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Effects of biogenic amines on the testicular development in mud crabs Scylla serrata

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Abstract

The regulation of three biogenic amines over the reproductive neuroendocrine activity of the male Scylla serrata was investigated by in vivo injection and in vitro incubation. The testicular index, the ratio of the mature sections in testes, and the ratio of Type B cells in androgenic gland were taken as the quantitative indexes. The in vivo injections indicated that: 5-HT can significantly promote the testicular development and the secretion of the androgenic gland in S. serrata; DA can inhibit the testicular development, but no influence on the secretion of the androgenic gland was found; no significant difference was observed between the OA-injected group and the concurrent control group. In vitro incubations showed that: 5-HT can stimulate the secretion of the brain and the thoracic ganglia, thus accelerating that of the androgenic gland; however, neither OA nor DA showed any significant influence on the secretion of the brain and the thoracic ganglionic mass. As to the optic ganglia, the three biogenic amines hardly have any effect on its secretion. It is the first time to report the regulation of biogenic amines over the reproductive neuroendocrine of male crustaceans through vitro experiments. Results corroborate that 5-HT activates the brain and the thoracic ganglia to secret GSH first, then promote the testicular development through the activity of the androgenic gland.

Key words: biogenic amines, Scylla serrata, neuroendocrine organs, androgenic gland, reproduction

1 Introduction

Biogenic amine, one of important bioactive substances in crustaceans, participates in extensive physiological activities, such as tegumentary color change, molting, muscle contraction, glucide metabolism, etc. (Fingerman et al., 1994). Furthermore, it has been reported that biogenic amines engage in the reproductive neuroendocrine activity of Procambarus clarkii and U. pugilator; in which serotonin (5-HT) can stimulate the brain and the thoracic ganglionic mass to secret the gonad stimulating hormone (GSH), thus promoting the ovarian development; dopamine can inhibit the ovarian development probably through its inhibition over the secretion of GSH and/or its stimulation over the secretion of gonad inhibiting hormone (GIH) (Fingerman, 1997). Our former study also showed that in the female S. serrata, 5-HT can stimulate the secretion of the brain and the thoracic ganglionic mass, thus accelerating the ovarian development (Ye, Li, Li, et al., 2003). However, by far, the studies on the regulation of biogenic amines over the reproduction of crustaceans have mostly focused on the females. In male crustaceans, only the injections of biogenic amines over the testicular development were reported (Sarojini et al., 1994, 1995). Much needs to be known about how biogenic amines affect the testicular development.

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Scylla serrata, which has great aquacultural potential, is one of commercially important crabs in China. We have found out that in the male S. serrata, the ratio of the mature sections in the testes can reflect the testicular development, while the ratio of Type B cells in the androgenic glandular cells can reflect the secretion of the androgenic gland (Ye et al., 2002, 2003a). On the basis of these results, the present paper studies the neuroendocrine regulation of 5-HT, octopamine and dopamine over the testicular development of S. serrata. The result will be of importance not only to the understanding of the reproductive neuroendocrine mechanism, but also to the exploring of reproductive regulation technologies with biogenic amines.

2 Materials and methods

2.1 Major reagents

5-hydroxytryptamine (5-HT) and octopamine (OA) were purchased from the Sigma Company, and dopamine (DA) from the Fluka Company. M199 was purchased from the Gibco Company, and bovine serum from the Hangzhou Sijiqing Biological Engineering Limited Company. 5-HT, OA and DA solutions are made in 0.01 mol/dm^3 phosphate buffer saline (PBS), containing 0.9% NaCl with pH 7.4.

2.2 In vivo injection

2.2.1 Maintaining mode

Scylla serrata, in the same developmental stage, with carapace length of 64~65 mm and body mass of 182~200 g, were purchased from local vendors in Xiamen. They were then maintained in cement pools (1 m×1 m×1 m) with a density of 2~3 ind./m^2 and fed daily with fresh clams, Ruditapes philippinensis. Shelters for the crabs were set in the pools. The containing seawater was of 10 cm depth, with the salinity of 24~26, at the temperature of 22.4~23.7 ℃. Well aeration was obtained with aerators. The containing water was changed periodically in order to deplete the pollutants.

