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L-3,4-Dihydroxyphenylalanine (L-DOPA) induces neuroendocrinological, physiological, and immunological regulation in white shrimp, *Litopenaeus vannamei*

Ratchaneegorn Mapanao[a], Hsin-Wei Kuo[b], Chin-Chuan Chang[b], Kuan-Fu Liu[c,**], Winton Cheng[b,*]

[a] Faculty of Applied Science and Engineering, Khon Kaen University, Nong Khai Campus, Nong Khai 43000, Thailand
[b] Department of Aquaculture, National Pingtung University of Science and Technology, Pingtung 91201, Taiwan, ROC
[c] Tungkang Biotechnology Research Center, Fisheries Research Institute, C.O.A., Pingtung 92845, Taiwan, ROC

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ABSTRACT

L-3,4-Dihydroxyphenylalanine (L-DOPA) is a precursor for dopamine (DA) synthesis. Assessments were conducted to analyze the effects of L-DOPA on mediating regulation of neuroendocrinological, immunological, and physiological parameters in the shrimp, *Litopenaeus vannamei* when they were individually injected with 0.01 N HCl or L-DOPA at 0.5 or 1.0 μmol shrimp^{-1} for 60, 120, and 240 min. For catecholamine synthesis evaluation, tyrosine hydroxylase (TH) and DA beta hydroxylase (DBH) activities, L-DOPA, DA, and norepinephrine (NE) levels in hemolymph were determined. The total hemocyte count (THC), differential hemocyte count (DHC), phenoloxidase (PO) activity, respiratory bursts (RBs), superoxide dismutase (SOD) activity, phagocytic activity, and clearance efficiency in response to the pathogen, *Vibrio alginolyticus* were assessed for immune responses, and plasma glucose and lactate levels were for physiological response. Results showed that the TH activity, THC, hyaline cells (HCs), and semigranular cells (SGCs) at 120 min, DA levels at 60–240 min, PO activity in hemocytes per 50 μL of hemolymph at 60–120 min, and PO activity per granulocyte (granular cells (GCs) + SGCs) at 60 min significantly increased, but TH activity, L-DOPA levels, GCs, SGCs, and respiratory bursts in hemocytes per 10 μL of hemolymph at 60 min, respiratory bursts per hemocyte and SOD activity at 120 min, phagocytic activity at 60–240 min, and the clearance efficiency at 60–120 min significantly decreased in shrimp injected with L-DOPA at 1.0 μmol shrimp^{-1}. In another experiment, 60 min after shrimp had received L-DOPA at 0.5 or 1.0 μmol shrimp^{-1}, they were challenged with an injection of *V. alginolyticus* at 2×10^5 colony-forming units (cfu) shrimp^{-1}. The injection of L-DOPA at 1.0 μmol shrimp^{-1} also significantly increased the cumulative mortality of shrimp by 16.7%, compared to the HCl-challenged control after 120 h. These results suggest that L-DOPA administration at 1.0 μmol shrimp^{-1} can mediate the transient regulation of neuroendocrinological, immunological, and physiologic responses resulting in immunosuppression, which in turn promoted the susceptibility of *L. vannamei* to *V. alginolyticus*.

1. Introduction

The white shrimp, *Litopenaeus vannamei*, is native to Eastern Pacific coasts from the Gulf of California to northern Peru, has been introduced into many countries for aquaculture, and has developed into a primary intensively cultured species. It is known that rapidly degraded environments in intensive culture systems may result in increased incidences of disease. Commercial shrimp farming has experienced serious economic losses due to epidemics associated with bacteria and viruses. Understanding the bidirectional communication of the neuroendocrine and immune systems is of primary concern in shrimp in order to establish efficient measures of stress and disease resistance.

In crustaceans, innate immunity is considered to be the primary defense mechanism, which includes both cellular and humoral components that perform in joint coordination to detect and eliminate all foreign organisms potentially hazardous to the host [1]. Cellular defense components include all of those reactions, i.e., phagocytosis, encapsulation, and nodule formation, directly performed by hemocytes, which are generally recognized as hyaline (HCs), semi-granular (SGCs), and large granular cells (GCs) [2]. On the other hand, humoral components include activation and release of molecules stored within hemocytes, such as anticoagulant proteins, agglutinins, phenoloxidase

* Corresponding author.
** Corresponding author.
E-mail addresses: kuanfu81@yahoo.com.tw (K.-F. Liu), winton@mail.npust.edu.tw (W. Cheng).

