Inhibition and reversal of myocardial infarction-induced hypertrophy and heart failure by NHE-1 inhibition

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Chen, Ling, Chang Xun Chen, Xiaohong Tracey Gan, Norbert Beier, Wolfgang Scholz, and Morris Karmazyn. Inhibition and reversal of myocardial infarction-induced hypertrophy and heart failure by NHE-1 inhibition. Am J Physiol Heart Circ Physiol 286: H381–H387, 2004; 10.1152/ajpheart.00602.2003.—Sodium/hydrogen exchange (NHE) inhibitors show promise as potential therapeutic agents for the treatment of heart failure, but it is not known whether they can reverse the maladaptive remodeling that results in heart failure. We sought to determine the effect of the NHE-1-specific inhibitor EMD-87580 (EMD) on heart failure produced by myocardial infarction in the rat and to assess whether up to 4 wk of treatment delay results in beneficial effects. Male Sprague-Dawley rats were subjected to coronary artery ligation (or a sham procedure) and followed for up to 3 mo, at which time hypertrophy and hemodynamic abnormalities were determined. EMD was provided in the diet, and treatment commenced immediately or 2–4 wk after ligation. EMD significantly reduced hemodynamic abnormalities, including the elevation in left ventricular end-diastolic pressure, and diminished the loss of systolic function with all treatment protocols. Left ventricular dilatation and hypertrophy, as assessed by heart weight, cell size, and atrial natriuretic peptide (ANP) expression, were similarly reversed to sham or near-sham levels. In addition, the increased plasma ANP and pro-ANP values were reversed to levels not significantly different from sham. Surprisingly, virtually all beneficial effects were identical with all treatment protocols. These effects were observed in the absence of infarct size reduction or blood pressure-lowering effects. Our results suggest that NHE-1 inhibition attenuates and reverses postinfarction remodeling and heart failure with a treatment delay of up to 4 wk after infarction. The effect is independent of infarct size or afterload reduction, indicating a direct effect on the myocardium.

SODIUM/HYDROGEN EXCHANGE (NHE) is a major proton extrusion pathway critical for intracellular pH regulation. NHE inhibition, especially with NHE-1-selective inhibitors, represents an effective approach toward reducing myocardial injury produced by ischemia and reperfusion (1, 11, 15). There is emerging evidence that NHE-1 could contribute to the heart failure process, especially in terms of mediating hypertrophy, an important component of the remodeling process that results in heart failure. For example, various hypertrophic factors, such as α1-adrenergic activation (28), endothelin-1 (17), and angiotensin II (20), have been shown to activate NHE-1. In isolated feline papillary muscles, stretch-induced intracellular alkalization occurs through activation of endothelin ETa and angiotensin II AT1 receptors and can be blocked by NHE-1 inhibition (4). These experiments were done using bicarbonate-free medium, thus precluding intracellular pH regulation through bicarbonate-dependent processes, which would be expected to occur under physiological conditions. Nonetheless, it has been suggested that NHE-1 is a common downstream mediator to angiotensin II and endothelin-1 in the cardiac cell and, thus, may represent an attractive target for intervention to reduce the remodeling process (6, 14–16). When administered before coronary artery ligation, the NHE-1 inhibitor cariporide reduced early (1 wk) and late (12 wk) cardiac hypertrophy and heart failure after myocardial infarction (18, 30). A number of other models of hypertension and heart failure also respond well to NHE-1 inhibition (3, 7). The present study was carried out to assess the effect of the newly developed and potent NHE-1-selective inhibitor EMD-87580 (EMD) on hypertrophy and hemodynamic abnormalities produced by 12 wk of coronary artery occlusion.

A major and novel component of the study was to assess whether NHE-1 inhibition reverses hypertrophy and heart failure when treatment is delayed by up to 4 wk after infarction.

METHODS

Drugs. EMD [N-[2-methyl-4,5-bis(methylsulfonyl)-benzoyl]-guanidine, hydrochloride] was synthesized in the Department of Medicinal Chemistry, Merck. Its structure is shown in Fig. 1 (inset). The drug was administered orally in rat chow at 200 ppm.

