Reduced Sodium Pump $\alpha_1$, $\alpha_3$, and $\beta_1$-Isoform Protein Levels and Na$^+$,K$^+$-ATPase Activity but Unchanged Na$^+$-Ca$^{2+}$ Exchanger Protein Levels in Human Heart Failure

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Background—Cardiac glycosides initiate an increase in force of contraction by inhibiting the sarcolemmal sodium pump (Na$^+$,K$^+$-ATPase), thereby decreasing Ca$^{2+}$ extrusion by the Na$^+$-Ca$^{2+}$ exchanger, which increases the cellular content of Ca$^{2+}$. In patients with heart failure the sensitivity toward cardiac glycosides is enhanced.

Methods and Results—Because the inotropic effect of cardiac glycosides may be a function of the sodium pump and Na$^+$-Ca$^{2+}$ exchanger (NCE) expression levels, the present study aimed to investigate protein expression of both transporters (immunoblot with specific antibodies against the sodium pump catalytic $\alpha_1$-, $\alpha_2$-, $\alpha_3$-, and glycoprotein $\beta_2$-isoforms and against NCE) in left ventricle from failing (heart transplantations, New York Heart Association class IV, n=21) compared with nonfailing (donor hearts, NF, n=22) human myocardium. The density of $^3$H-ouabain–binding sites ($B_{max}$) and the Na$^+$,K$^+$-ATPase activity were also measured. In NYHA class IV, protein levels of Na$^+$,K$^+$-ATPase $\alpha_1$ (0.62±0.06 of control), $\alpha_2$ (0.70±0.09), and $\beta_1$ (0.61±0.04) but not $\alpha_3$-isoforms were significantly reduced (P<0.01), whereas levels of NCE (0.92±0.13 of control) and calsequestrin (0.98±0.06) remained unchanged. Both Na$^+$,K$^+$-ATPase activity (NF: 1.9±0.29; NYHA class IV: 1.1±0.17 μmol ATP/min per milligram of protein) and the $^3$H-ouabain binding sites (B$_{max}$ NF: 15.9±1.9 pmol/mg protein; NYHA class IV: 9.7±1.5) were reduced in NYHA class IV and correlated significantly to each other ($r^2$=0.73; P<0.0001), as did $\beta_1$-subunit expression. In left ventricular papillary muscle strips from NYHA class IV compared with nonfailing tissue the Na$^+$-channel modulator BDF 9198 exerted an increase in force of contraction with unchanged effectiveness but enhanced potency.

Conclusions—The enhanced sensitivity of failing human myocardium toward cardiac glycosides may be, at least in part, attributed to a reduced protein expression and activity of the sarcolemmal Na$^+$,K$^+$-ATPase without a change in Na$^+$-Ca$^{2+}$ exchanger protein expression. (Circulation. 1999;99:2105-2112.)

Key Words: sodium ▪ heart failure ▪ myocardium ▪ receptors

Cardiac glycosides have been used in the treatment of patients with heart failure for about 100 years. Most recently, the effects of digoxin on mortality and morbidity in patients with heart failure have been the subject of a large, randomized, double-blind clinical trial. Most of the study patients (67%) had New York Heart Association class I or II heart failure, and digoxin reduced the rate of overall hospitalization as well as the rate of worsening heart failure. In vitro cardiac glycosides increase force of contraction in human failing and nonfailing myocardium to the same degree. Other compounds such as the Na$^+$-channel activator BDF 9148,5,6 which, like cardiac glycosides, use an increase in intracellular Na$^+$ concentration in their mode of action,6–8 have also been reported to be equally effective in failing and nonfailing human myocardium. Although effectiveness of cardiac glycosides and Na$^+$-channel activators are unchanged, both have been reported to increase force of contraction in human failing myocardium with higher potency than in human nonfailing myocardium.4,9

Cardiac glycosides are thought to act by inhibiting the membrane-bound sodium pump, leading to an elevation of the intracellular Na$^+$ concentration.7,10 An increase in intracellular Na$^+$ may produce a positive inotropic effect by either shifting the reversal potential of the Na$^+$-Ca$^{2+}$ exchanger to more negative potentials, reducing the outward transport of Ca$^{2+}$ at resting membrane potentials or by increasing Ca$^{2+}$ loading during depolarization when the Na$^+$-Ca$^{2+}$ exchanger is operating in a Na$^+$-efflux/Ca$^{2+}$ influx mode. Consequently, more Ca$^{2+}$ will be stored in the sarcoplasmic reticulum to be released during subsequent contractions.

