Aquaculture 313 (2011) 144–148

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Effect of selected biogenic amines on reproduction in the fresh water edible crab, *Oziotelphusa senex senex*

S.B. Sainath [a], P. Sreenivasula Reddy [a,b,*]

[a] Department of Biotechnology, S.V. University, Tirupati- 517 502, A.P., India
[b] Department of Zoology, S.V. University, Tirupati- 517 502, A.P., India

ARTICLE INFO

Article history:
Received 23 February 2010
Received in revised form 19 December 2010
Accepted 6 January 2011
Available online 15 January 2011

Keywords:
Dopamine
Serotonin
Melatonin
Ovary
Vitellogenin
Crab
Oziotelphusa senex senex

ABSTRACT

The increasing demand for high protein food from aquatic sources and the necessity to find an alternative for fisheries have given rise to a worldwide expansion of shellfish culture. The major bottleneck in crustacean aquaculture industry is the limited availability of quality seed. The present study was designed to elucidate the possible role(s) of selected biogenic amines on the regulation of reproduction in the fresh water edible crab, *Oziotelphusa senex senex*. Bilateral eyestalk ablation significantly increased ovarian index, mean oocyte diameter and ovarian vitellogenin levels. Injection of serotonin and melatonin also induced ovarian maturation in intact crabs. Injection of serotonin and melatonin into eyestalk ablated crabs did not further accelerate ovarian growth when compared with eyestalk ablated crabs. Given that serotonin and melatonin are able to stimulate ovarian growth and vitellogenin levels in intact crabs but not in eyestalk ablated crabs, it is clear that the stimulatory action of serotonin and melatonin is at the eyestalk through inhibition of release of vitellogenin inhibiting hormone from the X-organ-sinus gland complex. Injection of dopamine into intact crabs did not result in any change in reproduction. Whereas, injection of dopamine into eyestalk ablated crabs results in retarding ovarian growth when compared to eyestalk ablated crabs. From the results, we conclude that, dopamine regulated ovarian growth could be through inhibition of release of vitellogenin stimulating hormone from brain and thoracic ganglion or direct action of dopamine on ovaries but not mediated by eyestalk hormones.

1. Introduction

In recent years, there is a great potential for shellfish culture, and crustaceans are one of the nutritional aquatic food sources worldwide. The major bottleneck in crustacean aquaculture industry is the limited availability of seed. The commercial brood production through hatchery operation has successfully been carried out in shrimps, lobsters, and to certain extent in crabs through eyestalk ablation (ESX) (Huberman, 2000; Tsukimura, 2001; Wilder et al., 2002; Diwan, 2005). This operational process, though proved promising, is often associated with high mortality in the brood stock and also resulted in inferior quality of offspring produced (Benzie, 1998). To overcome the above problems, several non-surgical procedures have been attempted to induce ovarian maturation, such as injections of double-stranded RNA to knock off GIH mRNA, hormones and neurotransmitters (Sarojini et al., 1995; Vaca and Alfaro, 2000; Yano and Hoshino, 2006; Reddy et al., 2006; Wongprasert et al., 2006; Nagaraju, 2007; Treerattrakool et al., 2008). Though the results are promising, these methods are limited to a few crustaceans.

In decapods, it is well established that reproduction is primarily controlled by two antagonistic peptide hormones of different origin: a) gonad-inhibiting hormone (GIH) of the X-organ-sinus gland (XO/SG) complex, located in the eyestalks (Quackenbush, 1989) which inhibits ovarian development and b) gonad-stimulating hormone (GSH) secreted from the brain and thoracic ganglia (Otsu, 1963; Eastman-Reks and Fingerman, 1984) which stimulates the same process. Though the chemical nature, mode and site of action of GIH are well known (Huberman, 2000), such information for GSH has not been thoroughly established. Several *in vivo* and *in vitro* studies demonstrate the stimulating effects of brain and thoracic ganglia extract on ovarian maturation (see review Fingerman, 1997). Thus, it appears that, the coordination between these two hormones is crucial in the regulation of ovarian maturation. It has been reported that the synthesis and release of GIH and GSH from XO/SG complex and brain and thoracic ganglia, respectively are believed to be modulated by biogenic amines (Richardson et al., 1991; Fingerman, 1997). Biogenic amines are the conserved molecules and involved in the regulation of a wide array of physiological activities in decapods (Fingerman, 1997). They exert their effects on target tissues either functioning as

