Na\textsuperscript{+}/H\textsuperscript{+} exchange subtype 1 inhibition reduces endothelial dysfunction in vessels from stunned myocardium

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Symons, J. David, and Saul Schaefer. Na\textsuperscript{+}/H\textsuperscript{+} exchange subtype 1 inhibition reduces endothelial dysfunction in vessels from stunned myocardium. Am J Physiol Heart Circ Physiol 281: H1575–H1582, 2001.—Myocardial ischemia and reperfusion cause myocyte and vascular dysfunction, frequently termed “stunning.” We hypothesized that inhibiting the Na\textsuperscript{+}/H\textsuperscript{+} exchanger subtype 1 isoform (NHE\textsubscript{1}) during ischemia and reperfusion limits myocardial and coronary microvascular stunning. Anesthetized rats completed 2 × 10-min coronary artery occlusions separated by 5-min of reperfusion, followed by 15 or 60 min of reperfusion. Vehicle (saline) or the NHE\textsubscript{1} inhibitor cariporide (HOE-642) was administered 15 min before ischemia and was continued throughout each protocol. After reperfusion, hearts were excised, and the reactivity of resistance arteries (internal diameter, ~120 μm) was assessed. The first derivative of left ventricular (LV) pressure, LV developed pressure, and LV systolic wall thickening were depressed (P < 0.05) similarly in vehicle- and cariporide-treated rats during ischemia and after 15 or 60 min of reperfusion compared with sham-operated animals that were not exposed to ischemia (i.e., controls). In vessels obtained after 15 min of reperfusion, the maximal response to acetylcholine-induced relaxation (10\textsuperscript{−8}–10\textsuperscript{−4} M) was blunted (P < 0.05) in vessels from vehicle- and cariporide-treated rats (~35%) and cariporide-treated rats (~55%) compared with controls (~85%). However, the percent relaxation to acetylcholine was greater (P < 0.05) in cariporide-treated rats compared with vehicle-treated rats. Maximal contractile responses to endothelin-1 (10\textsuperscript{−11}–10\textsuperscript{−7} M) were increased (P < 0.05) similarly in vehicle- and cariporide-treated rats compared with controls. Relaxation to sodium nitroprusside (10\textsuperscript{−4} M) was not different among groups. Results were similar in vessels obtained from animals after 60 min of reperfusion. These findings suggest that NHE\textsubscript{1} inhibition before coronary occlusion lessens ischemia-induced microvascular dysfunction for 15–60 min after reperfusion but does not alter myocardial contractile function in the area at risk.

coronary resistance vessels; myocardial function; endothelium; vascular smooth muscle; acetylcholine; myocardial ischemia; myocardial stunning

The Na\textsuperscript{+}/H\textsuperscript{+} exchanger (NHE) is one of several pH-regulating systems that contributes to restoring intracellular pH during ischemia and early reperfusion (14).

This membrane-bound protein exchanges intracellular protons for extracellular Na\textsuperscript{+}, thereby limiting intracellular acidosis at the expense of elevating the intracellular Na\textsuperscript{+} concentration ([Na\textsuperscript{+}]\textsubscript{i}) (1, 9). If ischemia persists to an extent whereby extrusion of [Na\textsuperscript{+}]\textsubscript{i} by the ATP-dependent Na\textsuperscript{+}/K\textsuperscript{+} pump is compromised, elevated [Na\textsuperscript{+}]\textsubscript{i} stimulates the membrane-bound Na\textsuperscript{+}/Ca\textsuperscript{2+} exchanger, and intracellular Ca\textsuperscript{2+} concentration ([Ca\textsuperscript{2+}]\textsubscript{i}) overload ensues. This increase in [Ca\textsuperscript{2+}]\textsubscript{i} after ischemia and reperfusion has been implicated as an important mechanism that contributes to myocardial stunning. For example, ischemia-induced myocardial dysfunction is attenuated by limiting [Ca\textsuperscript{2+}]\textsubscript{i} directly (16) and/or by minimizing the stimulus for increased [Ca\textsuperscript{2+}]\textsubscript{i} (i.e., elevated [Na\textsuperscript{+}]\textsubscript{i}) (12, 24, 28). With regard to the latter mechanism, we (28) recently showed that inhibiting the subtype 1 isoform of NHE (NHE\textsubscript{1}) using 4-isopropyl-3-methylsulfonyl-benzoylguanidine methanesulfonate [cariporide (HOE-642), Aventis Pharmaceutical; Frankfurt, Germany] delays the onset and lessens the magnitude of ischemia-induced myocardial stunning in conscious pigs.

