Inhibition and Stimulation of K^+ Transport across the Frog Erythrocyte Membrane by Furosemide, DIOA, DIDS and Quinine

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Abstract. Frog erythrocytes were incubated in iso- or hypotonic media containing 10 mmol/l Rb^+ and 0 1 mmol/l ouabain and both Rb^+ uptake and K^+ loss were measured simultaneously Rb^+uptake by frog red cells in iso- and hypotonic media was reduced by 30–60% in the presence of 0 01–0 1 mmol/l [(dihydroindenyl)oxy] alkanoic acid (DIOA) or 0 5–1 0 mmol/l furosemide Furosemide inhibited K^+ loss from frog erythrocytes incubated in hypotonic media but did not affect it in isotonic media DIOA at a concentration of 0 05 mmol/l inhibited of K^+ loss from frog erythrocytes in both iso- and hypotonic media At the concentrations of 0 01 and 0 02 mmol/l DIOA significantly suppressed K^+ loss in a K^+-free chloride medium but not in a K^+-free nitrate medium The Cl^--dependent K^+ loss was completely blocked at a concentration of 0 1 mmol/l DIOA and the concentration required for 50% inhibition of K-Cl cotransport was ~0 015 mmol/l However, the inhibitory effect of DIOA on K-Cl cotransport was masked by an opposite stimulatory effect on K^+ transport which was also observed in nitrate medium Quinine in a concentration of 0 2–1 0 mmol/l was able to inhibit Rb^+ uptake and K^+ loss only in hypotonic media In isotonic media, quinine produced a stimulation of Rb^+ uptake and K^+ loss A three to five-fold activation of Rb^+ uptake and K^+ loss was consistently observed in frog erythrocytes treated with 0 05–0 2 mmol/l 4,4'-diisothiocyanatostilbene-2,2'-disulphonic acid (DIDS) In contrast, another stilbene derivative 4-acetamido-4'-isothiocyanatostilbene-2,2'-disulpho nic acid (SITS) had no effect on K^+ transport in the cells Thus, of these drugs tested in the present study only DIOA at low concentrations may be considered as a selective blocker of the K-Cl cotransporter in the frog red blood cells

Key words: Erythrocytes — K-Cl cotransport — Furosemide — DIOA — DIDS — Quinine

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Abbreviations: DIOA, [(dihydroindenyl)oxy] alkanoic acid; DIDS, 4,4'-diisothiocyanato-stilbene-2,2'-disulphonic acid; SITS, 4-acetamido-4'-isothiocyanatostilbene-2,2'-disulphonic acid.

Introduction

The plasma membranes of a wide variety of animal cells contain a K-Cl cotransport system that has been shown to play a crucial role in cell volume regulation(Lauf et al. 1992; Hoffmann and Dunham 1995). To date, most detailed studies on K-Cl cotransport have been done with erythrocytes of mammals and some other species, and they focused on the investigation of the kinetic properties and the regulation of its activity. However, no specific inhibitor of the K-Cl cotransport system has been found so far. The Cl^--dependent K^+ fluxes in human (Kaji 1986), sheep (Lauf 1984), and duck (Lytle and McManus 1987) erythrocytes have been found to be inhibited by millimolar concentrations of loop diuretics, furosemide and bumetanide. However, these diuretics had no effect on the Cl^--dependent K^+ fluxes in horse red blood cells (Gibson et al. 1994). Ten years ago Garay et al. (1988) have reported that [(dihydroindenyl)oxy] alkanoic acid (DIOA) may be used as a selective blocker of the K-Cl cotransport in human erythrocytes. The inhibitory effects of DIOA on K-Cl cotransport were confirmed in following studies on human (Olivieri et al. 1993) and equine erythrocytes (Gibson et al. 1994) and vascular smooth muscle cells (Saitta et al. 1990). On the other hand, DIOA has little effect on K-Cl cotransport in mouse red blood cells (Armsby et al. 1996). Anion transport inhibitors stilbene sulphonates (DIDS and SITS) have been shown to have the Cl^--dependent K^+ fluxes in human erythrocytes unaffected (Kaji 1986) but DIDS was able to inhibit K-Cl cotransport in sheep and fish red blood cells (Garcia-Romeu et al. 1991; Delpire and Lauf 1992). Adragna and Lauf (1994) have recently reported that K-Cl cotransport in sheep red blood cells was inhibited by quinine. However, quinine did not effect on K-Cl cotransport in human erythrocytes (Kaji 1986). Such controversial findings could be due to differences in methodology and dose of blockers used, as well as to species differences.

