Relationship between altered sympathetic innervation, oxidative metabolism and contractile function in the cardiomyopathic human heart

A non-invasive study using positron emission tomography

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Aims To identify functional and metabolic correlates of impaired presynaptic sympathetic innervation in the cardiomyopathic human heart using non-invasive correlative imaging.

Methods and Results In 10 patients with idiopathic dilated cardiomyopathy, presynaptic catecholamine uptake sites were quantified by positron emission tomography with C-11 hydroxyephedrine. Oxidative metabolism was measured using C-11 acetate. Global and regional function was assessed by tomographic radionuclide angiography. Left ventricular ejection fraction in patients was 19% ± 10%. Myocardial hydroxyephedrine retention was abnormally low in 58% ± 38% of the left ventricles. Globally and regionally, hydroxyephedrine retention was significantly correlated with ventricular function (r=0.67, P<0.01) and peripheral vascular resistance as a measure of afterload (r=−0.61, P=0.06), but did not correlate with hydroxyephedrine retention (r=0.08 for global, r=0.04 for regional parameters).

Conclusion Alterations of presynaptic sympathetic innervation in dilated cardiomyopathy are associated with impaired contractile function, suggesting a common pathogenic pathway. Overall oxidative metabolism, however, was not directly correlated with these findings. Normal regulatory mechanisms for oxidative metabolism were operational.

Key Words: Positron emission tomography, autonomic nervous system, oxidative metabolism, heart failure, left ventricular function.

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Introduction

In various animal and human studies, alterations of cardiac sympathetic innervation have been described as a feature of progressive heart failure[1–3]. Density and function of the uptake-1 carrier protein in nerve terminals, which are responsible for elimination of norepinephrine from the synaptic cleft, have been shown to be reduced[4–6]. This impairment of presynaptic sympathetic reuptake is a probable reason for increased local exposure of the failing heart to norepinephrine. In experimental models, excess catecholamine stimulation has been shown to be associated with alterations in adrenergic signal transduction, resulting in reduced diastolic and systolic contractile function[7,8]. Furthermore, direct toxicity of norepinephrine to myocytes has been demonstrated[9]. Other studies have suggested that catecholamines may also be associated with disturbed oxygen consumption and energy metabolism[10], and thereby further reduce performance of the failing heart.
In humans, altered presynaptic sympathetic innervation has been described non-invasively using radio-labelled norepinephrine analogues in combination with nuclear imaging techniques\(^{[4,6,11]}\). The degree of abnormality has been shown to be closely associated with patient outcome\(^{[12,13]}\). Little, however, is known about direct functional correlates at the myocardial level.

Thus, it was the aim of the present study to determine the global and regional relationship between sympathetic innervation, contractile function and the oxidative metabolism of the cardiomyopathic human heart, using a combination of non-invasive imaging techniques. Presynaptic catecholamine uptake sites were imaged quantitatively by positron emission tomography, with the radiolabelled norepinephrine analogue C-11 hydroxyephedrine. In addition, myocardial perfusion and oxidative metabolism were measured in the same positron emission tomography session using C-11 labelled acetate. Global and regional contractile function were assessed using tomographic radionuclide angiography.

### Methods

#### Patients and study design

Ten patients (eight men, two women; age 53 ± 11 years) who had had chronic idiopathic dilated cardiomyopathy and symptomatic heart failure for >6 months were studied. At the time of inclusion, seven patients were in NYHA class III and three in NYHA class II. Significant coronary artery disease and primary valvular disease were ruled out by cardiac catheterization and coronary angiography. Diabetes mellitus or medication known to interfere with presynaptic innervation such as tricyclic antidepressants, clonidine or reserpine were further exclusion criteria. All patients were in sinus rhythm, three had left bundle branch block, and five had a history of high-grade ventricular arrhythmia (Lown class IV). To keep patients in a stable clinical condition, a standard medication including ACE-inhibitors, beta-blockers and diuretics had to be continued on the study day.

All patients underwent tomographic radionuclide angiography for assessment of global and regional ventricular function at rest as part of a clinical routine follow-up. Additionally, positron emission tomography imaging of myocardial perfusion, oxidative metabolism and presynaptic sympathetic innervation was carried out on the same day. Prior to inclusion, all patients gave written informed consent. The study protocol was approved by the ethical committee of the medical faculty of the TU Muenchen.

#### Tomographic radionuclide angiography

Autologous erythrocytes were labelled with 800–1000 MBq of Tc-99m by a combined in vivo/in vitro technique, and reinjected after purification. After 5 min to allow for equilibrium, patients were positioned in a rotating triple-headed gamma camera (Multispect 3, Siemens, Erlangen, Germany), and electrocardiographically gated single photon emission computer tomographic acquisition was performed at rest (12 phases, 120° acquisition angle, 20 views, 40 s per view). Heart rate and blood pressure were continuously monitored throughout the imaging procedure by ECG and arm cuff measurements.

