Decreased activity of the red blood cell ATPase-dependent Na\(^+\) pump in patients with cardiac syndrome X

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ABSTRACT

Marked Na\(^+\)/Li\(^+\) countertransport hyperactivity and post-load hyperinsulinaemia have been described in 93\% of patients with cardiac syndrome X. We hypothesized that more complex abnormalities in Na\(^+\) traffic across the cell membrane are present in these patients. The aim of the present study was to evaluate the activities of the two main transporters responsible for transmembrane Na\(^+\) transport, i.e. the ATPase-dependent Na\(^+\) pump and the Na\(^+\)–K\(^+\)–2Cl\(^−\) co-transporter, in a selected group of patients with cardiac syndrome X. We evaluated 19 patients with cardiac syndrome X and 14 control subjects. The ATPase-dependent Na\(^+\) pump and Na\(^+\)–K\(^+\)–2Cl\(^−\) co-transport activities were assessed from Na\(^+\)-loaded red blood cells by using nystatine, in the presence of furosemide and ouabain, as appropriate. Erythrocyte Na\(^+\)/Li\(^+\) countertransport activity, serum lipid and post-load (75 g of oral glucose) insulin levels were also evaluated. The \(V_{max}\) of Na\(^+\)/Li\(^+\) countertransport activity, serum lipid and post-load insulin levels (120 min; \(P = 0.0001\)) were confirmed to be higher in patients with syndrome X than in controls. The \(V_{max}\) of Na\(^+\)–K\(^+\)–2Cl\(^−\) co-transport was similar in patients and controls. By contrast, the \(V_{max}\) of the ATPase-dependent Na\(^+\) pump was significantly lower (\(P = 0.002\)) in syndrome X patients (3.13 ± 0.87 mmol·h\(^−1\)·l\(^−1\)) than in controls (4.28 ± 1.10 mmol·h\(^−1\)·l\(^−1\)). Serum total cholesterol and triacylglycerol concentrations were also higher in patients with syndrome X than in control subjects (\(P < 0.0001\)). Thus decreased activity of the ATPase-dependent Na\(^+\) pump was present in patients with cardiac syndrome X. Such an abnormality has the biological potential to augment microvascular tone and the response to constrictor stimuli via increased intracellular free Ca\(^{++}\). Of note, syndrome X patients also manifested Na\(^+\)/Li\(^+\) countertransport hyperactivity which, in turn, is known to induce peripheral insulin resistance and consequent abnormalities in insulin secretion and lipid turnover. Thus cardiac syndrome X appears as a multifaceted syndrome presenting with either metabolic or cardiovascular symptoms, or both, based on the expression of complex abnormalities in Na\(^+\) traffic across the cell membrane.

INTRODUCTION

The term ‘cardiac syndrome X’ is used widely to identify patients with angina-like chest pain, angiographically normal epicardial coronary vessels with no evidence of ergonovine-inducible coronary artery spasm, and non-invasive tests suggestive of myocardial ischaemia in the absence of other known cardiovascular diseases [1]. Because of the absence of epicardial coronary lesions, either structural abnormalities [1,2] or altered responses

Key words: cell membrane, microvascular angina, Na\(^+\)-ATPase.

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to constrictor stimuli [1,3] of small coronary vessels, or both, have often been indicated as the cause of the angina and ischaemic-like abnormalities. Recent reports have demonstrated enhanced activities of red blood cell Na+/Li+ countertransport [4], an in vitro marker of in vivo Na+/H+ antiport [5,6], and of Na+/H+ antiport itself [7], as well as post-load (oral 75 g dose of D-glucose) hyperinsulinaemia [4], in the vast majority of patients with cardiac syndrome X. As enhanced Na+/Li+ countertransport activity has the potential to cause both glucose intolerance and smooth muscle cell hyper-reactivity [5,6,8], it might represent a common pathogenetic mechanism for the metabolic and vascular abnormalities frequently observed in cardiac syndrome X.

