Ca$^{2+}$ Permeability in Deoxygenated Sickle Cells

By M.D. Rhoda, M. Apovo, Y. Beuzard, and F. Giraud

Deoxygenation of sickle cells is known to increase cation permeabilities (Na$^+$, K$^+$, and Ca$^{2+}$). The possible mechanisms involved in the increased uptake of Ca$^{2+}$ were investigated: activation of Ca$^{2+}$ channels, involvement of the anion channel, and the formation of endocytic vacuoles. The Ca$^{2+}$ channel blocker nifedipine reduced the deoxygen-stimulated Ca$^{2+}$ uptake by about 30% to 40%. The anion channel inhibitor DIDS (4,4’ diisothiocyanate stilbene 2,2’ disulfonate) inhibited the deoxygen-stimulated Ca$^{2+}$ uptake by approximately 50%. Maximal possible endocytic uptake, measured by using an impermeant marker ([$^{3}$H] inulin), accounted for 6% to 9% of the total Ca$^{2+}$ uptake. These data indicate that the deoxygenation-induced increase in Ca$^{2+}$ permeability could result from both the activation of a Ca$^{2+}$ channel and of a transport system for cations involving interactions between polymerized hemoglobin S, band 3 and other membrane components. Endocytosis appears to play only a minor role in the Ca$^{2+}$ uptake of deoxygenated sickle cells.

© 1990 by The American Society of Hematology.

---

Ddeoxygenation of sickle cells induces the polymerization of hemoglobin S, sickling, and a variety of functional abnormalities of the red blood cell membrane, particularly an elevated permeability to Na$^+$, K$^+$, and Ca$^{2+}$.

While reoxygenation restores the normal shape and membrane properties of most cells (reversibly sickled cells), prolonged deoxygenation or cycles of deoxygenation-reoxygenation results in the presence of a subpopulation of cells that becomes permanently deformed, the so-called irreversibly sickled cells (ISC). These ISC exhibit a loss of cell volume associated with an increased monovalent cation permeability resulting in a K$^+$ loss exceeding the Na$^+$ gain. In addition, Ca$^{2+}$ accumulates in intracellular inside-out vesicles, increasing the total cellular calcium content (40 to 50 μmol/L instead of 5 to 10 μmol/L in normal erythrocytes). However, the concentration of the free cytosolic ionized Ca$^{2+}$ ([Ca$^{2+}$]) remains normal when determined in the oxygenated state (20 to 40 mmol/L). The increased Ca$^{2+}$ permeability upon deoxygenation of sickle cells could contribute to cell dehydration by activation of a Ca$^{2+}$-dependent K$^+$ channel during cycles of deoxygenation-reoxygenation.

The apparent Ca$^{2+}$-dependent formation of dense cells during such cycles, and the inhibition of this process by some Ca$^{2+}$ channel blockers, led us to examine, using nifedipine, the contribution of a putative Ca$^{2+}$ channel to the deoxygenation-induced Ca$^{2+}$ uptake. Additionally, the effect of DIDS (4,4’ diisothiocyanate stilbene 2,2’ disulfonate), an anion channel inhibitor, on the Ca$^{2+}$ influx was also investigated, since DIDS has been shown to inhibit the K$^+$ loss in deoxygenated sickle cells, and Ca$^{2+}$ could enter the cell through the anion channel, as a monovalent anion complex with chloride, as already reported for Zn$^{2+}$.

Finally, the extent of Ca$^{2+}$ uptake due to the formation of endocytic vacuoles was evaluated using an impermeant marker, [$^{3}$H] inulin.

MATERIAL AND METHODS

Heparinized blood samples were obtained from healthy, nontransfused adult patients homozygous for sickle cell hemoglobin (SS cells) and from normal adults (AA cells).

