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CALCIUM-INDUCED OSCILLATIONS IN K^+ CONDUCTANCE AND MEMBRANE POTENTIAL OF HUMAN ERYTHROCYTES MEDIATED BY THE IONOPHORE A23187

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The time-dependence of ionophore A23187-induced changes in the conductance of the Ca^{2+}-sensitive K^+ channels of the human red cell has been monitored with ion-specific electrodes. The membrane potential was reflected in CCCP-mediated pH changes in a buffer-free extracellular medium, and changes in extracellular K^+ activity and electrode potential of an extracellular Ca^{2+}-electrode were recorded. Within a narrow range of ionophore-mediated Ca^{2+} influx, the above-mentioned parameters were found to oscillate when ionophore was added to a suspension of glucose-fed cells. The period of oscillation was about 2 min/cycle depending on ionophore concentration, and the amplitude of hyperpolarization was about 60 mV, corresponding to a maximal g_{K^+} of the same magnitude as g_{Cl^-}. Without CCCP present no oscillation in K^+ conductance was observed. The Ca^{2+} affinity for the opening process was in the micromolar range. The closing of the K^+ channels was a spontaneous process in that the depolarization was well under way before the Ca^{2+}-ATPase-mediated Ca^{2+} net efflux started. Below the Ca^{2+} influx range for oscillations, no response was observed for up to 20 min after the addition of ionophore. Above the upper limit, a permanent hyperpolarization resulted with the extracellular K^+ activity increasing monotonically as a function of time. In experiments with ATP-depleted cells, responses of the latter type ensued at all ionophore concentrations above the lower limit. Addition of surplus EGTA to suspensions of hyperpolarized cells restored the normal membrane potential in the case of glucose-fed cells, whereas the K^+-channels in ATP-depleted cells remained open.

Introduction

The membrane of the vertebrate erythrocyte possesses Ca^{2+}-sensitive K^+-specific channels. Under physiological conditions almost all of these channels are closed, but if the intracellular Ca^{2+} activity increases above the physiological level the channels open and the K^+ conductance of the erythrocyte membrane increases drastically (cf. Ref. 1).

In the study of the characteristics of the Ca^{2+}-sensitive K^+ channels, variation in the intracellular Ca^{2+} activity of intact cells has been induced in different ways. In the original demonstration of the K^+ channels [2,3], the cells were depleted of ATP by preincubation in the absence of glucose and with various inhibitors of glycolysis added. The ATP depletion eventually leads to a cessation of the active Ca^{2+} efflux [4]. Since the passive influx of Ca^{2+} continues, the Ca^{2+} activity in the intracellular phase increases and a permanent increase in K^+ permeability of the cell membranes takes place.

The alternative way to increase net influx of Ca^{2+} into the cells would be to increase the pas-

Abbreviations: Mops, 4-morpholinepropanesulphonic acid; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone.

sive Ca^{2+} influx to a value exceeding that of the pump efflux. Thus, Lassen et al. [5] found that a sudden increase in the extracellular concentration of Ca^{2+} in a suspension of *Amphiuma* red cells at 17°C led to a transient hyperpolarization of the cell membranes based on a selective increase in the K^+ conductance of the membrane. Later it was shown that human red cells at 37°C and pH 8.0–8.5 responded to a sudden increase in extracellular Ca^{2+} concentration in a similar manner [6].

Lew and Ferreira [7] increased the Ca^{2+} influx of intact human red cells by the addition of the ionophore A23187. Suspending the cells in a K^+-equilibrium Ringer's solution (75 mM K^+) containing ^{45}Ca and ^{42}K they demonstrated a permanent increase in intracellular Ca^{2+} and a concomitant, drastic increase in ^{42}K equilibration rate across the membrane. By this method it was possible to obtain various well-defined stationary concentrations of intracellular Ca^{2+} as well as a quantitative relationship between K^+ permeability and intracellular Ca^{2+} activity. They found that the apparent K_m of the K^+ channel for Ca^{2+} was about 1 mM in normal cells, whereas the apparent K_m in ATP-depleted cells was in the micromolar range, and concluded that the low affinity response reflects the behaviour of the normal system [7,8].

