LEAD ACTIVATES CALMODULIN SENSITIVE PROCESSES

Gary W. Goldstein and Diane Ar

Departments of Pediatrics and Neurology
University of Michigan Medical School
Ann Arbor, Michigan 48109

Summary

The effect of lead on two calcium sensitive processes was deter-
mined. Micromolar concentrations of lead successfully replaced
calcium in the activation of calmodulin-sensitive phosphodiesterase
and in the promotion of potassium loss from erythrocytes. Both
actions of lead were blocked by trifluoperazine--an inhibitor of
calmodulin function. We propose that some of the toxic effects of
lead may be explained by its interaction with calmodulin.

Children poisoned with lead may develop brain injury, muscle weakness,
anemia, and renal tubular dysfunction (1). It is well established that
calcium influences the absorption and distribution of lead (2), but this
observation does not explain the cellular manifestations of lead toxicity. To
determine whether lead might alter cell function by substituting for calcium
in regulatory processes, we measured the effect of lead upon the activity of
two calcium-sensitive reactions. We found that lead could replace calcium in
the calmodulin-dependent activation of cyclic nucleotide phosphodiesterase
(PDE) and in the promotion of potassium efflux from erythrocytes. Both
effects were blocked by trifluoperazine (TFP), an inhibitor of calmodulin
action.

Materials and Methods

The activator deficient PDE and calmodulin used in these experiments were
purchased from Sigma Chemical, St. Louis, MO. PDE activity was determined by
measuring conversion of cAMP to adenosine (3). The reaction mixture contained
700 μ units of bovine heart, activator-deficient PDE with or without 200 ng of
phosphodiesterase 3':5'-cyclic nucleotide activator (calmodulin), 20 μM cAMP,
and 0.1 μCi of ^{3}H-cAMP in a total volume of 100 μl calcium-free buffer (100 mM
Tris, pH 8.0, 1 mM $MgSO_4$, 600 μM dithiothreitol, and 10 μM EGTA). The EGTA
was added to chelate endogenous calcium. The concentration of lead chloride
in the incubation varied from 0 to 100 μM. To inhibit calmodulin binding to
PDE, 20 μM TFP was added when the lead concentration was 50 μM.

The effect of calcium, lead and calmodulin on potassium permeability was
studied with red blood cells. Human red blood cells were prepared from fresh
heparized blood, washed three times in 20 volumes of ice cold 15 mM HEPES
buffered saline, pH 7.4, containing 2 mM $CaCl_2$. A cell pellet was prepared by
centrifuging red blood cells at 600 x g for 10 min and then resuspended in

3 volumes of ice cold buffer.   Incubations were initiated by adding 200 µl of the red blood cell suspension to 4.8 ml of buffer prewarmed to 37°C.   Calcium entry into the cells was produced with the specific calcium ionophore, A23187. The ionophore was dissolved in DMSO and added to the incubation at time zero in a volume of 5 µl.   The final concentration of A23187 was 0.2 µg/ml.   DMSO alone had no effect on potassium release.   To chelate calcium, the cells were preincubated 10 min at 37°C in 5 mM EGTA.   When present, lead chloride was added to the incubation at time zero at a final concentration of 10 µM.   To inhibit calmodulin action the cells were preincubated 5 min with 20 µM TFP at 37°C.   The reaction was stopped with 5 ml of ice cold buffer and centrifuga- tion for 10 min at 600 x g.   The concentration of potassium in the supernatant was measured using a flame photometer.

Results and Discussion

The role of calmodulin, an intracellular calcium receptor protein, in the regulation of several enzymes and transport systems, has only recently been discovered and the list of calcium-sensitive reactions modulated by the calmodulin-calcium complex is rapidly expanding (4, 5).   When the concentra- tion of calcium in the cytosol rises, calcium ions bind to high affinity sites on calmodulin causing the configuration of the protein to change (6).   The complex is then able to alter the activity of certain enzymes and transport systems.   TFP inhibits this cascade by binding to the calmodulin-calcium complex and preventing its interaction with calcium-sensitive enzymes (7). Since lead is known to have a kinetic behavior similar to calcium in intact cells (8) and to compete with calcium for absorption from the intestine (9), incorporation into bone (10), and accumulation by mitochondria (11, 12), we questioned whether lead could replace calcium in the activation of calmodulin.

