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Control by Circulating Factors of Mitochondrial Function and Transcription Cascade in Heart Failure: A Role for Endothelin-1 and Angiotensin II

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Control by Circulating Factors of Mitochondrial Function and Transcription Cascade in Heart Failure

A Role for Endothelin-1 and Angiotensin II

Anne Garnier, PhD; Joffrey Zoll, PhD; Dominique Fortin; Benoît N'Guessan, PhD; Florence Lefebvre; Bernard Geny, MD, PhD; Bertrand Mettauer, MD, PhD; Vladimir Veksler, MD, PhD; Renée Ventura-Clapier, PhD

***Background*—**Evidence is emerging to support the concept that the failing heart is "energy depleted" and that defects in energy metabolism are important determinants in the development and the progression of the disease. We have shown previously that depressed mitochondrial function in cardiac and skeletal muscles in chronic heart failure is linked to decreased expression of the gene encoding transcriptional proliferator-activated receptor-γ coactivator-1α, the inducible regulator of mitochondrial biogenesis and its transcription cascade, leading to altered expression of mitochondrial proteins. However, oxidative capacity of the myocardium of patients treated for chronic heart failure and pathophysiological mechanisms of mitochondrial dysfunction are still largely unknown.

***Methods and Results*—**In patients with chronic heart failure treated with angiotensin-converting enzyme inhibition, cardiac oxidative capacity, measured in saponin-permeabilized fibers, was 25% lower, and proliferator-activated receptor-γ coactivator-1α protein content was 34% lower compared with nonfailing controls. In a rat model of myocardial infarction, angiotensin-converting enzyme inhibition therapy was only partially able to protect cardiac mitochondrial function and transcription cascade. Expression of proliferator-activated receptor-γ coactivator-1α and its transcription cascade were evaluated after a 48-hour exposure of cultured adult rat ventricular myocytes to endothelin-1, angiotensin II, aldosterone, phenylephrine, or isoprenaline. Endothelin-1 (−30%) and, to a lesser degree, angiotensin II (−20%) decreased proliferator-activated receptor-γ coactivator-1α mRNA content, whereas other hormones had no effect (phenylephrine) or even increased it (aldosterone, isoprenaline).

***Conclusions*—**Taken together, these results show that, despite angiotensin-converting enzyme inhibition treatment, oxidative capacity is reduced in human and experimental heart failure and that endothelin-1 and angiotensin II could be involved in the downregulation of the mitochondrial transcription cascade. **(*Circ Heart Fail*. 2009;2:342-350.)**

Key Words: angiotensin II ■ endothelin-1 ■ mitochondrial biogenesis ■ heart failure ■ proliferator-activated receptor-γ coactivator-1α

Chronic heart failure (CHF) is a syndrome resulting from multiple origins, and, despite significant advances in therapy, it remains a leading cause of morbidity and mortality in developed countries. Evidence is emerging to support the concept that the failing heart is "energy depleted" and that defects in energy metabolism are important determinants in the development and the progression of the disease.[1–3] This includes a shift in substrate utilization from fatty acid to glucose, decreased oxidative and glycolytic capacity, and alterations in energy transfer and utilization. Despite a renewed interest for the energetic side of heart failure, our knowledge of the upstream events is still sparse.

Editorial see p 275
Clinical Perspective on p 350

Addressing the origin of decreased cardiac muscle oxidative capacity in the pathogenesis of heart failure, we previously showed in a rat model of CHF induced by pressure overload that the decrease in mitochondrial function in both cardiac and skeletal muscles is linked to altered expression of mitochondrial proteins associated with decreased expression of the transcriptional coactivator peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) and its downstream transcription factors.[4] PGC-1α is a master regulator of energy metabolism at the level of gene transcription. Through its interaction with multiple transcription factors, such as peroxisome proliferator-activated receptors, estrogen receptor-related receptor, or nuclear respiratory factor (NRFs), PGC-1α enhances mitochondrial capacity for fatty acid oxidation and oxidative phosphorylation and triggers the coordinate expression of nuclear and mitochondrial encoded genes driving mitochondrial biogenesis.[5–7] Decreased PGC-1α expression has been confirmed in other experimental

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Table 1. Patient Characteristics

Patients	Gender	Age	Pathology	ACE Inhibitors	β-Blockers	LVEF, %	ET-1, pg/mL
1	M	62	Nocm	Captopril	Carvedilol	13	13.3
2	M	54	Valve	Perindopril		35	nd
3	M	63	Nocm	Captopril	Metoprolol	15	nd
4	F	46	Congenital	Captopril		50	13.3
5	M	51	Nocm	Perindopril		19	20.5
6	M	60	Nocm	Captopril	Carvedilol	22	nd
7	M	42	Cad	Perindopril	Atenolol	45	2.3
8	M	50	Nocm	Perindopril	Carvedilol	15	16.2
9	M	49	Cad	Ramipril		15	19.2
10	M	57	Nocm	Quinalapril		20	6.4
11	M	60	Cad	Fosinopril		28	11.6
12	M	63	Cad	Enalapril		30	12.1
13	M	33	Hdcm	Ramipril	Carvedilol	15	nd
14	F	48	Hdcm	Perindopril	Carvedilol	23	6.1
15	M	31	Nocm	Fosinopril	Carvedilol	17	12.5
16	M	61	Nocm	Ramipril	Carvedilol	15	8.0
17	M	56	Nocm	Ramipril	Carvedilol	35	6.5
18	M	64	Nocm	Ramipril	Carvedilol	30	6.1
19	M	32	Familialcm	Ramipril	Carvedilol	24	8.4
20	M	60	Hcm	Ramipril		15	6.4

