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HORMONE-INDUCED DIURETIC-SENSITIVE POTASSIUM TRANSPORT IN TURKEY ERYTHROCYTES IS ANION DEPENDENT

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Summary

In turkey erythrocytes, the hormone-induced, diuretic-sensitive components of both unidirectional K^+ influx and net salt uptake were Cl^- dependent, with Br^- partially able, and NO_3^- and SO_4^{2-} unable to substitute as the main anion. Since the measured fluxes involve Na^+/K^+ cotransport, these observations indicate that the unifying concept of an $Na^+/K^+/2Cl^-$ cotransport system (Geck, P., Pietrzyk, C., Burckhardt, B.-C., Pfeiffer, B. and Heinz, E. (1980) Biochim. Biophys. Acta 600, 432—447) may be extended to hormone-induced ion transport in avian erythrocytes.

In avian erythrocytes, unidirectional cation fluxes can be stimulated by several special treatments such as activation by β-adrenergic hormones or exposure to hypertonic media [1—3]. Under suitable conditions, activation results in net salt transport followed by water: thus, at physiological Na^+ concentrations, cells swell at K^+ concentrations above, and shrink at K^+ concentrations lower than 2.5 mM [1—3]. During the last few years, evidence has been accumulated linking the stimulated cation fluxes to the action of an Na^+/K^+ cotransport system [1,4]. This system appears to be independent of the classical Na^+ pump [5] (see, however, Ref. 6): whilst insensitive towards ouabain, it is characteristically inhibited by diuretics such as furosemide (for a recent review, see Ref. 1).

Abbreviations: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; SF_{6847}, 3,5-di-*tert*-butyl-4-hydroxybenzylidenemalononitrile.

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For some time, diuretics have been known to inhibit other (co-) transport systems in different cell types, notably Cl^--dependent K^+ exchange diffusion in ascites cells [7,8] and Na^+/Cl^- cotransport in epithelia [9]. In a recent development, Geck et al. [10] have provided data suggesting a diuretic-sensitive electroneutral cotransport of $Na^+ + K^+ + 2Cl^-$ in Ehrlich cells: they postulate that the diuretic-sensitive transport activities demonstrated in other cells may represent different aspects of this same $Na^+/K^+/2Cl^-$ symport system.

In this communication, I present evidence that in turkey erythrocytes the hormone-induced, diuretic-sensitive components of both unidirectional K^+ influx and cellular volume response are Cl^- dependent, with Br^- partially able, and NO_3^- and SO_4^{2-} unable to substitute as the main anion. These data strongly suggest that the unifying concept of an $Na^+/K^+/2Cl^-$ symport system [10] may be extended to hormone-induced ion transport in avian erythrocytes.

Table I, Expt. A, shows that hormone-induced, diuretic-sensitive unidirectional K^+ influx was supported by Cl^- and to a lesser degree by Br^-, but not by NO_3^- or SO_4^{2-}. That the latter anions did not inhibit the activation process per se (known to involve adenylate cyclase [1—3]) can be appreciated from Table I, Expt. B; when cells were hormone-induced in media containing the substituting anions and subsequently transferred to Cl^--saline, diuretic-sen-

TABLE I

ANION DEPENDENCY OF HORMONE-INDUCED, DIURETIC-SENSITIVE $^{86}Rb^+$ INFLUX

Erythrocytes (obtained from the wing vein of two young female turkeys) were washed twice in a saline solution containing (mM): $NaNO_3$, 120; KNO_3, 2.5; $Mg(NO_3)_2$, 1; glucose, 10; Hepes, 20, neutralized with NaOH, 10 ('NO_3^--saline'). The final pH was 7.4—7.6 at 38°C. For Expt. A, cells were suspended at 4—6% in saline containing the indicated anions (final incubations contained 17 mM NO_3^- vs. 103 mM Cl^- or Br^-, or 68 mM SO_4^{2-}), and incubated at 38°C. After 45 min, epinephrin (1 μM) was added, and after 55 min the Na^+ pump was inhibited by 0.1 mM ouabain [5]; where indicated, the diuretic bumetanide [13] was added at 10 μM. At 60 min, $^{86}Rb^+$ as a tracer for K^+ [1,8,11] was added to the cells at 10—50 nCi/ml. 10 and 30 min after addition of the label, duplicate 1-ml samples were spun for 1 min in a Beckman microfuge, the supernatant was decanted and saved, and the tubes were blotted and swabbed to remove adhering medium. Cell pellets were extracted with 5% trichloroacetic acid, and radioactivity of cell extracts and supernatants was measured in a liquid scintillation system. Duplicates varied by maximally 10%. Influx was calculated from the difference in cellular $^{86}Rb^+$ content between the two time points and the activity of the supernatant. In all cases, extrapolation of uptake to zero time gave an $^{86}Rb^+$ uptake component corresponding to the extracellular space as determined from the distribution of [^{14}C]inulin [12] (12—15%): this shows that uptake was linear over the first 30 min (see also Ref. 3). Results are expressed per ml packed cells. For Expt. B, cells were preincubated for 2 h in the respective salines containing 1 μM epinephrin, spun down and washed twice in ice-cold isotonic $Mg(NO_3)_2$ solution. Pellets were taken up in Cl^--saline containing 0.1 mM ouabain and, where indicated, 10 μM bumetanide. Total manipulation time after hormone induction was 30 min. Then $^{86}Rb^+$ was added. Further details as for Expt. A.

