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Effects of Quinine on Ca^{++}-Induced K^+ Efflux from Human Red Blood Cells

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Summary. The Ca^{++}-mediated increase in K^+-permeability of intact red blood cells (Gardos effect) was initiated by exposing cells to known concentrations of Ca^{++} (using EGTA buffers) in the presence of the ionophore A23187. The potency of quinine, an inhibitor of the response, was found to depend on the external K^+ concentration. In K^+-free solutions the concentration of quinine to achieve 50% inhibition (K_{50}) was 5 μM, but at 5 mM K^+ the required concentration was increased 20-fold to 100 μM. An increase in internal Na^+ had the opposite effect, allowing a high potency of quinine despite the presence of external K^+. Alterations in the internal K^+ level, on the other hand, were without effect on the K_{50}, suggesting that the membrane potential is not a factor. This conclusion is supported by the lack of effect on quinine inhibition of substitution of Cl^- by NO_3^-, a considerably more permeant anion. The data are consistent with the hypothesis that quinine inhibits by competitively displacing K^+ from an external binding site, the reported K^+-activation site for the Ca^{++}-mediated K^+-permeability.

The phenomenon of Ca^{++}-controlled K^+-permeability changes was first observed in human red blood cells (Gardos, 1956). Although a number of agents such as iodoacetate and substrate (Gardos, 1956), fluoride (Romero & Whittam, 1971), propanalol (Manninen, 1970; Szasz, Sarkadi & Gardos, 1978), Pb^{++} (Riordan & Passow, 1971), and A23187 (Lew & Ferreira, 1976; Reed, 1976) are capable of inducing a selective increase in K^+-permeability in red cells; all, with the exception of Pb^{++}, act by causing an increase in internal Ca^{++}.

Ca^{++} exerts its effect only on the internal surface of the cell (Romero & Whittam, 1971; Simons, 1976a; Blum & Hoffman, 1972) causing a large increase in K^+-permeability with no effect on Na^+-permeability (Kregenow & Hoffman, 1972). External K^+ stimulates the permeability increase (Blum & Hoffman, 1971; Knauf et al., 1975; Simons, 1976a), while internal Na^+ inhibits it (Knauf et al., 1975; Hoffman & Blum, 1977). A large number of membrane-active drugs inhibit the induced K^+-efflux (Szasz et al., 1978; Lew & Ferreira, 1978) including quinine and quinidine (Armando-Hardy et al., 1975; Lew & Ferreira, 1978).

The present report is concerned with the characteristics of inhibition of Ca^{++}-activated K^+-efflux by quinine. External K^+ reduces and internal Na^+ increases the quinine inhibition, and it is postulated that quinine acts by displacing K^+ from its external activation site. A preliminary report of this study has been presented (Reichstein, 1980).

Materials and Methods

Cell Preparation

Banked blood collected in acid-phosphate dextrose (1–2 weeks old) was used in all experiments. The blood was centrifuged, the buffy coat removed, and the cells were washed three times at room temperature with 140 mM NaCl, 20 mM Tris pH 7.4, at 37 °C, and once with 140 mM KCl, 1 mM EGTA, 5 mM adenosine, 20 mM Tris, pH 7.4. The cells were then incubated overnight at 50% hematocrit at 37 °C in the K^+-rich buffer containing in addition 20–40 μCi $^{86}RbCl$/ml (Amersham Corp.). The adenosine was used to limit the variation in the initial intracellular ATP.

Modulation of Internal Ions

Internal K^+ was partially substituted with Na^+ by use of nystatin (Cass & Dalmark, 1973). Cells were incubated 10 min, 0 °C, at 10% hematocrit in 140 mM chloride salts (the desired KCl concentration and the balance in NaCl), 30 mM sucrose, 20 mM Tris at pH 7.4 plus 62.5 μg/ml nystatin (Mycostatin, E.R. Squibb & Sons). The cells were collected by centrifugation and resuspended and incubated a further 10 min in the same solution, then washed 5× at room temperature in the same solution without nystatin, allowing 2–

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3 min at room temperature between washes. The cells were washed once in 140 mM (KCl + NaCl), 30 mM sucrose, 5 mM adenosine, 20 mM Tris at pH 7.4 and loaded with ^{86}RbCl overnight in this solution.