However, the proposal that red blood cell Na+/Li+ countertransport does indeed represent the in vitro mode of operation of Na+/H+ antiport in vivo is not undisputed [9]. In addition, the Na+ ion is actively transported across the cell membrane mainly through the ATPase-dependent Na+ pump and a Na+-K+-2Cl- co-transporter rather than via Na+/H+ antiport [10]. As with Na+/H+ antiport, these Na+ transporters also manifest identical kinetics and Vmax values in red blood cells, endothelial cells [10,11] and vascular smooth muscle cells [10,12]. In these last cells, both the ATPase-dependent Na+ pump and Na+-K+-2Cl- co-transport indirectly modulate the intracellular free Ca2+ concentration and membrane depolarization, thereby regulating vascular responses to constrictor stimuli [10,13]. On this basis, we hypothesized that a complex abnormality in Na+ traffic across the cell membrane, i.e. involving not only Na+/H+ antiport but also the ATPase-dependent Na+ pump and Na+-K+-2Cl- co-transport, was responsible for abnormal microvascular contractility in patients with cardiac syndrome X.

METHODS

Study populations

Patients with cardiac syndrome X

This group consisted of 19 consecutive patients (18 women, one man; mean age 54 ± 6 years) referred to our institution for suspected coronary artery disease. Fifteen of these patients participated in a previous study by our group [4]. The entry criteria were: recurrent chest pain at rest and on effort; normal 12-lead ECG at rest; repeatedly positive exercise test for ischaemic-like ECG changes (horizontal or down-sloping ST-segment depression of > 1.5 mm at 60 ms after the J point in at least two contiguous leads lasting more than 1 min); normal left and right ventricular function at rest; absence of valvular heart disease and myocardial hypertrophy in M-mode and B-mode echocardiographies; sitting systolic/diastolic blood pressure levels of < 140/90 mmHg in the absence of anti-hypertensive medications; normal glucose tolerance, as evaluated by fasting and post-load (75 g oral dose of D-glucose) glucose levels; no family history of essential hypertension and/or diabetes; no concommitant diseases; no recent (< 3 months) use of drugs known to interfere with Na+ transporters across the cell membrane (i.e. cardiac glycosides, furosemide, glucocorticoids, etc.); and a recent (< 12 months) normal coronary angiogram without evidence of focal or diffuse coronary spasm after intracoronary infusion of ergonovine maleate. 201Tl scintigraphy was positive for adenosine-induced regional uptake abnormalities in 15 of the subjects. Menopause had occurred in nine of the women, seven of whom had undergone surgical hysterectomy.

Control group

As previously reported [4], 14 age-, sex- and weight-matched healthy volunteers (13 women, one man; mean age 47 ± 15 years) recruited among physicians and nurses of our institution served as controls. All subjects had no family history of either hypertension or diabetic heredities. Further, they had normal clinical examinations, had no concomitant diseases, were not under treatment for any pathological conditions, and did not voluntarily take any kind of drugs or dietary supplement, including vitamins, antioxidants, etc. Chest radiogram, rest ECG, echocardiogram and exercise stress tests were also normal. Menopause had occurred in six of the women.

The study protocol was evaluated and approved by our institutional Ethics Committee. Informed consent to take part in the study was obtained from patients and control subjects.

Laboratory measurements

All biochemical analyses were performed after 10 days of therapy discontinuation. Erythrocyte Na+/Li+ countertransport, ATPase-dependent Na+ pump and Na+-K+-2Cl- co-transport activities were measured in fresh red blood cells according to a previously described methodology [14]. Peripheral blood was collected in heparin-containing tubes. Red blood cells were separated within 3 h by centrifugation for 10 min at 300 g. Plasma and buffy coat were separated by suction. Red blood cells were washed three times in Na+-containing washing solution (125 mM NaCl, 75 mM MgCl2, 85 mM sucrose, 10 mM glucose, 10 mM Tris/Mops, pH 7.4) at 4 °C. The Vmax for Na+/Li+ countertransport was assayed by measuring external Na+-stimulated lithium efflux after lithium loading [14,15]. The Vmax of red blood cell Na+-K+-2Cl- co-transport was evaluated by measuring furosemide-sensitive Na+ and K+ efflux into choline media from cells loaded with Na+ by means of nystatin [14,16]. The Vmax of the red blood cell ATPase-dependent Na+ pump was assessed by determining ouabain-sensitive Na+ efflux from cells loaded with sodium by means of nystatin [16].
Sodium transmembrane transport and syndrome X

For oral glucose tolerance tests, plasma glucose was evaluated by the glucose oxidation method and a Glucose Analyzer II instrument (Beckman, Fullerton, CA, U.S.A.). Plasma insulin levels were assessed using a commercially available kit (Ares Serono, Milan, Italy). Serum total cholesterol, high-density lipoprotein cholesterol and triacylglycerol levels were assessed by enzymic methods (Boheringer Mannheim, Mannheim, Germany), in the case of high-density lipoprotein cholesterol after precipitation of low-density lipoprotein cholesterol by phosphotungstate. Serum low-density lipoprotein cholesterol levels were assessed by the Friedewald method [17]. Other laboratory tests were carried out using routine methods.

