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Immunological and biochemical responses in *Mya arenaria* (Mollusca Bivalvia) exposed *in vivo* to estradiol-17β

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Abstract

Soft-shell clams *Mya arenaria* were injected with 10, 20 or 40 nmol of estradiol 17β (E2). We observed a significant inhibiting effect of E2 on phagocytic activity of hemocytes from clams exposed to 10 and 20 nmol. A dose–response increase of the glycogen phosphorylase in the gonad tended to show a remobilisation of glycogen reserves involved in vitellogenesis although the exposure time must have been too short to observe a decrease in glycogen reserves or an increase in RNA concentration. Both results corroborate those of other studies about estrogen involvement in controlling immune capacity and energy metabolism related to vitellogenesis in bivalves. We can assume that immune parameters should now be taken into consideration in assessing endocrine disruption in bivalves. Nevertheless further studies are needed to understand the controlling pathways of E2 with a special regard on its interactions with other effectors involved in bivalve immunity and reproduction as well.

Keywords: Bivalves; *Mya arenaria*; Estradiol-17β; Phagocytosis; Energy metabolism; Glycogen

1. Introduction

Determining effects of endocrine disruptors in wildlife is one of the greatest challenges of ecotoxicology since in the last decade, studies were scarce on steroid and endocrine disruptor effects on bivalve physiology. Nevertheless, steroid concentrations were reported in gonad, whole tissue or hemolymph in *Mytilus edulis* (Reis-Henriques and Coimbra, 1990; Reis-Henriques et al., 1990), *Crassostrea gigas*, *Patinopecten yessoensis* (Matsumoto et al., 1997), *Ruditapes decussata* (Morcillo and Porte, 2000) and *Mya arenaria* (Siah et al., 2002; Gauthier-Clerc et al., 2006). Occurrence of sex steroids in bivalve tissues must therefore have a biological significance since estrogen-binding sites have been characterized in the gonad from *Elliptio complanata* (Gagné et al., 2001), in hemocytes from *Mytilus galloprovincialis* (Canesi et al., 2004a) and in pedal ganglia from *M. edulis* (Stefano et al., 2003). Viarengo et al. (2000) were the first to report an effect of E2 on immune cells from *M. galloprovincialis* involving a lysosomal membrane destabilization but more recently Canesi et al. (2004a,b) described in the same species the involvement of E2 on haemocyte signaling associated with morphological and functional effects. On the other hand, numerous papers reported effects of estrogens on sexual stimulation as evidenced by the enhanced growth of ovary and glycogenolysis in *M. edulis* (Mori, 1980), the increase of oocyte diameter as well as the vitellin content and the synthetic activity of the gonad in *C. gigas* (Li et al., 1998) or the level of a vitellin-like protein in the hemolymph of *M. arenaria* (Blaise et al., 1999) and *E. complanata* (Gagné et al., 2001). Sexual maturation requires important mobilisation of energy resources, especially through lipid and glycogen catabolism in bivalve (Bayne, 1976; Gabbott, 1975) to support, in part, nucleic acid synthesis and vitellogenesis (Robbins et al., 1990; Li et al., 2000). Because glycogen provides energy in the gonad during gametogenesis in *M. arenaria* (Gauthier-Clerc et al., 2002), its metabolism might be induced by E2 to promote synthesis of vitellin-like proteins.

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Studies about estradiol effect on bivalve vitellogenesis mostly investigate the vitellin-like or vitellogenin synthesis. They do not report effects on the energy mobilisation involved during the observed inductions. In this study, we challenged the hypothesis that estradiol-17β modulates the energy demand to support the induction of vitellogenesis in the gonad of *M. arenaria* since we do neither have any specific marker to assess the vitellin content nor a cDNA sequence to develop an expression analysis of vitellins. Clams were injected *in vivo* with different concentrations of E2 for a period of 48 h according to Blaise et al. (1999) who reported an increase of vitellin-like protein concentrations in the haemolymph. To assess glycogen mobilisation induced to support an energy demand in maturing gonad we determined glycogen content and glycogen phosphorylase activity. We also investigated the synthetic activity in the gonad to assess an estrogenic effect on gametogenesis and protein synthesis according respectively total DNA and total RNA concentrations. To interpret sex-related biochemical results, gender was determined in clams to eventually describe a potential variation in response to E2 exposure. On the other hand, since Blaise et al. (1999) observed this inductive effect of estradiol on vitellogenesis in the haemolymph we intent to investigate the estradiol effect on immune function of clams. Then we chose to assess the phagocytic capacity of immune cells since it is the main cellular defense of immune function in bivalves. No study about the estradiol effect on immune function of bivalve investigated this immune function before.

