SUPPLEMENTAL DATA

Cardiac Mitochondria in Heart Failure: Decrease in Respirasomes and Oxidative Phosphorylation

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Short title: Mitochondrial integrative function in heart failure

METHODS

The activity of the electron transport chain (ETC) complexes in heart tissue homogenates.

Samples of fresh heart tissue homogenate were suspended to 10 mg/ml MSM-EDTA buffer (75 mM mannitol, 220 mM sucrose, 5 mM Mops, and 2 mM EDTA, pH 7.4) supplemented with 1 mg cholate/1 mg heart wet wt and 1 μl/ml mammalian protease inhibitor cocktail. ETC complex activities were measured spectrophotometrically as specific donor-acceptor oxidoreductase activities in phosphate buffer 0.1M. Both donors and acceptors span specific regions in the ETC. NADH-cytochrome c reductase was measured as the rotenone-sensitive oxidoreductase and assesses complexes I and III. Succinate-cytochrome c reductase was measured as the antimycin A-sensitive oxidoreductase and assesses complexes II and III. Decylubiquinol-cytochrome c oxidoreductase was measured as the antimycin A-sensitive reductase to assess complex III. Cytochrome c oxidase (COX) was measured as the oxidation of reduced cytochrome c and expressed as the first order rate constant.

Separation of mitochondrial complexes by Blue Native PAGE electrophoresis

Individual mitochondrial complexes were extracted with dodecyl β-D-maltoside and separated on 6-13% acrylamide-bisacrylamide gradient gels at room temperature.

RESULTS

Oxidative phosphorylation

1. Oxidative phosphorylation in the presence of lipid substrates also was performed in order to investigate the changes in mitochondrial substrate preference in the failing heart. Our approach was to measure oxidative phosphorylation rates in the presence of CPT I-dependent and CPT I-independent substrates. Palmitoyl-CoA transported into the mitochondrial intermembrane space is converted to palmitoylcarnitine by the enzyme CPT I. Palmitoylcarnitine is transported into the mitochondrial matrix via the carnitine-acylcarnitine transporter bypassing CPT I. These two substrates dissect whether a change in fatty acid utilization by mitochondria is due to either modification of kinetic properties and maximal activity of CPT I or mitochondrial oxidation of fatty acids and their reducing equivalents. With palmitoyl-CoA (+carnitine+malate), both SSM and IFM isolated from HF hearts have state 3 rates of approximately 50% of control states 3. Heart SSM and IFM isolated from HF also have approximately 50% state 3 respiratory rates compared with the control in the presence of CPT-I independent substrates (palmitoylcarnitine+malate) (**Figure S1**). These results show that the decrease in fatty acid oxidation in both SSM and IFM is not localized at the level of CTP-I, but is consistent with the decreased oxidation of reducing equivalents in the mitochondrial ETC.

2. The adenine nucleotide translocase (ANT) was investigated polarographicaly in the presence of different substrates and saturating concentrations of ADP. Respiratory states 3 were not improved by the addition of saturating concentrations of ADP (**Figure S2**). These

data show that HF does not alter the kinetic properties of the ANT. Since the respiratory rates were not improved by the uncoupler (**Figure 3** in the main text), the data also indicate that the defect is localized in mitochondrial structures situated upstream from the ANT in the respiratory chain.

Activity of mitochondrial electron transport chain (ETC) enzymes in heart muscle homogenate

When measured in fresh heart muscle homogenate, the individual activity of antimycin A-sensitive decylubiquinol-cytochrome c oxidoreductase (complex III) was significantly increased in HF (**Figure S3**). However, the combined activities of complexes I/III (rotenone-sensitive NADH cytochrome c reductase, NCR) and complexes II/III (antimycin-sensitive succinate cytochrome c reductase, SCR) were unchanged, as well as the activity of cytochrome c oxidase (complex IV).

Mitochondrial individual ETC complexes

The amounts of individual mitochondrial complexes extracted with dodecyl β-D-maltoside and separated by BN-PAGE were unchanged in both heart SSM and IFM in HF (**Figure S4**). Also, all mitochondrial complexes shared the same electrophoretic mobility in both control and HF mitochondria, showing their subunit integrity.

