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Lead-induced activation and inhibition of potassium-selective channels in the human red blood cell

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The selective increase of net K^+ permeability in human red cells brought about by either Ca^{2+} or lead was studied using a light scattering technique to measure net K^+ fluxes in cell suspensions and the patch-clamp technique to study K^+ transport in individual K^+-selective channels of the red cell membrane. Using ultrapure solutions it was demonstrated that the effect of lead is neither the indirect consequence of a lead-induced increase of the accessibility of the receptor sites of the K^+-selective channels to traces of Ca^{2+} that are present as contamination in analytical grade reagents nor to the release of Ca^{2+} from intracellular Ca^{2+} stores. It is further shown that in cell-free membrane patches low concentrations of lead (10 μM) in Suprapur solutions evoke the same single-channel events as added Ca^{2+} and that this activity can be inhibited by high concentrations of lead (100 μM), similar to the net KCl efflux measured by means of the light scattering technique. It is concluded, therefore, that both Ca^{2+} and lead independently activate the same K^+-selective channels in the red cell membrane.

Introduction

In 1935, it was observed by Ørskov [1] that traces of lead induce a rapid efflux of potassium from the red blood cells of the human and a number of other animal species. The permeability change for potassium takes place without a significant increase of sodium influx. The osmotic content of the cells decreases and the cells shrink, indicating that the potassium ions carry with them an equivalent amount of chloride ions.

Later studies have shown that similar selective increases of K^+ efflux can be produced by a variety of treatments of the red blood cells [2–5]. All of these treatments involve the use of calcium [6] and it is now generally agreed that intracellular calcium ions are responsible for the permeability changes and the ensuing shrinkage of the cells [7].

A comparison of the available information on the effects of calcium and lead led to the suggestion that both metal ions activate the same transport system, although their effects on the system may differ in certain details [8].

One of the problems associated with the comparative studies on the effects of lead and calcium consisted of the fact that most analytical grade chemicals used for the preparation of physiological solutions contain contaminating calcium ions. It remained unclear, therefore, to what extent the permeability change induced by lead was the consequence of an increase of Ca^{2+} influx or of a liberation of Ca^{2+} from intracellular Ca^{2+} stores.

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Abbreviations: EGTA, ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid; H_2DIDS, 4,4′-diisothiocyanatodihydrostilbene-2,2′-disulfonate; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Mops, 4-morpholinepropanesulfonic acid.

The present paper addresses this question. In addition, it provides observations that may help to further clarify the similarities and differences between the effects of lead and calcium. Recently, it had been shown by means of the patch-clamp technique that calcium opens aqueous channels which are responsible for the selective increase of potassium permeability [9,10]. It seemed useful, therefore, to demonstrate that lead produces similar effects and that the specific details of the effects of lead on the K^+ flux as measured by chemical methods are the consequence of the behaviour of the individual channels as determined by the patch-clamp technique. The specific effect of lead investigated in the present context is the self-inhibition of potassium flux observed at high lead concentrations. The observations reported below are in complete agreement with the previously expressed view [8] that both calcium and lead are capable of activating the same K^+ selective channels in the red blood cell membrane.

Methods

Cell preparation and flux measurements

Human red blood cells freshly drawn with heparin as anticoagulant were washed two times in ice-cold Cl^- medium containing (mM), NaCl(150), EDTA(1), pH 7.4 and then three times in NO_3^- medium containing (mM), $NaNO_3$(150), KNO_3(1), Hepes(20), pH 7.4. They were then made up to a 10% cell suspension in the same medium.

The net K^+ efflux from red cells induced by lead or calcium, which is accompanied by anion and H_2O loss, was calculated from the decrease in volume of the cells as measured by the increase of right angular light scattering as described previously [11].

The photometric readings were transformed into cell volume by means of a standard curve, which was obtained by varying cell volume by the addition of small volumes of concentrated $NaNO_3$ (to NO_3^- medium) or NaCl (to Cl^- medium) to the cell suspension in the photometric cell. Cell volume was found to vary according to Ponder's equation [12] and hence the volume could be read off in relative units from a standard curve relating the light extinction to the reciprocal of the tonicity in the medium.

Experiments minimising Ca^{2+} in the media

6-day-old red cells were washed three times in a medium consisting of (mM), $NaNO_3$(50), KNO_3(100), Hepes(20), EGTA(1), pH 7.4 and suspended at 10% hematocrit in the same medium. The cells were then incubated with 0.66 μM A23187 at 37°C for 30 min. They were subsequently washed twice in the above medium containing 0.5% bovine serum albumin to remove A23187, and then a further three times in NO_3^- medium made from Suprapur $NaNO_3$. The Ca^{2+} concentration was determined with an atomic absorption spectrophotometer, Perkin-Elmer Model 372 equipped with a graphite furnace M6A-76B. The cells were then suspended at 10% hematocrit in the Suprapur $NaNO_3$ medium.

