Prostaglandin E₂ Stimulates a Ca²⁺-dependent K⁺ Channel in Human Erythrocytes and Alters Cell Volume and Filterability*

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To understand the mechanism by which human red blood cells (RBCs) contribute to hemostasis and thrombosis, we have examined the effects of metabolites released by activated platelets on intact RBCs. Prostaglandin E₂ (PGE₂), a signal molecule produced by activated platelets, was observed to lower the filterability of human erythrocytes by −30% at 10⁻¹⁰ M. PGE₂ also caused a reduction in mean cell volume of −10%. The shrinkage of red cells after PGE₂ treatment was confirmed by documenting a decrease in osmotic fragility and an increase in cell density following exposure to the hormone. Careful analysis, however, revealed that only ~15% of the erythrocytes responded to stimulation with PGE₂. Examination of the cause of cell shrinkage showed that induction of a PGE₂-stimulated K⁺ efflux pathway leading to rapid loss of cellular K⁺ was responsible. The PGE₂-stimulated K⁺ loss was also observed to be Ca²⁺-dependent, suggesting the possible involvement of the Gardos channel. Gardos channel participation was supported by the observation that two Gardos channel inhibitors, charybdotoxin and clotrimazole, independently blocked the PGE₂-stimulated K⁺ efflux. Further evidence for Gardos channel activation came from experiments aimed at characterizing the efflux pathway followed by the obligatory counterion. Thus, K⁺ efflux was readily stimulated even when NO₃⁻ was substituted for Cl⁻, suggesting that neither KCl cotransport nor Na/K/Cl cotransport plays a prominent role in the PGE₂-induced cell shrinkage. Further, the anion transporter band 3 was implicated as the counterion efflux route, since DIDS inhibited the PGE₂-stimulated cell volume change without blocking the change in membrane potential. Taken together, we propose that release of PGE₂ by activated platelets constitutes part of a mechanism by which activated platelets may recruit adjacent erythrocytes to assist in clot formation.

Red blood cells (RBCs) comprise 99% of the blood cells in circulation. RBCs are typically thought to function only in gas transport, facilitating movement of O₂ from the lungs to the tissues, and CO₂ (as free HCO₃⁻) from the tissues to the lungs. Although generally considered hemostatically inert, isolated observations have suggested that erythrocytes might also participate in clot formation. Thus, clinicians have periodically reported a correlation between low erythrocyte count and prolongation of bleeding time (1-3). Hellem et al. (3, 4) have also documented a dependence of platelet adhesion to glass beads on the presence of red cells, increasing as hematocrit is elevated. More recently, Saniabi and Lowe (5) reported that RBCs markedly enhance spontaneous platelet aggregation in vitro, and Santos and colleagues (6) have demonstrated that erythrocytes enhance platelet serotonin release as well as arachidonate/cyclo-oxygenase production and eicosanoid formation (6). The same group (7) has further demonstrated that collagen-stimulated platelets aggregate 3 times more effectively and discharge 7 times more ADP in the presence of RBCs than in their absence. Taken together, these observations argue for some type of communication between red cells and activated platelets, although at present the diffusible second messengers remain unknown.

To evaluate the mechanism by which red cells recognize an activated platelet and respond molecularly to platelet-generated signals, we have begun to examine the effects of metabolites released by activated platelets on intact RBCs. Because prostaglandin E₂ (PGE₂) is a potent signaling molecule produced by activated platelets, PGE₂ was selected for initial examination. PGE₂ is a metabolic product of arachidonic acid which is released by the action of phospholipase A₂ during platelet activation (8). PGE₂ mediates a broad range of biological activities in diverse tissues through specific plasma membrane receptors (9-11). In normal resting platelet-rich plasma, PGE₂ is essentially absent (8). However, during platelet activation, the level of PGE₂ in plasma increases to about 10⁻⁹ M (8). Allen and Rasmussen (12, 13) have reported that PGE₂ decreases the filtration rate of both human and rat RBCs, and they have consequently suggested that erythrocytes must contain some type of PGE₂ receptor. We report here that PGE₂ activates the Gardos channel in human erythrocytes and thereby induces K⁺ efflux, cell shrinkage, and reduced cell filterability. This reduced cell filterability could conceivably help retain the stimulated RBCs in the newly formed hemostatic plug.

