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OUABAIN-UNINHIBITED Na^+ TRANSPORT IN HUMAN ERYTHROCYTES: THE EFFECTS OF TRIFLOCIN

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SUMMARY

1. Triflocin, a new diuretic agent, was used to study ouabain-uninhibited Na^+ transport in human erythrocytes.

2. Triflocin inhibits ouabain-uninhibited $^{22}Na^+$ efflux and $^{22}Na^+$ influx and in this regard it resembles furosemide and ethacrynic acid. No effect on net Na^+ flux could be demonstrated.

3. The portion of the ouabain uninhibited $^{22}Na^+$ flux inhibited by triflocin, ethacrynic acid or furosemide appears to represent exchange diffusion of Na^+ rather than a net transport step.

INTRODUCTION

Recently, interest has developed in the ouabain-uninhibited Na^+ transport in human erythrocytes[1–4]. It has been known for some time that ouabain and related digitalis glycosides inhibited approximately 70 % of Na^+ efflux (an uphill process) and this ouabain-inhibited fraction of efflux has been considered synonymous with the active transport of Na^+ (ref. 5). Na^+ influx (a downhill process) is unaffected by ouabain unless extracellular K^+ is removed[6]. Through the use of ethacrynic acid and furosemide it has been recognized that the ouabain-uninhibited component of Na^+ efflux could be reduced and that Na^+ influx was also diminished in equimolar amounts[3]. Controversy exists as to whether this ethacrynic acid-inhibited or furosemide-inhibited component of Na^+ flux contributes net Na^+ transport or exchange diffusion[1–4]. The present studies utilized a new diuretic agent, triflocin, (trifluoro, toluidino nicotinic acid)[7] to examine further the ouabain-uninhibited Na^+ movements in the human erythrocyte.

METHODS

All experiments were conducted with fresh, heparinized blood obtained on the morning of study from normal human volunteers. The techniques of Na^+ efflux, Na^+ influx, and intracellular Na^+ determinations have been described previously[3,8]. All incubation solutions contained: 140 mM NaCl; 5 mM KCl; glycylglycine–$MgCO_3$ buffer, pH 7.4, at 37°, 27 mM and 4.4 mM, respectively; 1.2 mM phosphate as Na_2HPO_4–

NaH_2PO_4, pH 7.4; 10 mM glucose; and 0.1 g albumin per 100 ml. Ouabain was used in a concentration of 0.1 mM whereas triflocin and ethacrynic acid were used in concentrations of 1 mM. Triflocin and ethacrynic acid were dissolved in a small volume of 0.1 % NaOH and ethanol, respectively.

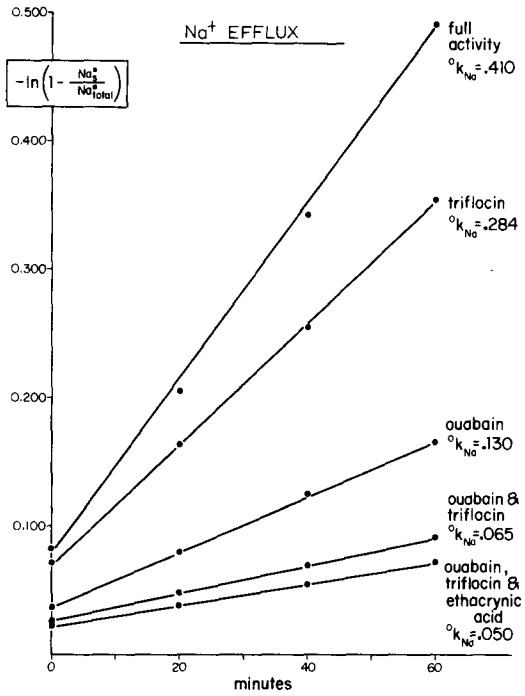

Fig. 1. A typical Na^+ efflux and the effects of triflocin. $-\ln(1 - Na^*_s/Na^*_{total})$ represents the amount of $^{22}Na^+$ appearing in the supernatant fluid (Na^*_s) divided by the total $^{22}Na^+$ present (Na^*_{total}). Values for the efflux rate constant ($^\circ k_{Na}$) for Na^+ were calculated as the regression equation for the lines. Electrolyte and inhibitor concentrations are given in METHODS.