#### Data analysis — positron emission tomography

(1-C-11)-acetate and C-11 meta-hydroxyephedrine were synthesized according to Pike et al.\(^{[14]}\) and Rosenspire et al.\(^{[15]}\). Positron emission tomography imaging was performed using an ECAT EXACT or ECAT 951 scanner (CTI/Siemens, Knoxville, TN, U.S.A.). After adequate positioning, a transmission scan of 10 to 15 min was acquired using external rod sources for correction of photon attenuation. Subsequently, 300–400 MBq of C-11 acetate were injected as a slow bolus over 30 s, and a dynamic imaging sequence of 21 frames over 30 min (10 × 10, 1 × 60, 5 × 100, 3 × 180, 2 × 300 s) was initiated. After a break of 50 min to allow for decay of radioactivity, 500–700 MBq of C-11 hydroxyephedrine were injected, and a second dynamic imaging sequence of 14 frames over 40 min (6 × 30, 2 × 60, 2 × 150, 2 × 300, 2 × 600 s) was acquired. Again, heart rate and blood pressure were monitored continuously throughout the study.

#### Data analysis — positron emission tomography

Tomographic data were reconstructed by filtered back-projection (Butterworth filter, 5th order, cutoff frequency 0·5 cycles . cm\(^{-1}\)). Subsequently, a previously validated volumetric sampling tool was used for three-dimensional detection of endocardial borders in all phases\(^{[16]}\). Global left ventricular end-systolic and end-diastolic volumes were obtained, and left ventricular ejection fraction, stroke volume as well as cardiac output were calculated. Systemic vascular resistance was estimated as mean arterial blood pressure divided by cardiac output and converted to dynes\(\cdot\)s . cm\(^{-5}\).

In addition to global parameters, regional wall motion was measured by endocardial shortening in 460 myocardial segments, expressed as a polar map of the left ventricle.

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Heart rate (min⁻¹/p1) tracer washout was measured as a measure of oxidative metabolism[20]. From the datasets for C-11 hydroxyephedrine retention and oxidative metabolism. Results were independently associated with myocardial hydroxyephedrine retention and oxidative metabolism. Results in groups were compared by the unpaired Student’s t-test. A P-value <0.05 was defined as significant.

Results

Haemodynamic and functional parameters

The patients’ left ventricular performance ranged from moderately to severely reduced. Haemodynamic and global functional parameters derived from radionuclide angiography are summarized in Table 1. Figure 2(a) depicts regional endocardial shortening in nine myocardial segments. Wall motion varied regionally, and was highest in basal anterior and lateral segments, and reduced towards the septum, inferior wall and apex. This regionally reduced septal shortening may be partially explained by the presence of left bundle branch block in three of the 10 patients.

Myocardial perfusion

Static images of early C-11 acetate uptake, as a qualitative measure of myocardial perfusion, were regionally

cutoff frequency. Similar to radionuclide angiography analysis, a modified version of the previously validated volumetric sampling tool[17] was applied to a summed dataset of frames 11–13 of the dynamic imaging sequence for C-11 acetate, to detect myocardial activity and create polar maps of static tracer distribution and arterial input function were obtained. Clearance kinetics of C-11 acetate have been shown to reflect tricarboxylic acid cycle flux[19]. Thus, the early phase of tracer washout was fitted monoexponentially to obtain the constant k(mono) as a measure of oxidative metabolism[20]. From the datasets for C-11 hydroxyephedrine, tracer retention as a measure of presynaptic catecholamine uptake sites, was calculated by dividing activity at 30–40 min by the integral of the arterial input function[21].

Global values were calculated as the average of the entire polar maps for k(mono) and hydroxyephedrine retention. In addition, a regional comparison of endocardial shortening (derived from radionuclide angiography), k(mono) and hydroxyephedrine retention was performed, using a model of nine regions of interest for the apex and both the basal as well as distal anterior, septal, inferior and lateral walls (Fig. 1).