2.2.2 Grouping

Five experimental groups were set with five crabs in each group. Crabs in the initial control group (Group IC) were dissected on the first day of the experiment. Crabs in the concurrent control group (Group PBS) was injected with 100 μL PBS from the base of the fifth pereiopod, on the first day, the fifth day and the tenth day of the experiment respectively, then dissected on the fifteenth day. Group 5-HT, Group OA and Group DA were injected with 100 μL 5-HT, OA and DA respectively, with the dose of 1 μg/g body mass; and they have no other difference between them and Group PBS. The experiment has been repeated once.

2.3 In vitro incubation

2.3.1 Experimental conditions

Scylla serrata, in the same developmental stage, with carapace length of 72~73 mm and body mass of 220~228 g, were purchased from the same aquaculture field. Their testes were all in the spermatocyte stage. They were then maintained for 4~6 d in aquatic tanks containing seawater with well aeration and fed with fresh clams, R. philippinensis. Before dissection, crabs were rinsed in 1% $KMnO_4$ for 0.5 h, and then they were superficially disinfected with 75% alcohol under sterile conditions. The brain, the thoracic ganglionic mass, the optic ganglia, the androgenic gland (AG) and the testes were quickly dissected out and washed in PBS containing 100 IU/cm^3 (1 IU=0.60 μg) penicillium for several times. The testes for incubation were cut into small pieces of about 1 mm^3 volume.

The samples for incubation were inoculated in vials, with 2 mL M199 cellular incubation medium, containing 20% Bovine Serum, 100 IU/cm^3 penicillium, 100 mg/dm^3 streptomycin and 50 mg/dm^3 kanamycin. They were then incubated in the oscillator at 26~27 ℃ in darkness for 24 h, with the oscillation frequency of 50~60 r/min. Three concurrent groups were set in the

experiment.

2.3.2 Organic incubation mode

The testicular pieces with PBS in each vial were incubated with 5-HT, OA and DA, respectively, with the dose of 1 μg/g body mass, in order to investigate whether these biogenic amines have direct influence on the testicular development.

Five millimetres long AG sections with PBS in each vial were incubated with 5-HT, OA and DA respectively, with the dose of 1 μg/g body mass, in order to investigate whether these biogenic amines have direct influence on the androgenic gland.

Five millimetres long AG sections with PBS in each vial were incubated with one brain, one thoracic ganglionic mass and a pair of optic ganglia from one crab, respectively. Then each vial was added with 5-HT, OA and DA respectively, with the dose of 1 μg/g body mass, in order to investigate the regulation of these biogenic amines over the secretion of AG through the neuroendocrine organs.

2.4 Observation and statistics

After 24 h incubation, the samples of the AG and the testes were fixed in Bouin's solution and then embedded in paraffin. Sections of 6~8 μm thickness were prepared and stained with Ehrlich's hematoxylin and eosin and finally observed with Olympus BH-2 light microscope.

The ratio of mature sections in testes: one hundred transverse sections of one testes sample were examined sporadically with the light microscope to calculate the ratio of transverse sections containing mature sperms, which was taken as one statistical datum. Twenty such data were obtained from each group, and they were eventually expressed as their mean values ($\bar{X}\pm SD$) (Ye et al., 2002).

The ratio of Type B cells in the AG: one hundred glandular cells from one section of AG were examined sporadically with the light microscope to calculate the ratio of Type B cells, which was taken as one statistical datum. Twenty such data were obtained from each group, and they were eventually expressed as their mean values ($\bar{X}\pm SD$) (Ye et al., 2003a).

The difference was analyzed by the student-test.

3 Result

3.1 In vivo injection experiments

The results are indicated in Table 1. Compared with Group IC, the testicular index and the ratio of matured sections in Group PBS both augment very significantly ($P<0.01$). Compared with Group PBS, these two indexes in Group 5-HT also augment very significantly ($P<0.01$). No significant difference was observed between these two indexes of Group OA and Group PBS ($P>0.05$). As to Group DA, the testicular index is a little less than that of Group PBS ($P>0.05$), but the ratio of matured sections is significantly less than that of Group PBS ($P<0.05$).