In addition to depressing myocyte contractility, myocardial ischemia can cause coronary vascular dysfunction (3, 23), thus limiting reperfusion at the microvascular level. The potential for NHE\textsubscript{1} inhibition to preserve coronary vascular function in vessels perfusing the stunned myocardium has never been evaluated. However, if NHE\textsubscript{1} inhibition has a beneficial effect on endothelial cells similar to that described for myocytes, endothelium-dependent relaxation should be preserved relative to the untreated condition. Results from our previous study (28) in conscious pigs suggest that NHE\textsubscript{1} inhibition is beneficial in limiting ischemia-induced coronary vascular dysfunction. Specifically, after repeated occlusions of the left circumflex (LCx) coronary artery, adenosine-induced increases in LCx blood flow velocity were greater in NHE\textsubscript{1}-inhibited compared with vehicle-treated pigs. Because adenosine-induced relaxation of pig arterioles is largely endothelium dependent (15), we speculated that endothelial function was preserved in these animals. The

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purpose of the present study was to evaluate whether NHE1 inhibition before ischemia 1) preserves coronary vascular function and/or 2) attenuates global and regional myocardial dysfunction in our rat model of myocardial and microvascular stunning. We hypothesized that myocardial dysfunction, coronary endothelial dysfunction, and receptor-mediated constriction would be less, and vascular smooth muscle function would be similar, in NHE1-inhibited rats compared with vehicle-treated rats. Furthermore, we hypothesized that the beneficial effects of NHE1 inhibition would be evident after relatively short (i.e., 15 min) and long (i.e., 60 min) periods of reperfusion.

METHODS

Experimental animals. All protocols used in this study were approved by the Animal Use and Care Committee at the University of California-Davis and conformed to the guidelines set by the American Physiological Society and Animal Welfare Act. Male Sprague–Dawley rats were housed individually under controlled temperature (23°C) and light conditions (12:12-h light-dark cycle) and were allowed access to food and water ad libitum.

Surgical procedures. Rats were anesthetized using ketamine (30–50 mg/kg im) and xylazine (3–5 mg/kg im). Supplemental doses of this mixture were given as required. A small incision was made in the neck, the trachea was intubated, and respiration was maintained artificially (model 661, Harvard Apparatus) using room air supplemented with 100% oxygen. Catheters were then inserted into the carotid artery for infusion of drugs and/or fluids and into the caudal artery to measure arterial pressure and obtain blood samples for gas analyses (Radiometer ABL-3; Westlake, OH). After the heart was exposed through a lateral thoracotomy and the pericardium was opened, a pressure transducer-tipped catheter (2-Fr, Millar Instruments; Houston, TX) was inserted through the apex into the left ventricle (LV) to measure LV pressures and the first derivative of LV pressure (LV dP/dt). A 6-0 suture was then passed loosely under the proximal portion of the left coronary artery, and both ends were threaded through a vinyl tube. This tube served as a snare to occlude the coronary artery and was used to evoke reversible myocardial ischemia. A 20-MHz single-transducer sonomicrometer was then sewn on the epicardium in the region perfused by the left coronary artery to measure LV systolic wall thickening (30). Throughout the surgical procedures and experimental protocols, rectal temperature was maintained at 37°C using a heating pad and lamp.

Preliminary in situ experiments. Our primary goal was to evaluate the effects of NHE1 inhibition on the microvascular function of vessels from the stunned myocardium. In preliminary studies, therefore, it was necessary to establish the extent of ischemia required to cause impairment of our primary endpoint, i.e., acetylcholine-evoked relaxation of coronary microvessels. Preliminary experiments using several bouts (i.e., 1–3) and durations (i.e., 1 × 5 min, 2 × 10 min, and 3 × 10 min) of ischemia, separated by various reperfusion periods (5–60 min), were completed. The ischemia and reperfusion protocols used in the present study were capable of evoking significant myocardial and microvascular dysfunction compared with sham-operated animals (30).

In situ assessment of myocardial function. When instrumentation was complete and blood gases and hemodynamic variables were stable, 36 rats were divided into one of three groups.

