Biochimica et Biophysica Acta, 776 (1984) 1–9
Elsevier

BBA 72254

K^+-VALINOMYCIN AND CHLORIDE CONDUCTANCE OF THE HUMAN RED CELL MEMBRANE

INFLUENCE OF THE MEMBRANE PROTONOPHORE CARBONYLCYANIDE *m*-CHLOROPHENYLHYDRAZONE

POUL BENNEKOU

Zoophysiological Laboratory B, August Krogh Institute, University of Copenhagen, 13, Universitetsparken, DK-2100 Copenhagen Ø (Denmark)

(Received May 1st, 1984)

Key words: Cl^- conductance; Valinomycin; Membrane potential; CCCP; (Erythrocyte membrane)

Chloride ion conductance of the human red cell membrane has been calculated, as the ratio between ion net charge flux and driving potential. The proton carrier CCCP was used to monitor changes in membrane potential following addition of valinomycin in sufficient quantities to raise the K^+ conductance to a level comparable to the Cl^- conductance. A K^+-specific electrode was used to monitor changes in extracellular K^+ concentration, and an H^+-sensitive glass electrode for changes in extracellular pH, reflecting changes in membrane potential. The effects of varied concentrations of valinomycin and CCCP upon K^+ and Cl^- conductances were studied. It was found that, within an experimental error of about 10% S.D., the chloride conductance was constant for valinomycin concentrations in the range $1.0 \cdot 10^{-8}$–$1.0 \cdot 10^{-6}$, and for CCCP-concentrations in the range $2.0 \cdot 10^{-7}$–$2.0 \cdot 10^{-5}$ mol per litre cell suspension, while at a constant concentration of valinomycin the induced K^+ conductance was considerably augmented by addition of CCCP.

Introduction

Measurement of membrane potential is a key element in the determination of the electrical parameters of cells, and it has become increasingly common to perform direct measurements with microelectrodes.

This method, however, becomes more difficult as the physical dimensions of the cells become smaller, and for the present it is not possible to make microelectrode measurements on cells as small as human red blood cells because the electrode introduces a shunt through which the membrane discharges too rapidly [1].

It is therefore necessary to use an indirect approach. By adding a net charged chemical probe which translocate rapidly across the membrane, changes in membrane potential are reflected through the redistribution of the probe.

Macey et al. [2] proposed to use a proton carrier, CCCP, for the determination of changes in membrane potential in red cells suspended in unbuffered media. This approach confers an immediate advantage, because the redistribution of CCCP with changes in membrane potential mediates a change in extracellular pH, to a new steady-state value which is readily measured.

The question which has to be answered,

Abbreviations: Mops, 4-morpholinepropanesulfonic acid; CCCP, carbonylcyanide *m*-chlorophenylhydrazone.

whenever probes are used to monitor the behaviour of a system, is to what extent the probe changes the properties of the system.

It has been observed [3] that valinomycin itself does not cause a substantial K^+ efflux from red blood cells, unless uncoupling agents (proton carriers or protonophores) are also present. The accelerated K^+ efflux was interpreted as a potassium/proton exchange process circumventing the anion-restriction of the efflux.

As a consequence a substantial fraction of the K^+ efflux in the presence of protonophores would not be accompanied by a flux of counter-ions, in this case chloride.

In the present paper it is demonstrated that potassium/proton exchange is not the only mechanism that underlies the flux acceleration, in that CCCP augments the valinomycin-induced potassium ion conductance. Furthermore it is shown that the chloride ion conductance does not depend on membrane potential in the interval from 25 to 80 mV inside negative.

Materials and Methods

Chemicals. All inorganic salts (p.a.), glucose and sucrose were purchased from Merck. Morpholinepropanesulfonic acid (Mops) and carbonylcyanide *m*-chlorophenylhydrazone (CCCP) were obtained from Sigma and valinomycin from Calbiochem. CCCP and valinomycin were administered as concentrated alcoholic solutions.

Membrane potential. Changes in membrane potential were determined according to the method of Macey et al. [2]. When CCCP, a weak acid, is added to a suspension of red cells, the highly permeable CCCP anion/free acid [4,5] mediates a proton distribution across the cell membrane according to the membrane potential. If the extracellular medium is unbuffered the cellular pH is constant, even with changing extracellular pH, in that the cytosol is heavily buffered [2].

Shift in membrane potential will thus be reflected by changing extracellular pH, where a change in steady-state extracellular pH of one unit corresponds to a change in membrane potential of 61 mV at 37 °C.

