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Elevated intracellular Ca^{2+} affects Li_i-Na_o countertransport in human red blood cells

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Changes in cytoplasmic Ca^{2+} concentration and in Li_i-Na_o countertransport activity have been shown to be associated with essential hypertension. Elevated intracellular free [Ca^{2+}], as well as abnormalities of Ca^{2+} binding and transport have been reported in cells from different tissues of hypertensive laboratory animals and essential hypertensive patients. Similarly, enhanced rates of Li_i-Na_o countertransport and the modified pattern of the temperature dependence of this activity in red blood cells from essential hypertensive patients have been previously demonstrated. The aim of the present study was to investigate possible interaction between changes in intracellular free [Ca^{2+}] and the Li_i-Na_o exchange in human red blood cells. The ionophore ionomycin was used to allow Ca^{2+} incorporation into the cells in a dose-dependent manner. The elevation of intracellular [Ca^{2+}], in turn, resulted in enhanced Li^+ efflux from the cells. At 3 μM, ionomycin selectively and significantly enhanced the Li_i-Na_o countertransport but not Li^+ leakage from the cells. EGTA totally abolished the effect of ionomycin, indicating that the effect is directly related to Ca^{2+}. As low as 0.4 μM Ca^{2+} caused a statistically significant effect. The maximal effect of Ca^{2+} on the Li_i-Na_o countertransport was achieved around the external pH range of 6.8–7.5. In contrast, the leakage of Li^+ was significantly enhanced by Ca^{2+} at a pH of 7.4 and above. Ca^{2+} did not affect the K_m of the Li_i-Na_o countertransport for Li^+. Amiloride, which inhibits Na^+/H^+ exchange, inhibited only 10% of the Ca^{2+}-enhanced countertransport. It is concluded that Ca^{2+} may play a role in the regulation of Li_i-Na_o countertransport in erythrocytes.

Introduction

Ca^{2+} is known to be involved in many biological functions. It operates alongside cyclic AMP as an intracellular second messenger [1], regulates smooth muscle contraction [2], and affects cytoskeleton in nonmuscular cells [3]. Furthermore, it controls protein phosphorylation [4], plays a role in prostaglandin and thromboxane synthesis [5], and is essential for neurotransmitter synthesis and release [6]. Elevated intracellular free Ca^{2+} levels, as well as abnormalities of Ca^{2+} binding and transport have been reported in cells of hypertensive laboratory animals and essential hypertensive patients [7–9]. Furthermore, calcium antagonists are drugs known to be powerful tools in the therapy of heart disease and hypertension [10].

The transport of monovalent cations across the red blood cell membrane has been widely studied in relation to essential hypertension [11–17]. In

Abbreviation Mops, 4-morpholinepropanesulfonic acid.

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particular, the rate of Li_i-Na_o countertransport was repeatedly shown to be significantly higher in essential hypertensive patients than in normotensive subjects. Previous studies in our laboratory confirmed this finding; furthermore, it was shown that the Li_i-Na_o countertransport in erythrocytes of essential hypertensive patients exhibits altered thermodynamic properties, which might serve for prediction and diagnosis of the disease [18–20].

It was therefore the aim of the present study to investigate possible interaction between changes in intracellular free Ca^{2+} levels and the Na-Li exchange. Such an interaction is indeed demonstrated.

Materials and Methods

Solutions (1) Na-medium contained 150 mM NaCl, 10 mM glucose, 0.1 mM ouabain and 10 mM Tris-Mops (pH 7.4). (2) K-medium — same medium as (1) but NaCl is replaced by 150 mM KCl. (3) Li-loading solution — 150 mM $LiHCO_3$, 5 mM KCl and 10 mM glucose brought to pH 7.4 with CO_2 at 37°C. (4) Na-washing solution — same as (1), but ouabain is omitted. Where stated (see Table II), another washing solution was used, in which NaCl was replaced by KCl. (5) Stock solution of ionomycin (free acid, 3 mM) was prepared in 70% ethanol.

Determination of Li efflux. Portions of 10 ml blood, drawn from healthy human volunteers, were anticoagulated with heparin (25 units/ml) and processed within 30 min. Preparation of cells loaded with Li and measurements of Li efflux were as described by Ibsen et al. [14]. In essence, the loading was in lithium bicarbonate medium. The cells were washed five times at 4°C with the Na-washing solution. In the experiments described in Table II the cells were washed, for comparison, either in Na- or K-washing solution. The washing procedure lasted for 60 min. The final intracellular Li^+ concentration was 8–12 mmol/l cells. The efflux was performed simultaneously into potassium medium (for the measurement of Li leakage) and sodium medium (for the measurement of total Li efflux) at 37°C for 30 min. The differences between the rate of lithium efflux into Na-medium and K-medium were taken as countertransport. Lithium was determined by means of an atomic absorption spectrophotometer (Perkin Elmer Model 2380), and calibrated by standards corresponding to the medium used.