Statistical analysis

Values are expressed as mean ± standard deviation. Simple linear regression analysis was used to describe correlations between pairs of continuous variables. Additionally, a multivariate model using stepwise linear regression analysis was applied to determine variables independently associated with myocardial hydroxyephedrine retention and oxidative metabolism. Results in groups were compared by the unpaired Student’s t-test. A P-value <0.05 was defined as significant.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
<th>Range</th>
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<tbody>
<tr>
<td>Heart rate (min⁻¹)</td>
<td>70 ± 13</td>
<td>51–85</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>106 ± 18</td>
<td>79–132</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>71 ± 14</td>
<td>49–96</td>
</tr>
<tr>
<td>Mean aortic pressure (mmHg)</td>
<td>83 ± 15</td>
<td>59–108</td>
</tr>
<tr>
<td>Rate pressure product (mmHg . min⁻¹)</td>
<td>7471 ± 1909</td>
<td>4187–9801</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>19 ± 10</td>
<td>7–37</td>
</tr>
<tr>
<td>LVEDVI (ml . m⁻²)</td>
<td>92 ± 27 ± 6</td>
<td>56.6–135.5</td>
</tr>
<tr>
<td>LVESVI (ml . m⁻²)</td>
<td>76 ± 29 ± 9</td>
<td>39.5–126.6</td>
</tr>
<tr>
<td>Stroke volume index (ml . m⁻²)</td>
<td>15.6 ± 5.8</td>
<td>8.8–25.7</td>
</tr>
<tr>
<td>Cardiac output (l . min⁻¹)</td>
<td>1.92 ± 0.70</td>
<td>1.22–3.61</td>
</tr>
<tr>
<td>SVR (dynes . cm⁻²)</td>
<td>3638 ± 1085</td>
<td>1595–5088</td>
</tr>
</tbody>
</table>

LVEF=left ventricular ejection fraction; LVEDVI=left ventricular end-diastolic volume index; LVESVI=left ventricular end-systolic volume index; SVR=systemic vascular resistance.
homogeneous (Fig. 2(b)). Perfusion defects, defined as regional uptake <50% of the maximum, were not surveyed in any individual.

**Oxidative metabolism**

In cardiomyopathy patients, the global clearance constant $k_{(\text{mono})}$ for C-11 acetate was $0.040 \pm 0.011 \text{ min}^{-1}$, ranging from $0.020$ to $0.054 \text{ min}^{-1}$. Regional values are depicted in Fig. 2(c), demonstrating homogeneity throughout the left ventricle.

Compared to a control group of 11 healthy normals (four men, seven women; age $51 \pm 9$ years; $k_{(\text{mono})}=0.060 \pm 0.015 \text{ min}$), global $k_{(\text{mono})}$ was significantly lower in cardiomyopathic patients ($P<0.01$), concordantly with lower values for the rate pressure product as a predictor of oxygen demand ($7471 \pm 1909$ for cardiomyopathy vs $10082 \pm 2190$ for normals, $P<0.01$). Simple linear regression analysis revealed a significant correlation between global $k_{(\text{mono})}$ and the rate pressure product ($r=0.78; P<0.01$). Afterload, as expressed by systemic vascular resistance, showed a correlation with $k_{(\text{mono})}$ which was of borderline statistical significance ($r=0.61; P=0.06$). Multivariate stepwise regression analysis including left ventricular end-diastolic and end-systolic volume, ejection fraction, systemic vascular resistance, rate pressure product, heart rate, mean arterial blood pressure, and global hydroxyephedrine retention in cardiomyopathy patients established rate pressure product ($F=10.4$) and systemic vascular resistance ($F=3.6$) as independent determinants of oxidative metabolism in the final model ($P=0.008$).

**Sympathetic innervation**

Global myocardial hydroxyephedrine retention in cardiomyopathy patients was $6.8 \pm 1.9 \% \text{ min}^{-1}$ (range 4.1 to 10.4% min$^{-1}$), and was significantly lower compared to a normal database of eight healthy adults (seven men, one woman; age $28 \pm 9$ years; $11.0 \pm 0.6\% \text{ min}^{-1}, P<0.01$). Regionally, mild heterogeneity with lowest retention in the apex was found in cardiomyopathy patients (Fig. 2(d)). Hydroxyephedrine retention was abnormally low (<2 SD of normals) in 58 ± 38% of the left ventricles. Figure 3 depicts images of hydroxyephedrine retention in a cardiomyopathy patient and a normal individual.

Regression plots for hydroxyephedrine retention and contractile function in cardiomyopathy are shown in Fig. 4. Global hydroxyephedrine retention correlated significantly with left ventricular ejection fraction ($r=0.67; P=0.03$). Furthermore, regional hydroxyephedrine retention in myocardial segments was weakly, but significantly correlated with regional endocardial shortening ($r=0.31; P<0.01$). Oxidative metabolism, on the other hand, was not directly associated with altered sympathetic innervation. Neither global $k_{(\text{mono})}$ ($r=0.08$) nor regional $k_{(\text{mono})}$ in myocardial segments ($r=0.04$) correlated significantly with hydroxyephedrine retention (Fig. 5).