Absolute values of the membrane potential were obtained by measurement of pH after haemolysis of the cells. Because the extracellular phase is unbuffered, the hemolyzate will attain the same pH value as the cytosol before lysis, and this pH corresponds to membrane potential zero [6].

Cells. Freshly drawn, heparinized human blood was centrifuged and plasma and buffy coat were removed by aspiration. The cells were washed once in Mops-Ringer (145.8 mM NaCl/1.0 mM KCl/0.15 mM $MgCl_2$/10.0 mM Mops/5.0 mM glucose, pH 7.2), resuspended in Mops-Ringer at a hematocrit of 20% and preincubated for at least 1 h at 37 °C.

Standard experimental procedure. 2500 μl unbuffered Ringer (145.8 mM NaCl/1.0 mM KCl/0.15 mM $MgCl_2$/15.0 mM sucrose) were pipetted into a plastic vessel, thermostatted at 37 °C, and 2.5 μl CCCP solution, concentration varied, added.

1000 μl cell suspension were centrifuged and the cells washed twice in 5 vol. of unbuffered Ringer. After the final centrifugation 100 μl packed cells with a trapped volume of 6% were transferred to the plastic vessel and vigorous stirring was begun.

Transfer of packed cells was done with a Transferpettor, with a direct displacing and scraping plunger, to avoid loss of cells caused by adherence to surfaces.

Within a few minutes after the addition of cells to the reaction vessel proton and temperature equilibration was achieved, and 2.5 μl valinomycin solution were added. Hemolysis was typically below 1‰, controlled by hemoglobin analysis on samples of extracellular medium.

At the end of the experiment 50 μl aqueous saponin solution (10 g saponin per litre) were added, and total hemolysis occurred within a few seconds.

Electrodes and amplifiers. The proton-sensitive electrode was a Radiometer G 2222 C glass electrode. The K^+-sensitive electrodes were prepared from glass tubes (Corning 7740) which were tapered, dried for 2 h at 200 °C and siliconized (dimethyl dichlorosilane). The tips were filled with a K^+-selective ion-exchanger (Corning 7740) embedded in a PVC matrix. The inner solution was 100 mM KCl and Ag^+/AgCl was used as the inner reference electrode.

A Radiometer K 4112 calomel electrode was

used as a common reference. KCl efflux through the porous plug amounted at most to about 2% of cellular efflux, and was thus negligible.

The glass electrode was connected to a Radiometer TTT2 pH-meter and the K^+-sensitive electrode to a Bioelectric NF 1 amplifier.

Analyses. Water content was determined as weight reduction of 50 μl packed cells after drying to constant weight (48 h at 80 °C).

Determination of cellular Na^+, K^+, Cl^- and hemoglobin. 50 μl packed cells were hemolyzed in 200 μl saponin solution. Hemoglobin was determined spectrophotometrically as cyanmethemoglobin in 10-μl samples of hemolysate. Na^+ and K^+ were determined by flame photometry, and Cl^- by electrometric titration on hemolysate samples, deproteinized by addition of perchloric acid.

Calculations

In order to calculate potassium and chloride ion conductances, ion currents and membrane potentials are measured. Phenomenologically, the electrical (ion) flux across the membrane can be described as:

$$i_j = g_j(V_m - E_j) \tag{1}$$

where i_j is the current density, g_j the conductance per unit area, V_m the membrane potential and E_j the Nernst potential, all for the jth ion [7].

The total current across the membrane is then:

$$I = \Sigma i_j = \Sigma g_j(V_m - E_j) \tag{2}$$

Eqn. 2 can be solved for V_m if $I = 0$, or equivalently stated $(dV_m/dt) = 0$, which gives:

$$V_m = \frac{\Sigma g_j E_j}{\Sigma g_j} = \Sigma T_j E_j \tag{3}$$

where T_j is the transference number for the jth ion [8]. Fluxes and membrane potential of red cells, where g_{K^+} has been raised to a value comparable to that of g_{Cl^-} by addition of valinomycin, and with CCCP added for proton equilibration, is determined by Cl^- and K^+ distribution. g_{Na^+} is negligibly low compared to other conductances (g_{Na^+} is about 10^{-3}-times g_{Cl^-}), and $E_{CCCP^-} = E_{H^+} = E_{OH^-} = E_{HCO_3^-} = V_m$, when $I = 0$. Consequently the terms dealing with these ions in the right-hand side of Eqn. 2 equal zero, and $i_{K^+} + i_{Cl^-} = 0$, i.e., the flux of chloride ions equals the flux of potassium ions.

