Biochimica et Biophysica Acta, 513 (1978) 292—295

BBA Report

BBA 71360

EFFECT OF ANIONS ON POTASSIUM SELF-EXCHANGE IN ASCITES TUMOR CELLS

TILLY BAKKER-GRUNWALD

Department of Physiology, University of Colorado Medical Center, 4200 E. Ninth Avenue, Denver, Colo. 80262 (U.S.A.)

(Received May 24th, 1978)

Summary

Potassium self-exchange in ascites cells was partially suppressed when chloride in the incubation medium was replaced by bromide, and completely abolished in iodide-, nitrate- or sulfate-containing saline. Thus, it appears that potassium self-exchange activity is anion-dependent.

One of the modes by which monovalent cations are transferred across cell membranes is self-exchange [1]. In this communication we present evidence that K^+ self-exchange in ascites cells is dependent on the anion composition of the medium.

Fig. 1A shows the effect of ouabain and furosemide on the influx of ^{86}Rb into ascites cells; this isotope serves as a tracer for K^+ in this system (e.g., ref. 2). In the absence of furosemide ouabain had a small inhibitory effect. Furosemide alone decreased the rate of ^{86}Rb influx by 50%. Both inhibitors together brought about a marked reduction to approximately 8% of the control value. These findings are similar to those of Tupper [2]. Ouabain caused a net loss of K^+ (Table I); we show elsewhere (unpublished) that the concentration of ouabain employed is sufficient to totally inhibit the $(Na^+ + K^+)$ ATPase. Furosemide did not influence the cellular K^+ content (Table I). In another experiment (Table II) the furosemide-sensitive component of ^{86}Rb influx was shown to be equal in absolute magnitude to that of efflux, both in the absence and in the presence of ouabain; in the presence of ouabain the furosemide-sensitive component was markedly stimulated, as in the experiment of Fig. 1.

Fig. 1B shows the results of a similar experiment carried out in a saline solution in which 100 mM $NaNO_3$ was substituted for NaCl. In this system furosemide had no effect. Moreover, all fluxes were reduced to the rates observed in the presence of furosemide in Cl^- saline (1.9 and 0.3 μmol $K^+ \cdot g^{-1}$

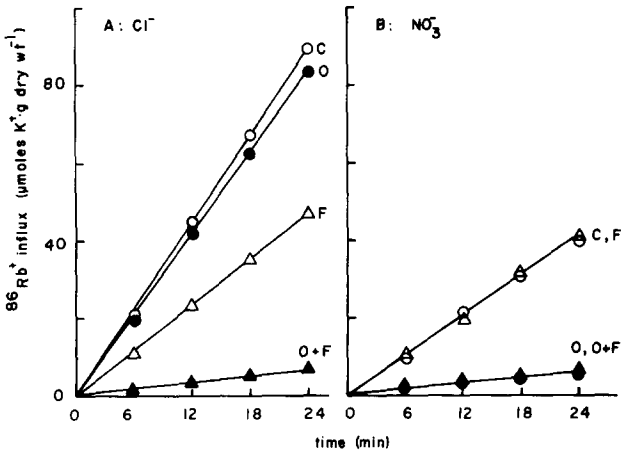

Fig. 1. Effect of inhibitors on ^{86}Rb influx into ascites cells in Cl^--saline and NO_3^--saline. Ascites cells were grown in the abdominal cavities of Swiss Webster mice, and harvested after 6—12 days. They were washed in a saline solution containing: 50 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) anion, neutralized with 20 mM NaOH and 4.4 mM KOH, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 20 mM glucose, and either 100 mM NaCl or 100 mM $NaNO_3$. The final pH was 7.5. They were then suspended at a concentration of 1.5% in the respective saline plus or minus furosemide (1.2 mM), and incubated for 90 min at 37° C to allow the ion content to stabilize. At time zero ^{86}Rb (20 nCi/ml) was added alone or together with ouabain (1.2 mM). At the indicated times, duplicate 3 ml samples of suspension were spun down at 1500 rev./min, the supernatant decanted and saved, and the cells extracted with 5% trichloroacetic acid. Radioactivity of cell extracts and supernatants was counted by liquid scintillation counting, and K^+ content determined by flame photometry (see Table I). Extracellular space correction was made by extrapolating the uptake curves to zero time. Points shown are average of duplicates; duplicates were equal within 5%. A, Cl^--saline; B, NO_3^--saline. ○ (C), control; ● (O), ouabain; △ (F), furosemide; ▲ (O + F), ouabain plus furosemide.

dry wt.·min^{-1}, in the absence and presence of ouabain, respectively). K^+ contents were comparable to those in the Cl^- system (Table I).

