Dysfunction of Mitochondrial Respiratory Chain Complex I in Human Failing Myocardium Is Not Due to Disturbed Mitochondrial Gene Expression

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OBJECTIVES Activity of mitochondrial respiratory chain complexes with and without mitochondrially encoded subunits was assessed in failing human myocardium together with parameters of mitochondrial gene expression.

BACKGROUND Mutations and deletions in mitochondrial genome (mtDNA) sporadically accumulate in the aging myocardium. In experimental heart failure, they are discussed to be a generalized problem resulting in disturbances of mitochondrial gene expression and mitochondrial function.

METHODS In left ventricular specimens from 43 explanted failing hearts and 10 donor hearts, enzyme activities of respiratory chain complexes, messenger ribonucleic acid (mRNA) expression of mitochondrially and nuclear encoded mitochondrial components (reverse transcriptase-polymerase chain reaction, Northern blot), undeleted wildtype mtDNA (Southern blot), and nuclear encoded mitochondrial transcription factor A (mtTFA) (Western blot) were quantified.

RESULTS Citrate synthase normalized activity of mitochondrial respiratory chain complex I, which contains seven mitochondrially encoded subunits, was decreased by 28% in terminally failing myocardium, whereas the activity of the exclusively nuclear encoded complex II was unchanged. However, the amount of intact mtDNA, the mRNA of all mitochondrially encoded subunits of the entire respiratory chain, the amount of mtTFA, and the enzymatic activity of complex III and complex IV, which also contain mitochondrially encoded subunits, were normal compared with donor hearts, excluding generalized disturbance of mitochondrial gene expression. Retrospective analysis of drug therapy before transplantation identified beta-blockers as one putative protection against this disturbance.

CONCLUSIONS In terminally failing human myocardium of patients receiving drug therapy, complex I depression is not caused by mtDNA damage and disturbed mitochondrial gene expression. The absence of mtDNA damage should facilitate recovery of the overloaded myocardium, if effective unloading could be achieved. (J Am Coll Cardiol 2002;40:2174–81) © 2002 by the American College of Cardiology Foundation

Hereditary or prenatally acquired mitochondriopathies, characterized by mitochondrial deoxyribonucleic acid (mtDNA) deletions and/or mitochondrial transfer ribonucleic acid (RNA) point mutations, are associated with peripheral myopathies or with multisystemic syndromes, sometimes including the myocardium with features of dilated cardiomyopathy (DCM) (1). Affected cells show disturbed aerobic energy production, enhanced oxidative stress, and exaggerated susceptibility for apoptosis. In a thorough analysis of 601 patients with DCM, 19 cases of heteroplasmic mtDNA mutations or deletions were found with depressed respiratory activity, suggesting that mitochondriopathies are rather rare among patients diagnosed for DCMs (2). In affected cases, activity of complex II, which contains only subunits encoded by the nucleus, was normal, whereas activities of complexes I and IV, which contain also mitochondrially encoded subunits, were depressed, yielding a disproportional respiratory chain (2).

Secondary disturbances of mitochondrial gene expression, not resulting from hereditary mtDNA defects, but with similarly disproportional respiratory chains, were also found in the glomeruli of an hereditary form of nephrotic syndrome (3) and sporadically in cells of aging organs with minimal cellular turnover (4). While the role of such secondary disturbances in mitochondrial gene expression in the process of aging of postmitotic cells is still under debate, it is remarkable that a severe, secondary disturbance of mitochondrial gene expression has been documented in the surviving myocardium of mice with postinfarction cardiac failure (5). In this model, the failing myocardium exhibited severely depressed mitochondrial gene expression, a disturbed mitochondrial respiratory chain, and substantial losses of undeleted mitochondrial wildtype deoxyribonucleic
METHODS

