Comparative Biochemistry and Physiology, Part A 165 (2013) 199–206

Contents lists available at SciVerse ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Heavy metals affect regulatory volume decrease (RVD) in nematocytes isolated from the jellyfish *Pelagia noctiluca*

R. Morabito [a], A. Marino [b], G. La Spada [b,*]

[a] Dept. of Human and Social Sciences, University of Messina, Italy
[b] Dept. of Biological and Environmental Sciences, University of Messina, Italy

ARTICLE INFO

Article history:
Received 6 December 2012
Received in revised form 26 February 2013
Accepted 3 March 2013
Available online 14 March 2013

Keywords:
Heavy metals
Cell volume regulation
Nematocytes
Pelagia noctiluca
Biomarkers

ABSTRACT

The environmental contamination caused by heavy metals raises the question of their effect on biological systems. Among bio-indicators useful to monitor the toxicological effects of these chemicals, Cnidarians offer a unique model. Cnidarians possess highly specialized stinging cells, termed nematocytes, which respond to hyposmotic solution with well established homeostatic parameters as an acute osmotic phase (OP), leading to cell swelling, and then a slower regulatory volume decrease (RVD) phase, causing cell shrinkage. Here we report the effect of 65% artificial sea water (ASW) containing heavy metals, such as Cd, La, Co, Cu and Zn (concentrations comprised between 100 and 0.1 μM) on both OP and RVD in nematocytes isolated from the jellyfish *Pelagia noctiluca* by 605 mM NaSCN plus 0.01 mM Ca^{2+}. The exposure of the cells to Co and La inhibited RVD but not OP. However, Cu, Cd and Zn prevented the OP in a dose-dependent manner and, hence, also the detection of RVD. These results suggest that, in isolated nematocytes, heavy metal pollutants impair RVD either directly or indirectly through interference with the OP, thus negating RVD. Although further studies need to clarify the exact mechanisms whereby heavy metals exert their toxicity, it is evident that nematocytes of Cnidarians could serve as a model for ecotoxicological investigations.

1. Introduction

Metal pollution impacts our ecological environment, provoking serious alterations to animals such as morphological abnormalities, neurophysiological dysfunction, alteration of cells associated with teratogenesis and carcinogenesis (Rainbow, 1995; Prozialeck et al., 2003; Waalkes, 2003). Research has focused on monitoring metal concentrations and toxic effects in marine environments and organisms (Roberts, 2012). Different organisms such as fish, nematodes, oyster and sea urchins have been often used as bioindicators (Schröder et al., 2005; Monserrat et al., 2007; Xu et al., 2011 and references therein), while, among invertebrates, Cnidaria have not been explored yet. Cnidaria may be exposed to pollutants by either uptake, or ingestion or contact with chemicals contained in marine waters (Tarrant, 2007; Downs et al., 2012), constituting an important model to monitor environmental quality. Interestingly, the closely related hydras were used in toxicity testing of pharmaceuticals and found sensitive to metals, including Cd, Zn, butyltin and toxaphene (Holdway et al., 2001; Harter and Matthews, 2005; Tarrant, 2007; Woo et al., 2012).

The phylum Cnidaria represents the first stage of metazoan evolution and comprises different classes, such as Anthozoa, Scyphozoa, Hydrozoa and Cubozoa, all are abundant worldwide. Cnidarians possess highly specialized stinging cells, referred to as nematocytes, producing an organoid, the nematocyst, with a three-layered capsule wall, occupying most of the cytoplasm, which is thus confined to a thin rim (Fig. 1). The nematocyst includes a tubule and venom, delivered under appropriate physico-chemical stimuli (Salleo, 1984). Nematocytes, that can be isolated from tentacles and acontia (mesenterial filaments) of jellyfish and sea anemones (Salleo et al., 1996; La Spada et al., 2001), exhibit cell volume regulation capability (La Spada et al., 1999; Marino and La Spada, 2004).