Eqn. 1 can now be solved for g_{K^+} and, following insertion in Eqn. 2, this can be solved for g_{Cl^-}. Permeabilities were calculated from the constant field netflux equation for singly charged ions:

$$i_j = P_j \frac{(z_j F)^2 V_m C_j^0 \left(1 - e^{\frac{z_j F}{RT}(V_m - E_j)}\right)}{RT\left(1 - e^{\frac{z_j V_m}{RT}}\right)} \tag{4}$$

where P_j is the permeability and C_j^0 the extracellular concentration for the jth ion. R, T, F and z have their usual meaning [9]. The cell membrane area was taken as $4.2 \cdot 10^7$ cm^2 per kg dry matter [10].

Permeabilities calculated according to Eqn. 4 have physical significance only as far as the ion fluxes conform to constant field conditions. If constant field requirements are not fulfilled the calculated P_j values become 'equivalent permeabilities'.

Equating the right-hand sides of Eqns. 1 and 4 gives the relationship between conductance and (equivalent) permeability [8].

Cellular concentrations and Nernst potentials

According to Freedman and Hoffman [11] the osmotic balance across the cell membrane can be expressed as:

$$\Phi_{Hb} C_0 = \phi_{Hb}[Hb] + \phi_s[s] \tag{5}$$

where Φ_{Hb} is the average osmotic coefficient of the extracellular solutes, C_0 the total concentration of extracellular solutes, $\phi_{Hb} = 1 + 0.0645[Hb] + 0.0258[Hb]^2$ the osmotic coefficient of hemoglobin, ϕ_s the average osmotic coefficient of intracellular solutes apart from hemoglobin, and [s] the corresponding intracellular solute concentration. For 150 mM salt solutions $\phi_s = \phi_0 = 0.93$.

Inserting the measured cellular Na^+, K^+, Cl^- and hemoglobin concentrations in Eqn. 5, the

concentration of remaining intracellular impermeable organic solute for a relative cellular volume, $V_{Rel} = 1$, that is the initial volume, can be calculated. At a relative cellular volume less than one, i.e., when the cells shrink after addition of valinomycin, the cellular concentration of Na^+, Hb and remaining impermeable organic solute can be calculated by multiplying the initial concentrations with a 'shrinkage' factor, $(H_2O_c^{t=0})/(H_2O_c^t) = (V_c^{t=0} \cdot Wf)/(V_c^{t=0} \cdot Wf - (V_c^{t=0} - V_c^t)) = Wf/(Wf - 1 + V_{Rel})$, where H_2O_c is cellular water volume, V_c is cellular volume and Wf is the initial cellular water fraction. Inserting these values, and the extracellular concentrations at a given time, Eqn. 5 can be solved for the sum $[K^+]_c + [Cl^-]_c$, in that the extracellular compartment, at a hematocrit of about 4%, can be regarded as an osmotic reservoir.

Maintainance of electroneutrality requires that the K^+ loss equals that of Cl^-. The difference between $[K^+]_c$ and $[Cl^-]_c$ at a given relative cellular volume can be calculated as the initial difference multiplied by $Wf/(Wf - 1 + V_{Rel})$. Because the extracellular compartment is unbuffered, the shift in H^+ reflecting membrane potential changes is very low, about $1 \cdot 10^{-5}$ mol/litre suspension, which is negligible compared to the net flux of potassium chloride (see Fig. 2).

Thus two equations have been established for the determination of the cellular concentrations of K^+ and Cl^-, and, at a given time, the Nernst potentials for K^+ and Cl^- can be calculated.

The ratio of intracellular to extracellular activity was taken to be unity.

Results

Immediately after the addition of valinomycin to the CCCP-equilibrated cell suspension, the K^+ efflux starts and simultaneously the extracellular pH increases, reflecting the hyperpolarization of the cell membranes.

After about 30 s the extracellular pH reaches a level, which is stable for a period of about 30 s or more, depending on valinomycin concentration, whereafter it declines; see Fig. 1. At the time, when the extracellular pH has reached the plateau, the increase in extracellular K^+ concentration is almost linear, and the K^+ net efflux is calculated from the slope of the time versus extracellular K^+ concentration curve.

