Dopamine depresses the immune ability and increases susceptibility to *Lactococcus garvieae* in the freshwater giant prawn, *Macrobrachium rosenbergii*

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**Abstract**

The total haemocyte count (THC), phenoloxidase activity, respiratory burst (release of superoxide anion), superoxide dismutase activity, and phagocytic activity and clearance efficiency to the pathogen *Lactococcus garvieae* were measured when freshwater giant prawns *Macrobrachium rosenbergii* (16.2±2.1 g) were individually injected with saline, or dopamine at 0.5, 5.0, or 50.0 pmol prawn\textsuperscript{-1}. The results show that a transient period of immunosuppression occurred between 2 and 8 h after injection of dopamine for all immune parameters except circulating haemocytes and all immune parameters returned to control values within 8–16 h after receiving dopamine. Injection of dopamine also significantly increased the mortality of *M. rosenbergii* challenged with the pathogen *L. garvieae*. These results suggest that stress-inducing dopamine suppresses the immune system, which in turn promotes the susceptibility to *L. garvieae* in *M. rosenbergii*.

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**Key words:** *Macrobrachium rosenbergii; Lactococcus garvieae; Dopamine; Phenoloxidase activity; Respiratory burst; Superoxide dismutase; Phagocytic activity; Clearance efficiency

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1. Introduction

The giant freshwater prawn *Macrobrachium rosenbergii* is commercially important globally and especially in Taiwan [1]. The farmed production of freshwater prawns reached a maximum of 16,196 tons in 1991, but subsequently decreased, and was 10,045 tons in 2003 in Taiwan. Disease outbreaks caused by yeast infections in the cool season and bacteria in the hot season have resulted in declining production of farmed prawns in Taiwan [2,3]. The bacterium *Lactococcus garvieae* isolated from diseased prawns has been documented to cause muscular necrosis and mass mortality of prawns in the laboratory [3,4].

The corticotropin-releasing hormone (CRH), adrenocorticotropin hormone (ACTH), of the biogenic amine axis is the main origin of the stress response in vertebrates and invertebrates (molluscs) [5]. In invertebrates, the process is simplified, for rather than several organs, such as the hypothalamus, pituitary, and adrenal glands, being involved, the response is concentrated in phagocytic haemocytes, which harbour all the relevant molecules. The release of biogenic amine is believed to be a proto-stress response [5].

The fact that neuroregulators, including neurotransmitters and neuromodulators, mediate and control a wide variety of physiological actions of neurohormones has been reported elsewhere. Classical neuroregulators are small biogenic amines, which in crustaceans, include acetylcholine, glutamate, gamma-aminobutyric acid, dopamine, histamine, 5-hydroxytryptamine, norepinephrine, and octopamine [6]. In prior studies, dopamine was reported to function as a neurotransmitter and to stimulate the release of both pigment-concentrating hormone [7,8] and distal retinal pigment dark-adapting hormone [9] in the fiddler crab *Uca pugilator*. It has also been demonstrated that dopamine can promote the release of crustacean hyperglycaemic hormone from the x-organ-sinus gland complex of the spinycheek crayfish *Orconectes limosus* and the shore crab *Carcinus maenas* [10], the tiger shrimp *Peneaus monodon* [11], and the freshwater giant prawn *M. rosenbergii* [12]. In addition, dopamine was shown to inhibit 5-hydroxytryptamine-stimulated testicular maturation in the fiddler crab *U. pugilator* [13], and the red swamp crayfish *Procambarus clarkii* [14], and ovarian maturation in *P. clarkii* [15].

