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THE ERYTHROCYTE MEMBRANE IN ESSENTIAL HYPERTENSION

MODIFIED TEMPERATURE-DEPENDENCE OF Li^+ EFFLUX

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The rate of ouabain-resistant Li^+-efflux was studied in erythrocytes of normal controls and of patients with essential hypertension. Despite variability in rate, erythrocytes from normotensive persons revealed a uniform pattern of temperature dependence of the efflux, with two slopes (K_a = 9.4 and 19.1 kcal/mol, respectively) and a transition at about 25°C. Erythrocytes from the patients showed both a higher rate of Li^+ efflux and significant changes in the temperature repsonse, with essentially a single slope (K_a = 14 kcal/mol). The data indicate localized changes in the membrane organization of hypertensive erythrocytes, involving lipid-protein interaction.

Erythrocytes of persons with essential hypertension are characterized by modified fluxes of monovalent cations [1,2]. Recent reports demonstrated a decrease in Na^+/K^+ co-transport [3] and an increase in Li^+/Na^+ countertransport [4]. Yet, the interrelationship between these parameters are obscure, since essential hypertensives appear modified either in Na^+/K^+ co-transport, Li^+/Na^+ countertransport or both [5–7]. Of particular interest are the membrane changes related to the modified fluxes which are associated, apparently genetically, with essential hypertension [8,9]. With respect to cation fluxes, two alternative interpretations are plausible. The differences in flux could be mainly quantitative (e.g. due to a change in number of transport sites), or they may reflect some alterations in the organization of the membrane. As an approach for distinguishing between such alternatives, we have selected to study the effect of temperature on lithium flux in erythrocytes. Profound differences are demonstrated in the temperature dependence of the flux in hypertensives, pointing to localized structural modifications in the membrane.

Blood was obtained from ten normotensive persons, aged 25 to 50, with no known personal or family history of hypertension and from ten persons with essential hypertension ranging in age from 28 to 56. Maximal blood pressure recorded during hospitalization of the hypertensives was 150–200 mmHg systolic (mean 170 mmHg), 100–120 mmHg diastolic (mean 107 mmHg). The patients had normal urea and creatinine levels and urinalysis was without pathological findings. Only one patient had hypertensive changes in the eye fundus and none had evidence of left ventricular hypertrophy. Seven patients previously treated for hypertension were off anti-hypertensive therapy for at least two weeks before and during the study, with the exception of one, who received a β blocking agent.

Blood was drawn into heparin solution (25 units

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per ml) and processed within 30 min. Separation of red blood cells, loading the cells with Li^+ and meausrements of Li^+ efflux were as described by Canessa et al. [4]. Efflux was performed simultaneously in an Na^+-containing medium (150 mM NaCl, 10 mM glucose, 0.1 mM ouabain and 10 mM Tris-Mops, pH 7.4) and in Na^+-free medium (same medium but NaCl replaced by 75 mM $MgCl_2$ and 85 mM sucrose). The media were equilibrated to the desired temperature prior to the addition of Li^+-loaded cells. Lithium was determined by means of an atomic absorption spectrophotometer (Varian Techtron Model AA6), and calibrated by standards corresponding to the medium used. The flux was computed from the linear regression of Li^+-loss within 30 min [4]. Acetylcholinesterase activity was measured according to the method of Ellman et al. [10] as modified by Livne and Bar-Yaakov [11].