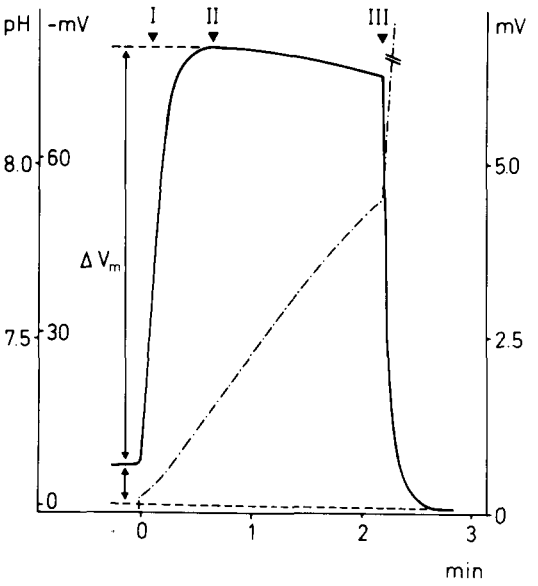

Fig. 1. Experiment under standard conditions. At $t = 0$ valinomycin was added to a final concentration of 10^{-6} M. CCCP concentration was $2.0 \cdot 10^{-5}$ M. K^+ efflux (·–·–·) begins immediately, and extracellular pH (solid line) rises concomitantly. Conductances and permeabilities are calculated at II, where the zero-current condition (see section on calculations) is fullfilled. Saponin is added at III. The ordinate to the left is extracellular pH and membrane potential, the axis to the right the K^+ electrode response in mV. The abscissa is time in minutes.

With initial effluxes of KCl exceeding 1000 mmol/litre cells per h, the Nernst potentials of K^+ and Cl^-, which determine the membrane potential, change fast, but as these changes are in opposite directions they tend to stabilize the membrane potential (see Fig. 2) as reflected by the slow change in extracellular pH after the plateau is reached.

In the absence of valinomycin, CCCP has no significant effect on the K^+ permeability of human red cells. Red cells treated with ouabain (10^{-4} M) were suspended in a sucrose-substituted Ringers solution (217 mM sucrose, 27.5 mM Cl^-, 1 mM K^+ and Na^+ to make up the balance), in order to increase the electrochemical potential difference for K^+. Potassium net efflux, determined by flame photometry, was followed for a period of 2 h. In experiments with and without CCCP ($2.6 \cdot$

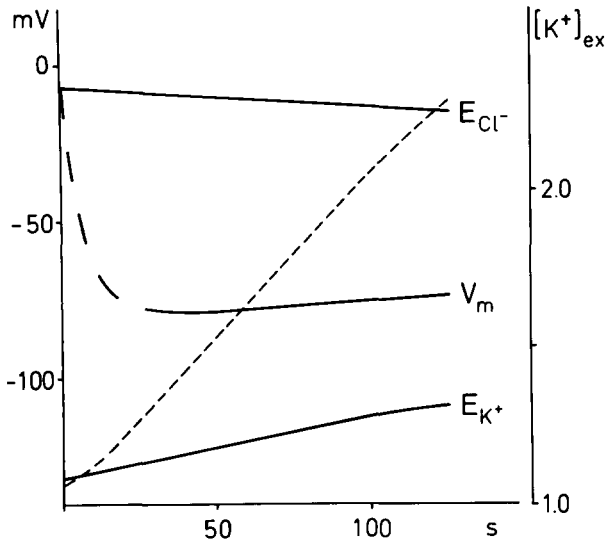

Fig. 2. Calculated time-dependent K^+ and Cl^- Nernst potentials and membrane potential corresponding to the experiment shown in Fig. 1. The first part of the line depicting the membrane potential is dashed, to indicate that CCCP equilibration is too slow to reflect the almost instantaneous voltage jump, indicated by the immediate onset of potassium efflux, after valinomycin addition. ------ indicates the extracellular K^+ concentration. The ordinate to the left is potential in mV, the axis to the right extracellular K^+ in mM, and the abscissa is time in seconds.

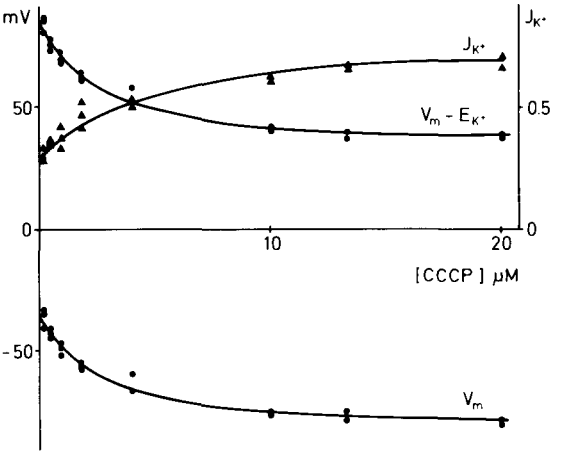

Fig. 3. CCCP dose-response curve. Membrane potential and driving potential for the K^+ efflux, and net K^+ efflux are shown (all at zero current). The ordinate to the left is potential in mV, the ordinate to the right K^+ efflux in mmol/kg dry matter per s. The abcissa is CCCP concentration in mol/litre total water. Valinomycin concentration is 10^{-6} mol/litre suspension.