Environmental stress is known to suppress the immune system and lead to an enhanced susceptibility to infectious disease agents in crustaceans [16,17]. The effects of stress on immunological responses are the consequence of the release of glucocorticoids and biogenic amines [5]. Stress induction of neuroendocrine responses involving the release of catecholamines, such as noradrenaline (NA) and dopamine, in the haemolymph and depression of the immune functions leading to enhanced susceptibility to pathogens, has been demonstrated in molluscs [18,19]. Dopamine is widely distributed in the crustacean nervous system and has a diverse array of physiological effects [20]. It has been reported that following cold shock in *M. rosenbergii*, dopamine, mediated through crustacean hyperglycaemic hormone (CHH) at the eyestalk, was one of the factors of stress-induced hyperglycaemia [12]. Moreover, dopamine was detected in the thoracic ganglia of *M. rosenbergii* at a concentration of 58.1 ± 20.7 pmol g tissue⁻¹ [21]. Therefore, we hypothesise that dopamine depression of the immune system of *M. rosenbergii* is inevitable.

In the present paper, we examined several immune parameters including the haemocyte count, phenoloxidase activity, respiratory burst, superoxide dismutase activity, phagocytic activity and clearance efficiency, and the susceptibility to *Lactococcus garvieae* of *M. rosenbergii* following an injection of dopamine.

2. Materials and methods

2.1. Culture of *L. garvieae*

A known pathogenic strain, *Lactococcus garvieae*, isolated from diseased *M. rosenbergii*, which displayed symptoms of opaque and whitish musculature, was used for the study. The pathogen was
cultured 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 it remained for 24 h at 30 °C as a stock culture for tests. The broth cultures were centrifuged at 7155×g for 15 min at 4 °C. The supernatants were removed, and the bacterial pellets were re-suspended in saline solution (0.85% NaCl) at 5.0×10^6 and 1.0×10^9 CFU ml^{-1} as stock bacterial suspensions for the susceptibility study, and for the studies of phagocytic activity and clearance efficiency in *M. rosenbergii*, respectively [22].

2.2. Experimental design

Dopamine hydrochloride (H8502, Sigma Chemical, Saint Louis, MO, USA), a neurotransmitter, was used in the study. Dopamine hydrochloride was dissolved in sterile saline (0.85% NaCl) to concentrations of 2.5×10^{-6}, 2.5×10^{-7}, and 2.5×10^{-8} M before injection [21].

About 300 prawns were harvested from a commercial farm in Pingtung, Taiwan and acclimated to room temperature (27.0±0.5°C) in the laboratory for 2 weeks before experimentation. During the acclimation period, prawns were fed twice daily with a formulated prawn diet (Shinta Feed Co., Pingtung, Taiwan). Only prawns in the intermolt stage (stage C) were used for the study. The molt stage was determined by examination of the uropoda in which partial retraction of the epidermis could be distinguished [23]. Three studies were conducted. For the study of susceptibility of prawn to *L. garvieae*, test and control groups were comprised of ten shrimp each. For the studies of haemocyte count, phenoloxidase activity, respiratory burst, and superoxide dismutase activity, test and control groups were comprised of eight prawns each. For the studies of phagocytic activity and clearance efficiency, another eight prawns were used in each test and control group. The prawns ranged from 14.5 to 18.3 g, averaging 16.2±2.1 g (mean±SD) with no significant size differences among treatments.

2.3. Effect of dopamine on the susceptibility of *M. rosenbergii* to *L. garvieae*

Dopamine hydrochloride solution (20 μl) at 2.5×10^{-6}, 2.5×10^{-7}, or 2.5×10^{-8} M was injected into the ventral sinus of the cephalothorax of individual *M. rosenbergii* to reach doses of 50.0, 5.0, and 0.5 pmol prawn^{-1}, respectively, for the initial stage. A challenge test was conducted in the second hour by injecting 20 μl of a bacterial suspension (5.0×10^6 CFU ml^{-1}) resulting in 1×10^5 CFU prawn^{-1} into the ventral sinus of the cephalothorax. Control prawns received saline (no dopamine), and then received *L. garvieae* at 1×10^5 CFU prawn^{-1}, which served as the challenged control. Prawns received dopamine at 50.0 pmol prawn^{-1}, and then received saline (20 μl), and this served as the unchallenged control (Table 1). Experimental and control prawns (ten prawns aquarium^{-1}) were kept in 60-l glass aquaria containing 40 l of freshwater. Therefore, there were five treatments. Each treatment used 30 prawns. Water was renewed daily, and the experiment lasted 6 days.