Ischemia and 15-min reperfusion. After a 5-min baseline period, the vehicle for cariporide (i.e., saline, n = 7) was administered first as a bolus (0.3 ml) and subsequently by constant infusion (0.05 ml/min) into the carotid artery. Next, a 15-min preischemic period preceded 2 × 10-min coronary occlusions that were separated by 5 min of reperfusion. This was followed by 15 min of reperfusion. Hemodynamic variables were measured at 5-min intervals and processed by a computer through an analog-to-digital interface card (R. C. Electronics; Santa Barbara, CA) that allowed for subsequent off-line quantitative analyses. In a second group of rats (n = 7), NHE1 inhibition using cariporide was administered to determine whether this agent limited the extent of myocardial and microvascular stunning. Procedures for animals completing the vehicle and cariporide protocols were identical except that after 5 min of baseline in this group, cariporide was administered first as a bolus (7 mg/kg, 0.3 ml total) and subsequently as a constant infusion (0.07 mg·kg⁻¹·min⁻¹, 0.05 ml/min) into the carotid artery. The dose and method of cariporide administration were chosen based on our previous study (28). Arterial blood gases (PO2 and PCO2) and pH were measured at baseline, 30 min, and 60 min.

Ischemia and 60-min reperfusion. Procedures were identical to those described earlier except that after the 2 × 10-min coronary occlusions (separated by 5 min of reperfusion), vehicle- (n = 6) and cariporide-treated animals (n = 6) completed 60 min of reperfusion. Arterial Po2 and PCO2 and pH were measured at baseline, 50 min, and 105 min.

Sham-operated controls. This protocol determined whether myocardial and microvascular function were altered as a result of surgical instrumentation or administration of vehicle (n = 5) or cariporide (n = 5). All procedures were similar to those described earlier except that the snare occluder placed around the left coronary artery was not tightened.

Preliminary in vitro experiments. First, only KCl was capable of evoking a stable preconstriction of 60–80% of maximal tension development (Lmax) for the time required (i.e., 20–30 min) to the complete concentration-relaxation responses comprising approximately eight doses. Other preconstrictors tested were the thromboxane A2 receptor mimetic U-46619, endothelin-1, prostaglandin F2α, and serotonin. Second, dose-response curves using several agents that produce relaxation by mechanisms dependent on a viable endothelium (i.e., acetylcholine, bradykinin, and substance P) were performed. Only acetylcholine produced repeatable relaxation responses. Third, acetylcholine-evoked relaxation after KCl preconstriction was abolished by 1) damaging the endothelium, 2) administering the muscarinic receptor antagonist atropine (10⁻⁶ M), and 3) administering the nitric oxide synthase inhibitor N⁵-monomethyl-L-arginine (10⁻⁶ M). These findings suggest strongly that acetylcholine-evoked relaxation 1) is dependent on a viable endothelium, 2) acts via muscarinic receptor stimulation, and 3) is dependent on the production of nitric oxide in rat coronary resistance vessels. Fourth, time-volume control experiments were performed whereby 8 × 10⁻¹⁰ M additions of normal physiological salt solution (NPSS; pH –7.40) rather than drug were added after KCl-evoked preconstriction. No significant changes from the original tension were observed, verifying the stability of the preconstrictor response to KCl.