Preparation of erythrocytes. Freshly drawn erythrocytes were washed three times in the following buffer: 140 mmol/L NaCl, 5 mmol/L KCl, 1 mmol/L MgCl$_2$, 10 mmol/L glucose, 1 mmol/L Na$_2$HPO$_4$/NaH$_2$PO$_4$, 10 mmol/L HEPES (pH 7.4) (buffer A). These cells were either immediately used for chelator loading and Ca$^{2+}$ flux measurements or kept overnight at 4°C and subsequently incubated in an adenosine triphosphate (ATP) regenerating system (2 mmol/L adenosine, 10 mmol/L inosine in buffer A, 45 minutes at 37°C, hematocrit 15%) before chelator loading.

Ca$^{2+}$ influx measurements. Ca$^{2+}$ fluxes were measured after incorporation of a Ca$^{2+}$ chelator, quin2, in order to increase the exchangeable intracellular Ca$^{2+}$ pool. Chelator loading can be carried out either in the absence or in the presence of Ca$^{2+}$ in the medium. In the first case, during the chelator loading, the endogenous Ca$^{2+}$ pool was depleted, and upon Ca$^{2+}$ addition, a unidirectional Ca$^{2+}$ influx was measured. In the second case, the endogenous pool was increased up to an equilibrium during the chelator loading, and upon Ca$^{2+}$ addition, bidirectional Ca$^{2+}$ fluxes were observed. Either one or the other procedure was used, as indicated in the text.

Cells were incubated in buffer A containing either 1 mmol/L CaCl$_2$ or no CaCl$_2$ (37°C, hematocrit 30%) and quin2-AM (Lancaster Synthesis, Morecambe, UK; 200 pmol/L of cells). After 45 to 60 minutes, the cells were washed three times in buffer A, with or without 1 mmol/L CaCl$_2$. The concentration of intracellular free Ca$^{2+}$ ([Ca$^{2+}$]$_i$) was calculated as previously described.

Chelator-loaded cells in the presence of CaCl$_2$ and resuspended in buffer A (hematocrit 10%) containing 1 mmol/L CaCl$_2$ were incubated at 37°C with $^{45}$Ca (CEA, Oris, France; 370 kBq/pmol). After 60 minutes’ incubation with $^{45}$Ca under air, deoxygenation experiments were done on a part of the cell suspension by flushing it with humidified N$_2$. The $^{45}$Ca content of the cells was measured from time zero corresponding to the addition of $^{45}$Ca up to 120 minutes, as described. When required, the cells were preincubated at 37°C for 10 minutes with or without nifedipine (N 7634; Sigma, St Louis, MO) before the addition of $^{45}$Ca. The effect of DIDS (D3514; Sigma) was determined in cells preincubated for 30 minutes at 37°C with DIDS and then washed and loaded with quin2-AM. The contribution of endocytosis to the $^{45}$Ca uptake was determined by

From INSERM CJF 8814, Centre hospitalier, Pointe-à-Pitre, Guadeloupe; INSERM U.91, Hôpital Henri Mondor, Créteil; and CNRS UA 646, Physiologie de la nutrition, Université Paris XI, Orsay, France.

Submitted July 3, 1989; accepted February 26, 1990.

Supported by research grants from INSERM and CNRS.

Address reprint requests to M.D. Rhoda, INSERM CJF 8814, Centre Hospitalier Régional et Universitaire, BP 465, Pointe-à-Pitre 97159, Guadeloupe, France.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1990 by The American Society of Hematology.

0006-4971/90/7512-0017$3.00/0
suspending the cells in buffer A containing [3H] inulin (2.6 MBq/mL of cells), an impermeant marker, and 45CaCl2 (1 mmol/L).

Chelator-loaded erythrocytes in the absence of CaCl2 were resuspended in buffer A (hematocrit 10%) and preincubated at 37°C for 10 minutes with or without nifedipine before the addition of 0.1 or 1 mmol/L 45CaCl2 (3.700 or 370 kBq/μmol). In some experiments, cells were deoxygenated at 37°C, 30 minutes before the addition of 45Ca. The 45Ca content of the cells was measured at different times up to 20 minutes.