Volume regulation under anisosmotic conditions is essential to cell survival and counteracts changes in external medium osmolarity (Dubois and Rouzaire-Dubois, 2012). When exposed to a hyposmotic medium, cells undergo osmotic swelling, known as osmotic phase (OP). Depending on the species and cell types, the cell volume returns either immediately or slowly toward control values, due to regulatory volume decrease (RVD). This homeostatic response has been mostly investigated in different mammalian cells (Aschner, 2011; Chen and Duan, 2011; Wormser et al., 2011), but also in cells of lower vertebrates (Chara et al., 2011) and in some invertebrates (Neufeld and Wright, 1996; La Spada et al., 1999; Peña-Rasgado et al., 2001; Amado et al., 2011). Recovery of the normal cell volume after

Abbreviations: ASW, artificial sea water; Cd, cadmium; Co, cobaltum; Cu, copper; ENaC, epithelial Na^+ channels; La, lanthanum; NaSCN, sodium thiocyanate; OP, osmotic phase; RVD, regulatory volume decrease; RVI, regulatory volume increase; ROS, reactive oxygen species; Zn, zinc.

* Corresponding author at: Dept. of Biological and Environmental Sciences, Viale F. Stagno D'Alcontres 31, 98166 Messina, Italy. Tel.: +39 090 6765209; fax: +39 090394030.
E-mail address: glaspada@unime.it (G. La Spada).

1095-6433/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.cbpa.2013.03.004

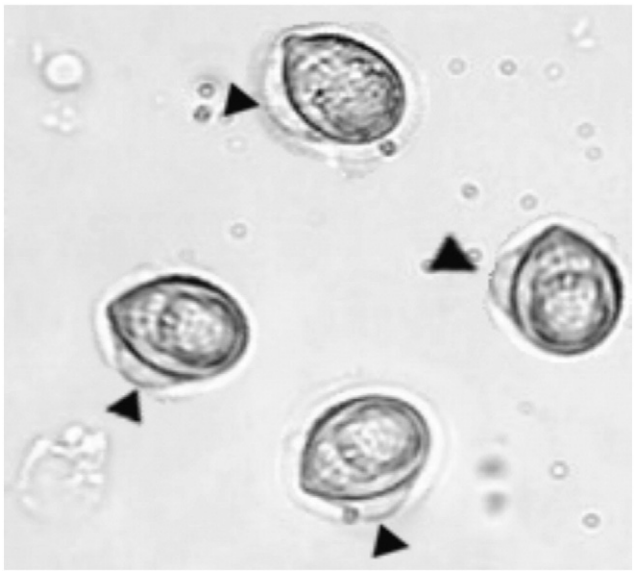

Fig. 1. Light microscope picture of nematocytes isolated from tentacles of *Pelagia noctiluca* by 605 mM NaSCN application. Note the cytoplasm as a rim (arrowhead). 400× magnification.
(Modified from Marino et al., 2011).

hyposmotic shock requires an outflow of K^+ and Cl^-,·K^+/H^+ exchange, cotransport of K^+ and Cl^-,·Na^+/Ca^{2+} exchange, extrusion of amino acids and re-arrangement of cytoskeleton (Wehner, 2006; Hoffmann and Pedersen, 2011), acting separately or synergistically. Moreover, cell signaling and regulatory pathways induce an increase in cytosolic free Ca^{2+}, either by influx through stretch-activated channels, release from intracellular stores or by activation of the arachidonic acid cascade or protein phosphorylation (Waldegger et al., 1998; Chara et al., 2011; Hoffmann and Pedersen, 2011; Pedersen et al., 2011; Dubois and Rouzaire-Dubois, 2012).

Cell volume regulation of isolated nematocytes during hyposmotic challenge has been investigated by La Spada and co-workers, showing that K^+ and Cl^- outflow, through both channels and cotransporters, is responsible for this homeostatic feature (La Spada et al., 1999, 2002; Marino et al., 2010). More recently, evidence has been shown for the role of aquaporins during the OP of nematocytes (Amado et al., 2011; Marino et al., 2011).

Since RVD accounts for maintenance of cell homeostasis (Lang et al., 1998; Hoffmann and Pedersen, 2011; Dubois and Rouzaire-Dubois, 2012) and in an attempt to verify whether pollutants in the external medium may affect cell function, we hypothesize that cell volume regulation under hyposmotic shock may be considered a biomarker for ecotoxicological studies. In particular, the present paper aims to verify the action of heavy metals such as Cd, La, Co, Cu and Zn on OP and RVD during 35% hyposmotic shock (produced by 65% artificial seawater, ASW, 22 psu) in nematocytes isolated from tentacles of the jellyfish *Pelagia noctiluca*. This species has been chosen because of its wide distribution in the Mediterranean Sea (Mariottini and Pane, 2010), particularly in the Strait of Messina, an exemplary habitat where anthropic factors may critically contribute to pollution.