Patch-clamp experiments

The gigaseal patch-clamp technique [13] was used to measure single-channel currents in cell-free, inside-out membrane patches where the internal membrane surface is exposed to the bath solution (for details, see Ref. 10). The solution inside the pipette was in contact with the external membrane surface. It contained (in mM) $MgCl_2$(1), KCl(150), Mops(10) and 1.36 μM Ca^{2+} as an impurity. The bath solution in contact with the internal membrane surface could be changed. The composition of the solutions used is indicated in the figure legends.

Materials

A23187, Ca^{2+} free, was from Calbiochem-Behring Corp., U.S.A. Suprapur $NaNO_3$ and KCl was from E. Merck, Darmstadt, F.R.G. Bovine serum albumin and valinomycin were from Sigma, Munich, F.R.G. H_2DIDS was a gift from Professor H. Fasold, Institute for Biochemistry, University of Frankfurt. All other chemicals were reagent grade.

Results

(1) Calcium does not contribute to the activation of K^+-efflux by lead

Analytical grade reagents contain sufficient calcium as a contaminant to activate the K^+-selective channels in the red cell membrane. This is

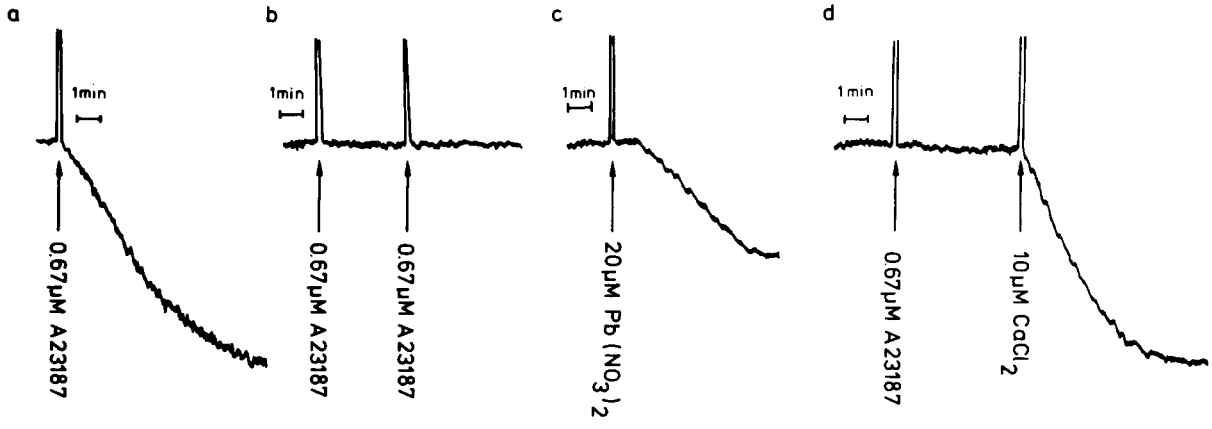

Fig. 1. The activation by A23187, calcium or lead of K^+ efflux. Red cells were suspended at a hematocrit of 0.06% in a medium containing (mM) $NaNO_3$(160), KNO_3(1), pH 7.0, 37°C made from either reagent grade (a) or Suprapur (b, c and d) $NaNO_3$. The Ca^{2+} concentration in the Suprapur solution was 0.2 μM and in the reagent grade solution 7–8 μM. At the times indicated A23187 (0.6 μM), Ca^{2+} (10 μM) or $Pb(NO_3)_2$ (20 μM) were added to the media and the ensuing K^+ efflux followed by the shrinkage of the cells. The sharp upward deflections in the traces of this and the subsequent figures indicate the opening and closing of the photometer cuvette during the various additions that are marked by the arrows.

illustrated by the experiment shown in Fig. 1a. Red cells had been suspended in a medium made up from $NaNO_3$, KNO_3 and Tris-NO_3. After addition of the calcium ionophore A23187 a rapid shrinkage occurs, indicating that KNO_3 leaves the red cells. Analysis of the medium by atomic absorption spectrophotometry revealed a Ca^{2+} concentration of 7.5 μM, which is known to suffice for the activation of the channels by combination with the receptor sites at the inner membrane surface [14].

When the analytical grade $NaNO_3$, which constitutes the bulk of the electrolyte in the medium, is replaced by $NaNO_3$ Suprapur, the addition of A23187 induces no KNO_3 efflux (Fig. 1b). This is to be expected from the analytically observed Ca^{2+} concentration of about 0.2 μM, which is below the threshold for the activation of the channels. Addition of 10 μM Ca^{2+} to the A23187-containing suspension immediately elicits KNO_3 efflux (Fig. 1d). When $Pb(NO_3)_2$ is added in place of calcium, an essentially similar response is observed (Fig. 1c). It is clear, therefore, that lead as such is capable of inducing the permeability change.