We have recently shown that the erythrocyte membrane of the frog *Rana temporaria* may serve as a convenient model for investigating the K-Cl cotransport mechanism (Gusev et al. 1995; Agalakova et al. 1997). The frog erythrocytes lack Na-K-2Cl cotransport as its selective inhibitor, bumetanide had no effect on K^+ and Na^+ transport in the cells. The Cl^--dependent K^+ transport across the frog erythrocyte membrane is about 50–70% of the total K^+ fluxes under isotonic conditions and it is significantly increased in hypotonic media. The present experiments were designed to test the ability of known inhibitors (furosemide, DIOA, quinine, DIDS, and SITS) to suppress K-Cl cotransport activity in frog red blood cells. We compared the effects of blockers on both inward and outward K^+ transport measuring simultaneously Rb^+ uptake and K^+ efflux. In addition, the influence of the blockers on K^+ (Rb^+) fluxes was studied either in isotonic or hypotonic conditions after activation of the K-Cl cotransport. The results of the present study indicate

that in frog erythrocytes the effects of the inhibitors studied are dependent on the direction of the K^+ (Rb^+) transport as well as on the swelling-induced stimulation of the K-Cl cotransport.

Materials and Methods

Animals

The experiments were carried out on erythrocytes of the frog *Rana temporaria* in the period from October to April. The animals were kept in an aquarium with a small amount of tap water at a temperature of 2–4 °C.

Cell preparation

The blood samples were taken from the heart into test tubes with heparin, washed immediately with physiological saline and sedimented by centrifugation (2300 × *g*, 5 min, 4 °C). The supernatant and the upper layer were discarded and the cells were washed three times with the same saline. The washed erythrocytes were suspended in standard medium to a hematocrit of 30–35%. The standard medium contained (mmol/l): 102 NaCl; 3 KCl; 1 $MgCl_2$; 10 Tris-HCl (pH 7.6 at 20 °C). The cell suspension was kept at room temperature for 60 min before measuring K^+ transport.

Measurement of K^+ fluxes

The K^+ fluxes in both directions were simultaneously determined from Rb^+ uptake and K^+ loss from the cells incubated in media with 10 mmol/l RbCl. The cell suspension was washed with isotonic or hypotonic medium and its aliquots were added into tubes with media containing different test substances to a final hematocrit of 2–3%. After 60 min incubation the red cells were sedimented and the supernatant was taken to measure K^+ concentration. The cells were washed three times with isotonic or hypotonic buffered $MgCl_2$ solutions. The washed cells were lysed in distilled water to determine cellular Rb^+, K^+ and Na^+ contents. The isotonic incubation medium contained (mmol/l): 55 NaCl; 10 RbCl; 1 $MgCl_2$; 10 Tris-HCl; 80 sucrose; and 0.1 mmol/l ouabain (pH 7.6). The hypotonic incubation medium had the same composition except for 80 mmol/l sucrose. In the K^+-free medium 10 mmol/l RbCl was replaced with 10 mmol/l NaCl. Previously we have shown (Gusev et al. 1995) that ^{86}Rb uptake by the frog red cells was linear up to 60 min and reflected unidirectional K^+ (Rb^+) influx. The fluxes of Rb^+ and K^+ were expressed as mmol per litre of original volume of packed cells per hour. K^+ and Na^+ contents in cell lysates were measured by a Flapho-40 flame photometer; K^+ concentrations in the flux media containing Rb^+ and Rb^+ concentration in cell lysates were determined by emission flame spectroscopy (Perkin-Elmer model Atomic absorption spectrophotometer).

Chemicals

Ouabain, furosemide, quinine sulphate and DIDS (4,4'-diisothiocyanatostilbene-2,2'-disulphonic acid) were obtained from Sigma Chemical Co (St Louis, MO, USA) SITS (4-acetamido-4'-isothiocyanatostilbene-2,2'-disulphonic acid) was from Serva (Heidelberg, Germany) R(+)-DIOA ([(dihydroindenyl)oxy] alkanoic acid) was from Research Biochemicals Incorporated (USA) All other chemicals were of the highest purity commercially available Stock solutions of DIDS, SITS (20 mmol/l), furosemide (100 mmol/l) were prepared with dimethylsulphoxide (DMSO), that of quinine (100 mmol/l) with acidified DMSO (pH 5), all other reagents were dissolved in standard saline The same volume of DMSO was added to the control samples

