RELEASE OF CADMIUM-COMPLEXED ATP AS A NOVEL DETOXIFICATION MECHANISM OF CULTURED RECTAL GLAND CELLS OF THE SHARK *SQUALUS ACANTHIAS*

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Exposure to environmental contamination by heavy metals triggers cellular mechanisms which reduce the intracellular pollutants by detoxifying the compounds and/or transporting them out of the cell. Such mechanisms include the γ-glutathione-S-transferase pump, the production of detoxifying compounds such as metallothioneins, and the expression of ATP-binding cassette (ABC) transporters, including P-glycoprotein (Pgp). This latter transporter, in particular, may be directly involved in the transfer of drugs, inorganic chemicals and possibly endogenous metabolites to the extracellular milieu. Cellular detoxification after heavy-metal adaptation is also known to involve the expression of ABC transporters such as the bacterial arsenic tolerance gene product, Pgp in *Leishmania sp.*, and the chloroquine-resistance gene product of the *Plasmodium falsiparum*. Pgp-expression in mammalian cells has also been implicated in Cd adaptation. The molecular steps linking Cd transport and cell detoxification are largely unknown.

We recently demonstrated the presence of electrodiffusional transport mechanisms for the release of cellular ATP in shark rectal gland (SRG) cells (Cantiello et al., Am. J. Physiol., vol. 272, 1997, C466-C475). Therefore, the possibility exists for heavy metal adaptation to be related to the presence of ATP-permeable transport mechanisms whereby Cd-ATP complexes are driven out of the cell. This may provide the first detoxifying step, further complemented by expression of Cd-binding peptides. In this study, SRG cells were adapted to grow for two weeks in the presence of $CdCl_2$ (1-10 μM). ATP release was then assessed in a Mg-free medium to determine the release of Cd-ATP. Further indication of the presence of CdATP-permeable channels was also determined by the excised inside-out patch-clamp technology. The data are consistent with the expression of Cd-ATP-permeable pathways, which may help detoxify the cells of this heavy metal.

Primary cultures of shark rectal gland cells were obtained from adult male *Squalus acanthias* as previously described (Valentich et al., Am. J. Physiol. 260: C813-C828, 1991) and grown on glass coverslips. Extracellular ATP was measured with a modified luciferin-luciferase assay as previously described (Abraham et al., Proc. Natl. Acad. Sci. USA, 90:312-316, 1993). Briefly, at the time of the experiment, the coverslips were placed in plastic cuvettes containing 0.1 ml of the luciferin-luciferase assay mix (Sigma Chem. Co., St Louis, MO) and 0.5 ml of a Ca^{2+}-, and Mg^{2+}-free solution containing 280 NaCl and HEPES 10, pH 7.4. The ATP release was

determined by the photon release of the luciferin-luciferase assay for 2 min before membrane permeabilization with alamethicin (10 μM) and sonication (30 sec, Ultrasonic sonicator, FS-14, Fisher Scientific) to measure total intracellular ATP. To determine the amount of ATP released from cells, known concentrations of Mg^{2+} were titrated. SRG cells were kept in culture for up to 3 weeks in the absence or presence of $CdCl_2$ (10 μM). Extracellular ATP was measured in a custom-made luciferin-luciferase assay mix devoid of Mg^{2+}, which was further titrated with a $MgCl_2$ (1 M) solution, after cells were added to the cuvette (Fig. 1). Cellular ATP release by the SRG cells was much higher in the Cd-adapted cells as indicated by the steeper slope and delayed increase in MgATP-elicited fluorescence (see Fig. 1). The possibility exists, however, for an underestimation of the differences as there is an exponential correlation between the arbitrary light units and the equivalents of ATP.

