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The modulation of catecholamines on immune response of scallop *Chlamys farreri* under heat stress

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ABSTRACT

Catecholamines (CAs) play key roles in mediating the physiological responses to various stresses. In the present study, the expression of CA-related genes were examined in the hemocytes of scallop *Chlamys farreri* under heat stress, and several immune or metabolism-related parameters were investigated after heat stress and adrenoceptor antagonist stimulation. After the scallops were cultured at 28 °C, the mRNA expression level of dopa decarboxylase (CfDDC) and α-adrenoceptor (CfαAR) increased significantly ($P < 0.01$), whereas that of monoamine oxidase (CfMAO) was down-regulated in the first 6 h ($P < 0.05$), and then up-regulated to the maximum level at 24 h ($P < 0.01$). In the hemocytes of scallops injected with adrenoceptor antagonist, the expression levels of peptidoglycan-recognition protein (CfPGRP-S1) and C-type lectin (CfLec-1) began to increase significantly at 2 and 3 h post propranolol and high temperature treatment, respectively ($P < 0.01$). While the up-regulation of heat shock protein 70 (CfHSP70) post heat stress was significantly inhibited by prazosin injection ($P < 0.01$), and that of hexokinase (CfHK) was inhibited by both prazosin and propranolol injection ($P < 0.01$). Moreover, the remarkable increase of relative specific activity of SOD in the hemolymph post heat stress ($P < 0.01$) was further up-regulated early after prazosin or propranolol injection ($P < 0.01$), while that of the relative anti-bacterial ability was down-regulated by prazosin or propranolol treatment ($P < 0.01$). These results collectively indicated that the catecholaminergic neuroendocrine system in scallop could be activated by heat stress to release CAs, which subsequently modulated the immune response and energy metabolism via α- and β-adrenoceptors.

1. Introduction

Chlamys farreri is one of the economic scallop species cultivated widely in the coastal provinces of north China. Since the summer of 1997, large-scale mortality of *C. farreri* has been reported in China and has caused catastrophic economic losses to scallop aquaculture. It was reported that the mortalities were probably caused by a combination of several factors including stress associated with high temperature, overcrowding and poor circulation in the grow-out cages, opportunistic invaders or pathogens (Xiao et al., 2005). Among these factors, stress caused by high water temperature was admitted as one of the most important environmental factors contributing to scallops summer mortality by affecting scallop immune defense (Chen et al., 2007).

Stress response is a series of coordinated physiological reactions increasing an organism's capacity to maintain homeostasis (Ottaviani and Franceschi, 1996). Neuroendocrine hormones play major roles in the regulation of both basal homeostasis and responses to internal or external adverse forces (Chrousos, 2009). Among the first hormones in response to stressors, catecholamines (CAs), including the dopamine (DA), noradrenaline (NA) and adrenaline (AD), are most crucial in stimulating actions in response to a perceived threat (Sabban and Kvetnansky, 2001; Kvetnansky et al., 2009). DA, converted from L-DOPA by dopa decarboxylase (DDC), is taken up from the cytoplasm into storage vesicles and converted into NA and AD. All the three kinds of CAs can be metabolized by monoamine oxidase (MAO) in the cytoplasm for degradation. After being released to synaptic cleft via exocytosis, NA and AD could diffuse into blood or bind to α- or β-adrenoceptor and be taken up into nerve terminal or nonneuronal cells (Kvetnansky et al., 2009). The binding of CAs to the adrenoceptors activates different signal transduction cascades, which subsequently shuts down certain processes such as growth, reproduction, metabolism and immunity to redirect bioenergetics resources to specific physiological functions that are immediately required for the adaptation and survival of the animal under various environmental stresses (Chen et al., 2008; Massarsky et al., 2011; Zhou et al., 2013). Taking into

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account the biological role of CAs and the severe summer mortality of scallop, the immunomodulatory mechanism of CAs in response to heat stress is of primary concern.

Upon environmental or pathogenic perturbations, the innate immune response of invertebrates is stimulated to reintegrate the physiological balances in order to regain homeostasis (Beutler, 2004; Ellis et al., 2011). Invertebrates discriminate self from harmful non-self by pattern recognition receptors (PRRs), such as peptidoglycan-recognition proteins (PGRP) (Yang et al., 2010) and C type lectins (Wang et al., 2007), and then the cellular and humoral immune responses are activated to eliminate non-self through phagocytosis, encapsulation or various immune effects (Beutler, 2004). After the invertebrates are infected by pathogen or exposed to stressful environments, a large amounts of superoxide anion (O_2^-) will be generated immediately and increasingly released into hemolymph (Ellis et al., 2011). Afterwards, the oxidative damage resulted from respiratory burst will be repaired by specific antioxidant enzymes such as superoxide dismutase (SOD) (Miller, 2004) and nonspecific molecular chaperones like heat shock protein 70 (HSP 70) (Pratt et al., 2010). Meanwhile, various stresses trigger energy metabolic alternation and reallocate energy distribution competing physiological and behavioral systems (Demas, 2004).

