# Monitoring magnesium efflux cyclic AMP-induced in HL60 cells by using a new hydroxyquinoline fluorescent chemosensor

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## Abstract

Cellular homeostasis of magnesium is still unclear. Several studies documented the occurrence of fluxes of magnesium across the plasmamembrane within minutes from the application of metabolic or hormonal stimuli. These fluxes, however, result in limited variation of free $Mg^{2+}$ intracellular concentration and large changes in total Mg content. It has been reported that a stimulation with cyclic AMP caused a movement of total magnesium within 10 min after treatment in cardiomyocytes. In this study we tested this hypothesis in HL60 leukemic cells, not excitable but highly proliferating cell model. We evaluated Mg flux by DCHQ5, the phenyl-derivative of hydroxyquinoline fluorescent probe family. We observed a drastic decrease of intracellular total magnesium in the first 3 min. We also verified that at least 10% of the total intracellular amount of magnesium moved in the supernatant of stimulated cells.

## Introduction

Cellular magnesium homeostasis is still unclear, although this cation is essential for numerous cell functions.[1,2] Several studies documented the occurrence of fluxes of magnesium across the plasmamembrane within minutes from the application of metabolic or hormonal stimuli. These fluxes, however, result in limited variation of free $Mg^{2+}$ intracellular concentration and large changes in total Mg content.[3] DCHQ5 probe, which is stable up to 30 min of incubation and highly retained within loaded cells, results to be the proper tool for monitoring magnesium content.[4]

It has been reported that a stimulation with cyclic AMP (cAMP) caused a movement of total magnesium within 10 min after treatment in cardiomycites, suggesting that this stimulus caused a magnesium efflux in excitable cells.[5] In this study we tested this hypothesis in HL60 leukemic cells, a not excitable but highly proliferating cell model. We evaluated Mg flux by DCHQ5 using spectrofluorimetric and cytofluorimetric assays.

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## Materials and Methods

HL60 cells were maintained in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 2 mM L-Glutamine, 10% foetal bovine serum (FBS) at 37°C and 5% CO2. Total magnesium was assessed incubating $5x10^5$ cells/mL in phosphate buffered saline (PBS) without $Mg^{2+}$ with DCHQ5 15 µM for 15 min in the dark. Then cells were treated with different concentration of cAMP and analyzed by cytofluorimetric and spectrofluorimetric assay. Magnesium efflux was evaluated treating $3x10^6$ cells/mL in PBS without $Mg^{2+}$ for 3 min with cAMP. The cells were then centrifugated and the magnesium in the supernatant was measured by DCHQ5 probe using a calibration curve. Intracellular free $Mg^{2+}$ were determined by Mag-Fluo4-AM staining: HL60 cells were incubated with 0.8 M fluorophore in medium without $Mg^{2+}$ for 30 min at 37°C and analyzed by flow cytometry. Dead cells were excluded by propidium iodide (PI) incorporation.

## Results

We evaluated Mg flux by using DCHQ5 spectrofluorimetric assay: the cells were treated with different cAMP concentrations and the fluorescence intensity was monitored for 10 min after the stimulation (Figure 1). A marked dose-dependent decrease of total intracellular magnesium was observed in the first 3 min after treatment; moreover, this effect continued in the next time of analysis, confirming that cAMP induced an extracellular Mg efflux.

We then quantified the intracellular magnesium content released in the supernatant after 3 min of 1.25 mM cAMP stimulation by DCHQ5: we found that magnesium in cAMP-treated cells is doubled respect to the physiological release observed in control cells (Figure 2). When we compared the magnesium released (5.3 nmol/$10^6$ cells) with the normal intracellular content of this cation (30 nmol/$10^6$ cells), we found that 1.25 mM cAMP stimulation released about 20% of this cation in the extracellular medium.

