Sodium/lithium countertransport and intracellular calcium concentration in patients with essential hypertension and coronary heart disease

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ABSTRACT

The present study was designed to test the hypothesis that enhanced intracellular calcium signalling and increased sodium/lithium countertransport (Na⁺/Li⁺ CT) activity may be associated with coronary heart disease (CHD) in non-diabetic patients with essential hypertension. Platelet-activating factor (PAF)-evoked rises in the intracellular calcium concentration ([Ca²⁺]i) were measured in Epstein–Barr-virus-immortalized lymphoblasts from 62 hypertensive patients with CHD and 34 patients without CHD. Na⁺/Li⁺ CT activity was assessed in erythrocytes from 80 hypertensive patients with CHD and 46 patients without CHD. Baseline values of unstimulated and PAF-stimulated [Ca²⁺]i were not significantly different between hypertensive subjects with (baseline, 126 ± 5 nmol/l; stimulated, 550 ± 43 nmol/l) and without (baseline, 125 ± 5 nmol/l; stimulated, 654 ± 105 nmol/l) CHD. Similarly, Na⁺/Li⁺ CT activity was not significantly different between the two groups (patients with CHD, 219 ± 8 l mol/l⁻¹ h⁻¹; patients without CHD, 234 ± 10 l mol/l⁻¹ h⁻¹). We conclude that intracellular signal transduction, as indicated by PAF-induced rises in [Ca²⁺]i, and Na⁺/Li⁺ CT activity, is not associated with an increased risk of CHD in non-diabetic patients with essential hypertension.

INTRODUCTION

Essential hypertension is associated with several abnormalities in cellular ion transport systems, including Na⁺/Li⁺ countertransport (Na⁺/Li⁺ CT) [1–8], Na⁺/H⁺ exchange [3,9–14], Na⁺–K⁺ co-transport [6,15], and intracellular Ca²⁺ signalling [9,12,15–17]. Several of these abnormalities have been shown to be particularly frequent in subsets of subjects with hypertension, giving rise to the hypothesis that they may be associated with hypertension-induced end-organ damage rather than with hypertension per se [18,19]. Thus a high intracellular calcium concentration ([Ca²⁺]i) and high activity of the Na⁺/H⁺ exchanger have been reported to be associated with a high proliferation rate and growth of vascular smooth muscle cells [13,20–23], as well as with left ventricular hypertrophy [24]. Furthermore, increased Na⁺/Li⁺ CT activity has been shown to be associated with left ventricular hypertrophy [18,19,24–26], coronary heart disease (CHD) [27], hyperlipidaemia [4,7,8]

Key words: calcium signalling, cellular ion transport system, coronary heart disease, hypertension, platelet-activating factor, sodium/lithium countertransport.

Abbreviations: BMI, body mass index; [Ca²⁺]i, intracellular calcium concentration; CHD, coronary heart disease; Na⁺/Li⁺ CT, sodium/lithium countertransport; PAF, platelet-activating factor.

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and urinary albumin excretion [18,28]. A relationship between Na+/Li+ CT activity and diabetic nephropathy is less evident [4,29,30].

In the present study we tested the hypothesis that increased Na+/Li+ CT activity and changes in intracellular Ca²⁺ signalling may be associated with CHD in non-diabetic patients with essential hypertension. To test this hypothesis, we compared erythrocyte Na+/Li+ CT activity and platelet-activating factor (PAF)-evoked rises in [Ca²⁺], in lymphoblasts from hypertensive patients with or without CHD.

**METHODS**

**Patients**

The study population consisted of 126 non-diabetic patients with essential hypertension who attended the University Hospital of Internal Medicine of the University of Greifswald between December 1997 and February 2001 for symptoms of CHD and who were subjected to coronary angiography. The study was approved by the institutional review committee of the University of Greifswald. Prior to inclusion in the study, all patients gave informed consent.