One could, nevertheless, argue that the lead that enters the red cells is releasing Ca^{2+} from intracellular stores. This possibility could be excluded by experiments with cells which had been depleted of intracellular Ca^{2+} by several incubations and washings with A23187 and extracellular EGTA. After removal of ionophore and EGTA by further washes, there was little or no K^+ loss when fresh A23187 was added (Fig. 2a). An increase of K^+

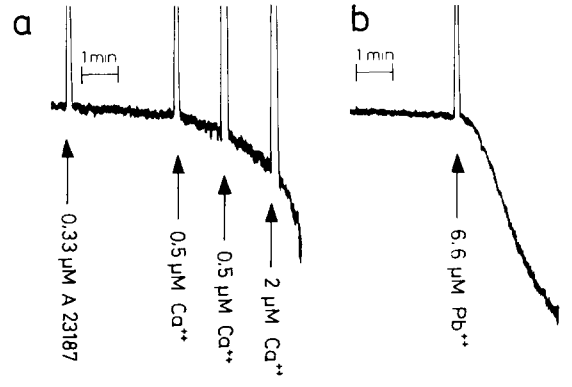

Fig. 2. Activation of K^+ efflux by calcium or lead from cells depleted of internal calcium. Red cells were depleted of internal calcium by incubation for 30 min at 37°C at a hematocrit of 10% in a medium containing $NaNO_3$(50), KNO_3(100), Hepes(20), EGTA(1), pH 7.4 plus 0.66 μM A23187. A23187 was then removed by washing in the same medium plus 0.5% bovine serum albumin. The cells were washed a further three times in Suprapur $NaNO_3$. K^+ efflux was elicited in the Suprapur $NaNO_3$ medium (see Fig. 1) by the addition at the times indicated of A23187, calcium or lead and the shrinkage of the cells recorded.

loss was observed, however, when sufficient calcium was added to the medium to obtain a final concentration of 3 μM. When instead of calcium, lead was added to the calcium-depleted cells, a similar enhancement of K^+ efflux could be demonstrated, indicating a genuine action of lead itself (Fig. 2b).

The experiments described above have been performed at low hematocrit (0.06%). At higher hematocrit, contamination of the media with Ca^{2+} becomes less significant since the Ca^{2+} that enters the cells will be sequestered by the naturally occurring complex forming compounds inside the red cells. At a hematocrit of about 10% or more contamination by Ca^{2+} of media made from analytical grade reagents produces only a minimal increase of K^+ efflux.

(2) Dependence of K^+ efflux on lead concentration

The K^+ efflux elicited by increasing $Pb(NO_3)_2$ concentrations is shown in Fig. 3. To describe the effects of lead on KNO_3 efflux from the red cells, three parameters need to be measured:

(1) The length of time elapsing between the addition of $Pb(NO_3)_2$ and the onset of KNO_3 efflux (lag period).

(2) The maximal rate of KNO_3 efflux.

(3) The amount of KNO_3 lost before the rate of loss returns to the same low level seen before the addition of lead. (This loss may be smaller than the loss required for complete equilibration of KNO_3 between the red cell and the medium.)

An analysis of the curves in Fig. 3 shows that the lag period decreases with increasing lead concentration and becomes too short to be measurable above 20 μM $Pb(NO_3)_2$ (Fig. 4c). The maximal rate of loss (i.e. the maximal slope of the curves relating volume to time) increases (Fig. 4a) and so does the amount of K^+ lost in the time period between the initiation of the permeability change and the cessation of the K^+ efflux (Fig. 4b). Above 10 μM $Pb(NO_3)_2$, both the maximal rate of loss and the amount of KNO_3 efflux are reduced, indicating a self inhibition. The inhibition lost in the time up to the cessation of KNO_3 is not due to blockage of the exit of the anion that accompanies the K^+ exit. This can be dem-