2. Materials and method

2.1. Clam collection and acclimation

Clams (*M. arenaria*, Mollusca: Bivalvia) were collected at Metis Beach (48°40′N, 68°00′W) on the south coast of the St. Lawrence Estuary (Quebec, Canada). This remote location is exempt from any direct sewage outputs or harbor activities and its contamination by persistent contaminants can be considered as baseline (Lebeuf et al., 1995). Clams ($n=80$), with a size range between 6.5 and 7.5 cm were collected during low tide and maintained in four aquaria filled with sediment collected at Metis Beach (sieved with a 0.9 mm mesh). Aquaria were supplied with filtered sea water from the St. Lawrence estuary at a flow rate of 1 to 1.25 L min^{-1} while salinity and temperature were maintained at 28 to 30 ppm and 7 to 9.5 °C, respectively. Clams were acclimated for a week, before the beginning of exposure treatments, under a 14 h light and 10 h dark period.

2.2. Exposure to estradiol 17β

This experience was conducted in September when clams have replenished their energy reserves heading to a new sexual maturation cycle and E2 concentrations are not yet increasing in female gonad (Gauthier-Clerc et al., 2002, 2006). After the acclimation period, clams were injected with E2 (25 μL) in the posterior adductor muscle. Three doses were selected: 10 nmol, 20 nmol and 40 nmol E2 (Sigma E8875) dissolved in 100% ethanol and 20 clams were exposed to each dose. Control clams ($N=20$) were injected with the same volume of solvent. Clams were placed in sediment for 48 h in an aquarium receiving seawater as described previously during the acclimation period and they were unfed. However, since seawater is filtered through coarse sand at the experimental laboratory, phytoplankton was available for bivalves during exposure periods. The experiment was repeated once more 10 days after the first trial. Table 1 shows that the duration as well as the dose we choose for this *in vivo* exposure is comparable other experiment realized with bivalves.

Table 1
In vivo injections of estradiol-17β with bivalves reported in the literature

Author	Organism	Injected organ	Injection frequency	Exposure duration	E2 concentration of injected solution	Volume injected	Number of mole per injection
Chronic exposure							
Mori et al., 1969; Mori, 1969	*Crassostrea gigas*	Gonad	3 times	2.5 months	3.6 mM	100 μL	360 nmol
Mori et al. (1972)	*Crassostrea gigas*	Gonad	Up to 8 times	2.5 months	3.6 mM	100 μL	360 nmol
Wang and Croll (2004)	*Placopecten magellanicus*	Muscle	1/month	3 months	3.6 mM	30 μL	108 nmol
Osada and Nomura (1990)	*Patinopecten yessoensis*	Gonad	Each 2 days	44 days Sampling 2 days after the last injection	1.8 mM	200 μL	360 nmol
Osada et al. (2003)	*Patinopecten yessoensis*	Gonad	1 injection/10 days	30, 60 and 90 days	1.8 mM	100 μL	183 nmol
Short term exposure							
Osada and Nomura (1989)	*Patinopecten yessoensis*	Gonad	Only 1	Up to 22 h	1.8 mM	400 μL	730 nmol
Blaise et al. (1999)	*Mya arenaria*	Muscle	Only 1	48 h	0.1, 0.5, 1, 10 and 1000 mM	25 μL	2.5, 12.5, 25, 250 or 2500 nmol
Our study	*Mya arenaria*	Muscle	Only 1	48 h	0.4, 0.8 and 1.6 mM	25 μL	10, 20 or 40 nmol