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Fish & Shellfish Immunology 21 (2006) 11–19

Fish & Shellfish Immunology

www.elsevier.com/locate/fsi

Noradrenaline modulates the immunity of white shrimp *Litopenaeus vannamei*

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Received 15 February 2005; revised 15 July 2005; accepted 5 September 2005
Available online 27 December 2005

Abstract

The total haemocyte count (THC), phenoloxidase activity, respiratory burst, superoxide dismutase (SOD) activity, phagocytic activity and clearance efficiency in response to pathogen *Vibrio alginolyticus* were measured when the white shrimp *Litopenaeus vannamei* (18.4 $\pm$ 1.2 g) were injected individually with noradrenaline at 10^{-8}, 10^{-7} and 10^{-6} mol shrimp^{-1}. For the shrimp that received noradrenaline at 10^{-8}, 10^{-7} and 10^{-6} mol shrimp^{-1}, the THC decreased by 15%, 21% and 32%, phenoloxidase activity decreased by 15%, 31% and 31%, respiratory burst decreased by 13%, 21% and 32%, and SOD activity decreased by 46%, 56% and 55%, respectively, after 2 h. The phagocytic activity and clearance efficiency of shrimp that received noradrenaline at either dose decreased significantly after 2 h. The THC, phenoloxidase activity, respiratory burst, SOD activity, phagocytic activity and clearance efficiency returned to normal values after 4, 4, 8, 24, 16 and 8 h, respectively, in the shrimp that received noradrenaline at either dose. In another experiment, *L. vannamei* which had received noradrenaline at 10^{-8}, 10^{-7} and 10^{-6} mol shrimp^{-1} were challenged after 1 h by injection with *V. alginolyticus* at 1.0×10^5 colony-forming units (cfu) shrimp^{-1} and then placed in seawater of 20‰. The cumulative mortality of shrimp that received noradrenaline at either dose was significantly higher than that of shrimp that received saline after 4 h, and at the termination of the experiment (48 h after the challenge). It is therefore concluded that noradrenaline administration at 10^{-6} mol shrimp^{-1} or less causes immune modulation of *L. vannamei*.

Keywords: *Litopenaeus vannamei*; Noradrenaline; Total haemocyte count; Phenoloxidase activity; Respiratory burst; Superoxide dismutase activity; Phagocytic activity; Clearance efficiency

1. Introduction

White shrimp *Litopenaeus vannamei*, which was introduced to the Eastern hemisphere in 1985, has since become the primary species currently being cultured in Thailand, Taiwan, and China [1]. Best survival of juveniles is between temperatures of 20 and 30 °C and salinity levels above 20‰ [2]. Farmers are likely to add freshwater to adjust salinity

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1050-4648/$ - see front matter
doi:10.1016/j.fsi.2005.09.003

levels lower than these levels, because they believe that the growth of shrimp in brackish water is better than that in seawater. Since 2001, shrimp farmers have experienced disease problems causing production declines in farmed *L. vannamei*. The symptoms differ from those of Taura syndrome virus (TSV) in Taiwan [3]. A bacterium *Vibrio alginolyticus* isolated from the diseased *L. vannamei* with whitish musculature and inactivity is considered to be a secondary and opportunistic pathogen, and causes mortality of shrimp under salinity change or ammonia stress [4–6].

In teleosts, the primary response to physiological stress involves the release of corticosteroids and catecholamines. These then induce hyperglycaemia as a secondary response [7]. Several biogenic amines which function mainly as neuroregulators (neurotransmitter and neuromodulators), including serotonin, dopamine, octopamine, histamine, noradrenaline (norepinephrine), adrenaline (epinephrine), tryptamine and tyramine, have been identified and quantitatively measured in crayfish *Pacifastacus leniusculus* and other decapod crustaceans [8–11]. It is known that shrimp *Upogebia littoralis* can synthesise noradrenaline from dopamine [12], and grass shrimp *Palaemonetes varians* and American lobster *Homarus americanus* can synthesise noradrenaline from tyrosine [13,14].

It is known that when Pacific oyster *Crassostrea gigas* are subjected to mechanical disturbance, noradrenaline and dopamine are released into the circulatory system, subsequently decreasing immune functions [15], and increasing susceptibility to *Vibrio splendidus* infection [16]. It is also known that, when the abalone *Haliotis tuberculata* are subjected to a 15-min mechanical disturbance, noradrenaline and dopamine are released, and immune parameters such as haemocyte counts decrease, as do migratory activity, phagocytic and respiratory burst capacity of the haemocytes [17]. Swamp crayfish *Procambarus clarkii* when subjected to constant exposure of illumination for 3 days, increases its noradrenaline level [18]. However, there is no knowledge on the release of biogenic amines and their potential roles in immunosuppression of penaeids shrimps under stress.

Environmental stressors like salinity, ammonia, nitrite and Cu^{2+} have been reported to cause reduction in immune ability of white shrimp *L. vannamei* [4,6,19,20]. Blue shrimp *Litopenaeus stylirostris* in hypoxic condition also decreases its release of superoxide anion [21]. Dopamine has been found to mimic the action of crustacean hyperglycaemic hormone (CHH) in increasing the level of glucose in tiger shrimp *Penaeus monodon* [11]. It is assumed that penaeid shrimp under such stress may increase their levels of biogenic amines including noradrenaline and dopamine, which subsequently leads to immunosuppressive effects and increases susceptibility to pathogen infection. Accordingly, the purpose of the present study is to examine (1) the effect of noradrenaline on the susceptibility of *L. vannamei* to *V. alginolyticus*, and (2) the immune response of *L. vannamei* injected with noradrenaline. For the latter purpose, we examined total haemocyte count (THC), phenoloxidase activity, respiratory burst, superoxide dismutase (SOD) activity, phagocytic activity and clearance efficiency of shrimp to *V. alginolyticus*.

2. Materials and methods

2.1. *L. vannamei*

L. vannamei juveniles (16–20 g) were obtained from a commercial farm in Pingtung, Taiwan, and acclimated in the laboratory for 2 weeks before experimentation. Only shrimp in the intermoult stage were used for the study. The moult stage was determined by the examination of uropods in which partial retraction of the epidermis could be distinguished [22]. For the susceptibility experiment, test and control groups were comprised of 10 shrimp each in triplicate. For the determination of immune parameters, tests were carried out in eight replicate test groups consisting of one shrimp each in 20 l PVC tanks containing 10 l aerated test solution. In all tests, the shrimp were fed twice daily with a formulated shrimp diet (Shinta Feed Company, Pingtung, Taiwan). No significant difference in weight was observed among the treatments. During the experiments, water temperature was maintained at 27 ± 1 °C, pH 7.8–8.2 while salinity was maintained at 20‰.

2.2. *V. alginolyticus*

A known pathogenic strain of *V. alginolyticus* (CH003), which had been isolated from diseased *L. vannamei* in Pingtung, Taiwan, was used for the study [5]. Stocks were cultured on tryptic soy agar (TSA supplemented with 2% NaCl, Difco) for 24 h at 28 °C and transferred to 10 ml tryptic soy broth (TSB supplemented with 2% NaCl, Difco) for 24 h at 28 °C as the culture for the test. The broth culture was centrifuged at $7155 \times g$ for 20 min