Comparative Biochemistry and Physiology, Part C 150 (2009) 261–269

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part C

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

Measuring Ca^{2+}-signalling at fertilization in the sea urchin *Psammechinus miliaris*: Alterations of this Ca^{2+}-signal by copper and 2,4,6-tribromophenol

Sabine Schäfer [a], Ulf Bickmeyer [b], Angela Koehler [a,*]

[a] Alfred Wegener Institute for Polar and Marine Research in the Helmholtz Association, Am Handelshafen 12, 27570 Bremerhaven, Germany
[b] Biologische Anstalt Helgoland, Alfred Wegener Institute for Polar and Marine Research in the Helmholtz Association, Kurpromenade 201, 27483 Helgoland, Germany

ARTICLE INFO

Article history:
Received 28 January 2009
Received in revised form 8 May 2009
Accepted 10 May 2009
Available online 18 May 2009

Keywords:
Calcium signalling
Copper
Fertilization
MK571
Sea urchin
2,4,6-tribromophenol
MRP

ABSTRACT

During fertilization, eggs undergo a temporary rise in the intracellular concentration of free Ca^{2+} ions. Using the membrane permeable acetoxymethylester of the fluorescent calcium indicator dye Fura-2, Fura-2 AM, the Ca^{2+}-signal at fertilization was not detectable in eggs of the sea urchin *Psammechinus miliaris*. However, after treatment of the eggs with Fura-2 AM in combination with MK571, an inhibitor for multidrug resistance associated proteins, clear Ca^{2+}-signals at fertilization could be measured without microinjection of the dye. We used this methodology to detect possible alterations of Ca^{2+}-signalling at fertilization by exposure of eggs to environmental pollutants. For this purpose, the heavy metal copper, the bromophenol 2,4,6-tribromophenol, the organic compound bisphenol A and the polycyclic aromatic hydrocarbon phenanthrene were tested for their potential to inhibit fertilization success of *P. miliaris*. Copper and 2,4,6-tribromophenol showed a dose-dependent effect on fertilization rates of *P. miliaris* and significantly inhibited fertilization at 6.3 µM Cu^{2+} and 1 µM 2,4,6-tribromphenol. Bisphenol A significantly inhibited fertilization success at 438 µM while phenanthrene had no effect up to 56 µM. 6.3 µM copper and 100 µM 2,4,6-tribromophenol significantly increased the Ca^{2+}-signal at fertilization. This alteration may contribute to the reduced fertilization rates of *P. miliaris* after exposure to copper and 2,4,6-tribromophenol.

1. Introduction

Free calcium ions are essential second messengers in cells from their origin at fertilization throughout their entire lifespan (Carafoli, 2002). Disruption of cellular Ca^{2+} homeostasis appears to mediate the toxicity of many chemicals (Nicotera et al., 1992). Sustained increase in intracellular Ca^{2+} can provoke cytotoxic mechanisms in various cells and tissues by activation of Ca^{2+}-dependent enzymes, alterations of the cytoskeleton, mitochondrial damage, and by the activation of irreversible catabolic processes which may ultimately result in cell death (Nicotera et al., 1992; Stohs and Bagchi, 1995; Nicotera and Orrenius, 1998). A diverse range of natural and anthropogenic chemicals such as divalent heavy metal ions, bromophenols, bisphenol A as well as polycyclic aromatic hydrocarbons have been shown to interfere with cellular Ca^{2+} signalling (e.g. Büsselberg et al., 1990; Davila et al., 1995; Stohs and Bagchi, 1995; Nielsen et al., 2003; Wozniak et al., 2005).

At fertilization, eggs undergo an increase in intracellular Ca^{2+} beginning at the point of sperm–egg fusion and crossing the egg to the antipode in a wave-like fashion (Santella et al., 2004; Whitaker, 2006). This calcium wave is the first event at fertilization triggering the quiescent egg into metabolic activity by posttranslational activation of enzymes, exocytosis of cortical granules for formation of the fertilization membrane and resumption of the cell cycle (Covian-Nares et al., 2004; Santella et al., 2004).

