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THE EFFECT OF pH ON THE K^+ MOVEMENTS ACROSS THE HUMAN PLATELET MEMBRANE DURING IN VITRO INCUBATION IN PLASMA AT 37°C

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Summary

(1) The K^+ concentration in human blood platelets, separated at room temperature from citrated platelet-rich plasma at pH 7.1, was 88 μmol/10^{11} platelets.

(2) Changing pH in the plasma altered immediately the intracellular K^+ concentration in platelets. An equilibrium was reached within 60—90 min and no further change was observed during the next 90 min. The maximum value was found at pH 6.0—6.4.

(3) The velocity of passive K^+ efflux varied with pH, having a minimum value at pH 6.0—6.4. Increasing the pH to 7.9 accelerated the velocity by a factor of 15. This was found in platelets incubated with ouabain to inhibit the active (Na^+, K^+)-pump.

Introduction

The majority of publications concerning ion transport across blood cell membranes deal with erythrocytes. Only few investigations have focused on blood platelets.

Like other cells, platelets have a high intracellular K^+ concentration maintained by an active (Na^+, K^+)-pump (ouabain-sensitive) and a passive K^+ influx and efflux [1—4]. Previous experiments [5] have shown that the K^+ concentration in platelets decreases by about 15% when stored at 37°C for 1 h in citrated plasma at pH 7.4. At pH 6.5 the decrease is negligible.

A reduced K^+ concentration in platelets results in reduced or irreversible

loss of their ability to aggregate [6]. K^+ is obviously of vital importance for their function. The influence of pH on intracellular K^+ concentration is reported in more detail in this paper. To find the pH optimum for keeping K^+ within the platelets, the effect of changing pH in the plasma was studied with the active (Na^+, K^+)-pump intact. The influence of pH on the passive K^+ efflux was studied with the pump inhibited with ouabain.

Methods

Human platelet-rich plasma was prepared from citrated blood obtained from apparently healthy donors, having received no medication during the week before blood sampling. 9 parts of blood were anticoagulated with 1 part of a solution containing 5.5 mM glucose, 38 mM citric acid and 75 mM trisodium citrate. Platelet-rich plasma was obtained by centrifugation for 15—20 min at 175 × *g*, at 20°C. The pH in the platelet-rich plasma varied from 7.00 to 7.19 (mean 7.12).

Platelet counts. These were performed by means of phase contrast microscopy after dilution (1 : 40) with a 0.2% trisodium citrate solution containing 1.6% HCHO. The counts in platelet-rich plasma varied from 4 to 6 · 10^{11}/l.

pH adjustment. To avoid variations due to changes in temperature, platelet-rich plasma was reheated to 37°C for 30 min (pH 7.0—7.19) before the pH adjustment and kept at 37°C throughout the experiment. The pH was adjusted by means of 1 part citric acid/citrate buffer (113 mM) to 4 parts of platelet-rich plasma. pH values above 7.5 were obtained by adding Tris (1 M) and pH values below 6.0 were obtained by means of HCl (1 M). The pH values after adjustment at 37°C were 5.6, 6.0, 6.4, 7.0, 7.5 and 7.9.

Incubation. Incubations were performed at 37°C with platelet-rich plasma from seven different donors. After pH adjustment the platelet-rich plasma was rotated (1 rev./min) in closed plastic syringes to avoid sedimentation of the platelets. When the passive K^+ efflux was estimated, the active (Na^+, K^+)-pump was inhibited with ouabain (0.1 mM). The incubations lasted for 30, 60, 90, 120 and 180 min with and without ouabain, in four experiments after pH adjustment to 5.6, 6.4 and 7.5, and in four other experiments after adjustment to pH 6.0, 7.0 and 7.9. In an additional four experiments, the platelet-rich plasma was adjusted to all six pH values and incubated without ouabain for 2 h. At the end of incubation, platelet-rich plasma was centrifuged at 37°C for 5 min at 20 000 × *g*. In none of the experiments did the pH change by more than 0.1 during the incubation.