Statistical analysis

All results were expressed as mean values ± S E M for the indicated number of experiments Data were compared using paired Student's *t*-test and were regarded as significant at $P < 0\ 05$

Results

In the first series of experiments, frog erythrocytes were incubated for 60 min at 20°C in isotonic and hypotonic media containing 55 mmol/l NaCl and 10 mmol/l RbCl Under these conditions, Rb^+ uptake by the red cells and K^+ efflux were simultaneously measured

Effect of blockers on Rb^+ uptake

The data on Rb^+ uptake in the presence of various agents are presented in Fig 1 Furosemide at a concentration of 0 5 and 1 0 mmol/l induced a significant reduction in Rb^+ uptake by red cells incubated in both iso- (Fig 1*A*) and hypotonic media (Fig 1*B*) Under isotonic conditions, the decrement in Rb^+ uptake in the presence of 0 5 mmol/l furosemide (0 80 ± 0 13 mmol/l cells h) was smaller than that produced by 1 mmol/l furosemide (1 21 ± 0 16 mmol/l h, $P < 0\ 02$) Thus furosemide at the concentrations of 0 5 and 1 0 mmol/l suppressed 34 2% and 51 1% of the total Rb^+ uptake by isovolemic cells, respectively A similar extent of Rb^+ uptake inhibition (~63%) was observed when swollen erythrocytes were treated with 0 5 and 1 0 mmol/l furosemide DIOA, a known inhibitor of Cl^--dependent pathways, at a concentration of 0 01, 0 05 and 0 10 mmol/l inhibited 27%, 46% and 44% of the Rb^+ uptake by frog red cells in isotonic medium, respectively (Fig 1*A*) In hypotonic medium, 0 05 mmol/l DIOA reduced Rb^+ uptake by 30% of the control level (Fig 1*B*) Exposure of frog red blood cells to 0 2–1 mmol/l quinine, an inhibitor of many ion transport pathways, caused a small, but statistically significant, increase in Rb^+ uptake in isotonic medium In contrast, quinine produced a marked reduction in Rb^+ uptake by swollen cells The inhibitory effect in swollen cells was 9 8%, 42 4% and 51 3% of the control level at quinine concentrations of

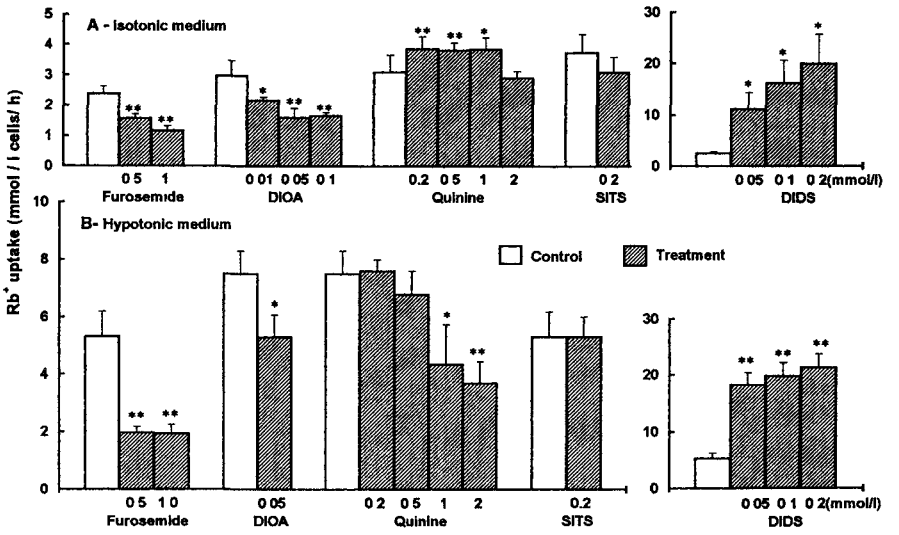

Figure 1. Comparison of the effects of drugs on Rb^+ influx Frog erythrocytes were incubated in isotonic (*A*) or hypotonic (*B*) media with 10 mmol/l Rb^+ for 60 min in the absence (open columns, control) and in the presence of different agents (hatched columns) The columns show means ± S E M for 5 6 separate experiments ($*P < 0\ 05$, $**P < 0\ 02$ significantly different from control, paired Student's *t*-test)