EXPERIMENTAL PROCEDURES

Materials—PGE₂, clotrimazole, and Drabkins solution for hemooglobin measurements were purchased from Sigma. Charybdotoxin was obtained from Peptides International (Louisville, KY). A23187 was purchased from Calbiochem Corp. (La Jolla, CA), and the anion transporter inhibitor, DIDS, was from ICN Biochemicals (Irvine, CA). 3,3'-Dipropylthiadicarbocyanine iodide (diSC₃(5)) was obtained from Molecular Probes, Inc. (Eugene, OR). Stractan, marketed under the trade name, Celsip Universal Solution, was purchased from Larex, Inc. (St. Paul, MN).

Preparation of Erythrocytes—Human blood was withdrawn from healthy volunteers in acid citrate-dextrose solution on the day of the experiment. After centrifugation and removal of the plasma, "buffy coat," and upper 20% of the red cells, the remaining red cells were washed three times in phosphate-buffered saline (150 mM NaCl, 5 mM glucose 5 mM sodium phosphate, pH 7.4), and twice again in buffer A (125 mM NaCl, 3 mM KCl, 1 mM MgCl₂, 2 mM CaCl₂, 1.2 mM sodium phosphate, 50 mM Tris-buffer, pH 7.4 (37 °C), 10 mM glucose) in order to
equilibrate the cells in a Ca\(^{2+}\)-containing medium. During these washing steps, essentially all of the white cells were aspirated away. The washed erythrocytes at 10\% hematocrit in buffer A were then treated with 0.001 volume of PGE\(_2\) dissolved in ethanol and incubated for the desired times.

**Cell Filtration Studies**—Cell filtration studies were performed as described by Ataullakhanov (14, 15). Briefly, a filtration device was used consisting of an upper plastic reservoir equipped with two electronic fluid level detectors separated vertically by 9 mm. At the bottom of the reservoir was a 1-cm diameter membrane filter with 3-\(\mu\)m cylindrical pores prepared by nuclear beam technology. The reservoir was filled either with an RBC suspension at 2\% hematocrit in buffer A containing 5 mg/ml bovine serum albumin or with the serum albumin-containing buffer alone. As the erythrocytes passed through the membrane filter under force of gravity, the two level detectors first activated and then deactivated an electronic chronometer that recorded the time of movement of the meniscus from the first detector to the second. For each membrane filter, the filtration time of the erythrocyte suspension was normalized to the filtration time of the bovine serum albumin solution. The relative filterability of the erythrocyte suspension was then calculated according to the equation: relative filterability = \(T_{RBC}/T_{BSA}\), where \(T_{RBC}\) and \(T_{BSA}\) represent the filtration times of erythrocyte suspensions without and with PGE\(_2\) treatment, respectively; \(T_{BSA}\) is the filtration time of the serum albumin buffer alone measured with the same filter used for the corresponding erythrocyte suspension.

The filtration device and nuclear membrane filters were gifts of Dr. F. I. Ataullakhanov, National Center of Hematology, Moscow, Russia.

**Cell Volume Evaluation**—Flow cytometric forward light scattering was used to estimate the relative sizes of the cells (16–18). Briefly, cell suspensions (10\% hematocrit) were aspirated directly into an EPICS Elite flow cytometer (Coulter Corp., Hialeah, FL) equipped with a 488 nm laser beam. Forward light scattering was recorded at the desired time points following PGE\(_2\) treatment, and mean cell diameter was determined using the computer software provided by the manufacturer. The flow cytometer was calibrated prior to each experiment using the standard beads (9.99 \(\mu\)m) provided by the manufacturer.

Open Filterability of RBCs—Cell suspensions at 10\% hematocrit were diluted 10-fold in media consisting of buffer A with decreasing NaCl concentrations. After incubating at 4°C for 4 min, cell suspensions were centrifuged (1,000 \(\times\) g for 5 min, cell suspensions were centrifuged (1,000 \(\times\) g, 5 min) and 100 \(\mu\)l of supernatant was mixed with 10 volumes of Drabkins solution. The hemoglobin concentrations in the supernatants were determined by measuring the absorbance at 540 nm. Percent hemolysis was defined as

\[
\text{Percent hemolysis} = \frac{A_{540\,nm} - A_{540\,nm\,cont}}{A_{540\,nm\,cont}} \times 100
\]

where \(A_{540\,nm}\) is the absorbance of the supernatant, and \(A_{540\,nm\,cont}\) is the absorbance of the buffer containing buffer alone. As the erythrocytes passed through the membrane filter containing buffer alone, the filtration time of the supernatant was measured (Fig. 1).