For the measurements of temperature dependence of the Li_i-Na_o countertransport, a graded temperature device was used to allow the simultaneous assays of Li efflux at the desired temperatures in the range of 15–40°C with 1–2°C increments [19]. The pH of the medium varied from 7.38 (at 15°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.

Treatments Ionomycin, and/or $CaCl_2$ and/or EGTA solutions were introduced directly at the beginning of the assay period within 3 min after resuspension of washed cells in Na- and K-assay medium. Up to 1 µl ionomycin/ml of cells suspension was used. The control was treated with the same concentration of ethanol. The extracellular Ca^{2+} concentrations were calculated with a computer program for solving the multiple equilibrium equation for Ca^{2+} and EGTA [21]. In the presence of ionomycin these were regarded as the upper values for the intracellular Ca^{2+} concentrations. Unless otherwise stated, the external Ca^{2+} concentration in the presence of ionomycin was 2.5 µM.

$^{45}Ca^{2+}$ incorporation 5% Li-loaded cells suspensions in Na-medium were supplemented with 50 µM $^{45}CaCl_2$ (200 µCi/µmol) and ionomycin at different concentrations. Incubation was carried out at 37°C. To follow the time dependence of $^{45}Ca^{2+}$ incorporation, 100-µl aliquots of the incubation mixture were transferred (in duplicates) at different time intervals into 10 ml of ice-cold 155 mM NaCl solution, the cells sedimented by centrifugation at $3000 \times g$ for 5 min at 4°C and three subsequent washes in the same solution were carried out. The radioactivity was counted in the final cell sediments as well as in equal volumes of the last supernatant to verify an effective removal of extracellular $^{45}Ca^{2+}$. The samples were suspended in the liquid scintillation cocktail Insta-Gel II and counted in a scintillation counter (Packard Instrument International, Downers Grove, IL).

Statistical analysis. The differences in means were analysed by Student's *t*-test Linear plots

were drawn according to the least-squares equation by a computer using 'Curve Fitter' version 1.2 by Paul K. Warme.

Materials. Ionomycin was from Calbiochem AG, Lucerne, $^{45}CaCl_2$ was from New England Nuclear, Stevenage, Hertfordshire. Insta-Gel II was from Packard Instrument Company, Inc., Downers Grove, IL. All other chemicals were purchased from Sigma, St Louis, MO.

Results

Effect of ionomycin

To study the effect of elevated intracellular free Ca^{2+} concentration upon the Li_i-Na_o countertransport in human erythrocytes, Ca^{2+} was introduced into the cells by the ionophore ionomycin [22]. The cells were first loaded with Li^+, then treated with ionomycin, and the Li_i-Na_o countertransport as well as Li^+ leakage were measured under conditions in which the $(Na^+ + K^+)$-ATPase is inhibited.

Fig. 1 depicts the effect of ionomycin concentration, at a constant extracellular Ca^{2+} concentration (8 μM), on the rate of the Li_i-Na_o countertransport and the leak of Li^+. A dose-dependent pattern was obtained. According to these results, a concentration of 3 μM ionomycin was chosen for the routine study, as it selectively and significantly enhanced the Li_i-Na_o countertransport but not Li^+ leakage from the cells. This is of particular importance since the Li_i-Na_o countertransport is measured as the difference between total Li efflux and its leak.

Fig. 2 demonstrates that the ionomycin indeed facilitates Ca^{2+} incorporation into the cells: the higher the ionomycin concentration (1, 3 and 10 μM) and the longer the period of incubation the more $^{45}Ca^{2+}$ is incorporated into the cells. With 3 μM ionomycin $^{45}Ca^{2+}$ uptake was increased 4-fold between 3 and 30 min. It had to be verified that the effect of ionomycin is related to Ca^{2+}. For this purpose, the effect of the Ca^{2+} chelator EGTA was studied. Table I demonstrates that ionomycin significantly stimulates the Li_i-Na_o countertransport but EGTA abolishes this effect. The changes in the leak under the same conditions are insignificant.