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The Na\(^+\),K\(^+\)-ATPase is a heterodimer composed of an \(\alpha\)-catalytic and \(\beta\)-glycoprotein subunit. Cardiac glycosides bind to the extracellular face of the \(\alpha\)-subunit with isoform-dependent affinity and inhibit both transport and enzymatic activity of the pump. In the human heart, \(\alpha_1\), \(\alpha_2\), and \(\alpha_3\) catalytic isoforms have been detected in atrial and ventricular tissue at both the mRNA and protein levels.\(^{4,11-13}\) \(\beta_1\) but not \(\beta_2\) glycoprotein subunit was detected, indicating that the human cardiac glycoside receptors are \(\alpha_1 \beta_1\), \(\alpha_2 \beta_1\), and \(\alpha_3 \beta_1\).\(^{10}\) The expression of these subunits and the Na\(^+\)-Ca\(^2+\) exchanger will influence the inotropic response to cardiac glycosides. The increased sensitivity toward cardiac glycosides during myocardial failure could be due to decreased sodium pump activity, a change in the isoform expression pattern that favors more cardiac glycoside sensitive isoforms, or an increase in NCE that could potentially increase outward Ca\(^2+\) loading during depolarization but would also potentially increase outward Ca\(^2+\) transport at resting potentials.

The present study aimed to determine whether or not changes in the Na\(^+\),K\(^+\)-ATPase isoform expression and/or the Na\(^+\)-Ca\(^2+\) exchanger expression influence the inotropic response of compounds increasing intracellular Na\(^+\). Previous reports addressing this aim have focussed on protein expression influences or were limited to a small number of nonfailing hearts. For example, in the only previous report comparing functional and protein-expression properties of the sodium pump, \(\alpha_1\) Na\(^+\)-pump activity, and Na\(^+\)-Ca\(^2+\) exchanger expression influence the inotropic response to the novel channel modulator BDF 9198 was measured throughout in left ventricular myocardium.

**Methods**

**Human Myocardium**

Myocardium from terminally failing human hearts was obtained from patients after cardiectomy during cardiac transplantation (n=21, 14 male, 7 female, age 42.4±2.3 years, range 18 to 58). The preoperative diagnosis was dilated cardiomyopathy in all patients. All patients had been classified as NYHA class IV. The preoperative diagnosis was dilated cardiomyopathy in all patients. All patients had been classified as NYHA class IV. None of the patients were receiving cardiac glycoside therapy. None of the patients had received Ca\(^2+\) channel antagonists nor agonists within 7 days of surgery or \(\beta\)-adrenoceptor agonists 48 hours before operation. None of the patients were receiving cardiac glycoside therapy. Drugs used for general anesthesia were flunitrazepam, fentanyl, and pancuronium bromide with isoflurane. Heart surgery was performed on cardiopulmonary bypass with cardioplegic arrest during hypothermia. Patients gave written informed consent before operation. Nonfailing human myocardium was obtained from donors with brain death caused by traumatic injury (n=22, 12 male, 10 female, age 41.5±2.8 years, range 15 to 58). The nonfailing hearts could not be used for transplantation for technical reasons. The cardioplegic solution used was a modified Bretschneider solution containing (in mmol/L): NaCl 15; KCl 10; MgCl\(_2\) 4; histidine HCl 180; tryptophane 2; mannitol 30; potassium dihydrogen oxoglutarate 1.