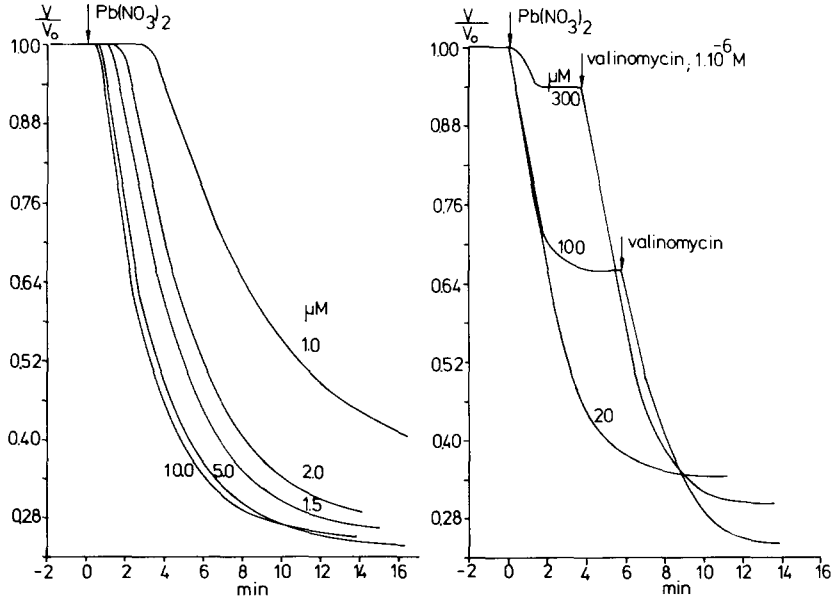

Fig. 3. Activation and inhibition of selective K^+ efflux by $Pb(NO_3)_2$. K^+ efflux was induced by the addition of $Pb(NO_3)_2$ at the times designated by arrows. The final concentrations are indicated on the respective curves. The medium contained (in mM) $NaNO_3$(140), Tris-NO_3(20), KNO_3(1.0), pH 7.6 at 37°C. The hematocrit was 0.07%. For clarity of representation, experiments at low and high lead concentrations have been plotted in separate panels. Ordinates: volume at time t (V) as a fraction of volume at time zero (V_0). Abscissas: time.

onstrated by the addition of valinomycin to the cell suspension after the cessation of KNO_3 efflux (Fig. 3). KNO_3 efflux is now resumed indicating that the K^+ efflux induced by the carrier peptide is accompanied by the efflux of nitrate. It would be tempting to calculate from the curve relating KNO_3 efflux to $Pb(NO_3)_2$ concentration half-saturation constants for activation and inhibition of the K^+ channels. However, such calculations are not meaningful since:

(1) Most of the lead is taken up by the red cells and bound to hemoglobin and other cell constituents [15,16]. Hence the concentration of free lead that is at equilibrium with lead bound to the channels is not known.

(2) A variation of the amount of K^+ lost in addition to the variation of the rate of loss suggests that different cells in the population show a different response. This phenomenon is either due to an unequal distribution of the lead amongst the cells of the population or to different thresholds in the individual cells. The implications of these interpretations have been discussed previously [8,17] but are not repeated again since they are not relevant to what follows below.

(3) Potentiation of the action of lead by the ionophore A23187

Besides Ca^{2+} and Mg^{2+}, the ionophore A23187 is capable of combining with other divalent and trivalent cations [18,19,28], including possibly lead.Experiments similar to those represented in Fig. 3, were made, except the $Pb(NO_3)_2$ was added to cell suspensions that contained in the nitrate medium 10 μM EGTA either with or without the subsequent addition of the ionophore A23187. The presence of the ionophore affects all three parameters that are required to describe the action of lead. As compared to a control with the same concentration of EGTA but without A23187, the shrinkage of the cells becomes apparent at a lower concentration of $Pb(NO_3)_2$ (Fig. 5a), the lag period is reduced to an immeasurably short length of time (Fig. 5c), the maximal enhancement of KNO_3 efflux is reached at a lower concentration (Fig. 5a) and the self inhibition at high lead concentration is more complete (Fig. 5b). Addition of valinomycin to red cells that showed self inhibition at lead

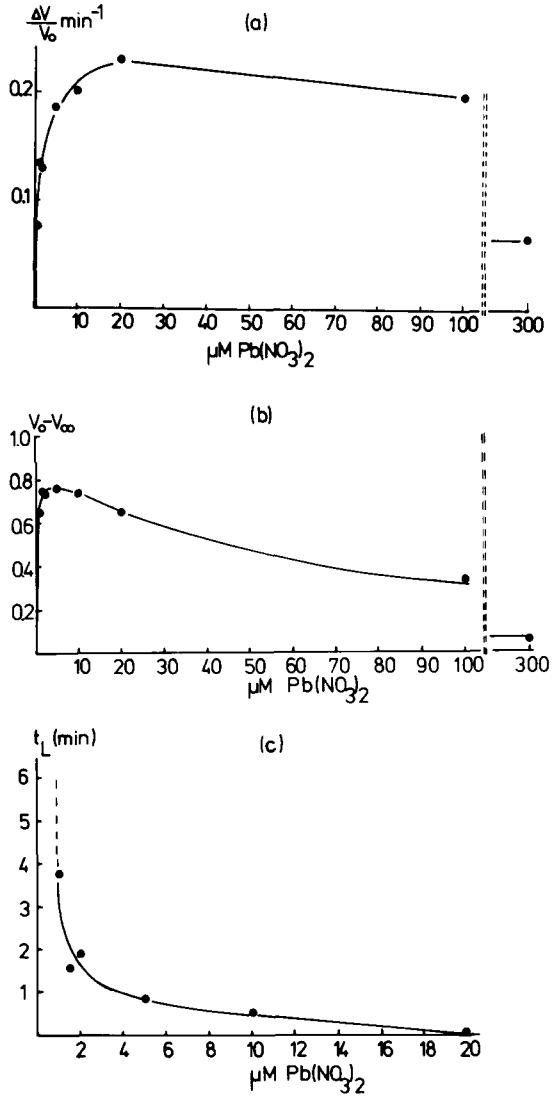

Fig. 4. (a) Maximal slopes of the curves represented in Fig. 3. These slopes are roughly equivalent to rate constants of KNO_3 net efflux.