10^{-5} M), K^+ conductance was found to be $6.0 \cdot 10^{-8}$ S/cm^2, or an equivalent permeability of $4.2 \cdot 10^{-10}$ cm/s. The membrane potential was initially 32 mV inside positive and declined 1.2 mV per hr.

The influence of CCCP upon the valinomycin mediated potassium efflux, however, is pronounced.

The K^+ efflux increases as the CCCP concentration is raised (see Fig. 3) with a $k_{1/2}$ of about $3 \cdot 10^{-6}$ M, and saturates above $(15 - 20) \cdot 10^{-6}$ M CCCP. The net K^+ efflux at saturation is about 3-times higher than the valinomycin mediated efflux without CCCP.

The membrane potential, as reflected by the pH change in the extracellular medium, becomes more and more negative as the CCCP concentration increases, and thus $(V_m - E_{K^+})$, the driving potential for the K^+ net efflux, becomes smaller. The effect saturates in the same concentration range as the K^+ efflux, and with a similar $k_{1/2}$ (see Fig. 3).

The valinomycin-K^+ conductance, and equivalent constant-field permeability, shows simple saturation behaviour with increasing CCCP concentrations, with a $k_{1/2}$ of $1.6 \cdot 10^{-5}$ M and a maximum conductance about 10-times higher than the extrapolated conductance at zero CCCP concentration (see Fig. 4). In the presence of $2.7 \cdot 10^{-5}$ M CCCP, the valinomycin-potassium conductance is found to be a simple saturating function of valinomycin concentration with a $k_{1/2}$ of $6.3 \cdot 10^{-7}$ M valinomycin, and a maximum conductance of about $5.0 \cdot 10^{-5}$ S/cm^2 (see Fig. 5).

The Cl^- conductance, and equivalent constant-field permeability, in contrast to the valinomycin-mediated K^+ conductance, show no dependence on either CCCP concentration (see Fig. 4) or valinomycin concentration (see Fig. 5).

The magnitude of the chloride conductance at $pH_{cell} = 7.0$ was found to be $2.35 \cdot 10^{-5}$ S/cm^2, as a mean value for three different donors; see Table I.

As the membrane potential varies with the concentration of valinomycin and CCCP, in these experiments from about 25 mV to 90 mV inside negative, this implies that the chloride conductance is not dependent upon the membrane potential in the range tested.

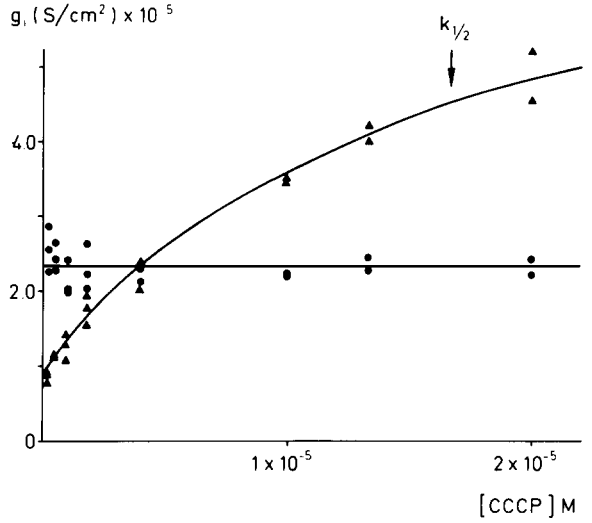

Fig. 4. Potassium (▲) and chloride (●) conductances as function of CCCP concentration, corresponding to the experiments shown in Fig. 3. Ordinate, conductance in S/cm^2; abscissa, CCCP concentration in mol/litre total water.

