EFFECTS OF CALMODULIN INHIBITORS ON THE
CELLULAR METABOLISM OF $^{45}$Ca AND $^{210}$Pb

J. G. Pounds and A. C. Nye
Dept. of Applied Science, Brookhaven National Laboratory
Upton, NY 11973

INTRODUCTION

Many Ca$^{++}$-mediated cell processes depend on the intracellular Ca$^{++}$ receptor protein, calmodulin, to exert regulatory effects on target metabolic and physiological pathways. In many cells, Ca$^{++}$ transport mechanisms are activated by the Ca$^{++}$-calmodulin complex. Several aspects of the cellular metabolism of Pb$^{++}$ and Ca$^{++}$ are similar. Lead is actively transported or diffuses through mitochondrial and plasma membranes via Ca$^{++}$ transporters and gates. Cellular lead is mobilized by hormones which also mobilize cell Ca$^{++}$ and has a kinetic distribution and behavior in cultured cells similar to Ca$^{++}$. Lead binds to calmodulin with greater affinity, and can substitute for Ca$^{++}$ in calmodulin-activated processes. However, the relative concentrations of free Ca$^{++}$ and Pb$^{++}$ ions in cytosol and the ability of Pb$^{++}$ to effectively alter calmodulin mediated processes in situ are not well established. The objective of this study was to characterize the regulation of Pb and Ca metabolism by calmodulin-dependent processes.

METHODS

Hepatocytes were obtained by collagenase perfusion of caudate liver lobes dissected from male Sprague-Dawley rats. Cells were plated at a density of $2 \times 10^5$ viable cells/cm$^2$ in Williams' E containing 10% fetal bovine serum, 2 mM glutamine, 2 mM/ml insulin, 20 µg/ml gentamicin, and 1 nM dexamethasone. Cultures were exposed to 0 or 3 µM Pb acetate for 17 hours prior to addition of calmodulin inhibitors and $^{45}$Ca and $^{210}$Pb. Following overnight adaptation in culture 25 µCi/ml $^{45}$Ca (1.8 µM total Ca), or 1 µCi/ml $^{210}$Pb (3 µM total Pb), and 60 µM W12 or W13 were added to the cultures for a 3 hr incubation period. After the labeling period, cultures were rinsed and subjected to a 210-minute washout procedure. Data obtained from each culture were plotted as the radioactivity $R(t)$ present in the cells at washout time, $t$, divided by the total radioactivity in the cells at washout time 0, $R(0)$. The data from each culture were fit to the polyexponential equation, $r(t) = B_1 \exp(-\lambda_1 t) + B_2 \exp(-\lambda_2 t) + B_3 \exp(-\lambda_3 t)$. The pool sizes, rate constants, halftimes, and fluxes describing the steady state cellular metabolism of Ca and Pb, normalized to 1 mg cell protein, were derived from the coefficients and exponents as previously described (Pounds et al., 1982). The data represent the mean of four cultures from a representative experiment, expressed...
as percent of the untreated control. The data were analyzed statistically by one-way ANOVA and Dunnett’s multiple comparison.

RESULTS

The inactive analog W-12 had little effect on the cellular metabolism of Pb or Ca. The calmodulin inhibitor W-13 did not alter total cell Pb or Ca but did affect the subcellular kinetic distribution of both Pb and Ca.

![Graphs](image)

**Figure 1.** Effect of the calmodulin inhibitors W-12 or W-13 on the kinetic distribution and behavior of $^{45}$Ca and $^{210}$Pb in cultured rat hepatocytes.

DISCUSSION

Evaluation of the calmodulin-dependence of cell Ca$^{++}$ and Pb$^{++}$ in situ is difficult for several reasons. Altered Ca$^{++}$ homeostasis may result from the direct inhibition of calmodulin-dependent or -independent transport processes. Changes in cell function not directly related to the transport of Ca, e.g., uncoupling of oxidative phosphorylation or altered membrane permeability also disrupt cell calcium metabolism. Thus, the effects of the calmodulin inhibitor W-13 on cell Pb$^{++}$ metabolism may be due to its direct effects on Pb$^{++}$ transporting Ca$^{++}$ pumps, or indirectly as a result of changes in Ca$^{++}$ homeostasis. Direct comparison of the effects of W-13 on the metabolism of Pb and Ca is impaired by differences in the kinetic distribution and behavior of Pb and Ca. A further complication is that the calmodulin-dependent processes are most active during periods of elevated intracellular Ca$^{++}$. The preliminary experiments reported here were conducted in unstimulated cells which have a low resting level of cytosolic Ca$^{++}$. Therefore, W-13 induced alterations in cell Ca$^{++}$ and Pb$^{++}$ may not reflect the changes which could occur in stimulated cells.

REFERENCES

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.