**Immunoblot Analysis**

Immunoblot analysis was conducted on samples of unfractionated homogenate to avoid the issue of different recoveries of membranes from failing and nonfailing heart as described previously.\(^{13}\) Protein concentration was determined by the method of Lowry et al.\(^{16}\) Membrane fractions from human brain and kidney and from rat brain and sarcolemmal membranes from dog heart and human heart were prepared as previously described\(^ {13}\) and used to verify isoform specificity of antibodies. Samples were resolved by SDS-PAGE, electrophoretically blotted onto Immobilon P membrane, 25 to 50 μg protein for \(\alpha_1\), \(\beta_1\), and \(\beta_2\) to 50 to 100 μg for NCE detection. Blots were probed with one of the following antibodies: 464.6, against \(\alpha_1\) (1:100) from M. Kashgarian (Yale University);\(^{17}\) Mcb2, against \(\alpha_2\) (1:100) from K. Sweadner (Harvard University);\(^{18}\) anti-TED, against \(\alpha_1\) (1:200) from T. Pressley (Texas Tech);\(^{19}\) spET \(\beta_1\), (1:2000) against \(\beta_1\) from P. Martin-Vassallo (Tenerife, Spain);\(^{20}\) and two different anti–Na\(^+\)-Ca\(^2+\) exchanger antibodies: \(\pi\) (1:100) from K. Czajkowski (Medical College of Wisconsin, Milwaukee);\(^{21},\) \(\pi\) (1:5000) from K. Philipson (University of California Los Angeles).\(^ {21,22}\) Each blot was probed with antibody only once. All blots were prepared and processed as described previously\(^ {23}\) using \(^{\text{125}}\text{I}\)-Protein A and autoradiography for quantitation of the antibody-antigen complexes.

For quantitation of immunoblot, the linearity of the signal as a function of the amount of antigen loaded was ensured as previously established.\(^ {13}\) Immunoblotts were quantified with Imaging Densitometer GS670 and Molecular Analyst software (Bio-Rad Laboratories).

**Membrane Preparation**

Myocardial tissue (5 to 10 g wet wt) was chilled in 30 mL ice-cold homogenization buffer (50 mmol/L Tris-HCl, 10 mmol/L EDTA, 1 mmol/L dithiothreitol, pH 8.0), trimmed of connective tissue, minced with scissors, and homogenized with a motor-driven glass-Teflon homogenizer for 1 minute then by hand for 1 minute with a glass-glass potter and spun at 480g. The supernatant was filtered through 2 layers of gauze (pellet discarded) and diluted with an equal volume of ice-cold 1 mol/L KCl, stored on ice for 15 minutes, then centrifuged at 100 000 g for 30 minutes. The resultant pellet was resuspended in homogenization buffer and centrifuged again at 100 000 g for 30 minutes. The final pellet was resuspended in 3 to 5 mL of storage buffer containing 50 mmol/L Tris-HCl and 10 mmol/L MgCl\(_2\) (pH 7.4) at 5 to 13 mg protein/mL. 5' Nucleotidase activity\(^ {24}\) was 15.7±2.4 μmol/min per milligram of protein in the nonfailing and 17.9±2.9 μmol/min per milligram of protein in the failing human myocardium.

**Ouabain Binding**

Membrane protein (300 μg) was incubated in 3 mmol/L MgCl\(_2\), 3 mmol/L phosphate/imidazole, 50 mmol/L imidazole/HCl, pH 7.4, and various concentrations of unlabeled g-strophantin (ouabain, 0 to 2 μmol/L) for 4 hours at 37°C. H\(^+\)-ouabain concentration was kept constant at 2.5 μmol/L. Ouabain binding sites were determined according to the method of Erdmann and Schoner.\(^ {25}\)