FIGURE LEGENDS

Figure S1. State 3 respiratory rates of heart SSM and IFM with lipid substrates. State 3 was induced by 200 μM ADP; PalCoA + Carn + Malate: Palmitoyl CoA + Carnitine + Malate; PalCarn + Malate: Palmitoylcarnitine + Malate. * $p<0.05$ control (N=5) *vs* heart failure (N=5). Mean ± SEM.

Figure S2. Maximal (state 3, 2 mM ADP) respiratory rates of heart SSM and IFM. Substrates that donate reducing equivalents to complexes I (Glutamate), II (Succinate + rotenone), III (Duroquinol, DHQ + rotenone) and IV *via* cytochrome c (TMPD ascorbate + rotenone). * $p<0.05$ control (N=5) *vs* heart failure (N=5). Mean ± SEM.

Figure S3. The activity of ETC enzymes in heart muscle homogenate. The activity of enzymes was measured in detergent solubilized fresh heart muscle from control (N=5) and heart failure (N=5) dogs, and expressed as micromol/min/g wet wt (U/g) or as the first-order rate constant (k=1/min/g wet wt) for complex IV. NCR=rotenone-sensitive NADH-Cytochrome c Reductase; SCR=antimycin A-sensitive Succinate-Cytochrome c Reductase. C III=Complex III; C IV= Complex IV. Mean ± SEM.

Figure S4. Separation of mitochondrial ETC complexes by 1D BN-PAGE in SSM and IFM of control and heart failure hearts. Dog heart mitochondria were solubilized with dodecyl β-D-maltoside. The solubilized ETC complexes were separated by 1D BN-PAGE. C I, C II, C III, C IV and C V indicate mitochondrial complexes I-V. 1D BN-PAGE, one dimension blue native PAGE.

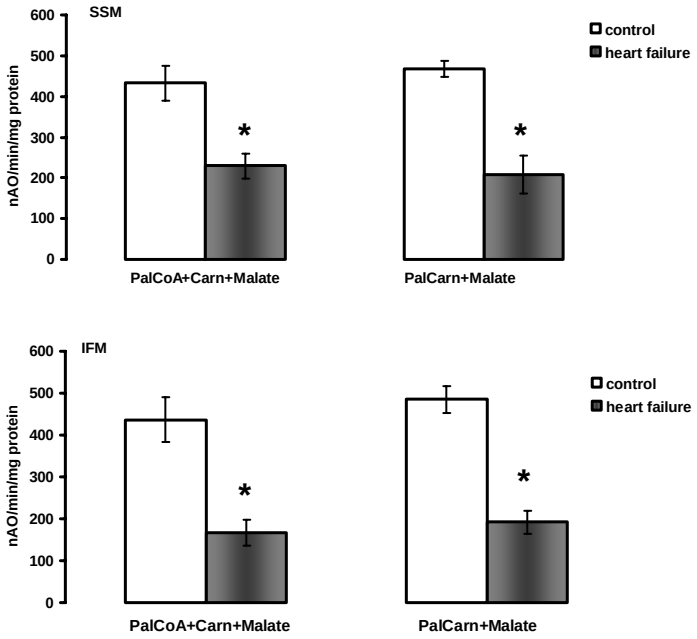
SSM
IFM
nAO/min/mg protein
600
500
400
300
200
100
0
control
heart failure
*
PalCoA+Carn+Malate
PalCarn+Malate