* Corresponding author. Department of Zoology, S.V. University, Tirupati- 517 502, A.P., India. Tel.: +91 9247593000; fax: +91 877 2249666.
E-mail address: reddy_1955@yahoo.co.in (P.S. Reddy).

0044-8486/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquaculture.2011.01.010

neuroregulators or neurohormones (Fingerman and Nagabhushanam, 1992; Lüschen et al., 1993; Sneddon et al., 2000).

Among the biogenic amines dopamine and serotonin were found in the central nervous system (CNS) of crustaceans (Laxmyr, 1984; Fingerman et al., 1994). It has been reported that they control and modulate the release of peptide hormones from eyestalks and brain (Chang, 1985; Kulkarni and Fingerman, 1986; Kuo and Yang, 1999) thereby regulating vital physiological processes including reproduction (Fingerman, 1997). It was demonstrated that serotonin stimulated ovarian maturation in red swamp crayfish *Procambarus clarkii* (Kulkarni et al., 1992) and dopamine inhibited serotonin-stimulated ovarian maturation (Sarojini et al., 1995). Thus, it appears that dopamine and serotonin have antagonistic actions on reproduction of the crayfish. Similar results were observed in the fresh water prawn *Macrobrachium rosenbergii* (Tinikul et al., 2008). Melatonin, another conserved indoleamine was also discovered in the CNS of crustaceans (Balzer et al., 1997; Maciel et al., 2008) and is involved in the regulation of hemolymph sugar levels, limb regeneration and molting (Tilden et al., 1997; 2001; Sainath and Reddy, 2010a). In as much as (a) melatonin, serotonin and dopamine are present in the crustaceans, (b) biogenic amines act as neurotransmitters in crustaceans and (c) crustacean reproduction is under the control of neurohormones, the present study was undertaken to examine the possibility that these amines have any role in regulating ovarian growth and maturation in fresh water edible crab, *Oziotelphusa senex senex* and if so, to determine whether these amines directly regulate reproduction or act as a neurotransmitter through the mediation of eyestalk neuropeptide hormones. The experimental model, *Oziotelphusa senex senex* selected for the present study is a fresh water edible crab abundantly available in Southern parts of India and commonly referred to "poor man's protein."

2. Materials and methods

2.1. Collection and maintenance of animals

Uninjured intact female crabs, *Oziotelphusa senex senex* were collected from the rice fields and irrigation canals around Tirupati (13° 36′ N, 79° 21′ E), Andhra Pradesh, India. Crabs were housed 6–8 per glass aquaria (length: width: height = 60: 30: 30 cm) with sufficient ambient medium (salinity: 0.5 ppt) and water was replaced daily. They were maintained under controlled laboratory conditions (temperature 27 ± 1 °C and a light period of 12 h) for at least one week. During their sojourn, the crabs were fed with sheep meat daily *ad libitum*. During the experiment, both intact and eyestalk ablated crabs were used.

Dopamine, serotonin and melatonin were used as the chemicals in the present study. Dopamine and serotonin were purchased from Sigma Chemical Co. (St Louis, MO) and melatonin was purchased from MP Biomedicals Inc., (France).