Internal K^+ was partially substituted with choline using *p*-chloromercuribenzene sulfonate (PCMBS) to increase the cation permeability (Garrahan & Rega, 1967; Sachs, 1977). Washed cells were incubated at 5% hematocrit, 4 °C, for 32 hr in 140 mM monovalent salts (KCl plus choline chloride). 1 mM $MgCl_2$, 10 mM glucose, 5 mM adenosine, 30 mM sucrose, 0.1 mM PCMBS. The medium was changed twice during incubation. To re-establish normal permeability, the cells were collected by centrifugation and incubated for 1 hr at 37 °C in the same solution without glucose and PCMBS but containing 4 mM dithiothreitol as a PCMBS scavenger. The cells were loaded overnight with ^{86}RbCl in the resealing solution.

For experiments in which the anion was substituted (NO_3^- for Cl^-), cells were incubated at 5% hematocrit in parallel in 140 mM KCl or KNO_3, 1 mM EGTA, 20 mM Tris, pH 7.4, at 37 °C for 1 hr. The cells were centrifuged and washed once in 140 mM KCl or KNO_3, 1 mM EGTA, 5 mM adenosine, 20 mM Tris at pH 7.4 and loaded with ^{86}RbCl in this solution.

Rb^+ Efflux Measurements

Ca^{++} was introduced into the cells to initiate the permeability response by means of the ionophore A23187 rather than by ATP depletion (Reed, 1976; Lew & Ferreira, 1976) and ^{86}Rb was used as a tracer for K^+-efflux. ^{86}Rb-loaded cells were washed 5–6 times with 140 mM (KCl plus NaCl), 1 mM EGTA, 20 mM Tris at pH 7.4 and suspended at 0.2% hematocrit in this solution. A23187 (Calbiochem-Behring Corp.) was added to a final concentration of 2 μM from a 1-mM stock dissolved in ethanol. Quinine hydrochloride (Sigma Chemical Co.) in the same buffer was added to the desired final concentration. At time zero, $CaCl_2$ was added to give a final concentration of 0.7 mM, unless stated otherwise. 1-ml samples of cell suspension were withdrawn every 3–4 min and centrifuged 10 sec in an Eppendorf model 5412 centrifuge. The supernatant was retained and counted; hemolysis was determined by comparison of the OD_{411} of supernatant and a hemolysed cell suspension. The Rb^+ remaining in the cell was calculated from the formula

$$Rb_{rem} = \frac{Rb_\infty - \text{cpm in supernatant}}{Rb_\infty \times (1\text{-fractional hemolysis})}.$$

Rb_∞, the total radioactivity per ml in the cell suspension, was determined by counting an aliquot of suspension and correcting for quenching by hemoglobin. The efflux rate constant was derived from a semi log plot of Rb_{rem} *vs.* time.

Determination of Anion Permeability

The net chloride and nitrate permeabilities were determined by a method developed by P.A. Knauf (*personal communication*). Briefly, the net KCl or KNO_3 efflux was determined over a range of valinomycin concentrations. At high concentrations of the antibiotic, the rate of efflux becomes independent of concentration and the net salt flow is limited by anion permeability.

Cells were incubated as described under anion substitution but without EGTA and without ^{86}RbCl. Net fluxes were determined at 10 mM K^+, 130 mM Na^+, 20 mM Tris, pH 7.4, and increasing valinomycin (0.025 to 1.66 μg/ml) (Calbiochem-Behring Corp.). Cell K^+ was determined by flame photometry using Li^+ as an internal standard (NILAB), hemoglobin was determined with Drabkin's reagent (Cyanmethemoglobin reagent dry pack, Hycel), cell water content was measured by the gravimetric method of Knauf et al. (1977) using ^{3}H-inulin to measure extracellular volume. The chloride distribution ratio was measured using ^{36}Cl. Fluxes were measured in mmol K^+ per liter cell water per min using the conversion factor, 0.7 liter cell water per liter of packed cells (Knauf et al., 1977) and 0.34 kg hemoglobin per liter packed cells. The value for P_K is taken from Knauf et al. (1977) and P_{Cl} and P_{NO_3} were calculated from Eq. (2) in that paper.

Uptake of Ca^{++}

Cells were washed in 140 mM NaCl, 20 mM Tris at pH 7.4 and suspended at 0.2% hematocrit in this medium at 37 °C with 2 μM A23187, and 0, 10 or 20 μM quinine at 37 °C. At time zero, 1–2 μCi/ml of $^{45}CaCl_2$ was added ($[Ca^{++}] < 1$ μM). 1-ml aliquots were layered on 0.4 ml silicone fluid SF-1023 (a generous gift of Mr. Edward Strohack, Canadian General Electric, Toronto) and centrifuged 15 sec in an Eppendorf centrifuge. The supernatant was removed and the space above the silicone fluid rinsed twice. The silicone was then removed, the excess absorbed with a cotton tipped swab, and the pellet lysed in 1 ml cold water. Hemoglobin was determined with Drabkin's reagent, and ^{45}Ca was counted after protein precipitation with trichloroacetic acid.