We also verified that the observed magnesium release was not due

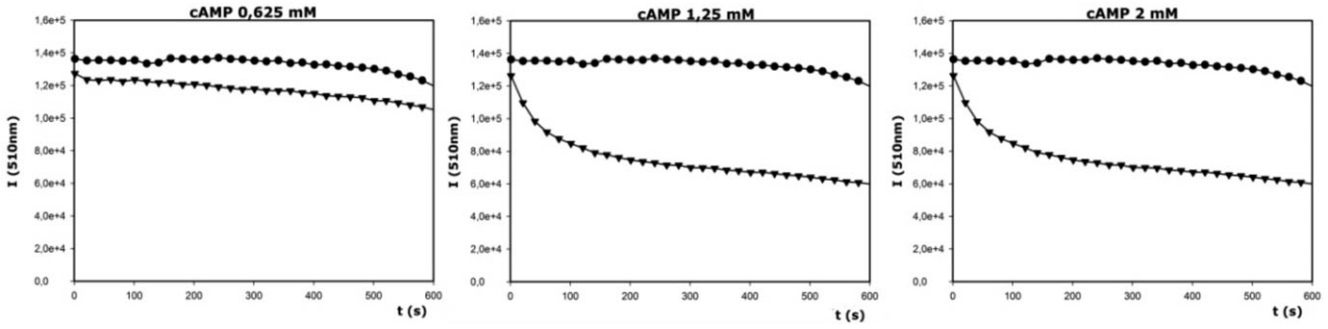


**Figure 1. Time course of DCHQ5 fluorescence intensity ($\lambda_{ex}$ 360 nm, $\lambda_{em}$ 510 nm), in stimulated (▼) and control cells (●).**

to cellular swelling, as shown by the vitality assay reported in Figure 3, where the plots of the fluorescence of DCHQ5 in function of PI fluorescence in treated and control samples are reported.

It is reported that in cardiomyocytes cAMP stimuli caused a variation in total intracellular magnesium but not in the free fraction: in HL60 cells, the analysis of free, ionized magnesium by Mag-Fluo-4 probe[3] did not show any difference between control cells and cells treated with cAMP, even at the higher dose (data not shown).

## Discussion

We demonstrated that cAMP stimulation promotes a magnesium release also in highly proliferating cancer cells and not only in excitable cells. The results obtained suggest a possible role for magnesium as second messenger as proposed in the past on theoretical basis.[2] Moreover, our results confirmed that DCHQ5 is a suitable tool to deepen our knowledge on magnesium homeostasis, as it can be used for the quantification of total intracellular magnesium, as well as for monitoring magnesium cellular fluxes.

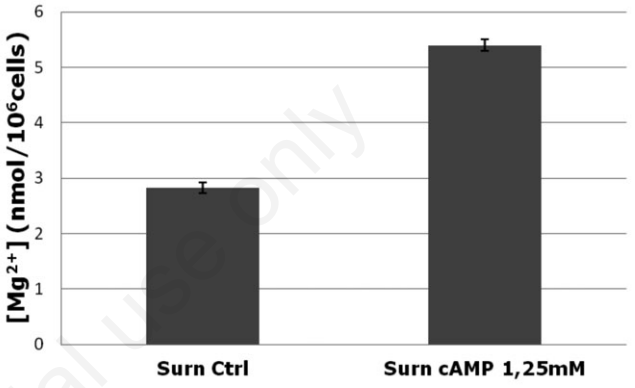


**Figure 2. Quantification by DCHQ5 of the intracellular magnesium content released in the supernatant after 3 min of 1.25 mM cyclic AMP stimulation.**

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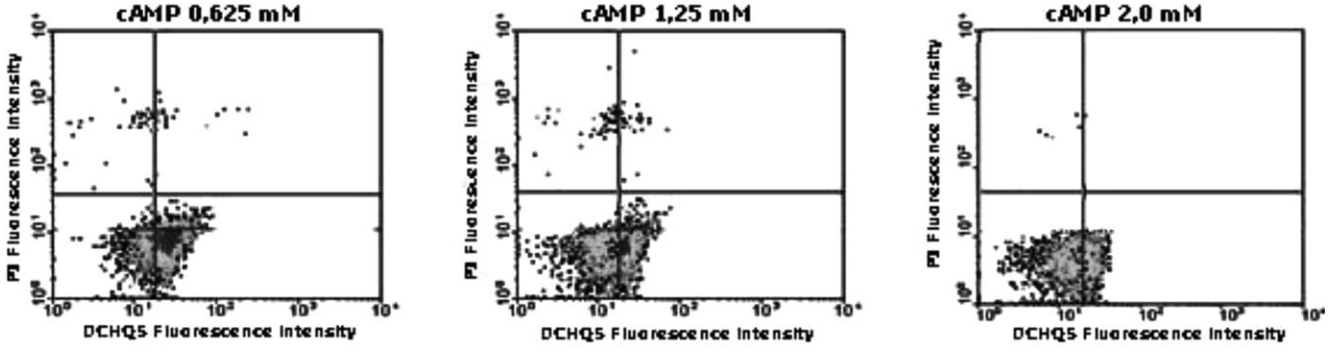


**Figure 3. Cytofluorimetric vitality assay with propidium iodide: the samples, treated with cyclic AMP, were stained with DCHQ5 and propidium iodide: damaged, propidium iodide-positive cells fall in the upper quadrants.**