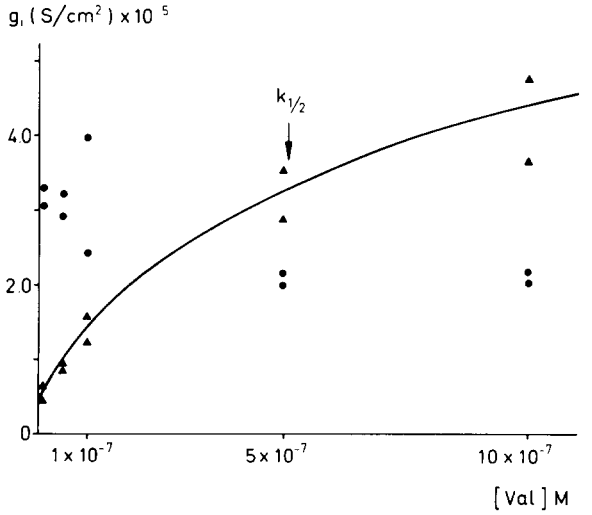

Fig. 5. Potassium (▲) and chloride conductances (●) as function of valinomycin concentration. Ordinate, S/cm^2; abscissa, valinomycin concentration in mol/litre total water.

TABLE I

RED CELL MEMBRANE CHLORIDE CONDUCTANCE, AT pH_{cell} = 7.0 FOR THREE DIFFERENT DONORS

Donor	g_{Cl^-} (S/cm^2) ($\times 10^5$)	P_{Cl^-} (cm/s) ($\times 10^8$)	n	S.E. (S/cm^2) ($\times 10^5$)
1	2.75	7.30	20	0.17
2	2.33	4.88	20	0.51
3	1.97	4.51	6	0.12

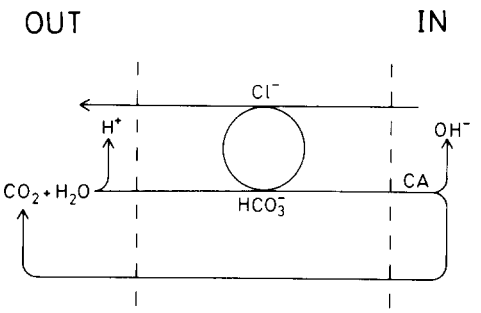

Fig. 6. Reaction scheme for carbon dioxide cycling across the red cell membrane, the Jacobs-Stewart cycle. HCO_3^- dissociates intracellularly, catalyzed by carbonic acid anhydrase (CA); CO_2 diffuses through the membrane and recombines spontaneously with water in the extracellular medium.

Valinomycin specificity in the presence of CCCP

It has been observed that, following addition of the lipid-soluble anions trinitrocresolate or picrate, valinomycin loses its selectivity towards K^+ [12]. If CCCP had a comparable effect, part of the observed K^+ efflux would be an exchange of extracellular sodium for cellular potassium down chemical gradients.

To test whether this was the case, $2.0 \cdot 10^{-5}$ M CCCP was added to ouabain (10^{-4} M) -treated cells suspended in a K^+ equilibrium Ringer (90 mM K^+, 60 mM Na^+), containing $1.25 \cdot 10^{-6}$ M valinomycin. Because $E_{K^+} = V_m$, the net efflux of K^+ equals zero, in spite of the addition of valinomycin, and volume changes are avoided, unless significant Na^+ fluxes are induced. Cellular and extracellular Na^+ concentrations was determined every 10 min for a period of 2 h. No acceleration in Na^+ fluxes could be observed compared to the control experiment without CCCP.

Discussion

Potassium efflux and membrane potential

As seen from Figs. 3 and 4, CCCP has a pronounced effect upon the valinomycin-induced K^+ efflux and the valinomycin-K^+ conductance, whereas no effect is seen upon the Cl^- conductance. Accelerations of ionophore-mediated K^+ efflux by proton carriers, comparable to those reported here, have previously been reported [2,3], but without quantification.

Harris and Pressmann [3] observed that addition of valinomycin to a buffered suspension of red cells caused only a limited loss of potassium, but the K^+ efflux was accelerated after addition of FCCP (trifluorometoxycarbonylcyanide phenylhydrazone, an uncoupler of the same class as CCCP) with a concomitant proton influx.

Macey et al. [2] reported that CCCP caused a substantial acceleration of the valinomycin-mediated K^+ efflux, and likewise an increased K^+ efflux through the Ca^{2+}-sensitive K^+ channels. They proposed that in both cases these effects might be due to a K^+/H^+ exchange.

Based on experiments with phospholipid liposomes, where initial proton-fluxes in the presence of valinomycin and protonophores (uncouplers) were measured, Yamaguchi et al. [13] have proposed that a K^+/H^+ exchange is mediated through formation of a ternary electroneutral complex between valinomycin-potassium and the anionic form of the protonophore, followed by backflux of protonated CCCP (the free acid).

In the case of the apparent acceleration of K^+ flux through the Ca^{2+}-sensitive K^+ channels it is likely that the effect of CCCP involves synchronization of the opening process, rather than an increased efflux through the individual channels [14]. Thus it has been shown [15] that A23187 Ca^{2+} influx, which triggers the K^+ channels, is increased by the proton concentration gradient, which is established by CCCP as a consequence of the initial hyperpolarization.