0 5, 1 0 and 2 0 mmol/l, respectively Unexpectedly, exposure of frog red cells to DIDS (0 05–0 2 mmol/l) resulted in a sharp enhancement of Rb^+ uptake by the cells incubated in iso- and hypotonic media The Rb^+ uptake by red cells in isotonic medium was increased from a control level of 2 5 ± 0 3 mmol/l h to 11 1 ± 3 2, 16 2 ± 4 4 and 19 9 ± 5 8 mmol/l h in the presence of 0 05, 0 1 and 0 2 mmol/l DIDS, respectively After treatment of swollen erythrocytes with DIDS Rb^+ uptake was about 20 mmol/l h as compared with the control level of 5 3 ± 0 9 mmol/l h On the other hand, addition of another stilbene derivative SITS (0 2 mmol/l) to incubation media had no detectable effect on Rb^+ entry into the red cells

Effect of blockers on K^+ efflux

Fig 2 illustrates net K^+ loss from frog erythrocytes during a 60 min incubation in iso- and hypotonic media There was no detectable change in K^+ loss when cells were exposed to 0 5–1 0 mmol/l furosemide in isotonic medium But treatment of red cells with furosemide in hypotonic medium significantly reduced K^+ efflux (Fig 2*B*) Furosemide at the concentration of 0 5 mmol/l caused a greater reduction in K^+ loss (5 5 ± 0 9 mmol/l h) than that at the concentration of 1 0 mmol/l (3 0 ± 0 8 mmol/l h) A statistically significant decrease in K^+ loss was observed in the cells treated with 0 05 mmol/l DIOA in both media However, there was a small increase in K^+ loss in isotonic medium at the DIOA concentration of 0 1

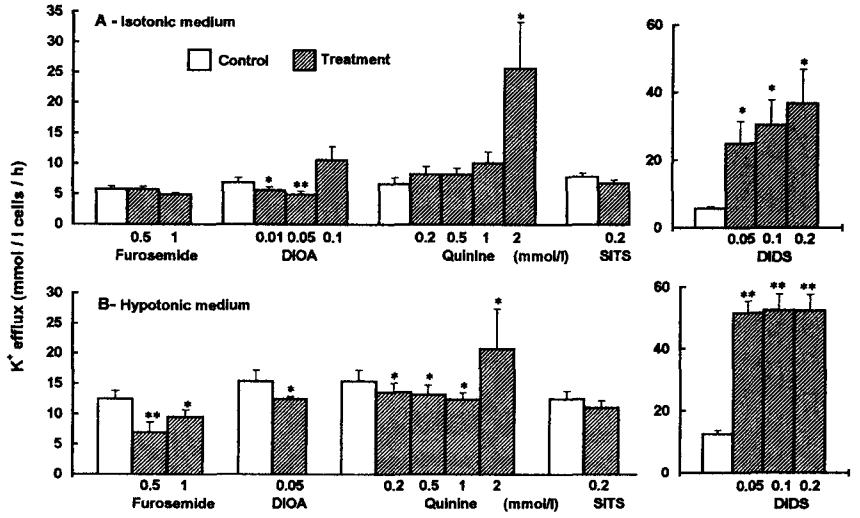

Figure 2. Effects of different drugs on K^+ efflux from frog red cells incubated in isotonic (*A*) or hypotonic (*B*) media. The K^+ effluxes were determined in the same experiments as illustrated in Fig. 1 for Rb^+ influx. All symbols are the same as in Fig. 1. The K^+ effluxes were calculated from changes in K^+ concentration in the media following cell incubation. Values are means ± S.E.M. for 5–6 separate experiments. ($^*P < 0.05$; $^{**}P < 0.02$ significantly different from control, paired Student's *t*-test).

mmol/l. Under isotonic conditions, 0.2, 0.5 and 1 mmol/l quinine did not alter K^+ efflux whereas 2 mmol/l quinine significantly stimulated K^+ transport from frog erythrocytes. In contrast, quinine at concentrations up to 1.0 mmol/l had a weak inhibitory effect on K^+ loss from swollen red cells. Again, under all experimental conditions, treatment of frog erythrocytes with DIDS (0.05–0.2 mmol/l) was associated with a dramatic increase in K^+loss (~4–6-fold). SITS at the concentration of 0.2 mmol/l had little effect on K^+ transport from the red cells in both iso- and hypotonic media.

Thus the results obtained indicate that some blockers had a nonspecific influence on the red cell membrane producing stimulation of K^+ transport in both directions. To ascertain the specificity of the ion transport changes induced by these inhibitors, intracellular ion composition was also investigated.