Myocardial specimens from cardiomyopathic and donor hearts. Samples from 10 organ donors (mean age 44.8 ± 4 years), whose hearts were not suitable for transplantation because of clinical reasons and who had no history of heart disease, were obtained in cardioplegic arrest. In addition, 43 explanted hearts were obtained from patients (mean age 52 ± 1 year) undergoing transplantation due to DCM (n = 23) or coronary artery disease (CAD) (n = 20), with a mean ejection fraction of 24 ± 1%, a mean pulmonary wedge pressure of 20 ± 1 mm Hg, and a mean cardiac index of 2.27 ± 0.1 l/min/m². Samples were immediately snap frozen in liquid nitrogen. Twenty-five of the failing patients had beta-blocker therapy—in 19 cases in combination with an angiotensin-converting enzyme (ACE) inhibitor—while the 18 patients without beta-blocker therapy received an ACE inhibitor in 17 cases. Nine patients were treated with a hepatic hydroxymethylglutaryl coenzyme A reductase inhibitor (statin). No patient required positive inotropic therapy in the form of phosphodiesterase inhibitors or catecholamines. Samples from all 53 hearts were used for measurement of mitochondrial respiratory chain enzyme activity and reverse transcriptase-polymerase chain reaction (RT-PCR). Out of 43 failing hearts, 12 were used for Southern blot and 9 for Northern blot and Western blot analysis, whereas 6 of the 10 donor hearts were used for the three mentioned analyses.

Furthermore, out of a series of diagnostic left ventricular biopsies performed for evaluation of causes of arrhythmias, left ventricular biopsies without histologic signs of local inflammation were available from three patients without dilative cardiomyopathy. Semiquantitative RT-PCR was performed in these biopsies.

The local ethics committee approved the study of these human cardiac tissues, and the subjects or their families gave informed consent.

RNA extraction and semiquantitative RT-PCR. Total RNA was isolated from ventricular specimens, and semiquantitative RT-PCR was performed in accordance with the protocol described previously (6). Primer sequences and specific characteristics of semiquantitative messenger ribonucleic acid (mRNA) analyses of the analyzed genes for ND1, ND2, ND3, ND4, ND4L, ND5, ND6, cytochrome b, cytochrome oxidase I (COX I), COX III, 16S ribosomal ribonucleic acid (rRNA), tumor necrosis factor (TNF)-alpha, inducible nitric oxide synthase, interleukin-6, atrial natriuretic peptide, brain natriuretic peptide, and manganese superoxide dismutase are available from the corresponding author. The polymerase chain reaction (PCR) products were quantified per 18S rRNA.

Northern blot analysis. Total RNA from left ventricular specimens was also used to assess mRNA levels of ND1, ND5, cytochrome b, COX I, COX III, and 16S rRNA by Northern blot analysis. The mRNA levels were normalized to 18S rRNA. Ten micrograms of total RNA per lane were separated by electrophoresis through 1.2% agarose gels after denaturation of the RNA with glyoxal and dimethylsulfoxide. The RNA was transferred to Hybond-N nylon membranes (Amersham Life Science Inc., Freiburg, Germany) by capillary blot with 20× saline sodium citrate buffer. The hybridization of digoxigenin-11-2′-deoxy-uridine-5′-triphosphate labeled probes of PCR products mentioned earlier was performed at 50°C according to the protocol of the PCR DIG Probe Synthesis Kit (Roche Diagnostics GmbH, Mannheim, Germany). Blots were developed by enhanced chemiluminescence (Amersham Pharmacia Biotech, Freiburg, Germany), and the intensity of hybridizing bands was quantified by AIDA imaging software (raytest, Berlin, Germany).

DNA isolation and Southern blot analysis of mtDNA. Genomic DNA and mtDNA were isolated from ventricular specimens according to the description in the Puregene DNA Isolation Kit (Genta Systems, Minneapolis, Minnesota). The DNA concentrations were calculated from the absorption at 260 nm, and DNA was digested with BamHI. Then, 3.5 μg of the cleaved DNA were resolved by electrophoresis through 0.8% agarose gel. After capillary transfer to Hybond-N+ nylon membrane (Amersham Life Science Inc.), the blot was hybridized at 50°C with digoxigenin-11-2′-deoxy-uridine-5′-triphosphate labeled
probes of PCR products for 16S rRNA and 18S rRNA
detection according to the description in the PCR DIG
Probe Synthesis Kit (Roche Diagnostics GmbH, Mann-
heim, Germany). Blots were developed as described pre-
viously for Northern blot.

**Immunoblot analysis.** Proteins were isolated from ventric-
ular specimens and electroblotted onto nitrocellulose mem-
brane in accordance with the protocol described previously (7).

