Biochimica et Biophysica Acta 854 (1986) 325–328
Elsevier

BBA 70260

BBA Report

Li_i-Na_o countertransport and Li leak in erythrocytes are differentially affected by membrane enrichment with cholesteryl hemisuccinate

Rachel Levy [a], Dana Hevroni [a], Z. Ioav Cabantchik [b] and Avinoam Livne [a,*]

[a] *Department of Biology, Ben Gurion University of the Negev, Beer Sheva and* [b] *Department of Biological Chemistry, The Hebrew University of Jerusalem, Jerusalem (Israel)*

(Received August 5th, 1985)

Key words: Na^+-Li^+ countertransport; Cholesteryl hemisuccinate; Hypertension; Erythrocyte membrane

Enrichment of erythrocytes with cholesteryl hemisuccinate caused a marked reduction in Li leak but did not change kinetic and thermodynamic properties of Li_i-Na_o countertransport of either normotensive persons or patients with essential hypertension. As cholesteryl hemisuccinate was shown to affect the membrane similarly to cholesterol, it is likely that the unique thermodynamic properties of erythrocyte Li_i-Na_o countertransport in essential hypertension are not caused by changes in cholesterol.

The erythrocyte transport system Li_i-Na_o countertransport shows inheritant differences in patients with essential hypertension [1–6]. In previous works we have shown that the temperature dependence of the Li_i-Na_0 countertransport differs in erythrocytes of hypertensive patients; its modification is localized in the countertransport protein, while its expression apparently involves associated lipids [7,8]. The observed alteration in the Li_i-Na_0 countertransport function may be related to changes in the fluidity of the membrane [9,10]. The effect of cholesteryl hemisuccinate on the Li_i-Na_o countertransport activity was studied in the present work as cholesterol is a major component of the red blood cell membrane and is known to affect passive carrier-mediated transport systems [11–15]. Furthermore, the ratio of cholesterol/phospholipid appears to be an important determinant in regulating the properties of biological membranes [16]. The cholesterol content of the red blood cell membrane can be greatly increased in certain diseases [14,17–19], after cholesterol feeding [20] and by suitable treatment in vitro [18,20].

In the present work we show that the Li_i-Na_o countertransport activity is essentially unaffected by an enrichment of the erythrocyte membrane by treatment with cholesteryl hemisuccinate, while Li leak is markedly reduced.

Blood was obtained by consent, either from healthy normotensive persons (both parents and all living relatives were demonstratable normotensive) or from age and sex-matched patients, whose sole disease was essential hypertension. The patients had an established family history of hypertension, namely, that at least one of the parents was a patient with essential hypertension. They did not receive medication at all or at least for a year. Blood was drawn into heparin solution (25 units/ml) and processed within 30 min. The erythrocytes were separated and washed three times with 155 mM NaCl.

The cells were incubated in LiCl-loading solu-

* To whom reprints requests should be addressed.

tion [1] for 2 h and then suspended, at 0.5% hematocrit, in a sterol enrichment medium for an additional hour at 37°C. The enrichment medium contained 3.5% polyvinylpyrolidone 40000, 1% bovine serum albumin, 5 mM glucose, 10 mM Tris-Mops (pH 7.4) and 145 mM lithium chloride. Cholesteryl hemisuccinate dissolved in ethanol was added to a final concentration of 20 μg/ml. Ethanol in 0.5 μl per ml was equally added to the control. Following the 3 h incubation, the intracellular Li concentration was 7–8 mM. The cells were washed and the Li efflux was measured as described [1]. Efflux was performed simultaneously into sodium-rich medium (150 mM NaCl, 10 mM glucose, 0.1 mM ouabain, 10 mM Tris-Mops) and into sodium-free medium (same medium but NaCl was replaced by 75 mM $MgCl_2$ and 85 mM sucrose). The difference between the rate of lithium efflux into sodium-rich medium and sodium-free medium was taken as countertransport [1]. A graded temperature device allowed the simultaneous assays of Li efflux at the various temperatures in the range of 14–40°C with 1–2 Cdeg increments. The pH of the medium varied from 7.40 (at 14°C) to 7.24 (at 40°C). The effect of this difference in pH on Li efflux rate when tested at constant temperature was found to be negligible, within the experimental error. Lithium was determined by means of an atomic absorption spectrophotometer (Perkin-Elmer model 2380), and calibrated by standards corresponding to the medium used. The flux was computed from the linear regression of Li loss within 30 min. The Arrhenius plots were drawn as follows: The 13 points were divided into 2 groups, one with k ($4 \leqslant k \leqslant 9$) points, the other with $(13 - k)$ points. A straight line was fitted by least squares to each group of points and the intersection point of the two lines calculated. The standard error of each line was found and from that the standard error of the intersection point, in the x axis. The computation was repeated for each value of k and the intersection point with the lowest standard deviation was selected. Measurements of osmotic fragility (as described in Ref. 21) were conducted concomitantly with the measurement of Li efflux.