2.4. Immune parameters of *M. rosenbergii* injected with dopamine

A dopamine hydrochloride solution was injected into the ventral sinus of the cephalothorax in individuals of *M. rosenbergii* to reach 50.0, 5.0, and 0.5 pmol prawn^{-1} in the initial stage. Some prawns received saline (20 μl), which served as the saline control group. There were four treatments (saline, and 50.0, 5.0, and 0.5 pmol prawn^{-1}) with six sampling times (2, 4, 8, 16, 24, and 48 h). Eight prawns from each treatment and each time were used for the studies. In addition, another eight prawns were used as the initial group.

Before and then 2, 4, 8, 16, 24, and 48 h after the injection, haemolymph (100 μl) was withdrawn from the ventral sinus of each shrimp into a 1-ml sterile syringe (25 gauge) containing 0.9 ml of an anticoagulant solution (30 mM trisodium citrate, 0.34 M sodium chloride, and 10 mM EDTA, pH 7.55, with the
osmolality adjusted with glucose to 780 mOsm kg\(^{-1}\)). Samples were divided into three parts. A drop of the anticoagulant–haemolymph mixture was placed on a haemocytometer to measure the total haemocyte count (THC) using an inverted phase-contrast microscope (Leica DMIL, Leica Microsystems, Wetzlar, Germany). The remaining portion of the haemolymph mixture was used for subsequent tests.

Phenoloxidase activity was measured spectrophotometrically by recording the formation of dopachrome produced from L-dihydroxyphenylalanine (L-DOPA) following published procedures [24]. The diluted haemolymph was centrifuged at 700 \(\times\) g (model 5403, Eppendorf, Hamburg, Germany) at 4 \(^\circ\)C for 20 min. The supernatant was discarded, the pellet rinsed, gently re-suspended in 1 ml cacodylate-citrate buffer (0.01 M sodium cacodylate, 0.45 M sodium chloride, and 0.10 M trisodium citrate, pH 7.0), and then centrifuged again. The pellet was then re-suspended in 200 \(\mu\)l cacodylate buffer (0.01 M sodium cacodylate, 0.45 M sodium chloride, 0.01 M calcium chloride, and 0.26 M magnesium chloride, pH 7.0). Aliquots (100 \(\mu\)l) were incubated for 10 min at 25–26 \(^\circ\)C with 50 \(\mu\)l of trypsin (1 mg ml\(^{-1}\)), which served as an elicitor. Fifty \(\mu\)l of L-DOPA (3 mg ml\(^{-1}\) in cacodylate buffer) were then added, followed by 800 \(\mu\)l of cacodylate buffer 5 min later. The optical density at 490 nm was measured using a Hitachi U-2000 spectrophotometer (Tokyo, Japan). The control solution was used for the background phenoloxidase activity, and consisted of 100 \(\mu\)l of cell suspension, 50 \(\mu\)l of cacodylate buffer (to replace the trypsin), and 50 \(\mu\)l of L-DOPA. The optical density of the background phenoloxidase activity was in the range of 0.03–0.04. The optical density of the prawn’s phenoloxidase activity was expressed as dopachrome formation per 50 \(\mu\)l of haemolymph.

Haemocyte respiratory burst was quantified using the reduction of nitroblue tetrazolium (NBT) to formazan as a measure of the superoxide anion (O\(_2^\cdot\)) level as described previously [25]. Briefly, 100 \(\mu\)l of haemolymph sample in an anticoagulant solution was deposited in triplicate in microplates, previously coated with 100 \(\mu\)l of a poly-L-lysine solution (0.2%) to improve cell adhesion. Fifty microlitres of L-DOPA (3 mg ml\(^{-1}\) in cacodylate buffer) were then added, followed by 800 \(\mu\)l of cacodylate buffer 5 min later. The optical density at 630 nm was measured in triplicate using a microplate reader (Model VERSAmax, Molecular Devices, Sunnyvale, CA, USA). Respiratory burst was expressed as NBT reduction per 10 \(\mu\)l of haemolymph.