2. Material and methods

2.1. Specimen collection

Specimens of *P. noctiluca* (Scyphozoa) were collected during summer 2011 along the Sicilian coast of the Strait of Messina (Italy), within a few meters from the shore. Once put in a tank filled with seawater and transferred to the laboratory, they were immediately used for experiments.

2.2. Nematocyte isolation

Eurytele nematocytes, according to the classification by Mariscal (1974), were isolated from tentacles of *P. noctiluca*, by treatment with an isosmotic solution of 605 mM NaSCN plus 0.01 mM Ca^{2+} (Salleo et al., 1996). Briefly, tentacles, once excised from the umbrella of at least ten jellyfish from the same collection, were repeatedly washed with low Ca^{2+} ASW to remove mucus and then treated with NaSCN, allowing nematocyte extrusion from the tissue. Substitution of the NaSCN solution with a Ca^{2+}-free ASW permitted cell isolation. Successively, restoration to physiological conditions was obtained with complete ASW. Isolated nematocytes were checked under a light microscope (400× magnification) to ensure morphological integrity and exclude cell shape damage. Cell integrity was confirmed by Trypan blue test and RVD tests were performed after a 1 h incubation at 14–16 °C, in any case within 3 h from isolation.

2.3. RVD tests

To perform the tests, after incubation, two thin strips of a double adhesive sided tape were placed to both the upper and lower edges of the slide with isolated nematocytes, to support a coverslip (24 × 32 mm). The experimental solutions were then added at one side of the coverslip and removed at the opposite side, by adsorbing them with strips of filter paper (Hidaka, 1993). In this way they were completely exchanged within a few seconds. Cell volume experiments, carried out at 18–20 °C in both treated and untreated cells, were performed on one nematocyte per each test chosen for its strong adhesion to the slide and constantly observed at 400× magnification under a light microscope. The RVD control test consisted of three periods: 1st period, isosmotic ASW ($\pi = 1100$ mOsm/kg_{H2O}, 35 psu) for 5 min; 2nd period, hyposmotic ASW (65% ASW, $\pi =$ 710 mOsm/kg_{H2O}, 22 psu) for 15 min; and 3rd period, isosmotic ASW for 5 min.

Experimental protocols with different heavy metals ($La(NO_3)_3$, $CoCl_2$, $CdSO_4$, $CuSO_4$, $ZnCl_2$), were performed as follows: period 1A, isosmotic ASW for 5 min; period 1B, isosmotic ASW plus heavy metal for 5 min; period 2, hyposmotic ASW plus heavy metal for 15 min; and period 3, isosmotic ASW for 5 min. The 1st period (1A) is needed to set a basal point describing the original shape and size of the untreated cell; 1B period accounts for possible cell changes in presence of heavy metals; the 2nd period describes the cell response to the hyposmotic challenge; and the 3rd period (isosmotic medium) is needed to restore the initial conditions. This latter period is particularly interesting in cells showing swelling but not RVD: in this case the application of an isosmotic final medium proves if the swollen cell is still able to go back to the basal point.

During the experiments, about 30 images/nematocyte were taken with a phase contrast microscope (Leica DMLS, Milan, Italy) connected to a video camera (JVC model TK-1180E) and to a computer equipped with suitable software (Apple Video Player, Adobe Photoshop). The sagittal area of each recorded image was successively measured (Image J, US) to assess cell volume changes as a function of time. The results were then expressed as the relative areas A/A_0: A and A_0 represent, respectively, the average sagittal areas of nematocyte at given time throughout the whole test, and the average area of sagittal section of untreated nematocyte at $t = 0$, before the hyposmotic challenge.

2.4. Experimental solutions and reagents

Isosmotic ASW had the following composition (mM) (McKay and Anderson, 1988): NaCl 520, KCl 9.7, $CaCl_2$ 10, $MgCl_2$ 24, $MgSO_4$ 28, imidazole 5, pH 7.65, $\pi = 1100$ mOsm/kg_{H2O}, and 35 psu. The hyposmotic solution (65% ASW, 22 psu) was obtained by reducing the NaCl concentration to $\pi = 710$ mOsm/kg_{H2O}. Stock solutions for heavy metals were dissolved in distilled water. Each compound was then added