Na\textsuperscript{+}/H\textsuperscript{+} exchange inhibition reduces hypertrophy and heart failure after myocardial infarction in rats

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Kusumoto, Keiji, James V. Haist, and Morris Karmazyn. Na\textsuperscript{+}/H\textsuperscript{+} exchange inhibition reduces hypertrophy and heart failure after myocardial infarction in rats. Am J Physiol Heart Circ Physiol 280: H738–H745, 2001.—We investigated the effect of sodium/hydrogen exchange inhibition (NHE-1) on hypertrophy and heart failure after coronary artery ligation (CAL) in the rat. Animals were subjected to occlusion (or sham) of the left main coronary artery and immediately administered a control diet or one consisting of the NHE-1 inhibitor cariporide for 13–15 wk. Hearts were separated by small [<30% of left ventricle (LV)] and large (>30% of LV) infarcts. CAL depressed change in left ventricular increase in pressure over time (LV +dP/dt) in small and large infarct groups by 18.8% (P < 0.05) and 34% (P < 0.01), respectively, whereas comparative values for the cariporide groups were 8.7% (not significant) and 23.1% (P < 0.01), respectively. LV end-diastolic pressure was increased by 1.225% in the control large infarct group but was significantly reduced to 447% with cariporide. Cariporide also significantly reduced the degree of LV dilation in animals with large infarcts. Hypertrophy, defined by tissue weights and cell size, was reduced by cariporide, and shortening of surviving myocytes was preserved. Infarct sizes were unaffected by cariporide, and the drug had no influence on either blood pressure or the depressed inotropic response of infarcted hearts to dobutamine. These results suggest an important role for NHE-1 in the progression of heart failure after myocardial infarction.

Congestive heart failure is an important and rapidly expanding clinical problem with 400,000 new cases diagnosed each year in the United States. Hypertrophy is an early maladaptive response in the heart failure process (14), and its attenuation is therefore, a principal therapeutic goal (3). Sodium/hydrogen exchange (NHE) is a major proton extrusion pathway, critical for intracellular pH (pHi) regulation. However, in addition to its role in pHi regulation, the antiporter also contributes to myocardial injury produced by both ischemia and reperfusion. Inhibitors of NHE, particularly newly developed NHE-1-specific inhibitors such as cariporide, and other agents, protect the ischemic myocardium in a wide variety of animal species (1, 6, 7, 12, 19, 22 and reviewed in 13). Although predominant attention is related to cardioprotection, recent evidence suggests NHE-1 may also be important in cardiac cell growth (2, 4, 9, 11, 30); and the activity of the antiporter is augmented by hypertrophic factors such as α1-adrenergic activation (32), endothelin-1 (15), and angiotensin II (8, 20). This led to the hypothesis that NHE-1 is the downstream mediator for at least some of these factors and that inhibiting NHE-1 would limit the cellular hypertrophy and, potentially, the heart failure process (4). NHE-1 inhibition could limit postinfarction responses as a result of infarct size reduction (29). We have recently shown that dietary administration of the NHE-1-specific inhibitor cariporide 1 wk before coronary artery occlusion attenuates early (1 wk) left ventricular (LV) myocyte hypertrophy and early hemodynamic abnormalities (33), in the absence of any infarct-reducing effects. The potential role of NHE-1 in chronic postinfarction responses is not known with certainty, particularly with respect to its direct influence independent of infarct size attenuation. Accordingly, the present study was carried out to assess the effect of cariporide in a chronic model of heart failure when administered immediately after infarction produced by sustained coronary artery occlusion. We assessed both in vivo hemodynamic responses and ex vivo myocyte characteristics after treatments.