In vitro assessment of microvascular function. At termination of the in situ protocols (i.e., at 60 or 105 min), hearts were excised and placed in oxygenated ice-cold NPSS. With the use of a dissecting microscope (Leica Stereo Zoom 5), placement of the suture around the left coronary artery was confirmed, and the vessel was traced toward the apex of the heart. Second- and third-order branches of this artery were...
then isolated, cut from the heart, and prepared for mounting on a microvessel myograph (Jules Osher; Pomona, CA) (20, 30). This apparatus allows direct determination of vessel wall force development while internal diameter is controlled. Two tungsten wires (outer diameter, 20 μm) were inserted in a parallel manner through the lumen of the vessel. One wire was attached to a force transducer (Fort10 Transducer, World Precision Instruments; Sarasota, FL) to measure tension development, whereas the other wire was fixed to a micrometer that was used to stretch the vessel in small increments. Tension data were recorded continuously (Gould Brush 260). Vessels were immersed in a temperature-controlled 8.5-ml reservoir (i.e., a tissue “bath”) containing oxygenated (95% O2, 5% CO2) NPSS (pH ∼7.40). Samples from all buffers and each tissue bath were analyzed frequently for PO2, PCO2, and pH. After the coronary resistance arteries were mounted, the tissue bath was warmed gradually to 37°C, and the vessels were equilibrated at zero tension for ∼30 min. Ten milligrams of tension were then applied to the artery, and the distance between the wires was measured to calculate the internal diameter of the vessel using the following formula: \( L_c = (2 + \pi) W_t + 2G \), where \( L_c \) is internal circumference, \( W_t \) is wire thickness, \( G \) is the distance between the wires, and vessel internal diameter = \( L_c / \pi \) (20). This formula assumes that the walls of the vessel are flat between the wires after applying a slight stretch. Next, a series of internal circumference-active tension curves were constructed to determine the vessel diameter that evoked \( L_{max} \) to 100 mM KCl as we have previously described (30). \( L_{max} \) was determined for every vessel, and this optimal resting tension was maintained throughout the study. An equilibration period of 30 min preceded the assessment of endothelium-dependent relaxation.

Acetylcholine-evoked relaxation. The isolated vessels were precontracted using 45 mM KCl. When tension development was stable, concentration-relaxation curves were constructed using cumulative additions (10 ml, 10⁻⁸–10⁻⁴ M) of the muscarinic receptor agonist acetylcholine to assess endothelium-dependent relaxation. Relaxation responses are presented as a percentage of KCl-induced preconstriction. After the response to the final dose of acetylcholine was recorded, NPSS was reintroduced into the tissue bath, and a 30-min equilibration period was initiated, during which time the bathing medium was reexchanged with NPSS several times.

Endothelin-1-induced constriction. Concentration-contraction curves were performed in response to cumulative additions of endothelin-1 (10⁻¹¹–10⁻⁸ M). Contractile responses evoked by endothelin-1 are presented as milligrams of developed tension.

Sodium nitroprusside-evoked relaxation. The contractile response to the last dose of endothelin-1 (i.e., 10⁻⁷ M) served as preconstriction for evaluating endothelium-independent relaxation to sodium nitroprusside (10⁻⁴ M). The relaxation response is presented as a percentage of endothelin-1-induced preconstriction.

**Drugs and solutions.** NPSS contained (in mM) 125 NaCl, 4.7 KCl, 1.2 KH₂PO₄, 1.2 MgSO₄, 2.5 CaCl₂, 18 NaHCO₃, 0.026 Na₂EDTA, and 11.2 glucose. The KCl concentration was increased in designated solutions (i.e., 45 and 100 mM) by isomolar exchange with NaCl. All solutions were maintained at ∼37°C and aerated with 95%O₂, 5%CO₂ at a rate sufficient to maintain pH at ∼7.40. NPSS and KCl solutions were prepared daily from stock solutions. Acetylcholine (Sigma; St. Louis, MO), nitroprusside (Sigma), and endothelin-1 (Peninsula Laboratories; San Carlos, CA) were purchased commercially and prepared daily from stock solutions using distilled deionized water. All doses are expressed as the final concentration of each drug in the vessel bath.

**Statistical analyses.** Animal (e.g., weight and age) and vessel characteristics (e.g., internal diameter at rest, \( L_{max} \), percent precontraction, and tension developed at \( L_{max} \)) were compared among groups using a one-way ANOVA (10).

**RESULTS**

Myocardial and microvascular responses were similar between the sham-operated animals treated with vehicle (\( n = 5 \)) or cariporide (\( n = 5 \)). We concluded that NHE1 inhibition has no effect on LV contractility or vascular function in the absence of myocardial ischemia and reperfusion. Therefore, data from the sham-operated animals treated with cariporide are not shown.