Uptake of Quinine

^{3}H-quinine was prepared by special order by Amersham Corporation. The compound was purified by thin-layer chromatography on silica gel plates using $CHCl_3/CH_3OH$ 1:3. The quinine was identified under UV light by comparison to a standard and scraped off the plate. The silica gel was extracted overnight with methanol, the gel removed by centrifugation, the methanol was evaporated and the residue dissolved in 0.1 N HCl. The concentration was determined by fluorescence in 0.1 N H_2SO_4.

To measure uptake, cells were incubated in 140 mM NaCl, 20 mM Tris at pH 7.4 plus quinine at 0 °C, 5% hematocrit for 1 min. 1-ml aliquots were centrifuged through silicone oil 1023 as described for Ca^{++} uptake. The radioactivity in the suspension and pellet were determined after protein precipitation with trichloracetic acid.

Results

In the presence of the ionophore A23187, Ca^{++} equilibrates relatively rapidly across the membrane of the red blood cell (Fig. 1), providing a convenient method for determining its effect on cation permeability. The response (measured by $^{86}Rb^+$-efflux) as a function of the Ca^{++} concentration is illustrated in Fig. 2. In one experiment (labeled high K^+) the external K^+ was 130 mM, approximately the same as cellular K^+, so that the possibility of hyperpolarization due to the increased K^+-permeability was avoided. Under these conditions it can be assumed that the internal free Ca^{++} approximates the external free Ca^{++}, and that the curve approximates the Ca^{++}-activation curve for the internal Ca^{++}-binding site. The half-maximum response was found at 0.17 μM and the maximum at 0.3 to 0.6 μM free extracellular

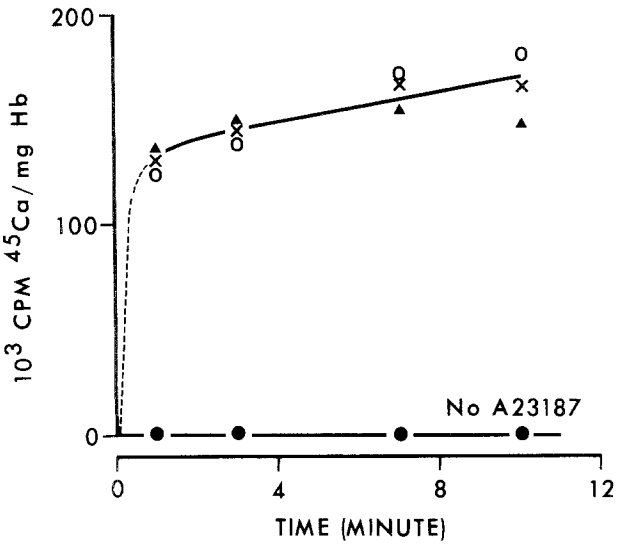

Fig. 1. Effects of ionophore A23187 and of quinine on the uptake of $^{45}Ca^{++}$. The medium contained 140 mM NaCl, 20 mM Tris, pH 7.4, at 37 °C. The concentration of A23187 if present was 2 μM. EGTA was not included and no nonradioactive Ca was added. ●—●, no A23187; ○—○, no quinine; ×—×, 10 μM quinine; ▲—▲, 20 μM quinine

