Na^+\text/H^+ exchange inhibition-induced cardioprotection in dogs: effects on neutrophils versus cardiomyocytes

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1Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, Wisconsin 53226; 2Division of Cardiology, Department of Medicine, University of Louisville, Louisville, Kentucky 40202; and 3Boehringer Ingelheim Pharma KG, D-88397 Biberach an der Riss, Germany

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**Gumina, Richard J., John Auchampach, Rongang Wang, Erich Buerger, Christian Eickmeier, Jeannine Moore, Juergen Daemmgen, and Garrett J. Gross.** Na*/H* exchange inhibition-induced cardioprotection in dogs: effects of neutrophils versus cardiomyocytes. *Am J Physiol Heart Circ Physiol* 279: H1563–H1570, 2000.—Numerous studies have examined the effect of Na*/H* exchanger (NHE) inhibition on the myocardium; however, the effect of NHE-1 inhibition on neutrophil function has not been adequately examined. An in vivo canine model of myocardial ischemia-reperfusion injury in which 60 min of left anterior descending coronary artery occlusion followed by 3 h of reperfusion was used to examine the effect of NHE-1 inhibition on infarct size (IS) and neutrophil function. BIIB-513, a selective inhibitor of NHE-1, was infused before ischemia. IS was expressed as a percentage of area at risk (IS/AAR). NHE-1 inhibition significantly reduced IS/AAR and reduced neutrophil accumulation in the ischemic myocardium. NHE-1 inhibition attenuated both phorbol 12-myristate 13-acetate- and platelet-activating factor-induced neutrophil respiratory burst but not CD18 upregulation. Furthermore, NHE-1 inhibition directly protected cardiomyocytes against metabolic inhibition-induced lactate dehydrogenase release and hypercontraction. This study provides evidence that the cardioprotection induced by NHE-1 inhibition is likely due to specific protection of cardiomyocytes and attenuation of neutrophil activity.

**METHODS**

**Materials.** To perform the present studies, BIIB-513 was used. All reagents were obtained from Sigma Chemicals (St. Louis, MO) or GIBCO-BRL (Gaithersburg, MD) unless otherwise indicated.

**Ischemia-reperfusion protocol.** A standard myocardial ischemia-reperfusion protocol was employed, as described previously (14). Dogs were assigned to one of two groups in a randomized fashion. Either saline (control group) or BIIB-513 (3.0 mg/kg) was infused intravenously for 15 min immediately before left anterior descending (LAD) artery occlusion (Fig. 1). All dogs were subjected to 60 min of LAD artery occlusion and 3 h of reperfusion. In both groups, hemodynamic measurements and arterial blood gas analysis were...
obtained before LAD artery occlusion, at 30 min during the 60-min occlusion, and every hour after reperfusion. Regional myocardial blood flows were determined at 30 min during the 60-min occlusion period and at the end of the experiment.

At the end of the 3-h reperfusion period, the anatomic area at risk (AAR) and the nonischemic area were differentiated as previously described (14). The hearts were electrically fibrillated, removed, and prepared for infarct size (IS) determination by 2,5,5-triphenyltetrazolium chloride histochemical staining and regional myocardial blood flow measurements. Infarcted and noninfarcted tissues within the AAR were separated and determined gravimetrically. IS was expressed as a percentage of the AAR. Regional myocardial blood flow was measured by the radioactive microsphere technique as described previously in this laboratory (14).

Dogs were excluded if 1) heartworms were found after the dogs were killed, 2) transmural collateral blood flow was >0.20 ml·min⁻¹·g⁻¹, 3) heart rate was >180 beats/min at the beginning of the experiment, or 4) more than three consecutive attempts were needed to convert ventricular fibrillation with low-energy direct current pulses.

Determination of myeloperoxidase activity. Neutrophil accumulation in the myocardium was measured by determining myeloperoxidase (MPO) activity according to previously published methods (22). The change in absorbance at 460 nm was measured spectrophotometrically. Each sample was tested in duplicate. MPO activity was determined by comparison with a standard curve generated using purified MPO (Sigma). The data are expressed as MPO activity (units) per gram weight of tissue.

