Erythropoietin Stimulates $^{45}$Ca$^{2+}$ Uptake in Friend Virus-infected Erythroid Cells*

Stephen T. Sawyer and Sanford B. Krantz

From the Department of Medicine, Division of Hematology, Vanderbilt University School of Medicine, and Veterans Administration Medical Center, Nashville, Tennessee 37232

It has been shown previously in this laboratory that in vitro infection of mouse bone marrow cells with the anemia strain of Friend leukemia virus leads to growth of large bursts of erythroid cells which are arrested in development prior to hemoglobin synthesis but can respond to erythropoietin (EP) to complete the late stage of erythroid differentiation. In this study, the effect of EP on the metabolism of $^{45}$Ca$^{2+}$ in these cells was examined. At 4°C, an increased rate of $^{45}$Ca$^{2+}$ uptake and efflux as well as an increase in the steady state level of $^{45}$Ca$^{2+}$ in treated cells was observed. Exchange of $^{45}$Ca$^{2+}$ from preloaded cells at 4°C indicated that treatment with EP increased the size of a rapidly exchanging pool of $^{45}$Ca$^{2+}$ from 5 to 12% of total $^{45}$Ca$^{2+}$ in the cell. The effect of treatment with EP can be seen as increased exchange of extracellular $^{45}$Ca$^{2+}$ with cellular Ca$^{2+}$; however, an effect of EP on the net level of Ca$^{2+}$ in these cells cannot be excluded. This investigation demonstrates one of the earliest effects of EP on erythroid cells and suggests that alterations in Ca$^{2+}$ metabolism may contribute to the progression of erythroid cells to their final development.

Erythropoietin is the normal physiological regulator of erythropoiesis (1). The mechanism by which this small, 34,000-dalton glycoprotein (2) interacts with its target cells and triggers erythroid differentiation is still largely unknown partly due to the lack of homogeneous cells which fully respond to the hormone. Recently, in this laboratory, a murine bone marrow culture method was developed which allows the isolation of a relatively homogeneous erythroid cell population which is arrested in erythroid differentiation prior to the onset of hemoglobin synthesis. Virtually all these cells proceed to full erythroid maturation when stimulated with EP, and they can be isolated in sufficient numbers to carry out biochemical experiments. Infection in vitro of the marrow with the anemia strain of Friend virus is used to promote erythroid cell growth up to the point of arrest; but, the final stage of erythroid maturation, induced by EP, appears to be very similar to normal erythrocyte development (3). Maturing FVA-infected erythroblasts undergo nuclear condensation and extrusion of the nucleus with synthesis of $a$- and $b$-globin mRNA and polypeptides, increased incorporation of iron into protoporphyrin, and synthesis of spectrin (3, 4).

In this study, the effect of EP on $^{45}$Ca$^{2+}$ metabolism in FVA-infected erythroid cells was studied. Free Ca$^{2+}$ levels of the cytosol are thought to act as a second messenger for a number of hormones, neurotransmitters, and mitogens which regulate the flow of Ca$^{2+}$ across the membranes encapsulating the cytosol (5-7). An increase in the cytosolic Ca$^{2+}$ concentration to 0.1 $\mu$M to 2.0 $\mu$M is believed to trigger lymphocyte mitogenesis (8, 9), muscle contraction (10), and many other effects of diverse hormones (see Ref. 11 for a review). In addition, a role for Ca$^{2+}$ has been implicated in the control of differentiation by cells in culture, such as murine epidermal cells undergoing keratinocyte differentiation (12), murine erythroid colony-forming cells (13), and murine erythroleukemia cells (14-16).

Experiments with murine erythroleukemia cells, induced into differentiation with dimethyl sulfoxide, demonstrated an increased rate of $^{45}$Ca$^{2+}$ uptake after induction (16). The effect of dimethyl sulfoxide on Ca$^{2+}$ translocation was linked to the function of the Na$^+$/Ca$^{2+}$ antiport (17) and the membrane potential of the mitochondria (18). An increase in the intracellular concentration of Ca$^{2+}$ seems to be the rate-limiting step in the commitment of the murine erythroleukemia to differentiation because the time lag between the exposure of the cells to inducer and the appearance of committed cells can be manipulated by the level of Ca$^{2+}$ in the medium (15) or compounds which affect the internal Ca$^{2+}$ concentration either directly (18) or indirectly (17, 18).

