Biochimica et Biophysica Acta, 646 (1981) 353–355
Elsevier/North-Holland Biomedical Press

BBA Report

BBA 71529

EVIDENCE AGAINST Na^+-PUMP MEDIATION OF Ca^{2+}-ACTIVATED K^+ TRANSPORT AND DIURETIC-SENSITIVE (Na^+/K^+)-COTRANSPORT

S.J.D. KARLISH [a], J.C. ELLORY [b] and V.L. LEW [b]

[a] *Biochemistry Department, Weizmann Institute, Rehovoth (Israel) and* [b] *Physiological Laboratory, University of Cambridge, Downing Street, Cambridge, CB2 3EG (U.K.)*

(Received January 30th, 1981)
(Revised manuscript received May 11th, 1981)

Key words: Na^+-pump; Ca^{2+} activation; K^+ transport; Na^+/K^+ cotransport; Diuretic sensitivity

Proteoliposomes reconstituted from purified Na^+ pumps show neither Ca^{2+} activation nor bumetanide inhibition of Rb^+ uptake, suggesting that the Na^+ pump does not mediate these passive fluxes.

The Na^+ pump has been postulated from time to time as mediating various passive cation fluxes. These include the Ca^{2+}-activated K^+ transport [1] and the diuretic sensitive Na^+/K^+ cotransport [2,3]. Na^+-pump mediation of both active and passive cation transport is an attractive unifying idea but the supporting evidence in intact cells and resealed red cell ghosts is indirect and admits alternative interpretations [3–5].

Purified Na^+ pumps reconstituted into liposomes provide a system in which Na^+-pump mediation of passive cation fluxes can be tested directly. We report here experiments in which we look for Ca^{2+} activation of K^+($^{86}Rb^+$) permeability and for bumetanide-sensitive or Cl^--dependent [2,6,7] $^{86}Rb^+$ uptake representing Na^+/K^+ cotransport in this preparation. The results suggest that neither of these fluxes is mediated by the Na^+ pump.

The reconstituted proteoliposomes were prepared from purified pig kidney ($Na^+ + K^+$)-ATPase and soya bean azolectin as recently described in detail by Karlish and Pick [8]. Control experiments showed that, in the presence of ATP in the medium, the vesicles used here can sustain high rates of ATP-dependent $^{22}Na^+$ uptake into K^+-containing vesicles and that about 50% of the pumps are in the inside-out configuration [8]. $^{86}Rb^+$ uptake was measured in proteoliposomes pre-equilibrated for 2 h at 25°C in a medium containing Tris-HCl (pH 7.4 at 25°C), 50 mM; NaCl, 50 mM and KCl, 50 mM. When present, the concentrations of $CaCl_2$ and calmodulin (purified according to Muallem and Karlish [9]) were 50 μM and 1.5 μM, respectively. The Ca^{2+}-free condition contained 50 μM of (Na)EGTA. At the indicated times (see Fig. 1) 50 μl of vesicle suspension (out of a total of 0.4 ml for each condition) were deposited on top of chilled Pasteur pipettes filled with Tris-neutralized, albumin-coated Dowex 50-X8-200 and rapidly eluted three times with 0.5 ml of ice-cold 250 mM sucrose into counting vials. These columns retained 99.998% of the ^{86}Rb counts in the extravesicular space. The measured vesicle-associated ^{86}Rb radioactivity was at least 20-times higher than the residual extravesicular count through the columns. Fig. 1a shows the time-course of tracer ^{86}Rb equilibration in the presence and absence of Ca^{2+} in conditions known to produce rapid Ca^{2+} activation of K^+(Rb^+) fluxes in red cells, i.e. ATP depletion [10,11], low-magnesium [12] and micromolar Ca^{2+} concentrations [13–15]. The effect of calmodulin was also tested in order to allow for possible activating effects [16] (Fig. 1b).