Effect of blockers on intracellular ion contents

We measured Na^+ and K^+ contents in washed erythrocytes after incubation of the cells for 60 min in the presence of the blockers studied. The Na^+ content in control cells in two series of experiments in isotonic medium was 6.4 ± 0.7 ($n = 10$) and 5.6 ± 0.5 mmol/l cells ($n = 6$). There was a significant rise in Na^+ concentration

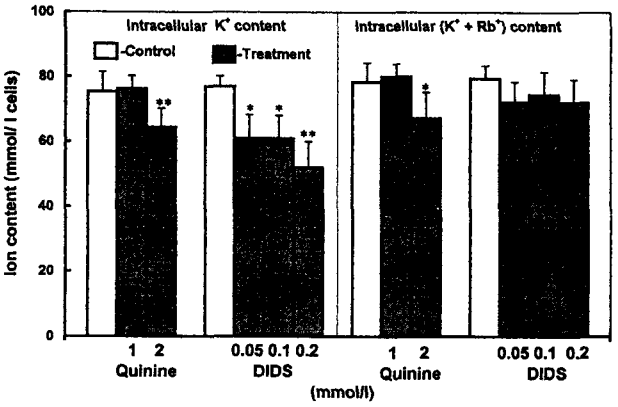

Figure 3. Effect of quinine and DIDS on intracellular K^+ and (K^+ plus Rb^+) content. Following a 60-min treatment, the cells were washed with buffered $MgCl_2$ solutions, and K^+ and Rb^+ concentrations were measured. Values are means ± S.E.M. for 5–6 separate experiments. * $P < 0.05$; ** $P < 0.02$ significantly different from control, paired Student's t-test).

in red cells treated with 2 mmol/l quinine to 7.2 ± 0.6 mmol/l as compared with 5.6 ± 0.5 mmol/l in control cells (paired t-test $P < 0.001$, $n = 6$). No significant changes in Na^+ content were observed when the red cells were incubated in the presence of furosemide, DIOA, DIDS or SITS. After exposure of the erythrocytes to furosemide, DIOA and SITS had no effects on the intracellular K^+ content as compared to the control levels. Treating red cells with 2 mmol/l quinine reduced the concentration of intracellular K^+ as well as total K^+ plus Rb^+ content (Fig. 3). A more appreciable decrease in K^+ content was found for erythrocytes incubated in the presence of 0.05, 0.1 and 0.2 mmol/l DIDS but the total K^+ plus Rb^+ intracellular content was not different from the control level. Similar changes in cellular ion contents were observed when frog erythrocytes were treated with these agents in hyposmotic medium (data not shown).

Effect of blockers on K^+ loss in a K^+-free medium

In our previous studies (Gusev et al. 1995; Agalakova et al. 1997) and other works (Garay et al. 1988; Delpire and Lauf 1992; Armsby et al. 1996) the effects of inhibitors on K-Cl cotransport were investigated in red cells incubated in a nominally K^+-free medium. Moreover, some authors (Lauf 1984; Kaji 1986; Lytle and McManus 1987) have reported that the inhibitory action of loop diuretics on K-Cl cotransport was dependent upon external K^+ concentration. Therefore in a separate series of experiments, we studied the effects of DIOA, quinine and DIDS on K^+ efflux from frog erythrocytes in a nominally K^+-free medium. To evaluate the specificity of action of these drugs on K-Cl cotransport, the frog erythrocytes were

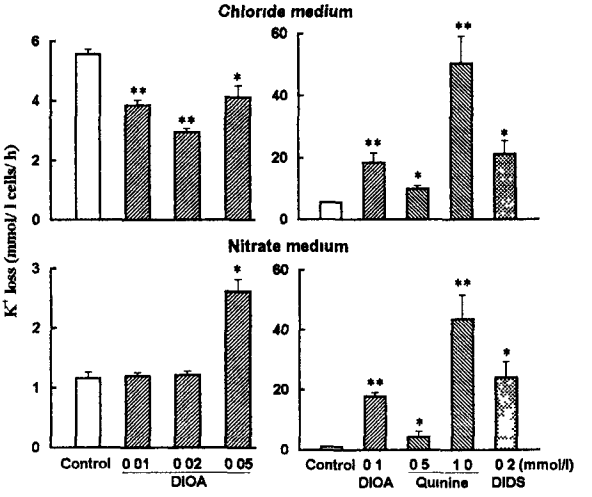

Figure 4. Influence of DIOA, quinine and DIDS on K^+ loss from frog erythrocytes in a K^+-free medium The cells were incubated for 60 min in nominally K^+-free isotonic chloride (*A*) and nitrate (*B*) media in the absence (open bars, control) and in the presence of the drugs (hatched bars) The values presented are means ± S E M of six separate experiments (*$P < 0$ 02, ** $P < 0$ 001 significantly different from control, paired Student's *t*-test)

