Developmental and Comparative Immunology 81 (2018) 19–32

Contents lists available at ScienceDirect

Developmental and Comparative Immunology

journal homepage: www.elsevier.com/locate/dci

Octopamine enhances the immune responses of freshwater giant prawn, *Macrobrachium rosenbergii*, via octopamine receptors

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ARTICLE INFO

Article history:
Received 24 August 2017
Received in revised form
9 November 2017
Accepted 9 November 2017
Available online 10 November 2017

Keywords:
Macrobrachium rosenbergii
Lactococcus garvieae
Octopamine
Immunity
Stress indices
Adrenergic-like octopamine receptors

ABSTRACT

Octopamine (OA) is known to play an important role in regulating insect immune responses. In *Macrobrachium rosenbergii* (18.0 ± 1.7 g), OA at 25.0 and 250.0 pmol/prawn significantly increased THC, semigranular cells (SGCs) and PO activity in hemocytes per 50 μL hemolymph, hyaline cells, granular cells (GCs) and RBs in hemocytes per 10 μL hemolymph, and RBs per hemocyte, and however, significantly decreased PO activity per granulocyte (GC + SGC), which returned to control levels after 4 h of injection. The significantly increased phagocytic activity and clearance efficiency of prawn received OA for 8 h returned to control levels after 16 h of injection. In addition, the significantly increased glucose and decreased lactate were observed within 1 h of OA injection. In the susceptibility test, prawn received OA at 25.0 or 250.0 pmol/prawn for 2 h then challenged with *Lactococcus garvieae* at 10^5 colony-forming units/prawn significantly increased the resistance of prawns by 23.3% and 30.0%, respectively, compared to the saline-challenged control after 144 h of challenge. In addition, the changes on immunocompetence induced by OA were observed to be blocked by adrenoceptors antagonists. These results suggest that OA administration at 250.0 pmol/prawn or less causes the mediate a transient up-regulation in immune and physiologic responses to promote the resistance of *M. rosenbergii* to *L. garvieae*, which are thought to be mediated by α- and β-adrenergic-like octopamine receptors.

1. Introduction

The giant freshwater prawn, *Macrobrachium rosenbergii,* has been introduced into many countries for aquaculture and is a primary inland cultured species worldwide. Commercial prawn farming in Taiwan has experienced serious economic losses caused by epidemics of microorganisms. Understanding the bidirectional communication of the neuroendocrine and immune systems is of primary concern for establishing efficient measures of stress and disease resistance in prawn.

In crustaceans, three types of circulating hemocytes are generally recognized as hyaline (HCs), semigranular (SGCs), and large granular cells (Tsing et al., 1989), which play important roles in regulating physiological functions including carbohydrate metabolism, coagulation, phagocytosis, and immunological defense (Ratcliffe et al., 1985; Martin et al., 1991). The prophenoloxidase (proPO) system, which is contained in granular hemocytes, is involved in encapsulation, melanization, and recognition (Johansson and Söderhäll, 1989). Phenoloxidase (PO) is the terminal enzyme in the proPO system and is considered a major immune indicator in crustaceans. It is activated by several microbial polysaccharides through the non-self recognition system (Söderhäll et al., 1996). Several reactive oxygen species (ROS) are produced during phagocytosis. The process known as a respiratory bursts (RBs) plays an important role in microbicidal activity (Muñoz et al., 2000). Superoxide anions are the first product released from RBs and are scavenged by superoxide dismutases (Bell and Smith, 1993).

Neuroendocrine hormones play critical roles in regulating homeostasis under stressful environments. Catecholamines (CAs) derived from tyrosine are required for many physiological processes in invertebrates, the release of which is the primary response to physiological stress in crustaceans, while subsequent induction of hyperglycemia and suppression of immunity are secondary responses (Chang et al., 2009; Kuo and Yang, 1999). Tyrosine is a precursor for the production of CAs, which can be metabolized to norepinephrine (NE) or octopamine (OA) via different pathways. Octopamine biosynthesis requires tyrosine decarboxylase activity to convert tyrosine to tyramine and tyramine β-hydroxylase (TBH) activity to convert tyramine to OA (Monastirioti et al., 1996). L-DOPA

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https://doi.org/10.1016/j.dci.2017.11.002
0145-305X/

is converted from tyrosine by tyrosine hydroxylase, and it can be metabolized to dopamine (DA) and NE in order by dopa decarboxylase (aromatic L-amino acid decarboxylase, AADC) and DA β-hydroxylase (DBH), respectively (Joh and Hwang, 1987). In white shrimp *Litopenaeus vannamei*, hypothermal stress caused the increase of DBH activity and induced the high level of DA and NE in hemolymph (Cheng et al., 2016). The significantly evaluated hemolymph NE and DA levels (mean ± SE) at 10.4 ± 0.3 pmol/mL and 0.18 ± 0.01 ng/mL, respectively, were observed in *M. rosenbergii* exposed to hypothermal stress for 120 min (Chang et al., 2015a; 2016), and furthermore, the reduced immunocompetence and resistance to pathogens were observed after prawn had received a high level of DA or NE (Li et al., 2005; Chang et al., 2011; Chang et al., 2015b).

Adrenoceptors, involving in the response to different neuro- and autocrine transmitters and mediating the signal of the transmitter into the cell, belong to the family of G protein coupled receptors, and they are classified into two main categories, alpha and beta, and can be divided into three main classes based on sequence similarity, receptor pharmacology, and signaling mechanisms: alpha1 (α1), alpha2 (α2), and beta (β) (Bylund, 1992, 2005; Meloni, 2008). In pharmacology, antagonist binding reduces or prevents the action of the agonist when both of the agonist and antagonist can be bound simultaneously (Hein and Kobilka, 1995). The pharmacological action of antagonists against α- and β-adrenoceptors had been used to evaluate the potential modulating pathway on immunocompetence in *M. rosenbergii* (Chang et al., 2011; Chang et al., 2015b) and in *L. vannamei* (Chang et al., 2012), on hyperglycemic response in *M. rosenbergii* (Hsieh et al., 2006), and on hemocyte phagocytosis in the oyster *Crassostrea gigas* (Lacoste et al., 2001).