Table I presents the rate of Li efflux into Na-rich medium, Na-free medium and Li_i-Na_o countertransport in untreated erythrocytes and in cholesteryl hemisuccinate-enriched erythrocytes. It is shown that the addition of cholesteryl hemisuccinate to the membrane caused a reduction in the rate of Li efflux both into Na-rich medium and into Na-free medium, whereas the rate of Li_i-Na_o countertransport was not affected. Thus the change in the rate of the Li efflux into Na-rich medium is due entirely to the reduction in Li leak, and not to the Li_i-Na_o countertransport activity.

TABLE I

EFFECT OF CHOLESTERYL HEMISUCCINATE ENRICHMENT ON ERYTHROCYTE Li EFFLUX

The values represent the averages ± S.E. of four experiments, each in triplicates, measured at 37°C. CHS, cholesteryl hemisuccinate.

Erythrocytes	Li(mmol·(l RBC·h)$^{-1}$))		
	Li efflux		Li_i-Na_o countertransport
	Na-rich medium	Na-free medium	
Normal	0.73 ± 0.05	0.28 ± 0.04	0.45 ± 0.06
CHS-enriched	0.60 ± 0.06	0.16 ± 0.03	0.44 ± 0.05
P	< 0.01	< 0.001	> 3

The enrichment of the membrane with cholesteryl hemisuccinate was followed by the change in red cell osmotic fragility (Cabantchik, Z.I., personal communications). Fig. 1 demonstrates that the osmotic fragility of cholesteryl hemisuccinate-enriched erythrocytes was lower than that of control erythrocytes. The concentra-

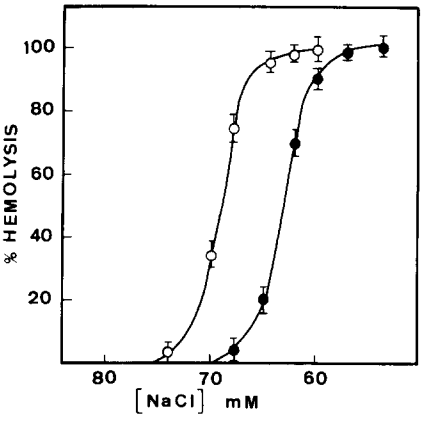

Fig. 1. Osmotic fragility of erythrocytes enriched with cholesteryl hemisuccinate (●) and untreated erythrocytes (○).

tion of NaCl solution which cause 50% hemolysis was 70 mM for untreated erythrocytes as compared to 63 mM for the sterol enriched erythrocytes.

The effect of cholesteryl hemisuccinate enrichment on temperature dependence of Li_i-Na_0 countertransport was studied in erythrocytes of three normotensive persons and three patients with essential hypertension. Arrhenius plots of representative results obtained for a normotensive and hypertensive are shown in Fig. 2. As already reported [7,8] the plots exhibit a characteristic break temperature for normotensives (at about 30°C) and for most of the hypertensive patients (at about 20°C). Yet, enrichment of the erythrocytes with cholesteryl hemisuccinate in either case did not affect the pattern of temperature dependence of Li efflux. Cholesteryl hemisuccinate partions into biological membranes much more efficiently than cholesterol and yet exerts similar effects as cholesterol [22–24]. It is therefore highly likely that cholesteryl hemisuccinate affects the erythrocyte membrane similarly to cholesterol. Thus, the modification in the thermodynamic properties of Li_i-Na_0 countertransport in erythrocytes of hypertensive patients [7,8] cannot be explained by possible alterations in the content of cholesterol in the membrane, as might be deduced from studies of fluorescence polarization in rat erythrocytes [9,10].

The enrichment of erythrocyte with cholesteryl hemisuccinate was shown to cause an increase in membrane microviscosity [25]. However, the change was not manifested as concomitant alteration in Li_i-Na_0 countertransport. Li_i-Na_0 countertransport thus appears to be insensitive to changes in membrane viscosity caused by the addition of cholesteryl hemisuccinate.

Unlike the Li_i-Na_0 countertransport, the leak of Li from the erythrocytes was reduced by the enrichment with the cholesteryl ester. Our data support the report of Kroes and Ostwald [12] who shoed that the diffusion of sodium and other permeants through the erythrocyte membrane is decreased by the enrichment with cholesterol.

The reduction of Li leak with no change in the Li_i-Na_0 countertransport activity may be explained by one of the following possibilities. (a) The existance of distinct pathways for the leak and the countertransport. (b) A common pathway for the leak and the countertransport, while the differential effect of cholesteryl hemisuccinate is due to an inhibition of the conformational change of the Na^+-free carrier. Additional study is required to resolve between these alternative explanations.