Cation fluxes vary considerably in the population, probably due to genetic factors [12–14]. Indeed, erythrocytes from normotensive individuals (Fig. 1) and from hypertensive ones (Fig. 2) exhibited variability in maximal Li^+ efflux rates in Na^+-containing medium. Fig. 3 presents average values ($N = 10$) for the temperature dependence of Li^+ efflux. For a direct comparison of the two groups, the rate at 37°C of the normotensive group was set as 100%. Despite the variability in maximal rates of Li^+ efflux, all the normotensive samples had the same pattern of temperature dependence: two distinct slopes were reproducibly seen, with a transition at, or near, 25°C. The corresponding energies of activation, deduced from Arrhenius plots were 9.4 ± 0.8 and 19.1 ± 0.5 kcal/mol (mean ± S.E.) for the temperature ranges below and above the transition, respectively. Li^+ efflux of erythrocytes from hypertensive persons showed temperature-dependence significantly different from normal in several respects. (a) Essentially a continuous plot was apparent over a wide range. Erythrocytes of hypertensives with a relatively lower Li^+ efflux rate (such as cases 1 and 2 in Fig. 2) exhibited some change in the slope of temperature dependene, but only above 33°C. (b) The slopes of hypertensive samples were intermediate, in between the normotensive ones, with energy of activation of 14 ± 0.3 kcal/mol over the temperature range of 12 to 33°C. (c) The dif-

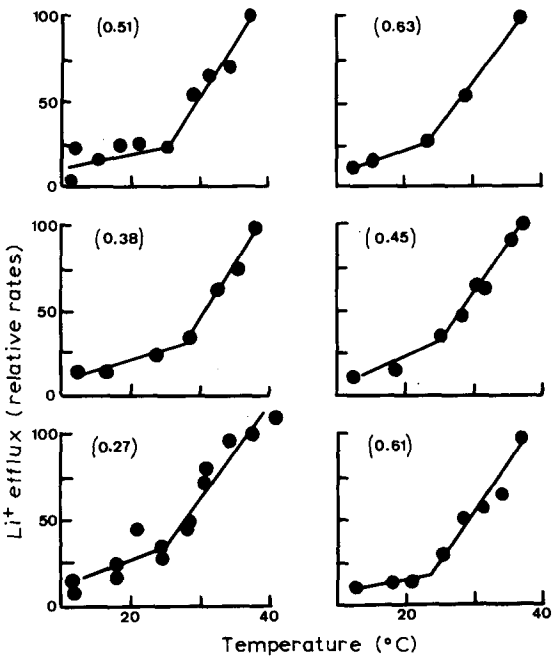

Fig. 1. Temperature dependence of lithium efflux from erythrocytes of normotensive persons. The rates at 37°C in mmol/litre red cell per h (given in parentheses) are set as 100% and relative values are presented.

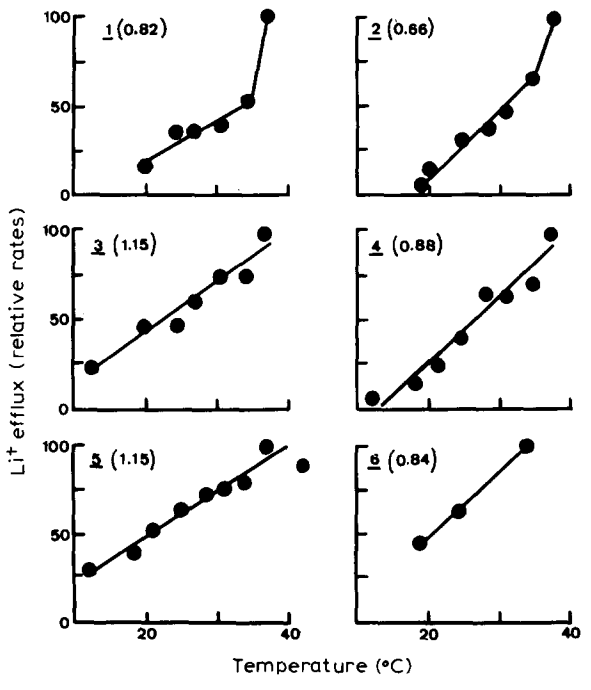

Fig. 2. Temperature dependence of lithium efflux from erythrocytes of hypertensive persons. Details as in Fig. 1.