The ATP content, measured after chelator loading (UV test combination, Boehringer-Mannheim, West Germany), was reduced by 40% in AA cells, but only by 30% in SS cells, as previously reported. The percentage of cell lysis was calculated by measuring the hemoglobin concentration in the supernatant and in the total cell suspension spectrophotometrically at 540 nm.

RESULTS

Stimulation of Ca2+ uptake in deoxygenated SS cells and inhibition by nifedipine. In the experiments shown in Fig 1, quin2 loading and Ca2+ uptake were done in the presence of 1 mmol/L CaCl2. Ca2+ uptake in SS cells slightly exceeded that in AA cells under oxygenated conditions, but was strongly enhanced upon deoxygenation (Fig 1A and B). Deoxygenation had no effect on the Ca2+ uptake in AA cells (Fig 1A). Ca2+ influx, under oxygenated conditions, was calculated from the plot of Log (45Caeq - 45Ca) = f(t), as previously described. Ca2+ influx in SS cells due to deoxygenation was estimated by the measurement of the initial rate of the 45Ca uptake. As reported in previous studies, the rate of influx in deoxygenated SS cells was found to be about five times that of oxygenated SS cells (Table 1).

Nifedipine was used to define the fraction of total Ca2+ influx due to the Ca2+ channel. After preincubation with nifedipine (10 minutes, 50 μmol/L), 45Ca2+ influx was inhibited in oxygenated AA and SS cells, as well as in deoxygenated SS cells (Fig 1A and B). Deoxygenation of SS cells enhanced both the nifedipine-sensitive and the nifedipine-resistant part of the Ca2+ influx (Table 1). However, the nifedipine-sensitive influx was increased by eightfold, whereas the nifedipine-resistant one was increased by only threefold. The extent of inhibition of Ca2+ uptake by nifedipine in AA cells in our experiments was found to be lower than previously reported data. Previous data were obtained with nitrendipine or verapamil in vanadate-treated erythrocytes, a condition that induces a carrier-mediated Ca2+ uptake.

The same experiments were done with erythrocytes loaded with quin2 in the absence of Ca2+. This procedure, which causes a decrease in the intracellular Ca2+ concentration, may induce a stimulation of the influx of Ca2+, as previously reported, and may increase the extent of inhibition by the Ca2+ entry blockers. In fact, under these conditions, the Ca2+ influx was not stimulated (Table 1), and the extent of inhibition by nifedipine remained low (30% to 40% maximum, Fig 2). Ca2+ uptake in these Ca2+-depleted SS cells, reincubated with 1 mmol/L CaCl2 (Table 1B), was stimulated by deoxygenation to a similar extent than under steady-state conditions of internal Ca2+ (Table 1A). Again, the nifedipine-sensitive fraction of the Ca2+ uptake in SS cells was much more stimulated by deoxygenation than was the nifedipine-resistant one. Ca2+ uptake was also investigated in SS cells that had been loaded with quin2-AM in the presence of 1 mmol/L CaCl2, washed, and reincubated with 1 mmol/L 46CaCl2. When required, nifedipine (50 μmol/L) was added 10 minutes before 46Ca. After 60 minutes under air, deoxygenation was induced by humidified N2. Data are means of duplicate determinations in a single experiment: three others gave similar values. In the experiment shown, the ATP content was 0.42 and 0.55 mmol/L of AA and SS cells, respectively, after quin2 loading. Intracellular quin2 concentration was 140 and 120 μmol/L of AA and SS cells, respectively. Hemolysis was 4% maximum after 1 hour of deoxygenation.