Dissing et al. [9] added the ionophore A23187 to a suspension of *Amphiuma* red cells in Ringer's solution with various EGTA-buffered low Ca^{2+} activities. Based on the membrane potentials measured at the various extracellular Ca^{2+} activities, they concluded that the K^+ channels open when the intracellular Ca^{2+} activity exceeds $2 \cdot 10^{-7}$ M.

In this paper we present oscillations in the CCCP-monitored membrane potential of human red cells. The conductance of the Ca^{2+}-sensitive K^+ channels was brought to oscillation by an ionophore-mediated, moderate increase in Ca^{2+} influx.

Materials and Methods

Chemicals

All inorganic salts (pro analysi), glucose and glycolic acid (for synthesis) were purchased from Merck. Mops, Trizma base and Trizma, iodoacetamide, EGTA and CCCP were from Sigma, and valinomycin from Calbiochem. CCCP was administered as a concentrated (20 mM) solution in ethanol.

The ionophore A23187 was from Calbiochem (Lot 803577). The stock solution of ionophore A23187 was a 2.00 mM solution of the compound in absolute ethanol for spectroscopy from BDH. From this stock solution, stored at −20°C, a diluted solution of ionophore was currently prepared by adding 1 vol. of stock solution to 19 vols. of 156 mM NaCl solution containing 1 g/l of bovine serum albumin (Provite).

Cells

Freshly drawn blood from healthy human donors was heparinized and centrifuged and the plasma and the buffy coat aspirated. The cells were washed once in 156 mM NaCl and then preincubated at 37°C in Mops/Ringer (144 mM NaCl/1 mM KCl/0.15 mM $MgCl_2$/5 mM glucose/10 mM Mops, pH 7.40) at a hematocrit of 20%.

ATP-depleted cells were prepared by preincubating the cells for 5 h in depletion Ringer's solution (75 mM NaCl/75 mM KCl/0.1 mM EGTA/10 mM inosine/6 mM iodoacetamide/15 mM sodium glycolate/10 mM Tris, pH 7.7) at 37°C and a hematocrit of 10%. Glycolate was included in the depletion Ringer's solution in order to ensure the breakdown of the large 2,3-diphosphoglycerate pool present in red cells [10].

Membrane potential

Changes in membrane potential were determined according to the method of Macey et al. [11]. The experiments were carried out with cells suspended in an unbuffered extracellular phase at a hematocrit of 3.7% in the presence of CCCP. This compound mediates a very fast electrochemical equilibration of protons across the cell membranes. Since the intracellular phase is heavily buffered, a change in membrane potential and accordingly in equilibrium potential of protons enforces a shift in the extracellular pH.

Electrodes

The proton sensitive electrode was a Radiometer glass electrode (G 2222C). The K^+- and Ca^{2+}-

selective electrodes were prepared from Corning glass tubes (7740; o.d. 2 mm) which were tapered and siliconized (dimethyldichlorosilane). Electrode tips were filled with cation-selective ion-exchanger embedded in a PVC-matrix [12]. The K^+-selective ion-exchanger was from Corning (477317) and the Ca^{2+}-selective ion-exchanger was a kind gift from Dr. W. Simon, Switzerland (ETH 1001/*o*-NPOE/TPB^-). As internal filling solutions, 100 mM KCl and respectively 5 mM Titriplex I (Merck), 2.5 mM $CaCl_2$ and 10 mM Tris (pH 7.40) were used. The inner reference electrodes were Ag|AgCl electrodes. A calomel electrode (Radiometer K 4112) was used as a common reference. The glass electrode was connected to a Radiometer TTT2 and the K^+- and Ca^{2+}-electrodes to Bioelectric NF1 amplifiers.