Effect of Ca^{2+} and H^+ concentration

In order to function as a physiological messenger, the threshold effective concentration of an effector should be low. Therefore, the dependence of the enhancement upon intracellular Ca^{2+} concentration was studied (Fig. 3). The mean rates of Li efflux are depicted as a function of the logarithm of intracellular Ca^{2+} concentration (assuming equilibrium with extracellular Ca^{2+} in the presence of 3 μM ionomycin based on the method of Liu and Hermann [22]). 0.4 μM Ca^{2+} already

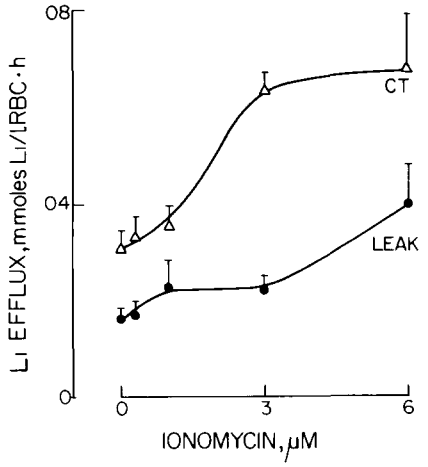

Fig 1 The enhancement of Li_i-Na_o countertransport (C.T.) and Li leakage in red blood cells mediated by ionomycin is dose-dependent The values are the average ± S.E of four experiments, each in duplicates

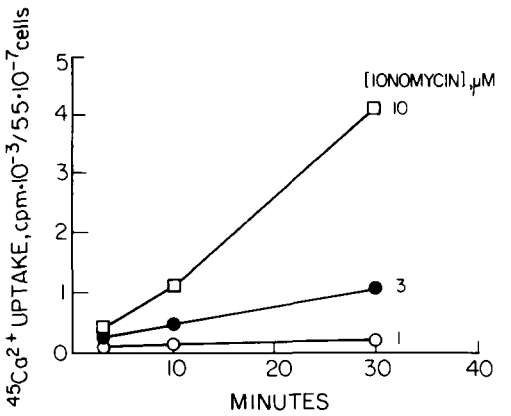

Fig 2 Ionomycin facilitates $^{45}Ca^{2+}$ incorporation into red blood cells in a time- and dose-dependent manner The results are means of duplicates of a given experiment

TABLE I

EFFECT OF ELEVATED Ca^{2+} ON Li EFFLUX

Numbers in parentheses are the number of experiments, each in duplicate * Significantly different from all the controls (no addition, EGTA or ionomycin + EGTA), $P < 0.001$ (Student's t-test of 15 experiments, each in duplicate)

Measurement	Addition (μM)				
	none	EGTA (500)	ionomycin (3) + EGTA (500)	ionomycin (3)	ionomycin + Ca^{2+} (10)
Intracellular free Ca^{2+} (μM) (computerized)	[a]	[a]	$5\ 10^{-4}$	6	16
Li_i-Na_o countertransport (mmol Li/lRBC per h)	0.19±0.02 (22)	0.15±0.02 (12)	0.17±0.02 (15)	0.49±0.06 * (22)	0.63±0.07 * (6)
Leak (mmol Li/l RBC per h)	0.14±0.01 (22)	0.18±0.04 (12)	0.19±0.22 (15)	0.23±0.02 (22)	0.30±0.09 (6)

[a] Basal.

causes a significant enhancement of Li_i-Na_o countertransport. A tendency towards saturation is achieved above 10 μM Ca^{2+}. An additional physiological aspect of the effect of Ca^{2+} on the Li_i-Na_o countertransport was tested by studying the pH profile of the enhancement (Fig. 4). It can be seen that the maximal effect of Ca^{2+} is exerted around the external pH range of 6.8–7.5. This is distinct from the effect of Ca^{2+} on Li leakage, which is

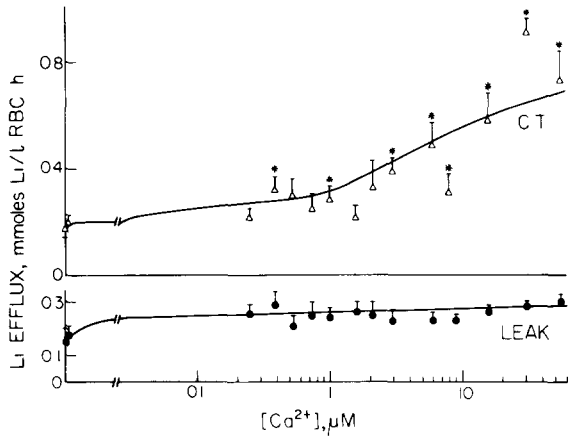

Fig 3 The enhancement of Li efflux by elevated intracellular Ca^{2+} concentration is dose-dependent Ca^{2+} concentration was manipulated by the use of 3 μM ionomycin and varying Ca^{2+} and EGTA concentrations and calculated as described in Materials and Methods. The concentrations given are the upper possible ones as considered in Discussion CT, Li_i-Na_o countertransport * Significantly different from the control, $P < 0.05$ or lower (Student's t-test of 13 experiments, each in duplicate).