Expression of NHE isoforms and determination of specificity of EMD in mouse fibroblasts. A mouse fibroblast cell line (LAP-1) deficient in NHE activity, as well as three LAP-1 cell lines expressing NHE-1, NHE-2, or NHE-3, was obtained from Prof. Jacques Pouységur (Nice, France). Expression of the three isoforms, cell culture procedure, and cDNA preparation were carried out as previously described (5, 22, 24, 27). The LAP-1 cell lines were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum in a humidified atmosphere of 10% CO2–90% air at 37°C. To assess NHE activity, cells were subjected to an acid load using the NH4Cl-prepulse technique, as described in detail previously (9), in the absence or presence of increasing concentrations of NHE inhibitors. The NHE-dependent 22Na+ uptake was defined as the difference between 22Na+ uptake in the absence and presence of 1 μM ethylisopropyl amiloride. The percent 22Na+ uptake data were plotted in a semilogarithmic plot against the concentration of the compounds. A sigmoidal curve according to the equation f(x) = 100/(1 + IC50/x) was fitted to the data by a nonlinear regression analysis, with assumption of a steepness of −1 for the curve.

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Animal groups and surgical procedures. All animals were studied in accordance with the guidelines of the Canadian Council on Animal Care (Ottawa, Ontario). Male Sprague-Dawley rats (275–300 g) were randomly assigned to the following five treatment groups: sham, myocardial infarction (MI) produced by coronary artery ligation, MI + EMD immediately after coronary ligation, MI + EMD started 2 wk after ligation, and MI + EMD started 4 wk after ligation. The surgical procedure was performed as previously described (18, 30). Rats were anesthetized with intraperitoneal pentobarbital sodium, intubated, and artificially ventilated using a rodent respirator (model BR-S601MU, JVC). A left thoracotomy was performed, and the heart was gently exposed. To induce MI, the left main coronary artery was ligated ~3 mm from its origin using a firmly tied silk suture (5-0). For sham operation, 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.

Determination of hemodynamics and mortality. Cardiac hemodynamics were obtained under anesthesia with pentobarbital sodium (40–50 mg/kg ip) 4 or 12 wk after surgery. Left ventricular (LV) pressures were obtained using a 3-Fr Millar pressure transducer catheter (model SPR 249). Another catheter (PE-50, Clay Adams) was also inserted into the femoral artery to measure systemic blood pressure. Heart rate was obtained from the LV pressure recordings using a tachometer (model 7P44B, Grass). Cardiac output was determined using thermodilution technique (model VGS, Baxter). Mortality was assessed immediately after completion of the surgery and daily thereafter.

Measurement of infarct size. Infarct size was determined with picrosirius red staining. Photographs were magnified, and epicardial and endocardial circumferences and the infarcted portion were measured by a planimeter. Infarct size was calculated by dividing the sum of the infarcted portion by the LV circumference.

Assessment of myocyte characteristics. To assess myocyte characteristics and function, cells were isolated from viable noninfarcted myocardium using standard collagenase dispersion. An aliquot of cells was mounted on the thermoregulated (35°C) stage of an inverted microscope (Axiovert 65, Zeiss) for 5 min and superperfused with HEPES solution containing 1 mM CaCl2 at a rate of 1 ml/min. The cell image was monitored on a video screen, and cell length and width were measured using an Argus 10 image processor (Hamamatsu, Tokyo, Japan). Cell area was calculated by the multiple of cell length and width. Field stimulation (0.5 Hz, 20–25 V, 5-ms duration) with bipolar platinum electrodes was initiated, and cell shortening was recorded on a medical-grade tape using an S-VHS videotape recorder (model BR-S601MU, JVC) and analyzed by an Argus 10 image processor. Cell shortening was expressed as the percent reduction of cell length from diastolic length. At least 10 cells from each heart were used to measure cell shortening, and an average was obtained for each value to yield n = 1.

Molecular and biochemical determinations. Semiquantitative RT-PCR was used to assess NHE-1 and atrial natriuretic peptide (ANP) expression, as described in detail previously (3). For NHE-1, competitive PCR was employed; for ANP, β-actin or GAPDH was used as the reference gene. Inasmuch as there were no changes in either of these genes, results are reported only for β-actin. Plasma ANP and prepro-ANP levels were determined after extraction using commercial radioimmunoassay (Phoenix Peptide, Mountain View, CA) according to the recommendations of the manufacturer. Plasma was assayed for EMD concentrations by high-pressure liquid chromatography using mass spectrometry (MS/MS) detection. The limit of quantification was 1 ng/ml.

Statistical analysis. Data were analyzed by ANOVA, except for mortality data, which were analyzed by χ2 test.

RESULTS

Selectivity and specificity of EMD for NHE inhibition. As summarized in Fig. 1, EMD IC50 values were 113 ± 6 nM, 5,830 ± 514 nM, and 1,330 ± 70 μM for inhibition of NHE-1, NHE-2, and NHE-3, respectively. Further investigations indicate that >1.0 mM EMD has no effect on NHE-4 (unpublished information); the NHE-5 isoform is inhibited by EMD, with an IC50 of 200 μM (unpublished information). Thus EMD is a selective inhibitor of NHE-1, showing a selectivity ratio of ~52 between NHE-1 and NHE-2 and a selectivity ratio of ~12,000 between NHE-1 and NHE-3. To confirm efficacy against NHE-1 in ventricular myocytes, NHE-1 activity was measured in freshly isolated adult rat ventricular myocytes exposed to NH4Cl pulsing. We found that 1 μM EMD completely prevented pH recovery after acidosis, indicating effective inhibition of NHE-1 (not shown).

Mortality. Mortality after surgical coronary occlusion was generally restricted to acute responses within the first 48 h after surgery. No animals died after sham surgery, whereas the average rate of mortality in those groups in which EMD was not immediately provided was 31% (pooled data for the 3 groups). However, when EMD was administered immediately after coronary occlusion, the acute mortality was reduced to 4% (P < 0.01). Post-48-h mortality rates during the 12-wk treatment were as follows: 0% for sham, 4.7% for MI control,
3.9% for MI + immediate EMD, 4.1% for MI + EMD delayed 2 wk, and 4.0% for MI + EMD delayed 4 wk.

Infarct sizes. Infarct sizes were unaffected by EMD. The following infarct sizes (%LV) were obtained: 0 for sham, 34 ± 2% for untreated MI, 31 ± 2% for MI + immediate EMD, 33 ± 2% for MI + EMD delayed 2 wk, and 31 ± 2% for MI + EMD delayed 4 wk.

Indexes of hypertrophy. There was a significant increase in total and LV weights in animals subjected to 3 mo of coronary artery occlusion but no significant elevation in right ventricular (RV) weight. Overall, EMD blunted (as for total heart weight) or significantly attenuated (as for LV weight) the increased tissue, even with treatment delay (Fig. 2). With respect to the latter, there were no differences in LV weight between these groups and animals that were treated immediately with EMD.

Surviving LV, as well as RV, myocytes exhibited increased cell area, which was significantly attenuated by EMD (Fig. 3). Animals subjected to 4 wk of coronary ligation demonstrated significantly increased LV weight (Fig. 2) as well as LV cell area (Fig. 3) compared with sham.

LV tissue exhibited a more than twofold increase in ANP mRNA expression, which was almost completely abrogated by EMD treatment, even when treatment was delayed by 4 wk (Fig. 4). NHE-1 expression also increased significantly in the LV, which was also inhibited by treatment with EMD (Fig. 4). RV gene expression of ANP or NHE-1 was unaffected by LV coronary artery ligation and was identical in all treatment groups (not shown).

Plasma ANP and pro-ANP levels were significantly increased in control coronary artery-ligated animals, but these responses were significantly inhibited by EMD with all treatment protocols (Fig. 5).

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**Fig. 2.** Heart weight (HW; A) and right (C) and left (B) ventricular weights (RVW and LVW) in rats 4 and 12 wk after coronary artery ligation or sham surgery. BW, body weight. Values are means ± SE of 10–14 animals per group. *P < 0.05 vs. sham; **P < 0.05 vs. myocardial infarction (MI) control.