Compared with Group PBS, the ratio of Type B cells in Group 5-HT increases very significantly ($P<0.01$), while this index of Group IC, Group OA and Group DA does not show any significant difference ($P>0.05$).

3.2 In vitro injection

3.2.1 Influence of biogenic amine over testis and AG

As enlisted in Table 2, neither the ratio of

Table 1. Status of testes and androgenic glands of *Scylla serrata* after injections of biogenic amines

	Group IC	Group PBS	Group 5-HT	Group OA	Group DA
Testicular index	0.12±0.02	0.15±0.02[2)]	0.24±0.05[2)]	0.15±0.03	0.14±0.04
Ratio of matured sections in testes	0.05±0.04	0.21±0.07[2)]	0.35±0.06[2)]	0.18±0.06	0.12±0.05[1)]
Ratio of Type B cells in AG	0.21±0.09	0.31±0.08	0.44±0.12[2)]	0.31±0.10	0.30±0.11

1) means $P<0.05$, significant. 2) means $P<0.01$, very significant.

matured sections in testes, nor the ratio of Type B cells in the AG shows significant difference between the four experimental groups ($P > 0.05$), which indicates that 5-HT, OA and DA have no direct promotion over the testicular development and the secretion of AG.

3.2.2 Regulation of amines over the neuroendocrine organs

After the brain or the thoracic ganglionic mass was co-incubated with the AG, the ratio of Type B cells in Group 5-HT is significantly larger than that in Group PBS ($P<0.05$); while no significant difference is observed in other three groups ($P > 0.05$). This result indicates that 5-HT might stimulate the secretion of AG cells through the brain and the thoracic ganglionic mass. After the optic ganglia were co-incubated with the AG, no significant difference was observed between the ratio of Type B cells of the AG in these four experimental groups ($P > 0.05$), as enlisted in Table 3.

4 Discussion

The maturation extent of the testis in crustaceans is usually depicted with different developmental stages. However, the lack of specific quantitative index to indicate the particular maturation extent during the same developmental stage has retarded the progress on the reproductive endocrinology of male crustaceans. On the basis of partition of the developmental stages of the testes in *S. serrata*, the diameter of the seminiferous tubules and the ratio of matured sections can be combined to reflect the developmental stage of the testes in *S. serrata* rather properly (Ye et al., 2002): the ratio of matured section in testes can be token of the testicular development to compare the matured extent during the same developmental stage. The androgenic gland is a specific endocrine organ of male crustaceans, and it can regulate the testicular development, stimulate and maintain the second sexual characteristic (Wu et al., 1999). The androgenic gland performs holocrine, and after secretion, the androgenic glandular cells exist as Type B cells. Thus, the ratio of Type B cells can be a quantitative index to indicate the secretion of androgenic gland (Ye et al., 2003a, b).

In Table 1, the difference between Group PBS and Group IC can reflect the in vivo development process of the testes of *S. serrata*, and meanwhile it indicates that the maintaining condition is suitable for in vivo injection. Compared with Group PBS, the testicular index, the ratio of matured sections and the ratio of Type B cells in Group 5-HT all increase very significantly (see Table 1). These show that 5-HT has promoted the secretion of AG and the testicular devel-

Table 2. In vitro effects of biogenic amines on testis and androgenic glands of *S. serrata*

	Group PBS	Group 5-HT	Group OA	Group DA
Ratio of matured sections in testes	0.23±0.07	0.23±0.07	0.22±0.06	0.21±0.05
Ratio of Type B cells in AG	0.35±0.13	0.39±0.12	0.40±0.14	0.38±0.16

Table 3. Ratio of Type B cells affected by biogenic amines during co-incubation of nervous organs with androgenic glands of *S. serrata*