Histopathological analysis. At the end of 180 min of reperfusion, tissue samples were obtained from the AAR. The tissues samples were snap-frozen in isopentane (maintained in liquid nitrogen) and embedded in Tissue-Tek optimum-cutting temperature compound (Miles, Elkart, IN) as previously described (2). Frozen sections of the myocardial samples were stained with hematoxylin and eosin and then were evaluated using a Zeiss Axioplan II microscope. Samples were visualized at X40 magnification.

Neutrophil activation studies. Canine whole blood was collected in 1% anticoagulant citrate dextrose (Miles, Ontario, Canada). Neutrophils were isolated using the Polymorphprep (Nycomed Pharma, Oslo, Norway) according to the manufacturer’s protocol for the isolation of human neutrophils. Residual red blood cells were removed via hypotonic lysis, and the remaining neutrophils were resuspended in Dulbecco’s PBS (DPBS) with 10 mM glucose and adjusted to 10⁷/ml. Cell preparations were >95% neutrophils as determined by Wright’s stain and forward scatter/side scatter profiles on the FACSscan (Becon-Dickinson, Palo Alto, CA).

BIIB-513, diluted in DMSO, was added to 1 ml of isolated cells to give final concentrations from 0.1 to 1000 μM. After incubation at room temperature for 10 min the cell suspensions were placed at 37°C for 15 min before the addition of 1,000 nM platelet-activating factor (PAF). Cells were incubated at 37°C for 30 min and then were placed on ice for 10 min. One microgram of FITC-conjugated anti-human CD18 monoclonal antibody clone MMH23 (Dako, Glostrup, Denmark), previously shown to cross-react with canine CD18 (31) or FITC-conjugated mouse IgG1 (isotype control), was added to the cell suspension and incubated at 4°C for 60 min. After incubation, the cells were diluted 1:2 in DPBS with 10 mM glucose and analyzed on a FACSscan.

For analysis of the respiratory burst, a flow cytometric assay was employed to quantify the oxidation of 2′,7′-dichlorodihydrofluorescein during the respiratory burst of neutrophils (1). Briefly, neutrophils (10⁶/ml) were incubated at 37°C for 15 min with 5 μM of 2′,7′-dichlorodihydrofluorescein diacetate (H₂DCF-DA; Molecular Probes, Eugene, OR). After being loaded with H₂DCF-DA, cells were washed one time in DPBS with 10 mM glucose and adjusted to 10⁶/ml. BIIB-513, diluted in DMSO, was added to 1 ml of isolated cells to give final concentrations from 0.1 to 100 μM. The final DMSO concentration was <0.005%. Cells were incubated at room temperature for 10 min and then were placed at 37°C for 15 min before the addition of equal volumes of either PAF at a final concentration of 1,000 nM, phorbol 12-myristate 13-acetate (PMA) at a final concentration of 400 ng/ml, or vehicle (100% DMSO or 0.1%)

Metabolic inhibition of rabbit cardiomyocytes. Ca²⁺-tolerant cardiac myocytes were isolated from adult New Zealand White rabbits according to previously published methods (24). Myocytes were subjected to metabolic inhibition by incubating the cells in glucose-free Krebs buffer (pH 6.5) containing (in mM) 137 NaCl, 3.8 KCl, 0.5 MgCl₂, 0.9 CaCl₂, 20 sodium lactate, 10 2-deoxyglucose, 0.75 sodium hydroxysulfite, and 10 HEPES (8). After incubation for 2 h at 37°C, the metabolic inhibition buffer was collected and replaced with culture media. The myocytes were “reoxygenated” for 3 h after which the cells were examined microscopically. The percentage of hypercontracted myocytes was calculated by counting the number of rod-shaped cardiomyocytes within the same three randomly chosen grids immediately before and after metabolic inhibition. Hypercontracted myocytes were identified as those with a length-to-width ratio less than 3:1 (32). Next, culture medium was removed and pooled with the metabolic inhibition buffer. After removal of the “reoxygenation” buffer, myocytes remaining on the plate were scraped into hypotonic lysis buffer (10 mM Na-HEPES and 2 mM EDTA, pH 7.4). As an additional index of cell injury, lactate dehydrogenase (LDH) activity was measured in the supernatants and lyses using a standard assay kit (Sigma), and the percentage of the total amount of LDH released into the supernatants in response to metabolic inhibition was calculated. Various concentrations of the NHE-1 inhibitor BIIB-513 or the equivalent vehicle (DMSO <0.1%)
Table 1. Hemodynamics in the different treatment groups