Methods

Experimental protocol. Male Sprague-Dawley rats weighing 275–300 g (Charles River; St. Constant, Quebec, Canada) were randomly assigned to four groups: sham surgery control diet; sham surgery cariporide diet (containing 3,000 parts per million of cariporide); coronary artery ligation (CAL) control diet; or CAL cariporide diet. Rats were anesthetized with intraperitoneal pentobarbital sodium (50 mg/kg), intubated, and artificially ventilated (10 ml/kg, 70 strokes/min) by using a rodent respirator (model 683, Harvard Apparatus). A lead II electrocardiogram was recorded by using a Grass electrocardiogram amplifier (model 7P6D, Grass Medical Instruments; Quincy, MA). A left thoracotomy was performed, and the heart was gently exposed. During surgery, rectal temperature was kept at 37°C. To induce myocardial infarction, the left main coronary artery was ligated ~3 mm from its origin.

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by using a firmly tied silk suture (5-0). Ischemia was con-
confirmed by changes in the S-T segment of the electrocardio-
gram and by visible blanching of the heart muscle. If both
parameters did not alter after ligation, reocclusion was im-
mediately performed. For sham operation, the ligature was
placed in an identical fashion but not tied. The incidence of
ventricular fibrillation was noted for the first 20 min after
ligation, and, if necessary, defibrillation was attempted by
gently touching the LV with a wet cotton-tipped applicator.
The chest was then closed in three layers (ribs, muscle, and
skin), and the animal was allowed to recover. For cariporide
treatment, an initial administration of the drug (30 mg/kg ip)
was made immediately after ligation or sham procedure and
again 8 h later. Regular eating generally resumed 10–12 h
after surgery. Identical saline injections were made for the normal
diet groups. Rats were given free access to rat chow and water
from the first day of surgery and for the duration of the study.

Measurement of hemodynamic parameters. In vivo hemo-
dynamic measurements were performed under anesthesia with
pentobarbital sodium (40–50 mg/kg ip) 3 mo after sur-
geries. A catheter (PE-50, Clay Adams) was also inserted into the femoral
to measure simultaneous changes in pressure. A catheter (PE-
50, Clay Adams) was also inserted into the femoral artery to
measure systemic blood pressure. The first derivative of LV
pressure was simultaneously monitored by using a Grass 7P2C
differentiator amplifier. Heart rate was obtained from the LV
pressure recordings by using a Grass 7P4B tachometer.

Measurement of myocardial infarct size. After hemody-
namic measurements were performed, the LV and right ven-
tricle (RV) were weighed, and the LV was fixed in 10%
buffered formalin (pH 7.4). Infarct size was determined as
reduction of cell length from diastolic length. At least 10
images were captured with the aid of an Argus 10
image processor. Cell shortening was expressed as the per-
percentage of rod-shaped cells was determined for each iso-
faction of the left ventricle. *P < 0.05 from respective sham; †P < 0.01 from

Assessment of myocardial characteristics. After these
experiments, rats were not subjected to either hemo-
dynamic assessments or infarct size determination but were
anesthetized with pentobarbital (50 mg/kg ip). The hearts
were immediately removed and placed in ice-cold Ca++-free
HEPES solution containing (in mM) 135 NaCl, 5.4 KCl, 1.0
MgCl₂, 0.33 NaH₂PO₄, 10 HEPES, and 10 glucose (pH 7.2,
4°C, bubbled with 100% O₂) and then rapidly mounted by the
aorta on a Langendorff perfusion system. Retrograde perfu-
sion was initiated with Ca++-free HEPES solution (37°C) for
5 min. The perfusate was then switched to Ca++-free HEPES
solution containing 1.8 mg/ml of collagenase (Type II, Wash-
ington Biochem), 0.1 mg/ml of protease (Type XIV, Sigma),
and 0.5 mg/ml of BSA (Sigma) for 12 min followed by perfu-
sion with HEPES buffer containing 0.2 mM CaCl₂ (37°C) for
5 min. The heart was then removed from the Langendorff
system, and, if necessary, the infarct area was discerned and
tissues were cut into small pieces and shaven for 15 min in a
water bath at 37°C. Cardiac myocytes were then filtered
through a 210 nylon mesh and gently centrifuged at 500 g for
45 s. The supernatant was aspirated, 35 ml of HEPES solu-
tion containing 0.5 mM CaCl₂ were added, and the suspen-
sion was left to stand for 10 min, after which the supernatant

Table 1. Infarct sizes, body weight, and ventricular weight in normal and cariporide-treated rats
with or without myocardial infarction

<table>
<thead>
<tr>
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<th>Sham</th>
<th>Infarcted</th>
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<tr>
<td>n</td>
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<tr>
<td>Normal diet</td>
<td>18</td>
<td>13</td>
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<tr>
<td>Cariporide diet</td>
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<td>14</td>
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<tr>
<td>Infarct size,</td>
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<tr>
<td>Normal diet</td>
<td>24 ± 2</td>
<td>36 ± 1</td>
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<td>Cariporide diet</td>
<td>22 ± 1</td>
<td>35 ± 1</td>
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<td>Body weight, g</td>
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<tr>
<td>Normal diet</td>
<td>539 ± 2</td>
<td>540 ± 12</td>
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<td>Cariporide diet</td>
<td>514 ± 11</td>
<td>512 ± 23</td>
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<td>LV weight, mg</td>
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<tr>
<td>Normal diet</td>
<td>1,017 ± 21</td>
<td>1,135 ± 22†</td>
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<td>Cariporide diet</td>
<td>987 ± 21</td>
<td>990 ± 26</td>
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<td>RV weight, mg</td>
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<td></td>
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<td>Normal diet</td>
<td>237 ± 8</td>
<td>244 ± 9</td>
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<tr>
<td>Cariporide diet</td>
<td>220 ± 7</td>
<td>224 ± 7</td>
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<tr>
<td>LV/BV weight ratio, mg/g</td>
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<tr>
<td>Normal diet</td>
<td>1.89 ± 0.03</td>
<td>2.11 ± 0.05†</td>
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<tr>
<td>Cariporide diet</td>
<td>1.85 ± 0.03</td>
<td>1.93 ± 0.044‡</td>
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<tr>
<td>RV/BV weight ratio, mg/g</td>
<td></td>
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<tr>
<td>Normal diet</td>
<td>0.44 ± 0.01</td>
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<tr>
<td>Cariporide diet</td>
<td>0.43 ± 0.01</td>
<td>0.52 ± 0.03§</td>
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</table>

Values are means ± SE; n, number of rats. LV, left ventricle; RV, right ventricle. †P < 0.05 from respective sham; ‡P < 0.01 from respective sham; †P < 0.05, §P < 0.01 from respective normal diet.
RESULTS

Effect of cariporide treatment on plasma drug levels. Serum cariporide levels averaged $381 \pm 44$ and $419 \pm 67$ ng/ml for the sham and CAL groups, respectively.

Table 2. Mean blood pressure and heart rates in normal and cariporide-treated animals with or without myocardial infarction

<table>
<thead>
<tr>
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<th>Infarcted</th>
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<tbody>
<tr>
<td></td>
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<td>Large</td>
<td>Sham</td>
<td>Small</td>
<td>Large</td>
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<tr>
<td>$n$</td>
<td>Normal diet</td>
<td>18</td>
<td>13</td>
<td>12</td>
<td>Cariporide diet</td>
<td>15</td>
</tr>
<tr>
<td>Mean blood pressure, mmHg</td>
<td>Normal diet</td>
<td>$119 \pm 4$</td>
<td>$114 \pm 5$</td>
<td>$105 \pm 4$</td>
<td>Cariporide diet</td>
<td>$117 \pm 3$</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>Normal diet</td>
<td>$379 \pm 9$</td>
<td>$358 \pm 8$</td>
<td>$363 \pm 8$</td>
<td>Cariporide diet</td>
<td>$375 \pm 14$</td>
</tr>
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Values are means $\pm$ SE; $n$, number of rats.

Fig. 1. In vivo hemodynamic characteristics in rats 3 mo after myocardial infarction produced by coronary artery ligation (CAL). Animals were divided according to the degree of infarct sizes as described in METHODS. The cariporide group represents those animals initially administered the sodium/hydrogen exchange (NHE-1)-specific inhibitor cariporide immediately after ligation and then maintained on rat chow containing 3 parts per million cariporide. Control animals were initially administered saline and maintained on an identical diet, although not containing the drug. LVSP, left ventricular systolic pressure; LVEDP, LV end-diastolic pressure; LV $+dP/dt_{max}$, LV maximal increase in pressure over time; LV $-dP/dt_{max}$, LV maximal decrease in pressure over time. *$P < 0.05$, **$P < 0.01$ from respective sham values. Percentages in parentheses indicate the mean percent change from sham. Numbers in parentheses at the bottom depict the number of animals in each group.

Early incidence of ventricular fibrillation and overall incidence of mortality. A major feature of this model is the relatively high incidence of initial ventricular fibrillation; 45% of control animals fibrillated, which was significantly ($P < 0.05$) reduced to 15% in those animals treated with cariporide. Total mortality during the subsequent observation period was 27% in control and 18% in the cariporide-ligated group, although this difference was not significant.

Infarct sizes and body and heart weights. These data are summarized in Table 1. Cariporide had no effect on infarct size in this permanent occlusion model. Body weights were not significantly affected by coronary artery occlusion but tended to be somewhat smaller in the cariporide group.

LV weight was significantly increased in the small infarct size group but not in those animals treated with cariporide. LV weight was not significantly affected in the large infarct size group, although when corrected for body weight, significant effects were observed. RV weight or RV-to-body weight ratios were elevated only
in animals exhibiting large infarcts, although this was attenuated significantly by cariporide (Table 1).

Hemodynamic characteristics. Because infarct size greatly influences the hypertrophic remodeling and heart failure processes (12, 13), we grouped the animals (except those used for isolated myocyte studies) into those rats showing small (<30% of LV) and moderate to large (>30% of LV) infarcts. These data are summarized in Fig. 1 by LV performance. Control animals with small infarcts exhibited moderate hemodynamic changes, although significant attenuations in maximal LV pressure increase over time (dP/dt max) were evident. However, this reduction was not seen in cariporide-treated animals exhibiting identical infarct size. In untreated animals exhibiting large infarcts, LV systolic pressure was reduced by 14% of sham values (P < 0.05), whereas this was significantly attenuated by the NHE-1 inhibitor. Moreover, LV +dP/dt max was reduced to a greater degree (34%, P < 0.05). However, the magnitude of reduction (23%) was significantly less with drug treatment, although this still represented a significant reduction from sham. A similar profile with respect to LV maximal decrease in pressure over time (−dP/dt max) was observed, including a prevention of significant attenuation in the small infarct group, and marked significant attenuation in animals with large infarcts.

Also evident in Fig. 1, LV end-diastolic pressure (LVEDP) was only moderately affected in animals with small infarcts; however, a marked elevation in LVEDP of 1.22 ± 0.3 to 19.6 ± 2.4 mmHg, P < 0.01 was evident in the large infarct group. This was markedly inhibited by more that 60% compared with the noncariporide group, although these values were still significantly greater than control. Neither heart rates nor blood pressures were affected by any treatment (Table 2).

Pressure-volume relationship. Pressure-volume relationships, an index of LV chamber volume in diastolic stage in vivo under various conditions, is shown in Fig. 2. Infarction resulted in a rightward shift in the pressure-volume at the end of the observation period depending on the size of the infarct region. With small infarcts, rightward displacement of the pressure-volume curves was unaffected by cariporide treatment, whereas with large infarcts, a significant attenuation of the rightward shift was observed.