K^+ concentration. The K^+ concentration within the platelets was determined after the centrifugation. The supernatant plasma was decanted and the test tube carefully wiped with cottonwool sticks to remove any remaining plasma. The platelet pellet was suspended in H_2O and freeze-thawed three times to lyse the cells. LiCl solution was added and the suspension centrifuged to remove insoluble fractions. K^+ was determined in the supernatant by means of flame photometry.

It was a striking observation that the platelet pellet, obtained after centrifugation of platelet-rich plasma, differed morphologically with respect to pH variations in the plasma. Platelets from plasma of low pH formed a firm pellet

which was difficult to resuspend in water, whereas platelets separed from platelet-rich plasma of pH 7.5—7.9 formed a loose jelly-like pellet. However, phase contrast microscopy at a magnification of 400X showed no altered platelet structure and no reduction in number after incubation, regardless of pH.

Trapped plasma. Trapped plasma in the platelet pellet was determined in four of the experiments in which the incubation lasted up to 180 min. ^{125}I-labelled human serum albumin (2.5 μCi/mg) was added to the platelet-rich plasma (0.05 μCi/ml platelet-rich plasma), and the trapped plasma was estimated from the ^{125}I content in the platelet extract. The mean volume was 0.78 ml/10^{11} platelets (S.E. 0.018), regardless of incubation time, pH or the presence of ouabain. With a K^+ concentration of 100—50 μmol/10^{11} platelets, the increase in [K^+] determination due to trapped plasma was within the error of the estimation, so no correction for trapped plasma was used.

Calculations and statistical methods. Due to significantly different K^+ concentrations in platelets from individual donors, the observed changes are given as relative values with references to the concentration measured in the platelets just before the pH adjustment. A two-sample *t*-test was used to test the significance of the variations. The significance level 5% was used ($P < 0.05$).

Results

K^+ concentration in platelets before and after incubation with the active (Na^+, K^+)-pump intact at different pH values

A K^+ concentration of 88 ± 2 μmol/10^{11} platelets (mean ±S.E., $n = 18$)

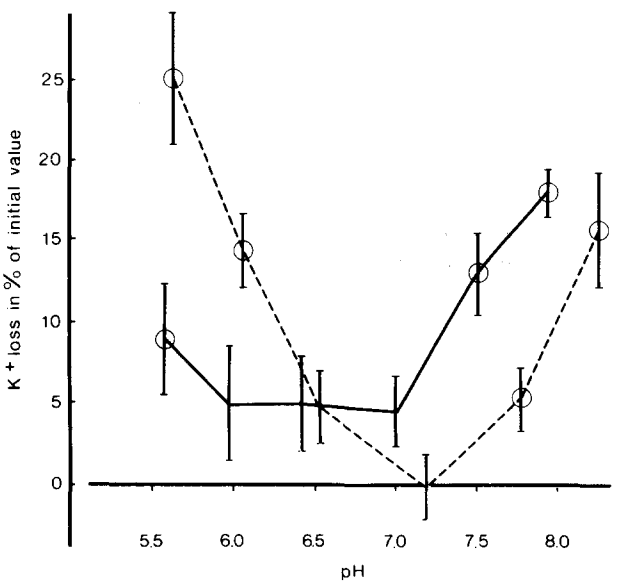


Fig. 1. K^+ loss in platelets due to changes in plasma pH. The values are calculated as % of concentrations. 4 parts of platelet-rich plasma were adjusted with 1 part of citrate buffer (113 mM). ------, platelet-rich plasma pH adjusted at 23°C (five experiments); ———, platelet-rich plasma pH adjusted at 37°C after reheating for 30 min at the same temperature (four experiments). Separation of the platelets was within 10 min after adjustment. The vertical bars indicate standard errors of the means. A circle indicates that the losses are statistically significant ($P < 0.05$).