Fig 1. 45Ca uptake by AA (A) and SS cells (B) under oxygenated or deoxygenated conditions and inhibition by nifedipine. Cells were loaded with quin2-AM in the presence of 1 mmol/L CaCl2, washed, and reincubated with 1 mmol/L 46CaCl2. When required, nifedipine (50 μmol/L) was added 10 minutes before 46Ca. After 60 minutes under air, deoxygenation was induced by humidified N2. Data are means of duplicate determinations in a single experiment: three others gave similar values. In the experiment shown, the ATP content was 0.42 and 0.55 mmol/L of AA and SS cells, respectively, after quin2 loading. Intracellular quin2 concentration was 140 and 120 μmol/L of AA and SS cells, respectively. Hemolysis was 4% maximum after 1 hour of deoxygenation.
CA²⁺ PERMEABILITY OF DEOXY SICKLE CELLS

Table 1. Effect of Nifedipine on CA²⁺ Influx

<table>
<thead>
<tr>
<th>CA²⁺ Influx (μmol/L of cells/h)</th>
<th>AA Cells</th>
<th>Oxy</th>
<th>Oxy</th>
<th>Deoxy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total influx</td>
<td>29.8 ± 2.6</td>
<td>42.9 ± 3.9</td>
<td>181 ± 11</td>
<td></td>
</tr>
<tr>
<td>Nifedipine-sensitive</td>
<td>4.8 ± 0.9</td>
<td>6.9 ± 1.5</td>
<td>58 ± 10</td>
<td></td>
</tr>
<tr>
<td>Nifedipine-resistant</td>
<td>25.1 ± 4.7</td>
<td>36.0 ± 6.7</td>
<td>124 ± 20</td>
<td></td>
</tr>
<tr>
<td>% Inhibition</td>
<td>16</td>
<td>16</td>
<td>32*</td>
<td></td>
</tr>
</tbody>
</table>

Cells were loaded with quin2-AM in the presence (A) or in the absence (B and C) of CaCl₂, washed and reincubated with either 1 mmol/L (A and B) or 0.1 mmol/L (C) CaCl₂ under air (oxy) or under humidified N₂ (deoxy). (A) Deoxygenation of SS cells was started after 60 minutes of equilibrium with ⁴⁰Ca under air, as described in the text (Methods) and in the legend of Fig 1. (B and C) SS cells were preincubated for 30 minutes before the addition of ⁴⁰Ca. When required, nifedipine (50 μmol/L) was added 10 minutes before ⁴⁰Ca. Values given are the means ± SE of five experiments or means of two experiments.

*P < .02.

In other experiments (data not shown), maximal inhibition of the CA²⁺ uptake (30% to 40%) by oxygenated AA or SS cells was also observed with 10 μmol/L nicardipine or 0.5 μmol/L nitrendipine.

Inhibition of deoxy-stimulated CA²⁺ uptake by DIDS. Pretreatment with DIDS (done before quin2-AM loading) had no effect on the ⁴⁰Ca uptake of oxygenated SS cells, but inhibited CA²⁺ uptake under deoxygenated conditions (Fig 3). This inhibition was concentration-dependent, and the maximum inhibition occurred at about 15 μmol/L of DIDS (Fig 4). Replacement of NaCl and KCl by NaNO₃ and KNO₃ did not modify the CA²⁺ uptake of SS cells under either oxygenated or deoxygenated conditions nor did it modify the extent of inhibition of DIDS of the deoxy-stimulated CA²⁺ uptake (data not shown).