Figure S1

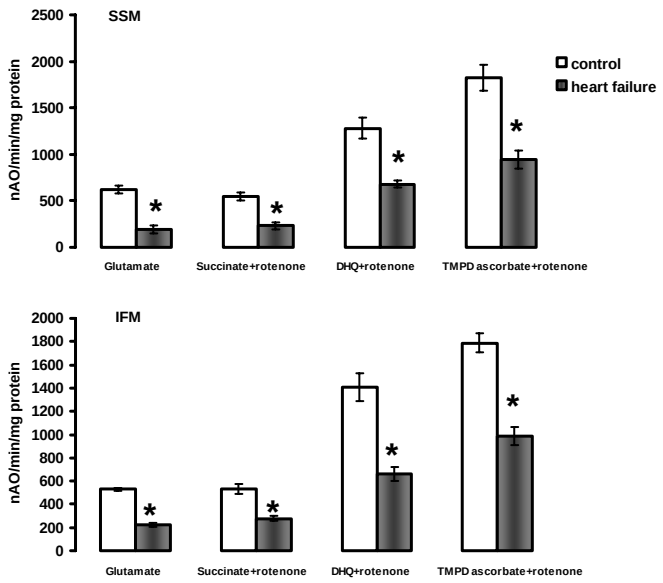
SSM
nAO/min/mg protein
2500
2000
1500
1000
500
0
control
heart failure
*
Glutamate
Succinate+rotenone
DHQ+rotenone
TMPD ascorbate+rotenone
IFM
nAO/min/mg protein
2000
1800
1600
1400
1200
1000
800
600
400
200
0
Glutamate
Succinate+rotenone
DHQ+rotenone
TMPD ascorbate+rotenone

Figure S2

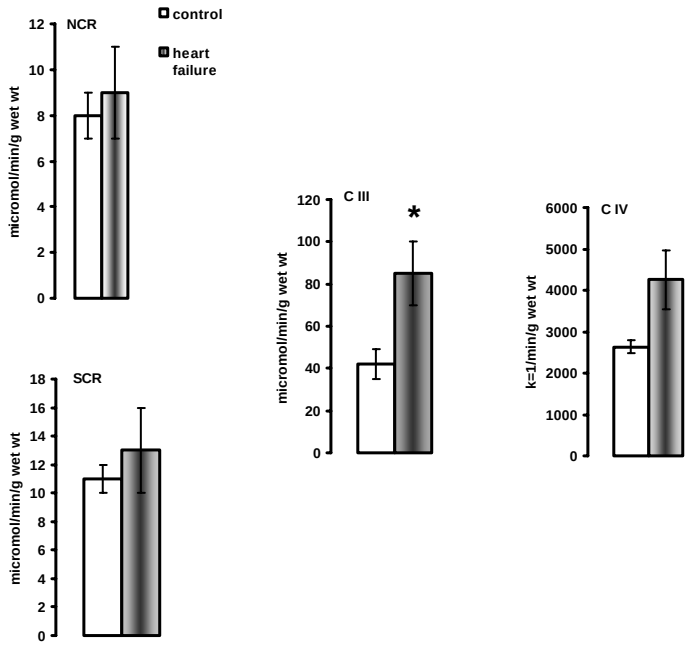
NCR
control
heart failure
micromol/min/g wet wt
12
10
8
6
4
2
0
SCR
micromol/min/g wet wt
18
16
14
12
10
8
6
4
2
0
C III
*
micromol/min/g wet wt
120
100
80
60
40
20
0
C IV
k=1/min/g wet wt
6000
5000
4000
3000
2000
1000
0

Figure S3

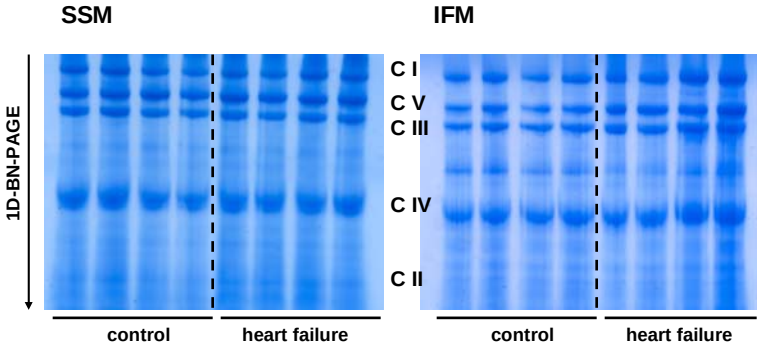
SSM
IFM
1D-BN-PAGE
C I
C V
C III
C IV
C II
control
heart failure
control
heart failure

Figure S4