Figure 1 shows the stimulation of PDE by calmodulin and lead.   The incubation buffer has no added calcium and contains 10 µM EGTA to remove endo- genous calcium.   PDE activity increased when the concentration of lead exceed- ed that of EGTA, a finding explained by the capacity of EGTA to bind lead on an equimolar basis.   The stimulation of PDE by calmodulin and lead was inhibited more than 50% by 20 µM TFP.   This is consistent with observations that TFP blocks calmodulin-calcium activation of PDE with an I_{50} of 10 µM (7). Lead had no effect on the activity of PDE in the absence of calmodulin.   When PDE was maximally activated by calmodulin and either lead or calcium, addition of the other divalent cation did not change the activity of the enzyme (exper- iments not shown).   From these results we cannot determine the mechanism by which lead activates PDE in the presence of calmodulin, but since divalent cations in addition to calcium (i.e., Sr^{++}, Ni^{++}, Co^{++}, Zn^{++}, Mn^{++} but not Ba^{++} or Mg^{++}) support the binding of TFP to calmodulin (13), we suspect that lead is acting directly at the high affinity calcium binding sites on calmodulin.
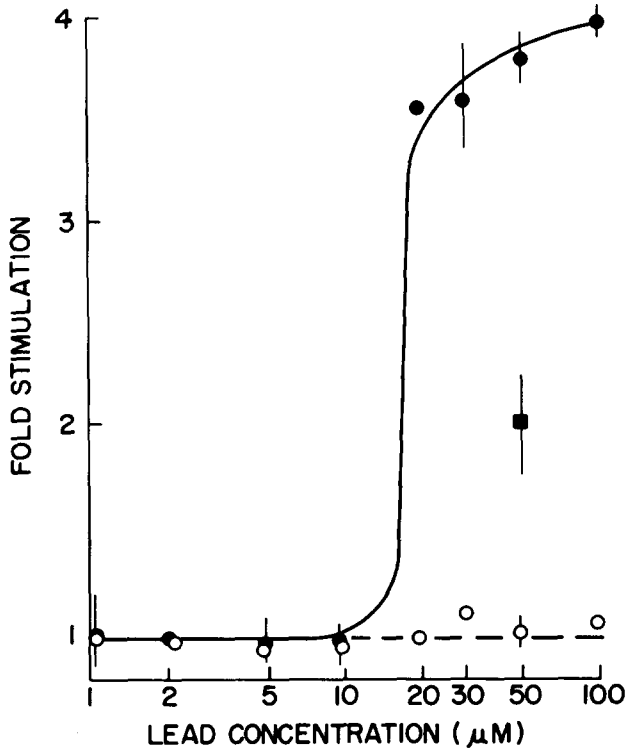
FIG. 1

Effect of increasing lead concentration upon PDE. Enzyme activity
was measured in the presence (●) or absence (○) of added cal-
modulin. To inhibit calmodulin binding, 20 µM TFP was added (■)
when the lead concentration was 50 µM. Incubations were performed
in triplicate and results are expressed as the fold-stimulation of
PDE + S.D. The basal activity of PDE was 180 pmol adenosine formed
per $\overline{1}0$ min. Where not shown, the deviations were less than 5% of
the mean. Stimulation of PDE by lead required the presence of
calmodulin and was inhibited by TFP.

    If lead functions as a calcium agonist in the activation of calmodulin,
it should alter calmodulin dependent cell functions that normally respond to
changes in the cytosol concentration of ionized calcium. By either energy
depleting erythrocytes or treating them with a calcium specific ionophore,
A23187, it is possible to raise their cytosolic concentration of calcium (14).
One response to such an elevation is a marked increase in the permeability of
the cell membrane to potassium (15).   The effect is selective and not
associated with loss of intracellular sodium. We found that potassium loss
produced in this fashion was largely prevented by preincubating the red blood
cells for 5 minutes with 20 µM TFP (Fig. 2). This is the same concentration
of TFP that inhibits calmodulin activation of calcium regulated enzymes (7).
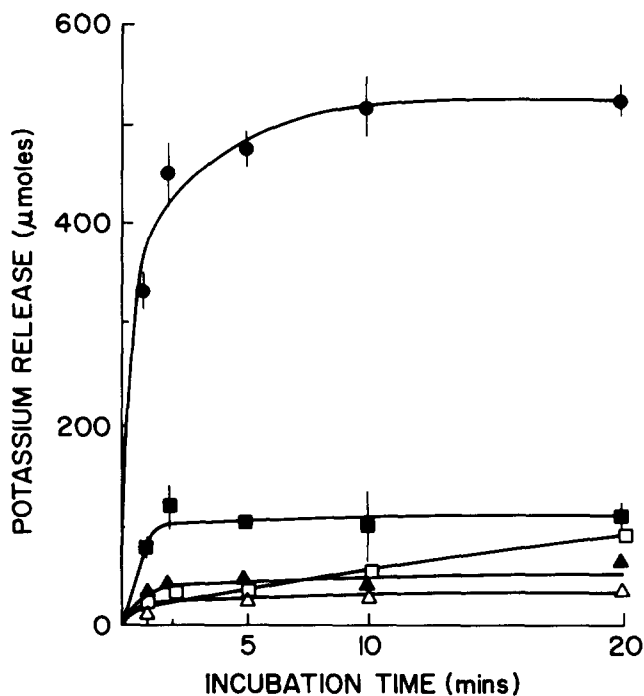
FIG. 2