**Statistical analysis**

Continuous normally distributed data are expressed as means ± S.D., and were analysed using two-tailed unpaired Student’s t-tests. Data on insulin, which were not normally distributed, are expressed as the median and interquartile range. The effects of oral glucose on plasma glucose and insulin levels were tested by analysis of variance for multiple comparisons; for \( P < 0.05 \), pairwise comparisons were performed by using the Scheffe \( F \)-test or the Mann–Whitney rank-sum test, as appropriate. Regression and correlation techniques were used to evaluate linear relationships between variables. Descriptive parameters were tested for significance by the \( \chi^2 \) method. Differences between groups were considered to be statistically significant when \( P < 0.05 \).

**RESULTS**

The demographic and clinical characteristics of the study population and the controls are given in Table 1. Patients with syndrome X and control subjects were similar with regard to age, gender, body mass index and blood pressure levels.

**Sodium transporters**

Red blood cell Na\(^+\)/Li\(^+\) countertransport activity was significantly greater in patients with syndrome X compared with controls (621 ± 182 and 324 ± 49 \( \mu \)mol·h\(^{-1} \)·l\(^{-1} \) of cells respectively; \( P = 0.0001 \)). Notably, 18 of the 19 patients with cardiac syndrome X (94.7%) presented Na\(^+\)/Li\(^+\) countertransport values higher than the mean ± S.D. value of the control group. Na\(^+\)–K\(^+\)–2Cl\(^−\) co-transport showed similar activities in red blood cells from patients with syndrome X and control subjects (388 ± 120 and 424 ± 128 \( \mu \)mol·h\(^{-1} \)·l\(^{-1} \) of cells respectively; not significant). In contrast, the \( V_{\text{max}} \) of the ATPase-dependent Na\(^+\) pump was lower in patients with syndrome X than in controls (Figure 1).

**Glucose tolerance test and plasma insulin**

According to entry criteria, no subject was affected by type II diabetes, i.e. fasting and post-load glucose levels were < 120 mg/dl and < 200 mg/dl respectively [18]. The fasting plasma insulin level was higher in patients with cardiac syndrome X than in controls (Table 2). Post-load insulin levels [4] were confirmed to be higher in patients with cardiac syndrome X than in controls at 30, 60, 90, 120 and 180 min after load (Table 2).

**Lipids**

As shown in Table 2, patients with cardiac syndrome X were markedly dyslipidaemic. Indeed, compared with

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**Table 1** General characteristics of the two study groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cardiac syndrome X ( n = 19 )</th>
<th>Controls ( n = 14 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>53 ± 7</td>
<td>47 ± 15</td>
</tr>
<tr>
<td>Gender (female/male)</td>
<td>18/1</td>
<td>13/1</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25 ± 3</td>
<td>24 ± 4</td>
</tr>
<tr>
<td>Smoker (yes/no)</td>
<td>8/11</td>
<td>6/0</td>
</tr>
<tr>
<td>Menopause (yes/no)</td>
<td>9/9</td>
<td>6/7</td>
</tr>
<tr>
<td><strong>Haemodynamic data</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>125 ± 10</td>
<td>128 ± 9</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>73 ± 10</td>
<td>76 ± 10</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>77 ± 8</td>
<td>72 ± 8</td>
</tr>
<tr>
<td>Ejection fraction (%)</td>
<td>73 ± 8</td>
<td>70 ± 9</td>
</tr>
<tr>
<td><strong>Exercise tests</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exercise duration (s)</td>
<td>702 ± 166</td>
<td>717 ± 134</td>
</tr>
<tr>
<td>( 10^2 \times \text{Peak-rate pressure product} ) (mmHg·beats·min(^{-1} ))</td>
<td>288 ± 90</td>
<td>278 ± 96</td>
</tr>
<tr>
<td>Time to 0.1 mV ST depression (s)</td>
<td>577 ± 174</td>
<td>–</td>
</tr>
</tbody>
</table>

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**Figure 1** Abnormal ATPase-dependent Na\(^+\) pump activity in patients with cardiac syndrome X

ATPase-dependent Na\(^+\) pump activities in red blood cells from patients with cardiac syndrome X and controls are shown. Mean ± S.D. values are indicated for each group (circles with error bars).
Table 2  Metabolic features of the two study groups

Plasma glucose (means ± S.D.) and insulin (median (interquartile range)) levels were measured before and at various times after oral glucose ingestion (75 g). Also shown are values for serum total cholesterol and cholesterol subfractions and for serum triacylglycerol levels (mean ± S.D.) in the two study groups.