Characteristics of the patients are presented in Tables 1 and 2. Intracellular Ca²⁺ signalling was determined in immortalized lymphoblasts from 96 patients (59 males and 37 females), 62 of whom suffered from CHD. Na+/Li+ CT activity was determined in erythrocytes from 126 patients (82 males and 44 females), 80 of whom suffered from CHD. All patients were under anti-hypertensive treatment with calcium channel blockers (27%), β-blockers (77%), angiotensin-converting enzyme inhibitors (75%), AT₁ receptor antagonists (7%) or α-blockers (8%), given alone or in combination. All clinical and biological parameters used to determine associated cardiovascular risk factors were estimated during the stay in hospital. PAF-evoked Ca²⁺ signals were determined in immortalized B lymphocytes isolated from 10 ml of blood. Na+/Li+ CT activity was quantified in isolated erythrocytes from an additional sample of 10 ml of blood.

**Cell culture**

Lymphocytes were isolated on a Ficoll density gradient (Lymphocyte Separation Medium, density 1.077 g/ml; Roche Diagnostics, Mannheim, Germany). B-lymphocytes were immortalized by incubation with Epstein–Barr-virus-containing supernatant from the B 95-8 cell line (Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany). The preparation and immortalization has been described in detail elsewhere [13]. Cells were routinely maintained in culture with RPMI 1640 medium (Gibco, Eggenstein, Germany) supplemented with 2 mmol/l l-glutamine, 100 units/ml streptomycin, 100 units/ml penicillin and 10% (v/v) fetal bovine serum (Gibco, Eggenstein, Germany). Passages were performed twice a week. After immortalization, lymphoblasts were grown for 16 weeks, then stock cultures were frozen and cells were used for measurements.

**Measurement of [Ca²⁺]i**

[Ca²⁺]i, was measured by the fura-2 fluorescence technique, as described previously [31].

**Preparation of erythrocyte suspension**

EDTA-treated blood was centrifuged at 1500 g. Erythrocytes were washed four times with iso-osmotic washing buffer (75 mmol/l MgCl₂, 80 mmol/l sucrose, 5 mmol/l glucose, 10 mmol/l Tris/Mops, pH 7.55) at room tem-

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**Table 1** Baseline characteristics of patients in the [Ca²⁺]i study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Without CHD</th>
<th>With CHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>34</td>
<td>62</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>17/17</td>
<td>42/20*</td>
</tr>
<tr>
<td>Age (years)</td>
<td>62.4 ± 1.6</td>
<td>63.9 ± 1.2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>28.7 ± 0.7</td>
<td>26.6 ± 0.5*</td>
</tr>
<tr>
<td>Creatinine (μmol/l)</td>
<td>91.8 ± 2.9</td>
<td>96.8 ± 2.8</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>6.0 ± 0.2</td>
<td>5.8 ± 0.2</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.4 ± 0.1</td>
<td>1.2 ± 0.4</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>3.8 ± 0.2</td>
<td>3.6 ± 0.1</td>
</tr>
<tr>
<td>Triacylglycerols (μmol/l)</td>
<td>2.2 ± 0.2</td>
<td>2.2 ± 0.2</td>
</tr>
<tr>
<td>Uric acid (μmol/l)</td>
<td>352 ± 21 (n = 10)</td>
<td>367 ± 15 (n = 57)</td>
</tr>
<tr>
<td>Lipoprotein (a) (mg/l)</td>
<td>210 ± 41 (n = 25)</td>
<td>372 ± 61 (n = 45)</td>
</tr>
</tbody>
</table>