Ca²⁺ uptake due to endocytosis. [³H] inulin, an impermeant marker, was used to measure the extent of endocytosis and to calculate the fraction of the CA²⁺ uptake due to this process. AA and SS cells, chelator-loaded in the presence of 1 mmol/L CaCl₂, were incubated in the same medium containing ⁴⁰Ca and [³H] inulin. After 60 minutes under air, a fraction of the suspension was deoxygenated. The uptake of each marker is shown in Fig 5. The volume of external medium incorporated in the cells (expressed as percent of cell volume) was calculated from [³H] radioactivity incorporated in the cells (cpm/μL of cells) and from the specific radioactivity of [³H] inulin in the external medium (cpm/μL of external medium). Under oxygenated conditions, a small incorporation of the [³H] marker was observed in normal AA cells and a slightly higher one in SS cells. In both cases a plateau level was reached after 10 minutes. Deoxygenation of SS cells resulted in a delayed increase in the incorporation of the [³H] marker up to a plateau level reached after 60 minutes. The difference between the oxy and deoxy plateau levels in SS cells could reflect the endovesiculation due to deoxygenation. This was found to be 1% of the cell volume (Fig 5B). Since the extracellular CA²⁺ concentration is 1 mmol/L, this incorporation of extracellular medium allowed the uptake of 10 μmol CA²⁺/L of cells. During the same time, the total exchangeable CA²⁺ in this experiment was about 110 μmol/L of cells, a value corresponding to the plateau level obtained upon deoxygenation. Thus, the contribution of endocytosis to the CA²⁺ uptake would be 10 μmol/L of cells, or 9% of the total ⁴⁰Ca uptake. In two additional experiments, endocytosis accounted for 6.5% and 6.8% of the total CA²⁺ uptake. In other experiments (data not shown), incorporation of [³H] inulin was observed both in light (1.068 < d < 1.078) and in dense (d > 1.096) SS cell fractions obtained from Stractan density gradients. ²³ In addition, when deoxygenation was done after addition of sufficient EGTA to reduce the extracellular CA²⁺ concentration to less than 50 nmol/L, [³H] inulin remained at the same level as that observed under oxy conditions, indicating that the process was dependent upon the presence of CA²⁺ in the medium (data not shown).

Fig 2. Inhibition of CA²⁺ uptake by nifedipine in oxygenated AA (○) or SS cells (●). Cells were loaded with quin2-AM in the absence of CaCl₂, washed, and reincubated with 0.1 mmol/L ⁴⁰CaCl₂ with or without nifedipine (added 10 minutes before ⁴⁰Ca). ⁴⁰Ca uptake, measured up to 20 minutes, increased linearly with time under these conditions (data not shown). The values shown are means ± SE of five experiments or means of two experiments.
DISCUSSION

The purpose of the present study was to investigate the possible mechanisms involved in the increased Ca\(^{2+}\) uptake noted in sickle cells upon deoxygenation. Experimental evidence was shown for the presence of a dihydropyridine-sensitive (30% to 40%) and a DIDS-sensitive Ca\(^{2+}\) influx (40% to 50%), as well as the direct endocytosis (6% to 9%) of Ca\(^{2+}\).

All the experiments were done after loading the erythrocytes with the calcium chelator quin2. Moderate concentrations of chelator were incorporated (100 to 140 pmol/L of cells) in order to prevent important ATP depletion.\(^{26}\) The level of ATP, measured after chelator loading, remained within values sufficient to maintain a normal activity of the Ca\(^{2+}\) pump.\(^{27}\)

The \(^{45}\)Ca content of incubated oxygenated SS cells increased gradually up to a pump-leak steady-state of 15 to 25 pmol/L of cells. From this value and that of the concentration of intracellular quin2, \([\text{Ca}^{2+}]\), was calculated (18 to 30 mmol/L). This value was not different from that in AA cells, in agreement with previous reports.\(^{8,10}\) Upon deoxygenation, \(^{45}\)Ca uptake increased up to a new steady-state of about 100 to 120 pmol/L of cells, a value exceeding that expected from the concentration of quin2 incorporated if \([\text{Ca}^{2+}]\), was maintained at its low level, as should be the case in SS cells with normal Ca\(^{2+}\) pump activity. The resealing of some lysed cells during deoxygenation could account for part of the observed Ca\(^{2+}\) uptake. However, assuming that all the uptake of the extracellular marker \(^{3}\)H inulin (1% of cell volume) was due to cell damage and resealing, the Ca\(^{2+}\) uptake that can be attributed to this process would be only 10 pmol/L of cells. Since SS cell populations are heterogeneous, it is possible that Ca\(^{2+}\) permeability is increased in some cells that do not contain quin2. In one experiment, in which SS cells were fractionated into light and dense populations (see...
results), sickling induced the same Ca\(^{2+}\) uptake in both fractions. A more likely explanation of our data is that this excess Ca\(^{2+}\) entering the cell is rapidly sequestered into an intracellular compartment lacking quin2, such as the endocytic inside-out vesicles previously described in SS cells. They can pump actively cytosolic Ca\(^{2+}\) but do not contain quin2, since this latter cannot cross the membrane in its free form.