Membranes were blocked with 5% nonfat dry milk in
Tris-buffered saline-Tween 20 (200 mM Tris [hydroxy-
methyl] aminomethane, 300 mM sodium chloride, 0.1% Tween 20; pH 7.5) and incubated with the anti-
mitochondrial transcription factor A (mtTFA) primary
antibody (dilution 1:1,000, provided by Prof. N.-G. Lar-
sen, Department of Medical Nutrition, Karolinska Insti-
tute, Sweden). Blots were subsequently washed in Tris-
buffered saline-Tween 20 and incubated with a specific
peroxidase-coupled secondary antibody (dilution 1:10,000,
anti-rabbit immunoglobulin G–horseradish peroxidase,
Amersham Pharmacia Biotech). Bound antibodies were
detected by enhanced chemiluminescence (Amersham
Pharmacia Biotech), and the intensity of hybridizing bands
was quantified by AIDA imaging software (raytest).

**Determination of enzyme activity.** Small pieces of frozen
tissues were homogenized (1/30 weight per volume) in a
solution containing 50 mM of Tris buffer (pH 7.5), 100
mM potassium chloride, 5 mM MgCl₂, and 1 mM ethyl-
endediaminetetraacetic acid using a glass/glass homogenizer
(Kontes Glass Co., Vineland, New Jersey, 2 ml, 0.025 mm
clearance). Enzyme activities were assayed at 30°C spectro-
photometrically using a DU 640 photometer (Beckmann
Instruments, Palo Alto, California) and related to citrate
synthase activity.

The analysis of the mitochondrial respiratory chain com-
plexes nicotinamide adenine dinucleotide (NADH):
coenzyme Q₁-oxidoreductase (complex I), NADH:
cytochrome c-oxidoreductase (complex I + III), succinate:
cytochrome c-oxidoreductase (complex II + III), ubiquinone:cytochrome c-oxidoreductase (complex III), cy-
tochrome c-oxidase (complex IV), and citrate synthase as
mitochondrial marker enzyme was performed in accordance
with the protocol described previously (8,9).

**Data analysis.** Western blots, Northern blots, Southern
blots, and RT-PCR analyses were evaluated by scanning of
blots or negatives of the gel-images using a computer-based
imaging system (AIDA). The optical density units are given
as mean ± SEM. The significance of comparison of mean
values was determined by the unpaired Student t test using
a significance level of p < 0.05.

**RESULTS**

**General observations.** The clinical characteristics of the
43 heart failure patients before heart explantation demon-
strated that patients with CAD (n = 20) were older (55.7 ±
1.3 years) than patients with DCM (49.0 ± 1.9 years, p <
0.01). The patients with DCM had a more deteriorated
cardiac function (ejection fraction = 21.8 ± 1.6% vs. 27.5 ±
2.0% in CAD, p < 0.05; pulmonary capillary wedge
pressure = 23 ± 2 mm Hg vs. 17 ± 2 mm Hg, p = 0.07)
and, accordingly, an increased expression of the overload
indicating factors atrial and brain natriuretic peptide (Table
1). Cardiac index and gender ratio (21 male/2 female in
DCM and 18 male/2 female in CAD) were similar in both
groups. Patients with DCM had angiographically normal
coronary arteries in all cases. The inflammatory activation
of the failing myocardium was characterized by assessment of
the mRNA expression of cytokines and inducible nitric
oxide synthase (Table 1). There was a positive correlation
between TNF-alpha mRNA and inducible nitric oxide
synthase mRNA (r = 0.29; p < 0.05; n = 53). Compared
with the biopsy samples, the inflammatory activation in
failing myocardium was substantial, both in patients with
DCM and in patients with CAD, which has been shown
previously (10).

**Mitochondrial enzyme activities.** The mitochondrial
electron transport chain consists of the partially mitochon-
drially encoded complexes I, III, and IV and the exclusively
nuclear encoded complex II. The enzymatic activity of
complex I was 28% lower in terminally failing ventricles
than in donor organs (Fig. 1A). This decrease was also
visible in the combined activity of complexes I + III (10.9
± 1.3 vs. 13.7 ± 1.9 in donors; p = 0.08). In contrast, there
was no decrease in the enzyme activity of the exclusively
nuclear encoded complex II, or in the partially mitochon-
drially encoded enzyme complexes III and IV (Fig. 1).
There was no difference in the respiratory chain enzyme
activities between myocardium from patients with DCM or
CAD (Table 1).