Murine erythroleukemia cells do not respond to EP, the normal inducer of erythroid maturation, or have only a limited response (19). Therefore, in this study, we have investigated the possibility that the induction of the erythroid maturation program in the FVA-infected erythroid cells by EP might proceed through alterations in the metabolism of Ca$^{2+}$.

**EXPERIMENTAL PROCEDURES**

**Materials**—$^{45}$CaCl$_2$ (4-30 Ci/g of Ca) and $[^3H]$inulin (100 mCi/g) were from New England Nuclear. $^{55}$FeCl$_3$ (3-20 Ci/g of Fe) was obtained from Amersham Corp. Pure human urinary EP (70,000 units/mg) was a gift from Dr. E. Goldwasser (University of Chicago), and human urinary EP (1,100 units/mg) was obtained from National Heart, Lung, and Blood Institute (Bethesda, MD). A23187 was purchased from Calbiochem-Behring. Other reagents were obtained from Fisher or Sigma. FVA (10$^5$ spleen focus-forming units/ml) was prepared and stored as described previously (20).

**Culture of Cells**—Bone marrow cells from phenylhydrazine-treated (21), 8-10-week-old CD1, mice were infected with FVA and cultured in a 1:1 mixture of $\alpha$ medium and Iscove’s modified Dulbecco’s medium (Gibco) with 0.8% methylcellulose and 30% fetal calf serum as described previously (22). On day 5 of culture, bursts of erythroid cells were plucked from culture using a dissecting microscope and a drawn-out Pasteur pipette. These isolated cells were judged to be...
greater than 90% erythroid on the basis of Wright-stained cytogen- 
trifuge preparations and subsequent hemoglobinization of recultured 
cells in the presence of EP (4). An average experiment using 12 mice 
produced 10–20 × 106 erythroid cells.

"Ca" Uptake Measurement—Isolated erythroid cells were sedi-
mented by low speed centrifugation and resuspended at 106 cells/ml in 
Dulbecco's modified Eagle's medium (Gibco) containing 20 mM 
Hepes, pH 7.4, 1.0% bovine serum albumin, and 10% fetal calf serum 
except where indicated. Experiments were carried out in medium with 
either the normal concentration of CaCl2 (1.8 mM) or 100 mM CaCl2. 
Experiments at 37°C were initiated by the addition of "Ca" at a final 
specific activity of 200 rpm/pmol. To measure "Ca" uptake into 
the cells, cells were separated from the radioactive medium by 
removing a 1.0-ml aliquot and centrifuging through 0.5 ml of a mixture 
of n-butyl phthalate oil and silicone oil (9:1) in a 1.5-ml microtube. 
EGTA was added immediately before centrifugation to a final 
concentration of 5 mM to reduce surface-bound "Ca" on the cells.

Preliminary experiments showed that La" and EGTA had a similar 
effect on reducing the total level of "Ca" associated with the cell. 
After centrifugation in a microfuge (Fisher) for 1 min, the tubes were 
frozen at −80°C. Tips of the frozen tubes were cut off just above 
the pellet. Then the pellet was disaggregated by vigorous vortexing in 1.0 
ml of 0.1% sodium deoxycholate and counted in 10 ml of ACS (Amersham Corp.). The volume of the medium trapped in the pellet 
was determined by performing the experiment with untransported 
["H]lumin or by adding "Ca" chelated with a large excess of EGTA 
(100 mM preincubation with tracer levels of "Ca" and 5 mM final 
concentration during centrifugation). Both methods gave identical 
results which showed that 90–95% of the total "Ca" in the pellet 
from a typical experiment was due to trapped medium. When the 
"Ca" uptake was determined at 4°C, the cells were preincubated 
with EP for varying times at 37°C before being chilled in an ice bath. 
After 15 min in ice, the uptake was initiated by the addition of "Ca" to 
50 rpm/pmol final specific activity and was terminated by centrif-
ugation of the cells through oil as described above.