(b) Volume changes between the time of addition of $Pb(NO_3)_2$ and the time after the curves become parallel to the abscissa. These volume changes are a measure of the amount of K^+ lost in the time-course of an experiment. In some of the experiments the time of observation extended beyond the time scale on the abscissa in Fig. 3 and thus permitted the determination of the differences without extrapolations.

(c) The lag period between the addition of $Pb(NO_3)_2$ to the medium and the onset of K^+ efflux derived from the data shown in Fig. 3. Ordinate: Lag period (t_L) in min. Abscissa: $Pb(NO_3)_2$ concentration (μM).

concentrations up to 300 μM caused a rapid shrinkage, indicating that the combined effects of lead and ionophore did not involve an inhibition of net anion transport (not shown).

(4) Inhibition of calcium or lead-induced KNO_3 efflux

When A23187 is added to a calcium-containing medium, then KNO_3 efflux is stimulated (Fig. 6a) and the cells shrink. However, when the addition of ionophore is closely followed by the addition of a high concentration of lead the KNO_3 efflux is inhibited (Fig. 6b). This inhibition of calcium-activated KNO_3 efflux is similar to the inhibition seen in the presence of cobalt (Fig. 6c). In both cases the subsequent addition of valinomycin results in further KNO_3 loss, indicating the inhibition by lead and cobalt is not due to an inhibition of anion transport, but represents a genuine action on the K^+-selective channels. Cobalt produces a similar inhibition of lead-induced KNO_3 efflux (not shown). However, the effects of Co^{2+} on lead and calcium-induced K^+ loss differ in some detail. $CoCl_2$ added after induction of the net K^+ efflux by Ca^{2+} plus A23187 leads to a rapid cessation of K^+ efflux (see Fig. 6c). When the efflux is induced by lead, several minutes are required for the inhibitory effect to develop (not shown). Thus, the lead-induced K^+ efflux continues up to the diffusion equilibrium even after addition of Co^{2+} since the length of time necessary for K^+ equilibration is shorter than the time required for the Co^{2+} to become fully inhibitory. However, when the cells are exposed to Co^{2+} for about 10 min prior to the addition of lead, the inhibition of the lead effect on the K^+ channels is virtually complete, provided the Co^{2+} had been added together with A23187. Without the ionophore, the Co^{2+} remains ineffective presumably because it cannot reach the site of channel blockage (not documented).

When Mg^{2+} is added to the medium, the K^+ efflux seen in response to Ca^{2+} is inhibited, while

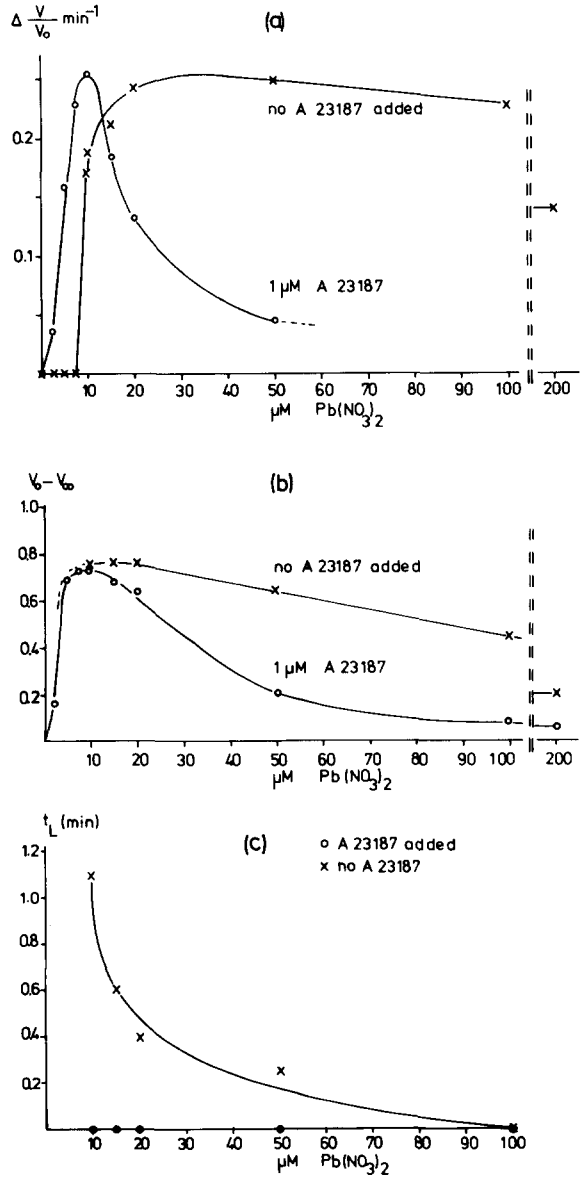

Fig. 5. The effect of A23187 on the parameters used to describe the lead activation of KNO_3 efflux.