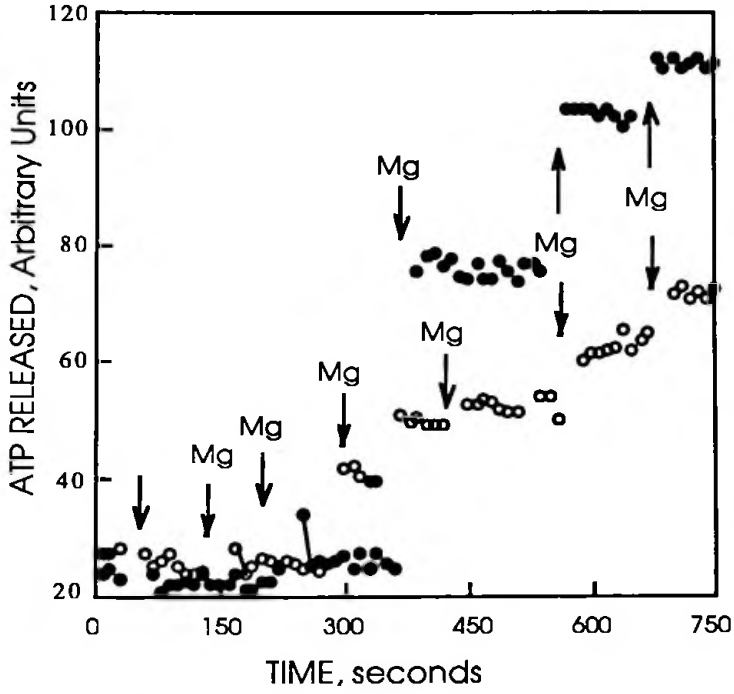

Fig. 1. Cellular ATP release was determined with a modified, Mg-free luciferin-luciferase assay. Known concentrations of Mg were titrated (arrows) after addition of cells to activate the assay mix. Cd-adapted cells (filled circles) released more ATP than control cells (open circles). Values are representative of two experiments under each condition. The first arrow indicates addition of cell attached coverslips. The next three arrows are matching additions for each cell group. Experiments were conducted independently.

CdATP currents were assessed in excised inside-out patches of control SRG cells under asymmetrical conditions, namely extracellular Mg-ATP (200 mM) and intracellular Cd-ATP (38 mM). Cadmium-ATP was made from dry $Cd(OH)_2$, which is completely insoluble in water, disolved into a solution of free acid ATP (50 mM, pH ~3.0), to maximal solubility, which was reached at a final concentration of CdATP of ~38 mM. The solution was adjusted to pH 7.00 by addition of HEPES (1 M) previously adjusted to pH 7.26 with N-methyl-glucamine (1M). Patches of control SRG cells were first obtained in normal bathing solution (high NaCl) under cell-attached conditions. Patches were excised, and PKA (10 μg/ml) and 1 mM MgATP were added to activate ATP-permeable channels prior to replacement with the Cd-ATP-containing solution. In 4 out of 4 experiments tested, ATP-permeable channels were observed under either spontaneous or PKA-activated conditions. In all cases,

single channel currents were observed for either form of ATP after replacement of the bathing NaCl with the Cd-ATP solution. Thus, ATP-permeable channels were also permeable to the Cd-ATP salt (Fig. 2).

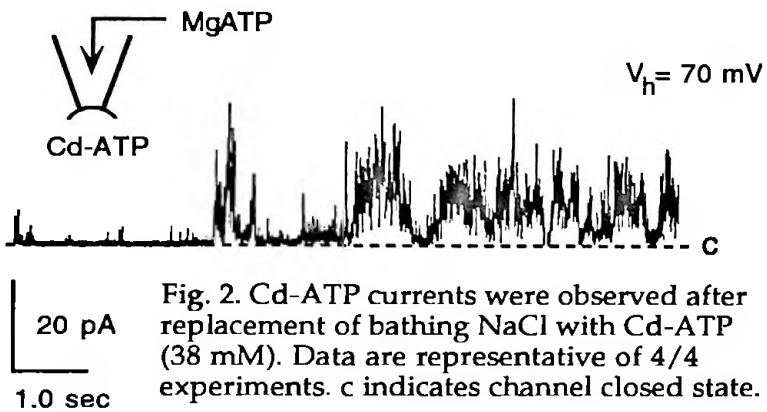

Fig. 2. Cd-ATP currents were observed after replacement of bathing NaCl with Cd-ATP (38 mM). Data are representative of 4/4 experiments. c indicates channel closed state.

The present study indicates that the movement of intracellular ATP in cultured SRG cells may serve as an adaptive mechanism to counter chronic exposure to heavy metals which may compliment other mechanisms, including the glutathione pump and the expression of metallothioneins. The presence of ATP-permeable channels that allow Cd-ATP complexes to be driven out of the cells, thereby reducing the toxic load of the Cd complexes, may be one of the first mechanisms for cell detoxification. It is possible, however, that Cd efflux by SRG cells may be accomplished by more than one ATP pathway.

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