**Myocardial function.** Body weight and age at the time of study were not different among groups (Table 1). Likewise, no differences in arterial blood gas status existed over time within or among groups during the in situ protocols (Table 2). Hemodynamic measures were similar among groups at baseline (i.e., 0–5 min). Moreover, hemodynamic variables during the preschismic

Table 1. Animal and vessel characteristics

<table>
<thead>
<tr>
<th>Age, days</th>
<th>Body Weight, g</th>
<th>Internal Diameter, μm</th>
<th>Baseline</th>
<th>Vessel length, μm</th>
<th>( L_{max} ), μm</th>
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</thead>
<tbody>
<tr>
<td>15-Min reperfusion</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>87 ± 7</td>
<td>380 ± 15</td>
<td>118 ± 4</td>
<td>223 ± 9</td>
<td>955 ± 52</td>
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<tr>
<td>Cariporide</td>
<td>111 ± 20</td>
<td>390 ± 33</td>
<td>129 ± 7</td>
<td>259 ± 19</td>
<td>906 ± 73</td>
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<td>Sham</td>
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<td>383 ± 25</td>
<td>130 ± 9</td>
<td>230 ± 10</td>
<td>946 ± 66</td>
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<td>60-Min reperfusion</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>95 ± 4</td>
<td>416 ± 23</td>
<td>108 ± 6</td>
<td>225 ± 10</td>
<td>960 ± 42</td>
</tr>
<tr>
<td>Cariporide</td>
<td>99 ± 2</td>
<td>436 ± 8</td>
<td>107 ± 4</td>
<td>214 ± 14</td>
<td>958 ± 45</td>
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</table>

Values are means ± SE. \( L_{max} \), internal diameter of vessel at the time of maximal tension development.
(or sham) period (i.e., 5–20 min) and final reperfusion periods were not different over time within the respective groups (Tables 3 and 4). Mean arterial pressure (MAP), LV developed pressure (LVDP), LV dP/dt, heart rate, and systolic wall thickening did not change from baseline over the 60-min protocol in sham-operated animals. However, MAP, LVDP, LV dP/dt, and systolic wall thickening were depressed similarly during the two ischemic episodes in all groups compared with shams (Table 3). During the 15- and 60-min reperfusion periods in the groups exposed to ischemia, LVDP, LV dP/dt, and systolic wall thickening remained depressed relative to their respective baseline measures, whereas MAP returned to control values.

**Vascular function.** Vessel characteristics were similar among groups (Table 1). Acetylcholine-evoked relaxation responses were blunted in vehicle- and cariporide-treated animals compared with sham-operated animals. However, the extent of acetylcholine-evoked relaxation was greater in cariporide-treated rats compared with vehicle-treated rats after both 15 min (Fig. 1A) and 60 min of reperfusion (Fig. 2A). Relaxation to sodium nitroprusside (10⁻⁴ M) was similar in vessels from vehicle- (~78%) and cariporide-treated (~75%) rats regardless of reperfusion time. This degree of smooth muscle relaxation was not different from sham-operated animals (~92%).

**Endothelin-1** caused dose-dependent contractile responses in all groups. Compared with sham-operated animals, however, absolute tension development (in mg) in response to the maximal dose of endothelin-1 (10⁻² M) was greater in vessels from cariporide- and vehicle-treated rats after both 15 min (Fig. 1B) and 60 min (Fig. 2B) of reperfusion.

**DISCUSSION**

We tested the hypothesis that NHE₁ inhibition lessens microvascular dysfunction of vessels perfusing the stunned myocardium. Consistent with an effect on endothelial-dependent relaxation, coronary resistance vessels from rats in which NHE₁ was inhibited using cariporide showed greater acetylcholine-evoked relaxation compared with vehicle-treated animals after both

### Table 2. Arterial blood gases during in situ protocols

<table>
<thead>
<tr>
<th>Variables</th>
<th>Protocol: 15-Min reperfusion</th>
<th>0 Minutes</th>
<th>5 Minutes</th>
<th>10 Minutes</th>
<th>20 Minutes</th>
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<tr>
<td></td>
<td>Time:</td>
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<tr>
<td></td>
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<td></td>
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</tr>
<tr>
<td>pH</td>
<td></td>
<td>7.42 ± 0.02</td>
<td>7.43 ± 0.02</td>
<td>7.43 ± 0.03</td>
<td>7.36 ± 0.02</td>
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<td>PCO₂</td>
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<td>34 ± 2</td>
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<tr>
<td>PO₂</td>
<td></td>
<td>222 ± 26</td>
<td>248 ± 48</td>
<td>212 ± 36</td>
<td>227 ± 29</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15-Min reperfusion</td>
<td>60 Minutes</td>
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<tr>
<td>pH</td>
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<td>7.37 ± 0.02</td>
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<td>7.37 ± 0.02</td>
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<td>PCO₂</td>
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<td>35 ± 3</td>
<td>37 ± 3</td>
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<td>PO₂</td>
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<td>260 ± 46</td>
<td>286 ± 36</td>
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Values are means ± SE.
15 and 60 min of reperfusion. In contrast, contractile responses to endothelin-1 and KCl and relaxation responses to sodium nitroprusside were similar among groups. These results suggest that when NHE1 inhibition is administered before ischemia, the endothelial function of vessels from stunned myocardium is preserved, but contractile (e.g., receptor and nonreceptor mediated) and dilatory responses of vascular smooth muscle are unaltered. Our second hypothesis, that NHE1 inhibition preserves endothelial and/or vascular function in vessels from stunned myocardium is precluded by 10.220.33.6 on June 28, 2017 http://ajpheart.physiology.org/ Downloaded from