Nocm indicates nonobstructive cardiomyopathy; Cad, coronary artery disease; Hdcm, hypertrophic dilated cardiomyopathy; Hcm, hypertrophic cardiomyopathy; LVEF, left ventricular ejection fraction; ET-1, plasma endothelin-1 level; nd, not determined.

models of heart failure,[8–12] showing that deactivation of the PGC-1α cascade leads to decreased mitochondrial activity through the downregulation of mitochondrial protein expression and contributes to the energetic failure of the failing heart. However, oxidative capacity and the implication of this pathway in human heart failure need further investigation.[13]

In contrast to experimental models,[4] in patients with severe CHF under angiotensin-converting enzyme inhibition (ACEi) therapy, skeletal muscle mitochondrial oxidative capacity and oxidative phosphorylation protein expression are preserved, despite lower exercise capacity.[14] In line with this maintained function, no evidence for deactivation of PGC-1α regulatory cascade was found, although, in healthy subjects, exercise performance is associated with improvement in mitochondrial function, PGC-1α activation, and increased expression of mitochondrial proteins.[15] This suggests a possible protective effect of ACEi on muscle energy metabolism. This seems to be true at least in skeletal muscle as in a rat model of myocardial infarction (MI), we showed that ACEi therapy could prevent the deleterious effects of CHF on skeletal muscle oxidative capacity through a preservation of the transcriptional PGC-1α regulatory cascade.[10] However, inhibition of angiotensin receptors by losartan in experimental model of MI led to contradictory results on cardiac PGC-1α expression.[11,12] Thus, whether ACEi therapy is also protective on cardiac mitochondrial protein expression and function is still an open question.

The generalized character of the metabolic myopathy that affects both cardiac and skeletal muscles in CHF and the protective effect of ACEi therapy described earlier in skeletal muscles suggest that humoral systemic factors could be involved.[16] In the progression from compensated hypertrophy to failure, there is a generalized hyperactivation of several neurohumoral systems, including renin-angiotensin-aldosterone axis, endothelin (ET)-1, and catecholamines, and these humoral factors might be responsible for the deactivation of the PGC-1α regulatory circuit and thereby for energetic dysfunction.

The purposes of the present study were to determine whether patients treated with ACEi exhibit decreased mitochondrial function; whether in an experimental model of heart failure, ACEi therapy may be protective for the mitochondrial function associated with an activation of PGC-1α expression; and finally to explore in isolated rat cardiomyocytes which hormonal signals can induce an altered mitochondrial function through a PGC-1α cascade.

Methods

Patient Population

Left ventricular myocardium was obtained from terminally failing human hearts of 20 patients (18 men, 2 women; mean age 52±2 years) at the time of transplantation mainly resulting from coronary artery disease or nonischemic cardiomyopathy at the "Hôpitaux Universitaires de Strasbourg" between 1998 and 2000. Patients had a mean ejection fraction of 24±2% (Table 1). All patients had medication with diuretics (furosemide) and ACE inhibitors at the time of heart transplantation. In addition, 12 patients in this group received β-blockers. As control, nonfailing human myocardium (normal ejection fraction) was obtained from 9 donors who were brain dead as a result of traumatic injury and from 8 donors during open heart surgery (16 men, 1 woman). Plasma ET-1 was measured by radioimmunoassay (Amersham, Buckinghamshire, United Kingdom) as described previously.[17] The study of these human cardiac tissues had been approved by Hôpitaux Universitaires de Strasbourg's ethics committee, and the subjects or their families gave informed consent.

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Table 2. Primers and Experimental Conditions for Real-Time PCR Amplification

Target Gene	GenBank Accession No.	Forward Primer Reverse primer (5′→3′)	PCR Product Size, bp	Annealing Temperature, °C
PGC-1α	NM_031347	CACCAAACCCACAGAGAACAG	206	58
		GCAGTTCCAGAGAGTTCCACA		
NRF-2α	XM_344002	CACCACACTCAACATTTCGG	244	58
		CCTTGGGGACCTTTGAAC TT		
mtTFA	NM_031326	GAAAGCACAAATCAAGAGGAG	175	55
		CTGCTTTTCATCATGAGACAG		
COX I	NC_001665	AGCAGGAATAGTAGGGACAGC	520	55
		TGAGAGAAGTAGTAGGACGGC		
COX IV	NM_017202	TGGGAGTGTTGTGAAGAG TGA	273	58
		GCAGTGAAGCCGATGAAG AAC		
ANF	NM_012612	GGGCTCCTTCTCCATCACCAA	203	60
		CTTCATCGGTCTGCTCGCTCA		
ET-1	NM_012548	TGCTCCTGCTCCTCCTTGAT	177	65
		CTGGCATCTGTTCCCTTGGT		
ECE-1	NM_053596	TACTACTCGCCCACCAAGAA	172	60
		TCCCATCCTTGTCATACTCC		
GCB	NM_000157	GCACAACTTCAGCCTCCCAGA	151	60
		CTTCCCATTCACCGTTCCATT		

PGC-1α indicates peroxisome proliferator-activated receptor γ coactivator 1α; NRF-2α, nuclear respiratory factor 2 DNA-binding subunit α; mtTFA, mitochondrial transcription factor A; COX I and COX IV, cytochrome *c* oxidase subunits I and IV; ANF, atrial natriuretic factor; ECE-1, endothelin-converting enzyme-1; GCB, glucocerebrosidase.