From these data it appeared that either removal of Cl^- or addition of NO_3^- abolished the furosemide-sensitive components of ^{86}Rb influx. In order to further examine this point we investigated the effect of several other anions in the presence of ouabain, where the size of the furosemide-inhibitable component was larger. Fig. 2 shows the results of these experiments. The dotted line gives the level of influx in the presence of furosemide and ouabain, i.e. the non-mediated influx. This level was the same in all saline systems tested (0.37 ± 0.02 μmol $K^+ \cdot g^{-1}$ dry wt.·min^{-1}). As Cl^- was gradually replaced by I^-, NO_3^- and SO_4^{2-}, the furosemide-sensitive component of the flux was reduced; at 2 mM Cl^-it was completely abolished. In contrast, Br^- at 90 mM suppressed

TABLE I

EFFECT OF INHIBITORS AND NITRATE ON K^+ CONTENT

Samples were obtained from the experiment shown in Fig. 1, 24 min after addition of ^{86}Rb plus or minus ouabain. Values given are the average of duplicates; duplicates were equal within 5%.

Major anion	K^+ content (mmol·g^{-1} dry wt.)			
	Cl^-		NO_3^-	
	− ouabain	+ ouabain	− ouabain	+ ouabain
− Furosemide	0.31	0.25	0.32	0.27
+ Furosemide	0.30	0.26	0.32	0.27

the furosemide-sensitive component by only 35%. Cellular K^+ content and the net loss induced by ouabain (not shown) were equal for all anions tested except SO_4^{2-}, and comparable to those in Table I. In SO_4^{2-} saline, values were approximately 24% lower on a dry weight-basis; this is probably due to shrinkage of the cells [3].

Tupper [2] interpreted his data on the effect of furosemide to mean that this compound inhibited K^+ self-exchange. For the experiments presented here this interpretation is supported by the observation that the furosemide-inhibitable fractions of influx and efflux were equal in absolute magnitude (Table II), as well as the fact that furosemide did not alter K^+ content (Table I). It thus seems justified to interpret the furosemide-inhibitable ^{86}Rb flux components as arising from K^+ self-exchange. Our data show that this self-exchange is influenced by the anionic composition of the medium. The concentration dependence of this effect was the same for I^-, NO_3^-, and SO_4^{2-} (Fig. 2). This suggests that Cl^- is required for K^+ self-exchange with a rather high apparent K_m. Br^- appears partially able to substitute for Cl^- in sustaining K^+ self-exchange. Effects of anions on unidirectional cation fluxes comparable to those described here have been reported by Funder and Wieth [4] for human red blood cells.