(a) Maximal slopes of the curves relating the decrease in volume of cells to time induced at the lead concentrations indicated. The cells were suspended in a medium containing (mM), $NaNO_3$(160), Tris-NO_3(1.0), KNO_3(1.0), and 10 μM EGTA, pH 7.4, 37°C. The last two data points in the curves presenting the volume changes after lead plus A23187 were omitted since their determination is too inaccurate to be meaningful.

(b) Volume changes between the time of addition of $Pb(NO_3)_2$ or $Pb(NO_3)_2$ and A23187 and the time after the curves become parallel to the abscissa for the cells in (a).

(c) The lag period between the addition of lead alone ('no A23187 added') or lead plus A23187 ('1 μM A23187') at the concentrations indicated and the initiation of cell shrinkage.

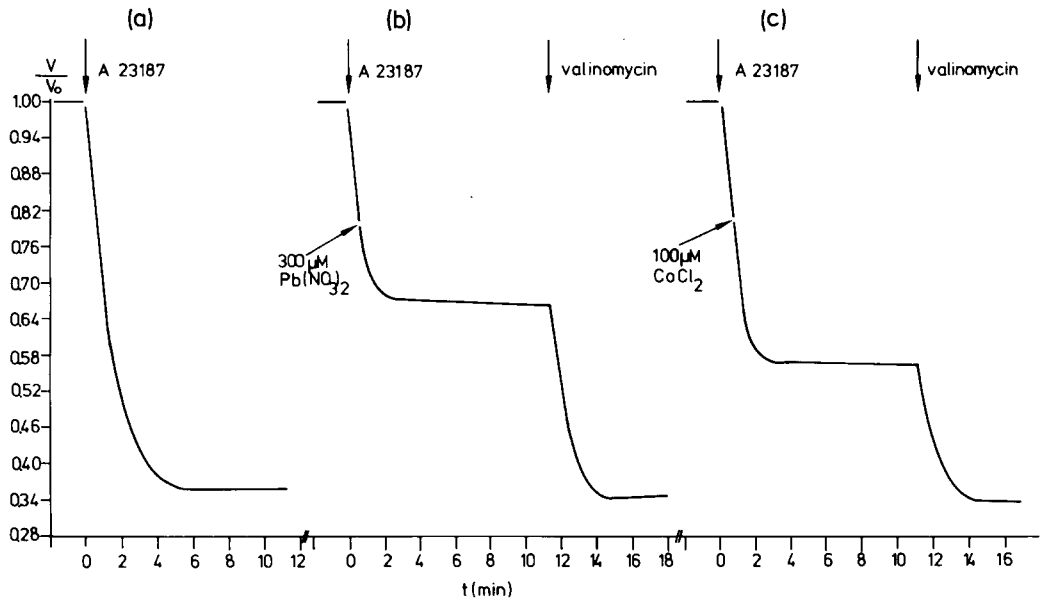

Fig. 6. Inhibition of Ca^{2+}-activated K^+-selective channels by high concentrations of cobalt and lead. K^+ efflux was induced at the times indicated by the addition of A23187 (final concentration 1 μM) to the Ca^{2+}-containing (20 μM) medium (mM) $NaNO_3$(140), Tris-NO_3(20), KNO_3(1.0), pH 7.6, hematocrit 0.07%, 37°C. At the times indicated $CoCl_2$, $Pb(NO_3)_2$ or valinomycin was added to give the final concentration of 1 μM. Ordinate: volume at time t (V) as a fraction of volume at time zero (V_0). Abscissa: time.

the efflux caused by lead remains unaffected (not documented). Mg^{2+} replaces Ca^{2+} on the ionophore and thus can be expected to prevent the penetration of Ca^{2+} into the red cells. A replacement by Mg^{2+} of the Pb^{2+} bound to the ionophore would not prevent the permeability change, since lead evokes its action even in the absence of the ionophore. The experimental results suggest, therefore, that external Mg^{2+} does not inactivate the K^+ channels; rather the inhibition of the Ca^{2+}-induced K^+ efflux is due to a replacement of Ca^{2+} on the ionophore.

In conclusion of this section it may be mentioned that the trivalent rare earth ion Tb^{3+} which is known to inhibit specifically the Ca^{2+}-induced K^+-channel activity [20] also inhibits the channel activity evoked by lead (not shown).