Rat Model of CHF

We used a rat model of heart failure induced by ligation of the proximal left coronary artery as described previously.[10] Four months of perindopril treatment (2 $mg \cdot kg^{-1} \cdot day^{-1}$ in the drinking water) started 1 week after coronary artery ligation. Treated (MI-PE, n=8) and untreated (MI, n=7) animals were studied. Control rats were sham operated without coronary ligation and had no medication (SHAM, n=7). The viable part of the left ventricle (LV) was isolated, part of which was immediately used for mitochondrial function measurement and the remaining part was rapidly frozen and kept at −80°C for subsequent analysis.

Functional Properties of Mitochondria

Oxygen consumption measurements of fresh saponin-skinned fibers from rat LV have been described previously,[18] and this protocol was modified slightly for human cardiac muscle fibers.[14] Basal (V_0) and maximal (V_{max}) respiration rates were expressed as μmol of $O_2 \cdot min^{-1} \cdot g^{-1}$ dry weight. For each human subject, the value is the mean of 2 to 3 separate measurements.

Preparation of Adult Rat Ventricular Myocytes

Adult rat ventricular myocytes (ARVM) were dissociated by retrograde perfusion of healthy isolated heart with collagenase as described previously,[19] with slight modifications. Freshly isolated cells were plated on laminin-coated culture dishes at a density of 2×10^5 to 3×10^5 cells/dish in minimal essential medium (M4780, Sigma) supplemented with 2.5% fetal bovine serum, penicillin (100 units/mL), streptomycin (100 μg/mL), and 2% HEPES (pH 7.4) for 1 hour, and switched to serum-free medium for 12 hours. Cells were then incubated for 48 hours with ET-1 (5.10^{-7} M), angiotensin II (Ang II, 10^{-6} M), aldosterone (Aldo, 5×10^{-8} M), phenylephrine (Phe, 5×10^{-5} M), isoprenaline (Iso, 10^{-8} M), or their diluents (control). Five independent cultures were used for each condition.

Metabolic Enzyme Activities

Lactate dehydrogenase activity was measured in cell culture medium to evaluate the cytotoxic effects of various drugs.[20] Rat and human frozen tissue samples were weighed and homogenized in ice-cold buffer, and citrate synthase (CS), cytochrome *c* oxidase (COX), and creatine kinase (CK) activities were determined using spectrophotometry at 30°C and pH 7.5, as described previously,[4] and expressed per gram of total protein.

Western Blot Analysis

Protein extracts (50 μg) of human control and failing myocardium were loaded onto a 10% SDS-polyacrylamide gel. Blots were first incubated with a specific antibody for mouse PGC-1α that also reacts with human because of sequence homology (dilution 1:1000, Chemicon International) and then with an anti-rabbit IgG, horseradish peroxidase-linked antibody (dilution 1:2500, Cell Signaling). PGC-1α protein was revealed with an enhanced chemoluminescent substrate (SuperSignal West Dura, Pierce Biotechnology), and α-actin (dilution 1:4000, Sigma) was used as reference protein.

Reverse Transcription–Polymerase Chain Reaction Analysis and Mitochondrial DNA Content

Standard procedures were used for total RNA extraction from rat LV or ARVM and reverse transcription. Real-time polymerase chain reaction was performed using the SYBR Green technology on a LightCycler rapid thermal cycler (Roche Diagnostics) as described.[4,15] Quantification results for each gene were normalized to glucocerebrosidase gene expression. Primers and polymerase chain reaction conditions are listed in Table 2. The mitochondrial DNA (mtDNA) content, expressed as the ratio of mtDNA to nDNA, was measured by a Southern blot analysis of DNA extracted from cultured myocytes.[4]

Statistical Analysis

Data are expressed as mean±standard error (SEM). Variables were checked for normal distribution. Significance was determined with Student's *t* test for human study or one-way analysis of variance followed by Newman–Keuls test or Kruskal–Wallis nonparametric test for animal studies when appropriate. For cell experiments, paired *t* tests were used. A value of $P<0.05$ was considered significant.