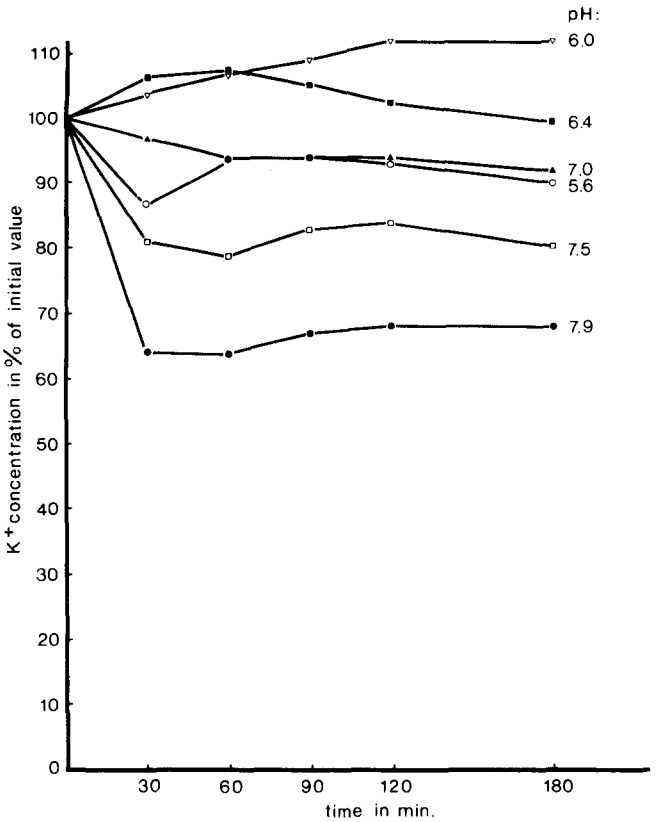


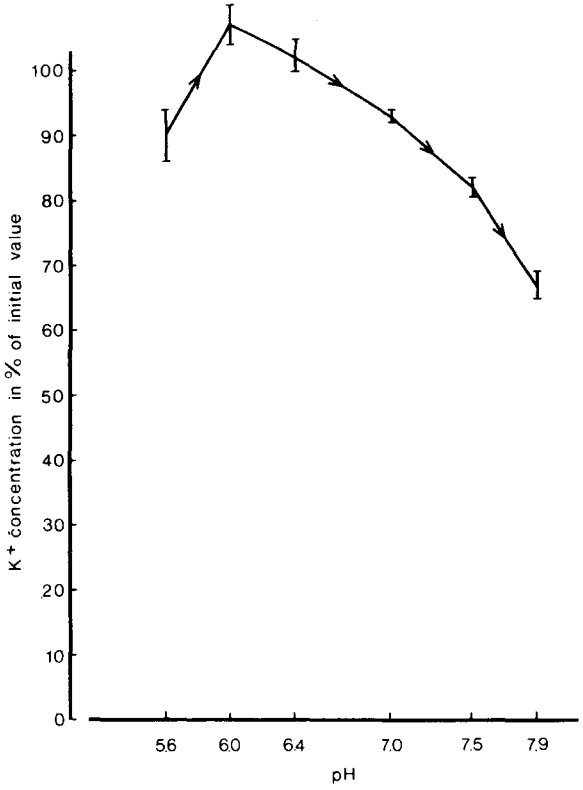
Fig. 2. K^+ concentration, as % of inital values, measured at 30-min intervals, in platelets incubated at 37°C and pH 5.6—7.9. The values are the means of four experiments.

was found when separation from platelet-rich plasma was performed at room temperature (23°C) and, pH 7.0—7.19, within 30—60 min after blood sampling. The mean value is based on the concentrations found in platelets from 18 donors. Nine of the donors were used more than once and the mean value for the individual donor applied.

The K^+ concentration measured in platelets kept at 23°C decreased significantly within a few minutes after the pH was adjusted to values below 6.5 and above 7.8. When platelet-rich plasma was reheated to 37°C, 30 min before adjustment, significant decreases were seen at pH values below 6.0 and above 7.0. Fig. 1 gives the relative K^+ loss related to changes in the pH at 23 and 37°C.

Fig. 2 depicts the relative K^+ concentration in platelets incubated at pH values between 5.6 and 7.9, measured at 30-min intervals. The major changes occurred within the first 30—60 min. After 90—120 min an equilibrium was reached.