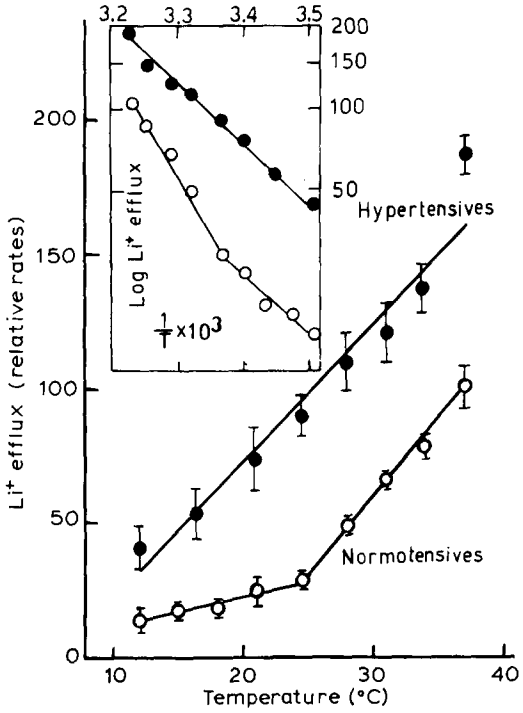

Fig. 3. Temperature dependence of lithium efflux from erythrocytes of normotensive and hypertensive persons. The rate of the normotensive samples (0.50±0.07 mmol/litre red cell per h) was set as 100%. Each curve represents average values of 10 individuals, including the samples shown in Figs. 1 and 2. The vertical bars represent ± standard error. Least square regression lines were computed and tested by analysis of variance ($P<0.001$). A single line for the hypertensive group and two lines (with a transition at 24.5°C) for the normotensives were accepted as the best fit. The rate of efflux for the normotensives could not be fitted by a straight line ($P<0.001$). Corresponding Arrhenius plots are given in the inset.

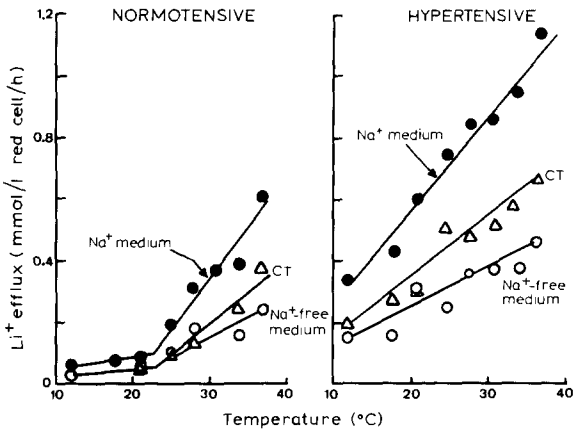

Fig. 4. Lithium fluxes from erythrocytes of normotensive and hypertensive persons as affected by temperature. Countertransport (CT) was calculated [4] by correcting the flux in the Na^+ medium for the flux in Na^+-free medium.

ference in temperature relates not only to Li^+ efflux in Na^+-medium, but also to Li^+/Na^+ countertransport (corrected for the flux in Na^+-free medium). Fig. 4 shows that the temperature plots of a normotensive are typically discontinuous, while the corresponding lines of a hypertensive are essentially continuous.