The calcium channel blocker nifedipine (50 μmol/L) inhibited partially the Ca\(^{2+}\) influx in deoxygenated SS cells and in oxygenated SS or AA cells preloaded with quin2-AM either in the absence or presence of Ca\(^{2+}\) in the medium. The extent of inhibition of Ca\(^{2+}\) influx by nifedipine was low (20% to 40%). Interestingly, when the external Ca\(^{2+}\) concentration was 1 mmol/L, nifedipine was a slightly more potent inhibitor of Ca\(^{2+}\) uptake in deoxy than in oxy SS cells or oxy AA cells, suggesting that deoxygenation of SS cells induces an activation of a Ca\(^{2+}\) channel. Although the concentration dependence of this process on external Ca\(^{2+}\) was not investigated over a wide range of external Ca\(^{2+}\) concentrations, the data show that at 0.1 mmol/L there was no activation. Nitrendipine (15 μmol/L) has been reported to inhibit 70% of the Ca\(^{2+}\) influx in vanadate-treated oxygenated AA cells. In addition to inhibiting Ca-ATPase, vanadate opens a carrier-mediated transport pathway similar to Ca\(^{2+}\) channels of excitable tissue. An inhibitory effect of nifedipine was also observed by Engelmann and Duhm in vanadate-treated but not in quin2-loaded erythrocytes. The lack of effect by nifedipine in the latter case was presumably due to the fact that, in their study, the rate of Ca\(^{2+}\) uptake was determined by atomic absorption spectrophotometry, a method less sensitive than \(\text{\textsuperscript{45}}\text{Ca}\) measurements and only between 0 and 15 minutes a period too short for an accurate determination of the small inhibition induced by nifedipine.

The possibility that deoxygenation in SS cells induces a transient increase in [Ca\(_i\)], triggering the activation of the Ca\(^{2+}\)-dependent K\(^+\) channel, resulting in K\(^+\) loss and cell dehydration has been suggested previously. However, the mechanism responsible for the increase in Ca\(^{2+}\) permeability has not been clearly understood. The fact that nifedipine, nitrendipine, diltiazem, and other calcium channel blockers have been shown to inhibit K\(^+\) loss and sickle cell dehydration, induced by prolonged deoxygenation or cycles of deoxygenation-reoxygenation has not been excluded. The concentration of DIDS required to inhibit 50% of the deoxy-stimulated Ca\(^{2+}\) uptake (15 μmol/L) was greater than that necessary to block the anion transport (~5 μmol/L). It is thus likely that the effects of DIDS on anion transport and Ca\(^{2+}\) influx are not directly related. In addition, chloride replacement with nitrate had no effect on the inhibition of Ca\(^{2+}\) uptake by DIDS, excluding the possibility of the entry of Ca\(^{2+}\) in the form of a monovalent anion complex with chloride, as described for Zn\(^{2+}\). Inhibition of the deoxy-stimulated Na\(^+\) and K\(^+\) fluxes in SS cells by similar doses of DIDS, also amounting to about 50%, have been reported previously, indicating that the DIDS inhibitory effect is not restricted to Ca\(^{2+}\). It is also known that the interaction of DIDS with band 3 results in changes in the binding properties of the cytoplasmic domain of band 3 with hemoglobin A. It may be assumed that as a consequence of sickling, the interaction of polymerized HbS with band 3 activates a cation transport system responsible for the increased permeability to Na\(^+\), K\(^+\), and Ca\(^{2+}\). Modifications of the conformation of band 3 after its interaction with DIDS may result in a diminution in the activation of the putative cation transport system, as previously suggested.