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>After 15 min Drug Treatment</th>
<th>Occlusion 30 min</th>
<th>Occlusion 60 min</th>
<th>Reperfusion 1 h</th>
<th>Reperfusion 2 h</th>
<th>Reperfusion 3 h</th>
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<tr>
<td><strong>HR, beats/min</strong></td>
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<tr>
<td>Control</td>
<td>151 ± 1</td>
<td>149 ± 1</td>
<td>148 ± 2</td>
<td>148 ± 2</td>
<td>145 ± 7</td>
<td>146 ± 7</td>
<td>122 ± 13</td>
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<tr>
<td>BIIB-513</td>
<td>150 ± 1</td>
<td></td>
<td>150 ± 1</td>
<td>148 ± 3</td>
<td>148 ± 7</td>
<td>151 ± 2</td>
<td>150 ± 2</td>
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<td><strong>MBP, mmHg</strong></td>
<td></td>
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<tr>
<td>Control</td>
<td>109.5 ± 11.6</td>
<td>128.3 ± 4.2</td>
<td>111.0 ± 8.8</td>
<td>108.0 ± 9.8</td>
<td>97.1 ± 11.7</td>
<td>104.2 ± 10.4</td>
<td>101.1 ± 11.2</td>
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<tr>
<td>BIIB-513</td>
<td>120.0 ± 7.2</td>
<td>114.8 ± 7.4</td>
<td>124.3 ± 8.2</td>
<td>116.6 ± 9.3</td>
<td>123.5 ± 8.2</td>
<td>118.8 ± 9.9</td>
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<td><strong>RPP, mmHg·min⁻¹·1,000⁻¹</strong></td>
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<tr>
<td>Control</td>
<td>18.6 ± 1.9</td>
<td>17.6 ± 18</td>
<td>15.9 ± 2.5</td>
<td>17.1 ± 2.3</td>
<td>14.3 ± 2.8</td>
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<tr>
<td>BIIB-513</td>
<td>20.2 ± 1.3</td>
<td>20.6 ± 1.4</td>
<td>19.2 ± 1.5</td>
<td>20.6 ± 1.4</td>
<td>20.0 ± 1.9</td>
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<tr>
<td><strong>LV dP/dt, mmHg/s</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Control</td>
<td>2,250 ± 462</td>
<td>2,025 ± 263</td>
<td>1,913 ± 290</td>
<td>1,463 ± 263</td>
<td>1,550 ± 95</td>
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<tr>
<td>BIIB-513</td>
<td>3,150 ± 357</td>
<td>2,662 ± 247</td>
<td>2,962 ± 206</td>
<td>2,362 ± 188</td>
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</table>

Values are means ± SE. HR, heart rate; MBP, mean blood pressure; RPP, rate-pressure product; LV dP/dt, left ventricular derivative of pressure vs. time. Amount of BIIB-513 used was 3 mg/kg.

were present in both the metabolic inhibition buffer and the reoxygenation buffer. All experiments were performed in triplicate.

**Statistical analysis.** All values are expressed as means ± SE unless otherwise noted. Differences between groups in hemodynamics and blood gases were compared by use of a two-way (for time and treatment) ANOVA with repeated measures. Differences between groups in MPO activity, LDH release, hypercontracture, tissue blood flows, AAR, and IS were compared with a two-tailed t-test. Analysis of covariance was used to determine whether the relation between transmural collateral blood flow and IS differed between the control and drug-treated groups. For all experiments, differences between groups were considered significant if the P value was < 0.05.

**RESULTS**

**Exclusions and hemodynamic and blood gas data.** A total of eight dogs were initially used in this study. None were excluded; thus, eight dogs successfully completed the protocol and were included in data analysis.