Regarding the valinomycin-mediated K^+ efflux in the presence of CCCP, an accelerated K^+ efflux coupled to a counter-transport of protons, regardless of mechanism, either requires a heavily buffered medium, as is the case under the experimental conditions used by Harris and Pressman [3], or will be seen only initially. If in the present system, however, K^+ net effluxes of the magnitude observed were accompanied by H^+ net influxes, the total extracellular buffer capacity would last for only a few seconds, after which the efflux should decrease sharply. Such a decrease in K^+ efflux is not observed in the present experiments (see Fig. 2).

Enhancement of K^+ effluxes from phospholipid vesicles is observed following addition of compounds which are far less acidic than CCCP, or aprotic [16], and for these systems lipid-soluble anions, which can participate in electroneutral complex-formation with valinomycin-K^+, are not available.

A more likely explanation for the accelerated K^+ efflux seems to be a direct influence of CCCP upon the valinomycin-K^+ conductance. The cause of this CCCP effect upon the valinomycin-K^+ conductance is not clear at the present, but increase of negative charge density, increased fluidity or lowering of the positive potential barrier in the membrane are all possible modes of action which could lead to the observed enhanced conductance.

The increasing steady-state extracellular pH with increasing CCCP concentrations thus reflects a direct CCCP influence upon the valinomycin-mediated K^+ conductance leading to an increased K^+ efflux and increasing hyperpolarization of the cell membranes, and is not a consequence of subminimal CCCP additions leading to incomplete pH equilibration, and therefore to calculated membrane potentials less negative than the true membrane potential.

CCCP-mediated pH equilibration and anion exchange

Determination of membrane potentials by the CCCP method could be biased by the Jacobs-Stewart cycle (see Fig. 5), mediated by the anion-exchange mechanism. Wieth has shown [17] that addition of small amounts of bicarbonate, 0.15 mM, pH 8.42, to an erythrocyte suspension treated with CCCP causes a drop in extracellular pH.

This is a result of the compromise between CCCP equilibrating protons according to the membrane potential, and the anion-exchange system equilibrating bicarbonate ions, and consequently OH^- (or H^+) ions according to the chloride distribution ratio.

If the anion-exchange mechanism under the present experimental conditions significantly interferes, the calculated membrane potentials will represent an underestimate relative to the true membrane potentials, in that the cycling of carbon dioxide provides extracellular protons for a K^+/H^+ exchange.

There is no indication, however, of interference of this kind under the present experimental condi-

tions, where the bicarbonate concentration is at least 50-times lower than the concentrations used for the demonstration of CO_2 cycling.

Experiments in which suspensions of cells have been CO_2-depleted by an argon gas stream show membrane potentials and K^+ flux accelerations comparable to determinations on cells in equilibrium with atmospheric air. This is not surprising, since the extracellular bicarbonate concentration is very low, and furthermore the flow through the Jacobs-Stewart cycle is rate-limited by the hydration of CO_2 in the extracellular medium, provided no hemolysis is present.

Cl^- permeability

The mean value of the Cl^- conductance is $2.35 \cdot 10^{-5}$ S/cm^2. The equivalent constant field permeability of $5.6 \cdot 10^{-8}$ cm/s is a little higher than, but in the same range as, other determinations, where the membrane potential has been indirectly estimated. Hunter [18,19] has estimated P_{Cl^-} as $(2-3) \cdot 10^{-8}$ cm/s at pH 7.40 and 37 °C, and Knauf et al. [20] as $2.8 \cdot 10^{-8}$ cm/s at pH 7.1 and 37 °C.

It is likely that the difference in the determinations of the chloride permeability is based upon the difference in experimental approach. In the previous determinations the membrane potential was estimated indirectly, under the assumption that the valinomycin-mediated K^+ fluxes conformed to a constant-field treatment, an assumption which is not necessarily fulfilled, (see Refs. 21 and 22). It should be noted that the conductance is defined as the ratio between ion current and driving potential, and is thus assumption-free regarding constant-field requirements [7].

Furthermore, the rapid changes in membrane potentials caused by the degradation of the chloride and potassium Nernst potentials under net flux conditions will result in a tendency to overestimate the driving potentials for the net effluxes of K^+ and Cl^-, and thereby underestimate the chloride permeability. This is avoided in calculations based on continuous monitoring of membrane potential and changes in extracellular K^+ concentration, under zero-current conditions.