Standard experimental procedure

2500 μl unbuffered, low-calcium Ringer's solution (155 mM NaCl/1 mM KCl/0.15 mM $MgCl_2$/5 mM glucose) were pipetted into a plastic vessel, maintained at 37°C. Ca^{2+} contamination in the inorganic salts resulted in a measured activity of Ca^{2+} of $5 \cdot 10^{-6}$ M in this solution. 2.5 μl CCCP solution were added to a final concentration of 20 μM.

1000 μl suspension of cells, preincubated in either Mops/Ringer or depletion Ringer's solution, was centrifuged and the cells washed twice with 5 vols. 156 mM NaCl with and without glucose, respectively. After the final centrifugation (20000 $\times g$ for 1 min), 100 μl packed cells with a trapped extracellular volume of 7% were transferred to the vessel and vigorous stirring was begun. Within a few minutes CCCP-mediated H^+ equilibration across the membranes and stabilization of the Ca^{2+} electrode with respect to CCCP (see Results section) were achieved. 20–30 μl of the diluted ionophore solution were then added.

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

Correction of the Ca^{2+}-electrode trace

The ordinate of the Ca^{2+}-electrode trace which is given in millivolts cannot be readily converted to Ca^{2+} activity. It is well known that lipid soluble anions in the sample cause interference with the cation-response from neutral-carrier liquid-membrane electrodes (cf. Ref. 13). Since the CCCP-anion distribution across the membrane reflects the membrane potential, the waves of hyperpolarization result in continuous changes in the extracellular CCCP-level and accordingly a continuous variation in interference. We have not yet been able to determine or eliminate this interference in general and, instead, another strategy has been chosen.

Cells were treated according to the standard procedure with the exception that valinomycin was added to the suspension to mimic the hyperpolarization caused by the Ca^{2+} ionophore. Assuming a constant extracellular Ca^{2+} activity this procedure provides a recording of the interference. In this way the effect of the varying extracellular CCCP level on the Ca^{2+}-electrode has been determined to be -10 mV/ΔpH_{ex}, and it is thus possible to establish an upper limit for the increase in intracellular Ca^{2+} activity. When the interference is subtracted from the peak value of a typical oscillatory calcium trace a change of only -1 mV remains. With an electrode sensitivity of 27 mV per 10-fold increase in Ca^{2+} activity, and an initial extracellular Ca^{2+} activity of $5 \cdot 10^{-6}$ M (standard experimental procedure), the above-mentioned -1 mV corresponds to a drop in extracellular activity of $0.5 \cdot 10^{-6}$ M. Assuming that all the Ca^{2+} transported into the cells remains free, the intracellular activity will increase to $2 \cdot 10^{-5}$ M.

According to Ferreira and Lew [14], however, the fraction of ionized calcium represents between 30% and 50% of the total intracellular calcium. Therefore, the upper limit of intracellular Ca^{2+} activity attained during the first wave of hyperpolarization should be about $1 \cdot 10^{-5}$ M. Applying the method outlined above to the Ca^{2+}-electrode trace shown in Fig. 1 results in an extracellular Ca^{2+} activity which is oscillatory but with a much smaller amplitude. Taking into account the lagtime of the Ca^{2+}-electrode, an analysis of the time relationship of the extracellular Ca^{2+} activity versus membrane potential indicates that the minimum in Ca^{2+} activity is reached in the depolarization phase.

Results

A typical oscillation from an experiment with glucose-fed erythrocytes in low-calcium Ringer's solution ($5 \cdot 10^{-6}$ M) is shown in Fig. 1. Under the standard procedure (see Materials and Methods) the oscillations normally started within 5–15 s after the addition of the ionophore. Within the time resolution of the recording system, the initial fall in the Ca^{2+}-electrode trace and the increase in extracellular K^+ activity and pH (membrane hyperpolarization) take place simultaneously. The stepwise increase in extracellular K^+ activity corresponds to net effluxes of the magnitude of 1000 mmol/l cells per h, resulting in a maximum hyperpolarization of 60–65 mV, corresponding to peak values in absolute membrane potential of about −75 mV.