**Na\(^+\),K\(^+\)-ATPase Activity**

Myocardial membranes were preincubated on ice for 1 hour in either storage buffer (above) with or without 5 mmol/L ouabain. Inhibition was maximal after 1 hour of incubation. The assay was started by adding 50 to 100 μg of the preincubated membranes to 750 μL of reaction solution containing (final concentration) in mmol/L: imidazole/HCl 100 (pH 7.25), NaCl 150, KCl 10, NH\(_4\)Cl 100, MgCl\(_2\) 5, ATP 5, NADH 0.5, phosphoenolpyruvate 2.5, LDH/PK-enzyme mixture (5000 U/100 mL). The decrease in absorbance was registered with a Beckmann DU 650 photometer.
and the kinetic values between 2 and 8 minutes were obtained for further evaluation. In this time range the reaction was linear. Activity was constant between 10 to 250 μg of protein. Na⁺,K⁺-ATPase activity was determined by subtracting activity with ouabain from maximal activity.26

Electrically Driven Human Papillary Muscle Strips
Each muscle strip was used for 1 concentration-response curve (BDF 9198, 0.001 to 30 μmol/L) only. Protocol has been described in detail elsewhere.27

Materials

'⁹⁻H-ouabain was obtained from Amersham Life Sciences, Amersham, England (specific activity: 43 Ci/mmol). ¹²⁵⁻I-protein A was from ICN (Irvine, Calif). Rabbit anti-mouse secondary antibody was from Calbiochem-Novabiochem (La Jolla, Calif). Ouabain was obtained from Herbert Pharma, Wiesbaden, Germany and BDF 9198 (4-(3-(1-Diphenylmethyl-azetidine-3-oxy)-2-hydroxy-propylamin)-1-H-indol-2-carbonitri) was kindly provided by Prof Mest, Beiersdorf-Lilly GmbH, Hamburg, Germany. Phosphoenolpyruvate and LDH/PK enzyme and proteolytic enzyme inhibitors were purchased from Sigma Chemicals (UK or USA). All other chemicals were either of analytical grade or the best grade commercially available.

Statistical Analysis
Because immunoblot signals yield data in arbitrary units, signals from both nonfailing and failing hearts were normalized to the mean signal of the nonfailing samples on a given blot, defined as 1.0. The normalized values of these measurements were used for analysis of combined data, data are expressed as mean±SEM, and statistical significance was determined with Student’s t test for nonpaired observations. Bₘₐₓ and Kᵣ values for ouabain binding were evaluated according to the method of Scatchard et al.28 Statistical significance was determined with Student’s t test; a value of P<0.05 was considered significant. Prism Graph Pad was used to determine correlation between the Bₘₐₓ, the sodium pump activity, and the β₁-subunit abundance.

Results
Detection of Sodium Pump Isoforms and Na⁺Ca²⁺ Exchanger in Human Failing and Nonfailing Myocardium

The isoform specificity of the anti-α antibodies for human heart α-subunits have been previously demonstrated.13 As in the brain, all 3 isoforms (α₁, α₂, and α₃) are detected in both human failing and nonfailing myocardium. We previously demonstrated that β₁ is detectable in human heart but that β₂ was detectable only after deglycosylation and only at negligible levels.13 The same finding was true in both failing and nonfailing heart samples (not shown). Thus all catalytic α-isofoms most likely form heterodimers with β₁ in both nonfailing and failing myocardium. The cardiac Na⁺-Ca²⁺exchanger was detected with both the polyclonal antibody π and the monoclonal antibody C2C12. Both the 120 and 70 kDa bands of the human cardiac Na⁺-Ca²⁺exchanger as previously reported in both canine and human myocardium.13,21,22 The 40 kDa band detected by Studer et al19 was also detected with the π but not with the C2C12 antibody in crude homogenate of human heart. This band was not included in the analysis because we have previously shown that it does not purify with the Na⁺/Ca²⁺exchanger.13

Expression of Sodium Pump Isoforms and Na⁺Ca²⁺ Exchanger in Human Failing and Nonfailing Myocardium

