Biochimica et Biophysica Acta, 352 (1974) 117–121

BBA 76638

THE INFLUENCE OF THE EXTRACELLULAR COUNTER-ION ON THE SODIUM-DEPENDENT, OUABAIN-UNINHIBITED SODIUM EFFLUX FROM HUMAN ERYTHROCYTES

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(Received December 27th, 1973)

SUMMARY

1. Progressive reductions of extracellular Na^+ (Na_o^+) reduced radioisotopic Na^+ efflux from human erythrocytes in the presence of ouabain. Nine different counter-ions were used to replace Na_o^+ and the results were qualitatively similar.

2. Ethacrynic acid always inhibited less Na^+ efflux in low Na_o^+ solutions than in 135 mM Na_o^+ solutions regardless of the counter-ion which replaced Na_o^+.

3. Na^+ efflux was stimulated in zero Na_o^+, ouabain solutions if sucrose served as the counter-solute; ethacrynic acid eliminated this effect.

4. We conclude that the reduction of ouabain-uninhibited Na^+ efflux as Na_o^+ is reduced from 135 mM to zero is truly the result of removal of Na_o^+ and not an inhibitory effect of the counter-ion used to preserve isosmolarity .Furthermore, this Na_o^+-dependent Na^+ efflux is practically eliminated by ethacrynic acid.

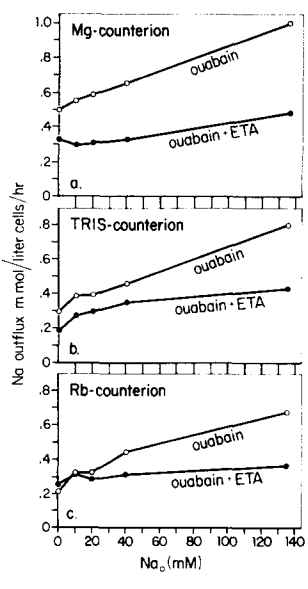
INTRODUCTION

There is general agreement that the ouabain-inhibited Na^+ efflux in human erythrocytes is an active process capable of net Na^+ transport and is dependent upon ATP for energy. However, substantial disagreement exists over the ouabain-uninhibited Na^+ efflux. This portion of Na^+ efflux has been attributed to a second pump [1,2], exchange diffusion [3–6], or a co-transport system [7]. Most workers have shown that ouabain-uninhibited Na^+ efflux is stimulated by extracellular Na^+ (Na_o^+) and a variety of counter-ions have been employed to replace Na_o^+ in these studies (chloride salts of Mg^{2+}, Ca^{2+}, Rb^+, Ca^+, K^+, Li^+ and $Tris^+$) [1–3, 6]. Results with choline [1, 8] and tetraethylammonium chlorides [1] were different in that no Na_o^+-stimulated, ouabain-uninhibited Na^+ efflux was observed when $choline^+$ or $tetraethylammonium^+$ replaced Na_o^+. Rettori and Lenoir [9] have recently concluded that Mg^{2+} and K^+, used as counter-ions to replace Na_o^+, inhibit a second Na^+ efflux pump. They contend that the decrement of Na^+ efflux after replacement of Na_o^+ with $MgCl_2$ or KCl was not the result of removal of the Na^+, but rather the inhibitory effects of the counter-ion on the Na^+ efflux mechanism. Because of these con-

flicting opinions, we conducted the experiments reported herein which systematically examined the interactions between nine different counter-ions as well as sucrose, Na_o^+ and ouabain-uninhibited Na^+ efflux in human erythrocytes. The results support our previous conclusion that ouabain-uninhibited Na^+ efflux is stimulated by Na_o^+ and this stimulation was observed to varying degrees with every counter-ion tested.

METHODS

All experiments utilized fresh human red cells from normal volunteers. Measurement of intracellular Na^+ and determination of $^{22}Na^+$ efflux have been described previously [4, 10]. High sodium flux solutions contained: 135 mM NaCl; 5.0 mM KCl; glycylglycine–$MgCO_3$ buffer, pH 7.4 at 37 °C, 27 and 4.4 mM, respectively; 10 mM glucose and 0.1 g/100 ml albumin. When extracellular Na^+ was varied, 295 milliosmolar solutions of the chloride salts of the cation to be considered were substituted accordingly for the NaCl. Ouabain was added as a water concentrate and ethacrynic acid was added as the dry powder. Ouabain was always present in a concentration of 10^{-4} M and all ethacrynic acid concentrations were 10^{-3} M.