In the following paragraph a selection of chemicals interfering with cellular Ca^{2+} signalling and homeostasis are presented: Cu^{2+} is an essential metal ion required for metabolic processes in all eukaryotes but can reach toxic levels in aquatic environments (Bryan and Langston, 1992; Stohs and Bagchi, 1995; Zorita et al., 2006). Cu^{2+} has been shown to alter Ca^{2+} signals in developing embryos of the macroalgae *Fucus serratus* (Nielsen et al., 2003). Bromophenols are industrially produced flame retardant intermediates and wood preservatives (Howe et al., 2005) which also occur naturally in the marine environment in algae (Whitfield et al., 1999) as well as in fish and invertebrates (Boyle et al., 1992; Fielman et al., 2001). Recently, bromophenols such as 2,4,6-tribromophenol have been shown to disturb cellular Ca^{2+}-signalling in neuroendocrine cells (Hassenklöver et al., 2006). Bisphenol A, an important key monomer in the production of polycarbonate plastics and epoxy resins, and endocrine disruptor, affects Ca^{2+} homeostasis by provoking Ca^{2+} influx via Ca^{2+} channels in mammalian tumor cell lines (Wozniak et al., 2005). Further, in goldfish bisphenol A significantly altered plasma Ca^{2+} levels (Suzuki et al., 2003). Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental pollutants contained in petroleum hydrocarbons and formed during combustion of fossil fuels and other products (Latimer and Zheng, 2003). PAHs and its metabolites have been shown to alter Ca^{2+}-associated signalling

* Corresponding author. Tel.: +49 471 4831 1407; fax: +49 471 4831 1425.
E-mail address: Angela.Koehler@awi.de (A. Koehler).

1532-0456/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpc.2009.05.004

pathways in immune (Davila et al., 1995) and nonimmune cells (Barhoumi et al., 2006) as well as in isolated membrane vesicles of mammalian skeletal muscles (Pessah et al., 2001).

Sea urchins are widely used to study the cellular events at fertilization (Santella et al., 2004; Whitaker, 2006). Further, some natural and anthropogenic chemicals have been tested on Ca^{2+} homeostasis in sea urchin eggs (Walter et al., 1989; Pesando et al., 1991, 1996; Girard et al., 1997). Thereby, the permeability of the plasma membrane to Ca^{2+} and other ions as well as the accumulation and release of sequestered Ca^{2+} were assessed (Pesando et al., 1991, 1996; Girard et al., 1997). Walter et al. (1989) investigated the Ca^{2+} content and uptake of Ca^{2+} as well as the role of mitochondrial damage in sea urchin eggs upon exposure to mercury chloride.

In sea urchins and some other organisms the calcium wave represents a single event which is followed by a few minor rises in the intracellular concentration of Ca^{2+} ions (Stricker, 1999). The mechanisms by which the sperm triggers Ca^{2+} release at fertilization are still under debate (Santella et al., 2004). In the most established model the sperm is believed to introduce a sperm factor into the egg promoting the formation of inositol-1,4,5-triphosphate ($InsP_3$) which initiates the activating Ca^{2+} wave (Jaffe et al., 2001; Santella et al., 2004). Studies indicate that in sea urchins there are two further messengers of Ca^{2+} signalling: nicotinic acid adenine dinucleotide phosphate (NAADP) and cyclic ADP ribose (cADPr) giving the fertilization calcium wave a boost and longevity (Steinhardt et al., 1977; Whitaker, 2006). Steinhardt et al. (1977) and Schmidt et al. (1982) have shown that the Ca^{2+} is released from intracellular stores, whereby later $InsP_3$ and cADPr were identified for mobilizing Ca^{2+} from the endoplasmic reticulum (reviewed by Galione, 1994, Jaffe et al., 2001). In contrast, NAADP is known to induce Ca^{2+} release from lysosomes (Churchill et al., 2002).

Calcium signals are mostly measured using fluorescent calcium indicator dyes (Whitaker, 2006). The ratiometric fluorescent dye Fura-2 has already been used for measuring the calcium wave at fertilization in eggs of the sea urchin *Lytechinus pictus* (Poenie et al., 1985; Swann and Whitaker, 1986) as well as in ascidians and mammals (Hyslop et al., 2001; Carroll et al., 2003). In general, the dyes are microinjected into the eggs. Indeed, Fura-2 is also available as membrane permeable acetoxymethylester Fura-2 AM. After crossing the membrane Fura-2 AM is quickly hydrolyzed by intracellular esterases to produce membrane impermeable Fura-2. Previously, the inhibitor for multidrug resistance associated proteins (MRP) MK571 has been shown to enhance uptake of fluorescent dyes in animal cells (Manzini and Schild, 2003; Bickmeyer et al., 2008) as well as in diatoms (Scherer et al., 2008). MRPs are efflux transporters of the ATP Binding Casette (ABC) superfamily actively transporting and sequestering endogenous and exogenous compounds (Holland and Blight, 1999; Leslie et al., 2001). In marine invertebrates MRPs have been demonstrated to be expressed in marine bivalve mollusks as well as in sea urchins (Hamdoun et al., 2004; Lüdeking et al., 2005).