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(PO), antimicrobial peptides, protease inhibitors, etc. [1,3]. Several reactive oxygen species (ROS) are produced during phagocytosis. The process known as respiratory bursts (RBs) plays an important role in microbicidal activity [4]. Superoxide anions are the first product release from the RBs, and they are scavenged by superoxide dismutases (SODs) [5]. The evaluation of total hemocytes counts (THCs), PO activity, free radical production, phagocytosis, clearance efficiency of the pathogen, and the survival rate from stress and challenge tests can be used as health status indicators of a host.

Neuroendocrine hormones play critical roles in regulating homeostasis in stressful environments. Catecholamines (CAs) are required for many physiological processes in invertebrates, the release of which is a primary response to physiological stress in crustaceans, while subsequent induction of carbohydrate metabolism and immune regulation are secondary responses [6,7]. Tyrosine is a precursor for the production of CAs, which can be metabolized to L-3,4-dihydroxyphenylalanine (L-DOPA) or tyramine via different pathways. L-DOPA is converted from tyrosine by tyrosine hydroxylase (TH), and it can be metabolized to dopamine (DA) and norepinephrine (NE) in order by dopa decarboxylase (DDC; aromatic L-amino acid decarboxylase, AADC) and dopamine β-hydroxylase (DBH), respectively [8]. In addition, tyrosine can also be hydroxylated to produce L-DOPA by tyrosinases, such as phenoloxidase (PO) [9].

Studies on insects indicated that L-DOPA is required for tanning of newly formed cuticle and the production of melanin during some types of immune responses. Gorman et al. [9] proposed that TH appears to be a better candidate for producing significant amounts of DOPA from tyrosine, DDC decarboxylates DOPA to DA, and DA is used as a substrate for PO to produce DA quinone in hemolymph, by a process activated in response to immune infection. We assume that L-DOPA is involved in physiological and immunological regulation by metabolizing to DA. Accordingly, the purposes of the present study were to examine the effect of L-DOPA on the susceptibility of *L. vannamei* to *Vibrio alginolyticus*, and determine the immunological, physiological, and neuroendocrinological responses after *L. vannamei* was injected with L-DOPA.

2. Materials and methods

2.1. Litopenaeus vannamei

Shrimp, *L. vannamei*, obtained from an aquafarm at National Pingtung University of Science and Technology (Pingtung, Taiwan) were acclimated in an indoor concrete pond (5 × 5 × 1 m) with 12 tons aerated seawater at 28 ± 1 °C and salinity of 20‰ for 2 weeks before experimentation. Only healthy shrimp in the intermolt stage (stage C) were used for the study. The molt stage was determined by examining the uropoda in which partial retraction of the epidermis could be distinguished [10]. During the acclimation and experimental periods, prawns were fed twice daily with a formulated shrimp diet (Grobest Feeds Corporation, Pingtung, Taiwan), and the water temperature was maintained at 28 ± 1 °C and the pH at 7.7–8.3.

Four studies were conducted. For the disease-resistance ability experiment, test and control groups were comprised of 10 prawns each, and tests were conducted in triplicate. To determine the THC, differential hemocyte count (DHC), PO activity, RBs, and SOD activity, analyses were carried out in six replicate test groups consisting of one prawn each in 20-L PVC tanks containing 10 L of aerated seawater (20‰). For studies of phagocytic activity and clearance efficiency, another six prawns were used in each of the test and control groups. To examine TH and DBH activities, and L-DOPA, DA, NE, glucose, and lactate levels, six different prawns were used in each of the test and control groups. No significant difference in weight was observed among the treatments.