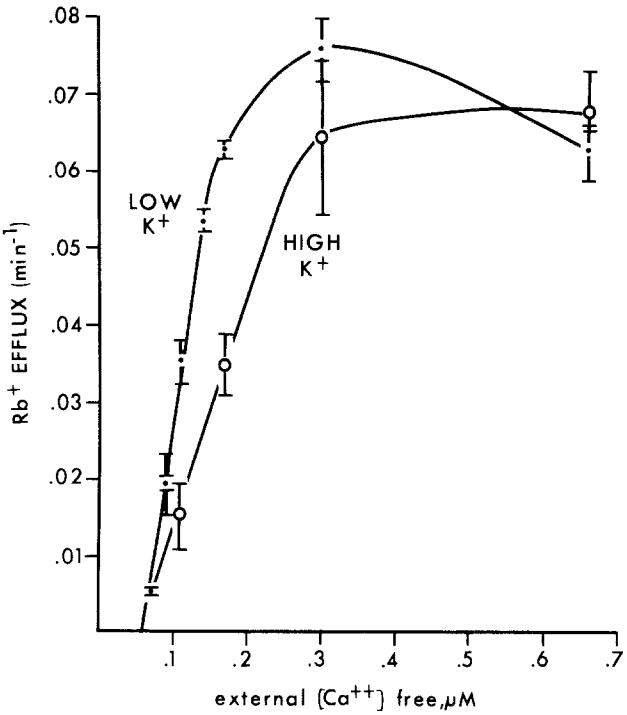

Fig. 2. Effects of external free Ca^{++} on Rb^+ efflux in high and low K^+ media. The efflux of Rb^+ from loaded cells at 37 °C in 140 mM NaCl (low K^+), or 130 mM KCl plus 10 mM NaCl (high K^+), 20 mM Tris, pH 7.4, 1 mM EGTA, 2 μM A23187 was measured at 0.5, 0.55, 0.6, 0.65, 0.7, 0.8 and 0.9 mM $CaCl_2$. The free Ca^{++} was calculated using the equilibrium constant $K_{CaEGTA} = 10^{7.13}$. Cell suspension were sampled at 4, 8 and 12 min to derive rate constants, and the points on the graph are the average results from 3 to 4 experiments

Ca^{++}.[1] These data agree reasonably well with those reported by Simons (1976*a*) using resealed ghosts without Mg^{++}, and Lew and Ferreira (1976, 1978) using high ionophore concentration in ATP-depleted cells. The sensitivity of the system to Ca^{++} is reduced by intracellular Mg^{++} (Simons, 1976*a*), but in our experiments A23187 has presumably transported Mg^{++} out of the cells (Reed, 1976).

In a similar experiment performed with cells suspended in a low K^+ medium, the half maximum external Ca^{++} concentration was lower (0.11 μM), suggesting that some degree of hyperpolarization has occurred. Under these circumstances, the internal free Ca^{++} would be expected to be somewhat higher than the external free Ca^{++}, resulting in the apparent increase in Ca^{++} sensitivity.

Quinine is an inhibitor of the Ca^{++}-activated K^+-permeability increase (Armando-Hardy et al., 1975; Lew & Ferreira, 1978). It has no effect on the ionophore catalyzed Ca^{++} ingress (Fig. 1), confirming the results of Armando-Hardy et al. (1975). It apparently prevents the cation permeability increase by a direct effect. It inhibits Rb^+ efflux if added before Ca^{++} (Fig. 3, top line, 96% inhibition) or if added after Ca^{++} has already initiated an increased permeability (center line, 86% inhibition) but with about a 1-min lag before inhibition becomes maximal. The control efflux is linear (on the semi-log plot) for the 12 min of the experiment, suggesting that a single homogeneous Rb^+ compartment is involved. In some experiments, the flux measurements were continued until over 75% of the Rb^+ had left the cells, with no apparent departure from linearity. Any inhomogeneity in response could, therefore, involve only a small fraction of the total Rb^+ compartment. Most of the cells in the population appear to respond equally.

The kinetics of quinine inhibition were examined at several external KCl concentrations and the results are shown in Fig. 4. With 5 mM KCl, the concentration of quinine required for 50% inhibition (K_{50}) is 98 μM, in the range of 0.1 to 0.2 mM reported by Armando-Hardy et al. (1975) and Lew and Ferreira (1978). As the K^+ concentration is decreased, however, there is a concomitant decrease in K_{50}. In "K^+-free" medium (K^+ is .01–.02 mM as measured by

[1] The extracellular free Ca^{++} concentration in the presence of 1 mM EGTA was calculated using the following stability constants: the formation constant for $CaEGTA^{2-}$ $K_{CaEGTA} = 10^{10.65}$ (Scharff, 1972), the acid association constants for $EGTA^{4-}$ and $HEGTA^{3-}$ K_1 and $K_2 = 10^{9.45}$ and $10^{8.85}$, respectively (Schwartzenbach & Flaschka, 1969) resulting in

$$K'_{CaEGTA} = \frac{[CaEGTA^{2-}]}{[Ca^{++}][EGTA_{TOT}]} = 10^{7.13}$$

at pH 7.4 where $EGTA_{TOT}$ is the total uncomplexed EGTA.