Multivariate stepwise regression analysis including ejection fraction, left ventricular end-diastolic and end-systolic volume, systemic vascular resistance, rate pressure product, heart rate, mean arterial blood pressure and $k_{(\text{mono})}$ confirmed ejection fraction as the closest independent determinant of global hydroxyephedrine retention in cardiomyopathy patients ($F=6.4; P=0.036$).
Discussion

The present data confirm alterations of presynaptic sympathetic innervation in the cardiomyopathic heart. For the first time, these alterations were correlated with myocardial function, geometry and metabolism in humans. Globally and regionally, altered presynaptic catecholamine uptake was closely linked with reduced...
contractile performance, but not with ventricular loading conditions. Furthermore, the status of sympathetic innervation was not directly associated with rates of oxidative metabolism in the failing heart. Cardiac work and ventricular afterload remained major determinants of myocardial oxygen consumption in the present study.

In dilated cardiomyopathy, the reduced density and function of the uptake-1 carrier protein, which is located on presynaptic sympathetic nerve terminals, have been described in previous studies using in vitro and non-invasive in vivo imaging techniques[6,11–13,22]. In the present study, a close correlation of these alterations with reduced contractile function was found both regionally and globally, while loading conditions were not independently associated at multivariate analysis. Because dilated cardiomyopathy is characterized by initial myocardial damage followed by secondary alterations of ventricular load due to inadequate tension generation, the origin of the loss of neurons or reduction of uptake-1 number per neuron, as reflected by reduced hydroxyephedrine uptake, may be a result of the same pathogenetic mechanism which also causes primary damage to myocytes. The lack of direct correlation between impaired innervation and end-diastolic volume or systemic vascular resistance does not suggest a major role of secondary impairments of ventricular loading in the pathogenesis of altered innervation.

In addition to potential damage due to a common pathogenetic factor, reduction of local presynaptic uptake sites due to cardiac dysinnervation may result in increased exposure of cardiomyocytes to catecholamines, and thereby further aggravate contractile dysfunction. First, catecholamines have been shown to exert direct toxic effects on myocytes, mediated by induction of apoptosis or calcium overload[23]. Secondly, the local hyperadrenergic state has been shown to be associated with various changes in the adrenergic signal transduction pathway, such as reduced density and function of beta-adrenergic receptors, increased activity of receptor kinases, and impaired levels of G-proteins and cyclic AMP, which ultimately result in altered calcium handling and thus in systolic and diastolic contractile dysfunction[23,24].

In a previous study, we validated the results of C-11 hydroxyephedrine positron emission tomography in the cardiomyopathic human heart in vivo against in vitro measurements of uptake-1 after explantation[4]. A regional heterogeneity with a gradient from base to apex and lowest values of hydroxyephedrine retention in the apical region was observed. This heterogeneity is confirmed by the present data. Interestingly, functional measurements also revealed regional heterogeneity with the highest endocardial shortening in the free anterior and lateral walls, and lower values in the apex, confirming the correlation between presynaptic catecholamine uptake and contractile function. No such regional heterogeneity was observed for perfusion and oxidative metabolism.

Disturbances of myocardial energy metabolism have also been suggested as a feature of cardiac failure. A state of energy starvation has been proposed[23,24], and alterations in high-energy phosphate levels[25] and cardiac enzymes involved in beta-oxidation[23] have been identified. It has been suggested that the metabolism of the failing heart is characterized by oxygen wastage[26], a state which may be aggravated by excess catecholamine exposure[10]. Therefore, we speculated that altered presynaptic innervation could influence oxidative metabolism in the cardiomyopathic heart. Our results, however, do not confirm this hypothesis. Globally and regionally, there was no correlation between innervation and oxygen consumption. The heart pressure product as an index of cardiac work and systemic vascular resistance as a measure of afterload remained major determinants of overall oxygen consumption in the failing heart. Oxygen consumption increased with higher peripheral resistance, but also decreased with lower work, suggesting that these normal regulatory mechanisms remain operational in the cardiomyopathic heart. Thus, the results do not support a role for alterations of oxidative metabolism in the primary pathogenesis of dilated cardiomyopathy. It needs to be pointed out, however, that kinetics of acetate reflect the turnover of the tricarboxylic acid cycle as a final common pathway of cardiac substrate metabolism. Potential disturbances of the utilization of specific substrates, such as glucose or long-chain fatty acids[27], and their relation to sympathetic innervation, are not identified in the present study and may need further investigation.

In conclusion, further insights into pathophysiological interrelations in the cardiomyopathic heart are provided by the present study. Alterations of presynaptic sympathetic innervation, as characterized by retention of C-11 hydroxyephedrine, are closely associated with impaired contractile function, suggesting a common pathogenetic pathway. The reduction of local catecholamine uptake sites, however, did not directly influence regulation of overall oxidative metabolism, which is at least partially maintained in dilated cardiomyopathy.

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References


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