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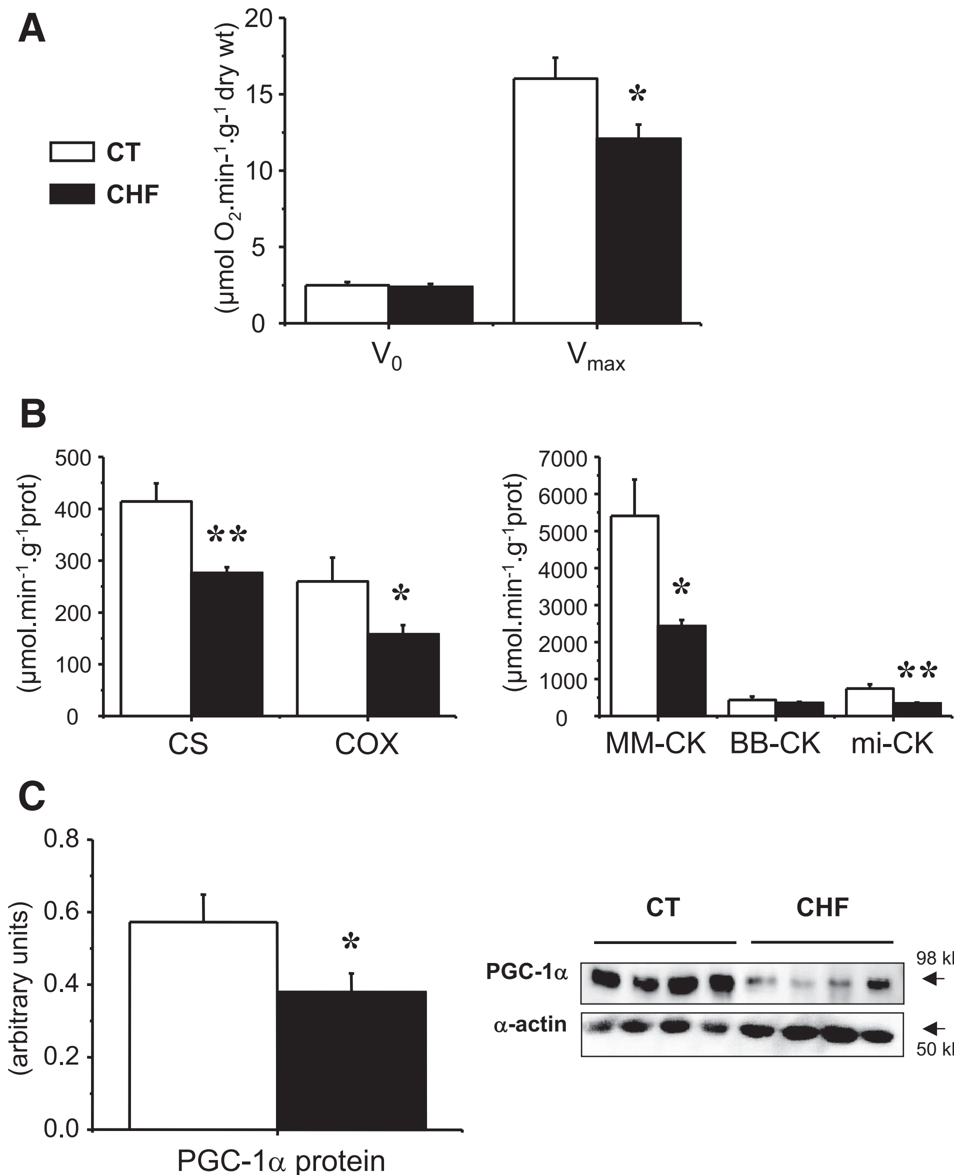

Figure 1. Mitochondrial function and PGC-1α protein expression in cardiac muscle of healthy subjects (CT) and patients with chronic heart failure. A, basal (V_0) and maximal respiration rates (V_{max}) were measured in saponin-skinned fibers (CT, n=8; CHF, n=20). B, enzymatic activities of MM-CK and BB-CK, cytosolic CK isoenzymes; mi-CK; CS; and COX were assessed by spectrophotometry (CT, n=12; CHF, n=20). C, Western blot analysis of PGC-1α protein expression. Values are mean±SEM. *$P<0.05$ and **$P<0.01$ for difference from control values.

Results

Mitochondrial Function and PGC-1α Protein Expression in Human Failing Hearts

In the failing hearts of patients treated for CHF, maximal mitochondrial oxidative capacity was significantly lower by 25%, associated with 40% lower activities of CS, a Krebs cycle enzyme, and of COX, complex IV of the respiratory chain (Figure 1A and 1B), suggesting an overall decrease in mitochondrial activity compared with the group of healthy subjects. Energy transfer was impaired as indicated by a lower total CK activity (CT: 6582±1111 versus CHF: 3124±199 $\mu mol \cdot min^{-1} \cdot g^{-1}$ prot, $P=0.004$) and mitochondrial (mi-CK) and cytosolic muscle (M) isoform of creatine kinase forming a dimer (MM-CK) isoenzyme activities (Figure 1B). No significant differences with the group of healthy subjects were observed whether or not patients were treated with β-blockers in addition to ACEi (Table 3). A 34% lower PGC-1α protein content was observed in patients with CHF (Figure 1C).

Effects of ACE Inhibition on Mitochondrial Function and the Transcriptional PGC-1α Regulatory Cascade in Heart of Infarcted Rats

As described previously, rats with MI exhibited a LV remodeling attested by a significant dilation (42% in LV end-diastolic diameter) and a high score of akinesia (approximately 50% of the LV), as well as a marked reduction in cardiac contractility (fractional shortening, −59%) compared with sham animals. ACE inhibition with perindopril improved survival and tended to increase fractional shortening (36%).[10] The effects of ACEi by perindopril treatment on cardiac energy metabolism were assessed in rats following MI. Maximal oxidative capacity (Figure 2) and activities of CS, COX, and mi-CK were lower by 20% to 30% in LV of MI rats (Table 4) compared with sham. These metabolic defects were associated with the downregulation of gene expression of PGC-1α, NRF-2α, and mitochondrial transcription factor A (mtTFA) and of COX subunits encoded by nuclear and mitochondrial genomes by 50% to 60% in failing hearts (Figure 2).

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Table 3. Effects of Patient Treatments on Metabolic Parameters

	ACEi	ACEi+β blockers	P
Gender	11 M/1 F	7 M/1 F	
Age	50±4	55±2	0.277
LVEF, %	22±3	27±4	0.437
V_0	2.5±0.2	2.3±0.4	0.695
V_{max}	12.0±1.3	12.2±1.4	0.909
CK	3088±275	3179±299	0.824
CS	283±18	267±10	0.434
COX	171±20	139±32	0.423

Values are means±SEM. Activities of CK, CS, and COX are expressed in $\mu mol \cdot min^{-1} \cdot g$ of $protein^{-1}$. V_0 indicates basal respiration rate and V_{max}, maximal respiration rate are expressed in μmol of $O_2 \cdot min^{-1} \cdot g$ dry $weight^{-1}$; LVEF, left ventricular ejection fraction.