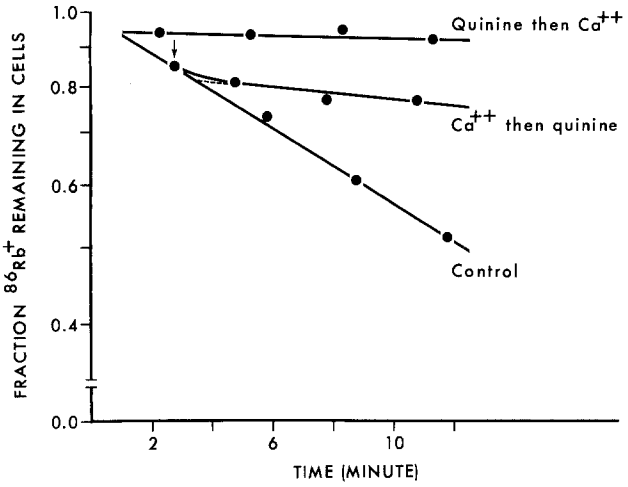

Fig. 3. Effect of order of addition of quinine and Ca^{++} on Rb^+ efflux. The medium contained 140 mM NaCl, 20 mM Tris, 1 mM EGTA, 0.7 mM $CaCl_2$, 2 μM A23187, and 20 μM quinine. Control – no quinine added; quinine then Ca – 20 μM quinine is added 2–3 min before $CaCl_2$ (which is added at time zero); Ca then quinine – 20 μM quinine is added 2-3/4 min after $CaCl_2$. Each point represents a duplicate determination. A representative experiment is shown of several performed

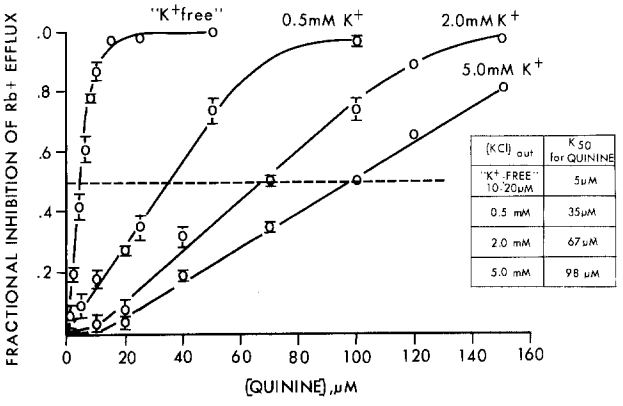

Fig. 4. Effect of external K^+ on the potency of quinine. Rb^+ efflux was measured in 140 mM NaCl, 20 mM Tris, pH 7.4, 1 mM EGTA, 0.7 mM $CaCl_2$, 2 μM A23187, and the indicated KCl and quinine concentrations. Rate constants for efflux varied from 0.03 to 0.11 min^{-1}. Points with error bars represent averages of at least three experiments; those without bars are single determinations. The broken line represents 50% inhibition and the values of K_{50} in the inset are taken directly from the intercepts with this line

flame photometry), the concentration of quinine required for 50% inhibition (K_{50}) is only 5 μM, over 20-fold less than that required at 5 mM K^+. At higher concentrations of external K^+, the curves are sigmoidal as previously reported (Lew & Ferreira, 1976), but the sigmoidicity is minimal at low K^+.

In the absence of quinine, the Rb^+ efflux is stimulated by external K^+ to the extent of 40 to 50%, with a maximal effect at about 2 mM cation (not shown). The concentration dependence of the activation in cells is similar to that reported in resealed ghosts, (Blum & Hoffman, 1971; Knauf et al., 1975; Simons, 1976*a*), but the extent of the activation is considerably less.

The membrane potential would vary with the external KCl concentration if the membrane potential is given by the Goldman constant field equation (Goldman, 1943)

$$V=\frac{-RT}{F}\ln B; \qquad B=\frac{P_{Cl}\,Cl_o+P_K\,K_i}{P_{Cl}\,Cl_i+P_K\,K_o}$$

where P_{Cl} and P_K are the permeabilities to Cl^- and K^+, and if it is assumed that P_K has been greatly increased by internal Ca^{++}.[2] In order to determine whether the modulation of the quinine potency by external K^+ (Fig. 4) was due to the K^+ concentration *per se* or to the membrane potential, the following two experiments were performed:

A) The external K^+ concentration was kept constant and the internal KCl was varied by rendering the membrane temporarily leaky by PCMBS so that internal KCl could be replaced by choline chloride, followed by restoration of cell impermeance by dithiothreitol (as described in Methods). At 0.5 mM external KCl, the potency of quinine was essentially the same in cells having internal concentrations of 25, 75, or 140 mM KCl (Fig. 5). Because the membrane potential would be modulated by alterations of *either* internal or external K^+, this lack of response to K^+-gradients suggests that the potential is not a factor and that the changes in quinine potency of Fig. 4 are directly related to the external K^+ concentrations *per se*.

B) Membrane potential changes that might arise from alterations of external K^+ were damped by substitution of Cl^- by NO_3^-. The measured Cl^- and NO_3^- net permeabilities (P_{Cl} and P_{NO_3}) were 0.0104 and 0.149 min^{-1}, respectively, a 15-fold difference.