(5) Effects of H_2DIDS on the action of lead

The specific anion transport inhibitor H_2DIDS could possibly interfere with the action of lead in two ways: (1) Lead is known to form anionic complexes including complexes with nitrate and chloride. It would seem feasible, therefore, that the metal is transferred into the red cell via the anion exchange system in the membrane. Hence H_2DIDS could possibly inhibit the access of lead to its site of action and thus retard or prevent K^+ efflux. (2) The rate of lead-induced K^+ efflux should not only depend on K^+ efflux through the activated K channels but also on the permeability of the membrane for the accompanying anions. Since H_2DIDS would inhibit the band 3-mediated component of net anion flux, a partial inhibition of K^+ efflux across the K^+ channels could be expected.

Experimentally it was observed that H_2DIDS, at a concentration at which the band 3-mediated anion transport is completely inhibited, does not interfere with the response of the cells to lead. Thus an inhibition of lead influx by H_2DIDS is too small to be detectable with the time resolution of our method. The K^+ efflux induced by lead is, however, partially inhibited by the agent (Table I). The inhibition is small in nitrate media [28] and more pronounced in chloride media, where it reaches a percentage (about 65%) similar to that reported in the literature for valinomycin-induced K^+ efflux [11,21,22].

TABLE I

THE EFFECT OF H_2DIDS ON THE RATE OF LEAD-INDUCED POTASSIUM EFFLUX FROM RED CELLS IN CHLORIDE OR NITRATE MEDIA

Cells were washed as described in the methods section in chloride or nitrate containing media and suspended in a medium containing either $NaNO_3$(160), KNO_3(1), Hepes(20) pH 7.4 at 37°C at 0.07% hematocrit with or without H_2DIDS(10 μM) or in a medium in which the nitrate salts were substituted by chloride salts. KCl or KNO_3 efflux was initiated by addition of $Pb(NO_3)_2$ (20 μM) and recorded as the shrinkage of the cells. The rate constant for lead-induced KCl or KNO_3 efflux was derived from the maximal rate of change in cell volume. Each value in the table is the mean of two estimates of the rate constants.

	Potassium efflux (rate constant, min^{-1})	
	Nitrate medium	Chloride medium
20 μM $Pb(NO_3)_2$	2.45	0.75
20 μM $Pb(NO_3)_2$ + 10 μM H_2DIDS	1.78	0.26
% inhibition	27	65

(6) Lead-induced single-channel events as measured by means of the patch-clamp technique

Stimulation of single (Ca^{2+}-activated) K^+ channels by 10 μM Pb^{2+} has been reported recently [23]. In these earlier experiments the bath solution also contained 1 mM EGTA in addition to 10 μM Pb^{2+}. To demonstrate that the channel activity is indeed produced by Pb^{2+} and not by contaminating Ca^{2+}, in some of the present experiments KCl Suprapur (Merck) was used in the bath solution while EGTA was omitted which reduced the contaminating Ca^{2+} levels to approximately 0.2 μM. In addition, the sensitivity of the K^+ channels for Ca^{2+} was reduced by incorporation of 5 mM $MgCl_2$ into the bath solution [24]. Fig. 7A demonstrates that without added Ca^{2+} or Pb^{2+} no single-channel activity occurs (trace b). However, when 10 μM lead are added to the bath solution, single-channel events appear (trace c) which have the same characteristics as those stimulated by added Ca^{2+} (trace a). Elevation of the concentration of Pb^{2+} to 100 μM blocks the channel activity reversibly (see Fig. 7B). This corresponds closely to the observations in cell suspensions.