incubated in Cl^-- and NO_3^-- media Results of these experiments are presented in Fig 4 Treatment of frog erythrocytes with 0 01 and 0 02 mmol/l DIOA significantly inhibited the K^+ loss by 31% and 47%, respectively, in Cl^--medium but did not affect K^+ transport in NO_3^--medium Increasing the concentration of DIOA to 0 05 mmol/l resulted in a decrease of its inhibitory potency in Cl^--medium and a stimulation of K^+ loss in NO_3^--medium DIOA at the concentration of 0 1 mmol/l induced a manifold activation of K^+ loss from frog erythrocytes incubated in both media Calculated from the data the Cl^--dependent component was progressively reduced up to a full inhibition at 0 1 mmol/l DIOA (Fig 5) giving a value for half-maximal inhibition (IC_{50}) ~0 015 mmol/l

Exposure of frog red cells to 0 5 mmol/l quinine led to a significant stimulation of K^+ loss in both Cl^-- and NO_3^-- medium (Fig 4 *A*, *B*) The K^+ loss induced by 0 5 mmol/l quinine was larger in Cl^--medium than in NO_3^--medium resulting in a small increase in Cl^--dependent K^+ loss (Fig 5) Quinine at the concentration of 1 0 mmol/l produced a sharp acceleration of K^+ transport from the red cells in both media causing a considerable variability in the Cl^--dependent component of K^+ efflux In the presence of 0 2 mmol/l DIDS there was a significant enhancement in K^+loss from the cells incubated in both media The Cl^--dependent component of K^+ loss from the DIDS-treated cells did not differ from zero (Fig 5)

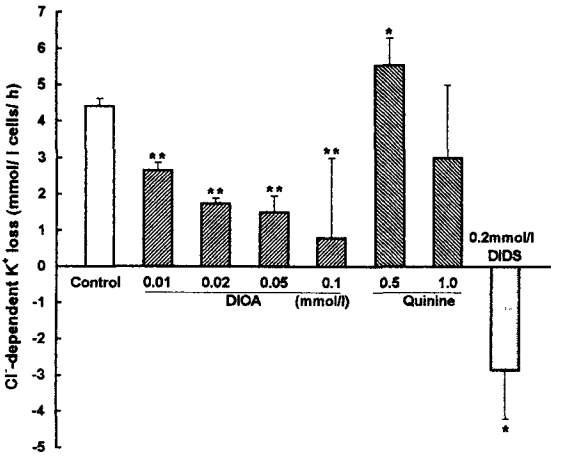

Figure 5. Cl^--dependent K^+ loss from data presented in Fig. 4. Values are means ± S.E.M. for six separate experiments. ($*P < 0.02$; $**P < 0.001$ significantly different from control, paired Student's t-test).

Discussion

Our previous studies (Gusev et al. 1995; Agalakova et al. 1997) have shown that at physiological external concentration of K^+(2.7 mmol/l) the K-Cl cotransporter provides for about 70% of the total K^+ (^{86}Rb) influx into frog erythrocytes. Under the conditions of our assay, at 10 mmol/l external $Rb^+$and in the presence of ouabain, the residual Rb^+ (K^+) influx in nitrate media was 0.21 ± 0.02 and 0.32 ± 0.02 mmol/l cells.h in isotonic and hypotonic media, respectively (unpublished data). Thus the contribution of Cl^--dependent K^+ transport was above 90% of the total Rb^+ influx in frog erythrocytes. Therefore, the inhibitory effects of the blockers tested on K^+ transport in this study are most probably to result from the inhibition of K-Cl cotransport in frog erythrocytes.