Main anion	$^{86}Rb^+$ influx (nmol K^+/min per ml)	
	Expt. A	Expt. B
Cl^-	147	47
Br^-	98	85
NO_3^-	3	63
SO_4^{2-}	10	69
Cl^- + bumetanide	4	16
Cl^- + epinephrin	3	—

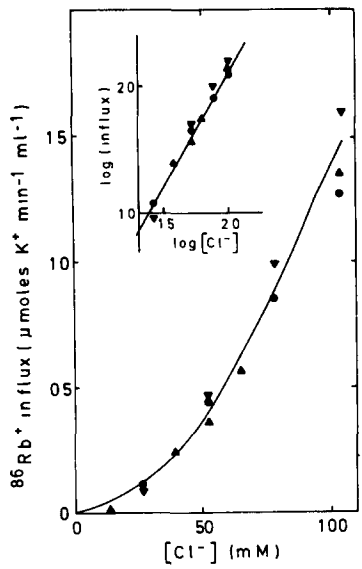

Fig. 1. Hormone-induced diuretic-sensitive $^{86}Rb^+$ influx as a function of Cl^- concentration. Cells were washed as in Table I, Expt. A, and suspended in mixtures of Cl^--saline and NO_3^--saline to give the indicated Cl^- concentrations. Uptake was determined as in Table I, Expt. A. Results of three experiments (indicated by different symbols) were pooled. $^{86}Rb^+$ influx was corrected for the small residual influx in the controls without hormone or with 10 μM bumetanide (3—12 nmol K^+/min per ml). Insert: double-logarithmic plot of main figure; slope, 1.86 (r = 0.99).

sitive K^+ flux was even higher than that in the cells induced in Cl^--saline. Moreover, dibutyryl cyclic AMP plus theophyllin induced a K^+ flux component of the same anion specificity (results not shown).

Fig. 1 shows the effect of gradual replacement of Cl^- by NO_3^-. Note that, as in human red blood cells [15] (see, however, Ref. 11), the dependence of the diuretic-sensitive K^+ flux on Cl^- concentration appears to be sigmoidal: the apparent Hill coefficient is 1.86 (Fig. 1, insert).

As mentioned above, hormone induction at elevated extracellular K^+ concentrations results in cellular swelling [1—3]. Table II, Expt. A, shows that also this phenomenon was specifically dependent upon the presence of Cl^-. For comparison, Table II, Expt. B, gives values for cell swelling induced by the addition of monensin (an ionophore exchanging Na^+ for H^+ [16]) together with the protonophore SF_{6847} [14]: effectively, this combination makes the cells permeable towards both Na^+ and H^+. In this set-up, Na^+ salts of Cl^-, Br^- and NO_3^- were equally effective, whereas in SO_4^{2-} the volume increase was partially suppressed.

It has been known for some time that the hormone-induced, diuretic-sensitive components of both unidirectional K^+ flux and cellular volume response are mediated by an Na^+/K^+ cotransport system [1,4]. The data presented here indicate that this system has a very specific anion requirement, surpassing the simple need for compensating negative charge movement: thus NO_3^-, whilst capable of supporting ionophore-induced cell swelling (Table II, Expt. B), was unable to substitute for Cl^- in either manifestation of hormone-induced

TABLE II

ANION DEPENDENCY OF CELL SWELLING

For Expt. A, cells were washed twice in NO_3^--saline and suspended to a hematocrit of 10—12% in the indicated salines; in all cases, the K^+ concentration was raised to 15 mM. Epinephrin (1 μM) was added to half of the incubations, and bumetanide (30 μM) was added where indicated. Triplicate 1-ml samples in preweighed microcentrifuge tubes were centrifuged after 60 min. Net volume changes were determined essentially as described in Ref. 12. the tubes were decanted and swabbed, reweighed, dried overnight at 90°C, and weighed again. Wet cell weight was corrected for 15% extracellular water. For Expt. B, cells were preincubated for 90 min in Cl^--saline, spun down and suspended in saline containing the indicated Na^+ salt at 150 mM (except from Na_2SO_4, 100 mM); Na-Hepes, 10 mM; ouabain, 0.1 mM; and bumetanide, 10 μM. The pH was 7.5 (38°C). SF_{6847} (1 μM) and monensin (10 μg/ml) were added to half of the incubations, and triplicate 1-ml samples were taken after 15 min. Further details as in Expt. A. Values in parentheses, S.D.; n.s., not significant ($P \leq 0.1$ in Student's t test).