<table>
<thead>
<tr>
<th>Variables</th>
<th>Protocol:</th>
<th>Baseline</th>
<th>Preischemia</th>
<th>Ischemia</th>
<th>Reperfusion</th>
<th>Ischemia</th>
<th>Reperfusion</th>
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<td>MAP, mmHg</td>
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<tr>
<td>Vehicle</td>
<td></td>
<td>79 ± 9</td>
<td>82 ± 8</td>
<td>72 ± 11*</td>
<td>97 ± 7</td>
<td>71 ± 7*</td>
<td>85 ± 8</td>
<td>87 ± 8</td>
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<td>Cariporide</td>
<td></td>
<td>90 ± 7</td>
<td>99 ± 12</td>
<td>70 ± 7*</td>
<td>87 ± 6*</td>
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<td>HR, beats/min</td>
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<td>249 ± 11</td>
<td>268 ± 7</td>
<td>290 ± 10*</td>
<td>257 ± 17</td>
<td>282 ± 8*</td>
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<td>278 ± 11*</td>
<td>293 ± 9*</td>
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<tr>
<td>LVDP, mmHg</td>
<td></td>
<td>255 ± 11</td>
<td>262 ± 19</td>
<td>277 ± 12*</td>
<td>260 ± 15</td>
<td>283 ± 16*</td>
<td>270 ± 16*</td>
<td>260 ± 11</td>
<td>267 ± 12*</td>
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<td>92 ± 7</td>
<td>95 ± 7</td>
<td>78 ± 9*</td>
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<tr>
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<td>95 ± 5</td>
<td>100 ± 8</td>
<td>68 ± 9*</td>
<td>87 ± 6</td>
<td>72 ± 7*</td>
<td>88 ± 8*</td>
<td>87 ± 7</td>
<td>82 ± 8*</td>
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<tr>
<td>LV dP/dt, mmHg/s</td>
<td></td>
<td>3,417 ± 288</td>
<td>3,617 ± 227</td>
<td>2,833 ± 232*</td>
<td>3,675 ± 111</td>
<td>2,850 ± 200*</td>
<td>3,100 ± 362*</td>
<td>3,117 ± 143*</td>
<td>3,142 ± 179*</td>
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<tr>
<td>Vehicle</td>
<td></td>
<td>3,608 ± 226</td>
<td>3,700 ± 331</td>
<td>2,467 ± 174*</td>
<td>3,758 ± 319</td>
<td>2,750 ± 149*</td>
<td>3,117 ± 339*</td>
<td>3,192 ± 337*</td>
<td>3,242 ± 387*</td>
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<tr>
<td>Cariporide</td>
<td></td>
<td>0.8 ± 0.4</td>
<td>3.1 ± 0.4</td>
<td>1.2 ± 0.2*</td>
<td>2.8 ± 0.7</td>
<td>1.1 ± 0.7*</td>
<td>2.0 ± 0.2*</td>
<td>1.8 ± 0.1*</td>
<td>1.6 ± 0.1*</td>
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<tr>
<td>SWth, %</td>
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<td>2.7 ± 0.3</td>
<td>2.9 ± 0.2</td>
<td>0.3 ± 0.2*</td>
<td>2.5 ± 0.3</td>
<td>0.9 ± 0.4*</td>
<td>1.6 ± 0.1*</td>
<td>1.6 ± 0.1*</td>
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</table>

Values are means ± SE. Preischemia denotes the average of variables obtained at 10, 15, and 20 min. *P < 0.05 vs. respective baseline value.
strictor agents. However, the lack of reproducibility of the dilators and the unstable nature of the constrictors rendered them unacceptable for our purposes. In any case, we refer to our assessment of endothelial function as “acetylcholine-induced vasorelaxation” and believe that nitric oxide release evoked by muscarinic receptor stimulation is largely responsible for the observed vasodilation (see Preliminary experiments). An alternative explanation for our results is that NHE1 inhibition upregulated cholinergic muscarinic receptor number and/or altered receptor subtypes or binding efficiency. While this possibility is unlikely, it cannot be ruled out with certainty because these variables were not quantified in the present study.