**Fig. 3.** Surface area of left (A) and right (B) ventricular cardiac myocytes from rats 4 and 12 wk after coronary artery ligation or sham surgery. Values are means ± SE of 10 animals per group, with 50 cells measured per animal. *P < 0.05 vs. sham; **P < 0.05 vs. MI control.
Hemodynamic responses. Hemodynamic responses were assessed in a separate group of animals that were not used for hypertrophy analysis. MI produced significant attenuations in cardiac output and stroke volume, although a significant effect in the latter was not seen at 4 wk (Fig. 6). In addition, there was a greater than threefold increase in LV end-diastolic pressure (Fig. 6), which was significantly attenuated by EMD. Mean arterial pressure for sham-operated rats was 135 /110 0.06 mmHg, which was reduced to 119 /110 0.06 2.9 mmHg (P < 0.05) in the untreated MI group. However, blood pressure was normalized to 128 /110 0.05 5, 131 /110 0.05 3.0, and 129 /110 0.05 3.2 mmHg in animals treated with EMD immediately and 2 or 4 wk after infarction, respectively.

Isolated myocyte function. An additional study was done in which function of surviving LV myocytes was determined ex vivo in terms of percent reduction of cell length from diastolic length. Values for cells from sham animals were 10.3 ± 0.4%; in cells from untreated infarcted hearts, this was significantly reduced to 6.7 ± 0.6% (P < 0.05, n = 10). However, with EMD, cell shortening was completely unchanged from sham: 9.7 ± 0.5, 10.1 ± 0.7, and 10.2 ± 0.7% in cardiac cells from infarcted animals treated with EMD immediately and after 2 or 4 wk of delay, respectively. A significant reduction in LV cell function was also observed 4 wk after coronary artery ligation (Fig. 7).

DISCUSSION

NHE is emerging as an important contributor to pathological processes, particularly during myocardial ischemia and reperfusion, where its activation and the resultant increase in intracellular sodium concentrations result in calcium overloading conditions and cell death (reviewed in Refs. 1, 11, and 15). Numerous studies with NHE inhibitors have shown exemplary cardioprotection against ischemia (1, 11, 15, 16), which appears to be superior to other cardioprotective strategies (10, 11) and has led to clinical trials in patients with coronary artery disease (23, 26, 31).

A rationale for implicating NHE in chronic postinfarction responses reflects observations in studies using diverse experimental approaches. First, various paracrine, autocrine, and hormonal factors that have been implicated in the ventricular remodeling-heart failure process also activate NHE-1 through receptor-dependent signal transduction processes: α1-receptor adrenergic agonists (28), endothelin-1 (17), and angiotensin II (20). Stretch-induced stimulation in protein synthesis in neonatal cardiac myocytes (29), as well as stretch-induced alkalization in feline papillary muscles superfused with bicarbonate-free medium (4), can be blocked by NHE inhibitors, as can norepinephrine-induced protein synthesis in cultured rat cardiomyocytes (13). It had been proposed that NHE-1 activation represents the downstream mediator for hypertrophic responses to angiotensin II and endothelin-1, suggesting that selective

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**Fig. 4.** Left ventricular atrial natriuretic peptide (ANP) and NHE-1 expression in rats 12 wk after coronary artery ligation or sham surgery (control). Values are means ± SE of 10 animals per group. *P < 0.05 vs. sham; †P < 0.05 vs. MI control. Top: representative agarose gels. Comp, competitor.

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**Fig. 5.** Plasma ANP and pro-ANP levels in rats 12 wk after coronary artery ligation or sham surgery. Values are means ± SE of 8 animals per group, except sham, where n = 6. *P < 0.05 vs. sham; †P < 0.05 vs. MI control. Cont, control.
inhibition of NHE-1 would represent a potentially effective approach toward mitigating myocardial remodeling and the subsequent evolution to heart failure (6, 14–16). In previous studies using the NHE-1 inhibitor cariporide, administration of the drug 1 wk before coronary artery occlusion resulted in significantly diminished early (1 wk after infarction) and late (3 mo after infarction) LV hypertrophy and hemodynamic abnormalities (18, 30). The following important question arises: Can NHE-1 inhibition also reverse the heart failure process, once it has been established? To this end, we delayed EMD treatment by 2–4 wk after MI. The plasma levels of EMD achieved at the end of the treatment period were approximately sixfold higher than the calculated IC$_{50}$ values observed in fibroblasts, suggesting effective NHE-1 inhibition in vivo. Our results demonstrate that EMD attenuates hypertrophy, ventricular dilatation, and heart failure, even with marked treatment delay, independently of infarct sizes. Indeed, as we showed in this study, by 4 wk, significant hypertrophy and cardiac dysfunction have already been established, thereby strongly implicating a reversal of the remodeling process. Moreover, afterload reduction appears to be unnecessary, because EMD had no effect on blood pressure, except to normalize pressures, such that they were not significantly different from sham. Our results also show that NHE-1 expression is increased in the surviving LV myocardium in heart failure. This finding is in agreement with other studies showing increased NHE-1 expression in the hypertrophied myocardium as well as its normalization by NHE-1 inhibition (7, 8). The ability of NHE-1 inhibitors to normalize NHE-1 expression levels may be secondary to inhibition of the hypertrophy per se, because it has been reported that the primary effect of chronic NHE-1 inhibition, at least in the normal myocardium, is to increase NHE-1 expression (2).

The precise mechanistic bases for the beneficial effects of NHE-1 inhibition are unknown. However, our results show that improved in vivo characteristics may be related to restoration of myocyte contractile function. A recent study using isolated myocytes suggests that the NHE-1-dependent hypertrophy produced by phenylephrine or isoproterenol can be dissociated from intracellular pH changes (25). It has been suggested that NHE-1-dependent intracellular sodium influx induces hypertrophy by stimulating various protein kinase C isoforms, which in turn upregulate a number of transcriptional factors (12). However, the salutary effects of NHE-1 inhibition on hemodynamics may also involve other mechanisms in addition to improved cell performance. In particular, a reduction in extracellular fibrosis is of particular potential importance, as has been demonstrated in a transgenic β_1-adrenoceptor overexpressor mouse model of heart failure (7).

The ability of EMD to reverse the remodeling process was indeed surprising, inasmuch as we anticipated, at the very most, diminished salutary effects. The concept of reversing the chronic maladaptive responses is clinically of major importance but one that has not been extensively studied pharmacologically. However, emerging evidence supports the concept that the heart failure process can be reversed. For example, Mulder and colleagues (21) reported that an up to 3-mo treatment delay with the angiotensin-converting enzyme inhibitor lisinopril reversed the severity of heart failure produced by myocardial infarction in rats, an effect associated with significant afterload reduction.
Although the theoretical conceptual basis and experimental evidence converge on a critical role of NHE-1 in the remodeling heart failure process, cariporide has recently been shown to have little effect in female rats subjected to coronary artery ligation for 49 days when administered 7 days after occlusion, at least in terms of hypertrophy and increased expression of various genes, including ANP and NHE-1, although improvement in surviving cell function was observed (19). These results are dramatically different from our findings. The differences are unlikely to be due to the NHE-1 inhibitor used, inasmuch as we found effects with cariporide that are virtually nonexistent. The drug interaction of NHE-1 inhibitors with other cardiovascular pharmacology is of high importance (20).

In conclusion, the present study indicates that reversal of myocardial remodeling and heart failure can be achieved through a process independent of afterload reduction and, thus, suggests that targeting myocardial NHE-1 represents an attractive approach toward reducing heart failure and reversing the postinfarction remodeling process in the absence of blood pressure-lowering effects. A major challenge is to unravel the molecular and cellular bases for prevention and reversal of the remodeling process by NHE-1 inhibition.

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DISCLOSURES

M. Karmazyn is a Career Investigator of the Heart and Stroke Foundation of Ontario.

REFERENCES