To test the hypothesis that the increased potency of cardiac glycosides in human heart failure is a function of altered expression of sodium pump isoforms and/or NCE, the pattern of expression of the isoforms in human failing and nonfailing myocardium was assayed in homogenate protein from the left ventricular myocardium (n=21 for failing, n=22 for nonfailing myocardium). The linearity of the autoradiographic signals for detection of sodium pump isoforms relative to the amount of protein loaded has been established.13 On the basis of these findings, 25-μg and 50-μg samples were each assayed for quantification of sodium pump isoforms and 50-μg and 100-μg samples were assayed for quantification of the Na⁺-Ca²⁺ exchanger. Figure 1 provides typical immunoblots for each of the antibodies assayed, and Figure 2 summarizes the mean values and variation in failing relative to nonfailing tissue. In failing myocardium both α₁ (0.62±0.06), α₂ (0.70±0.09), and β₁ (0.61±0.04) isoform expression were significantly reduced compared with nonfailing left ventricular myocardium (P<0.01). The expression of α₃ was not significantly different (0.88±0.09) in failing heart. Also unchanged were the membrane marker 5’nucleotidase activity and calsequestrin (0.98±0.06 in failing), as has been reported previously.29 In the same myocardial samples used to assay sodium pump pools, there was no detectable change in abundance of the Na⁺-Ca²⁺ exchange protein assayed as the sum of the densities of the 120 kDa and 70 kDa bands in each sample (0.92±0.13 in failing). These findings provide evidence that α₁, α₂, and β₁ isoform expression are reduced in failing compared with nonfailing human left ventricular myocardium, whereas α₃ expression remains unchanged and β₂ remains at negligible levels. Because of variable affinity for isoform specific epitopes, the antibody binding signals cannot be used to compare levels of expression of one isoform to another.
Human Myocardium
Left Ventricle
protein expression

Figure 2. Expression of sodium pump subunits, Na\(^+\)-Ca\(^{2+}\) exchanger, and calsequestrin in human heart left ventricles: nonfailing vs dilated cardiomyopathy (NYHA class IV). Autoradiograms from nonfailing (n=22) and dilated cardiomyopathy (n=21) for Na, K-ATPase subunits and NCE, and for CSQ (n=12 for nonfailing and NYHA class IV) were quantified by scanning densitometry. The data, expressed as mean±SEM, were collected in arbitrary units, processed as described in Methods, and normalized to nonfailing left ventricular protein abundance defined as 1 within each blot. \(P<0.05\) was considered significant.
Ouabain Binding and Sodium Pump Activity in Human Failing and Nonfailing Myocardium

To test the hypothesis that reduced sodium pump isoform expression leads to decreased expression of functional sodium pumps, ouabain binding \(^{3}H\)-ouabain: specific activity: 43 Ci/mmol) and sodium pump activity were measured in 10 nonfailing and 12 terminally failing myocardial samples overlapping with the set of samples assayed by immunoblot. Ouabain binding sites, a measure of the number of functioning sodium pumps, were significantly reduced in failing compared with nonfailing human heart samples whether binding was calculated relative to protein or relative to 5’nucleotidase in the sample (Figure 3 and Table). The \(K_D\) value for ouabain was not detectably different between nonfailing and failing samples, indicating that the affinity of ouabain to its receptor(s) was not significantly changed by the differential decrease in \(\alpha_1\) and \(\alpha_2\) relative to \(\alpha_1\) during heart failure. Sodium pump activity was significantly reduced in human failing myocardium \((P<0.025\), Table). Similarly, this holds true when measurements were normalized to 5’nucleotidase activity. The 40% decrease in Na, K-ATPase activity is indistinguishable from the 40% decrease in ouabain binding \((r^2=0.73; P<0.0001)\) and indistinguishable from the 40% decrease in \(\beta_i\) pool size \((r^2=0.36; P<0.005)\), a finding that is predicted if one assumes that all the cardiac sodium pump \(\alpha\)-catalytic subunits form heterodimers with \(\beta_i\). These relations hold true when measurements in only failing or only nonfailing tissue were correlated. However, no significant correlation emerged between individual \(\alpha\)-subunits and sodium pump activity or ouabain binding further supporting the hypothesis that \(\beta_i\) expression can serve as marker for total cardiac sodium pump expression.