Data from the present study suggest that furosemide appreciably inhibits K^+ influx into frog erythrocytes in both iso- and hypotonic media. However, furosemide did not affect K^+ efflux from the cells under isotonic conditions but it blocked K^+ loss from swollen cells. These data are consistent with previous studies (Gusev et al. 1995) that have shown the inhibitory effects of furosemide on K^+influx, but not K^+ efflux, in frog erythrocytes incubated in standard medium. Also in erythrocytes of the frog and other species (Lauf 1984) furosemide was found to have K^+transport unaffected in cells incubated in NO_3^--media. At the external K^+ (Rb^+) concentration of 10 mmol/l, the ouabain-insensitive Cl^--independent component of K^+ influx was about 0.2–0.3 mmol/l. cells/h and it was below the magnitude of K^+ influx in the presence of 1 mmol/l furosemide. Therefore, the treatment of frog red cells with 1 mmol/l furosemide resulted in an inhibition of

K^+ influx via K-Cl cotransporter by about 55% and 67% in isotonic and hypotonic media, respectively.

Furosemide in millimolar concentrations was found to be a potent inhibitor of Cl^--dependent K^+ transport in erythrocytes of some species (Lauf 1984; Kaji 1986; Lytle and McManus 1987; Garcia-Romeu et al. 1991) as well as epithelial tissues (Zeuthen 1994), hepatocytes (Bianchini et al. 1988), endothelial cells (Perry and O'Neill 1993) and neurosecretory cells (Dubreil et al. 1995). In a recent study ^{86}Rb efflux from human renal cell line HEK 293 expressing rabbit K-Cl cotransport protein (rbKCC1) has been shown to be blocked by 2 mmol/l furosemide (Gillen et al. 1996). On the other hand, the Cl^--dependent K^+ fluxes in horse red blood cells were insensitive to furosemide (Gibson et al. 1994). The inhibitory potency of furosemide on volume-activated Cl^--dependent K^+fluxes in human (Kaji 1986) and rabbit erythrocytes (al-Rohil and Jennings 1989) as well as NEM-activated K-Cl cotransport in sheep erythrocytes (Lauf 1984) was substantially enhanced by increasing external K^+ (Rb^+) concentration. In our experiments in frog erythrocytes, the lack of inhibitory effects of furosemide on K^+ loss in isotonic media was independent of the presence of 10 mmol/l external Rb^+ (data not shown).

Garay and co-workers (1988) have proposed [(dihydroindenyl)oxy] alkanoic acid (DIOA) as the most potent inhibitor of the K-Cl cotransport system in human red cells. These authors have found that the NEM-activated and volume-activated Cl^--dependent K^+ fluxes were blocked by DIOA with the 50% inhibitory concentration of ~0.01 mmol/l. The inhibitory effect of DIOA on the K-Cl cotransporter was confirmed in investigations in normal and sickle human erythrocytes (Olivieri et al. 1993) and horse red blood cells (Gibson et al. 1994). However, in the mouse strains studied, there was either modest or no significant inhibition of Cl^--dependent K^+ efflux by 0.1 mmol/l DIOA (Armsby et al. 1996). Similarly, conflicting evidence was reported for the DIOA effect on K^+ transport in other cell types (Saitta et al. 1990). The present study clearly demonstrates that DIOA can simultaneously exert two opposite effects on the frog erythrocyte membrane: inhibition of K-Cl cotransport and stimulation of K^+ transport across the cell membrane. Similarly as human erythrocytes, frog red cells display a high sensitivity to DIOA which, at a concentration as low as 0.01 mmol/l, caused a significant decrease in K-Cl cotransport (Figs. 1, 2 and 5). On the other hand, it is apparent that DIOA at the concentration of 0.05 mmol/l displays a small stimulatory effect on K^+ transport thereby masking the inhibitory action on K-Cl cotransport. DIOA at higher concentrations (0.1 mmol/l) largely induces the Cl^--independent K^+ transport pathway in the frog erythrocyte membrane. Nevertheless, DIOA produced a dose-related reduction in Cl^--dependent K^+ loss in K^+-free medium (Fig. 5). The concentration required for 50% inhibition (IC_{50}) appears to be ~0.015 mmol/l which is similar to that for human erythrocytes (Garay et al. 1988). In the above mentioned study, Garay et al. (1988) have also reported that 0.17 mmol/l DIOA activated K^+ loss from human red cells and 0.34 mmol/l DIOA produced a 5-fold increase in K^+ leak.