Table 1 summarizes the hemodynamic and blood gas data. There were no significant differences within or between the groups throughout the experiment with regard to heart rate, mean arterial pressure, rate-pressure product, and left ventricular (LV) derivative of pressure versus time. There also were no significant differences in pH, PO₂, and PCO₂ within and between groups at the times studied (data not shown).

**Infarct size measurements.** Figure 2 summarizes the effect of administration of the NHE-1 inhibitor BIIB-513 before ischemia on IS expressed as a percentage of the AAR (IS/AAR). Administration of BIIB-513 resulted in significant (P < 0.05) reductions in IS, IS/LV (Table 2), and IS/AAR (Fig. 2). There were no significant differences in LV weight, AAR, or AAR/LV between groups (Table 2). There also were no differences in transmural collateral blood flow between groups, indicating that both groups were subjected to equivalent degrees of ischemia (Table 2). However, a plot of transmural collateral blood flows versus IS/AAR demonstrated that the regression line describing this relationship in BIIB-513-treated animals was shifted down compared with the control group (data not shown), indicating that for any level of collateral blood flow, the IS/AAR would be predicted to be smaller in NHE-1-inhibited animals.

**Neutrophil accumulation and activity.** As shown in Fig. 3, a significant reduction in MPO activity within the infarcted myocardium was observed in dogs treated with 3.0 mg/kg of BIIB-513 when compared with control animals (P < 0.001). To ensure that the decrease in MPO activity observed was not due to direct effects of BIIB-513 on the assay, BIIB-513 (10 µM final concentration) was added directly to control and tissue samples. No attenuation of MPO activity was observed (data not shown). To visually confirm the decrease in

![Fig. 2. Effect of Na⁺/H⁺ exchanger (NHE)-1 inhibition on infarct size. BIIB-513 (3.0 mg/kg) or saline was administered 15 min before occlusion of the LAD artery for 60 min. Infarct size (IS)/area at risk (AAR) expressed as percentage. All values are means ± SE (n = 4 experiments). *P < 0.05 vs. the control group.](http://ajpheart.physiology.org/)}
neutrophil accumulation in BIIB-513-treated animals, hematoxylin- and eosin-stained frozen sections of AAR myocardium were examined. As shown in Fig. 4, although a marked neutrophil accumulation was observed in AAR from control animals, few neutrophils were found within the AAR in BIIB-513-treated animals.

To further investigate the effect of NHE-1 inhibition on neutrophil function, CD18 upregulation and respiratory burst were examined. Although BIIB-513 at concentrations ranging from 0.1 to 1,000 μM had no effect on CD18 upregulation (Fig. 5), a concentration-dependent inhibition of PAF-induced and PMA-induced neutrophil respiratory burst was observed (Fig. 6, A and B, respectively). Thus NHE-1 inhibition appears to attenuate neutrophil accumulation in the ischemic-reperfused myocardium and to inhibit neutrophil activation.

**Effect of NHE-1 inhibition on isolated cardiomyocytes.** To determine whether the cardioprotective effects observed in vivo were due to specific cardiomyocyte protection, isolated rabbit cardiomyocytes were subjected to metabolic inhibition-induced injury. Inhibition of NHE-1 with BIIB-513 resulted in significant cardiomyocyte protection. Both hypercontracture (Table 3 and Fig. 7) and LDH release were significantly attenuated compared with control cells (P < 0.001 and P < 0.05, respectively) at 0.1–3 μM BIIB-513. Thus NHE-1 inhibition directly protects cardiomyocytes.

