursicons,  $\alpha$  and  $\beta$  Burs, are initially identified to be responsible for cuticle hardening during the molting cycle and vitellogenesis stimulation in crustaceans. In prawn, Burs are expressed primarily in ventral nerve ganglia. Recently, they have been shown to involve in the crustacean immune system. We show that the significant increase of Burs expression is related to the increase of ammonia-N and salinity stress and the decrease of phenoloxidase (PO) activity and immune-related genes. The homodimeric form of Burs is proposed to regulate hemocyte granulation. We show significant differences in the proportions of agranulocytic and granulocytic hemocytes that corresponded with the upregulation of Burs in acute stress response. However, during chronic stress, the proportions of the two types of hemocytes returned to normal levels, which coincided with the downregulation of Burs. Therefore, changing hemocyte type is apparently under the influence of the Burs neurohormones. In addition, we show that Burs are likely under the control of ecdysteroid hormone, which controls molting. Although molting is essential to animal growth, animals at pre-and post-molt stages are more sensitive to stress and less resistant to bacterial infection. We found that ecdysteroid hormone could activate Burs expression and modulate immune gene expressions. Moreover, increases in hematopoietic cell proliferation and circulating hemocyte numbers, and increased crustacean hematopoietic factor (CHF) and antilipopolysaccharide factor (ALF) gene expression were demonstrated. Understanding hormonal effects on animal health may potentially provide a way to manipulate hormones to improve the animal's health in the future.