Superoxide dismutase (SOD) activity was measured by its ability to inhibit superoxide radical-dependent reactions using the Ransod Kit (Randox, Crumlin, UK). Briefly, the reaction mixture (1.7 ml) contained 0.05 mM xanthine and 0.025 mM 2-(4-iodophenyl)-3-(4-nitropheno)-5-phenyltetrazolium chloride (INT)

<table>
<thead>
<tr>
<th>Bacterial dose (CFU prawn(^{-1}))</th>
<th>Dopamine (pmol prawn(^{-1}))</th>
<th>No. of prawns</th>
<th>Cumulative mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>50.0</td>
<td>30</td>
<td>6 h 8 h 16 h 24 h 72 h</td>
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<tr>
<td>(1 \times 10^{-5})</td>
<td>30</td>
<td>30</td>
<td>0 0 0 0 0</td>
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<tr>
<td>(1 \times 10^{-5})</td>
<td>0.5</td>
<td>30</td>
<td>3.3±3.3c 43.3±3.3c 50.0±5.8b 60.0±5.8b 73.3±8.8b</td>
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<tr>
<td>(1 \times 10^{-5})</td>
<td>5.0</td>
<td>30</td>
<td>23.3±12.0b 53.3±6.7b 66.7±8.8ab 73.3±8.8b 73.3±8.8b</td>
</tr>
<tr>
<td>(1 \times 10^{-5})</td>
<td>50.0</td>
<td>30</td>
<td>50.0±5.8a 80.0±0.0a 83.3±3.3a 90.0±5.8a 90.0±5.8a</td>
</tr>
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Data in the same column with different letters significantly differ (\(p<0.05\)) among different treatments. Values are the mean±SE (\(n=30\) prawns in each case).

Table 1
Effect of dopamine administration on the mortality of *Macrobrachium rosenbergii* challenged with *Lactococcus garvieae*
dissolved in 50 mM 3-(cyclohexylamino)-1-propane sulfonic acid (CAPS) (pH 10.2) and 0.94 mM EDTA. In the presence of xanthine oxidase (80 U l\(^{-1}\), 250 µl), superoxide and uric acid were produced from xanthine. Then, the superoxide radical was reacted with INT to produce a red formazan dye. The optical density was measured at 505 nm and 37 °C, and the rate of the reaction was estimated from the absorbance readings at 0.5 and 3 min after adding xanthine oxidase. A reference standard SOD is supplied with the Ransod kit. One unit of SOD was defined as the amount required to inhibit the rate of xanthine reduction by 50%. Specific activity was expressed as SOD units ml\(^{-1}\) [26].

2.5. Phagocytic activity and clearance efficiency of *M. rosenbergii* to *L. garvieae*

*M. rosenbergii* received saline and dopamine as described above. Tests were carried out in eight replicate test groups consisting of one prawn in each treatment. For the tests of phagocytic activity and clearance efficiency, 20 µl of a bacterial suspension (1×10^9 CFU ml\(^{-1}\)) resulting in 2×10^7 CFU prawn\(^{-1}\) was injected into the ventral sinus, 1 h after it had received saline or dopamine. After the bacterial suspension injection, the shrimp were kept for 3 h in a separate tank containing 40 l of water at 27.5±0.5 °C. Then, 200 µl of haemolymph was collected from the ventral sinus and mixed with 200 µl of sterile anticoagulant. This mixture was divided into two equal sub-samples: one to measure phagocytic activity and the other to measure clearance efficiency.

Phagocytic activity was performed as previously described by [27]. Briefly, 200 µl of the diluted haemolymph sample was mixed with 0.2 ml of 0.1% paraformaldehyde for 30 min at 4 °C to fix the haemocytes. They were then centrifuged at 700×g at 4 °C, washed and resuspended in 0.4 ml of a saline solution. A 50-µl sample of the suspension was spread on a glass slide. The slide was placed in a cytospin centrifuge (Model Cytospin 3, Shandon, England) and centrifuged at 113×g for 3 min. The slide was then air-dried, stained with Diff-Quick, and observed under a light microscope. Two hundred haemocytes were counted. Phagocytic activity, defined as the phagocytic rate (PR), was calculated as follows: \[ PR = \left( \frac{\text{phagocytic haemocytes}}{\text{total haemocytes}} \right) \times 100. \]