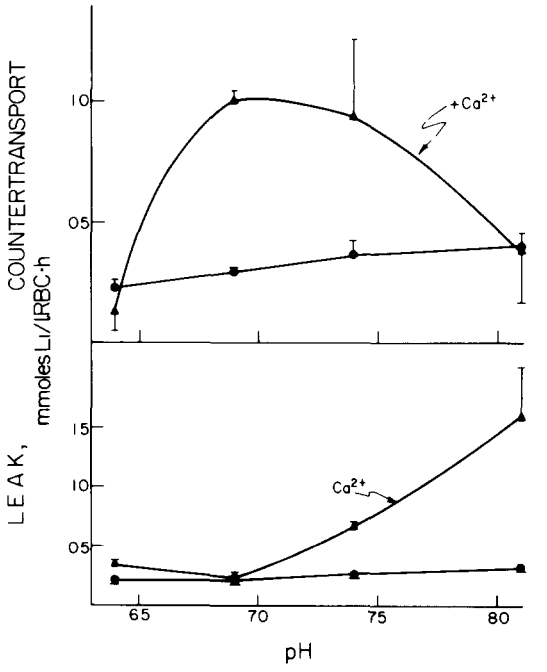

Fig 4 The effect of elevated intracellular Ca^{2+} concentration (2.5 μM) on Li efflux is dependent upon the pH of the external medium The Ca^{2+} concentration was obtained as indicated in Fig 3 Values depicted are mean ± S E of at least three experiments, each in duplicate

significantly enhanced at an external pH of 7.4 and above.

Comparison between external Na^+ and K^+ media

The Li_i-Na_o countertransport is known to undergo conformational changes in response to the external cationic environment. It has been shown that the Li_i-Na_o countertransport is more sensitive to inhibition by SH reagents, such as *N*-ethylmaleimide, when the reagents are introduced in Na-medium than in Na-free medium [23]. In parallel, it is shown in Table II that the cationic composition of the medium in which the cells were washed prior to the introduction of Ca^{2+} determines the extent of the effect of elevated intracellular Ca^{2+} concentration on the Li_i-Na_o countertransport: 63% enhancement was observed in cells washed in Na-washing solution compared with 29% in cells washed in K-washing solution. The lesser effect of Ca^{2+} in cells pretreated in the K-solution might have been attributed to the Gardos [24] effect — enhanced K^+ permeability by Ca^{2+} — leading to cell swelling and resulting in a lower effective Ca^{2+} concentration. This possibility was studied in a subsequent experiment as follows: first, the changes in cell volume due to introduction of Ca^{2+} in Na-medium or in K-medium were found to be approx. 20% shrinkage or 20% swelling, respectively. Then, volume changes of such magnitudes were caused osmotically using hyperosmotic medium (265 mM NaCl) and hypoosmotic medium (120 mM KCl) and the Li_i-Na_o countertransport assayed. It can be seen (Table III) that the change in the volume of the cells as such does not result in a significant change in the Li_i-Na_o countertransport. It is only when Ca^{2+} is introduced into the cells that the Li_i-Na_o countertransport is significantly enhanced.

TABLE II

EFFECT OF CATIONIC COMPOSITION OF THE WASHING SOLUTION ON THE DEGREE OF ENHANCEMENT BY INTRACELLULAR Ca^{2+} ON Li EFFLUX

Elevated intracellular Ca^{2+}	Na-washing solution		K-washing solution	
	−	+	−	+
Li_i-Na_o countertransport				
mmol Li/l RBC per h	0 48	0 78	0 47	0.61
% enhancement		63		29
Leak				
mmol Li/l RBC per h	0 28	0 29	0.14	0 14
% enhancement		3 5		0

Effect of elevated [Ca^{2+}] on kinetic and thermodynamic properties

Fig. 5 reveals that the V_{max} of the Li_i-Na_o countertransport is enhanced by Ca^{2+} while the K_m for intracellular Li^+ is unchanged.

It was previously shown [19,20] that the Arrhenius plots of the Li_i-Na_o countertransport are characterized by some distinct patterns. As shown in Fig. 6, the overall pattern of the plot is not changed by added Ca^{2+}, but the slope does change, apparently reflecting an increase in the energy of activation.

Effect of calmidazolium

The possible role of calmodulin in mediating the effect of Ca^{2+} was tested by the use of calmidazolium, a calmodulin inhibitor. The effect of this compound (5 μM) was selective: it significantly inhibited the Li_i-Na_o countertransport (by

TABLE III

PROBING FOR THE POSSIBLE INVOLVEMENT OF CELL VOLUME CHANGES DUE TO THE GARDOS [24] EFFECT ON L_i-Na_o COUNTERTRANSPORT

The data are mean ± S E. of six experiments, each in duplicate

Treatment	Cell volume (%)	Li_i-Na_o countertransport (mmol Li/ l RBC per h)
A Basal Ca^{2+} concentration		
Isoosmotic Na-medium	100	
Isoosmotic K-medium	100	0 19 ± 0 03
B Elevated Ca^{2+} concentration		
Isoosmotic Na-medium	75 ± 1.5	
Isoosmotic K-medium	120 ± 2 5	0 49 ± 0 05 [a]
C Basal Ca^{2+} concentration		
Hyperosmotic (265 mM) Na-medium	79 ± 1.5	
Hypoosmotic (120 mM) K-medium	120 ± 1 0	0.24 ± 0 01 [b]