The accumulated reports on marine invertebrates including crustaceans (Li et al., 2005; Cheng et al., 2006; Chang et al., 2007, 2012) and mollusk (Lacoste et al., 2001a,b,c,d, 2002; Malham et al., 2002, 2003; Chen et al., 2008; Franzellitti et al., 2011; Kuchel and Raftos, 2011; Zhou et al., 2011a) have preliminarily established the link between catecholaminergic neuroendocrine system and environmental stress or immune response. In crustaceans *Litopenaeus vannamei* and *Macrobrachium rosenbergii*, NA was found to regulate the prophenoloxidase system and depress the immunity and disease-resistance via α- and β-adrenoceptor (Cheng et al., 2006; Chang et al., 2012). While in oyster *Crassostrea gigas* and mussel *Mytilus galloprovincialis*, CA could induce stress response via β-adrenoceptor-cAMP signaling pathway (Lacoste et al., 2001a,b,c,d; Franzellitti et al., 2011). A catecholaminergic neuroendocrine system has been revealed in scallop *C. farreri* (Zhou et al., 2011b,c,d,e), which could be activated by bacteria challenge and negatively modulated the immune response (Zhou et al., 2011a). When exposed to high temperature, the concentration of NA and AD in scallop increased significantly (Chen et al., 2008), and the SOD activity and HSP gene expressions were both up-regulated (Wang et al., 2012). However, the relationship among CA, heat stress and immune response, as well as the energy metabolism during this course, are still far from well understood, and the knowledge about the signal pathway to regulate the heat stress-induced NA/AD modulation on immune functions in scallop is also very limited. The purposes of this study were (1) to ascertain the activation of scallop catecholaminergic neuroendocrine system after heat stress, (2) to determine the contribution of catecholamine and receptors to the immune modulation and and energy metabolism in response to heat stress, and (3) to provide insights to the complex neuroendocrine-immune regulatory network of scallops in response to heat stress.

2. Materials and methods

2.1. Scallop

Healthy adult scallops *C. farreri* with average wet weight of 14.37 ± 1.51 g were collected in July from a shellfish farm (longitude: 120.41° E and latitude: 36.16° N, located at Qingdao, China) and acclimated in a fiberglass tank at salinity 30 ± 0.1 ‰, temperature 18 ± 0.1 °C, dissolved oxygen above 6.0 mg L^{-1} and pH from 7.7 to 8.2 for two weeks. The seawater was changed 100% daily to ensure high water quality.

2.2. Heat-stress and adrenoceptor antagonist stimulation

Five hundred and twenty-five scallops were equally divided into 5 groups, with three replicate tanks for each group. The scallops in the control group were maintained in acclimated environment, and those in the group for heat treatment (designated as heat stressed group) were directly transferred to 28 °C seawater, which was near the maximum sea surface temperature in near-shore regions of Shandong province, China during summer (high frequency URI/NASA AVHRR Pathfinder 1km satellite data, http://satdat1.gso.uri.edu/opendap/Pathfinder/Pathfinder1km/pathfinder_1km.html). The scallops in the rest three groups received an injection of 50 μl sterilized phosphate buffered saline (PBS, 136.89 mmol L^{-1} NaCl, 2.68 mmol L^{-1} KCl, 8.10 mmol L^{-1} Na_2HPO_4, 1.47 mmol L^{-1} KH_2PO_4, pH 7.4), 1.0 mmol L^{-1} prazosin (α-adrenoceptor antagonist, Sigma, in sterilized PBS) and 1.0 mmol L^{-1} propanolol (nonselective β-adrenoceptor antagonist, Sigma, in sterilized PBS), respectively, and then maintained at 28 °C. These three groups were designated as heat + PBS, heat + prazosin and heat + propanolol groups, respectively. Fifteen scallops were randomly sampled from each group at 0, 1, 2, 3, 6, 12 and 24 h post treatment, respectively. The hemolymph from three scallops (about 0.5 ml per individual) at the same time point was collected using a syringe from the adductor muscles and pooled together as one replicate. Five replicates were employed for each sampling time point. The hemolymph was immediately centrifuged at 500×g, 4 °C for 10 min to harvest the hemocytes, and both hemocytes pellets and supernatant were stored at −80 °C.

2.3. RNA isolation and cDNA synthesis

Total RNA was isolated from the hemocytes of scallops using TRIzol reagent (Invitrogen). The first-strand cDNA synthesis was carried out based on Promega M-MLV RT Usage information using the DNase I (Promega)-treated total RNA as template and oligo (dT)-adaptor primer or gene specific primers (Table 1). The reaction mixtures were incubated at 42 °C for 1 h, terminated by heating at 95 °C for 5 min. The cDNA mix was diluted to 1:100 and stored at −80 °C for subsequent SYBR Green fluorescent quantitative real-time PCR.

2.4. The examination of mRNA expression of genes in hemocytes after heat stress and adrenoceptor antagonist stimulation

The mRNA expression level of CA system-related genes, including DDC, MAO and α-adrenoceptor of *C. farreri* (named as CfDDC,

Table 1
Primers used for real-time PCR.

Primer name	Forward/reverse sequence (5′–3′)
Oligo $(dT)_{17}$	GGCCACGCGTCGACTAGTACTTTTTTTTTTTTTTTTVN
CfDDC	TGTTAGCCAGACCGTCAG
	ATTATCTCCTTCTTCGTCCTCC
CfMAO	ATCACCGAACCAGAACATAAGA
	GGCAGACACAAGACCCAAGAAC
CfαAR	GCCAGAAAGCACATCCGA
	AATAGCCCACCAATATACTGACTG
CfPGRP-S1	GTATCAGCATCGTCAAAAGCATTC
	TGATCCTACGGTCTTCCAGCCA
CfLec-1	CAACCTGTTCTATATCTGCGAG
	GATCTGTTGGCTGATTTCAC
CfHSP 70	GCTCCTTTGTCCTTGGGTATTG
	AATGTTTGGGTCTGCTTGGTG
CfHK	CTTCCACCCTCACTTTCACGA
	TGTTACTATGGCAGCACCCTT
CfActin	CAAACAGCAGCCTCCTCGTCAT
	CTGGGCACCTGAACCTTTCGTT