Because endothelial dysfunction may become more severe as the duration of reperfusion increases (32), we assessed vascular reactivity after both relatively short (i.e., 15 min) and long (i.e., 60 min) reperfusion times. With the use of these methods, we hoped to determine whether the hypothesized protection afforded by NHE1 inhibition persisted in vessels exposed to a longer period of reperfusion. Our findings indicate that the extent of ischemia-induced vascular dysfunction in the vehicle-treated animals was similar after both 15 and 60 min of reperfusion. Likewise, the improvement in endothelium-dependent function in response to NHE1 inhibition was similar after both reperfusion periods. These findings suggest that treatment with cariporide before ischemia protects coronary microvascular function over a relatively long time period (i.e., 15–60 min) after reperfusion.

Fig. 1. A: acetylcholine-evoked relaxation in coronary resistance vessels from control, cariporide-treated, and vehicle-treated rats occurred in a dose-dependent manner. Relaxation was calculated as the percent reduction from precontraction evoked by 45 mM KCl. Responses in sham-operated animals were greater \((*P < 0.05)\) compared with cariporide- and vehicle-treated rats. Relaxation of vessels from vehicle-treated animals was less \((**P < 0.05)\) than cariporide-treated animals. B: contractile responses to endothelin-1 occurred in a dose-dependent manner in all groups. The response to \(10^{-7} \text{M}\) endothelin-1 was greater \((***P < 0.05)\) in vessels from ischemic (i.e., vehicle- and cariporide-treated animals) compared with sham-operated animals. All values are means \(\pm\) SE.

Fig. 2. A: acetylcholine-evoked relaxation in coronary resistance vessels from control, cariporide-treated, and vehicle-treated rats occurred in a dose-dependent manner. Relaxation was calculated as the percent reduction from precontraction evoked by 45 mM KCl. Relaxation was greater \((**P < 0.05)\) in vessels from cariporide- versus vehicle-treated rats. B: contractile responses to endothelin-1 were not different in vessels from cariporide- versus vehicle-treated rats. All values are means \(\pm\) SE. Note the similarity of responses to acetylcholine and endothelin-1 from vessels exposed to 15 min of reperfusion (Fig. 1).
Greater acetylcholine-evoked relaxation in coronary resistance vessels from cariporide-treated animals compared with vehicle-treated animals did not result from increased vascular smooth muscle responsiveness. Evidence supporting this is provided by our results showing similar maximal relaxation to the endothelium-independent vasodilator sodium nitroprusside among groups. These findings indicate that NHE1 inhibition does not alter nitric oxide/cGMP-dependent intracellular signaling pathways or second messenger systems within vascular smooth muscle cells of rat coronary resistance vessels isolated from the stunned myocardium.

Several explanations for the beneficial effects of cariporide on ischemia-induced microvascular dysfunction exist. In a previous study (27), isolated rat hearts treated with cariporide had improved postischemic contractile function, which was associated with reduced ([Ca$$^{2+}$$]$$\text{I}$$) overload and prolonged acidosis in cardiomyocytes. In the present study, the mechanisms of protection may be similar in endothelial cells of coronary microvessels. Furthermore, by maintaining intracellular acidosis and preventing [Na$$^{+}$$]$$\text{I}$$ and [Ca$$^{2+}$$]$$\text{I}$$ accumulation, NHE1 inhibition reduces the stimuli responsible for phospholipase A2 activation of the arachidonic acid cascade. This is relevant concerning vascular injury because ischemia and reperfusion produce superoxide anion through several mechanisms, one of which is by phospholipase A2 activation of arachidonic acid (6). Finally, studies performed in vitro (8) and in vivo (11) show that NHE1 inhibition attenuates neutrophil activity. This is important because neutrophils that accumulate in the ischemic myocardium upon reperfusion may contribute to the subsequent impairment of the coronary vasodilator reserve (18). These possibilities are only speculative, however, because they were not evaluated directly in the present study.