[a] The difference of this value from the values of A and C is statistically significant ($P < 0\ 001$)

[b] The difference between this value and that of A is non-significant ($P > 0\ 2$)

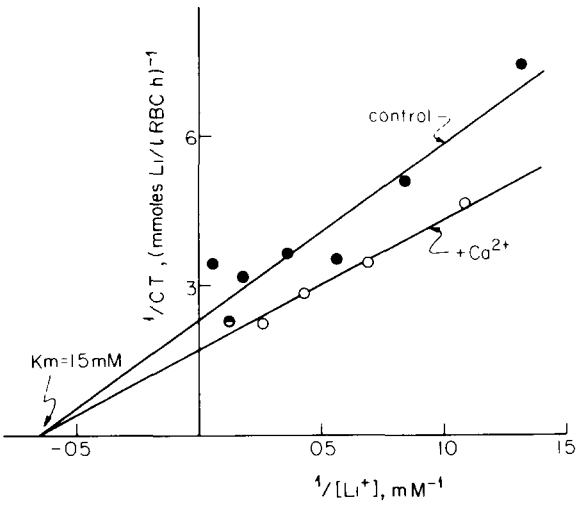

Fig 5. A Lineweaver-Burk plot of the dependence of the rate of Li_i-Na_o countertransport upon intracellular $[Li^+]$ at basal (●) and elevated (○) intracellular $[Ca^{2+}]$ The figure depicts the results of two experiments (each in duplicate) calculated according to the least-squares equation (see Materials and Methods) averaging two sequential points The correlation coefficients (r) are 0 94 for the control and 0 99 for elevated Ca^{2+} concentration

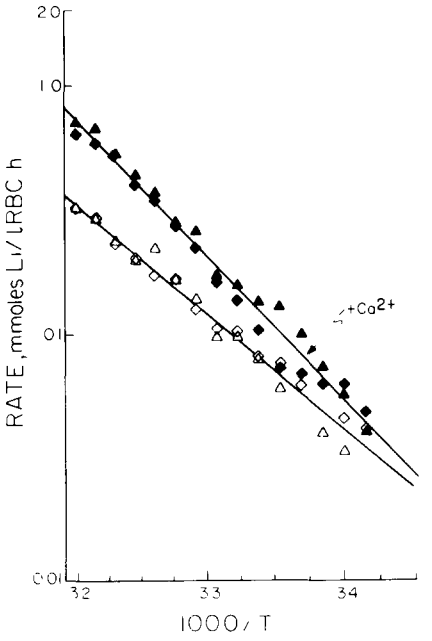

Fig 6 An Arrhenius plot of the dependence of the rate of Li_i-Na_o countertransport upon the temperature of the assay at basal (△◇) and elevated (▲◆) intracellular Ca^{2+} concentration

41%, $P < 0.02$, $n = 5$) at elevated intracellular Ca^{2+} concentration but did not affect it at basal Ca^{2+} level. Furthermore, Li leakage in these experiments was enhanced by 50% ($P < 0.001$) at both basal and elevated intracellular Ca^{2+} concentration.

Effect of amiloride

Na^+/H^+ exchange in red blood cells was reported recently to be enhanced by Ca^{2+} [25]. Therefore, the possibility that the enhancement of the Li_i-Na_o countertransport by Ca^{2+} reflects enhanced Na^+/H^+ exchange was studied. The extent of the Na^+/H^+ antiport activity was estimated by the sensitivity to amiloride. In the absence of added Ca^{2+} 22% of the Li_i-Na_o countertransport is amiloride-sensitive. When Ca^{2+} is added, the Li_i-Na_o countertransport is enhanced over 100%, but the amiloride-sensitive fraction remains restricted (17%). If one subtracts the amiloride-inhibitable fraction obtained with no added Ca^{2+} from the total Ca^{2+}-induced, amiloride-inhibitable portion, the difference obtained is the net fraction which is specifically Ca^{2+}- and amiloride-sensitive. This fraction exhibits only 13% of the Ca^{2+}-induced enhanced Li_i-Na_o countertransport.

Discussion

The central finding of the present work is that elevation of intracellular free Ca^{2+} concentration in human erythrocytes results in enhancement of the membranal exchange activity of Li^+ and Na^+ through a specific pathway. Elevated Ca^{2+} levels add to many other factors that have been shown to change the activity of the Li_i-Na_o countertransport. These include an accelerated Na^+-Li^+ exchange in pregnancy [26]; altered transport rates associated with elevated plasma triacylglycerols, reduced high-density lipoprotein-cholesterol levels and elevated total cholesterol [27]; inhibition of the exchange by external Li^+ after a few days of treatment [28,29]; decreased activity in hyperthyroidism [30] and after exercise [31].