When CCCP was omitted, but otherwise standard conditions maintained, addition of $5 \cdot 10^{-6}$ M

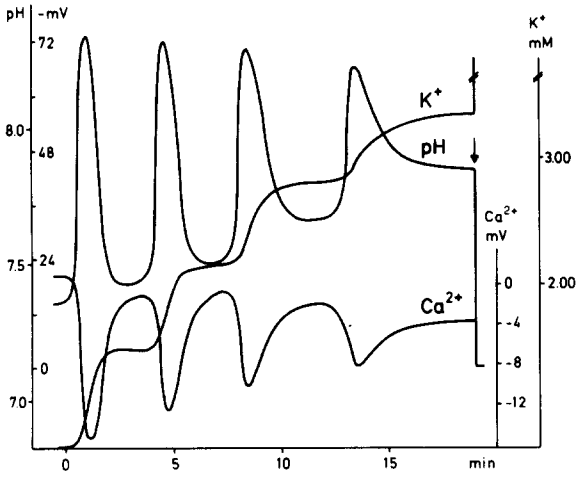

Fig. 1. Extracellular pH, K^+ activity and Ca^{2+}-electrode traces as functions of time. At zero time ionophore A23187 was added to a suspension of glucose-fed cells, to a final concentration of $2.3 \cdot 10^{-6}$ mol/l cell suspension. Saponin was added at the end of the experiment (arrow). pH values and calculated corresponding membrane potentials are shown on the double ordinate axis to the left and the K^+ activity on the ordinate axis to the right. The Ca^{2+}-electrode potential is indicated on the inserted ordinate axis. Note that a large part of the changes in Ca^{2+}-electrode potential reflects varying CCCP interference with the electrode. For a detailed discussion of the problems concerning conversion of the measured Ca^{2+}-electrode potential to Ca^{2+} activity see special section in the text. The abscissa is time in minutes after addition of ionophore.

of ionophore induced a low (about 20 mmol/l cells per h) permanent K^+ net efflux without the stepwise increase in extracellular K^+ activity characterizing the system in the case of oscillations.

As indicated in Fig. 1, 50 µl saponin solution was added at the end of the experiment. Since the extracellular phase was without buffering capacity, the pH of the hemolysate assumed the value of the intracellular phase. The difference between this value and the initial extracellular pH gives the value of the membrane potential (−12 to −16 mV) before the addition of ionophore and hence the absolute scale of membrane potential.

Oscillations in K^+ conductance and membrane potential as shown in Fig. 1 could be induced at an extracellular Ca^{2+} concentration of 2 mM, in this case, however, with only $1 \cdot 10^{-9}$ M ionophore present ($2.8 \cdot 10^{-8}$ M/l cells).

The influence of variation in the concentration of ionophore on the oscillations is shown in Fig. 2. At an extracellular Ca^{2+} activity of $5 \cdot 10^{-6}$ M (standard experimental procedure) and an ionophore concentration of about $8 \cdot 10^{-6}$ M or higher, a permanent hyperpolarization ensued. Below this value the periods between each cycle of hyperpolarization increased with decreasing concentration of ionophore whereas the duration of the single cycle of hyperpolarization was little affected.

With sub-threshold concentrations of ionophore at a given extracellular Ca^{2+} activity, oscillations could be induced by addition of Ca^{2+}. However, with respect to extracellular Ca^{2+} activity an absolute lower limit exists (attainable by EGTA addition) below which oscillations cannot be triggered by addition of excess ionophore.