**mtDNA copy number, mtTFA, and mitochondrial
mRNA expression.** A potential reason for the decreased
complex I enzyme activity could be a disturbed mitochon-
drial gene expression due to oxidative stress-induced dam-
age with subsequent deletions in the mtDNA, as docu-
mented in a murine model of postinfarction heart failure
(5). However, there was no detectable decrease in the
concentration (copy number) of undeleted wildtype
mtDNA in failing myocardium (not shown). Similarly, the
protein expression of the mtTFA was not different between
the failing ventricles and the donor hearts (1.3 ± 0.2 optical
density units vs. 1.0 ± 0.1 optical density units, respectively,
p = NS). Furthermore, the mitochondrial ribonucleic acid
(mtRNA) levels (obtained by RT-PCR) of all mitochondri-
ally encoded genes of complex I (ND1, ND2, ND3,
ND4, ND4L, ND5, and ND6), of complex III (cytochrome
b), and of complex IV (COX I, COX III) as well as of 16S
rRNA were not different between failing and donor myo-
cardium (Fig. 2A). Northern blot analysis of selected
transcripts of mitochondrially encoded subunits, e.g. ND1
(Fig. 2B), ND5, cytochrome b, COX I, or COX III
confirmed that there was no decline of any mtRNA in
failing myocardium. Similarly, the mRNA expression of manganese superoxide dismutase, a typical example for a nuclear encoded mitochondrial matrix enzyme, was not lowered in failing myocardium (Fig. 2A). In the left ventricular biopsies without histologic signs of inflammation, relative interleukin-6 mRNA expression (0.03 ± 0.01 U) was lower (p = 0.07) than in donor hearts (0.09 ± 0.01 U, Table 1). However, mRNA expression of ND1, ND5, or cytochrome b and of the nuclear encoded gene manganese superoxide dismutase was not lowered (data not shown). In failing myocardium, there was no difference in any mtRNA concentration between DCM and CAD (Table 1).

Effects of drug treatment on mitochondrial enzyme activity and gene expression. The retrospective analysis of terminally failing explanted hearts from patients undergoing beta-blocker therapy (n = 25) before explantation and those without this therapy (n = 18) showed that both groups had a similar degree of cardiac overload, indicated by the fourfold elevation of atrial natriuretic peptide mRNA expression in both groups (0.66 ± 0.18 in beta-blocker–free patients vs. 0.61 ± 0.16 in beta-blocker–treated patients compared with donors [0.15 ± 0.07; p < 0.05]). Furthermore, both subgroups had no differences in age, ejection fraction, pulmonary wedge pressure, and cardiac index. However, the beta-blocker–treated patients had a 20% higher complex III enzyme activity compared with the patients without this treatment, whereas this difference in the other complex activities did not reach the level of significance (Fig. 3).

Retrospective subgrouping of the terminally failing hearts into those from patients undergoing ACE inhibitor therapy (n = 37) before explantation and those without this therapy (n = 6) demonstrated a lower TNF-alpha mRNA expression in patients undergoing ACE inhibition (0.20 ± 0.02

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**Table 1.** Left Ventricular mRNA Expression (Per 18S rRNA) and Respiratory Chain Complex Activities