It has been shown previously that oxygenated SS cells contain internal endocytic vesicles enriched in calcium. Evidence was also provided that these vesicles were formed by endocytosis upon deoxygenation. In order to quantify this process under our experimental conditions, \[^{3}H\] inulin, an impermeant extracellular marker, was used to measure the extent of endocytosis induced by deoxygenation in SS cells. The data show that \[^{3}H\] incorporation into oxygenated AA and SS cells amounted to 0.8% of the total cell volume. In oxygenated cells, the incorporation of \[^{3}H\] inulin most likely did not indicate the formation of endocytic vesicles but only reflected the presence of leaky cells that then resealed upon reincubation at 37°C. This is further indicated by the very rapid saturation of the process (10 minutes). In contrast, the increase in \[^{3}H\] incorporation upon deoxygenation observed only in SS cells and only after a lag time could represent endocytosis. Similar data were obtained by Bookchin et al., who have used \(^{45}\)Co-EDTA or \(^{45}\)Ca-EGTA as extracellular marker and slightly different experimental conditions: (1) SS cells in which Ca\(^{2+}\) pump was inhibited and that were deoxygenated for 3 hours in autologous plasma and (2) SS cells subjected to four repeated 20-minute cycles of deoxygenation-reoxygenation. As mentioned by these authors, the uptake of an extracellular marker does not necessarily represent endocytosis, since lysis and resealing of a small fraction of the cells could also cause the same uptake. However, using an impermeant fluorescent marker, Rubin et al. have observed the presence of brightly staining vesicles in SS cells that increase in number upon deoxygenation. Although this methodology does not permit quantification of the process in terms of its contribution to Ca\(^{2+}\) uptake, it provides direct evidence for endocytosis. Omitting Ca\(^{2+}\) from the medium blocked both the stimulation of Ca\(^{2+}\) influx and the endocytosis. Ben-Bassat et al. have reported that Ca\(^{2+}\) was not required for endocytosis in erythrocytes. However, in the same laboratory it was shown that, in resealed ghost, internal Ca\(^{2+}\) at a low concentration (~1 mmol/L) stimulated vacuole formation. It may be suggested that the deoxy-stimulated endocytosis was caused by the transient increase in [Ca\(_i\)], resulting from the stimulated Ca\(^{2+}\) influx. The extent of endocytosis induced by deoxygenation in SS cells, assuming that all the marker uptake is due to this process (and calculated from the difference between the deoxy and the oxy conditions, amounted to approximately
0.8% to 1% of the total cell volume. Interestingly, we have observed that the formation of the vesicles occurred in all fractions, light or dense, of SS cells, as also reported by Rubin et al. The contribution of endocytosis to the Ca$^{2+}$ uptake upon deoxygenation was found to account for only 6% to 9% of the total Ca$^{2+}$ uptake.

In conclusion, the increase in Ca$^{2+}$ permeability induced by deoxygenation of sickle cells may be linked to the activation of a Ca$^{2+}$ channel. The inhibitory effects of DIDS on Ca$^{2+}$ uptake in deoxygenated SS cells indicates the presence of an additional pathway that merits further work to determine the mechanism underlying its mode of action. Finally, endocytosis was found to play only a minor role in the Ca$^{2+}$ uptake of deoxygenated sickle cells.

Acknowledgment

We thank Dr F. Galacteros of INSERM U.91, Hôpital Henri Mondor, Créteil, France, and Drs C. Berchel, H. Lorent, and L. Foucan of Centre Hospitalier Régional, Pointe-à-Pitre, Guadeloupe, for providing blood samples from their patients.

References

18. Rhoda MD, Galacteros F, Beuzard Y, Giraud F: Ca$^{2+}$ permeability and cytosolic Ca$^{2+}$ concentration are not impaired in $\beta$-thalassemic and hemoglobin C erythrocytes. Blood 70:804, 1987
Ca2+ permeability in deoxygenated sickle cells

MD Rhoda, M Apovo, Y Beuzard and F Giraud

Updated information and services can be found at:
http://www.bloodjournal.org/content/75/12/2453.full.html
Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml