NHE-1 inhibition improves cardiac mitochondrial function through regulation of mitochondrial biogenesis during postinfarction remodeling

Sabzali Javadov, Daniel M. Purdham, Asad Zeidan, and Morris Karmazyn

Department Physiology and Pharmacology, University of Western Ontario, London, Ontario, Canada

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Javadov, Sabzali, Daniel M. Purdham, Asad Zeidan, and Morris Karmazyn.

The energy fuel shift and mitochondrial oxidative dysfunction in heart failure are driven by the dysregulation of FAO and glucose-oxidizing enzyme genes (12, 13, 27).

Mitochondrial proteins are encoded by both nuclear and mitochondrial genomes. The mitochondrial genome is comprised of a closed circular DNA [mitochondrial DNA (mtDNA), ~16.6 kb] containing 37 genes that encode 13 subunits of respiratory chain complexes I, III, IV, and V, 2 rRNAs for mitochondrial ribosome, and 22 tRNAs for translation. The rest of the subunits of respiratory chain complexes and other mitochondrial proteins are encoded by nuclear DNA (nDNA). Mitochondrial function is regulated by the coordinated expression of nuclear and mitochondrial genes encoding mitochondrial proteins.

Recently, a number of transcription factors regulating the expression of both nuclear and mitochondrial genes responsible for mitochondrial biogenesis and function were discovered. Peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) is expressed in mitochondria-rich tissues such as skeletal muscle, heart, liver, and adipose tissue and serves as a key regulator of oxidative energy metabolism (12, 25, 34, 41). PGC-1α coactivates transcription factors nuclear respiratory factor 1 (NRF-1) and 2 (NRF-2), which are involved in regulating mitochondrial biogenesis and respiratory function. NRF-1 and NRF-2 in turn regulate downstream genes, including mitochondrial transcription factor A (MTF-A), which is responsible for the maintenance as well as replication and transcription of mtDNA. Expression of nuclear genes encoding proteins of mitochondrial respiratory chain and oxidative phosphorylation are also regulated by NRF-1 and NRF-2.

Na⁺/H⁺ exchange-1 (NHE-1) inhibition has been shown to offer beneficial effects in the postinfarcted myocardium in terms of cardiac hypertrophy, remodeling, and heart failure. Administration of the potent NHE-1-specific inhibitors cariporide (18, 32, 44) or EMD-87580 (EMD; see Ref. 5) results in attenuation and reversal of postinfarction remodeling and heart failure independently of infarct size or afterload reduction. Recent studies have demonstrated an anti-hypertrophic effect of cariporide in vitro in response to neurohormonal adrenoreceptor (29) and mechanical stretch-induced (42) stimuli. Despite emerging evidence for the anti-remodeling effect of NHE inhibition, potential mechanisms explaining these effects still are far from being fully elucidated. Recently, we have demonstrated that an anti-remodeling effect of NHE-1 inhibition by EMD is mediated, at least in part, by the improvement of mitochondrial respiratory function and prevention of mitochondrial permeability transition (MPT) pore opening (14).

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However, it is not known whether NHE-1 inhibition-induced protection of mitochondria against postinfarction remodeling is mediated through regulation of the posttranscriptional abnormalities of mitochondrial proteins or as a result of defects in the transcriptional network responsible for mitochondrial metabolism. In this study, we hypothesized that the preservation of mitochondrial function in the presence of NHE-1 inhibition might be a result of normalization of the expression of transcription factors regulating mitochondrial biogenesis and respiratory function. Accordingly, the effects of EMD were examined in hearts subjected to 12 and 18 wk of coronary artery ligation (CAL) or a sham-operated procedure. We quantified mRNA expression of transcription factors PGC-1α, NRF-1, NRF-2 and MTF-α as well as one nuclear (COXNUC- SUB IV)- and one mitochondrial (COXMTSUB 1)-encoded subunit of cytochrome c oxidase in the complex IV of the respiratory chain. Linear regression analysis was carried out to elucidate whether changes in mRNA level of transcription factors correlate with mitochondrial respiration rates and the expression of hypertrophic marker gene.

**METHODS**

**Animal groups and surgical procedures.** Animals were maintained in the Health Sciences Animal Care Facility of the University of Western Ontario in accordance with the guidelines of the Canadian Council on Animal Care (Ottawa, Ontario, Canada). Rats were randomly assigned to the following four treatment groups: 1) sham group; 2) sham + EMD group; 3) CAL group, and 4) CAL + EMD group. The surgical procedure was performed as previously described (5, 18). Briefly, male Sprague-Dawley rats weighing 285–320 g, purchased from Charles River (St. Constant, Quebec, Canada), were anesthetized with intraperitoneal pentobarbital sodium, intubated, and artificially ventilated by using a rodent respirator (model 683; Harvard Apparatus). A left thoracotomy was performed, and the heart was gently exposed. To induce myocardial infarction, the left main coronary artery was ligated ~3 mm from its origin by using a firmly tied silk suture (5-0). For the sham procedure, the ligature was placed in an identical fashion but not tied. The chest was then closed in three layers (ribs, muscle, and skin), and the animal was allowed to recover. The animals were then followed for either 12 or 18 wk after surgery. Previously, we have shown that the myocardial infarction induced by CAL produces heart failure with a significant attenuation in cardiac output and stroke volume at 12 wk after ligation (5).

**Diets.** Rat chow containing the NHE-1 inhibitor EMD at a concentration of 200 parts/million or EMD-free identical chow were generously provided by Merck KGaA (Darmstadt, Germany). Details of EMD characteristics in terms of potency and selectivity vs. other NHE isoforms have been published recently (5).

**In vitro anoxia/reoxygenation.** Mitochondria (1 mg of protein) were placed and stirred at 30°C in an air-tight closed oxygraph chamber with 1.8 ml of incubation buffer containing (in mM) 125 KCl, 20 MOPS, 10 Tris, 0.5 EGTA, and 2 KH2PO4, pH 7.2, that was supplemented with 2.5 mM 2-oxoglutarate and 1 mM L-malate as substrates.

To obtain kinetic parameters for ADP, mitochondrial respiration rates were measured in the presence of the increasing concentrations (from 10 to 500 μM) of ADP. To determine the apparent K_m for ADP and the maximal respiration rate (V_max), the ADP-stimulated oxygen consumption was plotted as a function of ADP.

**Measurement of citrate synthase.** Citrate synthase (CS) activity was assayed spectrophotometrically at 412 nm by measuring the rate of 5,5′-dithio-bis-(2-nitrobenzoic acid)-reduced coenzyme A formation (30°C, pH 7.4) as previously described (33).

**Real-time PCR analysis.** Total RNA was isolated from frozen myocardial samples (80–100 mg) using the TRizol reagent according to the manufacturer instructions (Invitrogen, Carlsbad, CA). Total RNA (1 mg) was used to synthesize the first strand of cDNA using random hexamer primers (Invitrogen) and SuperScript II Rnase H RT (Invitrogen) as per the manufacturer’s instructions. The cDNA was diluted 10-fold, and 1 μl of the diluted cDNA was used in a 20-μl reaction. Real-time PCR was performed using SYBR green Jumpstart Tag ReadyMix DNA polymerase (Sigma-Aldrich, Oakville, ON, Canada), and fluorescence was measured and quantified using DNA Engine Opticon 2 System (MJ Research, Waltham, MA). Forward and reverse primers were designed individually by a basic local alignment search tool (BLAST) search to obtain a sequence homology only for the target gene. Table 1 shows sequences of the primers used for amplification. PCR conditions and cell cycle number were optimized for each set of primers. Melting curve analysis showed a single PCR product for each gene amplification. PCR conditions to amplify all gene expression data obtained in this study. Multiple comparisons between groups were determined by using three-way ANOVA followed by Tukey’s post hoc test. An unpaired two-tailed Student’s t-test was used to compare mean differences between groups. Linear regression analysis was used to determine the relations between transcription factor genes and mitochondrial respiratory parameters, hypertrophic gene markers, or nuclear- and mitochondrial-encoded protein genes. All values are presented as means ± SE. Differences were considered to be statistically significant at a level of P < 0.05.

**RESULTS**

**Gene expression of atrial natriuretic peptide and NHE-1 and mitochondrial function.** All gene expression data obtained in this study were expressed relative to the mRNA level of β-actin as the housekeeping gene. There was no effect of any treatment on β-actin expression. Thus β-actin values (in arbitrary units) for the 12-wk ligated group were 0.17 ± 0.02, 0.16 ± 0.03, 0.15 ± 0.02, and 0.16 ± 0.02 for sham, sham + EMD, CAL, and CAL + EMD groups, respectively, whereas corresponding values for animals subjected to 18 wk of CAL were 0.14 ± 0.02, 0.13 ± 0.01, 0.12 ± 0.01, and 0.12 ± 0.01, respectively. CAL induced an increase in expression of the hypertrophic gene marker atrial natriuretic peptide (ANP) by 7.8 (P < 0.05) and 3.0 (P < 0.05)-fold 12 and 18 wk after ligation, respectively, which was significantly attenuated by EMD (Fig. 1A). NHE-1 mRNA levels were also significantly enhanced by CAL, although this was seen only after 12 wk ligation, with values returning to near-control levels after 18 wk. The increase in NHE-1 expression at 12 wk was significantly attenuated by EMD. It should be noted that CAL-induced increases in ANP and NHE-1 gene expression were associated with a significant increase in heart weight-to-body weight ratios (mg/g) at 12 wk (from 2.91 ± 0.08 to 3.69 ±
0.03, $P < 0.01$) and 18 wk (from 2.82 ± 0.09 to 3.45 ± 0.09, $P < 0.01$) after ligation. There were no significant differences between the 12 and 18 wk postligation groups, although the response was prevented by EMD treatment at both time points (12 wk: 3.17 ± 0.08 CAL + EMD vs. 2.83 ± 0.08 sham + EMD; 18 wk: 3.01 ± 0.07 CAL + EMD vs. 2.86 ± 0.05 sham + EMD).

As shown in Fig. 2, mitochondria isolated from the hearts subjected to CAL exhibited altered oxygen consumption rates in response to ADP addition in concentrations from 10 to 500 μM. CAL for 12 and 18 wk significantly decreased $V_{\text{max}}$ of mitochondria in the presence of 500 μM ADP from 207 ± 9 and 199 ± 7 nmol oxygen·min$^{-1}$·mg protein$^{-1}$ in sham-operated hearts to 147 ± 9 ($P < 0.001$) and 172 ± 7 ($P < 0.01$) nmol oxygen·min$^{-1}$·mg protein$^{-1}$ in the CAL group 12 and 18 wk after ligation, respectively (Fig. 2, A and B). EMD treatment significantly preserved the oxygen consumption capacity, increasing $V_{\text{max}}$ in ligated hearts at 12 and 18 wk to 175 ± 10 ($P < 0.05$) and 192 ± 7 ($P < 0.05$ vs. CAL group) nmol oxygen·min$^{-1}$·mg protein$^{-1}$, respectively, thus improving oxidative phosphorylation in mitochondria. Double-reciprocal (Lineweaver-Burke) plots of 1/O$_2$ consumption rates vs. 1/[ADP] demonstrated no significant effect of ligation at either 12 or 18 wk CAL on $K_m$ values (Fig. 2, C and D). $K_m$ values for ADP in mitochondria isolated from sham, sham + EMD, CAL, and CAL + EMD groups were 33, 31, 30, and 30 μM 12 wk and 32, 32, 29, and 31 μM 18 wk after ligation or sham procedure, respectively.

**Gene expression of transcription factors regulating mitochondrial biogenesis and function.** Gene expression of four transcription factors (PGC-1α, MTF-A, NRF-1, and NRF-2) was quantified using real-time PCR to examine the possible contribution of these factors to the remodeling in the presence or absence of EMD. As shown in Fig. 3A, CAL induced a 52% ($P < 0.01$) and 50% ($P < 0.01$) decrease in the mRNA level of PGC-1α 12 and 18 wk after ligation, respectively. EMD treatment had no effect on its own in sham animals but significantly preserved the mRNA level of PGC-1α 12 and 18 wk after CAL. EMD treatment significantly preserved the mRNA level of MTF-A 12 and 18 wk after CAL, and CAL + EMD vs. CAL group.$^*$

Table 1. **Primer sequences used for real-time PCR amplification**

<table>
<thead>
<tr>
<th>Gene</th>
<th>GenBank Accession Number</th>
<th>Primer (5’-3’)</th>
<th>PCR Product, bp</th>
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<tr>
<td>ANP</td>
<td>X00665</td>
<td>F: CTGCTAGACCCCTGGAGGA</td>
<td>319</td>
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<tr>
<td>β-Actin</td>
<td>NM_03144</td>
<td>R: AAGCTCTTGCAAGCTAGTC</td>
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<td>COXMITSUB I</td>
<td>X14848</td>
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<td>244</td>
</tr>
<tr>
<td>COXNUCSUB IV</td>
<td>X15029</td>
<td>R: CCTGCTACGTGTCCTTG</td>
<td>171</td>
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<td>MCIP1</td>
<td>AB075973</td>
<td>F: GGCAAAATCGCAACAAAGT</td>
<td>263</td>
</tr>
<tr>
<td>MTF-A</td>
<td>AJ312746</td>
<td>R: CCCTCGAGGCTAGATAGAT</td>
<td>168</td>
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<td>M85299</td>
<td>F: TCTGCTAGACCTGGTAAAG</td>
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<td>NRF-1</td>
<td>U27700</td>
<td>R: TGACTGAGGAGGATTGTA</td>
<td>124</td>
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<td>NRF-2</td>
<td>M74515</td>
<td>F: TGGCATGAGGAGGATGAA</td>
<td>208</td>
</tr>
<tr>
<td>PGC-1α</td>
<td>NM_031347</td>
<td>R: GCGAATTTTGGAGTCTG</td>
<td>241</td>
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**ANP** atrial natriuretic peptide; **COXMITSUB I** and **COXNUCSUB IV**, mitochondrial- and nuclear-encoded subunits of cytochrome c oxidase in complex IV of the respiratory chain; **MCIP1**, myocyte-enriched calcineurin-interacting protein 1; **MTF-A**, mitochondrial transcription factor A; **NHE-1**, Na$^+$/H$^+$ exchange 1; **NRF-1** and **NRF-2**, nuclear respiratory factors 1 and 2, respectively; **PGC-1α**, proliferator-activated receptor-γ coactivator 1α; F, forward primer; R, reverse primer.
prevented the decrease in PGC-1α gene expression in hearts both 12 and 18 wk after CAL. Linear regression analysis demonstrated a strong positive correlation between PGC-1α mRNA expression and $V_{\text{max}}$ ($r = 0.892, P < 0.001$) as well as CS ($r = 0.843, P < 0.001$) in mitochondria isolated from these hearts (Fig. 3, B and C).

As shown on Fig. 4, postinfarction remodeling was associated with significant reductions in gene expression of NRF-1, NRF-2, and MTF-A, which were found to be relatively identical for the 12- and 18-wk postligation period. EMD treatment attenuated CAL-induced reduction of the gene expression of transcription factors both 12 and 18 wk after CAL, although the difference for MTF-A in the CAL/EMD group with 18 wk ligation was not statistically significant from the respective CAL value.

Gene expression of COXMTSUB I and COXNUCSUB IV.

One nDNA-encoded (COXNUCSUB IV) and one mtDNA-encoded (COXMTSUB I) subunit of cytochrome c oxidase (complex IV of the respiratory chain) were studied to elucidate whether the postinfarction remodeling process with or without EMD treatment can affect gene expression of respiratory chain complexes. The results demonstrate parallel decreases in the expression of mRNA for both subunits by 25% ($P < 0.05$) and 16% ($P < 0.05$) for 12 wk postinfarcted hearts and 33% ($P < 0.01$) and 21% ($P < 0.05$) for 18 wk postinfarcted hearts, respectively (Fig. 5, A and B). Under most conditions, EMD prevented the decreased expression of both COXNUCSUB IV and COXMTSUB I in hearts subjected to both durations of CAL, although with respect to COXNUCSUB IV no effect was observed in hearts subjected to 18 wk of ligation (Fig. 5B). There was a positive correlation between the gene expression of COXNUCSUB IV or COXMTSUB I and PGC-1α, a key transcription factor regulating the synthesis of both nDNA- and mtDNA-encoded mitochondrial proteins (Fig. 5, C and D).

Gene expression of myocyte-enriched calcineurin-interacting protein 1.

Activity of PGC-1α is upregulated by numerous signaling pathways, including Ca$^{2+}$-dependent, nitric oxide, mitogen-activated protein kinase, and β-adrenergic pathways (12). One of the regulators of the PGC-1α pathway is calcineurin, a Ca$^{2+}$-sensitive phosphatase. A recent study has shown that both calcineurin and Ca$^{2+}$/calmodulin-dependent kinase activate PGC-1α expression in cardiomyocytes and induce distinct effects on downstream targets of the PGC-1α pathway (28). Studies to determine the role of calcineurin in cardiac hypertrophy and heart failure have produced contradictory results. For example, one report demonstrated that calcineurin is activated (21), but others found either no change (45) or a decrease (7) of calcineurin activity. Myocyte-enriched calcineurin-interacting protein 1 (MCIP1) is used as a marker of calcineurin activation since it has been shown that expression of this protein is induced by calcineurin (43). We measured the expression of mRNA for MCIP1 in sham-operated and ligated hearts in the presence and absence of EMD. CAL for 12 and 18 wk tended to reduce MCIP1 gene expression by 27% (sham: 4.71 ± 0.93 and CAL: 3.43 ± 0.48 AU, P < 0.22) and 31% (sham: 7.91 ± 1.02 and CAL: 5.44 ± 1.19 AU, P <
Moreover, mRNA levels in the CAL/H11006 EMD group (4.16 ± 0.69, P/H11021 0.38 and 7.99 ± 1.39 AU, P/H11021 0.16 at 12 and 18 wk, respectively) tended to be higher than in CAL group, although differences in both cases were not statistically significant.

Respiratory function of mitochondria during in vitro anoxia/reoxygenation. To study a possible direct effect of NHE inhibition on mitochondria, isolated mitochondria were subjected to in vitro anoxia/reoxygenation in the presence or absence of 5 μM EMD in medium at pH 6.4, 6.8, and 7.2. As shown in Fig. 7, anoxia/reoxygenation induced a significant decrease of state 3 respiration at all three pH values. However, EMD was unable to preserve mitochondrial function. These results demonstrate that EMD exerts an indirect influence on mitochondrial function.

DISCUSSION

The aim of the present study was to investigate the potential contribution of the transcriptional network regulating cardiac mitochondrial biogenesis and respiratory function to the anti-remodeling effect of NHE-1 inhibition. Our results demonstrate that CAL induces downregulation of the expression of PGC-1α, NRF-1, NRF-2, and MTF-A genes as well as mitochondrial- and nuclear-encoded subunits of complex IV (COXMITSUB I and COXNUCSUB IV) of the respiratory chain. Moreover, we show that alterations in gene expression of transcription factors and subunits of the respiratory chain complex IV were attenuated in mitochondria from hearts of animals treated with the NHE-1-specific inhibitor EMD. Our

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Fig. 3. Effect of EMD on gene expression of peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) in hearts of sham-operated and CAL rats. A: mRNA level of PGC-1α. The values were normalized to β-actin expression and are presented as AU. B and C: correlations between the mRNA level of PGC-1α and maximal mitochondrial respiration rate (Vmax) or enzymatic activity of mitochondrial citrate synthase (CS) in hearts 12 wk (open symbols) and 18 wk (filled symbols) after CAL and sham procedure with or without EMD treatment. Vmax of isolated mitochondria was measured in the presence of 500 μM ADP. P < 0.05 CAL vs. sham group (*) and CAL + EMD vs. CAL group (#); n = 9.

Fig. 4. Effect of EMD on the mRNA expression level of nuclear respiratory factor 1 (NRF-1; A), nuclear respiratory factor 2 (NRF-2; B), and mitochondrial transcription factor A (MTF-A; C) in hearts of sham-operated and CAL rats. The values were normalized to β-actin expression and are presented as AU. *P < 0.05 and **P < 0.01 CAL vs. sham group. #P < 0.05 and ##P < 0.01 CAL + EMD vs. CAL group; n = 9.
results demonstrate a significant relationship between levels of the transcription factor PGC-1α and the maximal mitochondrial respiratory rate, mitochondrial CS activity, and expression of COXMITSUB I and COXNUCSUB IV. Last, we also show that the protective effect of EMD on mitochondria likely represents an indirect effect of this agent.

Our study also shows a general lack of effect of prolonging coronary occlusion from 12 to 18 wk on either gene expression changes (ANP, NHE-1, or the mitochondrial transcription factors NRF-1 and MTF) or mitochondrial respiration rates. The reasons for this are uncertain, but these results agree with our previous observation that neither cardiac hypertrophy nor MPT pore opening is further affected when the period of CAL is extended from 12 to 18 wk (14). Although one initial possibility for the lack of further effect is the lower yield of

Fig. 5. Effect of EMD on gene expression of mitochondrial DNA (mtDNA)-encoded subunit of cytochrome c oxidase (COXMITSUB I) and nuclear DNA (nDNA)-encoded cytochrome c oxidase subunit IV (COXNUCSUB IV) in hearts of sham-operated and CAL rats. A and B: mRNA levels of COXMITSUB I and COXNUCSUB IV normalized to β-actin transcription. The values were normalized to β-actin skeletal expression and are presented as AU. C and D: correlations between the mRNA levels of COXMITSUB I or COXNUCSUB IV and PGC-1α in hearts 12 wk (open symbols) and 18 wk (filled symbols) after CAL and sham procedure with or without EMD treatment. *P < 0.05 and **P < 0.01 CAL vs. sham group. P < 0.05 CAL + EMD vs. CAL group (#) and CAL + EMD vs. sham + EMD (+).

Fig. 6. Effect of EMD on mRNA expression of calcineurin-interacting protein 1 (MCIP1) in heart of sham-operated and CAL rats. The values were normalized to β-actin expression and are presented as AU; n = 9.

Fig. 7. Effect of EMD on state 3 of mitochondrial respiration rate during in vitro anoxia/reoxygenation. Isolated mitochondria of rat heart were subjected to 20 min anoxia followed by 20 min reoxygenation in oxygraph chamber in the absence or presence of 5 μM EMD at pH 6.4, 6.8, and 7.2. Rates of oxygen consumption were measured in the absence (state 2) and presence (state 3) of 1 mM ADP. *P < 0.05 and **P < 0.01 vs. control group; n = 5.

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viable mitochondria after 18 wk CAL due to more severe mitochondrial damage in situ, it should be noted that CS activity in total tissue homogenate from both 12 and 18 wk ligated hearts tended to be reduced (22%, $P < 0.07$ and 17%, $P < 0.08$, respectively) compared with sham. In contrast, the decrease in CS activity in mitochondria isolated from both 12 wk and 18 wk ligated hearts was statistically significant, thereby suggesting that the CAL-induced mitochondrial dysfunction reflected a direct effect on mitochondrial integrity rather than a decrease in mitochondrial content. In other words, the mitochondrial content of the hearts was not decreased in ligated hearts, but mitochondria became more fragile and were thus lost to a greater degree during the isolation procedure. We found no difference in myocardial CS activity between 12 wk (98 ± 10 μmol·min⁻¹·g wet wt⁻¹) and 18 wk (104 ± 9 μmol·min⁻¹·g wet wt⁻¹) ligated hearts; thus, it is unlikely that the latter were more damaged than 12 wk ligated hearts. Moreover, although CAL induced a significant decrease in CS activity in mitochondria 12 and 18 wk after ligation, there was no significant difference between 12-wk (2.11 ± 0.10 μmol·min⁻¹·mg protein⁻¹) and 18-wk (1.97 ± 0.03 μmol·min⁻¹·mg protein⁻¹) CAL groups. It is more likely that the basis for the lack of effect of continued coronary occlusion on various parameters reflects an insufficient period of time to demonstrate exacerbation of functional and molecular parameters and the unexplored role of adaptive responses with chronic occlusion, which may counteract the relatively earlier responses to CAL. Indeed, it has been shown previously that abnormalities in mitochondrial respiratory function represent a late phenomenon in the development of heart failure (8, 37). In fact, the association between mitochondrial function and hemodynamic parameters is unlikely to represent a clear one-to-one relationship. In this regard, we were unable to find a significant effect on mitochondrial respiratory function in hearts subjected to 6 wk of CAL (14) even though remodeling and hemodynamic abnormalities are evident 4 wk after initiation of ligation (5).

An increasing number of studies have documented an important pathophysiological role of NHE-1 activity to the development of myocardial dysfunction during ischemia/reperfusion, cardiac hypertrophy, and heart failure (1, 16, 22). NHE-1 inhibitors are able to block hypertrophic responses of isolated myocytes to various stimuli (29, 42), and these agents have been shown to prevent and reverse remodeling and heart failure postinfarction and in genetic animal models (5, 18, 32, 44). The potential mechanisms underlying the protective effect of NHE-1 inhibition are not completely understood. Recent studies have implicated a potential contribution of mitochondria in the beneficial effect of NHE-1 inhibition. For example, cariporide has been shown to prevent mitochondrial membrane potential loss and delay ATP depletion in adult ischemic cardiomyocytes (26) and neonatal cardiomyocytes subjected to oxidative stress (36). MPT pore opening and mitochondrial respiratory dysfunction were attenuated significantly by EMD during postischemic remodeling in rats subjected to CAL for 12 and 18 wk (14). We hypothesized that the protective effect of NHE-1 inhibition on mitochondria may be mediated by normalization of the expression of the transcriptional network regulating mitochondrial biogenesis and respiratory function. The present results demonstrate for the first time that the downregulation of the gene expression of the key transcription factor PGC-1α during the postinfarction remodeling process is attenuated by the NHE-1 inhibitor EMD, both 12 and 18 wk after ligation. The reduction in PGC-1α is in agreement with other reports using varied models of cardiac hypertrophy and heart failure demonstrating the reduction in expression of PGC-1α in both heart and hypertrophied cardiomyocytes in culture (9, 10, 15, 38). Overall, these results suggest that a key mechanism involved in the energy substrate switches from fatty acid to glucose oxidation in the hypertrophied and failing heart could involve deactivation of PGC-1α and its downstream target genes at both transcriptional and posttranscriptional levels (12).

EMD also induced a marked preservation of the expression levels of NRF-1 and NRF-2 genes, which are downstream targets for PGC-1α. NRF-1 and NRF-2 regulate expression of MTF-A, a nuclear-encoded transcription factor that binds regulatory sites on mtDNA and is crucial for replication and transcription of the mitochondrial genome. Downregulation of the MTF-A gene causes depletion of mtDNA with subsequent loss of mtDNA-encoded proteins and severe respiratory chain dysfunction (19). Heart-specific inactivation of MTF-A gene expression was shown to induce a dilated cardiomyopathy with atrioventricular heart conduction blocks and an impairment of respiratory chain function (20, 40). Indeed, our results show a significant preservation of MTF-A gene expression levels by EMD, suggesting that preservation of MTF-A by preventing the reduction in NRF-1 and NRF-2 contributes to the anti-hypertrophic effect of the drug during chronic postinfarction remodeling.