β1-Adrenergic responses. Heart failure is associated with decreased myocardial response to β1-adrenergic agonists, and we recently reported that in 1-wk postinfarcted hearts, diminished response to isoproterenol in isolated myocytes from infarcted hearts can be attenuated in animals treated with cariporide. This may suggest that some of the potential beneficial effects of this treatment could involve an attenuation of resistance to catecholamines. Here, we studied whether similar salutary effects of cariporide can be observed in the failing myocardium in vivo 3 mo after infarction by using the β1-selective agonist dobutamine. As summarized in Fig. 3, dobutamine (0.3–10 µg/kg iv) dose-dependently increased LV +dP/dt max in all groups. However, the amplitude of responses to dobutamine was significantly reduced in animals with large infarcts. Half-maximal effective dose values in sham, small infarct, and large infarct groups maintained on a
normal diet were $1.0 \pm 0.1$, $1.3 \pm 0.2$, and $4.7 \pm 1.0$ mg/kg ($P < 0.01$), respectively, indicating a significantly depressed response in the latter. Corresponding values in animals given cariporide were $1.0 \pm 0.2$, $2.2 \pm 1.1$, and $3.8 \pm 1.4$ mg/kg ($P < 0.05$), indicating that cariporide had no effect on diminished responsiveness to dobutamine in this particular model.

Characteristics and function of surviving myocytes. To further assess the influence of NHE-1 inhibition, we characterized properties of surviving myocytes by cell dimension and shortening. Because these cells are quiescent, shortening was determined during electrical stimulation. There were no differences in the percentage of rod-shaped viable cells obtained from the various treatment groups, averaging about 80% of the total cell yield. Because it was not possible to isolate myocytes from hearts subjected to infarct size measurements, cells for these studies should be considered as originating from groups exhibiting varied infarct sizes. The data for myocyte dimensions are summarized in Fig. 4. The average myocyte length was significantly increased in control infarcted hearts to about 127% of the respective sham controls. This was attenuated by cariporide to 115%, a value significantly less than in cells from the infarcted group maintained on a control diet. Cell width was significantly increased to 113% of sham values. However, in hearts from cariporide-treated animals, this was almost completely abrogated.

Coronary ligation in untreated rats resulted in a decreased shortening of surviving myocytes of about 27% compared with their respective sham controls. However, myocytes isolated from hearts of cariporide-treated rats showed no diminution in function (Fig. 5).

DISCUSSION

In this study, we presented evidence that NHE-1 inhibition attenuates the adaptive hypertrophic response and congestive heart failure in a rat myocardial infarction model. Our results support and extend the general concept that NHE-1 is an important determi-
NHE-1 mediates intracellular alkalinization caused by mechanical stretch (2). These investigators (2) proposed that stretch stimulates angiotensin AT$_1$ and endothelin ET$_A$ receptors which increases phosphoinositol hydrolysis and activates protein kinase C (PKC), resulting in increased NHE-1 activity. However, in the case of endothelin-1, recent evidence suggests NHE-1 activation by this peptide involves mitogen-activated protein (MAP) kinase pathway (21).

Irrespective of precise mechanisms underlying NHE-1 activation, these studies suggest NHE-1 inhibition has effects similar to those of endothelin or angiotensin II blockade. However, it is important, and potentially clinically relevant, to note apparent differences with NHE-1 inhibition. For example, angiotensin-convert-
ing enzyme inhibitors and endothelin receptor antagonists reduce afterload, which forms the basis for their antihypertensive effects; however, no blood pressure-
lowering influence of cariporide was seen in our study, effectively ruling out afterload reduction as a contributing factor.

It is important to note also that cariporide failed to improve the reduced inotropic response to dobutamine. This would suggest that desensitization of the myocardial $\beta_1$-adrenergic system in the failing heart is unaffected by cariporide and that the salutary effect of cariporide is unrelated to this pathway.