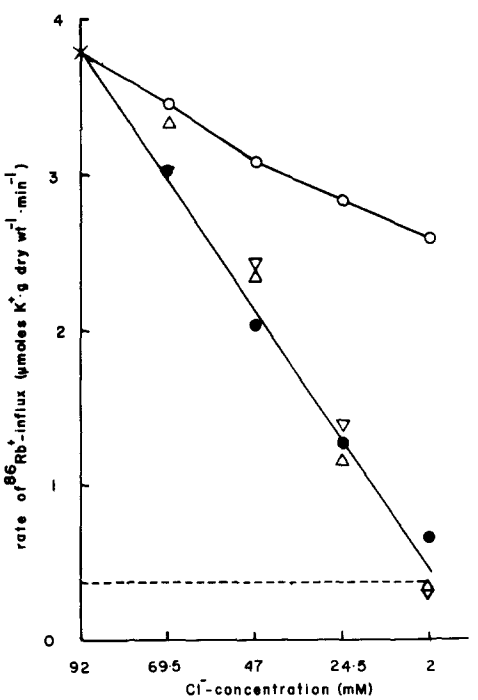

Fig. 2. Effects of different anions on the ouabain-inhibited influx of ^{86}Rb. Saline solutions were similar to that described in the legend to Fig. 1.with either NaBr, NaI, $NaNO_3$ (100 mM) or Na_2SO_4 (50 mM) substituted for NaCl. Cl^--saline was mixed with the other salines to give the indicated Cl^- concentrations. The experiment was performed as described for Fig. 1. Rates were calculated from the difference in cellular radioactivity between 30 min and 10 min after the addition of ^{86}Rb (20 nCi/ml) plus ouabain (1.2 mM). The dotted line gives the influx rate for the five unmixed saline solutions in the presence of furosemide (1.2 mM). I^-, which was found to be a strong quencher in our scintillation system, was precipitated with $AgNO_3$ prior to counting of radioactivity. ○, Br^-; ●, I^-; △, NO_3^-; ▽, SO_4^{2-}.

TABLE II

EFFECT OF INHIBITORS ON ^{86}Rb INFLUX AND EFFLUX IN Cl^- SALINE

For the rate of ^{86}Rb efflux, ascites cells were equilibrated for 90 min in the presence of the isotope (10 nCi/ml). At the end of this period cells were spun down for 1 min at 1000 rev./min, and at time zero the pellet was resuspended at a concentration of 1.5% in Cl^- saline in the presence or absence of inhibitors (concentration, 1.2 mM). Radioactivity remaining in the cells at a given time was determined as described in the legend to Fig. 1. Efflux rate constants (k_e) were obtained by linear regression from semilogarithmic plots of the decrease in cellular radioactivity with time. Correlation coefficients (r) exceeded 0.94 in all cases. Total efflux was calculated from the product of k_e and the average of the cellular K^+ contents at zero time and 24 min. Influx was determined in the same experiment by methods described in the legend to Fig. 1; the external K^+ concentration was 2.5 mM. The values for the furosemide-sensitive components were obtained by subtraction from the values given for the unidirectional fluxes; the error in these derived values can be estimated at about 10%.

	Efflux rate constant k_e (min^{-1})	Unidirectional fluxes ($\mu mol\ K^+ \cdot g^{-1}$ dry wt. $\cdot min^{-1}$)		Furosemide-sensitive components ($\mu mol\ K^+ \cdot g^{-1}$ dry wt. $\cdot min^{-1}$)	
		Influx	Efflux	Influx	Efflux
—	0.018	7.1	6.8	3.9	4.0
+ Furosemide	0.007	3.2	2.8		
+ Ouabain	0.034	7.1	10.8	6.8	8.2
+ Furosemide + ouabain	0.008	0.3	2.6		

The nature of the anion effect on K^+ self-exchange is not clear. Apparently the passive permeability of the anion is not involved since Cl^- and NO_3^- have comparable passive permeabilities (unpublished data), whereas that of SO_4^{2-} has been reported to be considerably lower [3]. A clue to the mechanism of K^+ self-exchange and its susceptibility to the anion composition of the medium may possibly be found in the observation of Heinz et al. [5] that K^+ and Cl^- are co-transported under certain conditions.

The fact that K^+ self-exchange was stimulated in the presence of ouabain suggests a close relation between pumping and exchange activities. This relation (often of a complementary nature) has been observed in a number of systems [6].

This research was supported by NIH grant AM 15807 to Dr. Margaret C. Neville, whom I would like to thank for her invaluable input.

References

1 Glynn, I.M. and Karlish, S.J.D. (1975) Annu. Rev. Physiol. 37, 13—55
2 Tupper, J.T. (1975) Biochim. Biophys. Acta 394, 586—596
3 Levinson, C. and Villeral, M.L. (1974) J. Cell. Physiol. 85, 1—14
4 Funder, J. and Wieth, J.O. (1967) Acta Physiol. Scand. 71, 168—185
5 Heinz, E., Geck, P., Pietrzyk, C., Burckhardt, G. and Pfeiffer, B. (1977) J. Supramol. Struct. 6, 125—133
6 Mills, B. and Tupper, J.T. (1976) J. Cell. Physiol. 89, 123—132