In experiments with ATP-depleted cells oscillations could not be induced (Fig. 3). Instead, a permanent hyperpolarization with a concomitant, initial K^+ net efflux of about 1000 mmol/l cells per h took place. In order to determine whether a decrease in intracellular activity of Ca^{2+} in this case would initiate closing of the K^+ channels, 50 µl 20 mM Na_4EGTA solution, with a pH value equal to that of the extracellular phase at maximum hyperpolarization, were added to the suspension at the peak of hyperpolarization (not shown). The trace of the Ca^{2+}-electrode shifted towards the detection limit, indicating that the extracellular

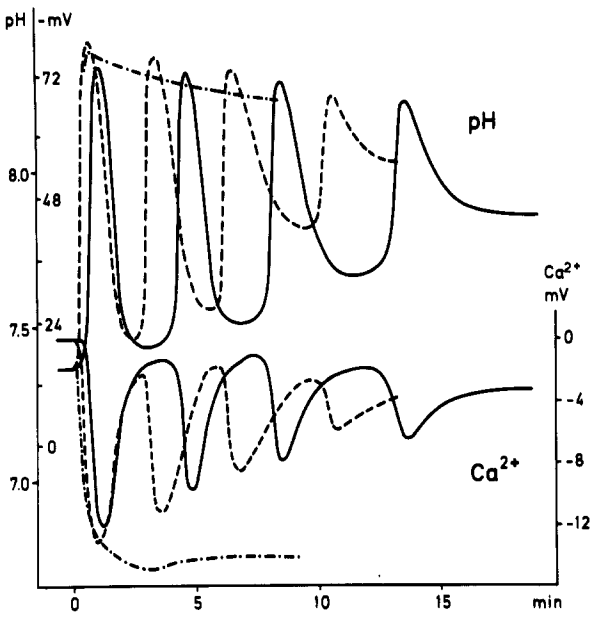

Fig. 2. Oscillations in a suspension of glucose-fed cells as a function of ionophore-concentration. At zero time ionophore A23187 was added to a final concentration of $2.3 \cdot 10^{-6}$ (————), $3.8 \cdot 10^{-6}$ (------) and $15 \cdot 10^{-6}$ M/l suspension (·–·–·). Changes in extracellular K^+ activity are not shown, otherwise the coordinate axes are as in Fig. 1.

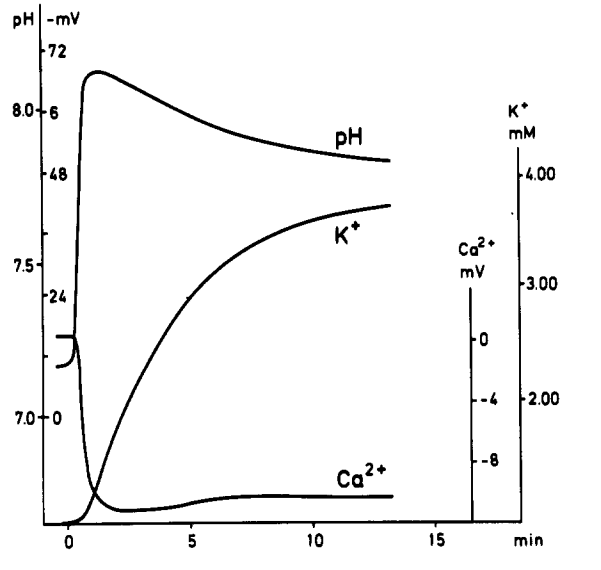

Fig. 3. Extracellular pH, K^+ activity and Ca^{2+}-electrode trace in a suspension of ATP depleted cells as functions of time. At zero time ionophore A23187 was added to a final concentration of $3.8 \cdot 10^{-6}$ mol/l cell suspension. Otherwise coordinate axes are as in Fig. 1.

activity of Ca^{2+} fell to 10^{-8}–10^{-9} M, but the hyperpolarization and K^+ net efflux persisted. In order to accelerate the decrease in intracellular Ca^{2+} activity, the concentration of ionophore A23187 in some experiments was increased simultaneously with the addition of EGTA but the K^+ channels remained open.