**Table 2** Baseline characteristics of patients in the Na⁺/Li⁺ CT study

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Without CHD</th>
<th>With CHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>46</td>
<td>80</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>20/26</td>
<td>62/18*</td>
</tr>
<tr>
<td>Age (years)</td>
<td>63.5 ± 1.2</td>
<td>65.4 ± 1.0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.0 ± 0.6</td>
<td>27.0 ± 0.4*</td>
</tr>
<tr>
<td>Creatinine (μmol/l)</td>
<td>95.9 ± 2.8</td>
<td>100.3 ± 3.4</td>
</tr>
<tr>
<td>Cholesterol (mmol/l)</td>
<td>5.9 ± 0.2</td>
<td>5.8 ± 0.1</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.3 ± 0.1</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>3.7 ± 0.1</td>
<td>3.6 ± 0.1</td>
</tr>
<tr>
<td>Triacylglycerols (μmol/l)</td>
<td>2.2 ± 0.2</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>Uric acid (μmol/l)</td>
<td>353 ± 16 (n = 39)</td>
<td>369 ± 12 (n = 71)</td>
</tr>
<tr>
<td>Lipoprotein (a) (mg/l)</td>
<td>239 ± 39 (n = 38)</td>
<td>442 ± 69 (n = 55)</td>
</tr>
</tbody>
</table>
perature. The erythrocyte suspension was kept at room temperature and used within 1 h.

**Loading with lithium bicarbonate**

A sample of 4 ml of washed erythrocytes was incubated for 15 min at 37 °C with shaking in 20 ml of lithium bicarbonate medium (150 mmol/l LiHCO₃, 10 mmol/l glucose, 10 mmol/l Tris/Mops, gassed with CO₂ until the pH value had adjusted to 7.55) [32]. To remove extracellular lithium, cells were washed five times with washing buffer.

**Measurement of lithium efflux**

Lithium efflux was measured by incubating 1.8 ml of cell suspension (haematocrit 0.05–0.08) in a sodium-free medium (150 mmol/l KCl, 1 mmol/l MgCl₂, 10 mmol/l glucose, 0.1 mmol/l ouabain, 10 mmol/l Tris/Mops, pH 7.55) or in a sodium-containing medium (KCl in the efflux medium was replaced by 150 mmol/l NaCl). After 60 min, incubation in either medium was stopped by cooling the tubes on ice for 2 min and subsequent centrifugation at 1500 g for 4 min at 4 °C. Then 1 ml of the supernatant was mixed with 10 μl of 20% (w/v) CsCl₂ solution, and lithium concentrations were measured by atomic absorption spectrophotometry (Unicam Model Solaar 939).

**Measurement of Na⁺/Li⁺ CT activity**

Na⁺/Li⁺ CT activity (μmol·l⁻¹·h⁻¹) was determined as the difference between sodium-stimulated lithium efflux and passive lithium efflux in the sodium-free medium over time.

**Statistics**

Data were analysed using the statistical package Statgrafics Plus (STSC Inc., Rockville, MD, U.S.A.). Data in the text are presented as means ± S.E.M. Differences between the two groups were compared by a non-parametric two-sample test (Mann–Whitney U test). Differences in gender distribution between the groups were analysed using chi-squared analysis. Stepwise multiple linear regression analyses were carried out to identify independent determinants of Na⁺/Li⁺ CT activity and PAF-stimulated [Ca²⁺]i. Values of P < 0.05 were considered statistically significant.

**RESULTS**

Baseline clinical and laboratory data of patients are shown in Tables 1 and 2. The proportion of male compared with female patients was significantly higher in the group of hypertensive patients with CHD than in the group without CHD. Hypertensives without CHD had a significantly higher body mass index (BMI) than patients with CHD. All other clinical and biological parameters were not significantly different between both groups.

Data on PAF-evoked rises in [Ca²⁺], are shown in Figure 1. Baseline values of [Ca²⁺], were not significantly different between patients with (126 ± 5 nmol/l) and without (125 ± 5 nmol/l) CHD. PAF stimulation led to significant increases in [Ca²⁺], in both groups. PAF-stimulated [Ca²⁺]i values were not significantly different between hypertensives with CHD (550 ± 43 nmol/l) and those without CHD (654 ± 105 nmol/l). Similarly, there were no significant differences in Na⁺/Li⁺ CT activity between patients with (219 ± 8 μmol·l⁻¹·h⁻¹) and without (234 ± 10 μmol·l⁻¹·h⁻¹) CHD.