This study was supported in part by funds from the Oelbaum Family and from Barrie and Amelia Rose.

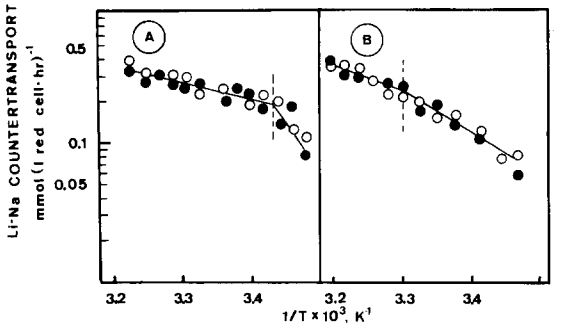

Fig. 2. The temperature dependence of Li_i-Na_0 countertransport in erythrocytes enriched with cholesteryl hemisuccinate (●) and in untreated erythrocytes (○). (A) Erythrocytes from a patient with essential hypertension; (B) erythrocytes from a normotensive donor. Vertical dashed lines indicate the Arrhenius plots 'break' temperature.

References

1 Canessa, M.L., Adragna, N.C., Solomon, H.S., Connolly, T.M. and Tosteson, D.C. (1980) N. Engl. J. Med. 302, 772–776
2 Cusi, D., Barlassina, C., Ferrandi, M., Lupi, P., Ferrari, P. and Bianchi, G. (1981) Clin. Exp. Hypertension 3, 871–884
3 Cusi, D., Barlassina, C., Ferrandi, M., Palazzi, P., Lelega, E. and Bianchi, G. (1981) Clin. Sci. 61, 33s–36s
4 Woods, J.W., Falk, R.J., Pittman, A.W., Klemmer, P.J., Watson, B.S. and Namboodiri, K. (1981) N. Engl. J. Med. 306, 593–595
5 Ibsen, K.K., Jensen, H.A., Wieth, J.O. and Funder, J. (1982) Hypertension 4, 703–709
6 Canoli, M., Borghi, L., Sani, E., Gurti, A., Montanari, A., Novarini, A. and Borghetti, A. (1981) Clin. Sci. 51, 13s–15s
7 Levy, R., Paran, E., Keynan, A. and Livne, A. (1983) Hypertension 5, 821–827
8 Levy, R. and Livne, A. (1984) Biochim. Biophys. Acta 769, 41–48
9 Montenay-Garestier, T., Aragon, I., Devynck, M.A., Meyer, P. and Helene, C. (1981) Biochem. Biophys. Res. Commun. 100, 660–665
10 Orlov, S.N., Gulak, P.V., Litvinov, I.S. and Postnov, Yu.V. (1982) Clin. Sci. 63, 43–45

11 Bruckdorfer, K.R., Demel, R.A., De Gier, J. and Van Deenen, L.L.M. (1969) Biochim. Biophys. Acta 183, 334–345
12 Kroes, J. and Ostwald, R. (1971) Biochim. Biophys. Acta 249, 647–650
13 Grunze, M. and Deuticke, B. (1974) Biochim. Biophys. Acta 356, 125–130
14 Jackson, P.A. and Morgan, D.B. (1982) Clin. Sci. 62, 101–107
15 Oldfield, E. and Chapman, D. (1972) FEBS Lett. 23, 285–297
16 Cooper, R.A. (1972) New Engl. J. Med. 297, 371–377
17 Cooper, R.A. (1970) Semin. Haematol. 7, 296–322
18 Ostwald, R. and Shannon, A. (1964) Biochem. J. 91, 146–154
19 Owen, J.S. and McIntyre, N. (1978) Biochim. Biophys. Acta 510, 168–176
20 Cooper, R.A., Arner, E.C., Wiley, G.S. and Shaltil, S.G. (1975) J. Clin. Invest. 55, 115–126
21 Livne, A., Kuiper, P.J.C. and Meyerstein, N. (1972) Biochim. Biophys. Acta 255, 744–750
22 Wilbrandt, W. (1978) in Cell Membrane Receptors for Drugs and Hormones, A Multidisciplinary Approach (Struab, R.W. and Bolis, L., eds.), pp. 243–249, Raven Press, New York
23 Lyte, M. and Shinitzky, M. (1979) Chem. Phys. Lipids 24, 45
24 Shinitzky, M., Borochov, H. and Wilbrandt, W. (1980) in Membrane Transport in Erythrocytes (Lassen, V.V., Using H.H. and Wieth, J.O., eds.), pp. 91–99, Munksgaard, Copenhagen
25 Yuli, I., Wilbrandt, W. and Shinitzky, M. (1981) Biochemistry 20, 4250–4256