[2] The approximate variation in the membrane potential of Ca^{++}-stimulated cells with internal K^+ concentration can be calculated with these assumptions: the unidirectional outward rate constant is expressed by the equation derived by Hunter (1977)

$$k_K^o=P_K\frac{\ln B}{B-1}; \qquad k_K^o,\ P_K \text{ in units of } min^{-1};$$

and the rate constant for Rb^+ efflux approximates the rate of K^+ efflux. In the outdated cells, the measured P_{Cl} is 0.0104 min^{-1}, in Fig. 5, k_K at 140 mM internal KCl is 0.034 min^{-1}; P_K is calculated to be 0.18 min^{-1}. Thus at 75 and 25 mM KCl the calculated rate constants are 0.050 and 0.089 min^{-1}, respectively; the observed rate constants were 0.061 and 0.075. The membrane potentials calculated from the P_K and P_{Cl} above at 140, 75, and 25 mM internal KCl are −73, −58, and −34 mV. In the presence of quinine, the K^+ fluxes are substantially reduced and the P_K and calculated potentials will be substantially lower, approaching at high quinine the values of the "normal" cell.

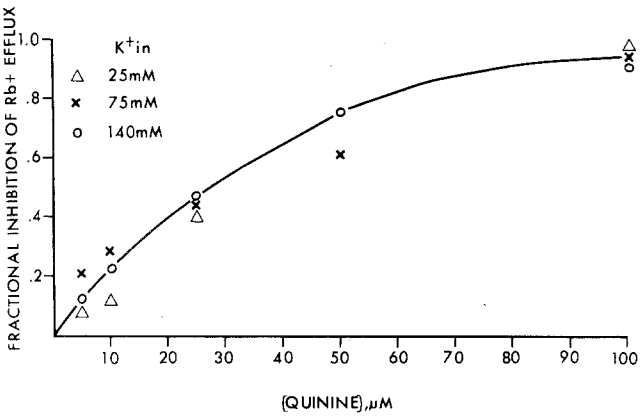

Fig. 5. Effects of internal K^+ on the potency of quinine. Internal KCl was varied using PCMBS (*see* Methods). Cells were loaded with 140 mM KCl, or 75 mM KCl, 65 mM choline chloride or 25 mM KCl, 115 mM choline chloride. Effluxes were measured in 139.5 mM NaCl, 0.5 mM KCl, 20 mM Tris, pH 7.4, 1 mM EGTA, 0.7 mM $CaCl_2$, 2 μM A23187 and the indicated quinine concentrations. Samples were taken at 4, 8 and 12 min. A representative experiment is shown

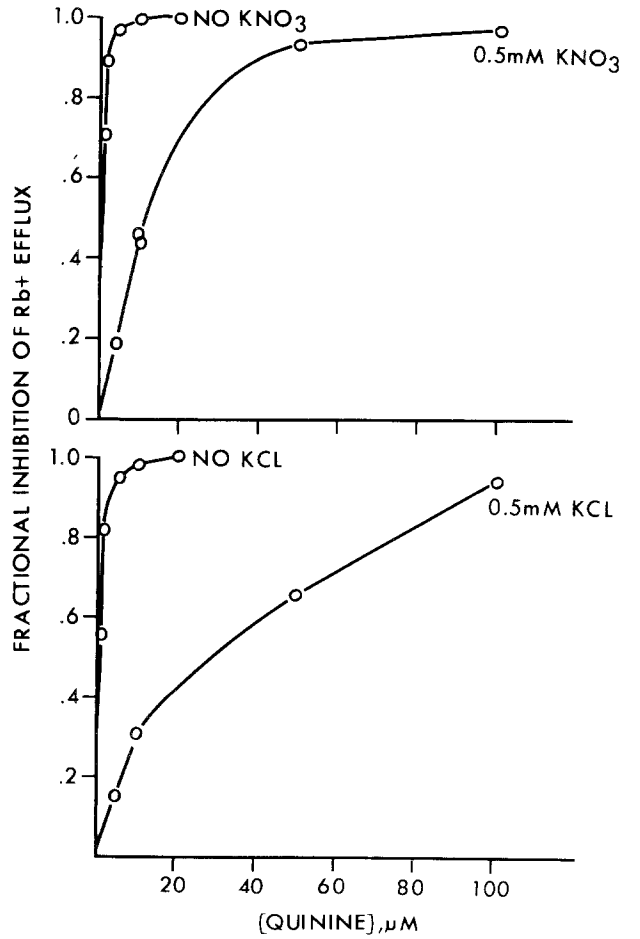

Fig. 6. Effect of anion situation on Rb^+ efflux. Cells were equilibrated with KNO_3 or KCl and loaded with $^{86}Rb^+$. Conditions were those described for Fig. 4. A representative experiment is shown