"Ca" Exchange—Isolated erythroid cells were resuspended in 
the medium described above, which contained 1.8 mM CaCl2 and 
5.0 μCi of "Ca"/ml at 106 cells/ml. The incubation with "Ca" was carried 
out at 37°C for 4 h. One-half of the replicates received EP during 
the last 60 min of incubation. The cells were sedimented by low speed 
centrifugation (1000 rpm × 5 min), and the exchange of "Ca" with 
"Ca" was initiated by resuspending the cells in incubation medium at 
4°C which lacked "Ca". One-ml aliquots were removed at intervals, 
and the "Ca" remaining in the cell was determined after centrif-
ugation through oil to separate the cells from the medium as described 
above.

"Ca" Efflux—Erythroid cells were loaded with "Ca" as described 
above. Half the replicates were treated with EP during the last 60 
min of incubation. The efflux was initiated by the addition of EGTA 
(100 mM preincubation with tracer levels of "Ca" and 5 mM final 
concentration during centrifugation). Both methods gave identical 
results which showed that 90–95% of the total "Ca" in the pellet 
from a typical experiment was due to trapped medium. When the 
"Ca" uptake was determined at 4°C, the cells were preincubated 
with EP for varying times at 37°C before being chilled in an ice bath. 
After 15 min in ice, the uptake was initiated by the addition of "Ca" at 
50 rpm/pmol final specific activity and was terminated by centrif-
ugation of the cells through oil as described above.

"Fe Incorporation into Protoporphyrin—To measure the synthesis 
of heme in FVA-infected erythroid cells, the incorporation of ["Fe] 
into protoporphyrin was determined. Human transferrin labeled with 
["Fe]heme was added to each of the cultures for 8 h. Following this incubation, 
the cells were washed twice, and cyclohexanone-extractable ["Fe]
heme was measured as described previously (23). Greater than 95% 
of ["Fe]heme which was extracted by this method chromatographed 
with mouse hemoglobin on CM-cellulose (23).

RESULTS

Erythropoietin Stimulates Uptake of "Ca" by FVA-infected 
Erythroid Cells at 37°C—In order to determine if the mech-
anism of action of EP might include alterations in Ca" 
metabolism of the cells, the effect of EP on "Ca" uptake in 
FVA-infected erythroid cells was examined. "Ca" uptake 
at 37°C in the presence and absence of 2.0 units of pure human 
urinary EP/ml is illustrated in Table I. During incubation for 
1 min, these cells in the presence of EP accumulated 50% 
more "Ca" than control erythroid cells. However, uptake was 
hyperbolic; and, as a steady state level of "Ca" was reached 
(40 pmol of "Ca"/106 cells), the difference in "Ca" uptake 
between erythroid cells in the presence and absence of EP 
became small. Factors present in fetal calf serum had no significant 
effect on "Ca" uptake in FVA-infected erythroid cells under the conditions of this experiment. This experiment 
suggests that the interaction of EP with the EP-response, 
FVA-infected cells is very rapid and alters the metabolism of Ca" in 
the cell in a manner that increased the initial uptake of "Ca".