Although the underlying cellular mechanisms that account for remodeling and the evolution to heart fail-
ure are exceedingly complex (3, 14, 29), our data sup-
port a role for NHE-1 in the process. The exact mech-
nisms for NHE-1 involvement, however, remains to be determined, although these mechanisms may in-
volve a permissive effect of NHE-1 activity on protein synthesis, perhaps through pH$_i$-dependent processes. Thus a potential scenario may involve activation of NHE-1 by various growth factors resulting in hypertrophic responses (reviewed in Ref. 13). We were un-
able to measure pH$_i$ by using the current protocol, and therefore, the validity of this hypothesis remains uncertain. In view of the multiplicity of pH$_i$-regulatory mechanisms in the cardiac cell, it is doubtful that intracellular acidosis would be markedly greater in hearts from cariporide-treated animals during sustained occlusion because other mechanisms would compensate for the inability of NHE-1 to remove pro-
tons. This is supported by acute ischemia studies where it was observed that pH$_i$, under conditions of NHE-1 inhibition, generally does not fall lower than
values seen in the absence of NHE-1 blockade (23) or, if pH is reduced, the reduction does not occur until late in the ischemic period (16).

It is also important to note that sodium ions are important mediators of cell hypertrophy (5, 10); therefore, the accompanying reduction in sodium entry occurring during NHE-1 inhibition may represent the major basis for salutary effects of cariporide on hypertrophy and heart failure. In a recent study using neonatal rat ventricular myocytes, it was proposed that NHE-1-dependent sodium influx is a major contributor to hypertrophy produced by various agonists, including α1-adrenergic stimulation, endothelin-1, or phorbol ester by activating various protein kinase C (PKC) isoforms, particularly PKC-δ and PKC-ε (10). This concept was reinforced by the ability of PKC inhibitors to reduce the hypertrophic response and by the NHE-1 inhibitor HOE-694 to attenuate both the hypertrophy and PKC activation (10). However, the role of NHE-1 in mediating hypertrophic responses in vitro may also involve more extensive cell-signaling systems. For example, stretch-induced cardiac cell hypertrophy was also associated with Raf-1 and MAP kinase activation with both the hypertrophy and kinase being inhibited by HOE-694, leading the authors to conclude that NHE-1 activates both kinases through a undetermined manner leading to cell growth (30). These authors reported that HOE-694 did not affect upregulation of either Raf-1 or MAP kinases by either endothelin-1 or angiotensin II, although hypertrophic responses were not reported (30). As noted above, in feline papillary muscle, stretch-induced intracellular alkalization was found to be NHE-1-dependent and linked to the activation of both endothelin ET_{A} and angiotensin II AT_{1} receptors via a PKC-dependent process (2). It is clear that unraveling the intracellular processes that mediate NHE-1-dependent cardiac hypertension will be challenging in view of the apparent complexity of the process.

In conclusion, our study demonstrates that a NHE-1-selective inhibitor cariporide attenuates the hypertrophic process, and heart failure, in the postinfarcted rat myocardium. This occurs in the absence of infarct size reduction or any effect on blood pressure. Moreover, the resistance to hypertrophic responses was unaltered by cariporide. When taken together, these findings suggest a direct influence of the drug on remodeling of surviving myocytes, a finding supported by myocyte analysis showing reduced hypertrophy and preservation of ex vivo function. The degree of attenuation of postinfarction responses was, roughly speaking, ~50% compared with values seen in the nontreated group. The failure to completely abrogate the remodeling-heart failure process was not surprising in view of the underlying complexity of postinfarction remodeling, hypertrophy, and heart failure, that is unlikely to be amenable to one therapeutic intervention. Nonetheless, it is possible that a higher dose of cariporide could exert greater beneficial effect, although this needs to be determined. Overall, however, our results suggest that in principle, NHE-1 inhibition represents a desirable approach to reduce the postinfarction heart failure process and could represent an attractive therapeutic approach. It can also be suggested that the benefits of NHE-1 inhibitors could be accentuated when used in combination with other therapies for the treatment of heart failure.

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