2.2. Vibrio alginolyticus

The bacterium, *V. alginolyticus* (CH003) isolated from diseased *L. vannamei* (Pingtung, Taiwan), which displayed symptoms of anorexia, inactivity, poor growth, and necrotic musculature, was used in the present study [11]. Stocks were plated on tryptic soy agar (TSA supplemented with 2% NaCl, Difco) for 24 h at 28 °C before being transferred to 10 mL of tryptic soy broth (TSB supplemented with 2% NaCl, Difco), where they remained for 24 h at 28 °C before being centrifuged at 7155*g* for 15 min at 4 °C. The supernatant was removed, and the bacterial pellet was suspended in a saline solution (0.85% NaCl) at concentrations of 10^7 and 10^9 colony-forming units (cfu) mL^{-1} as respective stock bacterial suspensions for the susceptibility study, and for the phagocytic activity and clearance efficiency studies.

2.3. Effect of L-DOPA on the susceptibility of shrimp to V. alginolyticus

L-DOPA (D-9628, Sigma, St. Louis, MO, USA) was dissolved in sterile 0.01 N HCl to concentrations of 2.5×10^{-2} and 5.0×10^{-2} mol L^{-1} and was injected into (20 μL) the ventral sinus of the cephalothorax of individual *L. vannamei* (9.1 ± 1.0 g) to reach respective doses of 0.5 and 1.0 μmol $shrimp^{-1}$ in the initial stage. At 60 min after the injection, a challenge test was conducted by injecting 20 μL of a bacterial suspension (10^7 cfu mL^{-1}) resulting in 2×10^5 cfu $shrimp^{-1}$ into the ventral sinus of the cephalothorax. Shrimp that received 20 μL of 0.01 N HCl and then *V. alginolyticus* at 2×10^5 cfu $shrimp^{-1}$ served as the HCl-challenged controls. Shrimp that received L-DOPA at 1.0 μmole $shrimp^{-1}$ and then were injected with 20 μL of saline served as the unchallenged controls (Table 1). Test and control prawn (10 shrimp $aquarium^{-1}$) were kept in 60-L glass aquaria containing 40 L of seawater at 28 °C and a salinity of 20‰. There were four treatments, each of which was conducted with 30 shrimp, and 120 shrimp were used for the susceptibility test. The experiment lasted 7 days.

2.4. Effect of L-DOPA on the immune parameters of L. vannamei

Shrimp (19.6 ± 1.4 g) were individually injected in the ventral sinus of the cephalothorax with 2.5×10^{-2} and 5.0×10^{-2} mol L^{-1} of 20 μL L-DOPA solutions to reach respective doses of 0.5 and 1.0 μmol $shrimp^{-1}$. Shrimp that received 20 μL of 0.01 N HCl served as the control. There were three treatments (0.01 N HCl (0), 0.5, and 1.0 μmol $shrimp^{-1}$) with three sampling times (60, 120, and 240 min) for determining immune parameters. Six shrimp from each treatment and

Table 1
Effect of L-3,4-dihydroxyphenylalanine (L-DOPA) on the susceptibility of *Litopenaeus vannamei* challenged with *Vibrio alginolyticus*.

Bacterial dose (cfu $shrimp^{-1}$)	L-DOPA (μmol $shrimp^{-1}$)	No. of shrimp	Cumulative mortality (%), time after challenge (h)							
			12	24	48	72	96	120	144	168
Saline	1.0	30	0	0	0	0	0	0	0	0
2×10^5	0.01 N HCl	30	56.7 ± 5.8^a	60.0 ± 10.0^a	60.0 ± 10.0^a	60.0 ± 10.0^a	60.0 ± 10.0^a	60.0 ± 10.0^b	60.0 ± 10.0^b	60.0 ± 10.0^b
2×10^5	0.5	30	50.0 ± 17.3^a	53.3 ± 11.6^a	53.3 ± 11.6^a	53.3 ± 11.6^a	53.3 ± 11.6^a	56.7 ± 5.8^b	56.7 ± 5.8^b	56.7 ± 5.8^b
2×10^5	1.0	30	46.7 ± 23.1^a	60.0 ± 20.0^a	63.3 ± 15.3^a	66.7 ± 11.6^a	70.0 ± 10.0^a	76.7 ± 5.8^a	76.7 ± 5.8^a	76.7 ± 5.8^a

Data in the challenge groups in the same column with different superscripts are significantly different ($p < .05$) among treatments. Values are mean ± S.E. (n = 30 shrimp in each case).