Clearance efficiency was measured as previously described [28]. Three 50-µl portions of each diluted haemolymph sample were spread on separate TSA plates and incubated at 26 °C for 12 h. After this period, a colony counter was used to count the colonies. Clearance efficiency, defined as the percentage inhibition (PI) of *L. garvieae*, was calculated as follows: \[ PI = 100 - \left( \frac{\text{CFU in the test group}}{\text{CFU in the control group}} \right) \times 100. \]

2.6. Statistical analysis

A multiple comparison (Tukey) test was conducted to compare significant differences among treatments using the SAS computer software (SAS Institute, Cary, NC, USA). Before analysis, the percentage data (susceptibility study) were normalised using an arc sine transformation. Statistically significant differences required that \( p < 0.05 \).

3. Results

3.1. Effect of dopamine on the susceptibility of *M. rosenbergii* to *L. garvieae*

All unchallenged control shrimp survived. By contrast, death occurred after 6 and 8 h for the challenged prawns which had received dopamine and saline, respectively. After 8–72 h, the cumulative mortality of prawns receiving dopamine was significantly higher than that of saline-challenged control prawns. The
cumulative mortality of prawns directly increased with dopamine concentrations from 0.5 to 50.0 pmol prawn⁻¹. Cumulative mortality over 72 h was 10.0%, 60.0%, 73.3%, and 90.0% for prawns which received saline, or dopamine at 0.5, 5.0, or 50.0 pmol prawn⁻¹, respectively (Table 1).

3.2. Immune parameters of M. rosenbergii injected with dopamine

Although the THC of prawns which received dopamine at 0.5, 5.0, and 50.0 pmol prawn⁻¹ indicated slight increases, these values did not significantly differ from values of the saline control group due to the high inter-animal variability. After 8 h, in prawns which received dopamine at 5.0 and 50.0 pmol prawn⁻¹, the THC had significantly increased \( (p<0.05) \) by 83.7% and 81.0%, respectively. However, no significant difference in the THC was observed among the four treatments from 16 to 24 h (Fig. 1A).

The phenoloxidase activity of prawns which received dopamine at 50.0 pmol prawn⁻¹ was significantly lower than that of prawns which received saline control from 2 to 4 h. The decrease in phenoloxidase activity following dopamine treatment appeared to be dose dependent. However, no significant difference in phenoloxidase activity was observed among the four treatments from 8 to 24 h (Fig. 1B).

The respiratory burst of prawns which received dopamine at 5.0 and 50.0 pmol prawn⁻¹ were significantly lower than those of prawns which received the saline control after 2 h. Respiratory burst of shrimp which received dopamine at 50.0 pmol prawn⁻¹ were significantly lower than those of shrimp which received saline control after 4 h. The decrease in respiratory burst of prawns following dopamine treatment appeared to be dose dependent. However, no significant difference in respiratory burst was observed among the four treatments from 8 to 24 h (Fig. 2A).

Superoxide dismutase activity of prawns which received dopamine at 50.0 pmol prawn⁻¹ was significantly lower than that of prawns which received saline control from 2 to 4 h. Eight hours following dopamine treatment, the superoxide dismutase activities of prawns which received dopamine at 5.0 and 50.0 pmol prawn⁻¹ were significantly lower than that of prawns which received the saline control. However, no significant difference was observed among the four treatments from 16 to 24 h (Fig. 2B).

3.3. Phagocytic activity and clearance efficiency of L. garvieae by M. rosenbergii

Phagocytic activity was significantly lower in prawns which received dopamine at 5.0 and 50.0 pmol prawn⁻¹ than in prawns which received dopamine at 0.5 pmol prawn⁻¹; its level after 2 h was lower than that of prawns which received saline control. Phagocytic activities after 2 h were 5.0%, 5.3%, 6.8%, and 8.2% in prawns which received 50.0, 5.0, and 0.5 pmol prawn⁻¹, and the saline control, respectively. However, no significant difference in phagocytic activity was observed among the five treatments from 4 to 24 h (Fig. 3A).