As a control, in experiments with ionophore A23187 in concentrations above the upper limit (see Fig. 2), EGTA was added to glucose-fed cells at the peak value of the permanent hyperpolarization. This resulted in a rapid decrease in both K^+ net efflux and hyperpolarization, restoring the original conditions within 1 min.

Experiments carried out at standard conditions, but with K^+ substituted for Na^+ in the low-calcium Ringer's solution resulted in a transient depolarization of 7–8 mV, indicating a transient K^+ net influx. No oscillations occurred under this condition but the transiency was of the usual duration.

Discussion

An oscillation such as that shown in Fig. 1 is the result of a complex interplay between the opening and closing processes of the K^+ channels, cyclic changes in ionophore-mediated Ca^{2+} influx, and the Ca^{2+} pump system. Since characteristics of the opening and closing of the K^+ channels can be analyzed on their own, these processes will be dealt with first and in the final part of the discussion the characteristics of all these systems will be gathered in an attempt to give an integral explanation of the mechanism behind the oscillation phenomenon.

Opening of the K^+ channels

At an extracellular Ca^{2+} activity of $5 \cdot 10^{-6}$ M or greater, the addition of sufficient ionophore resulted in a large net efflux of cellular K^+, simultaneous with a hyperpolarization of the membrane, culminating within 30–45 s in a peak value.

As discussed in detail in Results, the estimated maximum increase in intracellular Ca^{2+} activity during the first wave of hyperpolarization is $1 \cdot 10^{-5}$ M. At the peak value of the permanent hyperpolarization of ATP-depleted cells the figure

may be slightly higher. In both cases the observed maximum K^+ net efflux was about 1000 mmol/l cells per h. These results indicate that in glucose-fed as well as ATP-depleted cells the apparent K_m value of the opening mechanism for Ca^{2+} is in the micromolar range.

Lew and Ferreira [7] report an apparent K_m value of 1 μM Ca^{2+} for the K^+ channel in experiments with ATP-depleted cells, ghosts and glucose -fed cells exposed to high concentrations of ionophore ($1 \cdot 10^{-4}$ M), whereas they find an apparent K_m value of about 1 mM Ca^{2+} in experiments with fed cells at low ($1 \cdot 10^{-6}$ M) ionophore concentrations. They conclude that the high affinity found is an artifact introduced during ATP depletion, ghosting or by the high concentration of ionophore, and that the low affinity of the channels found in case of fed cells at lower concentrations of ionophore is the physiologically relevant figure.

Under present experimental conditions we cannot confirm this result, and since identical oscillations were induced by adding ionophore to a concentration as low as $1 \cdot 10^{-9}$ M to cells suspended in a 2 mM Ca^{2+}-Ringer's solution, the high affinity seen in this study can hardly be ascribed to an artifact caused by high concentrations of ionophore.

Closing of the K^+ channels

In the experiment presented in Fig. 1 the closure of the K^+ channels is particularly noteworthy. This closing process could be the result of a number of factors: the hyperpolarization of the membrane (voltage dependence); the high extracellular pH value accompanying the hyperpolarization; a decrease in intracellular Ca^{2+} activity; an effect of ionophore and/or CCCP; or the reflection of one or more time constants of the gating processes.

Most probably, the closing process is neither voltage-dependent, nor influenced by the high extracellular pH at the peak of the hyperpolarization. Thus, a transient depolarization was induced by the addition of ionophore A23187 to a suspension of cells in 155 mM KCl Ringer's solution. In this case a net influx of K^+ is turned off in a depolarized membrane at a decreased extracellular pH.

Obviously, the level of intracellular Ca^{2+} activity is of importance for the closing process, since the addition of excess EGTA, which changes a net influx of Ca^{2+} to a net efflux, during a permanent hyperpolarization in glucose-fed cells resulted in a closure of the K^+ channels.

In a similar experiment with ATP-depleted cells, the opening of the K^+ channels took place at the same rate as before, but EGTA addition did not result in a reversal of the hyperpolarization. It must therefore be concluded that the intracellular level of Ca^{2+} activity is not the only determining factor in the closing process.