The inhibitory effect of the stilbene derivative DIDS on the Cl^-dependent K^+

fluxes has been observed in erythrocytes of some species (Lytle and McManus 1987; Garcia-Romeu et al. 1991; Delpire and Lauf 1992). The data of the present study clearly indicate that treatment of frog erythrocytes with relatively low concentrations of DIDS (0.05–0.2 mmol/l), but not by another stilbene derivative SITS, was associated with a manifold acceleration of both Rb^+ (K^+) influx and K^+ efflux (Figs. 1, 2, 4). At 10 mmol/l external Rb^+ concentration, DIDS induced appoximately equal Rb^+ influx and K^+ efflux so that there was little alteration in total K^+ plus Rb^+ intracellular content (Fig. 3). In a K^+-free medium, 0.2 mmol/l DIDS considerably increased K^+ loss from frog erythrocytes incubated in both Cl^-- and NO_3^--media so that the Cl^--dependent component of K^+ efflux was completely inhibited (Figs. 4 and 5). It is possible that the stimulatory effect of DIDS exceeds its inhibitory influence on K^+ transport in the frog red blood cells. Most probably, DIDS is able to exert a nonspecific effect on the frog erythrocyte membrane causing activation of monovalent cation permeability. This proposal is supported by the fact that similar stimulation of K^+ and Na^+ influxes under the influence of DIDS (but SITS) was observed in the erythrocytes of another amphibian species, salamander (Kregenow et al. 1985).

There is some evidence for a stimulatory effects of DIDS on ion transport in a number of epithelial tissues (Gogelein and Pfannmuleer 1989; Wangemann et al. 1995) and hepatocytes (Wehner et al. 1993). In this context, of special interest is the recent study by Chambrey et al. (1997) which has shown that namely DIDS, but not other stilbenes like SITS and DNDS, induced Na-H exchange isoform NHE4 activity in mutant fibroblasts. Notably, the DIDS-activated NHE4 exchanger operated in K^+-H^+ exchange mode. Maldonado and Cala (1994) have suggested that DIDS may alter the K-Na selectivity of alkali metal/H^+ exchanger in Amphiuma erythrocytes, causing it to mediate net Na^+ uptake and K^+ loss. On the basis of this and other findings, we have suggested that DIDS is able to cause cation conductance, possibly preferentially for K^+, in plasma membranes of some cell types.

Quinine had little or no effect on Cl^--dependent K^+ influx in human red blood cells (Kaji 1986) and trout hepatocytes (Bianchini et al. 1988). More recently, however, quinine in relatively high concentrations has been found to inhibit Cl^--dependent K^+ fluxes activated by either swelling or by NEM in LK sheep red blood cells (Adragna and Lauf 1994). These discrepant data as for the quinine influence on K-Cl cotransport in erythrocytes of different species are supported by this study in the frog red cells. Quinine caused only a small inhibition of Rb^+ influx and K^+ efflux in swollen frog erythrocytes during activation of K-Cl cotransport. In isotonic media, quinine was able to activate significantly K^+ transport via the frog erythrocyte membrane, especially in K^+-free Cl^-- and NO_3^--media (Fig. 4). These data are in agreement with recent studies by Tsunenari et al. (1996) who found quinine to activate a nonselective cation channel in taste receptor cells from the bullfrog *Rana catesbeiana*.

Taken together, this study provide evidence that the inhibitory effects of the agents studied on K-Cl cotransport in frog erythrocytes is dependent on both the direction of K^+ movement and the volume activation of the K-Cl cotransporter.

The most potent inhibitor, DIOA in the concentration of 0 02 mmol/l blocked only 30–60% of the Cl^--dependent K^+ fluxes in frog red blood cells. However, 0.05 mmol/l DIOA can activate a Cl^--independent K^+ transport pathway via the cell membrane and partially overcome the inhibitory influence of DIOA on K-Cl co-transport. Furosemide in millimolar concentrations (0.5–1 0 mmol/l) caused ~50% inhibition of Cl^--dependent K^+ influx in iso- and hypotonic media whilst Cl^--dependent K^+ efflux was blocked only in swollen cells High quinine concentrations (1–2 mmol/l) also inhibited Cl^--dependent K^+ fluxes only in swelling-activated frog erythrocytes. These results can, in part, account for the discrepant effects of blockers on K-Cl cotransport reported for erythrocytes of other species and other cell types in which different methodological approaches were used Another possibility accounting for the controversial findings could be due to the presence of several isoforms of the K-Cl cotransporter in different cell types or in the same cell In recent years, first steps have been made towards the understanding of the molecular nature of the K-Cl cotransport system (Gillen et al. 1996) On the basis of our findings and data of other investigators, one would not consider the blockers tested to have a selective effect on K-Cl cotransport It should be noted that all the blockers tested in our work, especially DIDS, are able to increase K^+ transport via the frog erythrocyte membrane. Clearly, further studies will be needed to find an agent which more specifically inhibits K-Cl cotransport.

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