<table>
<thead>
<tr>
<th>mRNA</th>
<th>DCM (n = 23)</th>
<th>CAD (n = 20)</th>
<th>p Value</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pro-ANP</td>
<td>0.85 ± 0.11</td>
<td>0.36 ± 0.07</td>
<td>&lt;0.001</td>
<td>0.15 ± 0.05</td>
</tr>
<tr>
<td>Pro-BNP</td>
<td>0.56 ± 0.07</td>
<td>0.30 ± 0.06</td>
<td>&lt;0.01</td>
<td>0.23 ± 0.08</td>
</tr>
<tr>
<td>TNF-α</td>
<td>0.21 ± 0.02</td>
<td>0.21 ± 0.02</td>
<td>NS</td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td>iNOS</td>
<td>0.87 ± 0.09</td>
<td>0.86 ± 0.10</td>
<td>NS</td>
<td>0.70 ± 0.07</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.14 ± 0.01</td>
<td>0.12 ± 0.02</td>
<td>NS</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td>ND1</td>
<td>1.12 ± 0.07</td>
<td>1.05 ± 0.06</td>
<td>NS</td>
<td>1.09 ± 0.09</td>
</tr>
<tr>
<td>ND2</td>
<td>1.34 ± 0.04</td>
<td>1.40 ± 0.07</td>
<td>NS</td>
<td>1.41 ± 0.10</td>
</tr>
<tr>
<td>ND3</td>
<td>0.82 ± 0.08</td>
<td>0.95 ± 0.10</td>
<td>NS</td>
<td>0.92 ± 0.09</td>
</tr>
<tr>
<td>ND4</td>
<td>1.45 ± 0.05</td>
<td>1.64 ± 0.10</td>
<td>NS</td>
<td>1.46 ± 0.08</td>
</tr>
<tr>
<td>ND4L</td>
<td>0.93 ± 0.04</td>
<td>0.91 ± 0.06</td>
<td>NS</td>
<td>0.88 ± 0.08</td>
</tr>
<tr>
<td>ND5</td>
<td>1.29 ± 0.06</td>
<td>1.16 ± 0.05</td>
<td>NS</td>
<td>1.20 ± 0.07</td>
</tr>
<tr>
<td>ND6</td>
<td>1.26 ± 0.07</td>
<td>1.40 ± 0.08</td>
<td>NS</td>
<td>1.19 ± 0.10</td>
</tr>
<tr>
<td>Cytochrome b</td>
<td>1.49 ± 0.07</td>
<td>1.46 ± 0.06</td>
<td>NS</td>
<td>1.38 ± 0.12</td>
</tr>
<tr>
<td>COX I</td>
<td>1.50 ± 0.07</td>
<td>1.39 ± 0.05</td>
<td>NS</td>
<td>1.44 ± 0.09</td>
</tr>
<tr>
<td>COX III</td>
<td>1.19 ± 0.06</td>
<td>1.11 ± 0.04</td>
<td>NS</td>
<td>1.02 ± 0.09</td>
</tr>
<tr>
<td>16S rRNA</td>
<td>1.72 ± 0.09</td>
<td>1.61 ± 0.06</td>
<td>NS</td>
<td>1.62 ± 0.11</td>
</tr>
<tr>
<td>MnSOD</td>
<td>0.65 ± 0.04</td>
<td>0.63 ± 0.03</td>
<td>NS</td>
<td>0.64 ± 0.05</td>
</tr>
</tbody>
</table>

**Enzyme activity**

<table>
<thead>
<tr>
<th>Complex</th>
<th>DCM (n = 23)</th>
<th>CAD (n = 20)</th>
<th>p Value</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex I</td>
<td>11.3 ± 0.56</td>
<td>11.7 ± 0.66</td>
<td>NS</td>
<td>15.6 ± 1.27</td>
</tr>
<tr>
<td>Complex I /</td>
<td>11.3 ± 0.95</td>
<td>10.5 ± 0.93</td>
<td>NS</td>
<td>13.7 ± 1.44</td>
</tr>
<tr>
<td>Complex II +</td>
<td>20.0 ± 1.19</td>
<td>18.3 ± 1.57</td>
<td>NS</td>
<td>18.4 ± 0.79</td>
</tr>
<tr>
<td>Complex III</td>
<td>100.6 ± 6.19</td>
<td>93.4 ± 5.28</td>
<td>NS</td>
<td>98.2 ± 5.17</td>
</tr>
<tr>
<td>Complex IV</td>
<td>41.1 ± 3.03</td>
<td>39.9 ± 3.62</td>
<td>NS</td>
<td>46.4 ± 3.49</td>
</tr>
<tr>
<td>Citrate synthase</td>
<td>40.5 ± 1.70</td>
<td>39.6 ± 2.69</td>
<td>NS</td>
<td>44.5 ± 1.51</td>
</tr>
</tbody>
</table>

*Complex activities in % of citrate synthase (Units/g/ww). Data are given as mean ± SEM.

CAD = end-stage coronary artery disease; COX = cytochrome oxidase; DCM = dilated cardiomyopathy; IL-6 = interleukin-6; iNOS = inducible nitric oxide synthase; MnSOD = manganese superoxide dismutase; mRNA = messenger ribonucleic acid; ND = not significant; Pro-ANP = atrial natriuretic peptide; Pro-BNP = brain natriuretic peptide; rRNA = ribosomal RNA; TNF-α = tumor necrosis factor alpha.
DISCUSSION

This study of left ventricular myocardium from explanted hearts demonstrates that the activity of mitochondrial complex I, containing seven mitochondrially encoded subunits, is similarly lowered in patients suffering from DCM or ischemic heart disease. On the other hand, however, the activity of complex II, containing exclusively subunits encoded by the nucleus, is unchanged in both groups of patients. At first glance, this result appears compatible with dysfunction of mtDNA expression, as has been shown previously in a murine postinfarction model of heart failure (5).

