Relationship between altered Na⁺-K⁺ cotransport and Na⁺-Li⁺ countertransport in the erythrocytes of 'essential' hypertensive patients

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Summary

1. The maximal rate of activity of sodium extrusion by the sodium pump, Na⁺-K⁺ outward cotransport, passive permeability to sodium and potassium, Na⁺-Li⁺ countertransport, and passive permeability to lithium were measured in 45 essential hypertensive patients, 24 young normotensive subjects with at least one hypertensive parent and 24 normotensive subjects with both parents normotensive.

2. The maximal rate of activity of the sodium pump and the rate constants for passive permeability to sodium, potassium and lithium were similar in the three groups.

3. The mean value for Na⁺-K⁺ outward cotransport was significantly lower and that for Na⁺-Li⁺ countertransport significantly higher in the hypertensive patients than in the normotensive subjects without a family history of hypertension, but there was a great overlap between individual values. The offspring of hypertensive parents had intermediate values.

4. A highly significant positive correlation existed between Na⁺-K⁺ cotransport and Na⁺-Li⁺ countertransport both in the normotensive and in the hypertensive subjects, indicating that in the latter there were some with high Na⁺-Li⁺ countertransport and others with low Na⁺-K⁺ cotransport.

5. In nine hypertensive families studied, if an alteration of the transport system was detected in a hypertensive propositus, the same type of alteration was also found in his still normotensive offspring, thus indicating a familial tendency for the alteration to occur.

Key words: cotransport, countertransport, lithium, sodium.

Introduction

One of the possible approaches to understanding the mechanisms involved in the pathogenesis of essential hypertension is the study of sodium transport at the cell membrane level. Several investigators have reported alterations of sodium transport in erythrocyte membranes both in hypertensive humans and in spontaneously hypertensive rats [1-9], in leucocytes from hypertensive humans [10-11] and in vascular smooth muscle from spontaneously hypertensive rats [12-13], without reaching conclusive results. Two of the alterations observed in sodium transport in the erythrocytes of essential hypertensive subjects, i.e. Na⁺-K⁺ cotransport [14] and Na⁺-Li⁺ countertransport [4], have been described in detail. The relationship between the two systems has not yet been studied, nor is the role of these alterations in the pathogenesis of essential hypertension clear.

In an attempt to elucidate these points we measured simultaneously the maximal rates (V_max.) of sodium extrusion by the sodium pump, of Na⁺-K⁺ outward cotransport, of Na⁺-Li⁺ countertransport and the rate constant for passive permeability for sodium, potassium and lithium in the erythrocytes of essential hypertensive subjects, of some of their young normotensive offspring, and of normotensive control subjects without hypertensive first-degree relatives.

Methods

Patients

Forty-five essential hypertensive patients (diastolic pressure > 95 mmHg in at least three
consecutive readings) in whom known causes of blood pressure elevation had been excluded, 24 young normotensive offspring of essential hypertensive patients (aged 16–30 years) and 24 normotensive control subjects with both parents normotensive, matched for sex and age with the hypertensive group, were studied.

**Cation outflux determinations**

\( \text{Na}^+ - \text{Li}^+ \) countertransport and passive permeability to lithium were determined by the method of Canessa *et al.* [4] with minor modifications (MgCl\(_2\) 110 mmol/l was used as washing solution and the supernatant samples were removed at 0, 10, 20 and 30 min of incubation at 37°C). Sodium extrusion by the sodium pump, \( \text{Na}^+ - \text{K}^+ \) cotransport and the passive permeability to sodium and potassium were determined by a method recently developed [15], with minor modifications consisting of the use of a slightly different 'sodium and choline loading medium' [NaCl 40 mmol/l; KCl 5 mmol/l; choline chloride 256 mmol/l; sodium phosphate buffer, pH 7.35 (4°C), 2.5 mmol/l; \( p \)-chloromercuribenzenesulphonic acid (Na salt; Sigma) 0.02 mmol/l, and 'potassium enriched medium' (KCl, 6 mmol/l; MgCl\(_2\) 72 mmol/l; sucrose 85 mmol/l; Tris-MOPS buffer, pH 7.35 (37°C), 10 mmol/l) and the removal of supernatant samples at 0, 10, 20 and 30 min for the determination of total sodium efflux and at 0, 30 and 60 min for the determination of ouabain-sensitive and ouabain + frusemide-sensitive sodium and potassium effluxes.