Erythropoietin Stimulates Uptake of "Ca" by FVA-infected 
Erythroid Cells at 4°C—To examine the effect of EP on 
"Ca" accumulation by these cells under conditions that 
diminish the ability of the Ca"-ATPase to excrete calcium 
(24), "Ca" uptake was studied in the cold (4°C). "Ca" 
accumulation in FVA-infected erythroid cells preincubated in 
the presence and absence of 1.0 unit of EP/ml (human urinary 
EP, 1100 units/mg) for 1 h at 37°C is illustrated in Fig. 1. 
Uptake of "Ca" at 4°C was determined in medium containing 
100 mM CaCl2 (Fig. 1A) and 1.8 mM CaCl2 (Fig. 1B). In 
low CaCl2 medium, the uptake of "Ca" was linear for 8 min. 
This rate of uptake was 12.0 pmol of "Ca"/min for controls 
but increased to 18.0 pmol of "Ca"/min for 106 cells treated 
with EP. In contrast to the experiment measuring "Ca" 
uptake at 37°C, erythroid cells treated with EP achieved a 
higher steady state level of "Ca" than control cells at 4°C. 
This steady state level of "Ca" at 4°C is 2–3-fold higher 
than the corresponding level at 37°C. When "Ca" uptake 
was determined in medium containing the normal CaCl2 
concentration of 1.8 mM, "Ca" uptake was very rapid for 30 
s and then reached a near steady state level by 2 min. 
Erythroid cells treated with EP accumulated 80% more "Ca" 
at the steady state plateau than control erythroid cells during 
the course of the experiment.

Maximum enhancement of "Ca" accumulation at the steady state plateau required 1.0 unit of EP/ml during a 60-
min preincubation at 37°C, as illustrated in Fig. 2. Half-
maximum enhancement of "Ca" uptake occurred after a 
preincubation of 0.5 unit of EP/ml. A minimum of 0.1 unit 
of EP/ml during the preincubation was required to see any 
enhancement of "Ca" uptake. Pure EP had the same effect 
on "Ca" accumulation in erythroid cells on ice as the less 
pure EP, as shown by the data in Table II. Endotoxin, a 
potential contaminant of EP preparations (25), had no effect 
on "Ca" accumulation in this experiment. The effect of EP 
on non-erythroid cells was tested by determining the "Ca" 
uptake in cells from bone marrow cultures infected with FVA
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**Fig. 1.** Effect of EP on $^{45}$Ca$^{2+}$ uptake at 4 °C by FVA-infected erythroid cells. Accumulation of $^{45}$Ca$^{2+}$ was determined in uptake medium containing 100 μM CaCl$_2$ (A) and 1.8 mM CaCl$_2$ (B). Cells suspended in uptake medium were preincubated with or without EP (1.0 unit/ml) for 60 min at 37 °C and then cooled on ice for 15 min. Uptake was initiated by the addition of $^{45}$Ca$^{2+}$ at 50 cpm/pmol final specific activity. After the indicated interval, the $^{45}$Ca$^{2+}$ accumulated by the cells was determined by centrifugation through oil as described under "Experimental Procedures." Trapped medium in the pellet contributed 30% of $^{45}$Ca$^{2+}$ in the experiment shown in A and 12% of $^{45}$Ca$^{2+}$ shown in B; these counts were subtracted from the data.

**Fig. 2.** Relationship of the concentration of EP to enhanced $^{45}$Ca$^{2+}$ uptake in FVA-infected erythroid cells. Cells in uptake medium containing 1.8 mM CaCl$_2$ were preincubated for 60 min at 37 °C with the indicated concentration of EP. The cells were cooled on ice for 15 min, then $^{45}$Ca$^{2+}$ was added at 50 cpm/pmol final specific activity. After 4 min, the $^{45}$Ca$^{2+}$ accumulated by the cells was determined by centrifugation through oil as described under "Experimental Procedures." Symbols represent the average of four determinations from two pooled experiments ± S.D.

or mock-infected. Five-day cultures of FVA-infected bone marrow cells contain from 20 to 50% erythroblasts, while mock-infected cultures have very few identifiable erythrocyte cells. EP enhanced $^{45}$Ca$^{2+}$ uptake in FVA-infected cells by 40% but increased the uptake of $^{45}$Ca$^{2+}$ in mock-infected cells by less than 10% (data not shown). These experiments suggest that the effect of these preparations of EP on $^{45}$Ca$^{2+}$ uptake is specific to erythroid cells and not due to factors other than EP.