**RESULTS**

**Effects of PGE\(_2\) on Erythrocyte Filterability**—It has been shown previously that PGE\(_2\) decreases the filterability of human and rat erythrocytes with maximum potency at \(10^{-10}\) M PGE\(_2\) (12, 13). We have confirmed these results (data not shown) and extended them by evaluating the time course of the change in erythrocyte filterability. Red cells were treated with \(10^{-10}\) M PGE\(_2\) for various times at 37°C, and their rates of passage through a 3-\(\mu\)m membrane filter were measured (Fig. 1). Maximal decrease in filterability occurred 40 min after exposure to PGE\(_2\), by which time the relative rate of flow through the filter had diminished ~30%. Longer incubation times resulted in a gradual return to normal filterability. Unstimulated cells incubated at 37° C for up to 60 min showed no significant change in filterability (data not show), indicating the observed changes were not simply a consequence of prolonged incubation.

**Effect of PGE\(_2\) on Cell Volume, Osmotic Fragility, and Density**—Reductions in cell filterability can be caused by a decrease in cell surface to volume ratio, an increase in viscosity of the cytoplasm, or an elevation of membrane rigidity. Since the former two sources can arise from a change in cell volume, we evaluated the effect of PGE\(_2\) on erythrocyte filterability. As shown in Fig. 2, red cells treated with \(10^{-10}\) M PGE\(_2\) exhibited a gradual decrease in size that reached a maximum of ~10% decrease by 40 min of PGE\(_2\) treatment.

To confirm the observed PGE\(_2\)-stimulated shrinkage, we also evaluated the effect of PGE\(_2\) on cell osmotic fragility. Osmotic fragility is a measure of the ability of red cells to resist osmotic lysis and is generally accepted as a sensitive assay of an eryth-
Prostaglandin E<sub>2</sub> Stimulates a Red Cell Gardos Channel

Protein potential-sensitive dye frequently employed in red cell
value might be anticipated. To explore this possibility, a mem-
counterion, a change in membrane potential to a more negative
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primarily the youngest cells of the population, since such cells
When untreated red cells were exposed to hypotonic buffer, they began to hemolyze at -175 mosm and were almost completely lysed by 70 mosm (Fig. 3a). However, when red cells treated with 10<sup>-10</sup> M PGE<sub>2</sub> were similarly examined, onset of hemolysis occurred normally but completion of osmotic lysis was shifted to much lower osmolarities. At 100 mosm, for example, the number of lysed PGE<sub>2</sub>-treated cells was reduced by -15% relative to controls (Fig. 3a). These data confirm that PGE<sub>2</sub> can promote erythrocyte shrink-
age, and they also suggest that only a fraction of the cells may participate in the PGE<sub>2</sub>-triggered response.

To further evaluate the fractional participation of erythro-
cytes in the PGE<sub>2</sub>-induced cell shrinkage, the density distribu-
tion of erythrocytes was measured before and after treatment
with 10<sup>-10</sup> M PGE<sub>2</sub>. As seen in Fig. 3b, hormone treatment promotes a shift in -15% of the population to higher density. Curiously, the sensitive fraction of cells seems to reside in the middle of the gradient, suggesting the responsive cells are not the young erythrocytes that are the first to be observed in density gradients, thus validating the PGE<sub>2</sub>-triggered response.

**Effect of PGE<sub>2</sub> on K<sup>+</sup> Efflux and Membrane Potential**—A decrease in red cell volume usually results from cell dehydration that accompanies loss of cellular electrolytes. Since K<sup>+</sup> is the main osmotic regulatory ion in human red cells (24), we undertook to look for a change in K<sup>+</sup> efflux following addition of PGE<sub>2</sub>. As shown in Fig. 4a, all three PGE<sub>2</sub> concentrations tested stimulated a loss in cellular K<sup>+</sup>. Also, in agreement with previous observations, 10<sup>-10</sup> M PGE<sub>2</sub> elicited the greatest efflux among the concentrations examined. In fact, when the rate of loss of cellular K<sup>+</sup> was quantitatively measured over the first 5 min of stimulation, 10<sup>-10</sup> M PGE<sub>2</sub> was found to promote an average decrease of 36 mEq/h in intracellular K<sup>+</sup> content (Fig. 4b). In general, however, initial efflux rates were sustained only for 10-20 min, after which they gradually declined to control levels (data not shown).