Clearance efficiency was significantly lower in prawns after 2 h which had received dopamine at 50.0, 5.0, and 0.5 pmol prawn⁻¹. Clearance efficiency after 2 h decreased by 16.8%, 10.2%, and 10.5% in prawns that had received dopamine at 50.0, 5.0, and 0.5 pmol prawn⁻¹, respectively, as compared to saline control prawns. Clearance efficiency after 4 h was significantly lower in prawns which had received 50.0 pmol prawn⁻¹ dopamine than in prawns which received the saline control. However, no significant difference in clearance efficiency was observed among the four treatments between 8 and 24 h (Fig. 3B).

4. Discussion

Stress-induced neuroendocrine changes are thought to divert an organism’s energy resources away from physiological functions such as reproduction, growth, and certain immune processes to allow metabolic and
behavioural adaptations that may help the animal overcome a threat and survive [5,29]. However, under certain circumstances, redirecting internal energy to specific physiological functions may weaken an animal’s defences against a preexisting threat such as the presence of pathogenic or invading microbes. So, the ability of animals to resist a pathogen is an integrated, whole-organism response, incorporating features of numerous physiological systems.

Lacoste et al. [18] indicated that oysters *Crassostrea gigas* infected with the oyster pathogen *Vibrio splendidus* carry higher bacterial loads and experience higher mortalities following a 15-min mechanical

Fig. 1. Mean (± SE) total haemocyte count (A) and phenoloxidase activity (B) of *Macrobrachium rosenbergii* which received dopamine at 50.0, 5.0, or 0.5 pmol prawn⁻¹, or saline. Each bar represents the mean value from eight determinations with the standard error (SE). Data at the same elapsed time with different letters significantly differ (p<0.05) among treatments.
disturbance than do infected unstressed animals. They also indicated that injection of noradrenaline (NA) or an adrenocorticotropic hormone, two key components of the oyster neuroendocrine stress response system, caused higher mortality and increased accumulation of *V. splendidus* during the mechanical disturbance stress period. Lacoste et al. [19] also found that both NA and dopamine significantly increased in the haemolymph of oysters, which reveals a transient state of stress. Our previous studies showed that *M. rosenbergii* injected with the pathogen *L. garvieae* revealed increased mortality following pH and temperature stress [30]. In the present study, *M. rosenbergii* injected with dopamine at a dose of 0.5–50.0 pmol prawn\(^{-1}\) showed decreased resistance against *L. garvieae* after 8 h. These facts

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**Fig. 2.** Mean (±SE) respiratory burst (A) and superoxide dismutase activity (B) of *Macrobrachium rosenbergii* which received dopamine at 50.0, 5.0, or 0.5 pmol prawn\(^{-1}\), or saline. See Fig. 1 for statistical information.
suggest that stress-inducing dopamine and the disease outbreak are linked in both *M. rosenbergii* and *C. gigas*.

In the oyster *C. gigas*, the number of circulating haemocytes, haemocyte migration, phagocytic activity, and reactive oxygen species production significantly decreased, and the NA and dopamine concentrations significantly increased during application of a stress (15 min of shaking). Following application of the stressor, NA and dopamine concentrations gradually decreased and had returned to basal values 120 min after the beginning of the experiment. Nevertheless, all immune parameters had significantly increased above the initial value. The increase continued until the next sample at 60 and 120 min before all immune parameters began to show a notable decrease at 240 and 480 min [19]. A similar phenomenon was also
observed in the abalone Haliotis tuberculata [31]. In addition to their roles in immune defence, mollusc haemocytes are involved in nutrient transport [32]. It has been suggested that circulating haemocytes play a role in redirecting bioenergetic resources and that they leave the main haemolymph vessels to convey nutrients to certain tissues involved in adaptation and survival which would necessitate a down-regulation of immune functions [19,31].