In that experimental study, several observations suggested disturbed expression of the mitochondrial genome: in the nonischemic part of failing ventricles, concentrations of transcripts of mitochondrially encoded subunits were significantly lower, the activities of complexes III and IV (which also contain mitochondrially encoded subunits) were even more depressed than the activity of complex I, the amount of the non-deleted mitochondrial wildtype DNA was depressed to 44% of control myocardium, and the ventricular protein content of mtTFA was also depressed to 65% (5).

An enhanced radical formation was demonstrated in the mitochondria of failing ventricles, and the loss of undeleted wildtype mtDNA was considered as a consequence of DNA damage by oxidative stress, resulting in lowered mitochondrial transcript availability and lowered mtTFA content, which is degraded more rapidly in cells with a deficit of wildtype mtDNA (11).

None of these alterations, except for the complex I depression, could be confirmed in our study in failing human myocardium: The content of undeleted wildtype mtDNA (measured by Southern blotting, not shown) was normal, as was mtTFA protein and the amount of all mitochondrially encoded transcripts (Fig. 2A). A general impairment in the translation of mitochondrial transcripts in human hearts is also unlikely, because complexes III and IV, which contain mitochondrially encoded subunits, were normal (Fig. 1).

A critical aspect in studies on failing human myocardium from explanted hearts is the nonfailing donor myocardium used as control. Subsequent to brain death before organ removal and during cardioplegia, donor hearts experience severe inflammatory activation, indicated by enhanced expression of interleukin-6 and other cytokines (12,13). Such activation might conceivably interfere with mitochondrial gene expression and function. Compared with the biopsies without histologic signs of inflammation, our donor hearts had elevated interleukin-6 expression, compatible with

vs. 0.27 ± 0.02 in patients without this treatment, p < 0.05), but no hints of any difference in mitochondrial complex activities or gene expression.
some inflammatory activation. However, mitochondrial gene expression in these biopsies was not higher than in the donor hearts, arguing against inflammation-associated depression of mitochondrial gene expression in our donor hearts.

Several factors might be considered as explanations for these striking discrepancies between human and murine heart failure. A basic difference between heart failure in patients and the murine model is the history of the disease and the degree of decompensation, which is probably maximal in the rapidly progressing experimental model with extremely large infarcts (5). In our study, the patients with DCM had significantly higher atrial natriuretic peptide expression and exhibited more hemodynamic decompensation compared with patients with ischemic heart disease (Table 1 and the “General observations” section under the “Results” heading). However, there was no difference between these two patient groups in mitochondrial transcript concentrations and in the activities of all mitochondrial respiratory chain complexes (Table 1). Whether drug treatment of heart failure might have been protective against dysfunction of mitochondrial gene expression in our patients remains open. Retrospective subgrouping of patients according to treatment with ACE inhibition/angiotensin II type 1 receptor blocker or without such treatment revealed higher TNF-alpha mRNA expression in the latter group but no differences in parameters of mitochondrial gene expression. Retrospective subgrouping of patients according to treatment with beta-blockers (Fig. 3) revealed some protection of mitochondrial respiratory function, significant only for complex III activity in beta-blocker–treated patients (Fig. 3C). This might be considered as a vague hint of some protective effect of beta-blocker treatment on mitochondrial function in failing myocardium. A similar impression of beta-blocker–mediated mitochondrial protection was previously obtained in our retrospective analysis of apoptotic activation in human failing myocardium (7).