Effect of TFP on the release of potassium from human red blood cells
treated with calcium ionophore, A23187.   Incubations were performed
in the presence of 0.2 µg/ml ionophore alone (●), ionophore with
10 µM EGTA (▲), ionophore with 20 µM TFP (■), EGTA alone (△),
or TFP alone (□).   The results are averages of three determina-
tions $\pm$ S.D.   Where not shown, the deviations were less than 5% of
the mean.   The release of K^+ promoted by the calcium ionophore,
A23187, was inhibited by TFP.   In addition, it was largely prevented
by chelation of calcium with EGTA prior to addition of the ionophore.

Although TFP has effects in addition to calmodulin blockade, other biologic
events which are inhibited by micromolar concentrations of TFP have been
assumed to be mediated by calmodulin (5, 16).   Thus, this observation
implicates calmodulin in the regulation of potassium permeability by the
cytosolic concentration of calcium in red blood cells.   Exposing red blood
cells to lead also rapidly leads to selective potassium loss (17).   Unlike the
calcium effect, there is no need to energy deplete the cells or add an iono-
phore since lead readily enters red blood cells.   We found that the same 20 µM
concentration of TFP that inhibited the loss of potassium from red blood cells
with a raised calcium concentration (Fig. 2) also prevented the loss of
potassium from red blood cells exposed to lead (Fig. 3).   These findings
suggest that both calcium and lead produce potassium loss from erythrocytes by
activating calmodulin.   The possible significance of this observation to lead
poisoning is supported by the similarity between the threshhold for the lead-
induced permeability change in erythrocytes (5-10 µM) and the concentration of
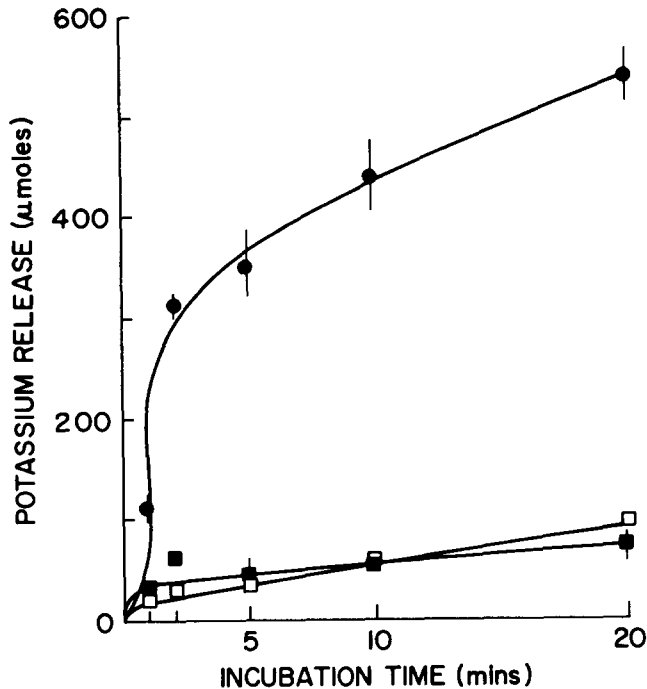lead in the blood of children with symptoms of lead poisoning (18).

FIG. 3

Effect of TFP on the release of potassium from human red blood cells
treated with lead. Incubations were performed in the presence of
10 μM lead (●), lead with 20 μM TFP (■) or TFP alone (□). The
results are averages of three determinations $\pm$ S.D. Where not shown,
the deviations were less than 5% of the mean. The release of K^+
promoted by lead was blocked by TFP.

In a study of heavy metal interaction with cyclic nucleotide metabolism,
Nathanson and Bloom described a marked inhibition of adenyl cyclase by lead
and other heavy metals (19). Competition at the calmodulin binding site seems
unlikely for this effect since high concentrations of calcium did not reverse
the inhibition. In addition, they observed a 22% stimulation of PDE by lead
while other heavy metals were without effect. The relation of calcium and
calmodulin to the stimulation of PDE by lead was not investigated.

We found that lead can replace calcium in the activation of calmodulin-
sensitive PDE and in the promotion of potassium loss from red blood cells.
Both processes were inhibited by low concentrations of TFP. Taken together,
these observations support a molecular model for lead toxicity in which lead
alters the function of a tissue by entering the cell and occupying calcium
binding sites on calmodulin. Since calmodulin regulates multiple cell
activities and is present in virtually all tissues, this mechanism may

underlie some of the diverse effects of lead poisoning. Tissue differences in lead permeability, calmodulin concentration, calmodulin-sensitive functions, and internal calcium concentration might explain in part the different thresholds and responses of various organs to the toxic effects of lead.

Acknowledgements

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