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Effect of $CdCl_2$ on Regulatory Volume Decrease (RVD) in *Mytilus galloprovincialis* digestive cells

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ABSTRACT

This study investigated the role of cadmium, a widespread heavy metal in the aquatic environment, on cell volume regulation of digestive cells isolated from the digestive gland of *Mytilus galloprovincialis*. These cells when exposed to a rapid change (from 1100 to 800 mOsm/kg) of the bathing solution osmolality swelled but thereafter underwent a Regulatory Volume Decrease (RVD), tending to recover the original size. This homeostatic response is altered by cadmium, as suggested by experiments performed both on isolated cells pre-incubated with cadmium (10^{-5} M) and on cells isolated from animals exposed to sub-lethal concentrations of the metal (40 μg/l for 21 days). It is suggested that cytoskeleton and Na^+/K^+ATPase are the possible targets of cadmium which impairment is responsible of the altered homeostatic response.

1. Introduction

Mussels of genus *Mytilus* are among the most widespread marine molluscs. They are sessil and filter feeding organisms filtering large amount of water to obtain food. They dwell coastal waters characterized by a wide array of salinities and temperatures (Gosling, 1992). For these reasons they are suitable organisms for biomonitoring coastal water quality. Their ability to develop consistent responses make these organisms excellent sentinel organisms employed in a large number of bio-monitoring programs (Goldberg and Bertine, 2000; Viarengo et al., 2007).

Heavy metals are among the major contaminants in the aquatic environment. Many studies have been performed using *Mytilus* spp. to assess cellular responses to these contaminants (Canesi et al., 1998, 2000, 2001; Dailianis and Kaloyianni, 2004). In the present paper the effect of cadmium on cell volume regulation of isolated digestive cells of hepatopancreas of *Mytilus galloprovincialis* is investigated.

Cell volume regulation is essential for many physiological functions such as metabolism, constancy of cell pH and membrane transport (Wehner et al., 2003). Cell can undergo anisotonic volume changes due to variations of extracellular osmolarity or isosmotic volume changes due to variations of intracellular solute content (Hoffmann et al., 2009; Strange, 2004). Most cells are equipped with mechanisms able to avoid excessive volume changes thus maintaining a proper function. If the osmotic stress produces a swelling the cells exhibit a homeostatic response called Regulatory Volume Decrease (RVD) by activating the efflux of inorganic or organic osmolytes from the cell. If the osmotic stress produces a shrinkage, the cells undergo a Regulatory Volume Increase (RVI) due to the uptake of extracellular solutes followed by water intake (Hoffmann and Dunham, 1995; Lang et al., 1998). One of our recent paper (Torre et al., 2012) showed that the digestive cells of the digestive gland of *M. galloprovincialis* exhibit a partial RVD. When exposed to a sudden decrease of extracellular osmolality (from 1100 to 800 mOsm/kg) they rapidly swelled but thereafter showed a small but significant decrease of cell size.

It is known that the digestive cells are the most abundant cells of bivalve digestive gland. They are involved, besides intracellular digestion, in detoxification, xenobiotic biotransformation and antioxidant defence (Gosling, 2003). Consequently they are possible targets of contaminants included heavy metals. The uptake of metals is mainly achieved via the digestive tract by endocitosis; thereafter they are transferred to lysosomes and then to residual bodies, especially in the digestive cells (Marigomez et al., 2002).

The aim of this paper is to investigate the effect of cadmium in a vital cell function such as the regulation of volume after a hypotonic stress. A lack of RVD leading to a dilution of cellular content could have detrimental effects by altering the activity of some or all enzymes (Lang and Waldegger, 1997) thus impairing the proper functions of the digestive cells.

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Table 1
Ionic composition of the solutions (concentration in mM).

	CMFI (1)	Isotonic control (2)	Hypotonic (3)	Hypotonic Ca^{2+}-free (4)
NaCl	600	550	350	350
KCl	12.5	12.5	12.5	12.5
$MgSO_4$	–	8	8	8
$CaCl_2$	–	4	4	–
$MgCl_2$	–	40	40	40
Glucose		10	10	10
Hepes	20	20	20	20
EDTA	–	–	–	0.1

CMFI: isotonic solution without added Ca^{2+} and Mg^{2+}; 1N NaOH was added to the solutions to obtain pH = 7.3; π = 1.100 ± 10 mOsm/kg (sols. 1 and 2), π = 800 ± 10 mOsm/kg (sols. 3 and 4).