Studies on invertebrates indicated that OA is involved in immunomodulation, and such studies were reviewed by Adamo (2008). The distribution of OA in the central nervous system and ovaries of *M. rosenbergii* was investigated by Tinikul et al. (2009), and OA level at 4 ± 0.35 nmol/mL can be detected in hemolymph of *M. rosenbergii* in our preliminary test following the OA determination reported by Châtel et al. (2013) (unpublished data). In insects, OA enhances phagocytosis and modulates activities of hemocytes, accelerates clearing of circulating bacteria from hemolymph, and increases resistance to pathogen infection (Baines et al., 1992; Dunphy and Downer, 1994). Evans and Maqueira (2005) proposed that the OA receptor family can be further subdivided into α-adrenergic-like octopamine receptors, β-adrenergic-like octopamine receptors, and OA/TA type receptors in insects. A putative octopamine/tyramine receptor in ganglia of *M. rosenbergii* had been cloned by Reyes-Colón et al. (2010), and its sequence clustered with the octopamine/tyramine family in insect closely. Although there was no research on α-adrenergic-like and β-adrenergic-like octopamine receptors in *M. rosenbergii*, the two receptors in insect were suggested to show structural similarities to vertebrate adrenoceptors, and they can clearly be distinguished on pharmacological grounds (Evans and Maqueira, 2005; Qi et al., 2017). These bring an insight that traditional antagonists against adrenoceptors might be used to evaluate the OA modulating pathways in *M. rosenbergii*.

Accordingly, the purposes of the present study were to examine (1) the effect of OA on the susceptibility of *M. rosenbergii* to *Lactococcus garviae*, and (2) the immune response of *M. rosenbergii* injected with OA. For the latter purpose, the total hemocyte count (THC), differential hemocyte count (DHC), PO activity, RBs, phagocytic activity, and the clearance efficiency of prawn toward *L. garvieae* were used as indicators. Furthermore, glucose and lactate levels of hemolymph were used to evaluate the effects of OA on physiologic functions, and the antagonistic effects of various adrenoceptor antagonists were used to evaluate the pharmacological modulation of OA on the immunological parameters.

2. Materials and methods

2.1. M. rosenbergii

Prawn (18.0 ± 1.7 g), obtained from an aquafarm of National Pingtung University of Science and Technology in Pingtung, Taiwan, were acclimated in an indoor concrete pond (5 × 5 × 1 m) with 12 tons of aerated fresh water at 27 ± 1 °C for 12 days before experimentation began. Only prawn in the intermolt stage (stage C) were used for the study. The molt stage was determined under a stereomicroscope according to retraction of the epithelium within the setal base interface of the antennal scale (Peebles, 1977).

Six studies were conducted. For the disease-resistance ability experiment, test and control groups were comprised of 10 prawn each, and tests were conducted in triplicate. For determination of THC, DHC, PO activity, and RBs, tests were carried out in six replicate test groups consisting of one prawn each in 20-L PVC tanks containing 10 L of aerated fresh water. For studies of phagocytic activity and clearance efficiency, another six prawn were used in each of the test and control groups. For examination of glucose and lactate, six prawns were used in each of the test and control groups. For determination of pharmacological modulation in immunological parameters, tests of THC, DHC, PO activity and RBs were performed in six replicate test groups consisting of one prawn, and another 6 prawns were used to determine the phagocytic activity and clearance efficiency. No significant difference in weight was observed among the treatments. During the acclimation and experimental periods, prawn were fed twice daily with a formulated shrimp diet (Grobest Feeds, Pingtung, Taiwan), and the water temperature was maintained at 27 ± 1 °C and pH at 7.1–7.6.

2.2. Culture of L. garvieae

The bacterium L. *garvieae* isolated from diseased *M. rosenbergii* (Pingtung, Taiwan), which displayed symptoms of an opaque and whitish musculature, was used in the present study (Cheng and Chen, 1998; Chen et al., 2001). Stocks were plated on tryptic soy agar (TSA, Difco) for 24 h at 30 °C before being transferred to 10 mL of tryptic soy broth (TSB, Difco), where they remained for 24 h at 30 °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 5.0×10^6 and 10^9 colony-forming units (cfu)/mL as respective stock bacterial suspensions for the susceptibility study and for the phagocytic activity and clearance efficiency studies.

2.3. Effect of OA on the susceptibility of prawn to L. garvieae

Octopamine (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in sterile saline (0.85% NaCl) to concentrations of 1.25×10^{-5} and 1.25×10^{-6} mol/L and 20 μL of OA solution was injected into the ventral sinus of the cephalothorax of individual *M. rosenbergii* to reach respective doses of 250.0 and 25.0 pmol/prawn in the initial stage. At 2 h after the injection, a challenge test was conducted by injecting 20 μL of a bacterial suspension (5×10^6 cfu/mL) resulting in 10^5 cfu/prawn into the ventral sinus of the cephalothorax. Prawn that received saline and then *L. garviae* at 10^5 cfu/prawn served as the saline-challenged controls. Prawn that received OA at 250.0 pmol/prawn and then were injected with 20 μL of saline served as the unchallenged controls (Table 1). Test and control prawn (10 prawns/aquarium) were kept in 60-L glass aquaria containing 40 L of fresh water. Therefore, there were four treatments. Each