**DISCUSSION**

The results of the present study demonstrate in an in vivo canine model of myocardial ischemia-reperfusion injury that administration of the specific and selective new NHE-1 inhibitor BIIB-513 results in a reduction of myocardial infarct size. Although a causal relationship between the attenuation of neutrophil activation and

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Table 2. Infarct size data and transmural blood flow data

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>LV, g</th>
<th>AAR, g</th>
<th>IS, g</th>
<th>Calculated Data, %</th>
<th>Transmural Blood Flow</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>AAR/LV</td>
<td>IS/AAR</td>
<td>IS/LV</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>113 ± 6</td>
<td>39.9 ± 4.3</td>
<td>9.3 ± 1.4</td>
<td>35.4 ± 2.0</td>
<td>23.2 ± 1.8</td>
</tr>
<tr>
<td>BIIB-513 (3.0 mg/kg)</td>
<td>4</td>
<td>91 ± 11</td>
<td>28.2 ± 3.5</td>
<td>0.2 ± 0.05*</td>
<td>31.0 ± 1.4</td>
<td>0.6 ± 0.2*</td>
</tr>
</tbody>
</table>

All values are means ± SE; n, no. of experiments. LV, left ventricle; AAR, area at risk; IS, infarct size; Occ, occlusion. *P < 0.05 vs. the control group.

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Fig. 3. Effect of NHE-1 inhibition on neutrophil accumulation as measured by myeloperoxidase (MPO) activity. Data are expressed as MPO activity (units)g weight of tissue. Each assay was performed in duplicate; n = 4 for control group and n = 4 for group treated with 3.0 mg/kg of BIIB-513. *P < 0.05 vs. the control group.

Fig. 4. Effect of NHE-1 inhibition on neutrophil accumulation by histology Representative photomicrographs are shown. A: control animal; B: BIIB-513-treated animal. A marked increase in neutrophil accumulation was observed in the control animals; n = 4 for control group and n = 4 for group treated with 3.0 mg/kg of BIIB-513.
accumulation and the reduction in infarct size has not been definitely proven, the observations that BIIB-513 protects cardiomyocytes and reduces activation of neutrophils at a similar range of concentrations in vitro and that BIIB-513 reduces neutrophil accumulation in vivo as assessed histologically and by MPO activity suggest that its effect on neutrophils may be at least partially responsible for the protection observed.

NHE in the myocardium during ischemia-reperfusion. With myocardial ischemia, an increase in intracellular $H^+$ ensues that activates the NHE, resulting in the extrusion of $H^+$ and the influx of $Na^+$ (10). Increases in intracellular $Na^+$ correlate with increases in intracellular $Ca^{2+}$, presumably through effects on the $Na^+/Ca^{2+}$ exchanger (10, 30). The net effect of increasing intracellular $Na^+$ is an accumulation of $Ca^{2+}$ in the ischemic myocardium that contributes to cellular damage, resulting in arrhythmias and contraction band necrosis (29). With reperfusion, extracellular $H^+$ rapidly decreases again, establishing a large intracellular-to-extracellular $H^+$ gradient that activates the NHE again, leading to an abnormally large accumulation of $Ca^{2+}$ during reperfusion (30) that contributes to reperfusion arrhythmias, myocardial contracture, and necrosis (29). Interestingly, although early reperfusion of ischemic myocardium leads to salvage of the jeopardized tissue, reperfusion also exacerbates the injury sustained during the ischemic period via the mechanisms outlined above and via a subsequent inflammatory response. With reperfusion, leukocytes adhere to the coronary microvasculature (25) and subsequently transmigrate into the myocardium, followed by the release of various inflammatory mediators such as oxygen radicals, which induce further damage of the myocardium (4–7, 15). NHE inhibition with less specific inhibitors of NHE-1 also has been shown to inhibit neutrophil function in vitro (9, 11, 26–28). Thus the current study examined the effects of specific inhibition of NHE-1 on cardiomyocyte protection and neutrophil activity.

Cardiomyocyte protection from metabolic inhibition. To confirm that the cardioprotection observed in vivo was due to direct cardiomyocyte protection by NHE-1 inhibition, metabolic inhibition/reoxygenation of isolated rabbit cardiomyocytes was employed. Inhibition of NHE-1 via treatment with increasing concentrations of BIIB-513 resulted in a concentration-dependent reduction of cardiomyocyte damage that was significant at concentrations $\geq$100 nM. As shown in Table 3 and Fig. 7, inhibition of NHE-1 with BIIB-513 resulted in a significant reduction of LDH release and a profound attenuation of hypercontracture, respectively. Previous studies have demonstrated that administration of