Perindopril treatment did not fully prevent the decrease in mitochondrial activity and expression of genes involved in the molecular control of mitochondrial protein expression, except for CS and mitochondrial CK activities and COXI gene expression (Table 4 and Figure 2), evidencing that ACEi therapy was not sufficient to preserve myocardial energetics.

Effects of Humoral Factors Involved in CHF on the Transcriptional Control of Mitochondrial Protein Expression

The next step was to identify the potential effects of circulating hormones on mitochondrial protein expression and transcription cascade in isolated adult rat ventricular myocytes. No effect of drug treatment was observed on cell viability (estimated by the release of lactate dehydrogenase in the culture medium; results not shown). Atrial natriuretic factor gene expression was significantly higher with all hormones except Ang II (Figure 3A). Activity of COX, the complex IV of the respiratory chain, was significantly lower by ET-1 (−40%), whereas Ang II only tended to lower it (Figure 3B) compared with nontreated cells. ET-1 exposure also induced an alteration in mtDNA content (−20%; Figure 3C). We then assessed the expression of PGC-1α and its transcription cascade involved in mitochondrial biogenesis. No significant effect was obtained with Phe, whereas Iso treatment induced a significant increase in PGC-1α and mtTFA mRNA levels (Figure 4). Effectors of the renin-angiotensin-aldosterone system seem to regulate PGC-1α gene expression differentially because we observed an upregulation by Aldo (+60%) and a downregulation by Ang II (−20%; Figure 4). A significant downregulation of PGC-1α (−30%), NRF-2α (−50%), and mtTFA (−20%) gene expression was observed with ET-1, associated with a lower COXI subunit mRNA level (−30%; Figure 4).

Because of the major effects of ET on mitochondrial biogenesis, we then assessed in our experimental model of CHF, the status of the ET system. ET-converting enzyme-1 mRNA expression was higher in myocardium of MI rats compared with sham, and ACEi therapy prevented the upregulation of cardiac ET-converting enzyme-1 (Table 4). Importantly, in patients, circulating ET-1 level was as high as 10.6±1.3 pg/mL (n=16).

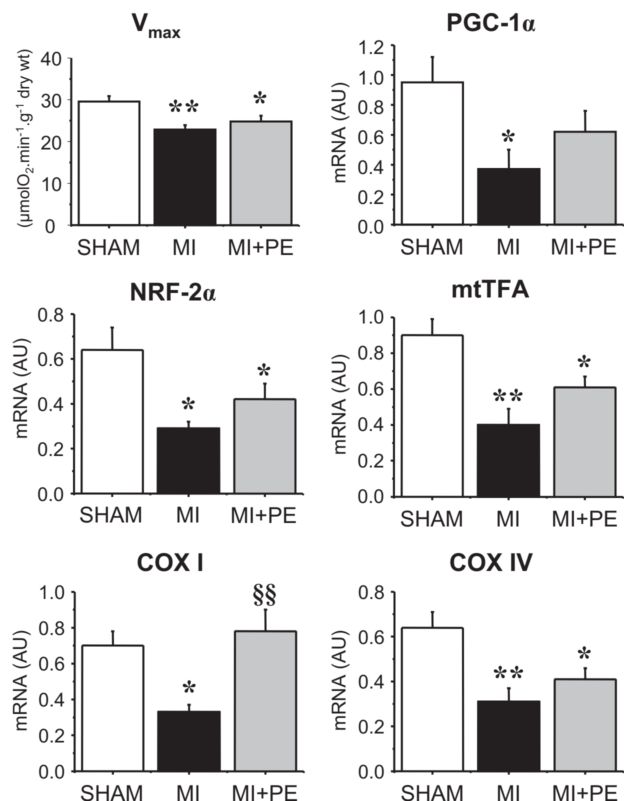

Figure 2. Mitochondrial oxidative capacity and transcriptional PGC-1α regulatory cascade in heart of sham-operated (SHAM), myocardial infarction rats with no medication (MI), or MI rats treated with perindopril (MI+PE). Maximal respiration rates of saponin-skinned fibers (V_{max}), peroxisome PGC-1α, NRF-2 DNA-binding subunit α, mtTFA, cytochrome *c* oxidase subunits encoded by nuclear (COXIV) or mitochondrial (COXI) genomes were measured. Values are mean±SEM (n=7 to 8). *$P<0.05$ and **$P<0.01$ for difference from SHAM group; §§$P<0.01$ for difference from MI group.

Discussion

In this study, we investigated whether patients with CHF treated with ACEi exhibit decreased cardiac mitochondrial function, whether ACEi therapy may be protective for the mitochondrial function in association with the activation of PGC-1α expression in experimental model of heart failure, and which hormonal signals can induce an altered mitochondrial activity through a PGC-1α transcription cascade. We show that (1) in patients with CHF treated with ACEi, cardiac oxidative capacity and PGC-1α protein content are decreased; (2) in a rat model of MI, ACEi therapy is only able to partially prevent mitochondrial function and mitochondrial transcription cascade; (3) in adult cardiomyocytes, ET-1 and, to a minor degree, Ang II can decrease the mitochondrial transcription cascade, whereas other circulating hormones had either no effects (Phe) or positive (Aldo, Iso) effects. Taken together, these results show that oxidative capacity is reduced in human and experimental heart failure, despite ACEi treatment, and that ET-1 and Ang II could be involved in the downregulation of the mitochondrial transcription cascade in heart failure.