The influence of the time of preincubation with EP on $^{45}$Ca$^{2+}$ uptake in FVA-infected erythroid cells at 4 °C was investigated. As shown by the data in Fig. 3, enhancement of $^{45}$Ca$^{2+}$ uptake in these cells was maximal after a preincubation with 1.0 unit of EP for 5 min. This alteration in $^{45}$Ca$^{2+}$ uptake due to EP probably occurs within 1 min but cannot be determined, since an interval of 3-5 min is necessary to either cool the cells on ice or spin out the cells and resuspend them in cold uptake medium.

Varying the time of preincubation of the cells with 1.0 unit of EP from 5 to 120 min resulted in increased $^{45}$Ca$^{2+}$ uptake.

**Table II**

<table>
<thead>
<tr>
<th>Addition</th>
<th>$^{45}$Ca$^{2+}$ uptake</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>120 ± 12</td>
<td></td>
</tr>
<tr>
<td>1.0 unit pure EP/ml (70,000 units/mg)</td>
<td>223 ± 19*</td>
<td>184</td>
</tr>
<tr>
<td>1.0 unit EP/ml (1,100 units/mg)</td>
<td>219 ± 7b</td>
<td>182</td>
</tr>
<tr>
<td>10 ng endotoxin/ml</td>
<td>115 ± 15</td>
<td>95</td>
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*Average of three determinations ± S.D.

*Significantly different from control (p ≤ 0.01).
of similar magnitude for each time, as shown by the data in Fig. 3. These erythroid cells were found to decline in viability during preincubation in the liquid medium, such that experiments in which the time of preincubation with EP was longer than 4 h were not meaningful. When EP was added to methylcellulose cultures of FVA-infected bone marrow cells 20 h before the erythroid cells were isolated, the erythroid cells from cultures treated with 0.1 unit of EP/ml accumulated 160 ± 22 pmol of 45Ca2+/106 cells while control cells accumulated 116 ± 4 pmol of 45Ca2+/106 cells during a 10-min uptake experiment at 4°C. Apparently, EP affects the metabolism of Ca2+ for at least 20 h.

**Erythropoietin Stimulates Exchange of 45Ca2+ from FVA-infected Erythroid Cells**—To investigate the possibility that 45Ca2+ was exchanging with cellular Ca2+, the effect of EP on the release of preloaded 45Ca2+ from erythroid cells was studied. As shown by the data in Fig. 4, the release of 45Ca2+ from erythroid cells at 4°C preloaded with 45Ca2+ for 4 h was hyperbolic in medium containing 1.8 mM CaCl2; a rapid loss of 45Ca2+ occurred during the first 2–4 min which was followed by a slower, linear loss of 45Ca2+ for at least 8 min. Treatment of erythroblasts with 1.0 unit of EP/ml for 60 min prior to the start of this experiment resulted in a 2.5-fold increase in the release of 45Ca2+ during the rapid phase of 45Ca2+ release. Comparing the magnitude of the rapid phase of 45Ca2+ loss in Fig. 4 suggests that the interaction of EP with these cells increased the size of a pool of rapidly equilibrating 45Ca2+ from 5% of the total 45Ca2+ loaded in the cell to 12%.

**Erythropoietin Stimulates 45Ca2+ Efflux from FVA-infected Erythroid Cells**—The net efflux of 45Ca2+ was studied at 37 and 4°C. At 37°C, the release of 45Ca2+ from preloaded cells was biphasic. A rapid phase of 45Ca2+ release occurred within 1 min which accounted for 50% of the total 45Ca2+ loaded during a 4-h preincubation. The remaining 45Ca2+ was very slowly released. Prior treatment of these cells with EP had little apparent effect (data not shown). However, reducing the temperature of the erythroid cells to 4°C extended the rapid phase of 45Ca2+ release to 10 min during which the release of 45Ca2+ was linear (data shown in Fig. 5). The rate of net efflux of 45Ca2+ from cells preincubated in the presence of 1.0 unit of EP/ml was 2-fold higher than the rate of efflux from control cells. These data indicate that the interaction of EP with