Elevated intracellular Ca^{2+} concentration was obtained in this study by the use of the Ca^{2+} ionophore, ionomycin. The enhancement of the Li_i-Na_o countertransport and, to some extent, of the leakage of Li^+ were dependent upon the

ionomycin concentration. Therefore, it could be argued that the ionophore acts not merely by elevating Ca^{2+} concentration, but also directly in a pharmacological manner. Such a possibility is ruled out by the facts that (a) EGTA, a highly specific Ca^{2+} chelator, abolishes the effect of ionomycin and (b) at a given ionomycin concentration — the higher the Ca^{2+} concentration the greater the acceleration of the Li_i-Na_o countertransport. Hespel et al. [32] found that short-term Ca^{2+} antagonism with felodipine increases plasma Ca^{2+} concentration but does not affect erythrocyte Li_i-Na_o countertransport. However, they did not report on the state of the intracellular Ca^{2+} concentration, which is crucial for the elevation of the Li_i-Na_o countertransport rate.

The K_m for intracellular Li^+ obtained in the present study (1.5 mM) is close to the value previously reported [33]. The fact that the K_m is unchanged by Ca^{2+} while the V_{max} is enhanced suggests that intracellular Ca^{2+} behaves as an accelerating modulator of the Li_i-Na_o countertransport. The arrhenius plots of the Li_i-Na_o countertransport indicate an increase in the apparent energy of activation of the process caused by elevated Ca^{2+} concentration. Furthermore, it seems that the conformational state of the exchanger which is imposed by extracellular Na^+ [23,34] is more susceptible to Ca^{2+} than the conformational state which predominates in the presence of external K^+.

Stimulation by Ca^{2+} of both Na^+ influx and efflux in human red blood cells has been previously reported by Escobales and Canessa [25]. They attributed the effect to the Na^+/H^+ exchanger. The present investigation documents the effect of Ca^{2+} on the Na^+-dependent Li^+ efflux. It is thus of interest to compare these two studies. In the study of Escobales and Canessa, Ca^{2+} concentration was modified by the Ca^{2+} ionophore A23187 and was raised to 100 μM and above — much higher than the physiological range. They report 30–60% inhibition of the Ca^{2+}-dependent Na^+ transport by amiloride (up to 10^{-3} M). Yet, they do not discuss the possible nature of the remaining 40–70% amiloride-resistant fraction of the Na^+ flux. In our study, when intracellular Ca^{2+} concentration was elevated just up to 2.5 μM, only 17% of the Ca^{2+}-induced enhancement was inhibited by amiloride. At basal Ca^{2+} concentration we found that 1 mM amiloride inhibits 22% of the Li_i-Na_o countertransport, and they found that Na^+ influx and efflux were absolutely amiloride-insensitive. It may be concluded that at relatively high Ca^{2+} concentration Na^+/H^+ exchange is a major path for Na^+ flux, while at physiological Ca^{2+} concentration the contribution of the Na^+/H^+ exchange is minute. This conclusion is in line with the observations in several cells that the Na^+/H^+ exchange is quiescent under normal conditions but may be activated under certain conditions [35–37].

The concentrations of the extracellular Ca^{2+} were derived from calculating Ca^{2+} concentration in the different Ca^{2+}/EGTA buffers. In the presence of ionomycin equilibration with the intracellular medium occurs [22]. The data in Fig. 2, showing increasing $^{45}Ca^{2+}$ uptake in the presence of ionomycin, may be interpreted in two ways. One is that internal $^{45}Ca^{2+}$ concentration is not equilibrated with the external. The second possibility is that equilibrium of free Ca^{2+} concentration does take place [22], but the increasing incorporation reflects a continuous intracellular Ca^{2+} binding (e.g., to membranes, phosphate molecules, Ca^{2+}-binding proteins). If the first assumption is valid, then the actual free intracellular Ca^{2+} concentration is even lower than the outer concentration. Thus, 0.4 μM or less intracellular Ca^{2+} significantly enhances the Li_i-Na_o countertransport This concentration lies within the physiological range of the intracellular Ca^{2+} of red blood cells [38,39]. Interestingly, 10^{-7}–10^{-6} M is also the sensitive range of vascular smooth muscle fibers concerning the effect of free Ca^{2+} concentration on their tension [40]. An additional indication for the possible physiological role of elevated Ca^{2+} concentration on the rate of the Li_i-Na_o countertransport emerges from its pH profile — a maximal effect within pH 6.8–7.5.

Acting as an intracellular mediator, regulator and/or messenger, it is believed that Ca^{2+} is reversibly bound to calmodulin and/or other calcium-modulated proteins [3]. Thus, if calmodulin mediates the effect of Ca^{2+} on the countertransport, then one would expect a calmodulin inhibitor to diminish selectively the effect of Ca^{2+} on the countertransport but not on the leak. The

results indeed support this expectation.