That the chloride permeability is not influenced by variations in valinomycin concentration with changes in membrane potential up to 80 mV inside negative is in accordance with observations on sheep red cells [23] and human red cells [20].

An increase in chloride conductance and equivalent permeability with increasing membrane potential as reported by Knauf et al. [24] has not been found in the interval -30 to -80 mV inside negative.

Conclusion

Changes in membrane potential and absolute membrane potential are reflected by extracellular pH changes, mediated by CCCP in the concentration range $2 \cdot 10^{-6}$–$2 \cdot 10^{-5}$ M.

The chloride conductance of the red cell membrane has been found to be constant with membrane potentials ranging from -30 to -80 mV.

Although CCCP does not influence the chloride conductance, there is a significant interaction between this membrane potential probe and the valinomycin-mediated potassium netflux, and it is demonstrated that CCCP directly affects the valinomycin-potassium ion conductance.

Acknowledgement

The author wishes to thank Dr. J.O. Wieth for stimulating discussions and helpful suggestions.

References

1 Lassen, U.V. and Rasmussen, B.E. (1978) in Membrane Transport in Biology (Giebisch, G., Tosteson, D.C. and Ussing, H.H., eds.), pp. 169–203, Springer-Verlag, Berlin
2 Macey, R.I., Adorante, J.S. and Orme, F.W. (1978) Biochim. Biophys. Acta 512, 284–295
3 Harris, E.J. and Pressman, B.C. (1967) Nature 216, 918–920
4 LeBlanc, O.H., Jr. (1971) J. Membrane Biol. 4, 227–251
5 Macey, R.I. and Orme, F.W. (1980) in Membrane Transport in Erythrocytes: Alfred Benzon Symp. 14 (Lassen, U.V., Ussing, H.H. and Wieth, J.O., eds.), pp. 498–510, Munksgaard, Copenhagen
6 Funder, J. and Wieth, J.O. (1966) Acta Physiol. Scand. 68, 234–245
7 Hodgkin, A.L. and Huxley, A.F. (1952) J. Physiol. Lond. 116, 449–472
8 Lassen, U.V., Pape, L. and Vestergaard-Bogind, B. (1978) J. Membrane Biol. 39, 27–48
9 Sten-Knudsen, O. (1978) in Membrane Transport in Biology (Giebisch, G., Tosteson, D.C. and Ussing, H.H., eds.), pp. 5–113, Springer-Verlag, Berlin
10 Wieth, J.O., Funder, J., Gunn, R.B. and Brahm, J. (1974) in

Comprehensive Biochemistry and Physiology of Transport (Bolis et al., eds.), pp. 317–338 North-Holland Publishing Company, Amsterdam
11 Freedman, J.C. and Hoffman, J.F. (1979) J. Gen. Physiol. 74, 157–185
12 Tosteson, D.C. (1973) in Transport Mechanisms in Epithelia (Ussing, H.H. and Thorn, N.A., eds.), pp. 28–43, Munksgaard, Copenhagen
13 Yamaguchi, A. and Anraku, Y. (1978) Biochim. Biophys. Acta 501, 136–149
14 Vestergaard-Bogind, B. and Bennekou, P. (1982) Biochim. Biophys. Acta 688, 37–44
15 Vestergaard-Bogind, B. and Stampe, P. (1984) Biochim. Biophys. Acta 775, 328–340
16 Clement, N.C. and Gould, J.M. (1981) Biochemistry 20, 1539–1543
17 Wieth, J.O. (1980) in Membrane Transport in Erythrocytes: Alfred Benzon Symp. 14 (Lassen, U.V., Ussing, H.H. and Wieth, J.O., eds.), pp. 512–520, Munksgaard, Copenhagen
18 Hunter, M.J. (1971) J. Physiol. 218, 49p–51p
19 Hunter, M.J. (1977) J. Physiol. 268, 35–49
20 Knauf, P.A., Fuhrmann, G.F., Rothstein, S. and Rothstein, A. (1977) J. Gen. Physiol. 69, 363–386
21 Läuger, P. (1980) J. Membrane Biol. 57, 163–178
22 Stark, G. and Benz, R. (1971) J. Membrane Biol. 5, 133–153
23 Tosteson, D.C., Gunn, R.B. and Wieth, J.O. (1973) in Erythrocytes, Thrombocytes, Leucocytes (Gerlach, E., Moser, K., Deutsch, E. and Wilmanns, W., eds.), pp. 62–66, Georg Thieme, Stuttgart
24 Knauf, P.A. and Marchant, P.J. (1977) Biophys. J. 17, 165a