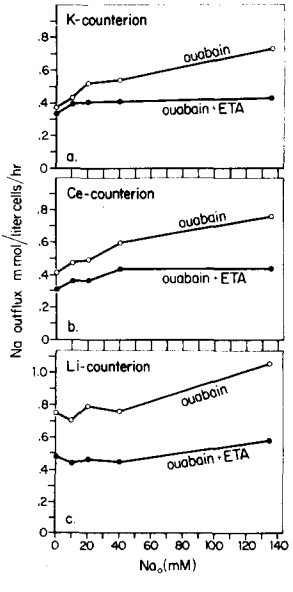
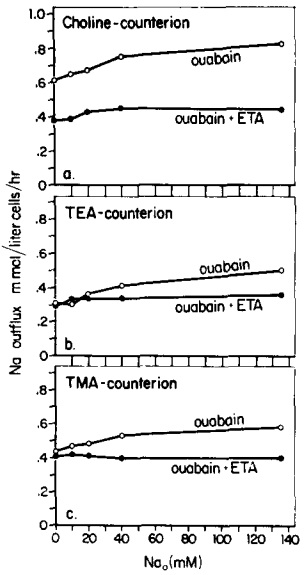


Fig. 1. The effects on erythrocyte Na^+ efflux of replacing extracellular Na^+ (Na_o^+) with chloride salts of Mg^{2+}, $Tris^+$ and Rb^+. Isosmolarity (295 mosM) was preserved at all times. The concentrations of ouabain and ethacrynic acid were 0.1 and 1.0 mM, respectively. The data are from individual experiments. The flux solution is described in the Methods section. ETA, ethacrynic acid.

Fig. 2. The effects on erythrocyte Na^+ efflux of replacing Na_o^+ with the chlorides of K^+, Ce^+ and Li^+. The basic experimental design was identical to Fig. 1 except that K^+, Ce^+ and Li^+ were used as counter-ions to replace Na_o^+. The data are from individual experiments except for Li^+ ($n = 2$). ETA, ethacrynic acid.



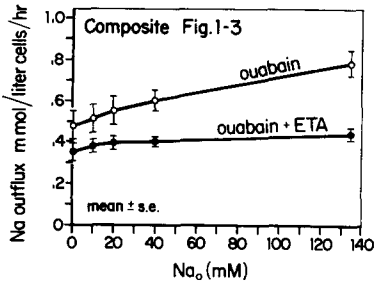


Fig. 3. The effects on erythrocyte Na^+ efflux of replacing Na_o^+ with the chlorides of choline$^+$, tetraethylammonium$^+$ and tetramethylammonium$^+$. These experiments were identical to those of Figs 1 and 2 except for the use of choline$^+$, tetraethylammonium$^+$ and tetramethylammonium$^+$ as counter-ions to replace Na_o^+. The plots are from individual studies except for choline ($n = 2$). TEA, tetraethylammonium$^+$; TMA, tetramethylammonium$^+$.

Fig. 4. Summary of counter-ions experiments. This is a composite drawing of the data from the eleven individual experiments using nine different counter-ions shown in Figs 1–3. ETA, ethacrynic acid.

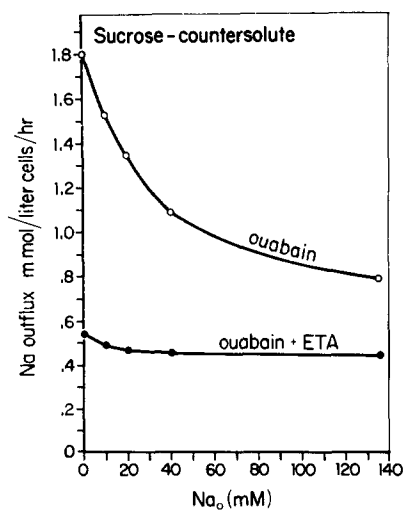


Fig. 5. The effects of extracellular sucrose on Na^+ efflux. Isosmolar sucrose (295 mosM) substituted for Na_o^+ in two experiments. Ethacrynic acid inhibited the increase of Na^+ efflux when sucrose totally replaced Na_o^+ in ouabain solutions. ETA, ethacrynic acid.