If K<sup>+</sup> were to exit stimulated erythrocytes faster than its counterion, a change in membrane potential to a more negative value might be anticipated. To explore this possibility, a membrane potential-sensitive dye frequently employed in red cell studies (DiS-C<sub>3</sub>)<sub>(5)</sub> was used (23). DiS-C<sub>3</sub> is a hydrophobic cation that partitions into erythrocytes in proportion to their negative membrane potential, thereby allowing the use of the Nernst equation and the equilibrium ratio of dye activities inside and outside the cell to estimate changes in membrane potential (23). In our studies, changes in membrane potential were calculated based on the assumption that fresh control erythrocytes have a membrane potential of -9 mV (23). Pre-treatment of cells with PGE<sub>2</sub> (10<sup>-10</sup> M) for 40 min at 37°C changed the membrane potential from -9 mV to -16 mV (Table I), reflecting a partially uncompensated loss of cations.

**Identification of the PGE<sub>2</sub>-stimulated K<sup>+</sup> Efflux Pathway**—The partially uncompensated loss of cations from PGE<sub>2</sub>-stimulated erythrocytes suggests that the cation efflux is not mediated by either KCl or Na/K/2Cl cotransport, since these efflux pathways are electrically silent (25, 26). In contrast, RBCs known to be equipped with a Ca<sup>2+</sup>-activated K<sup>+</sup> channel (Gardos channel), whose stimulation leads to cellular dehydration and a decrease in membrane potential (24, 27, 28). To examine the possible involvement of the Gardos channel in the PGE<sub>2</sub> signaling pathway, we looked to see whether the stimulated K<sup>+</sup> efflux might be dependent on extracellular Ca<sup>2+</sup>. For this purpose, 8 mM EGTA was added to the efflux buffer and PGE<sub>2</sub>-stimulated K<sup>+</sup> efflux was again monitored. As revealed in Fig. 5, removal of external Ca<sup>2+</sup> abolished the K<sup>+</sup> efflux, suggesting

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2 P. K. Lauf and N. Adragna, personal communication.
The PGE2-initiated K⁺ suspension participate in the stimulated responses. On the PGE2-activated K⁺ specific Gardos channel inhibitors, ChTX and clotrimazole, the impact of the membrane potential measurements were also obtained. The membrane potential for the control samples was assumed to be -9 mV. These data represent the average of two experiments ± S.E. of the mean.

**Table I**

| Treatment | Estimated membrane potentiala (mV) |
|-----------|-----------------------------------|
| Control   | -9                                |
| PGE2      | -16 ± 1                           |
| PGE2 + ChTX | -11 ± 1          |
| PGE2 + DIDS | -17 ± 0.5                     |
| A23187    | -40 ± 1                           |
| A23187 + ChTX | -11 ± 1          |

a Membrane potential measurements assume all erythrocytes in the suspension participate in the stimulated responses.

The PGE2-initiated K⁺ loss requires Ca²⁺.

To further explore the possible involvement of the Gardos channel in the PGE2-mediated volume changes, the impact of two specific Gardos channel inhibitors, ChTX and clotrimazole, on the PGE2-activated K⁺ efflux was examined (22, 29–31). Importantly, both inhibitors have been previously shown to block the K⁺ loss and dehydration induced in RBCs upon addition of the Ca²⁺ ionophore, A23187 (30, 31). In our studies, we adopted the inhibitory conditions for ChTX of Brugnara et al. (29, 30), who found it useful to lower the extracellular Ca²⁺ concentration to 50 μM to avoid Ca²⁺ inhibition of ChTX binding. In our hands, this decrease in extracellular Ca²⁺ content simultaneously reduced the magnitude of the PGE2-stimulated K⁺ efflux, consistent with the observed Ca²⁺ dependence of the efflux pathway. Further, pretreatment of red cells with 100 nmol/liter ChTX was found to largely abolish the effect of PGE2 on K⁺ loss (Fig. 6). This inhibitory effect of ChTX on the Gardos channel was also observed in related studies of erythrocyte membrane potential. As shown in Table I, ChTX also blocked the hyperpolarization of erythrocytes treated with either PGE2 or A23187, i.e. confirming that the majority of the membrane potential change derives from K⁺ efflux through the Gardos channel.

In related studies, we employed a structurally distinct type of Gardos channel inhibitor, clotrimazole, and observed a similar blockade of PGE2-induced K⁺ efflux (Fig. 6).