**Figure 3.** Concentration-dependent binding of \(^{3}H\)-ouabain in human failing and nonfailing myocardial preparations. Nonspecific binding was 0.05 pmol/mg protein in nonfailing and 0.03 pmol/mg protein in failing myocardium. Inset shows the ratio of the radioligand bound relative to free plotted as a function of the radioligand bound (according to Scatchard\(^{28}\)). Whereas \(B_{max}\) was reduced in human failing myocardium, the \(K_D\) remained unchanged.

**Table 1.** \(^{3}H\)-Ouabain Binding and Activity of Na\(^+\),K\(^+\)-ATPase in Human Failing and Nonfailing Myocardium

<table>
<thead>
<tr>
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<th>NFLV ((n=10))</th>
<th>DCMLV ((n=12))</th>
<th>(P)</th>
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<tbody>
<tr>
<td><strong>(^{3}H)-ouabain binding</strong></td>
<td></td>
<td></td>
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<tr>
<td>(B_{max})/protein, pmol/mg</td>
<td>15.9±1.9</td>
<td>9.7±1.5(^*)</td>
<td>0.02</td>
</tr>
<tr>
<td>(B_{max})/nucleotidase, pmol (\mu)mol(^{-1}) (\cdot) min(^{-1})</td>
<td>1.2±0.2</td>
<td>0.5±0.15(^*)</td>
<td>0.01</td>
</tr>
<tr>
<td>(K_D), pmol/mg</td>
<td>9.9±0.7</td>
<td>11.7±1.9</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Na(^+),K(^+)-ATPase activity, (\mu)mol (\cdot) min(^{-1}) (\cdot) mg protein(^{-1})</strong></td>
<td></td>
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<tr>
<td>Activity/protein</td>
<td>1.9±0.29</td>
<td>1.1±0.17(^*)</td>
<td>0.02</td>
</tr>
<tr>
<td>Activity/nucleotidase</td>
<td>0.14±0.02</td>
<td>0.07±0.02(^*)</td>
<td>0.02</td>
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</tbody>
</table>

\(NFLV\) indicates nonfailing left ventricle; DCMLV, dilated cardiomyopathy, left ventricle; \(B_{max}\), maximal binding; nucleotidase, 5’ nucleotide activity; activity, maximal Na\(^+\),K\(^+\)-ATPase activity. Values are mean±SEM.

\(\ast P<0.05\) vs nonfailing.

**Figure 4.** Concentration-response curves of the Na\(^+\) channel modulator BDF 9198 in left ventricular papillary muscle strips from human nonfailing and failing hearts. Basal force of contraction was 1.2±0.2 mN in the nonfailing \((n=5)\) and 1.6±0.2 mN in the failing myocardium \((n=10)\). Maximal increase in force of contraction was 5.2±1.0 mN and 4.7±0.3 mN, respectively.

**Inotropic Effect of the Na\(^+\) Channel Modulator in Human Failing and Nonfailing Myocardium**

To test the hypothesis that reduced sodium pump activity and protein expression in failing myocardium has a functional impact on cardiac contractility, the inotropic effect of the novel Na\(^+\) channel modulator BDF 9198 was studied. BDF 9198 increases intracellular sodium and alters the reversal potential of the Na\(^+\)-Ca\(^{2+}\) exchanger, so that Ca\(^{2+}\) efflux is decreased and Ca\(^{2+}\) influx is increased. As Na\(^+\)-Ca\(^{2+}\) exchanger protein levels are unchanged in failing ventricle, the inotropic sensitivity to BDF 9198 will be a function of the number and activity of both Na\(^+\) channels and sodium pumps. The maximal positive inotropic effect of the Na\(^+\) channel modulator BDF 9198 was unchanged in terminally failing (4.7±0.3 mN) versus nonfailing (5.2±1.0 mN) myocardium. However, BDF 9198 was more potent in increasing force of contraction in failing (\(EC_{50}\) 0.03 \(\mu\)mol/L) versus nonfailing tissue (\(EC_{50}\) 0.26 \(\mu\)mol/L) (Figure 4).
Discussion

Sodium Pump Expression and Activity in Failing and Nonfailing Human Myocardium

This study demonstrates conclusively that there is reduced protein expression and activity of 2 isoforms of the sarcolemmal Na⁺,K⁺-ATPase without a change in Na⁺-Ca²⁺ exchanger protein expression, changes that are likely to enhance the sensitivity of failing human myocardium toward cardiac glycosides and Na⁺ channel modulators.