Mitochondrial Function in Human Failing Hearts

Oxidative capacity of the myocardial tissue has been assessed in situ by measuring oxygen consumption of permeabilized cardiac

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Table 4. Metabolic Enzyme Activities and mRNA Expression in Rat LV

	SHAM (n=7)	MI (n=7)	MI-PE (n=8)	*P*
Enzyme activity				
Citrate synthase	502±44	398±20*	588±47‡	0.004
Cytochrome *c* oxidase	1645±75	1198±126†	1733±277	0.033
Mitochondrial creatine kinase	528±66	363±45	538±121	0.219
mRNA expression				
Endothelin-1	0.96±0.06	1.14±0.05	1.17±0.09	0.093
Endothelin-converting enzyme	0.85±0.05	1.09±0.10†	0.83±0.04§	0.020

Values are mean±SEM and are expressed in μmol·min^{-1}·g of protein^{-1} for enzyme activity and in arbitrary units for mRNA expression. MI indicates myocardial infarction without treatment; MI-PE, myocardial infarction treated with perindopril.
**P*=0.09 and †*P*<0.05 for difference from SHAM group; ‡*P*<0.01 and §*P*<0.05 for difference from MI group.

fibers of end-stage heart failure patients. Decreased oxidative capacity and metabolic enzymes together with decreased PGC-1α protein content clearly evidence an overall decrease in mitochondrial activity in CHF. Clinical studies of CHF have shown marked cardiac mitochondrial respiratory enzyme dysfunction, albeit variable in the extent and the specific enzymes affected.[21–24] Although a downregulation of adult metabolic gene transcript profile has been observed in failing human heart,[25] another study found altered activity of respiratory chain complexes, without alteration in gene expression in terminally failing human myocardium, identifying β-blockers as one putative protection against this disturbance.[24] Drug treatment of CHF protecting against mitochondrial damage in patients might be considered as explanations for this disparity. Although pharmacological therapy with ACE inhibitors has proved to be effective in patients with heart failure, the basis for these effects is still incomplete. In the present study, patients were all under ACEi therapy and still exhibited decreased oxidative capacity and mitochondrial enzymes, with no difference whether or not they were additionally treated with β-blockers. Although clearly established in experimental heart failure (see ref. 26 for review), the issue of whether mitochondrial transcription cascade is

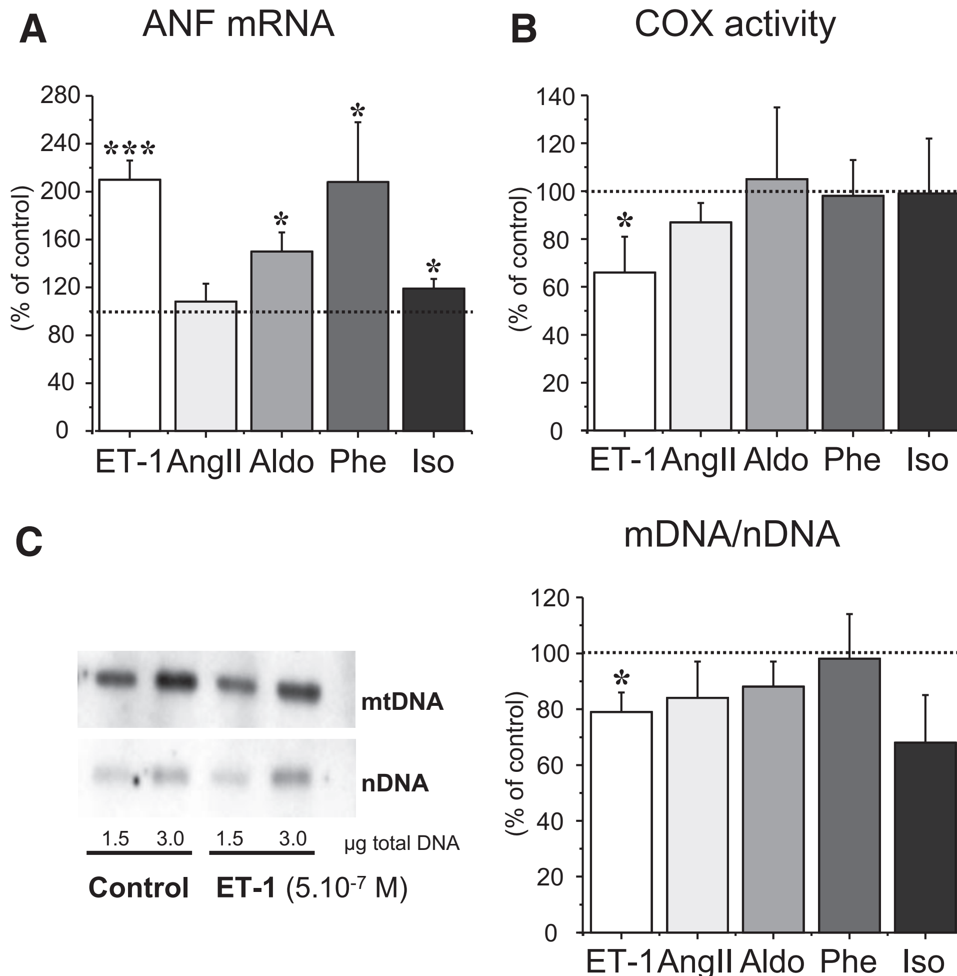

Figure 3. Effects of humoral factors on adult rat ventricular myocytes. Cells were incubated for 48 hours with ET-1 (5×10^{-7} M), Ang II (10^{-6} M), Aldo (5×10^{-8} M), Phe (5×10^{-5} M), or Iso (10^{-8} M). Atrial natriuretic factor gene expression (A), COX activity (B), and mtDNA content (C) were measured. mtDNA content was assessed by Southern blot analysis and expressed as the ratio of mtDNA to nDNA. Values are mean±SEM of 5 independent cultures per experimental condition and are expressed as percentage of control values. **P*<0.05 and ****P*<0.001 for difference from control values.