To further confirm that the PGE2-induced cell volume change is dependent on the Ca²⁺-activated K⁺ channel, we have taken advantage of the prediction that a measurable volume decrease cannot occur unless a counterion, i.e. Cl⁻, can accompany K⁺ in exiting the cell. Since Gardos channels do not cotransport Cl⁻, any accompanying anion must move through an alternate pathway. The anion transport protein, band 3, represents an obvious candidate for this counterion movement; hence, we examined the impact of the anion transport inhibitor, DIDS, on the PGE2-induced cell volume and membrane potential changes. Pretreatment of red cells with 100 μM DIDS at 37 °C for 1 h completely blocked anion transport activity (data not shown) as well as PGE2-induced cell shrinkage (Fig. 7). In contrast, the hyperpolarization of the membrane potential induced by PGE2 was not significantly affected by DIDS treatment (Table I), changing as usual to -17 mV. We interpret these data to suggest that the PGE2-induced volume change can occur only if a functional anion transporter is available to carry counterions across the membrane. Otherwise, the decrease in membrane potential will effectively prohibit osmotically relevant quantities of K⁺ from leaving the cell.

**FIG. 5.** Blockage of PGE2-stimulated K⁺ efflux by EGTA. Red cell suspensions were incubated with or without 10⁻¹⁰ M PGE2 at 37 °C and K⁺ efflux was monitored as a function of time: ○, control; □, 10⁻¹⁰ M PGE2; △, 10⁻¹² M PGE2 + 8 mM EGTA. Rates of decrease in intracellular K⁺ content are calculated assuming all erythrocytes participate in the efflux.

**FIG. 4.** Stimulation of K⁺ efflux by PGE2 in human erythrocytes. Red cell suspensions were incubated with or without PGE2 at 37 °C. The external K⁺ concentration was measured continuously using a K⁺-sensitive electrode, and these data were used to calculate the loss of intracellular K⁺. Panel a, loss of cellular K⁺ from erythrocytes treated with 0 mM (□), 10⁻¹¹ M (▲), 10⁻¹⁰ M (●), or 10⁻⁹ M (■) PGE2, as a function of time. Panel b, rate of loss of cellular K⁺ over the first 5 min. Each point represents the average value (± S.E. of the mean) obtained from two different experiments. Variations among the data are sometimes smaller than the size of the symbol and therefore do not appear on the graph. Rates of K⁺ loss are calculated assuming all erythrocytes participate in the efflux.

**FIG. 3.** Effect of PGE2 on membrane potential of human erythrocytes. Red cell suspensions were incubated with or without 10⁻¹⁰ M PGE2 at 37 °C for 40 min, and membrane potential was measured as described under "Experimental Procedures." In the inhibition studies, cells were pretreated with or without ChTX (100 nmol/liter) or DIDS (100 μmol/liter), as described in Figs. 6 and 7. Inhibitor-treated cells were also treated with or without 19 M A23187 for 5 min, following which membrane potential measurements were also obtained. The membrane potential for the control samples was assumed to be -9 mV. These data represent the average of two experiments ± S.E. of the mean.

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**Evaluation of the involvement of other K⁺ Efflux Pathways—**

The data presented above argue strongly for a PGE2-activated Gardos channel, but they do not exclude the possible minor participation of a Cl⁻-dependent K⁺ transport pathway, e.g.
KCl or Na/K/2Cl cotransport. To evaluate this possibility, we substituted NO₃⁻ for Cl⁻ in the incubation buffer and re-examined PGE₂ activation of K⁺ efflux. As shown in Fig. 8, removal of external Cl⁻ did not inhibit the PGE₂-stimulated K⁺ efflux. Thus, with reasonable confidence it can be concluded that the Gardos channel is the only volume regulatory channel activated by PGE₂.

**DISCUSSION**

We have demonstrated that PGE₂ reduces human erythrocyte filterability by stimulating K⁺ efflux leading to a loss of osmotic water and cell shrinkage. We have further shown that the discharged K⁺ exits the cell through the Gardos channel. Evidence for this latter contention derives from (i) the dependence of channel activation on external Ca²⁺, (ii) the inhibition of K⁺ efflux by two specific Gardos channel inhibitors (i.e. charybdotoxin and clotrimazole), (iii) the retention of channel function upon substitution of NO₃⁻ for Cl⁻, and (iv) the absence of electrical neutrality that is characteristic of the more prominent KCl and Na/K/2Cl cotransport pathways. To the best of our knowledge, this is the first documentation of ligand activation of a Gardos channel in any cell type. Nevertheless, several papers have reported PGE₂ activation of K⁺ efflux from nonerythroid cells without identifying the channel involved (32, 33), raising the possibility that our observations on red cells may not be an isolated phenomenon.