In a multiple regression analysis with Na⁺/Li⁺ CT activity as the dependent variable and gender, age, BMI, plasma triacylglycerol concentration and plasma cholesterol concentration as independent variables, only age (P < 0.05) and plasma triacylglycerol concentration (P < 0.01) emerged as significant and independent determinants of Na⁺/Li⁺ CT activity. In this model, age and plasma triacylglycerol concentration explained 17.7% of the variation in Na⁺/Li⁺ CT activity. In a further multiple regression analysis using the same independent variables and PAF-stimulated [Ca²⁺], as the dependent variable, none of the independent variables had a statistically significant effect.

**DISCUSSION**

PAF-evoked increases in both [Ca²⁺], and Na⁺/Li⁺ CT activity have been reported to be enhanced in patients with essential hypertension compared with normotensives [3,5,16,17,33–35]. One study [31] showed that only a subgroup of hypertensive patients was affected by increased Na⁺/Li⁺ CT activity and enhanced PAF-evoked increases in [Ca²⁺]. Since a high [Ca²⁺], and enhanced Na⁺/Li⁺ CT activity have been shown to be associated with hypertension-induced end-organ damage, such as left ventricular hypertrophy [18,24,25]
and CHD [27], we hypothesized that enhanced PAF-evoked increases in [Ca\(^{2+}\)], and increased Na\(^+/\)Li\(^-\) CT activity may be useful markers to distinguish between non-diabetic hypertensive patients with and without CHD. To test this hypothesis, measurements of PAF-evoked cytosolic calcium transients and Na\(^+/\)Li\(^-\) CT activity were performed in immortalized lymphoblasts and erythrocytes respectively obtained from patients with essential hypertension. Patients were grouped according to the presence or absence of CHD, as diagnosed by coronary angiography. PAF-evoked cytosolic calcium transients and Na\(^+/\)Li\(^-\) CT activity were not different between hypertensive patients with and without CHD. Thus our results argue against an association between these membrane ion transport abnormalities and CHD in non-diabetic patients with essential hypertension.

Our results may have been influenced by a number of factors that have been reported to affect membrane ion transport systems, including gender [6,36], BMI [6,7,36], plasma cholesterol concentration [7,8,37] and plasma triacylglycerol concentration [35,38,39]. To test for potentially confounding effects of these factors, we performed multiple regression analyses with Na\(^+/\)Li\(^-\) CT activity and PAF-stimulated [Ca\(^{2+}\)], as dependent variables. Na\(^+/\)Li\(^-\) CT activity was significantly affected by age and plasma triacylglycerol concentration, but not by gender, BMI or plasma cholesterol concentration. PAF-stimulated [Ca\(^{2+}\)], was not affected by any of the above-mentioned potential confounders.

Our failure to demonstrate a significant difference in Na\(^+/\)Li\(^-\) CT activity between hypertensive patients with and without CHD cannot be explained by the effects of age and plasma triacylglycerol concentrations, since our groups were well matched with respect to these factors. Other factors, such as gender distribution and BMI, which showed significant differences between the groups with and without CHD, did not have independent effects on Na\(^+/\)Li\(^-\) CT activity in a multiple regression analysis, and can therefore be excluded as confounders in the present study.

The finding that none of the factors under consideration had a significant and independent effect on PAF-stimulated [Ca\(^{2+}\)], may be due in part to the fact that measurements of [Ca\(^{2+}\)], were performed in Epstein–Barr-virus-transformed lymphoblasts after prolonged cell culture. Under these conditions, many potentially confounding effects of variables related to the homoeostatic environment of the circulating blood, such as neurohumoral factors or plasma lipid concentrations, can be excluded. In the present study it is particularly important that none of the factors that differed between the groups with and without CHD, i.e. gender and BMI, showed an independent effect on PAF-stimulated [Ca\(^{2+}\)].

In summary, we have failed to demonstrate an association between PAF-evoked increases in [Ca\(^{2+}\)], in Epstein–Barr-virus-transformed lymphoblasts or Na\(^+/\)Li\(^-\) CT activity in freshly obtained erythrocytes and CHD in non-diabetic patients with essential hypertension. Our results do not support the hypothesis that these membrane ion transport systems contribute to CHD in essential hypertension.

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**REFERENCES**


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