![Fig. 5. Effect of NHE-1 inhibition on CD18 upregulation on neutrophils. Change in relative fluorescence intensity of the linear mean of the population for CD18 expression on canine neutrophils unstimulated (−) or stimulated (+) with 1,000 nM platelet-activating factor (PAF) in the absence or presence of 10 μM BIIB-513. Each assay was performed in duplicate. Data shown are derived from 1 of 4 separate, comparable experiments. *P < 0.05 vs. the control group.](http://ajpheart.physiology.org/)

![Fig. 6. Effect of NHE-1 inhibition on neutrophil respiratory burst. Canine neutrophils were isolated and stimulated with 1,000 nM PAF (A) or 400 ng/ml of phorbol 12-myristate 13-acetate (B) in the absence or presence of 0.1–100 μM BIIB-513. Relative fluorescence intensity of the linear mean of the population for the respiratory burst is shown. [BIIB-513], BIIB-513 concentration; CONT, control. Each assay was performed in duplicate. Data shown are derived from 1 of 4 separate, comparable experiments. *P < 0.05 vs. the control group.](http://ajpheart.physiology.org/)
NHE-1 inhibitors prevented postsischemic myocardial contracture and Ca\(^{2+}\) clumping (12, 16, 18). Cardiomyocyte hypercontracture results from an abnormal accumulation of Ca\(^{2+}\) during ischemia (20, 30). Although several studies have demonstrated that NHE inhibition reduces Ca\(^{2+}\) accumulation, more recent data suggest that NHE blockade does not result in differences in the recovery of Ca\(^{2+}\) or Na\(^{+}\) levels but rather that the persistent acidosis acts to prevent hypercontracture due to reduced Ca\(^{2+}\) sensitivity of the myofibrils (20, 33). Furthermore, NHE inhibition attenuates phase-2 cytosolic Ca\(^{2+}\) oscillations (20), which are shifts in Ca\(^{2+}\) between the cytosol and sarcoplasmic reticulum that occur when Ca\(^{2+}\) load exceeds the capacity of the sarcoplasmic reticulum. Cytosolic Ca\(^{2+}\) oscillations contribute to reperfusion arrhythmias, and Ca\(^{2+}\) overload leads to eventual cardiomyocyte death (10, 29, 30). Thus prevention of Ca\(^{2+}\) overload/oscillation may result in the prevention of contraction band necrosis, which translates in vivo to the decreased infarct size observed in the current study.

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**NHE-1 inhibition differentially affects neutrophil activation.** This report demonstrates for the first time that NHE-1 inhibition attenuates the accumulation of neutrophils within the ischemic-reperfused myocardium (Figs. 3 and 4) and suggests that the reduction in myocardial infarct size observed in vivo was due in part to the effects of NHE-1 inhibition of neutrophil function. However, the interpretation of this observation is complex. Because BIIB-513 treatment markedly reduced myocardial infarct size, the ensuing inflammatory response might also be diminished, resulting in decreased neutrophil accumulation. Alternatively, in vitro, NHE inhibition has been reported to block neutrophil chemotaxis (28) and respiratory burst (27); therefore, the decreased accumulation may be due to direct effects on neutrophils. To determine whether specific inhibition of NHE-1 affects neutrophil activation, isolated canine neutrophils were stimulated with PAF, which is increased in coronary sinus blood after myocardial ischemia-reperfusion (19), or PMA, an activator of protein kinase C. Both PAF and PMA activate neutrophils, causing upregulation of the CD11b/CD18 integrin complex that facilitates neutrophil binding to endothelial cells and cardiac myocytes (6), and both stimulate the neutrophil respiratory burst (7, 19). NHE-1 inhibition via pretreatment of neutrophils with up to 1,000 μM of BIIB-513 did not attenuate the PAF-mediated induction of CD18 expression (Fig. 5). Similar data were obtained with PMA-stimulated neutrophils (data not shown). However, a concentration-dependent attenuation of both PAF- and PMA-induced respiratory burst was observed (Fig. 6, A and B, respectively). Interestingly, the dose of PAF employed is approximately 10 times the concentrations found in