<table>
<thead>
<tr>
<th></th>
<th>Cardiac syndrome X</th>
<th>Controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n = 19)</td>
<td>(n = 14)</td>
<td></td>
</tr>
<tr>
<td>Plasma glucose (mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>94 ± 10</td>
<td>79 ± 8</td>
<td>0.073</td>
</tr>
<tr>
<td>30 min</td>
<td>138 ± 26</td>
<td>120 ± 44</td>
<td>0.054</td>
</tr>
<tr>
<td>60 min</td>
<td>141 ± 33</td>
<td>116 ± 47</td>
<td>0.065</td>
</tr>
<tr>
<td>90 min</td>
<td>127 ± 38</td>
<td>102 ± 44</td>
<td>0.098</td>
</tr>
<tr>
<td>120 min</td>
<td>124 ± 36</td>
<td>98 ± 34</td>
<td>0.059</td>
</tr>
<tr>
<td>180 min</td>
<td>101 ± 34</td>
<td>78 ± 34</td>
<td>0.064</td>
</tr>
<tr>
<td>Plasma insulin (pmol/l)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting</td>
<td>163 (87–157)</td>
<td>86 (68–94)</td>
<td>0.014</td>
</tr>
<tr>
<td>30 min</td>
<td>571 (364–676)</td>
<td>244 (158–323)</td>
<td>0.005</td>
</tr>
<tr>
<td>60 min</td>
<td>683 (360–843)</td>
<td>352 (251–502)</td>
<td>0.019</td>
</tr>
<tr>
<td>90 min</td>
<td>659 (399–727)</td>
<td>366 (208–445)</td>
<td>0.009</td>
</tr>
<tr>
<td>120 min</td>
<td>599 (359–700)</td>
<td>258 (144–298)</td>
<td>0.001</td>
</tr>
<tr>
<td>180 min</td>
<td>378 (173–491)</td>
<td>122 (66–201)</td>
<td>0.001</td>
</tr>
<tr>
<td>Serum lipids (mg/dl)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol</td>
<td>324 ± 49</td>
<td>242 ± 29</td>
<td>0.0001</td>
</tr>
<tr>
<td>Low-density lipoprotein</td>
<td>170 ± 26</td>
<td>132 ± 25</td>
<td>0.0002</td>
</tr>
<tr>
<td>High-density lipoprotein</td>
<td>39 ± 8</td>
<td>42 ± 6</td>
<td>–</td>
</tr>
<tr>
<td>Very-low-density lipoprotein</td>
<td>33 ± 9</td>
<td>17 ± 5</td>
<td>0.0001</td>
</tr>
<tr>
<td>Triacylglycerol</td>
<td>164 ± 49</td>
<td>84 ± 27</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

control subjects they displayed higher serum concentrations of total cholesterol, low-density lipoprotein cholesterol, very-low-density lipoprotein cholesterol and triacylglycerol. In contrast, the high-density lipoprotein cholesterol concentration did not differ between the two groups.

DISCUSSION

Very little is known about cardiac syndrome X. Thus the current findings may represent a step towards comprehension of the pathogenesis of this disease. Indeed, as well as confirming our recent report [4] with regard to the presence of post-load hyperinsulinaemia and increased red blood cell Na+/Li+ countertransport activity in patients with microvascular angina, our data demonstrate that the same patients also display a decreased activity of the ATPase-dependent Na+ pump and dyslipidaemia, in the presence of a normal V_max for Na+-K+-2Cl- cos- transport. These findings indicate that important metabolic alterations are present in cardiac syndrome X, and that a complex disturbance of ion compartmentalization into the extracellular/intracellular fluids may play a pivotal role in the pathogenesis of microvascular dysfunction. In fact, the two most important pumps regulating Na+ traffic across the cell membrane in the whole body are malfunctioning in the majority of patients with cardiac syndrome X.