The effect of elevated Ca^{2+} concentration on membranal transport systems should be considered in relation to essential hypertension. This is based on the following: elevated intracellular Ca^{2+} concentrations have been found in a variety of tissues and cells of essential hypertensives, including red blood cells [41], platelets [9,42], adipocytes [43] and, of particular importance, vascular smooth muscle cells [44] and kidney [45]. These might explain the well-established accelerated Li_i-Na_o countertransport in erythrocytes of essential hypertensives [11–20] as well as the elevated rates of platelet Na^+/H^+ antiport in these patients, demonstrated recently by Livne et al. [46]. In view of the fact that Na^+/Na^+ exchange, measured as Li_i-Na_o exchange, does not give rise to any net movement of ions, it has no pathophysiological implications unless another exchange partner can be found. This could be the hydrogen ion. Funder et al. [47] found evidence suggesting that sodium and protons compete for the same membrane site in the Li_i-Na_o countertransport system. A net sodium influx across the luminal membrane of the proximal tubular cell, which was shown to be increased in hypertensives [48], occurs via electroneutral Na^+/H^+ exchange [49,50] but has also many features in common with the red cell sodium exchange pathway [51]. Moreover, Escobales and Canessa [52] have recently presented findings that are consistent with the presence of a Na^+/H^+ exchange system in human red blood cells. In addition, it has been postulated that the Li_i-Na_o countertransport may represent a modified form of the Na^+/H^+ exchange [47,51,53]. However, the amiloride-insensitivity of the Li_i-Na_o countertransport in erythrocytes shown by others [54] and confirmed in the present study is not compatible with such a possibility. Yet, the identity and the relationship between Li_i-Na_o countertransport and the Na^+/H^+ exchange systems should be further studied.

In conclusion, it is demonstrated that elevated intracellular free Ca^{2+} concentration enhances Li_i-Na_o countertransport at physiological H^+ and Ca^{2+} concentrations. The effect fits the kinetics of an accelerating modulator, affecting preferentially the Na^+-induced conformational state. Finally, since Ca^{2+} and Li_i-Na_o countertransport are both implicated in hypertension, further study of their interaction may lead to a better understanding of this disorder.

References

1 Rasmussen, H., Zawalich, W. and Kojima, J. (1985) in Calcium and Cell Physiology (Marme, D., ed.), pp 1–17, Springer-Verlag, Berlin
2 Walsh, M.P (1985) in Calcium and Cell Physiology (Marme, D., ed.), pp 170–203, Springer-Verlag, Berlin
3 Kakiuchi, S. (1985) in Calcium and Cell Physiology (Marme, D., ed.), pp 227—237, Springer-Verlag, Berlin
4 Walaas, S.I. and Nairn, A.C (1985) in Calcium and Cell Physiology (Marme, D., ed.), pp 238–264, Springer-Verlag, Berlin
5 Ballou, L.R and Cheung, W.Y (1985) in Calcium and Cell Physiology (Marme, D., ed.), pp 285–297, Springer-Verlag, Berlin
6 DeLorenzo, R.J (1985) in Calcium and Cell Physiology (Marme, D., ed.), pp 265–284, Springer-Verlag, Berlin
7 Orlov, S.N., Pokudin, N.I and Postnov, Y.V (1983) Pflugers Arch 397, 54–56
8 Orlov, S.N and Postnov, Y.V (1982) Clin Sci 63, 281–284
9 Erne, P., Bolli, P., Burgisser, E. and Buhler, F.R (1984) N. Engl J. Med. 310, 1084–1088
10 Godfraind, T (1985) in Calcium and Cell Physiology (Marme, D., ed.), pp. 204–226, Springer-Verlag, Berlin
11 Adragna, N.C., Canessa, M.L., Solomon, H., Slater, E. and Tosteson, D.C (1982) Hypertension 4, 795–804
12 Brugnara, C., Corrocher, R., Foroni, L., Steinmays, M., Bonfanti, E. and De Sandre, G. (1983) Hypertension 5, 529–534
13 Canessa, M., Adragna, N., Solomon, H.S., Connoly, T.M and Tosteson, D.C (1980) N Engl J Med 302, 772–776
14 Ibsen, K.K., Jensen, H.A., Wieth, J.O and Funder, J (1982) Hypertension 4, 703–709
15 Smith, J.V., Ash, K.O., Hunt, S.C., Hentschel, M.S., Sprowell, W., Dadone, M.M. and Williams, R.R (1984) Hypertension 6, 159–166
16 Cusi, D., Barlassina, C., Freeandi, M., Palazzi, P., Celega, E. and Bianchi, G (1981) Clin Sci. 61, 33s–36s
17 Trevisan, M., Ostrow, D., Cooper, R., Liu, K., Sparks, S., Okonek, A., Stevens, E., Marquardt, J and Stamler, J (1983) Hypertension 5, 363–367
18 Levy, R., Zimlichman, R., Keynan, A and Livne, A. (1982) Biochim Biophys Acta 685, 214–218
19 Levy, R., Paran, E., Keynan, A and Livne, A (1983) Hypertension 5, 821–827
20 Levy, R. and Livne, A (1984) Biochim Biophys. Acta 769, 41–48
21 Orentlicher, M., Reuben, J.P., Grundfest, H and Brandt, P.W. (1974) J Gen Physiol 63, 168–186
22 Liu, C.M and Hermann, T.E (1978) J Biol Chem. 253, 5892–5894
23 Levy, R and Livne, A (1984) Biochim Biophys Acta 777, 157–166
24 Gardos, G (1958) Biochim. Biophys Acta 30, 653–654