![Image of Figure 3](http://ajpheart.physiology.org/)  **Fig. 3.** Effect of NHE-1 inhibition on metabolic inhibition-induced hypercontracture. Rabbit cardiomyocytes were subjected to the metabolic inhibition/reoxygenation protocol and then were photographed. A: cardiomyocytes before protocol; B: control cardiomyocytes after protocol; C: NHE-1-inhibited cardiomyocytes after protocol (1 μM BIIB-513).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cells Hypercontracted, %</th>
<th>LDH Release, %</th>
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<tbody>
<tr>
<td>Control</td>
<td>94.2 ± 1.2</td>
<td>30.9 ± 3.2</td>
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<tr>
<td>BIIB-513</td>
<td></td>
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<tr>
<td>100 nM</td>
<td>88.4 ± 1.4*</td>
<td>26.7 ± 2.2</td>
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<tr>
<td>300 nM</td>
<td>72.4 ± 1.3*</td>
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<tr>
<td>1,000 nM</td>
<td>42.8 ± 3.4*</td>
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<tr>
<td>3,000 nM</td>
<td>42.8 ± 3.2*</td>
<td>20.6 ± 3.0†</td>
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Values are means ± SE of 4 separate experiments conducted in triplicate. NHE-1, Na\(^+\)/H\(^+\) exchanger-1. *P < 0.001 and †P < 0.05 vs. the control group.
ischemia-reperfused coronary sinus samples (19); thus, the inhibition of respiratory burst in vivo may in fact be greater. Thus neutrophils may be able to bind but not exert detrimental effects on the cells with which they interact. However, because CD18 upregulation does not translate to adherence, NHE-1 inhibition may decrease neutrophil adhesion. Furthermore, chemotaxis of human neutrophils stimulated by formyl-methionyl-leucyl-phenylalanine (fMLP) has been shown to be inhibited by NHE-1 inhibition. Thus NHE-1 blockade in vivo may inhibit chemotaxis, a postulate supported by the marked attenuation in neutrophil accumulation within the ischemic-reperfused myocardium that occurred with BIIB-513 treatment. Recently, in a model of canine myocardial ischemia-reperfusion injury employing 90 min of ischemia, it has been demonstrated that a significant proportion of myocytes within the infarct region are viable at the time of reperfusion and lose viability during reperfusion, suggesting specific cell death due to reperfusion injury (21). Consistent with this study is the fact that, after myocardial ischemia-reperfusion injury, neutrophils accumulate in the ischemic myocardium where they contribute to myocardial damage (3, 6, 7). The current in vivo data, together with the data on the effects of NHE-1 inhibition on isolated cardiomyocytes and neutrophils, suggest that NHE-1 inhibition may protect myocardial cells within the AAR that otherwise may have been irreversibly injured upon reperfusion and that NHE-1 inhibition also may reduce neutrophil-mediated myocardial damage that occurs after reperfusion.

Numerous studies examining Na\(^+\)/H\(^+\) exchange during myocardial ischemia and reperfusion suggest that inhibition of NHE-1 ultimately prevents Ca\(^{2+}\) overload/oscillations and its deleterious consequences in cardiomyocytes. Although the current data support this hypothesis, the data also demonstrate for the first time that the cardioprotection observed in vivo is more complex. Inhibition of NHE-1 also attenuates neutrophil accumulation and activation, suggesting that the reduction in myocardial infarct size observed in vivo also may be due, in part, to effects on neutrophil activity.

We thank Dr. Rongang Wang and Dr. Peter J. Newman (Blood Research Institute, Blood Center of Southeastern Wisconsin) for assistance with the FACScan instrument. We also thank Anna Hsu (Medical College of Wisconsin) and Jim Tarara (Mayo Clinic and Foundation) for excellent technical assistance. We also thank Anna Hsu (Research Institute, Blood Center of Southeastern Wisconsin) for assistance with the FACScan instrument. We also thank Anna Hsu (Medical College of Wisconsin) and Jim Tarara (Mayo Clinic and Foundation) for excellent technical assistance.

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