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**Fig. 4.** Effect of EP on exchange of 45Ca2+ from preloaded FVA-infected erythroid cells. Cells were suspended in uptake medium containing 1.8 mM CaCl2 and 5.0 μCi of 45Ca2+/ml and incubated for 4.0 h. EP was added to 1.0 unit/ml to half the cells during the last 60 min of incubation. The cells were then sedimented by low speed centrifugation (1000 rpm for 5 min) and resuspended in cold (4°C) uptake medium without 45Ca2+. Aliquots containing 10⁶ cells were removed at the indicated intervals, and the cell-associated 45Ca2+ was determined after centrifugation through oil as described under "Experimental Procedures." Triplicate determinations were done for the time 0 data points; control contained 5100 ± 200 cpm (± S.D.), while cells treated with EP contained 5600 ± 300 cpm (± S.D.). The data were standardized to these values. ○—○, minus EP; ——, plus EP.

**Fig. 5.** Effect of EP on efflux of 45Ca2+ from preloaded FVA-infected erythroid cells. Cells were suspended in uptake medium containing 1.8 mM CaCl2 and 5.0 μCi of 45Ca2+/ml and incubated for 4 h at 37°C. One unit of EP/ml was added to half the cells for the last 60 min of incubation. Efflux was measured at 4°C after the uptake medium was depleted of free Ca2+ by the addition of EGTA to a final concentration of 10 mM. After the indicated interval, cell-associated 45Ca2+ was determined after centrifugation through oil as described under "Experimental Procedures." Triplicate determinations were performed for time 0 points and showed no difference between control cells and cells treared with EP. Data from two separate experiments are shown. Symbols represent a single determination of 45Ca2+ in 10⁶ cells. ○—○, minus EP; ——, plus EP.

**Fig. 6.** Relationship between the concentration of EP and 59Fe incorporation into protoporphyrin in isolated FVA-infected erythroid cells. Erythroid bursts were plucked from cultures and recultured at 10⁶ cells/ml in normal culture medium. The indicated concentrations of EP were added. After 40 h, human transferrin saturated with 59Fe was added to the culture (1.0 μCi of 59Fe/75 μg of transferrin/5 × 10⁶ cells). At 48 h, the cells were washed and 59Fe heme was extracted as described under "Experimental Procedures." Symbols represent the mean of three determinations ± S.D. At 48 h, all wells contained 1.6 × 10⁶ cells/ml.
FVA-infected erythroid cells alters the Ca\textsuperscript{2+} metabolism of the cell such that the rate of Ca\textsuperscript{2+} release is increased.

**Erythropoietin Stimulates Heme Synthesis**—In order to compare the amount of EP required to affect Ca\textsuperscript{2+} metabolism in FVA-infected erythroid cells with the amount of EP required for late erythroblast differentiation, the effect of increasing concentrations of EP on the incorporation of \(^{45}\)Fe into protoporphyrin in isolated erythroblast cells was tested. Data shown in Fig. 6 illustrate that heme synthesis was stimulated by 100-fold after 48 h in the presence of 1.0 unit of EP/ml. Heme synthesis was stimulated over a wide range of EP concentrations, and increased heme synthesis after a 48-h exposure to EP was observed with concentrations of EP (0.01-0.1 unit/ml), which had no significant effect on Ca\textsuperscript{2+} metabolism after a 1-h exposure to EP (Fig. 2). It is of interest that the net level of Ca\textsuperscript{2+} uptake and release in the absence of EP was increased by more than 45Ca\textsuperscript{2+} uptake in these cells. However, this increase may not indicate an effect of EP on the net level of Ca\textsuperscript{2+} in the cell.