In crustaceans, haemocytes not only play an important role in immune defence but are also involved in physiological functions including carbohydrate metabolism, transport and storage of proteins and amino acids [33,34]. An increase in the THC provides enhanced immune capability during periods of stress [35] leading to disease resistance in decapod crustaceans [36]. In the present study, after injecting dopamine, the circulating haemocytes of M. rosenbergii and its susceptibility to L. garvieae had significantly increased at 8 h. These facts suggest that stress, inducing the release of dopamine, causes mobilisation of the reserve pool of sessile haemocytes, enhanced carbohydrate metabolism and nutrient transport towards adaptive physiological functions and/or modulates immunological functions in prawns M. rosenbergii.

Both phagocytic activity and the clearance efficiency of M. rosenbergii to L. garvieae decreased in prawns submitted to stressful conditions, such as exposure to 35 and 20 °C, pH 9.3 and 4.3, or a salinity of 15 ppt [37]. The present study indicates that phagocytic activity, clearance efficiency, and superoxide anion production of haemocytes of M. rosenbergii had decreased in prawns 2 h after they had received dopamine at higher than 5.0 pmol prawn⁻¹, and returned to control values after 8 h. These data suggest that stress-induced neuroendocrine changes such as dopamine secretion may be responsible for the immunosuppressive effects of stress on prawn immune parameters. Further research is needed to study the mechanisms by which stress produces reduced immune functions.

Both phenoloxidase activity and the THC of M. rosenbergii were significantly higher at pH 7.5–7.7 and 30–31 °C, and were significantly lower at pH 4.6–5.0 and 9.0–9.5 and 33–34 °C [30]. Fifty-six hours after exposure to copper sulfate, potassium permanganate, and benzalkonium chloride, the phenoloxidase activity of M. rosenbergii had decreased, while its THC was maintained [38,39,40]. It is reported [22] that the THC and phenoloxidase activity were significantly lower in M. rosenbergii following hypoxia exposure. In the present study, phenoloxidase activity was significantly lower for prawns injected with 50 pmol dopamine prawn⁻¹ after 2 and 4 h, but then recovered to the initial value by 8 h after the injection. THC was significantly higher in prawns injected with 5 and 50 pmol dopamine prawn⁻¹ after 8 h, and then recovered to the initial value by 16 h after the injection. These facts indicate that the decreased phenoloxidase activity in M. rosenbergii exposed to hypoxia, copper sulfate, potassium permanganate, and benzalkonium chloride, and injection with dopamine is not a consequence of a reduction in the THC.

NBT staining has been used for both qualitative and quantitative analyses of O₂⁻ (superoxide anions) generated by haemocytes, which are the first product of the respiratory burst and can be scavenged by superoxide dismutase (SOD). In the present study, M. rosenbergii receiving 50.0 pmol dopamine prawn⁻¹ showed decreased release of superoxide anions from 2 to 4 h, and decreased SOD activity from 2 to 8 h. This fact indicates that the activity of NADPH oxidase which is responsible for the release of superoxide anion decreased together with a decrease in the activity of SOD which is responsible for scavenging superoxide anions.

Following transient immunosuppression, a period of immunostimulation was observed between 0.5 and 2 h after stress application in the oyster C. gigas [19], and abalone H. tuberculata [31]. This stimulation may occur in order to compensate for the earlier decrease in immune responses [19,31]. In the present study, except for THC, all immunocompetence parameters decreased in the period of 2–8 h after injection of dopamine, and then all parameters returned to normal values after 16 h. Following immunosuppression, no immunostimulation was observed between 16 and 48 h after injection of dopamine. These facts suggest that there are different immunomodulation mechanisms for stress between prawns and molluscs.

In conclusion, the present study documented that the immune ability of M. rosenbergii after injection of dopamine showed a transient immunosuppression by decreases in phenoloxidase activity, respiratory burst,
SOD activity, phagocytic activity, and clearance efficiency, and these led to a temporary susceptibility to *L. garvieae*. It may be possible to manage environmental parameters in prawn ponds to minimise stress-induced dopamine depressing the immune system and preventing disease outbreak in cultured prawns.

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