Main anion	Expt. A			Expt. B		
	Cell H_2O (ml/g dry wt.)		% swelling	Cell H_2O (ml/g dry wt.)		% swelling
	Control	+Epinephrin		Control	+Ionophores	
Cl^-	1.59 (0.05)	1.92 (0.02)	21	1.66 (0.06)	2.88 (0.07)	73
Br^-	1.48 (0.02)	1.60 (0.04)	8	1.51 (0.06)	2.74 (0.10)	81
NO_3^-	1.69 (0.03)	1.65 (0.03)	n.s.	1.53 (0.01)	2.71 (0.04)	77
SO_4^{2-}	1.58 (0.01)	1.58 (0.01)	n.s.	1.44 (0.01)	2.00 (0.02)	39
Cl^- + bumetanide	1.49 (0.05)	1.52 (0.01)	n.s.	—	—	—

transport activity (Table I and II, Expts. A). It should be mentioned that, in contrast to this conclusion, previous observations have been interpreted to indicate a lack of anion specificity [1,2]: at least in one case [1], this apparent contradiction may be explained by the fact that in those experiments only part of the Cl^- was replaced by other anions.

From Fig. 1, there is a sigmoidal relationship between hormone-induced K^+ transport and Cl^- concentration, with an apparent Hill coefficient close to 2. It is tempting to consider this value as evidence for a stoichiometry of 1 Na : 1 K^+ : 2 Cl^-, such as that found [10] in Ehrlich cells. However, it should be stressed that under the conditions applied, intracellular Cl^- will vary together with the external Cl^- concentration, which makes such a straightforward interpretation impossible. Be that as it may, Cl^--dependent cation transport may well be ubiquitous: similar anion specificities have been reported for K^+ transport components in human, ruminant and duck red blood cells, as well as in dog kidney cells [11,15,17—19].

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References

1 McManus, T.J. and Schmidt, W.F., III (1978) in Membrane Transport Processes (Hoffman, J.F., ed.), Vol. 1, pp. 79—106, Raven Press, New York

2 Kregenow, F.M. (1977) in Membrane Transport in Red Cells (Ellory, J.C. and Lew, V.L., eds.), pp. 383—426, Academic Press, London
3 Gardner, J.D., Mensh, R.S., Kiino, D.R. and Aurbach, G.D. (1975) J. Biol. Chem. 250, 1155—1163
4 Wiley, J.S. and Cooper, R.A. (1974) J. Clin. Invest. 53, 745—755
5 Glynn, I.M. and Karlish, S.J.D. (1975) Annu. Rev. Physiol. 37, 13—55
6 Beaugé, L. and Lew, V.L. (1977) in Membrane Transport in Red Cells (Ellory, J.C. and Lew, V.L., eds), pp. 39—51, Academic Press, London
7 Tupper, J.T. (1975) Biochim. Biophys. Acta 394, 586—596
8 Bakker-Grunwald, T. (1978) Biochim. Biophys. Acta 513, 292—295
9 Frizzell, R.A., Field, M. and Schultz, S.G. (1979) Am. J. Physiol. 236, F1—F8
10 Geck, P., Pietrzyk, C., Burckhardt, B.-C., Pfeiffer, B. and Heinz, E. (1980) Biochim. Biophys. Acta 600, 432—447
11 Chipperfield, A.R. (1980) Nature 286, 281—282
12 Rudolph, S.A., Schafer, D.E. and Greengard, P. (1977) J. Biol. Chem. 252, 7132—7139
13 Palfrey, H.C., Feit, P.W. and Greengard, P. (1980) Am. J. Physiol. 238, C139—C148
14 Muraoka, S. and Terada, H. (1972) Biochim. Biophys. Acta 275, 271—275
15 Dunham, P.B., Stewart, G.W. and Ellory, J.C. (1980) Proc. Natl. Acad. Sci. U.S.A. 77, 1711—1715
16 Bakker, E.P. (1979) in Antibiotics (Hahn, F.E., ed.), Vol. V/1, pp. 67—97, Springer-Verlag, Berlin
17 Lauf, P.K. and Theg, B.E. (1980) Biochem. Biophys. Res. Commun. 92, 1422—1428
18 Kregenow, F.M. and Caryk, T. (1979) Physiologist 22, 73
19 McRoberts, J.A. and Saier, M.H. (1980) J. Supramol. Struct. Suppl. 4, 86