Because the sodium pump subunits α1, α1, and β₁ were significantly reduced in terminally failing left ventricular myocardium and because β₂ is detectable at only negligible levels and α₁ and calsequestrin levels were unchanged, we conclude there is an isoform specific decrease in expression of α₁β₁ and α₁β₂ heterodimers in human heart failure. Consistent with this finding, the density of ouabain binding sites was also reduced, whereas the affinity of the cardiac glycoside for its receptors was unchanged. In a previous study from this laboratory a change in the density of ouabain binding sites was not detected. However, only 3 nonfailing hearts were available for that study, significantly less than the 10 nonfailing and 12 failing hearts that have been investigated in the present study. In support of the findings of the current study, a study on human myocardial biopsies obtained during left heart catheterization demonstrated Na⁺ pump expression, measured by [H]-ouabain binding, was reduced in 19 patients with impaired left ventricular function compared with 5 patients with normal cardiac function. However, this study did not address the question of isoform composition. Moreover, Shamraj et al measured enhanced sensitivity of failing human myocardium to cardiac glycosides together with a 30% to 40% decrease in the number of ouabain binding sites in 13 failing heart samples, but compared these with only 2 nonfailing human heart samples studied functionally. Thus the present study provides more conclusive evidence that the enhanced sensitivity of failing human myocardium toward cardiac glycosides may be attributed to a reduced protein expression and activity of the sarcolemmal Na⁺,K⁺-ATPase. Consistently, cardiac glycoside binding sites are reduced in terminally failing myocardium due to dilated cardiomyopathy compared with nonfailing tissue as are the number of β₁-adrenoceptors.

The reduced number of ouabain binding sites observed in the present study in the failing myocardium correlated significantly with lower β₁-expression, not surprising given the assumption that all cardiac sodium pumps contain the β₁-subunit in both nonfailing and failing human heart. The fact that the Kᵢ for ouabain does not significantly change with heart failure, coupled to the isoform-specific decreases in expression, supports the hypothesis that the increase in cardiac glycoside sensitivity is secondary to a decrease in the total number of pumps rather than a shift in isoform ratios.

Na⁺-Ca²⁺ Exchanger in Failing and Nonfailing Human Myocardium

Na⁺-Ca²⁺ exchanger expression in human heart failure was investigated in the same large panel of samples (n=21 from failing and n=22 from nonfailing) used to characterize the isoform specific decrease in sodium pump subunit expression. The analysis did not provide evidence for altered immunoreactive pools of exchanger protein in the failing left ventricle. The protein abundance was unchanged whether immunoreactivity was related to total protein, calsequestrin levels, or to 5'-nucleotidase activity and unchanged whether either antibody was used for the detection. This finding contrasts that of Studer et al, who reported a 2-fold increase in immunoreactive Na⁺-Ca²⁺ exchanger in failing compared with nonfailing cardiac tissue using one of the same antisera used in this study. A discussion of the detection of NCE in human heart is warranted because of this discrepancy. In samples from any region of the human heart, we detect a strong signal at 120 kDa and a variable signal at 70 kDa with either of the 2 antibodies against the Na⁺-Ca²⁺ exchanger, the polyclonal antiserum called π, or the monoclonal called C2C12. Philipson et al have previously reported that the 70 kDa is derived from the 120 kDa exchanger and that both exhibit Na⁺-Ca²⁺ exchanger activity when reconstituted. However, in the study of human heart by Studer et al using the same π antiserum, bands were detected at 120 and 40 kDa, and the investigators concluded that the 40 kDa band was analogous to the 70 kDa band seen in other species. We also saw a band at 40 kDa with this antiserum in both heart homogenate and membranes prepared from heart homogenate but concluded that the 40 kDa band was not a fragment of the exchanger because: (1) purification of human sarcolemmal membranes resulted in the enrichment of the 120 and 70 kDa bands and the loss of the 40 kDa band, (2) a monoclonal antibody (C2C12) to the exchanger detected 120, 70 but not 40 kDa bands, and (3) the intensity of the 40 kDa band in the starting human heart homogenate was very strong-equivalent to the signal obtained in purified dog sarcolemma. We conclude that this 40 kDa protein may have a similar antibody binding epitope to a region of the Na⁺-Ca²⁺ exchanger but that it is unlikely to be a component or a proteolytic fragment of the exchanger. At the RNA level, Flesch et al reported an increase in NCE mRNA in ischemic and diluted cardiomyopathy (8 nonfailing hearts were examined), whereas Komuro et al reported levels unchanged. The results of the present study suggest that increasing sensitivity to cardiac glycosides is not due to altered NCE expression but to decreased numbers of sodium pumps with worsening of heart failure.