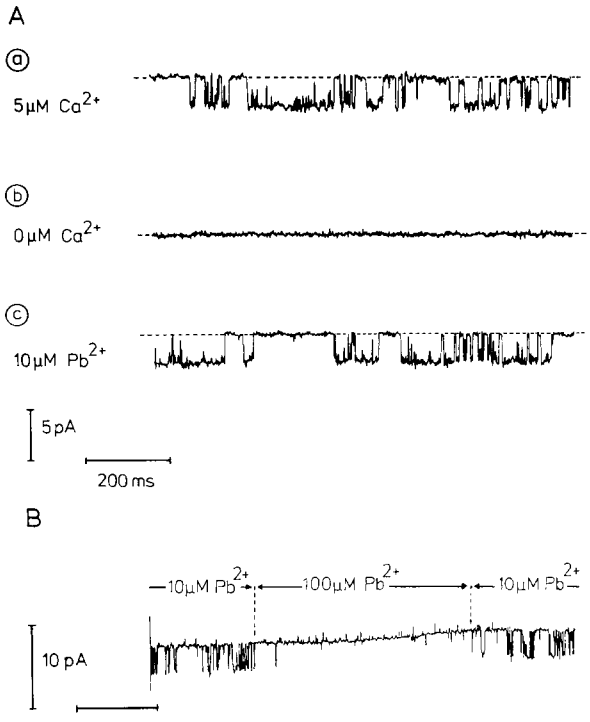

Fig. 7. Single-channel currents of calcium or lead-activated K^+ channels measured in a cell-free inside-out membrane patch. After gigaseal formation the cell was destroyed by briefly touching the bottom of the chamber which exposes the internal surface of the patch to the bath solution. The pipette solution contained in all cases (mM) KCl(150), $MgCl_2$(1), Mops(10). The bath medium contained in (A) Suprapur KCl(150), $MgCl_2$(5), Mops(10), while in (B) instead of Suprapur KCl the reagent grade KCl and 1 mM EGTA were used. All recordings were made at a holding potential of −100mV and a sampling rate of 1 kHz in (A) and 100 Hz in (B). (A) shows the similarity of the responses to Ca^{2+} and lead and (B) demonstrates that the channels activity induced by low concentrations of lead is reversibly inhibited at a higher lead concentration.

Discussion

The presence of micromolar concentrations of calcium in the physiological solutions commonly used for the study of the effects of lead raises the question as to whether or not lead could produce its effects indirectly by potentiating the actions of the ubiquitous calcium. Thus, the easily penetrating lead blocks the sodium-potassium ATPase [25] as well as the calcium ATPase (Schatzmann, H.J., personal communication) and hence should influence the balance between calcium influx and

efflux in favour of the former. Moreover, lead binding to receptors at the inside of the red cell could lead to a release of additional calcium from calcium stores. Our experiments with ultrapure solutions, complexing agents and cell-free membrane patches leave little doubt, however, that such secondary effects play little if any role and that the permeability change is a genuine effect of lead. The experiments also show that at high hematocrit, the complex forming capacity of the cell interior is large enough to bind most of the Ca^{2+} that enters the cells by ionophore-mediated diffusion across the membrane. The past studies on the effects of lead on red cells have, as a rule, been performed at hematocrits high enough to suppress artifactual results due to contamination of the solutions by Ca^{2+}.

High concentrations of lead produce an inhibition of potassium efflux (Figs. 3, 4 and 5).The effect is quite distinct from the activating effect. This does not only follow from the fact that biphasic effects cannot be explained by the action of the same agent on a single site. It is further confirmed by the observation that Co^{2+} produces an inhibition like high concentrations of lead (Fig. 6c) but is incapable of opening the potassium channels. It seems to be significant that high concentrations of lead not only inhibit lead-induced potassium efflux but also the calcium-induced permeability change (Fig. 6b). Thus, the inhibition of K^+ movements by high concentrations of lead represents a diagnostic tool for the demonstration of the participation of the calcium-activated K^+ channel in the permeability change. This is pertinent with respect to the patch-clamp experiments.

In the cell-free membrane patches, lead evokes single-channel events that resemble essentially those seen after exposure to calcium. When the lead concentration is raised beyond the activating level, an inhibition occurs. The effect is fully reversible. This result strongly suggests that (1) the channels observed under the patch-clamp are identical to the transport system that is responsible for the lead-induced potassium efflux and (2) that the effect of lead is due to a reaction with the same channels that can be activated by calcium.

Activation and self-inhibition by lead are facilitated by the addition of the ionophore A23187. This suggests that the presence of the ionophore facilitates the access to the lead binding sites for both activation and inhibition.

In summary, the present experiments confirm the previously expressed view [8] that lead is capable of activating the same channels that are activated by calcium but that it has the additional capacity of inhibiting the activity of the channels regardless of whether the activation had been brought about by its own presence or the presence of calcium.

In conclusion of the discussion, a comment may be added on the mode of penetration of lead across the red cell membrane. In isotonic salt solutions lead exists primarily in the form of complexes with the predominant anion species in the medium. It was thought, therefore, that lead reached its site of action at the inner membrane surface by a band 3 protein-mediated transport of negatively charged anionic complexes and the observation of a reduction of the rate of lead-induced KCl efflux into a chloride medium by the specific anion transport inhibitor H_2DIDS had been attributed to an inhibition of lead uptake via the anion transport system [26]. However, the reduction of the rate of K^+ efflux is more likely to reflect an inhibition of net K^+ efflux through the activated channels due to an impediment of the net transport of the accompanying chloride ions. This is suggested by our finding that lead-induced net KNO_3 transport is much less inhibited by H_2DIDS [21,28] than lead-induced KCl efflux, which is similar to observations on valinomycin-induced net K^+ efflux in nitrate and chloride media [21,28] (See Table I). Our observations also show that the length of the lag period preceding the onset of the permeability changes is independent of the presence or absence of H_2DIDS. Thus the actions of lead on the K^+ channels seems to be essentially unaffected by the rate of which an anionic complex of lead is carried across the red cell membrane via the band 3 protein. In the old literature it has been claimed that the electrically neutral complexes $Pb(NO_3)_2$ and $PbCl_2$ penetrate easily across the lipid phase [27].

Acknowledgement

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