Tension development in response to receptor-mediated (i.e., endothelin-1) and nonreceptor-mediated (i.e., KCl, data not shown) contractile agents also was assessed. Endothelin-1 was chosen for study because this endothelium-derived constricting factor 1) is the most potent mammalian vasoconstrictor known (33), 2) is released during myocardial ischemia and could contribute to coronary vasospasm (31), and 3) regulates myocardial blood flow in both the native and collateral-dependent myocardium (29). Originally, we hypothesized that vasoconstriction would be blunted in cariporide versus vehicle-treated rats because of preserved endothelial function and, therefore, greater endogenous opposition from endothelium-derived relaxing factors. Instead, even though acetylcholine-evoked relaxation was greater in cariporide- versus vehicle-treated animals, contractile responses to endothelin-1 were similar between groups. Likewise, the tension development to activation of voltage-gated calcium channels using 100 mM KCl was not different between the two groups of vessels isolated from the stunned myocardium. These results suggest that endothelial preservation by cariporide was not accompanied by an increased ability to oppose receptor- and nonreceptor-mediated constrictors.

Compared with vessels from control animals (i.e., not exposed to ischemia), contractile responses to the highest dose of endothelin-1 were exaggerated in arteries obtained from the stunned myocardium of cariporide- and vehicle-treated rats. Because acetylcholine-evoked relaxation was blunted in vessels from animals exposed to ischemia, the greater responses to endothelin-1 in these two groups may reflect less opposition from endothelium-derived relaxing factors compared with control animals. Other potential explanations for these findings are that myocardial ischemia 1) increased the density and/or sensitivity of the endothelin receptor subtypes (i.e., endothelin type A/type B receptors) on vascular smooth muscle that are responsible for vasoconstriction and/or 2) impaired the function of endothelin type B receptors on the endothelium that mediate vasodilation. Although these possibilities exist, pursuit of the mechanisms explaining the effects of ischemia per se on microvascular function was not the focus of the present study.

Our secondary hypothesis, that NHE1 inhibition lessens myocardial contractile dysfunction evoked by ischemia and reperfusion, was not supported. Instead, we observed that reductions in regional (i.e., LV systolic wall thickening) and global (i.e., LVDP and dP/dt) myocardial function were similar regardless of whether NHE1 inhibition was administered before the ischemia and reperfusion protocol. These data are strengthened by several factors. First, afterload was similar among groups during all protocols. This is important because alterations in this variable can influence both global and regional myocardial contractility (4). Second, because all indexes of myocardial function measured in the present study were similar in the sham-operated animals, changes from baseline observed in the vehicle- and cariporide-treated rats were due to ischemia and reperfusion rather than to a deteriorating experimental preparation. These data, however, do not confirm our previous report in conscious pigs showing that NHE1 inhibition using cariporide delays the onset and reduces the severity of myocardial dysfunction. Potential explanations for the discrepant findings include species differences (rats vs. pigs), anesthetic regimen (anesthetized vs. conscious), and/or the protocol to induce myocardial dysfunction (2 × 10-min occlusions, followed by 15 or 60 min of reperfusion vs. 25 cycles of 2-min occlusion/8-min reperfusion).

Concern regarding the clinical relevance of our experimental paradigm may be raised because NHE1 inhibition was administered before ischemia. However, there are several clinical situations in which an oxygen supply-demand mismatch could be predicted to occur. Examples include procedures during cardiac surgery, cardiac arrest during cardiopulmonary bypass, acute coronary occlusion during balloon angioplasty, and after cardiac transplantation. Because each of these situations include varying periods of ischemia and subsequent reperfusion, NHE1 inhibition before their onset may help to preserve microvascular function. Moreover, chronic
NHE$_1$ inhibition via oral administration may be beneficial for patients with diagnosed coronary artery disease who possess a high likelihood of experiencing periodic bouts of myocardial ischemia. Therefore, while extrapolation of results from experimental animals to in vivo pathophysiological conditions in humans should be done cautiously, our data suggest that NHE$_1$ inhibitors have important therapeutic potential in settings where ischemia and reperfusion are likely to occur.

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