Increased Sensitivity of Failing Human Myocardium to the Na⁺ Channel Modulator BDF 9198

The enhanced sensitivity of the failing heart toward cardiac glycosides and enhanced potency of BDF 9198 (consistent with previously report on BDF 9148) may result from (a) different protein and/or isoform expression of the sodium pump (present study), from (b) changes in the expression of Na⁺ channels, from (c) altered functional properties of the Na⁺ channel, or from (d) the intracellular Na⁺ concentration, or from (e) an increased Ca²⁺ sensitivity of the contractile filaments in diseased versus nonfailing myocardium, or from a combination of these possibilities. While the overall affinity of the cardiac tissue to ouabain did not change...
with failure, the precise affinities of the individual isoforms have not been determined. When these affinities are known, the impact of a shift in isoform ratios on sensitivity can be assessed. The physiological differences between isoforms are not completely understood, but $\alpha_1$ appears to have a lower affinity for intracellular Na$^+$ than $\alpha_2$ isoform.\(^{35}\)

Although ouabain binding, Na$^+$-K$^+$-ATPase activity, and Na$^+$-K$^+$-ATPase $\beta$-subunit pools are all depressed in the failed heart samples, we must consider the possibility that the functional changes in activity and ouabain binding may not be solely due to a change in pool size, because pool size of immunoreactive subunits was determined in total homogenate and ouabain binding and ATPase were measured in membrane fractions. Thus this study did not evaluate whether there were changes in the proportion of the pumps in the membrane or changes in activity or ouabain binding per pump unit in the failed heart samples. However, assays of Na$^+$,K$^+$-ATPase activity in homogenates and membranes from the same samples (however from a different set of hearts) support the notion that the decrease in abundance contributes to the decrease in activity. In homogenates as well as membrane preparations the activity of the Na$^+$,K$^+$-ATPase was significantly decreased in failing myocardial tissue when compared with human nonfailing control hearts (homogenates 43% and preps 48% of control).

We previously observed lower sodium pump expression and a slight decrease in Na$^+$–Ca$^{2+}$ exchanger protein pool size in human right atria accompanied by enhanced sensitivity toward BDF 9148,\(^{13}\) analogous to the association between lower sodium pump number and increased inotropic sensitivity in the failing left ventricle measured in this study, supporting the postulate that sensitivity to inotropic stimulation by sodium modulating agents increases as total pump number decreases. In failing human heart, the higher sensitivity to interventions that increase intracellular Na$^+$ may be due to a higher baseline cell sodium, secondary to depressed sodium pump expression. In fact, it is possible that the decrease in sodium pump expression is a compensatory mechanism to maintain cardiac contractility in the face of failure. Whether compensatory or pathological, this study establishes there is significant isoform specific depression of sodium pump expression in heart failure associated with increased sensitivity to inotropic agents.

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References


Reduced Sodium Pump \( \alpha_1 \), \( \alpha_3 \), and \( \beta_1 \)-Isoform Protein Levels and Na\(^+\),K\(^+\)-ATPase Activity but Unchanged Na\(^+\)-Ca\(^{2+}\) Exchanger Protein Levels in Human Heart Failure

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