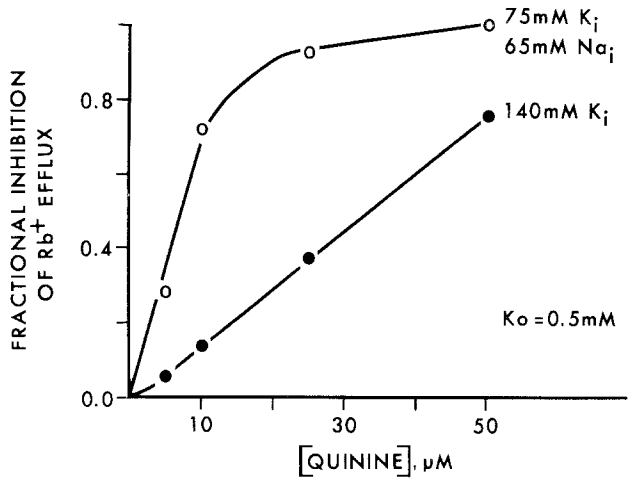

Fig. 7. Effect of internal Na^+ on the potency of quinine. Internal Na^+ and K^+ were varied using nystatin (*see* Methods). Conditions were those described for Fig. 5

Thus when internal and external chloride are replaced by the more permeant nitrate, the membrane potential should vary little when external K^+ is raised from 0.01–0.02 mM ("K^+-free" buffer) to 0.5 mM. Nevertheless, as shown in Fig. 6 there is still a substantial shift of the K_{50} for quinine even in the case of NO_3^-. This effect can be largely attributed to the effect of external K^+ *per se* rather than to a change in membrane potential. The absolute rate constants for Rb^+ efflux in NO_3^--substituted suspensions are about twice as high as those for Cl^- cells, which is consistent with the observation that $P_{NO_3} \gg P_{Cl}$. The membrane potential will be less negative for NO_3^--loaded cells, and the anion "drag" on the rate of cation efflux will be less. The K_{50} for quinine in the presence of 0.5 mM K^+ was less in the NO_3^--loaded cells than in Cl^--loaded cells. It cannot be excluded that this difference is in part due to differences in membrane potential.

In contrast to replacement of Cl^- by NO_3^- or partial replacement of K^+ by choline which had little effect on the potency of quinine, replacement of internal K^+ by Na^+ results in a substantial increase (Fig. 7). The K_{50} for quinine with 140 mM KCl inside without NaCl (at 0.5 mM KCl outside) was over 30 μM. With 75 mM KCl, 65 mM NaCl inside, the potency was increased fourfold to a K_{50} of 7 to 8 μM. At the same time, the absolute value of the K^+-flux (without quinine) decreased. At 75 mM KCl, 65 mM NaCl the rate constant is about 75% of that measured at 140 mM KCl and at 25 mM KCl, 115 mM NaCl the rate constant is barely above that for the EGTA control. Inhibition of Ca^{++}-stimulated K^+ efflux by internal NaCl has previously been reported by Knauf et al. (1975) in resealed ghosts and by Hoffman and Blum (1977) in intact cells.

^{3}H-quinine uptake was also measured (data not shown). Quinine appeared to be taken up nonspecifically by the cells, perhaps in the bilayer, with no evidence of saturation up to 1 mM quinine and no apparent KCl-sensitive component of binding.

Discussion

The characteristics of the Ca^{++}-mediated increase in K^+ permeability of intact cells described in this paper are in essential agreement with those reported in the literature: (i) the K^+- or Rb^+-efflux is stimulated by internal free Ca^{++} at levels below 1 μM as previously demonstrated in resealed ghosts without Mg^{++} (Simons, 1976*a*) and ATP-depleted cells using ionophore (Lew & Ferreira, 1976, 1978); (ii) the activation of the K^+-efflux by external K^+ requires similar cation concentrations, but the extent of the effect found in cells (40 to 50%) is less than that reported in resealed ghosts (Blum & Hoffman, 1971; Knauf et al., 1975; Simons, 1976*a*); (iii) the inhibitory effect of internal Na^+ is similar to that reported for resealed ghosts (Knauf et al., 1975) and intact cells (Hoffman & Blum, 1977); and (iv) the concentration of quinine for half maximal inhibition (K_{50}) of 0.1 mM at 5 mM external K^+ agrees with reported values of 0.1 to 0.2 mM (Armando-Hardy et al., 1975; Lew & Ferreira, 1978).