Enhanced Na+/Li+ countertransport activity could decrease insulin sensitivity [19,20], and increase microvascular tone and responses to constrictor stimuli [5,6,8,21]. Thus abnormal Na+/Li+ countertransport activity might also be responsible for decreased ATPase-dependent Na+ pump activity. Although this hypothesis is intriguing, several reports disagree with such an interpretation. Indeed, in red blood cells and vascular smooth muscle cells, the ATPase-dependent Na+ pump is regulated by the transmembrane Na+ and K+ gradient [10,22], whereas it is not influenced by the activities of other Na+ transporters, including Na+/H+ antiport [23,24]. Extremely small changes in the transmembrane Na+ or K+ gradient (< 0.1 μmol) induce immediate changes in affinity for external Na+ and internal K+, and in the density of ATPase-dependent Na+ pumps [10]. In particular, each ATPase-dependent Na+ pump is the physiological counterpart of Na+/K+-ATPase, a membrane-bound enzyme which consumes one ATP molecule and simultaneously transports two K+ ions in and three Na+ ions out of the cell [10,22–24]. In vascular smooth muscle cells, this cycle of ATP hydrolysis is not dependent on intracellular pH or Ca2+ [10,22–24]. Ac-
and a decrease in the transmembrane Na\(^{+}\)/H\(^{+}\) antiporter, but not ATPase-dependent Na\(^{+}\) pump activity [25]. In keeping with this, patients with type II (non-insulin-dependent) diabetes [5] or essential hypertension [14,16,26] that show increased Na\(^{+}/Li\(^{+}\) countertransport usually manifest normal ATPase-dependent Na\(^{+}\) pump activity. In turn, patients with diabetes and hypertension who manifest a significantly decreased \(V_{\text{max}}\) for the ATPase-dependent Na\(^{+}\) pump present with normal Na\(^{+}/Li\(^{+}\) countertransport activity [5,26,27]. Thus an increased \(V_{\text{max}}\) for Na\(^{+}/Li\(^{+}\) countertransport does not influence the activity of the ATPase-dependent Na\(^{+}\) pump. In this regard, Na\(^{+}/Li\(^{+}\) countertransport hyperactivity could decrease ATPase-dependent Na\(^{+}\) pump activity by favouring insulin resistance and the consequent elevation of circulating insulin levels [5,28]. Indeed, insulin is known to affect Na\(^{+}\) metabolism both directly and indirectly, i.e. by acting on Na\(^{+}\)-modulating hormones and Na\(^{+}\) transporters [22,28]. In contrast with this hypothesis, controversial data have been reported on the action of insulin on the ATPase-dependent Na\(^{+}\) pump [29], suggesting that activity is either unaffected or stimulated by insulin.

Further, insulin itself induces Na\(^{+}/Li\(^{+}\) countertransport hyperactivity [30], suggesting that such hyperactivity might be the consequence of hyperinsulinaemia rather than a pathogenic factor in cardiac syndrome X. Thus we suggest that neither Na\(^{+}/Li\(^{+}\) countertransport hyperactivity nor the consequent hyperinsulinaemia affects the \(V_{\text{max}}\) of the ATPase-dependent Na\(^{+}\) pump.