RESULTS

Na^+ efflux

An initial dose response curve showed no effect on Na^+ efflux with triflocin 0.01 mM and less effect with 0.1 mM than with 1 mM. Therefore all subsequent experiments utilized a concentration of triflocin of 1 mM. Fig. 1 depicts a representative outflux experiment and Fig. 2 summarizes the data. Triflocin, used alone, inhibited 1.30 ± 0.08 mmoles Na^+ efflux per l cells per h in 3 experiments. Compared to ouabain (2.95 ± 0.11) triflocin is a weak inhibitor of efflux; however, triflocin continued to exert an inhibitory effect in the presence of ouabain, *i.e.* on ouabain-uninhibited Na^+ efflux. In 8 studies triflocin caused a decrement in the ouabain-uninhibited efflux of 0.51 ± 0.05 mmole, $P < 0.001$. The magnitude of the fall in the ouabain-uninhibited efflux was similar to that reported previously with furosemide and with ethacrynic acid[3]. In order to investigate the possibility of a similar locus of action for triflocin and ethacrynic acid, the drugs were used simultaneously with ouabain. With triflocin present

ethacrynic acid had a negligible effect ($0.15 \pm 0.04, n = 5, P > 0.05$) upon the ouabain-uninhibited fraction of Na^+ efflux. These results suggested major similarities in the site of action of these transport inhibitors.

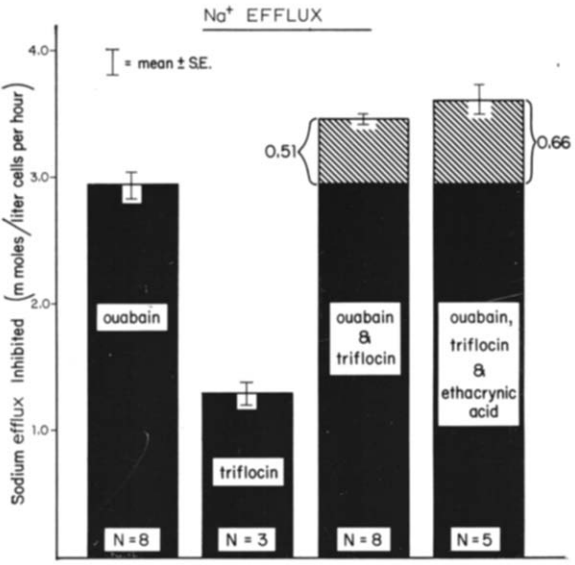

Fig. 2. Summary of efflux experiments. Triflocin always exerted an inhibitory action despite the ouabain effect. The effect of adding ethacrynic acid to ouabain and triflocin- was negligible (0.15 mmole decrement) and not significant in 5 studies.

Na^+ influx

Although Na^+ influx is a passive, downhill process, a portion of the influx may be carrier-mediated and thereby susceptible to inhibition. Earlier studies have shown that furosemide reduces Na^+ influx to the same extent that it reduces outflux in the presence of ouabain[3]. Ouabain alone does not influence Na^+ influx if extracellular K^+ is present[6]. Triflocin inhibited Na^+ influx in the present studies despite the presence of 5.0 mM extracellular K^+. Influx was reduced from 2.08 ± 0.12 in ouabain solutions to 1.75 ± 0.12 mmoles/l cells per h in ouabain and triflocin media ($n = 5$, $P < 0.001$). A representative influx experiment is depicted in Fig. 3. Although triflocin always diminished efflux to a greater extent than influx the mean difference between the fluxes was small (0.22 mmole) and not statistically different from zero.

Net fluxes

If the bidirectional inhibition by triflocin of ouabain-uninhibited Na^+ transport defines an exchange diffusion process then no alterations of net Na^+ flux would be expected[9]. Net fluxes were determined on the 5 paired influx–outflux studies over a 6-h incubation. Human erythrocytes incubated with 0.1 mM ouabain, gained 6.8 ± 0.45 mmoles Na^+ per l cells whereas cells incubated with ouabain and triflocin gained 7.2 ± 0.32 mmoles Na^+. There is no significant difference between these results. If triflocin acted upon a carrier which accomplished net transport of Na^+ under the circumstances of these experiments it would be predicted that cells would gain more Na^+ in ouabain and triflocin solutions than in ouabain solutions.