**Discussion**

We report here one of the earliest effects of EP yet described, an enhancement of Ca\textsuperscript{2+} accumulation after incubation of the FVA-infected erythroid cells with EP for 1 min at 37°C. A simple explanation of the data reported here is that a gate or pore which allows Ca\textsuperscript{2+} to pass the plasma membrane is opened when EP interacts with the cell. However, it is conceivable that alterations in the intracellular mobilization of Ca\textsuperscript{2+} (mitochondrial or endoplasmic reticulum) occur when EP interacts with the cell such that transport of Ca\textsuperscript{2+} into and out of the cell is increased. Moreover, alterations in the uptake of 45Ca\textsuperscript{2+} at 37°C in the FVA-infected cells which result from treatment with EP may be explained by an increase in the exchangeability of a pool of Ca\textsuperscript{2+} with extracellular Ca\textsuperscript{2+}. Since the steady state levels of Ca\textsuperscript{2+} accumulated by cells treated with EP and control cells were the same, the effect of EP appears to be an acceleration of the equilibration of Ca\textsuperscript{2+} with cellular Ca\textsuperscript{2+}. It is not clear from this experiment how the Ca\textsuperscript{2+} regulatory system of the cell was affected by treatment with EP.

Reducing the temperature at which Ca\textsuperscript{2+} uptake is determined has been used in several laboratories to measure changes in Ca\textsuperscript{2+} accumulation under conditions that limit the excretion and mobilization of Ca\textsuperscript{2+} within the cell (16, 24). A 2-fold increase in the steady state level of Ca\textsuperscript{2+} in control cells resulted from reducing the temperature at which uptake was determined from 37 to 4°C. This most likely indicates that the total level of Ca\textsuperscript{2+} accumulated by cells at 4°C was increased because the efflux of Ca\textsuperscript{2+} was reduced at 4°C. After the FVA-infected erythroblast cells were preincubated with EP at 37°C, the steady state level of Ca\textsuperscript{2+} accumulated at 4°C increased to 180% of the level in control cells. However, this increase may not indicate an effect of EP on the net level of Ca\textsuperscript{2+} in the cell.

In order to test if exchange of extracellular Ca\textsuperscript{2+} with intracellular Ca\textsuperscript{2+} occurred in these cells at 4°C, we examined the release of Ca\textsuperscript{2+} from preloaded cells. Cells preincubated in the presence of EP showed enhanced release of Ca\textsuperscript{2+} over control cells. This effect represents the increase of a rapidly exchanging pool of Ca\textsuperscript{2+} from 5 to 12% of the radioabeled cellular Ca\textsuperscript{2+}. The similarity of the effect of EP on the exchange of Ca\textsuperscript{2+} from preloaded cells with the effect on enhanced uptake of Ca\textsuperscript{2+} suggests that exchange of intracellular Ca\textsuperscript{2+} with extracellular Ca\textsuperscript{2+} contributes to the enhanced accumulation of Ca\textsuperscript{2+} at 4°C after treatment of the cells with EP. However, the extent to which treatment of the cell with EP changes the net level of cellular Ca\textsuperscript{2+} at 4°C cannot be determined since we did not establish the stoich-ometry of the exchange of Ca\textsuperscript{2+} with cellular Ca\textsuperscript{2+}. It is not clear if the increased rate of entry and higher steady state level of Ca\textsuperscript{2+} which follow treatment of the FVA-infected cells with EP result from an increased net Ca\textsuperscript{2+} entry, acceleration of a Ca\textsuperscript{2+}/Ca\textsuperscript{2+} exchange mechanism, or the availability of cellular Ca\textsuperscript{2+} for exchange with extracellular Ca\textsuperscript{2+}.