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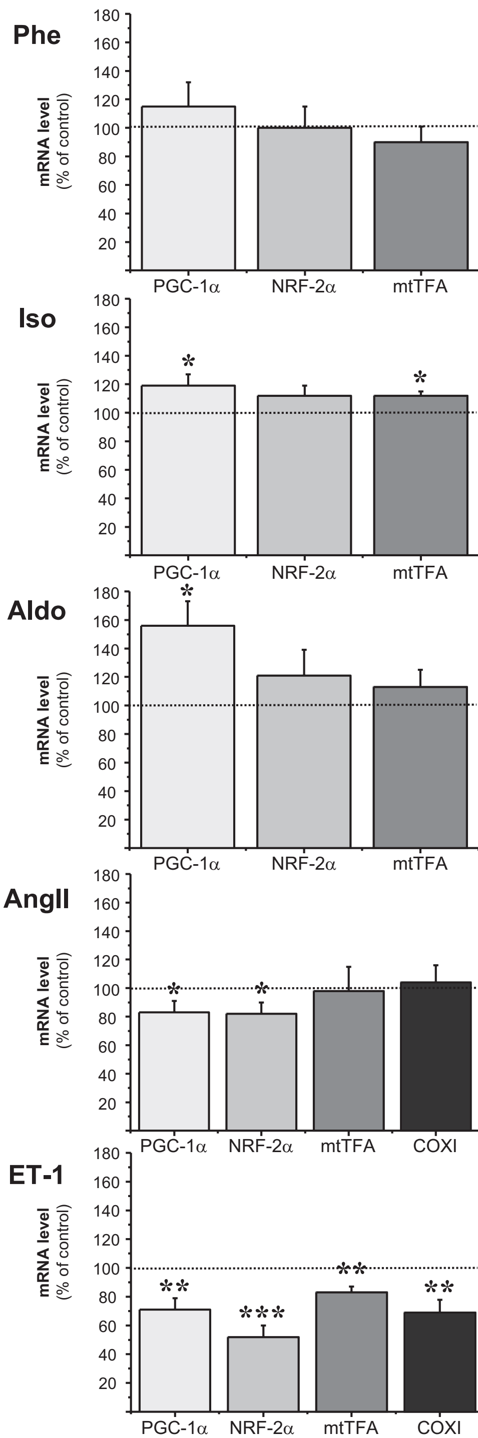

Figure 4. Neurohumoral regulation of the PGC-1α transcriptional cascade in adult rat ventricular myocytes. Cardiac myocytes were exposed for 48 hours to Phe (5×10^{-5} M), Iso (10^{-8} M), Aldo (5×10^{-8} M), Ang II (10^{-6} M), or ET-1 (5×10^{-7} M). mRNA expression of peroxisome PGC-1α, NRF-2 DNA-binding subunit α, mtTFA, and mitochondrial genome-encoded COXI were measured. Values are mean±SEM of 5 independent cultures per experimental condition and are expressed as percentage of control values. *$P<0.05$, **$P<0.01$, and ***$P<0.001$ for difference from control values.

altered in humans is still a matter of debate. Although it was found to be unchanged in terminally failing myocardium,[24] in a very recent study, decreased gene expression of PGC-1α and transcription cascade involved in mitochondrial biogenesis, fatty acid metabolism, and glucose transport was clearly observed in both dilated cardiomyopathy and ischemic heart disease patients.[27] This last result agrees with the decrease in PGC-1α protein content reported here. Thus, despite recent improvement of CHF therapy, myocardium of patients with CHF still exhibits defective mitochondrial function and biogenesis as well as energy transfer whose origin needs to be elucidated.

Angiotensin-Converting Enzyme Inhibition and Mitochondrial Function and Biogenesis in Rats Following Myocardial Infarction

We showed previously that ACEi could totally prevent alterations in mitochondrial function and biogenesis in skeletal muscle of rats with MI.[10] In myocardium of Syrian myopathic hamsters with advanced heart failure, ACEi treatment increases cardiac performance and energy reserve via the CK reaction.[28] Long-term ACEi therapy improves mitochondrial function in rats following MI.[29] To understand better the molecular and cellular effects of ACEi therapy on cardiac mitochondrial function and biogenesis, CHF was induced in rats by MI. Cardiac muscle of MI rats showed a decrease in oxidative capacity together with alterations of mitochondrial protein expression and activity, associated with downregulated expression of PGC-1α and its transcription cascade. Whereas in skeletal muscles, ACEi therapy totally prevented the adverse effects of MI on mitochondrial function and expression of genes involved in the molecular control of oxidative phosphorylation proteins,[10] this protective effect was only partial in cardiac muscle. Similarly, losartan, an Ang II type 1 receptor antagonist, was shown to partially reverse the MI-induced downregulation of PGC-1α expression in male rats[12] but not in female rats.[11] These partial cardiac and total "peripheral" beneficial effects of ACEi therapy on mitochondrial function and biogenesis could thus explain the different results observed in human failing skeletal[14,15] and cardiac muscle (present results). Understanding the events mediating the decreased oxidative capacity in failing heart seems to be of major significance. Although ACE inhibitors exert their beneficial effects by reducing the synthesis of Ang II, there are clinical and experimental evidences that they also prevent the upregulation of other circulating factors, such as ET, cytokines, Aldo, and catecholamines.[30–33]