As discussed in the section on the Ca^{2+}-electrode trace the minimum in extracellular Ca^{2+} activity (Fig. 1) most probably falls in the depolarizing phase. Accordingly, the K^+ channels close while the intracellular Ca^{2+} activity is close to maximum. Fig. 2 shows that at an intracellular Ca^{2+} activity above a certain threshold the K^+ channels remain open.

The fact that the results in Fig. 2 could equally well have been obtained at a higher extracellular Ca^{2+} level and a corresponding lower ionophore A23187 level indicates that the Ca^{2+} net influx and not the concentration of ionophore is the determining factor. Thus, the ionophore probably exerts no direct influence on the opening and closing processes of the K^+ channels.

The possible influence of CCCP on the opening and closing processes is more difficult to evaluate. The combination of CCCP-monitored membrane potential and ionophore A23187-facilitated Ca^{2+} net influx has been reported previously by Macey et al. [11]. They found no oscillations in K^+ conductance. This fact is easily explained, however, by the high Ca^{2+} net influx in their experiments. With an extracellular Ca^{2+} concentration of 0.5 mM they added $2 \cdot 10^{-6}$ M ionophore per litre cells as compared to the about $1 \cdot 10^{-8}$ M used in our experiments at this Ca^{2+} level.

Macey et al. [11] proposed that CCCP enhanced the Ca^{2+}-mediated permeability change, as manifested in an increased K^+ net efflux and a greater hyperpolarization in the presence of CCCP. Although the possibility of a direct effect of CCCP on the K^+-conductance of the single K^+ channel cannot be excluded, the fact that the maximum K^+ net effluxes observed in our experiments agree well with those obtained in experiments without CCCP [7], makes it seem more likely that the

synergistic effect is between CCCP and ionophore A23187.

As mentioned in the introduction, transient increases in conductance of the Ca^{2+}-sensitive K^+ channels of *Amphiuma* and human red cells exposed to a sudden increase in extracellular Ca^{2+} concentration have been reported previously [5,6,15]. In the experiments with human red cells it was shown, moreover, that K^+ channels closed while the concentration of total cellular calcium increased [17]. These results show that spontaneous closing of the K^+ channels can readily take place in the absence of CCCP. Preliminary results from experiments with varying CCCP concentrations indicate that CCCP induces an enhanced synchronization of the cells with respect to opening and closing of the channels.

Most probably, therefore, the closing of the K^+ channels seen in Fig. 1 is a spontaneous process. Regarding the mechanism behind this process very little can be said at present, but it might result from a sudden shift from the high- to low-affinity state reported by Lew and Ferreira [7,8].

In conclusion, the K^+ channels probably close spontaneously in cases of moderate increments in intracellular Ca^{2+} activity of glucose-fed cells. At Ca^{2+} activities above an upper threshold the K^+ channels remain open, but if the Ca^{2+} level is subsequently reduced the channels then close. In contrast, the closing process cannot proceed at all in ATP-depleted cells.

The oscillations

That oscillations in conductance of the Ca^{2+}-sensitive K^+ channel and intracellular Ca^{2+} level can be induced, is indicative of a system consisting of a number of sequential processes with feed-forward and -backward regulations.

Probably the ionophore-mediated Ca^{2+} transport and the Ca^{2+}-sensitive K^+ channel constitute one such system. Wulf and Pohl [16] have shown that the formation of the transport complex betwen ionophore A23187 and Ca^{2+} is strongly pH-dependent. The ionophore-mediated transport across a Müller-Rudin membrane was thus increased by a factor of 2 by an increase in pH from 7.0 to 8.0. Accordingly, the following sequence can be visualized. At the start of an oscillation the initial influx of Ca^{2+} triggers the opening of the K^+ channels. Mediated by CCCP, the accompanying hyperpolarization results in an increase in extracellular pH which in turn eventually doubles the Ca^{2+} influx. If the increase in intracellular Ca^{2+} activity is of moderate magnitude the K^+ channels close spontaneously and with progressing depolarization and decrease in pH_{ex}, the ionophore-mediated Ca^{2+} influx decreases.

