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Aquatic Toxicology 76 (2006) 59–68

AQUATIC TOXICOLOGY

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Adenylyl cyclase activity and its modulation in the gills of *Mytilus galloprovincialis* exposed to Cr^{6+} and Cu^{2+}

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Received 11 July 2005; received in revised form 23 September 2005; accepted 25 September 2005

Abstract

The adenylyl cyclase (AC)/cAMP system regulates a large number of physiological functions in bivalve mussels, although its basal properties and the potential effects of environmental pollutants are scarcely studied. We characterized some properties of AC and measured both the enzyme activity and the cAMP levels in the gills of the filter-feeding sea mussel *Mytilus galloprovincialis*. Basal AC activity was 5.6 ± 0.8 pmol cAMP $10\,min^{-1}$ mg protein^{-1} and showed a K_m value of 0.82 ± 0.06 mM for ATP in the presence of 5 mM Mg^{2+}. It was stimulated up to 2.5- and 3.5-fold by 5-HT and GTPγS, respectively. Similarly to what was found in other bivalves, forskolin is a poor activator that reached significant stimulation only at 100 μM. Both basal and 5-HT-stimulated AC activity were significantly increased in the gills of mussels exposed for 7 days in aquaria to Cr^{6+} (10 ng/l) and Cu^{2+} (5 μg/l). The cAMP content of the gill under these conditions was also significantly higher than in control animals. In vitro exposure of gill membrane preparations to Cr^{6+} and Cu^{2+} induced a bimodal effect. Cu^{2+} significantly stimulated AC activity at nanomolar concentrations, but a strong inhibition was displayed in the micromolar range. A similar bell-shaped curve was obtained in the presence of Cr^{6+}, with maximal AC stimulation at 10^{-8} M and inhibition at 10^{-5} M. Overall, these data suggest that the mussel AC/cAMP system can be affected with different patterns by heavy metals. AC activity is strongly affected by acute exposure to heavy metals in vitro, probably through a direct interaction of the pollutants with the enzyme molecule, while AC activity and cAMP content increase in organisms exposed for 7 days in vivo, probably as a defense response to acclimate the physiological functions to the environmental challenge.
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Keywords: *Mytilus galloprovincialis*; Gills; Adenylyl cyclase; 5-HT; cAMP; Chromium; Copper

1. Introduction

A broad range of extracellular signals, including hormones, neurotransmitters, odorants, and autocrine/paracrine regulators produce their physiological res-

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0166-445X/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.aquatox.2005.09.007

ponses, at least in part, through modulation of adenylyl cyclase (AC) activity, the plasma membrane enzyme that catalyzes the formation of cAMP from ATP (Gilman, 1995). The AC/cAMP system, comprising also a heterotrimeric G protein coupled to a heptahelical receptor, is ubiquitous and plays a pivotal role in signal transduction in vertebrates (Taussig and Gilman, 1995).

Nine AC isoforms are recognised in mammals. They may be specific to one tissue, such as the type III AC specific for the olfactory membranes, or expressed in variable amounts in a wide variety of tissues as, e.g. AC IV, VII and IX (Simonds, 1999). AC isoforms share a primary structure consisting of two transmembrane regions each containing six predicted membrane-spanning helices and two cytosolic regions. After the structure of the catalytic core was discovered (Zhang et al., 1997), specific binding sites for the substrate ATP, the essential cofactor Mg^{2+}, and the modulating Gi and Gs proteins were identified. Guanine nucleotides like GTP, GTPγS, GDPβS, and guanylyl imido bisphosphate, regulate AC through G proteins. The strongest activator of mammalian AC is forskolin, a hydrophobic compound that greatly stimulates all membrane AC except for the type IX isoform.

Much less information is available regarding AC structure and properties in invertebrates. Thorough investigations have been carried out only in a few organisms, including the freshwater snail *Limnea* and the marine mollusc *Aplysia*, where the first clear evidence for a basic role of AC in non-vertebrate organisms was obtained, and where cAMP was proposed to have an important role in learning and memory mechanisms (Kandel and Schwartz, 1982).

Nevertheless, data obtained from different species ascribe several roles to cAMP in the regulation of bivalve mollusc physiology. The cyclic nucleotide shows modulatory effects on the cardiovascular system (Ono et al., 1992), regulates the movement of the mantle and siphon (Ram et al., 1993), induces rapid relaxation of the adductor muscle after the catch response (Yamada et al., 2001), activates the beating of quiescent lateral cilia (Sanderson et al., 1985), and induces spawning (Khotinchenko and Deridovich, 1989). The AC/cAMP system is also involved in gonadal maturation with an important metabolic effect, as it induces the breakdown of glycogen stores in the mantle to make glucose available as fuel for gametogenesis (Diaz Enrich et al., 2003).

Despite its wide spectrum of physiological actions in bivalve molluscs, the basal properties of AC have been studied only in the mantle of *Mytilus galloprovincialis* (Mancebo et al., 1991) and in the gills of *Tapes philippinarum* (Valbonesi et al., 2004). Recently some interest has developed to examine the potential effects of environmental pollutants on the AC/cAMP system: cAMP levels were found to be higher in resident mussels, *M. galloprovincialis*, collected from polluted sites along the Greek coast than from reference sites (Dailianis et al., 2003). However, AC activities were not different between resident mussels collected from contaminated sites along the Tyrrenian Sea and reference mussels; however, in vitro exposure to Pb^{2+} did lead to a strongly decreased AC activity in mussel mantle membranes (Giannaccini et al., 2004).

In the present study, we characterized some basal properties of the AC system in the gills of *M. galloprovincialis* to fill the gaps that emerged from the previous AC studies. We measured both AC activity and cAMP levels in the gills of mussels kept in aquaria and exposed for 7 days to chromium or copper. Moreover, the in vivo effect of the same heavy metals on AC in gill membrane preparations was compared with the in vitro effect.

Various biogenic amines, prostaglandins and different bioactive peptides, including FMRFamide, small cardioactive peptide B and calcitonin gene-related peptide, are well known regulators of cell functions and their action is mediated by an increase of cAMP levels (Lloyd et al., 1985; Yamane and Gelperin, 1987). An important role was ascribed to 5-hydroxytryptamine (5-HT or serotonin) on many aspects of bivalve mollusc physiology (Stommel and Stephens, 1985; Dietz et al., 1992; Ram et al., 1993, 1999; Diaz Enrich et al., 2003). 5-HT was implicated as the excitatory transmitter in the gill muscles as a complex network of serotoninergic fibers is associated with septa, blood vessels and muscles in the gills of the venerid clam, *Mercenaria mercenaria* (Gainey et al., 2003). A membrane-bound AC, coupled to Gs proteins and to a specific class of serotoninergic receptors, has recently been reported in gills of *T. philippinarum* (Valbonesi et al., 2004). Regulation of gill function in bivalve molluscs inhabiting estuarine coastal areas is of crucial importance to animal fitness, as the gills are directly affected by

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