At present, it is difficult to precisely delineate the mechanism underlying the protective effect of NHE-1 inhibition on the transcription network regulating cardiac mitochondrial biogenesis and respiratory function, since little is known concerning the molecular and cellular pathways leading to a downregulation of PGC-1α and downstream targets in hypertrophy and heart failure. One potential mechanism of action of EMD could reflect attenuation of Na⁺ and/or Ca²⁺ overload in the cytoplasm. NHE-1 activity is increased in the postinfarcted heart because of stimulation by various autocrine and paracrine factors, such as α₁-adrenoreceptor agonists, endothelin-1, and ANG II, which are upregulated in these hearts (5, 18, 44). Activation of NHE-1 leads to the elevation of intracellular Na⁺ concentration which, in turn, induces cytoplasmic Ca²⁺ overload through activation of sarcolemmal Na⁺/Ca²⁺ exchanger in reverse mode. Regulation of cytoplasmic Ca²⁺ and Na⁺ levels may attenuate a release of cytokines, including interleukins and tumor necrosis factors (TNF), and thus the inflammatory process in heart. It has indeed been demonstrated that the elevated level of TNF-α, endothelin-1, and ANG II in failing heart can induce a marked activation of the protein kinase B (Akt) pathway (23). Elevated Akt activity has been demonstrated in the failing heart (11), and overexpression of Akt has been shown to induce a threefold downregulation of PGC-1α gene expression (6). As we have demonstrated earlier, the model of postinfarction remodeling used in our experiments develops heart failure with a significant attenuation in cardiac output and stroke volume at 12 wk after ligation (5). Thus a protective effect of EMD could potentially occur through regulation of Akt activity as a result of the attenuation of TNF-α release. This suggestion is supported by a recent study demonstrating that NHE-1 activation during cardiac remodel-
ing is associated with the Akt activation, which is prevented by cariporide (17). In view of the lack of significant modulation of MCIP1 by CAL, it is unlikely that the calcineurin system contributes a major role toward the downregulation of PGC-1α during postinfarction myocardial remodeling.

Nine NHE isoforms have been identified in mammalian cells, with NHE-1–NHE-5 apparently restricted to the plasma membrane, whereas NHE-6 might be located in the mitochondrial droplet (24). However, it has later been shown that NHE-6 is found in recycling endosomes of cells, not in mitochondria (4). Results of the present study demonstrated no effect of EMD on isolated mitochondria in vitro anoxia/reoxygenation, thus excluding its possible direct influence on mitochondria. The protective effect of NHE-1 inhibition on transcription factors may be mediated through the antiporter’s direct effect on nuclear receptors. Recent studies using immunofluorescence and three-dimensional confocal microscopy demonstrated that, in addition to the sarcolemma, NHE-1 is also localized at the nuclear membranes of cardiomyocytes (3). Although the uptake of NHE-1 inhibitors through plasma membranes is a slow process, the presence of EMD for a prolonged period (12 and 18 wk) could provide its accumulation in the cytoplasm with further direct effect on the nuclear membrane, inducing regulation of the expression of genes responsible for mitochondrial biogenesis and respiratory function.

In conclusion, the present study demonstrates that the downregulation of mitochondrial transcription factors induced by postinfarction remodeling can be significantly attenuated by the NHE-1-specific inhibitor EMD. Protection of the transcriptional network regulating mitochondrial biogenesis is strongly correlated with an improvement of mitochondrial function and downregulation of hypertrophic marker gene expression in these hearts. These data highlight the role of mitochondria in the mechanism of the anti-hypertrophic effect of NHE-1 inhibitors and further suggest that targeting mitochondria offers substantial promise for modulation of postinfarction remodeling and heart failure.

GRANTS

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REFERENCES