2. Materials and methods

2.1. Animal collection

Mussels were collected in Faro Lake (Messina). It is a small meromictic marine lake (~26 ha), is a circular basin with a 500 m diameter, and is deeper in its central part (~30 m), whereas its mean depth ranges from 0.5 to 5 m. The lake is characterized by sandy-muddy bottoms seasonally covered by green algal mats, although primary production here is mainly sustained by phytoplankton (Manganaro et al., 2009). Together with Ganzirri, Faro Lake was declared of a ethno-anthropological interest particularly important as the historical seat of traditional manufacturing activities related to shellfish breeding. In fact, Faro is for the most part exploited for bivalve cultivation mainly mussels (*M. galloprovincialis* Lamarck, 1819).

2.2. Animal maintenance

In the laboratory, mussels *M. galloprovincialis* were kept in 30 L aquaria filled with continuously aerated seawater (t = 18 °C ± 1, 1100 mOsm/kg) for acclimatization for 1 week before the onset of any experimental procedure. Water was changed every 2 days to assure natural sea food and avoid starvation. In the Cd^{2+} exposure experiments one group of mussels was exposed to 40 μg/l cadmium as $CdCl_2$. When water was changed cadmium concentration was re-established. No mortality was registered during the exposure.

2.3. Isolation of cells

The digestive gland cells were isolated following the method described in Dailianis and Kaloyianni (2004). Digestive glands were cut into pieces and washed with a solution Ca^{2+} and Mg^{2+} free (sol. 1, Table 1). Tissue samples were cut into small pieces and transferred to a test-tube containing 0.01% collagenase (type IV-activity ⩾125 CDU/mg; CDU = collagenase digestion units, Sigma Aldrich, St. Louis, MO, USA) dissolved in sol. 1 (Table 1). The test-tube was gently stirred for 60 min at 18 °C in a thermostatic bath. Afterwards the suspension was filtered through 200 μm and 75 μm nylon filters. The cells were suspended in physiological saline (sol. 2, Table 1) and washed twice by centrifugation (500 rpm/10 min/4 °C) and then re-suspended in sol. 2. Before the experiments the cells were maintained in physiological saline (sol. 2, Table 1) at 18 °C for 1 h to re-establish ionic concentration on either side of cell membrane. Afterward cell viability was tested using the Trypan blue exclusion method. The viability of the cells was tested at the end of each experimental condition too.

2.4. Experimental conditions

The isolated cells were visualized and measured by the method described in a previous paper (Torre et al., 2012). One drop of cell suspension was placed on a glass slide pre-treated with poly-lysine to facilitate cell adhesion. Two thin strips of double-sided adhesive were placed at the upper and lower edges of the glass slide to support the cover slip and to create an inter-space in which the experimental solutions were added. They were placed at one side of the cover slip with a pipette and were absorbed at the opposite side with strips of filter papers. This allowed a rapid change (a few second) of the solution in the inter-space. Cells were observed with a light microscope (Leitz-Diaplan); the videometric measurements were performed on the digestive cells, the most abundant type in all preparations. Cell images were digitized using a color video camera (Sony) which was connected to a PC. Individual cells were selected, images were taken at various time intervals, as it will be described below, and recorded on PC.

2.5. Experiments in cells isolated from both control and cadmium exposed animals

In RVD experiments performed in cells isolated from both control and cadmium exposed animals the images were taken at 0 and 5 min in isotonic solution, afterwards the solution was rapidly changed with a hypotonic solution and the images were taken every 1 min for the first 10 min after the change of the solution and then every 5 min for 20 min.

In isotonic control tests of cells isolated from cadmium exposed animals the same procedure was performed with the difference that isotonic solution was changed with an identical isotonic solution.

2.6. Experiments in cells exposed to cadmium in vitro

In one set of experiments cells isolated from control animals were pre-incubated with 10^{-5} M $CdCl_2$ for 30, 75 and 120 min. Preliminary the viability of the cells after the incubation period was tested by the Trypan blue exclusion methods. Both RVD experiments and isotonic test were performed as described above.

In another set of experiments cells isolated from control animals were used but 10^{-5} M $CdCl_2$ was added to the solution used for the rapid changes described above, isotonic for isotonic control tests, hypotonic for RVD experiments.

2.7. Experiments with inhibitors of possible targets of cadmium

In RVD experiments in which inhibitors were used, the test consisted of the following periods: isotonic (5 min), isotonic + inhibitor (10 min), hypotonic + inhibitor (30 min).

The drugs were prepared as concentrated stock and dissolved in the experimental solution (pH 7.3) to obtain the final concentration. Ouabain and verapamil were dissolved in sol. 2. Cytochalasin D was dissolved in ethanol. The final concentration of the solvent in the experimental solutions was 0.1%, it did not alter neither cell