In Fig. 3 the curve presents the relative K^+ concentrations in the platelets after 2 h incubation at 37°C, i.e., when equilibrium was reached. The maximal K^+ concentration was found at pH 6.0—6.4. The concentration was signifi-



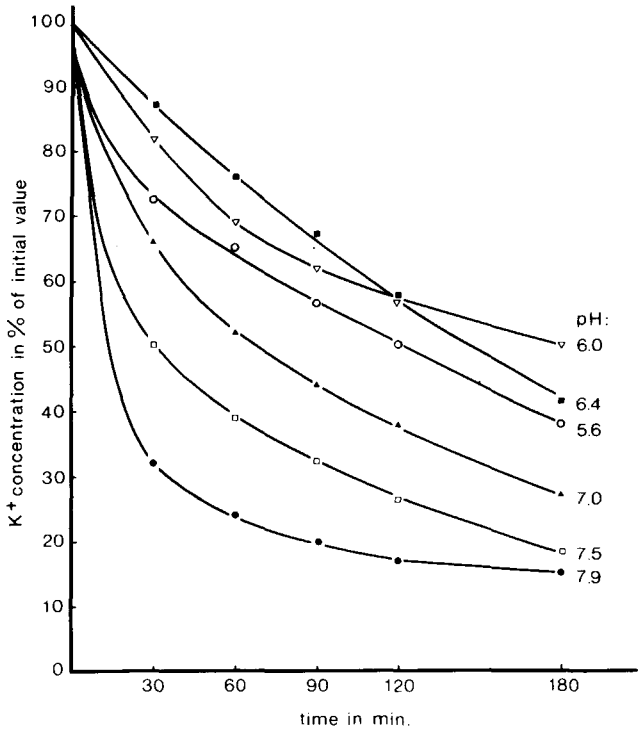


Fig. 3. K^+ concentration, as % of initial values, in platelets after 120 min incubation at 37°C and pH 5.6—7.9. The values are the means of eight experiments. The vertical bars indicate standard errors of the means. ⟶ indicates that the difference between two values is significant ($P < 0.05$).

Fig. 4. K^+ concentration, as % of initial values, measured at 30-min intervals in platelets incubated with ouabain (0.1 mM) at 37°C and pH 5.6—7.9. The values are the means of four experiments.

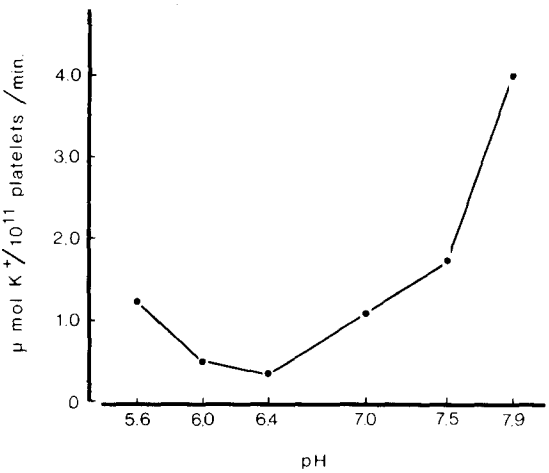


Fig. 5. Velocity of passive K^+ efflux measured for each pH value at the equilibrium concentration, i.e., the concentration measured after 120 min of incubation without ouabain. The velocities, % decrease/min, are measured by means of the slopes of the curves in Fig. 4 at the equilibrium concentrations. The results are converted to $\mu mol/10^{11}$ platelets by means of the initial K^+ concentration, 81 $\mu mol/10^{11}$ platelets (the mean value of all 12 experiments).

cantly lower ($P < 0.05$) at pH 5.6 and also at pH values above 6.4. At pH 7.9 the K^+ concentration was only 63% of that at pH 6.0.

Passive K^+ flux across the membrane

As ouabain inhibits the active transport of K^+ and Na^+ across the membrane, K^+ movements during incubation with ouabain are due to passive diffusion. The velocity depends on the concentration gradient for K^+ across the membrane, the membrane potential, and properties of the membrane.