Acetylcholinesterase activity of normal erythrocytes shows a temperature dependence with a transition at about 24°C [15,16]. It was therefore of interest to examine its temperature response in erythrocytes of hypertensives, as a clue to whether the membrane modifications revealed by Li^+ fluxes are general and widespread. Acetylcholinesterase is particularly suitable for such a comparison, due to a unique combination of properties. It is a membrane bound enzyme which can be assayed colorimetrically in the intact cell [10] and which can probe the dynamics of the membrane integrity [15]. Furthermore, of the enzymes recognized in the membrane of the human red cell, alterations in activity associated with pathological conditions are found regularly only with acetylcholinesterase [17]. Acetylcholinesterase purified to homogeneity is a lipid-glycoprotein [18]. Such a ternary complex is compatible with the assymetric orientation of the enzyme on the outer surface of the erythrocyte [19] along with the dependence of the allosteric properties of the enzyme on the fatty acid composition of the erythrocyte membrane [20,21]. The hydrophobic association with the membrane apparently accounts for the sensitivity of acetylcholinesterase to added lipids in intact cells, conditioned also by a transmembrane potential [11]. Fig. 5 presents acetylcholinesterase activity in intact erythrocytes from hypertensives as affected by the temperature. The activities were within the normotensive range and the temperature dependence was indistinguishable from that already observed for normal red blood cells [15,16], characterized by two slopes and a transition at about 24°C. It is thus concluded that the membrane changes expressed

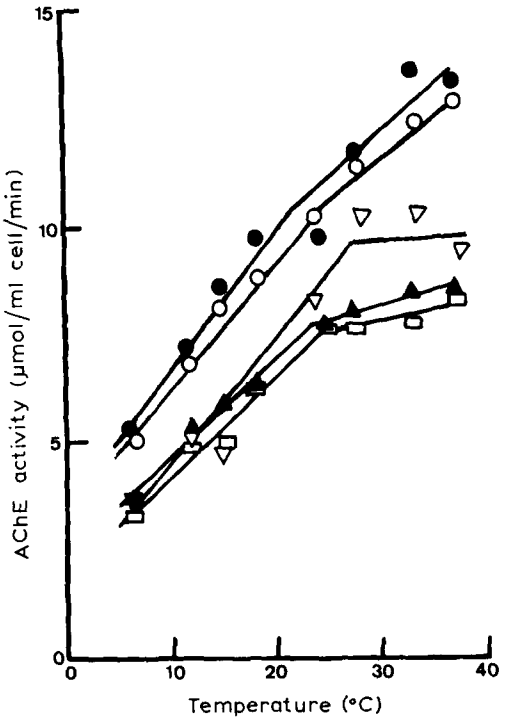

Fig. 5. Temperature dependence of acetylcholinesterase activity in erythrocytes of five hypertensive persons.

by the Li^+ efflux of hypertensive erythrocytes are likely to be localized.

The discontinuous temperature dependence of Li^+ efflux in normotensive samples indicates the involvement of lipid-protein interactions in the influx system studied. Indeed, a discontinuity in Arrhenius plots of transport systems is typical for proteins which require a mobile state of the lipids for activity [22–24]. Furthermore, the discontinuous temperature plots may indicate that cholesterol is not present at the immediate environment of the Li^+ 'openings', since cholesterol is well known to attenuate the transition in temperature dependence in membrane systems [25,26]. Several studies indicate that protein-lipid boundary regions in the membrane are typically deficient in cholesterol. Warren et al. [27] demonstrated that $(Ca^{2+} + Mg^{2+})$-ATPase (from sarcoplasmic reticulum) with a complete phospholipid annulus is not inhibited by cholesterol. However, as cholesterol replaces phospholipids in the annulus, ATPase activity is progressively inhibited. It was thus concluded that cholesterol is excluded from the first shell of the lipid bilayer surrounding the protein [27]. Using paramagnetic quenching of protein fluorescence by nitroxide lipid analogues, Bieri and Wallach [28] concluded that much of the cholesterol in the erythrocyte ghost membrane is in clustered state, distributed away from penetrating proteins. A change in the distribution of cholesterol close to the Li^+ efflux 'openings' in erythrocytes of hypertensives could explain the typical slope but not the elevated rate: the enrichment of the membrane with cholesterol decreases Na^+ efflux by either the active or the passive process [29], while erythrocytes low in cholesterol show increased permeability to Na^+ [30].

In conclusion, the erythrocytes of patients with essential hypertension are characterized by some profound, localized changes in the membrane. Further study is of course required to explore the possible relevance of such changes in the pathogenesis or the manifestation of the disorder.

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