Uninjured intact and eyestalk ablated crabs were randomly divided into nine groups of 10 crabs each. Crabs in group 1 did not receive any treatment. Crabs in group 2 were injected with 10 μL of crustacean saline (Van Harreveld, 1936), through the base of the chelae with a microsyringe (Hamilton) and served as controls. Crabs in groups 3, 4 and 5 were independently injected with 10^{-6} mol. dopamine, 10^{-6} mol. serotonin, and 10^{-7} mol. melatonin, respectively in a 10 μL solution. Bilateral eyestalk ablated crabs were allocated into groups 6 to 9. In order to deprive the animal of the eyestalk hormones, both the eyestalks were extirpated by cutting off the stalks at the base, without prior ligation, but with cauterization of the wound after operation. No mortalities were observed after operation and the crabs were used for experimentation 24 h post operation. Crabs in group 6 served as ablated crabs and did not receive any treatment. Crabs in groups 7, 8 and 9 were independently injected with 10^{-6} mol. dopamine, 10^{-6} mol. serotonin and 10^{-7} mol. melatonin, respectively. The test doses were selected based on our earlier dose–response studies (Pushpalatha and Reddy, 2007; Sainath and Reddy, 2010b).

The selected test doses of dopamine, serotonin and melatonin were administered to intact and eyestalks ablated crabs on days 1, 7, 14 and 21 and were sacrificed on day 28. The ovarian index, oocyte diameter, histology of the ovary and ovarian vitellogenin (OV) levels were determined.

2.2. Determination of ovarian index

The crabs from control and experimental groups were weighed and the ovaries were excised, rinsed in crustacean saline, blotted on filter paper and weighed wet to the nearest milligram. The ovarian index was determined using the following formula:

Ovarian index

= [wet weight of the ovary (g) / weight of the crab (g)] X 100

2.3. Histological studies of the ovary and measurement of oocyte diameter

Ovaries were collected after 28 days and fixed in aqueous Bouin's solution. After 24 h of fixation the ovaries were dehydrated through an alcohol series and then embedded in paraffin (m.p. 58–60 °C). Sections were cut at 7 μm and affixed to gelatinized slides. After being deparaffinized in xylol the ovarian sections were stained with hematoxylin and counterstained in eosin (Bancroft and Stevens, 1982). The diameter of 50 oocytes from each ovary was measured using an ocular micrometer under a compound microscope (Olympus, Model-BX41TF HB, Japan). The measurements were made on the longest and shortest axes of each oocyte, both dimensions were added and the mean was taken as mean oocyte diameter.

2.4. Estimation of vitellogenin and authentication by double immunodiffusion

Ovarian vitellogenin was isolated and purified using the protocol described by Tsukimura et al. (2000) and modified by Reddy et al. (2006). The ovarian vitellogenin content in each purified sample was estimated by the method of Bradford (1976) by using bovine serum albumin as standard. The vitellogenin content was expressed as mg/g tissue. Double immunodiffusion was performed according to the method described by Ouchterlony (1958) to authenticate vitellogenin in ovary, using anti-vitellin antibodies raised in the rabbits using purified ovarian vitellogenin from the crab, *Oziotelphusa* (Reddy et al., 2006).

2.5. Statistical analysis

Statistical analyses were necessary to determine the level of significance of the effect of treatment on the experimental group of animals. Data were expressed as mean ± SD. Statistical analysis was performed using an analysis of variance (one way ANOVA) followed by Tukey's post test using SPSS (Student version 7.5, SPSS Inc, Chertsey., UK).

3. Results

3.1. Effect of dopamine, serotonin and melatonin on ovarian index, oocyte diameter and ovarian vitellogenin levels in intact and ESX crabs

The ovarian index and oocyte diameter in initial control crabs and control crabs were 0.37 ± 0.03 (WW %), 20.41 ± 2.34 μm and 0.38 ± 0.01 (WW %), and 21.13 ± 4.21 μm, respectively (Table 1). No significant changes were observed neither in the ovarian index nor in the oocyte diameter in both initial control and saline injected crabs in a 28-day experimental period.