In Fig. 4 the relative K^+ concentrations are depicted as a function of time during incubation with 0.1 mM ouabain. The initial efflux velocity is minimal at pH 6.4 and increases more than 15-times when the pH is raised to 7.9. The velocity of the efflux can be measured from the slopes of the smoothed-out curves at any point. At the equilibrium concentrations found during incubation without ouabain, i.e., at the concentrations depicted in Fig. 3, the velocity measured as % decrease of initial value/min has been estimated for each pH value by means of the curves in Fig. 4. The results have been converted to real values and are depicted in Fig. 5. This rate of passive net efflux must be equal to the rate of active net influx during incubation without ouabain, since the K^+ concentrations are in a steady state.

Discussion

In platelets separated from citrated blood at pH 7.1, the K^+ concentration is 88 $\mu mol/10^{11}$ platelets. This is somewhat higher than that reported by Born [1] (74 $\mu mol/10^{11}$) and by Cooley and Cohen [2] (73 $\mu mol/10^{11}$). The discrepancy may reflect different separation procedures, both authors using 2°C and pH 7.6. It is well known from studies of other cells [7—9] that temper-

ature and extracellular pH have an effect on intracellular cations, as is also displayed in Figs. 1 and 2.

It has previously been reported [5] that platelets lose K^+ during incubation at 38°C and pH 7.4, whereas the loss is almost negligible at pH 6.5. The present investigations demonstrate the marked influence of pH on the K^+ concentration of human platelets. Due to a high K^+ exchange rate, as shown by Born [1], it is possible to estimate alterations in intracellular [K^+] immediately after the change in external pH. However, the platelets do not continuously lose K^+. After 60—90 min, a steady state is reached. Maximal K^+ content in platelets is seen after incubation at pH 6.0—6.4 where the value is approx. 105% of the initial concentration. At pH 7.9 the concentration is reduced to 67%.

The intracellular K^+ concentration is regulated by properties of the membrane similar to those of other cell membranes, i.e., an active regulation by an (Na^+, K^+)-ATPase system tending towards counterbalancing the loss of K^+ by passive diffusion [2—4]. When the steady state is reached the passive efflux is in equilibrium with the active influx.

The rate of passive K^+ efflux is determined by the concentration gradient for K^+ across the membrane, the membrane potential, the equilibrium potential for K^+, and the properties of the membrane. When the active (Na^+, K^+)-pump is blocked with ouabain the velocity of the passive K^+ efflux is found to be minimal at pH 6.0—6.4. Changing the pH to 5.6 or 7.0 increases the initial velocity 3-times. At pH 7.9 the velocity is accelerated by a factor of 15, indicating an increase in the passive membrane permeability to K^+. The pH dependence of the state of the lipids and proteins in the platelet membrane is unknown. However, the fact that after centrifugation the platelets formed firm pellets at low pH and loose jelly-like substances at high pH indicates that the membrane may undergo morphological changes.

The direct influence of pH on the active (Na^+, K^+)-pump has not been investigated presently. Assuming that ouabain only has effect on the pump activity, the passive K^+ efflux measured in the ouabain experiments must be the same as when the pump is in function. Since a steady state is reached within 90—120 min after changing the pH, the active K^+ net influx is equal to the passive K^+ net efflux. The (Na^+, K^+)-ATPase system, regulating the active transport, has a pH optimum at 7.5—7.6 when isolated from mammalian tissue [10]. The results show, however, that the active K^+ net influx is remarkably increased at pH 7.9 as compared to the lower pH values.

It is well known from studies on the active cation transport in human red blood cells that the pump mediates a K^+-K^+ exchange and a K^+-Na^+ reversal in addition to the 'normal' Na^+-K^+ exchange [11,12]. Similar functions are likely to exist in the active transport system in platelets. They are probably influenced by pH and may be the explanation for the discrepancy between the pH optimum for the (Na^+ + K^+)-ATPase and for the active K^+ net influx.

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