A putative explanation for the depression in complex I might be derived from observations in macrophage cell lines (14,15). In these cells, nitric oxide (NO) induces a competitive, rapidly reversible inhibition of complex IV, resulting in enhanced radical formation by the respiratory chain upstream this inhibition. In situations with lowered antioxidant defense, this combination of NO and enhanced mitochondrial superoxide anion formation results in a slowly developing, irreversible depression of complex I activity, probably via peroxynitrite formation and/or S-nitrosylation of not yet identified targets within complex I, whereas other complexes are not affected (14,15). Although this sequence has not yet been analyzed in failing myocardium, all conditions for this pattern of reactions are given: enhanced NO formation in failing myocardium has been documented (16) and can be assumed in the hearts in this study with enhanced TNF-alpha expression correlating to inducible nitric oxide synthase expression; NO is a potent inhibitor of complex IV in myocardium (17); and enhanced oxidative stress in failing myocardium due to mitochondrial and extramitochondrial mechanisms has been documented (18–20).

The complex I deficiency in human failing myocardium should have functional consequences for respiratory adenine triphosphate production and for mitochondrial radical formation. Inhibition of mitochondrial complex I to various degrees and by different interventions allowed titration of the effect of complex I deficiency for maximal respiratory capacity (21). From these data it can be derived that the 28% depression in complex I activity of the failing human heart (Fig. 1) results in a 15% decline in maximal respiratory capacity (21). This respiratory capacity is further attenuated in the failing human heart by the mitochondrial loss of cytochrome c (7), and probably by some reversible inhibition of complex IV by enhanced NO formation (17) (the latter inhibition is not detectable during the applied analysis of the complex IV activity in vitro because of NOS inactivation due to lack of L-arginine and calcium, see “Methods” section). Thus, several mechanisms for a primary attenuation in mitochondrial adenine triphosphate synthesis are now identified, which explain the reduced respiratory capacity in skinned fibers from failing human myocardium under state-3 respiration supported by substrates for complex I (22). This reduction in mitochondrial respiratory capacity of failing myocardium is considered as causal for the reported lowered myocardial concentrations of phosphocreatine and adenine triphosphate (23), for enhanced glycolytic activity (24), and it may be the reason for the tendency toward enhanced endothelin-1 formation (25,26). This enhanced endothelin-1 expression results from hypoxia-inducible factor-1alpha induction due to impaired energy metabolism and can be mimicked by complex I inhibition (25,27). Heart failure–associated mitochondrial alterations downstream to the respiratory chain complexes, such as reduction in mitochondrial creatine kinase (28) and creatine transporter (29), might probably be compensated by an enhanced adenylate kinase–catalyzed phosphotransfer (30,31).

Complex I deficiency due to inhibition by rotenone or due to attenuated mitochondrial gene expression in mitochondriopathies results in enhanced mitochondrial production of superoxide anion (32,33). Similarly, in experimental heart failure with complex I activity depressed to the same extent as in our human failing myocardium, complex I is the source of enhanced mitochondrial radical formation (20), although the mechanisms of complex I deficiency appear different.

Apart from cases of mitochondriopathies, comparisons in respiratory chain activities between failing and non-failing myocardium have seldom been performed. In explanted left ventricular myocardium from patients with DCM, a dramatic depression of NADH:cytochrome c-oxidoreductase (complex I + III activity) by 61% compared with donor myocardium has been reported, whereas NADH:CoQ1-oxidoreductase (complex I) was normal (34). This is difficult
to understand even in the presence of attenuated complex III activity, because the NADH cytochrome c-oxidoreductase activity almost exclusively depends on complex I activity (35). In an experimental model of tachypacing-induced heart failure without any drug treatment, a depression in the activities of all complexes containing mitochondrial encoded subunits, including the mitochondrial adenosine triphosphatase, was described (36), indicating disturbed mitochondrial gene expression similar to the data in the murine failure model mentioned previously (5). Thus, these heart failure models in two different species both without drug treatment (5,36) exhibit a disproportional respiratory chain as it results from disturbed mitochondrial gene expression (5). On the other hand, terminally failing explanted human myocardium of patients undergoing established drug treatment for heart failure suggests a protective role of drug treatment against disturbances of mitochondrial gene expression.

Conclusions. Although failing human myocardium exhibit-s a significant deficit in the activity of complex I, this deficit is not due to generalized damage of mtDNA and disturbed mitochondrial gene expression. Other complexes of the respiratory chain have normal activity regardless of whether they contain mitochondrially encoded subunits. The protective role of drug treatment, specifically by beta-blockers, against global damage of mtDNA remains to be proven. The absence of global mtDNA damage should facilitate recovery of the overloaded myocardium, provided that primary causes of heart failure could be adequately corrected.

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