Efflux of Ca\textsuperscript{2+} from FVA-infected cells preincubated with EP was increased compared to control cells. Whereas some, if not all, of the effect of treatment with EP on Ca\textsuperscript{2+} uptake and release can be seen as increased exchange of intracellular Ca\textsuperscript{2+} with extracellular Ca\textsuperscript{2+}, the effect of EP on the efflux of Ca\textsuperscript{2+} from these cells should be independent of this exchange. This increased rate of efflux of radiolabeled Ca\textsuperscript{2+} from cells treated with EP could result from the activity of a transport mechanism for Ca\textsuperscript{2+} on the cell surface or an alteration in the internal pools of Ca\textsuperscript{2+} such that the net efflux of Ca\textsuperscript{2+} was increased. Since the experiments which investigated the effect of EP on the uptake of Ca\textsuperscript{2+} in these cells failed to clearly demonstrate an effect on the net increase of Ca\textsuperscript{2+} transported into the cell but indicated an increase in exchangeable pools of Ca\textsuperscript{2+}, there is some suggestion that the effect of EP on efflux of Ca\textsuperscript{2+} is due to increased availability of cellular Ca\textsuperscript{2+} rather than an effect on a transport mechanism. However, a direct effect of EP on the Ca\textsuperscript{2+} transport mechanism in these cells cannot be excluded from these experiments.

The cellular location of the exchangeable pool of Ca\textsuperscript{2+} which was increased after treatment of the FVA-infected erythroblast cells with EP was not identified in this investigation. However, the possibility that this pool of Ca\textsuperscript{2+} on the cell surface seems unlikely since the cells were treated with 5 mM EGTA during centrifugation through oil to reduce surface-bound Ca\textsuperscript{2+}, and the necessity of reducing the temperature to observe an effect of EP on the increased accumulation of Ca\textsuperscript{2+} suggested the involvement of intracellular organelles which mobilize Ca\textsuperscript{2+} rather than cell surface binding sites for Ca\textsuperscript{2+}.

Higher concentrations of EP were required to stimulate uptake of Ca\textsuperscript{2+} in the cell than the lower concentrations of EP which were sufficient to induce erythroblast maturation; however, these measurements were temporally distant by 47 h. Treatment of the cells with 0.1 unit of EP for 20 h resulted in a greater effect on the accumulation of Ca\textsuperscript{2+} than the effect observed after treatment of the cells with EP for 1 h (38% increase in Ca\textsuperscript{2+} uptake compared to an increase of 20%). This suggests that a longer time of treatment can increase the effect of suboptimal concentrations of EP on the metabolism of Ca\textsuperscript{2+} in these cells. Since at least a 12-h preincubation with EP is necessary to see any commitment of the FVA-infected cell to erythroblast maturation, the exposure of the cell to concentrations of EP less than 0.1 unit/ml may alter the metabolism of Ca\textsuperscript{2+} in a less apparent but necessary manner during this interval to trigger erythroblast maturation.

We have investigated the effect of agents which block Ca\textsuperscript{2+} entry and facilitate Ca\textsuperscript{2+} entry into cells on the EP-induced erythroblast maturation of these FVA-infected erythroblast cells. In these studies to be published elsewhere, Ca\textsuperscript{2+} ionophore, A23187, and dimethyl sulfoxide increased the uptake of Ca\textsuperscript{2+} and enhanced the ability of suboptimal levels of EP to induce hemoglobin synthesis in these cells. Reducing the concentration of CaCl\textsubscript{2} in the medium and treatment with drugs which block the entry of Ca\textsuperscript{2+} into cells blocked or reduced the effect of EP on the maturation of the FVA-infected erythroblast cells. These results are consistent with the idea that an effect of...
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EP on the metabolism of Ca$^{2+}$ in the FVA-infected erythroid cells is a necessary event in erythroid maturation. These studies are similar to prior studies of normal murine erythropoiesis (13) and murine erythroleukemia cells (15-18) which have provided evidence that changes in the metabolism of Ca$^{2+}$ are an integral part of erythroid maturation.

In summary, treatment of the FVA-infected erythroid cell with EP results in a rapid effect on the size of exchangeable pools of $^{45}$Ca$^{2+}$ within these cells. It is not clear from this study how the alterations in Ca$^{2+}$ metabolism which result from treatment of the FVA-infected cells with EP are related to the final erythroid maturation induced by EP, but changes in the Ca$^{2+}$ metabolism are likely to be involved in the mechanism of action of EP.

REFERENCES


Erythropoietin stimulates 45Ca2+ uptake in Friend virus-infected erythroid cells.
S T Sawyer and S B Krantz


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