DISCUSSION

Triflocin is a new and chemically novel diuretic agent[7,10]. It is structurally dissimilar from ethacrynic acid and furosemide although their natriuretic potency and site of action in the kidney appear similar. *In vitro* triflocin does not have sulfhydryl-inhibitory properties[10]. In this regard it resembles furosemide and contrasts with ethacrynic acid. Since chemically dissimilar agents such as furosemide and ethacrynic acid have reasonably similar effects on ouabain-uninhibited Na^+ transport in erythrocytes[3], it appeared useful to study triflocin in this context.

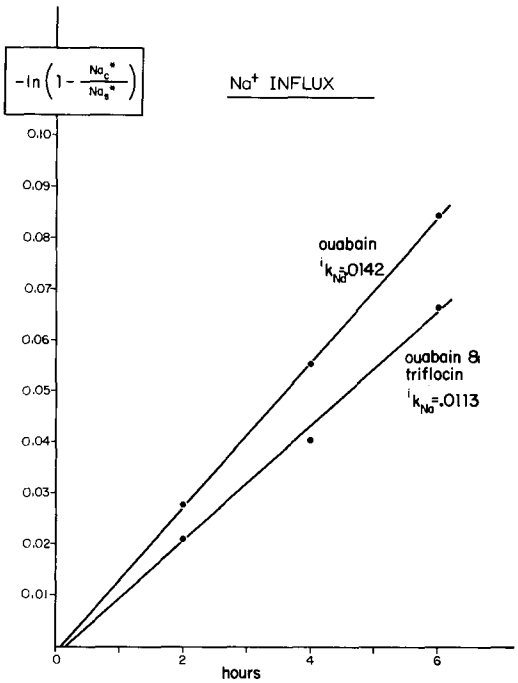

Fig. 3. A representative Na^+ influx. The ordinate $-\ln(1-Na^*_c/Na^*_s)$, shows the $^{22}Na^+$ which was transported into the cells (Na^*_c) divided by the $^{22}Na^+$ in the supernatant media (Na^*_s). Electrolyte and inhibitor concentrations are given in METHODS.

Controversy exists over the meaning of ouabain-uninhibited Na^+ movement in erythrocytes. HOFFMAN AND KREGENOW[1] described originally an inhibitory action of ethacrynic acid on ouabain-uninhibited Na^+ efflux[1]. They concluded that a second active transport system for Na^+ existed. SACHS[4] has recently reached the same conclusions utilizing furosemide rather than ethacrynic acid to define the second efflux mechanism. On the other hand LUBOWITZ AND WHITTAM[2] and DUNN[3] have concluded that the ouabain-uninhibited, furosemide or ethacrynic acid-inhibited, Na^+ fluxes represent exchange diffusion. Exchange diffusion differs fundamentally from active transport in that it is a bidirectional flux without net Na^+ transport[9]. The present experiments, utilizing triflocin, appear to confirm the conclusions that exchange diffusion occurs in human red blood cells. Triflocin inhibits bidirectional Na^+ flux (efflux and influx, Figs. 1 and 3) and did not change intracellular sodium concentration over a

6-h incubation. If the triflocin-inhibited Na^+ efflux contributed net, active transport of Na^+, cells incubated with triflocin and ouabain should accumulate more Na^+ than with ouabain alone. Such was not the case. If the triflocin-inhibited portion of Na^+ efflux is exchange diffusion, no triflocin effect should be demonstrable in solutions free of Na^+. Although this specific experiment was not done, recent work with furosemide and ethacrynic acid confirms that these agents show no substantial inhibitory effect upon Na^+ efflux in Na^+-free ouabain solutions containing the same other ionic constituents (Mg^{2+} as replacement ion for Na^+) as the present experiments (unpublished observations). A final point should be made concerning the similarity or identity of the aforementioned ouabain-uninhibited Na^+ transport mechanisms. It was shown previously that ethacrynic acid and furosemide inhibited this fraction of efflux at the same locus as judged by the lack of additive effects[3]. The results in Fig. 2 show that triflocin and ethacrynic acid do not have additive effects in the presence of ouabain and presumably are affecting the same outflux carrier. Triflocin apparently inhibits some of the classic, ouabain-inhibited pump since it reduced Na^+ efflux 1.30 ± 0.08 mmoles when ouabain was not included in the media. Furosemide and ethacrynic acid have similar effects[1-4].

ACKNOWLEDGEMENT

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