The aim of the present study was to test if chemicals may alter the calcium wave at fertilization in sea urchins. For this purpose, the heavy metals Cu^{2+} and Pb^{2+}, the bromophenol 2,4,6-tribromophenol, bisphenol A, and the polycyclic aromatic hydrocarbon phenanthrene

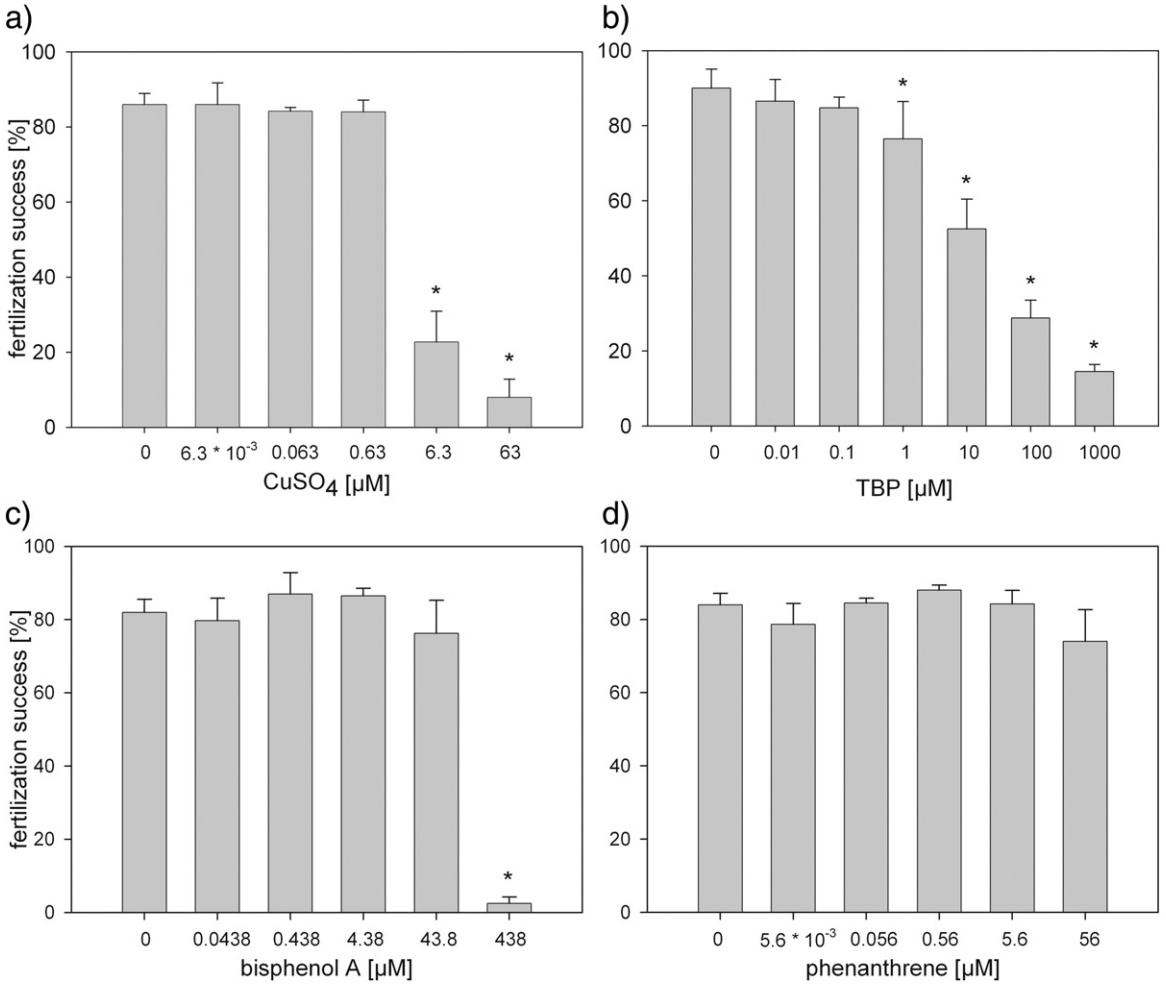

Fig. 1. Fertilization success of *Psammechinus miliaris* during exposure to set concentrations [µM] of a) copper sulfate, b) 2,4,6-tribromophenol (TBP), c) bisphenol A and d) phenanthrene. Asterisks indicate significant differences in comparison to controls ($CuSO_4$: one-way ANOVA $p<0.001$, Dunnett's test $p<0.05$; TBP: one-way ANOVA, $p<0.001$, Dunnett's test $p<0.05$; Bisphenol A: one-way ANOVA $p<0.001$, Dunnett's test $p<0.05$; Phenanthrene: Kruskal–Wallis ANOVA on ranks $p=0.074$).