The results showed that Ca^{2+} had no effect on the

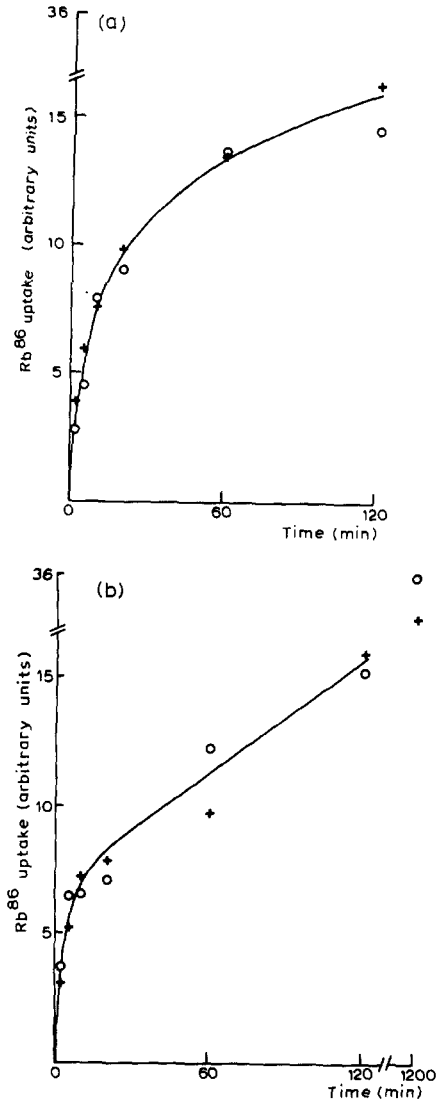

Fig. 1. Effect of calcium (a) and calmodulin (b) on the $^{86}Rb^+$ uptake by reconstituted Na^+-pump proteoliposomes. Panel (a): ○, controls, containing 50 μM (Na)EGTA; +, in the presence of 50 μM $CaCl_2$. Panel (b): ○, without and +, with, calmodulin. In both conditions 50 μM $CaCl_2$ was present in the medium. The effect of calmodulin in the absence of calcium was not investigated.

Rb^+ uptake curve in the presence or absence of calmodulin. This indicates that Ca^{2+} failed to activate $^{86}Rb^+$ fluxes through the inside-out pumps. Activation of right-side out pumps would have required Ca^{2+} to enter the vesicles. This could only have accelerated $^{86}Rb^+$ equilibration had there been any Ca^{2+} activation at all.

In order to test Na^+-pump mediation of Na^+/K^+ cotransport, $^{86}Rb^+$ uptake was measured as before into proteoliposomes containing Na^+, 75 mM and K^+, 75 mM in either Cl^- or NO_3^--containing media of the same composition, in the presence and absence of bumetanide (10^{-4} M) (Fig. 2a). The Na^+/K^+ cotransport system is known to be Cl^--dependent and bumetanide inhibitable ($K_{1/2}$ $2 \cdot 10^{-7}$ M, [6,7]). In a similar experiment fluxes were again measured in NO_3^- or Cl^- media with and without bumetanide, but the proteoliposomes were reconstituted with ouabain-treated pump and suspended in a medium containing $MgCl_2$ or 0.45 mM $Mg(NO_3)_2$, and 0.45 mM ATP to