	Group PBS	Group 5-HT	Group OA	Group DA
Brain plus AG	0.53±0.16	0.69±0.19[1)]	0.59±0.12	0.60±0.16
Thoracic ganglionic mass plus AG	0.60±0.09	0.70±0.15[1)]	0.62±0.09	0.61±0.11
Optic ganglia plus AG	0.42±0.14	0.47±0.15	0.45±0.12	0.47±0.19

1) means significantly different from Group PBS.

opment at the individual level of the animals. As we can learn from Table 2, 5-HT has no direct influence on the testes and the androgenic gland, and in other words, the testes and the androgenic gland are not the target organ of 5-HT. We have found out that the secretion of the brain and the thoracic ganglionic mass in S. serrata can stimulate the androgenic glandular cells to secrete[1)]. In Table 3, the ratio of Type B cells in Group 5-HT increases very significantly, which indicates that through the regulation over the secretion of the brain and the thoracic ganglionic mass, 5-HT can indirectly stimulate the secretion of androgenic gland. 5-HT neuroendocrine cells have been detected from the brain, the thoracic ganglionic mass and optic ganglia of S. serrata, which provides morphological proofs for the involvement of 5-HT in reproductive endocrinology (Huang et al., 2003, 2005). Sarojini et al. (1994) once put forward the regulation axis of 5-HT over the testicular development as "5-HT-GSH-AGH-testes". The present study approves this supposition, and validates for the first time that in vitro, 5-HT can regulate the testicular development through the secretion of the brain and the thoracic ganglionic mass of S. serrata.

In the male U. pugilator, the testicular development was inhibited after injection of DA, and the inhibition was dependent on the injection dose. The mechanism of DA is considered as restraining the secretion of GSH, or stimulating the secretion of GIH, or both (Sarojini et al., 1995). In the present study, no obvious regulation of DA over the neuroendocrine organgs was observed. In the in vivo injection experiments, the testicular index in Group DA has no significant difference with that of Group PBS, but the ratio of matured sections is significantly less than that of Group PBS. These indicate that DA has inhibition over the testes at the individual level of the animals. However, the ratio of Type B cells in Group DA has no significant difference with that in Group PBS, which implies that the inhibition of DA over the testes might not root in the androgenic gland. In in vitro studies, neither the ratio of matured sections in testes, nor the ratio of Type B cells in Group DA has significant difference with that in Group PBS. This further indicates that at the organic level, Group DA has no direct regulation over the androgenic gland and the testes. Group DA might hasten the testicular maturation indirectly through other endocrine organs, such as the mandibular organ and the Y-organ, which is different from that in U. pugilator.

In the present in vivo and in vitro experiments, none of the testicular index, the ratio of matured sections and the ratio of Type B cells in Group OA has significant difference with that of Group PBS. This indicates that Group OA has no regulation over the testicular development at either the individual level of the animals or the organic level. Few studies have focused on the influence of Group OA on the development of sexual gonads in crustaceans. Kulkarni et al. (1992) has reported no influence of Group OA on the ovary maturation in P. clarkii, and Ye et al. (2003) also found no significant regulation of Group OA over the reproductive neuroendocrinology in the female S. serrata.

In our in vitro study, no inhibition of the optic ganglia over the androgenic gland was observed in S. serrata, which might be related to the weak activities of GIH in the optic ganglia during the spermatocyte stage. None of the three biogenic amines has distinct regulation over the optic ganglia, which is similar to the results in the female S. serrata (Ye et al., 2003). By far, researches on the regulation of biogenic amines over the reproduction of crustaceans, have basically been done at the individual level of the animals and the organic level, and not yet at the cellular level. Many experiments have shown that, the signal conduction of neurons and the secretion of neuro-hormones are based on the electrical activities formed by the

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flow of ions on the cellular membrane (Meyers et al., 1992; Sun et al., 2001). It is a pity that the influence of biogenic amines on the ionic channel of neurosecretory cells and the release of neuro-hormones has not been reported, since such studies will be helpful to further understanding the regulative mechanism of biogenic amines over the reproductive neuroendocrine activities of crustaceans at the cellular level.

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