Effects of Humoral Factors Involved in Chronic Heart Failure on the Transcriptional Control of Mitochondrial Protein Expression

We thus examined the effects of the main hormones known to be activated in heart failure on adult rat ventricular myocytes. Circulating catecholamines are elevated in heart failure; however, neither α nor β-adrenergic stimulation led to decreased expression of the PGC-1α transcription cascade. Indeed, the PGC-1α promoter contains a positive cAMP response element[34] that could explain the increased expression of PGC-1α observed with Iso incubation. It is well known that the renin-angiotensin-aldosterone system is overactivated in heart failure. Interestingly, Ang II decreased PGC-1α expression, whereas Aldo had an opposite effect. Plasma levels of ET-1 are increased in patients with heart failure and correlate with the severity of the disease,[35] and ET substantially contributes to left ventricular remodeling and progression of heart failure.[36] We demonstrate that ET-1 also induced a downregulation of PGC-1α and its

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transcription cascade, NRF-2α and mtTFA. Ang II and ET-1 were shown to play essentially different pathophysiological roles in states of chronic hypertrophy and subsequent CHF. The local ET-1 system remains in the basal state at the stage of compensatory hypertrophy and shows de novo activation during the transition to CHF, whereas Ang II increases at the phase of compensated hypertrophy and remains stable thereafter.[37] Local Ang II and ET-1 systems participate in an autocrine/paracrine manner to their physiological effects. Not only cardiac ET-1 but also ET receptors and ET-converting enzyme are increased in heart failure,[35] and ACEi therapy induces a decrease in circulating and tissue ET-1 levels.[38–41] ET-converting enzyme-1 expression was similarly upregulated in myocardium of MI rats and reversed by PE treatment, showing a partial protective effect on the ET system.[33] Taken together, these results support a causal link between ET-1 and Ang II activation, and decreased PGC-1α expression and oxidative capacity in cardiac muscle and suggest that ACEi could be only partially protective by a complex action on the renin-angiotensin-aldosterone and ET systems.

Limitation of the Study

It was not possible to perform all biochemical and functional measurements for human studies on all subjects. Indeed, functional data need to have freshly collected biopsies. That was possible only on donors on open heart surgery in the control group. In this group, the small amount of collected tissue precluded from performing all of the measurements on the same subjects. Another possible limitation is that the cause of heart failure is multiple in patients, with coronary artery disease and nonobstructive cardiomyopathy being the main causes of heart failure. Heart failure in rats resulted from artificial coronary obstruction. In this model, the nonischemic part of the ventricle, remote from the ischemic region, was used, and heart failure mainly resulted from increased workload on the viable part of the myocardium. Finally, all patients were not under the same ACE inhibitor. However, all of them have demonstrated a clear effect on mortality so that at least class effects are expected.

Conclusion

Interventions that modulate myocardial energy metabolism have been proposed as a new strategy for treatment of CHF, and one possible way might be a direct stimulation of mitochondrial oxidative phosphorylation.[3,26,42] In this way, moderately improving PGC-1α expression or activity in vivo has been proposed to be a promising approach as a metabolic therapy in CHF.[13,26,43,44] Long-term treatment with ET-1 receptor blockers improves survival and cardiac function and decreases LV hypertrophy and fibrosis.[45] It also partially reverses myocardial dysfunction and specific mitochondrial enzyme deficiencies in pacing-induced CHF,[46] underlining the importance of this hormone for the control of cardiac energy metabolism. In view of the synergistic and supplemental role of Ang II and ET-1, long-term therapy with a combination of ACEi and ET antagonism could be beneficial for heart failure patients.[37,47] Although promising in terms of improvement of heart failure and remodeling in several animal models, ET-1 receptor antagonists in patients with CHF have shown neutral effects in terms of mortality and symptoms. However, selectivity toward ET receptors as well as time or duration of administration in these trials could be questioned,[35,48] and their effects on energy metabolism and mitochondrial biogenesis need to be assessed.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Heart failure is still a major cause of mortality, and a better knowledge of its pathophysiology will be useful to optimize patient medical treatments. This study supports a key involvement of endothelin-1 and angiotensin II in the impaired energetic capacities of the human failing myocardium. Accordingly, angiotensin-converting enzyme inhibition reduced cardiac mitochondrial dysfunction and improved mitochondrial biogenesis. Such improvement was only partial, but the results of this study (and of other works demonstrating a beneficial effect of endothelin-1 antagonists in the setting of heart failure) support that simultaneous inhibition of angiotensin-converting enzyme and endothelin-1 activities might be synergistic and might significantly improve cardiac energetic in patients with heart failure. These data support the view that myocardial energy metabolism modulation could be a pertinent strategy for treating heart failure. Although still a matter of debate, large-scale clinical studies further investigating the effect of endothelin-1 antagonists on top of renin-angiotensin system inhibitors (using appropriate doses for enough time) might be warranted to improve both cardiac energetic and heart failure patients prognosis.

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