The new findings reported here are the dependence of the quinine potency (measured by the K_{50}) on external K^+ and internal Na^+ concentrations. As the external K^+ is reduced, the inhibition curves shift substantially toward lower quinine concentrations (Fig. 4). In "K^+-free" solutions (0.01 to 0.02 mM) the potency is 20 times as high as at 5 mM K^+. Raising the internal Na^+ also shifts the inhibition curves toward lower quinine concentrations. For example, replacement of about half of the internal K^+ by Na^+ increased the potency fourfold (Fig. 7). The K_{50} was shifted from 35 to 8 μM, almost as low as that for K^+-free solutions (Fig. 4) even though K^+ (0.5 mM) was present in the medium.

None of the effects of quinine or of K^+ can be attributed to any effect on the rates of Ca^{++} distribution. The Ca^{++} is largely equilibrated across the membrane by ionophore in about 1 min, with the rate of uptake independent of the presence of quinine (Fig. 1). Furthermore, the effects of quinine are independent of its time of addition relative to the ionophore (Fig. 3).

The effects of external K^+ and internal Na^+ on quinine potency might be indirect due to changes in membrane potential. To explore this possibility the potential was modulated in two ways, (i) by changing the internal K^+ concentration and (ii) by substitution of a more permeant anion NO_3^- for Cl^-. Neither the extent of the Ca^{++}-activated K^+-permeability increase nor the quinine inhibition was substantially altered (Figs. 5 and 6). The uninhibited rate constants for efflux at 75 and 25 mM internal KCl could be approximated using the calculated value for P_K at 140 mM KCl[3], suggesting the value of K^+ permeability is to the first approximation independent of potential.

The simplest explanation for the capacity of K^+ to reduce the potency of quinine is the assumption that quinine inhibits by competitively displacing K^+ from its external activating site, and that conversely, quinine can be competitively displaced from the same site by raising the external K^+ concentration. The common external site would presumably be negatively charged, with quinine binding involving its cationic group. A quinine-K^+ competition is consistent with the curves of Fig. 4 and is also supported by the evidence that internal Na^+ enhances the quinine inhibition, increasing its apparent affinity (Fig. 7). Internal Na^+ is reported to reduce the K^+-activation, so that higher concentrations of external K^+ are required to produce the permeability change (Knauf et al., 1975; Hoffman & Blum, 1977). The observed increase in the potency of quinine in the presence of internal Na^+ could result from the reduced effectiveness of K^+ as a quinine competitor at the external binding site.

In quantitative terms, addition of quinine and removal of external K^+ are not completely comparable. Addition of quinine results in an inhibition of over 95% of the Ca^{++}-mediated K^+ permeability, whereas removal of external K^+ inhibits by only 25 to 35%. In previous studies with resealed ghosts (Blum & Hoffman, 1971; Knauf et al., 1975), on the other hand, the dependence in the external K^+ was considerably greater. A possible explanation is based on the fact that the rates of K^+ efflux are considerably higher (by a factor of four to five times) in the experiments reported here on intact cells, probably due to use of the ionophore. This might result in a local elevation of the K^+ concentration in the vicinity of the activating sites. The efflux stream contains about 120 mM K^+, and concentrations of only 1 to 2 mM can produce substantial activations. Alternatively, part of the quinine effect may involve a different mechanism of inhibition.

The proposed mechanism assumes that quinine exerts its action at a site located at the outside of the membrane. A case can be made that quinine does not act at the cytoplasmic face of the membrane. In the experiment of Fig. 5 in which 140 mM internal KCl is

[3] *See* footnote 2, p. 60.

compared to 75 and 25 mM KCl at low quinine concentration, the calculated P_K is larger than the P_{Cl} so that at higher internal KCl concentrations the membrane potential should be more negative inside[4]. Quinine, which is a cation, would be expected to attain a higher internal concentration and therefore a larger inhibition. There is no evidence, however, of any substantial enhancement of inhibition.

Other inhibitors of the Gardos effect are also sensitive to external K^+, in particular oligomycin (Hoffman & Blum, 1977) and the carbocyanine dye 3,3′-diethylthiadicarbocyanine iodide (diS-C_2(5)) (Simons, 1976*b*, 1979). Oligomycin is not sensitive to external K^+ concentrations under 2 mM (Hoffman & Blum, 1977), but diS-C_2(5), like quinine, is sensitive to low K^+ concentrations. Simons suggested that the cyanine dyes bind to open channels only at the inner half of the membrane. Quinine binds to the open channels in that it inhibits even if added after Ca^{++}-enhanced K^+ efflux has been initiated (Fig. 3), but it is unknown whether it binds to closed channels. It seems to differ from the cyanine dyes, however, in terms of the apparent location of its effector site at the outer surface.

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[4] *See* footnote 2, p. 60.

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