An alternative explanation for our findings is that decreased ATPase-dependent Na\(^{+}\) pump activity itself could increase the contractility of vascular smooth muscle cells. Consistent with this, a reduced rate of Na\(^{+}\) extrusion through the cell membrane results in an elevation in the intracellular Ca\(^{2+}\) concentration via three distinct mechanisms: membrane depolarization, which in turn activates voltage-operated Ca\(^{2+}\) channels [31]; inhibition of intracellular Ca\(^{2+}\) exchange with extracellular Na\(^{+}\) due to an increased cytosolic Na\(^{+}\) concentration; and a decrease in the transmembrane Na\(^{+}\) gradient [32,33], which would augment disposal of free Ca\(^{2+}\) in the sarcoplasmic reticulum because of the high-affinity cytoplasmic ATPase-dependent Na\(^{+}\) pump [34]. In vascular smooth muscle cells, inhibition of the ATPase-dependent Na\(^{+}\) pump leads to increased vascular tone and responsiveness to vasoconstrictors, such as vasopressin, noradrenaline and angiotensin II [10,34,35]. In sympathetic nerve endings [32,34,36], the same inhibition increases presynaptic noradrenaline release and decreases its re-uptake [10,37,38]. As a consequence, a decrease in ATPase-dependent Na\(^{+}\) pump activity increases sympathetic tone [34,35,39], and might explain the massive regional defects in myocardial uptake of the catecholamine analogue \([\text{I}^{[13]}]\)metaiodobenzylguanidine that have recently been described in patients with cardiac syndrome X [40]. Thus decreased ATPase-dependent Na\(^{+}\) pump activity has the biological potential to sensitize small coronary arteries to vasoconstrictor stimuli and to cause coronary pre-arteriolar dysfunction, thereby leading to coronary microvessel spasms. Nevertheless, a defect of the ATPase-dependent Na\(^{+}\) pump cannot explain simultaneous Na\(^{+}/Li\(^{+}\) hyperactivity, post-load hyperinsulinaemia and dyslipidaemia, which have been observed in most patients with syndrome X. On the other hand, the present study is not the first to describe abnormal insulin responses to glucose ingestion in patients with cardiac syndrome X [4,41]. Therefore, while it could be argued that our data on dyslipidaemia require confirmation in further studies, the linkage between hyperinsulinaemia and cardiac syndrome X is already a well-established one. As a consequence, we think it is reasonable to hypothesize that a multisystem abnormality in Na\(^{+}\) traffic across the cell membrane is present in patients with syndrome X. Such a malfunction in Na\(^{+}\) traffic could be responsible for a multifaceted syndrome, presenting with abnormalities of both glucose and lipid metabolism, as well as cardiovascular symptoms. Whether or not these alterations in Na\(^{+}\) transmembrane carriers share a common aetiology remains to be determined.

Study limitations should be taken into account when interpreting our results. Effects of cardioactive drugs and/or other medications are not relevant, as patients discontinued therapy 10 days before entering the study. Further, the study was performed using red blood cells, i.e. a cell type in which Na\(^{+}\) transporters work in the same way as in nucleated cells, such as renal tubular, endothelial and vascular smooth muscle cells [5,10,20–22], and after repeated passages in washing solutions, i.e. after careful elimination of all endogenous and exogenous circulating substances. However, membrane-bound Na\(^{+}/K\(^{+}\)-ATPase is known to exist in various isoforms, depending on the amino acid sequences of the \(\alpha\) and/or \(\beta\) subunits [42]. Since each isoform has a specific affinity for external Na\(^{+}\), the reduced \(V_{\text{max}}\) of the ATPase-dependent Na\(^{+}\) pump observed in patients with cardiac syndrome X could reflect an abnormal prevalence of the low-affinity isoform in their red blood cells. Thus molecular studies are required to elucidate whether the reduced ATPase-dependent Na\(^{+}\) pump activity is simply due to a lower number of Na\(^{+}\) pumps or reflects more complex abnormalities in ATPase isoform distribution. Finally, the Na\(^{+}/H\(^{+}\) antiporter also exists in different isoforms, whereas punctiform mutations of Na\(^{+}/K\(^{+}\)-ATPase have been recently reported to markedly influence pump affinity for Na\(^{+}\) [43,44]. As a consequence, DNA cloning is necessary in order to evaluate the amino acid sequences of both the Na\(^{+}/H\(^{+}\) antiporter and the ATPase-dependent Na\(^{+}\) pump in patients with cardiac syndrome X.
In conclusion, the present study demonstrates decreased ATPase-dependent Na⁺ pump activity in patients with cardiac syndrome X, in addition to increased Na⁺/Li⁺ countertransport activity, post-load hyperinsulinemia and hyperdyslipidaemia. We speculate that this abnormality contributes to increases in microvascular tone and in responses to constrictor stimuli. Further, we hypothesize that simultaneous abnormalities of Na⁺ traffic across the cell membrane are present in cardiac syndrome X and are responsible for (or at least contribute to) the progressive onset of a complex syndrome, presenting with abnormal glucose and lipid metabolism as well as cardiovascular symptoms. The possibility also exists that Na⁺/Li⁺ countertransport hyperactivity is simply a consequence of such metabolic abnormalities. In any case, our findings shed new light on the pathogenesis of cardiac syndrome X. Molecular studies are necessary in order to elucidate whether abnormalities in transmembrane Na⁺ traffic are simply a consequence of a reduced number of Na⁺ exchangers, or are due to an abnormal prevalence of low-affinity isoforms and/or punctiform mutations in Na⁺ transporter subunits.

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Received 11 February 1999/28 April 1999; accepted 28 May 1999