**Results**

The maximal rate of sodium extrusion by the sodium pump and the passive permeability to sodium, potassium and lithium were similar in the hypertensive group and in the normotensive offspring of essential hypertensive and normotensive, matched for sex and age with the hypertensive group, was 337 ± 34.1; potassium 372 ± 22.1; \( \text{Na}^+ - \text{Li}^+ \) countertransport: 234 ± 18.7), the individual values ranging from those typical of the hypertensive patients to those typical of the normotensive subjects. In spite of the great overlap between the individual values for the normotensive and hypertensive groups both for cotransport and countertransport (Fig. 1) we selected arbitrary 'normal values' (275–650 μmol h\(^{-1}\) l\(^{-1}\) of erythrocytes for \( \text{Na}^+ - \text{K}^+ \) countertransport and 150–300 μmol h\(^{-1}\) l\(^{-1}\) of erythrocytes for the \( \text{Na}^+ - \text{Li}^+ \) countertransport). 'Normal values' were selected in such a way that of the values for normotensive subjects fell between such limits. In this way 20 (44.4%) of the hypertensive patients had abnormally low cotransport and 14 (31.1%) had abnormally high countertransport. Moreover, 10 (41.6%) of the normotensive offspring of hypertensive parents had low cotransport and three (12.5%) had high countertransport. Five hypertensive patients had abnormally high cotransport values.

A highly significant positive correlation was observed between \( \text{Na}^+ - \text{K}^+ \) cotransport and \( \text{Na}^+ - \text{Li}^+ \) countertransport, both in the hypertensive and the normotensive groups (hypertensive, \( y = 0.42x + 174, r = 0.59, P < 0.001); normotensive, \( y = 0.45x + 36, r = 0.70, P < 0.001\), where \( x \) = cotransport and \( y \) = countertransport. [We do not have data to show which of the two variables (cotransport and countertransport) is the dependent one, or if one really depends on the other. The choice of cotransport as the independent variable (\( x \)) is arbitrary.] The angular coefficients of the two regression lines were similar, thus indicating that they were roughly parallel. The intercepts on the y axis were significantly different (\( P < 0.01 \)).

Nine hypertensive families were studied in depth. So far, we have observed in all of them a familial tendency for one type of abnormality to occur (low cotransport or high countertransport), regardless of whether the members were hypertensive or normotensive. In this way, we have identified four 'low cotransport' families and three 'high countertransport' families.

**Discussion**

Our main findings were: (1) a positive correlation between \( \text{Na}^+ - \text{K}^+ \) cotransport and \( \text{Na}^+ - \text{Li}^+ \) countertransport and the fact that for any given level of \( \text{Na}^+ - \text{K}^+ \) cotransport the hypertensive patients had significantly higher levels of \( \text{Na}^+ - \text{Li}^+ \) countertransport; (2) the hypertensive patients had significantly higher \( \text{Na}^+ - \text{Li}^+ \) countertransport and lower \( \text{Na}^+ - \text{K}^+ \) cotransport
than the normotensive subjects, but there was great overlap; (3) the lack of difference in the $V_{\text{max}}$ of the sodium pump; and (4) the familial tendency for both types of alteration to occur.

(1) The highly significant correlation between $\text{Na}^+-\text{K}^+$ cotransport and $\text{Na}^+-\text{Li}^+$ countertransport suggests that the two systems are related in some way. We have to bear in mind that our knowledge of these transport systems is very small. At present we can only describe a phenomenon (inhibition of sodium and potassium efflux in the presence of frusemide and trans-stimulation of lithium efflux in the presence of sodium in the medium), and the underlying molecular mechanisms are still unclear [16]. Some authors doubt the existence of a cation transport system in addition to the sodium pump: the fluxes observed in the presence of ouabain...
may depend on different modes of operation of the sodium pump when different pharmacological agents are added [17]. Recent evidence suggests that Na\(^+\)-K\(^+\) cotransport and Na\(^+\)-Li\(^+\) countertransport are different transport systems, Na\(^+\)-Li\(^+\) countertransport possibly 'reflecting the synthesis, activation or degradation of a more complex sodium transport unit' [18]. These observations do not explain the higher Na\(^+\)-Li\(^+\) countertransport found for any given level of Na\(^+\)-K\(^+\) cotransport in the hypertensive patients. Only one fact seems clear: the erythrocyte membranes of the hypertensive patients appear different from those of the normotensive subjects. Such a difference seems to precede the development of hypertension, since it can also be found in some of the normotensive offspring of hypertensive parents.

(2) The great overlap observed in the values of Na\(^+\)-Li\(^+\) countertransport and Na\(^+\)-K\(^+\) cotransport between hypertensive and normotensive subjects indicates that both systems need to be investigated in the same patient, in order to show at least one abnormality.

(3) In hypertensive patients, the lack of difference between the maximal rate of activity of the sodium pump, taken together with the equal number of ouabain-binding sites and with the high activity when measured at other than \(V_{\text{max}}\), which have been previously reported by others [14], suggest that the apparent affinity of one of the cations for the sodium pump is different in the hypertensive patients.

(4) The familial tendency of these abnormalities suggests, but does not prove, that they might be inheritable. Moreover, if environmental factors are present, they are not related to sodium consumption, since the latter is similar in high Na\(^+\)-Li\(^+\) countertransport and low Na\(^+\)-K\(^+\) cotransport subjects.

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References