The importance of the calcium pump is stressed by the fact that oscillations cannot be induced in ATP-depleted cells or glucose-fed cells exposed to ionophore A23187 concentrations above an upper threshold (compare Fig. 2). The activation or inactivation of the Ca^{2+} pump by increasing respectively decreasing intracellular Ca^{2+} activity constitutes, however, another sequence of processes with feedback regulation. About 4000 Ca^{2+} pump sites have been reported to be present in the human red cell membrane, corresponding to a V_{max} of about 20 mmol Ca^{2+}/l cells per h [17]. Only a small fraction of these pumps operate under physiological conditions. This situation can be described by the equilibrium

$$E + Ca^{2+} + Z \rightleftarrows (E\text{-}Ca\text{-}Z)$$

Here E represents inactive Ca^{2+}-ATPase, Z is the 17 kDa polypeptide activator calmodulin, and (E-Ca-Z) is the active form of the Ca^{2+}-ATPase which effectuates the translocation of Ca^{2+} across the membrane at the expense of ATP [20]. At physiological intracellular activities of Ca^{2+} ($1 \cdot 10^{-7}$ M) the equilibrium is displaced strongly to the left [19]. As the Ca^{2+} activity increases, E associates with Z and Ca^{2+} resulting in an increase in the number of active pump units. Scharff [18] has shown, however, that the reaction between E, Ca^{2+} and Z in vitro can be very slow. Thus the dissociation of (E-Z-Ca) takes place with a $t_{1/2}$ of about 2.5 s at p*Ca* 8, whereas at p*Ca* 6 $t_{1/2}$ is about 10 min. Thus, a tendency to overshoot is built into the system. During increasing intracellular Ca^{2+} activity a large number of inactive pumps is activated with a certain delay. The pump flux increases strongly and the net influx of Ca^{2+} is shifted to a net efflux. This results in a decrease in Ca^{2+} activity which, however, at a p*Ca* of about 6 is of little immediate consequence for the number of pumps and a high pump rate is therefore main-

tained. The high pump rate combined with the above-mentioned decrease in ionophore-mediated Ca^{2+} influx might result in so low an intracellular Ca^{2+} activity that the number of active Ca^{2+} pump units suddenly decreases sharply towards the physiological value. This in turn creates the condition for a new wave of hyperpolarization.

If the increase in intracellular Ca^{2+} activity takes place slowly, the peak value of this parameter as well as the degree of overshoot of the pump should be of moderate size. Therefore the periods between the waves of hyperpolarizations should be relatively long (compare Fig. 2).

Conclusion

Oscillation in the conductance of the Ca^{2+}-sensitive K^+ channels of human erythrocytes can be elicited only within a narrow range of ionophore A23187-mediated Ca^{2+} net-influxes. The occurrence of oscillations is probably based on two physiologically important characteristics: the Ca^{2+}-sensitive K^+ channels, once opened by a micromolar increase in intracellular Ca^{2+}-activity, close spontaneously within about 30 s.

The Ca^{2+} pump responds to an increase in intracellular Ca^{2+} activity after a delay of several seconds. Once activated, the high rate of pumping results in a sharp decrease in Ca^{2+} activity which is brought back to the original $1 \cdot 10^{-7}$ M or probably to a lower level. At this Ca^{2+} activity the number of active pump units drops suddenly, and the initial situation characterized by a pump rate which is too low to counteract the ionophore-mediated Ca^{2+} influx is reestablished. A new wave of Ca^{2+} net influx then takes place.

Oscillations in K^+ conductance could not be induced in ATP-depleted cells. Not only was the Ca^{2+} pumping obliterated but the K^+ channels remained open even if the intracellular activity of Ca^{2+} was diminished by addition of surplus EGTA.

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