With the limited information available, it is difficult to evaluate the physiological relevance of the PGE₂-stimulated volume changes. Arguments suggesting that erythrocyte shrinkage might contribute to hemostasis include (i) cell shrinkage significantly impacts cell filterability, (ii) the observed volume changes are maximal at PGE₂ concentrations released by activated platelets (8), and (iii) some type of communication between erythrocytes and activated platelets has already been well documented (5–7). The major argument against a physiological role for the PGE₂-induced volume decrease is that it appears to proceed too slowly to significantly impact clot formation, i.e. clotting times for whole blood treated with procoagulant ranges from 1 to 5 min. However, many physiological regulators are missing from the simplified red cell suspension employed in these studies, and it is conceivable that co-stimulation by other signals surrounding activated platelets could greatly synergize the effect of PGE₂. Indeed, we have recently observed that thrombin elicits a similar group of signaling
intermediates in red cells as PGE\(_2\). Whether synergy between platelet-derived signals actually occurs in vivo must obviously await further experimentation, but it would indeed be surprising if a cell that constitutes >99% of the total blood cell mass did not participate in some manner in hemostasis.

The data we have presented obviously require PGE\(_2\) activation of a regulated Ca\(^{2+}\) influx channel in human erythrocytes. At present, Ca\(^{2+}\) is believed to enter red cells primarily via passive leaks, especially in sickle cells where membrane deformation is thought to permit transient ion fluxes in and out of the cell (34). However, the normal impermeability of erythrocytes to Ca\(^{2+}\) plus the absolute requirement of the PGE\(_2\)-stimulated Gardos channel for extracellular Ca\(^{2+}\) argues that PGE\(_2\) somehow induces a regulated Ca\(^{2+}\) uptake. In nonerythroid cells, PGE\(_2\) is thought to bind a classical G protein-coupled receptor that functions via a pertussis toxin-sensitive GTP-binding protein to activate phospholipase C (11, 33, 35). In this pathway, the subsequent release of inositol trisphosphate leads to Ca\(^{2+}\) channel opening and a transient elevation of cytoplasmic Ca\(^{2+}\). It will be important to learn whether a similar signal transduction pathway operates in erythrocytes, and whether red cells express an inositol trisphosphate-activated Ca\(^{2+}\) channel.

One major question raised by our data concerns the nature of the erythrocyte subpopulation that participates in the PGE\(_2\)-stimulated cell shrinkage. Thus, both the osmotic lysis and density gradient analyses suggest that up to 85% of erythrocytes may remain refractory to PGE\(_2\) (Fig. 3a). Unfortunately, the flow cytometric analyses are too insensitive to allow further confirmation or rebuttal of this estimate (Figs. 2 and 7). Furthermore, the cell filterability assays (Fig. 1), the K\(^+\) efflux experiments (Figs. 4–6 and 8), and the membrane potential measurements (Table I) report only average values for the entire population of cells examined. Indeed, if we were assumed that only 15% of the erythrocytes were stimulated by PGE\(_2\), then the PGE\(_2\)-induced changes in membrane potential and K\(^+\) efflux rates of participating red cells would have to be increased by a factor of ~6. Furthermore, the ability of the Ca\(^{2+}\) ionophore A23187 to protect more red cells against osmotic lysis than PGE\(_2\) (Fig. 3a) and to lower average erythrocyte membrane potential more dramatically than PGE\(_2\) (Table I) also suggests that a larger fraction of cells can respond to direct stimulation with Ca\(^{2+}\) than to PGE\(_2\). Where the signaling pathway between PGE\(_2\) binding and Gardos channel activation fails in nonparticipating erythrocytes is obviously an important question, but without more information it will have to await further scrutiny.

Finally, we undertook these studies to explore how erythrocytes might facilitate both platelet secretion and aggregation, as previously reported by others (5–7). We have observed that a hormone released by activated platelets can indeed stimulate erythrocytes, but we have learned nothing regarding how stim-

\(^3\) Q. Li, V. Jungmann, A. Kiyatkin, and P. S. Low, unpublished observations.

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