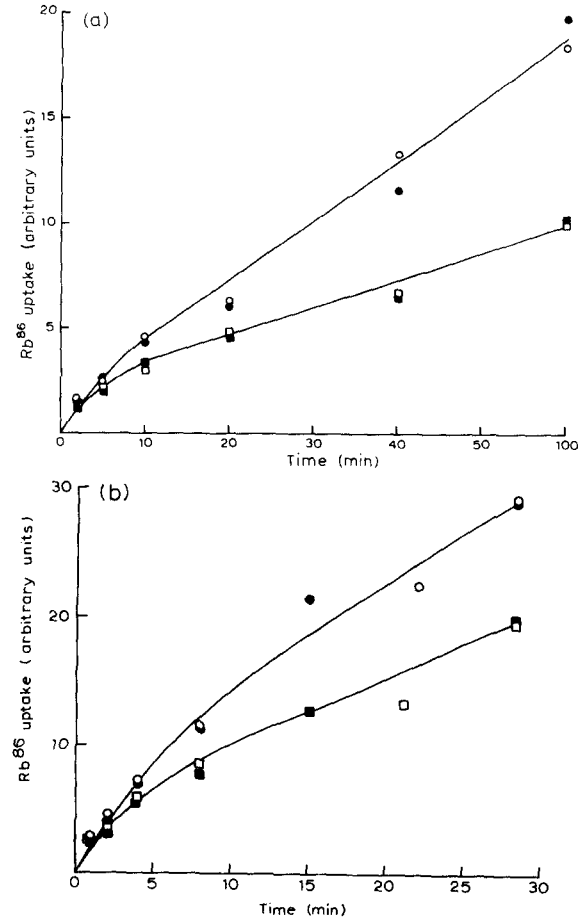

Fig. 2. Effect of NO_3^- and bumetanide on the $^{86}Rb^+$ uptake by reconstituted Na^+-pump proteoliposomes. In Panel (b), Na pumps were treated with ouabain; $MgCl_2$ and ATP were present in the medium. ■, Cl^-, controls; □, Cl^-, + bumetanide; ●, NO_3^-, controls; ○, NO_3^-, + bumetanide.

explore conditions more likely to reveal Na^+-pump mediation of passive fluxes [3]. The results from the two experiments were very similar (Fig. 2b). Bumetanide had no effect on the $^{86}Rb^+$ uptake. Unlike in intact red cells [6,7], NO_3^- stimulated $^{86}Rb^+$ influx, but the origin of this effect remains obscure.

There is previous evidence supporting the genetic independence of the Na^+ pump from the Ca^{2+}-activated K^+-channel in red cells [17–19] and from the furosemide-sensitive pathway in mutant L cells [20, 21] and, except for unknown inactivating effects resulting from Na^+-pump purification or reconstitution, the present results also support exclusion of Na^+-pump mediation of Ca^{2+}-activated K^+ fluxes and diuretic sensitive Na^+/K^+ cotransport.

References

1 Blum, R.M. and Hoffman, J.F. (1971) J. Membrane Biol. 6, 315–328
2 Wiley, J.S. and Cooper, R.A. (1974) J. Clin. Invest. 53, 745–755
3 Lew, V.L. and Beaugé, L.A. (1979) in Membrane Transport in Biology II (Giebisch G., Tosteson, D.C. and Ussing, H.H., eds.), pp. 81–115, Springer Verlag, Heidelberg
4 Lew, V.L. (1974) in Comparative Biochemistry and Physiology of Transport (Bolis, L., Bloch, K., Luna, S.E. and Lynen, F., eds.), pp. 310–316, North-Holland, Amsterdam
5 Lew, V.L. and Ferreira, H.G. (1978) in Current Topics in Membranes and Transport, Vol. 10 (Kleinzeller, A. and Bronner, F., eds.), pp. 217–277, Academic Press, New York
6 Dunham, P.B., Stewart, G.W. and Ellory, J.C. (1980) Proc. Natl. Acad. Sci. USA 77, 1711–1715
7 Chipperfield, A.R. (1980) Nature (Lond.) 186, 281–282
8 Karlish, S.J.D. and Pick, U. (1981) J. Physiol. 312, 505–530
9 Muallem, S. and Karlish, S.J.D. (1979) FEBS Lett. 107, 209–212
10 Hoffman, J.F. (1966) Am. J. Med. 41, 666–680
11 Lew, V.L. (1971) Biochim. Biophys. Acta 233, 827–830
12 Lew, V.L. and Ferreira, H.G. (1976) Nature (Lond.) 263, 336–338
13 Blum, R.M. and Hoffman, J.F. (1972) Biochem. Biophys. Res. Commun. 46, 1146–1152
14 Lew, V.L. (1970) J. Physiol. 206, 33–36P
15 Simons, T.J.B. (1976) J. Physiol. 256, 227–244
16 Sarkadi, B., Szebeni, J. and Gardos, G. (1980) in Membrane Transport in Erythrocytes (Lassen, U.V., Ussing, H.H. and Wieth, J.O., eds.), pp. 220–231, Munksgaard, Copenhagen
17 Jenkins, D.M.G. and Lew, V.L. (1973) J. Physiol. 234, 41–42P
18 Brown, A.M., Ellory, J.C., Young, J.D. and Lew, V.L. (1978) Biochim. Biophys. Acta 511, 163–175
19 Richhardt, H.W., Fuhrman, G.F. and Knauf, P.A. (1979) Nature (Lond.) 279, 248–250
20 Gargus, J.J. and Slayman, C.W. (1980) J. Membrane Biol. 52, 245–256
21 Gargus, J.J., Miller, I.L., Slayman, C.W. and Adeberg, E.A. (1978) Proc. Natl. Acad. Sci. USA 75, 5589–5591