25 Escobales, N. and Canessa, M (1985) J Biol Chem 260, 11914–11923
26 Worley, R J., Hentschel, W.M., Cormier, C , Nutting, S , Pead, G., Zelenkov, K., Smith, J B , Ash, K O and Williams, R.R (1982) N. Engl. J. Med. 307, 412–416
27 Duhm, J and Behr, J (1986) Scand. J. Clin Lab. Invest 46 (suppl. 180), 82–95
28 Dunner, D.L , Meltzer, H L. and Fieve, R R (1978) Am J Psychiat 135, 1062–1064
29 Ehrlich, B E , Diamond, J M., Fry, V and Meier, K (1983) J. Membrane Biol 75, 233–240
30 Sutterlin, V , Gless, K.H , Schatz, K , Hufner, M , Schutz, V and Hunstein, W (1984) Klin. Wchschr 62, 598–601
31 Adragna, N C , Chang, J N , Morey, M C and Williams, R.S (1985) Hypertension 7, 132–139
32 Hespel, P , Lijnen, P , Fiocchi, R , Lissens, W and Amery, A (1986) J Hypert 4, 767–772
33 Duhm, J. and Becker, B F (1977) Pflugers Arch. Eur J Physiol 370, 211–220
34 Becker, B.F. and Duhm, J. (1979) J. Membrane Biol 51, 287–310
35 Grinstein, S., Clarke, C.A and Rothstein, A (1983) J Gen Physiol 82, 619–638
36 Grinstein, S., Cohen, S and Rothstein, A (1984) J. Gen Physiol 83, 341–370
37 Doppler, W , Maly, K. and Grunicke, H (1986) J Membrane Biol. 91, 147–155
38 Schatzmann, H.J (1975) in Topics in Membranes and Transport (Bronner, F. ad Kleinzeller, A , eds.), Vol 6, p 125, Academic Press, New York
39 Shalev, O., Leida, M N., Hebbel, R.P , Jacob, H S and Eaton, J W. (1981) Blood 58, 1232–1235
40 Blaustein, M.P (1977) Am. J Physiol 232, C165–C173
41 Zidek, W., Vetter, H., Dorst, K G., Zumkley, H and Losse, H (1982) Clin. Sci 63, 413–435
42 Bruschi, G , Bruschi, M.E., Caroppo, M., Orlandini, G , Spaggiari, M and Caotatorta, A (1985) Clin Sci 68, 179–184
43 Postnov, Y.V , Orlov, S.N and Pokudin, N I (1980) Pflugers Arch. 388, 89–91
44 Orlov, S N and Postnov, Y V (1980) Clin Sci. 59, 207s–209s
45 Park, C S. and Malvin, R L (1978) Am J Physiol 235, F22–F25
46 Livne, A , Balfe, J W , Veitch, R , Marquez-Julio, A , Grinstein, S and Rothstein, A (1987) Lancet i, 533–536
47 Funder, J., Wieth, J.O , Jensen, H A E and Ibsen, K.K (1984) in Topics in Pathophysiology of Hypertension (Villarreal, H. and Sambhi, M.P., eds), pp 147–161, Martinus Nijhoff Publishers, Boston
48 Weder, A B. (1986) N Engl J Med 314, 198–201
49 Murer, H , Hopfer, V and Kinne, R (1976) Biochem J 154, 597–604
50 Kinsella, J L and Aronson, P.S (1980) Am J Physiol 238, F461–F469
51 Aronson, P S. (1982) N Engl J. Med 307, 317
52 Escobales, N and Canessa, M. (1986) J Membrane Biol 90, 21—28
53 Mahnensmith, R.L. and Aronson, P.S (1985) Circ Res 56, 773–788
54 Sarkadi, B , Alifinoff, J.K., Gunn, R B and Tosteson, D C (1978) J Gen Physiol 72, 249–265