RESULTS

Figs 1–5 depict the results. The data for a given counter-ion are grouped arbitrarily on the basis of the magnitude of the Na_0^+-dependent Na^+ efflux in the presence of ouabain. In every experiment as Na_0^+ decreased, the concentration of the counter-ion increased reciprocally to preserve isosmolality. Regardless of the counter-ion studied, Na^+ efflux diminished in ouabain solutions as Na_0^+ decreased from 135 mM to zero. This Na_0^+ dependent efflux varied in its magnitude with the different counter ions in the following descending relationship: $Mg^{2+} > Tris^+$, $Rb^+ > K^+$, Ce^+, $Li^+ > choline^+ > tetraethylammonium^+$, $tetramethylammonium^+$. Fig. 4 is a composite drawing of the eleven individual experiments with the nine counter-ions shown in Figs 1–3. The complete removal of Na_0^+ in the presence of ouabain reduced Na^+ efflux to the same extent as the addition of ethacrynic acid in solutions with $Na_0^+ = 135$ mM. In addition the Na_0^+-dependent flux was practically eliminated in solutions of ouabain and ethacrynic acid. The results in the two experiments with sucrose differed from those with the counter-ions because replacement of Na_0^+ with sucrose diminishes the ionic strength of the solutions and thereby changes membrane potential difference [11]. In the sucrose experiments, ouabain-uninhibited Na^+ efflux more than doubled as Na_0^+ was replaced by sucrose and this increment was abolished by ethacrynic acid (Fig. 5). The effects of ethacrynic acid were studied with the other counter-ions since ethacrynic acid has been reported to inhibit the Na_0^+-dependent, ouabain-uninhibited Na^+ efflux if Mg^{2+} is the extracellular counter-ion [1, 3, 4]. Two points should be made about the results with ethacrynic acid. First, ethacrynic acid substantially diminished or completely inhibited the Na^+ stimulation of Na^+ efflux in ouabain solutions regardless of the counter-ion. Second, the effects of ethacrynic acid when Na_0^+ was zero depended upon the counter-ion used to replace Na_0^+. Ethacrynic acid inhibited 0.1 mmole or less of Na^+ efflux in zero Na_0^+ solutions if the counter-ion was $tetramethylammonium^+$, $tetraethylammonium^+$, K^+, Rb^+, Ce^+ or $Tris^+$. Ethacrynic acid reduced Na^+ efflux from 0.15 to 0.3 mmoles at zero Na_0^+ if the counter-ion was Mg^{2+}, Li^+ or $choline^+$. In sucrose solutions with zero Na_0^+, the ethacrynic acid-inhibited Na^+ efflux increased 4-fold when compared with 135 mM Na_0^+ solutions.

DISCUSSION

These experiments were designed to examine the relationship between Na_0^+ and ouabain-uninhibited Na^+ efflux. Nine different counter-ions and one counter-solute were used to replace Na_0^+ in order to examine the possibility that a decrement of Na^+ efflux may be due to the positive (inhibitory) influence of the counter-ion chosen rather than to the reduction or elimination of Na_0^+. Since the effect of decreasing Na_0^+ was qualitatively similar for all the counter-ions used to substitute for Na_0^+, we conclude that the concentration of Na_0^+ was the important variable. Rettori and Lenoir [9] concluded differently and stated that Mg^{2+} and K^+ inhibited Na^+ efflux if they substituted for Na_0^+. These authors reported, in agreement with others [1, 8], that when $choline^+$ replaced Na_0^+, there was no decrement of ouabain-uninhibited Na^+ efflux. Our results with $choline^+$ differ since we observed small but sequential decrements of Na^+ efflux as $choline^+$ replaced Na_0^+ in ouabain solutions.

Sachs [2] has found a similar decrement of Na^+ efflux (0.3 mmoles) if Na_o^+ was totally replaced by choline chloride.

Under all conditions ethacrynic acid reduced substantially or eliminated this Na_o^+-stimulated, ouabain-uninhibited Na^+ efflux. This suggests but does not prove, that the ethacrynic acid-inhibited Na^+ efflux and the Na_o^+-stimulated Na^+ efflux were identical. If ethacrynic acid did not inhibit any Na^+ efflux when Na_o^+ was zero regardless of the counter-ion, then the identity of the two fluxes would be more certain. With four of the counter-ions (K^+, Rb^+, tetraethylammonium$^+$ and tetramethylammonium$^+$) ethacrynic acid had no discernible effects on Na^+ efflux in Na^+-free solutions. If Mg^{2+}, Li^+ or choline$^+$ replaced Na_o^+ then ethacrynic acid inhibited 0.15–0.30 mmoles of Na^+ efflux in ouabain solutions. The explanation for these different results is not apparent.

The results of the two experiments utilizing sucrose to preserve isosmolarity (Fig. 5) show the expected stimulation of Na^+ efflux as extracellular ionic strength is reduced. Donlon and Rothstein [11] have shown that the potential difference across the membrane and the membrane permeability increase under these circumstances. Since ethacrynic acid almost eliminated this phenomenon, it is possible the drug prevented the potential difference and permeability changes although inhibition of a carrier-mediated process cannot be excluded.

These experiments show that removal of Na_o^+, regardless of the counter-ion used to preserve isosmolality, reduces Na^+ efflux from human erythrocytes. This reduction of Na^+ efflux cannot be attributed to an inhibitory action of the counter-ion since nine different cations could be used with qualitatively similar results. We prefer the interpretation based upon our previous work that the ouabain-uninhibited, Na_o^+-stimulated Na^+ efflux is Na^+ exchange diffusion [4–6].

ACKNOWLEDGEMENT

This work was supported by the United States Public Health Service Grant AM 14264 and by an unrestricted grant from the Markle Foundation. The secretarial assistance of Jennifer Pastore was invaluable.

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