Phosphodiesterase 5A Inhibition Induces Na$^+$/H$^+$ Exchanger Blockade and Protection Against Myocardial Infarction

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Abstract—Acute phosphodiesterase 5A inhibition by sildenafil or EMD360527/5 promoted profound inhibition of the cardiac Na$^+$/H$^+$ exchanger (NHE-1), detected by the almost null intracellular pH recovery from an acute acid load (ammonium prepulse) in isolated papillary muscles from Wistar rats. Inhibition of phosphoglycerate kinase-1 (KT5823) restored normal NHE-1 activity, suggesting a causal link between phosphoglycerate kinase-1 increase and NHE-1 inhibition. We then tested whether the beneficial effects of NHE-1 inhibitors against the deleterious postmyocardial infarction (MI) remodeling can be detected after sildenafil-mediated NHE-1 inhibition. MI was induced by left anterior descending coronary artery ligation in Wistar rats, which were randomized to placebo or sildenafil (100 mg kg^{-1} day^{-1}) for 6 weeks. Sildenafil significantly increased left ventricular phosphoglycerate kinase-1 activity in the post-MI group without affecting its expression. MI increased heart weight/body weight ratio, left ventricular myocyte cross-sectional area, interstitial fibrosis, and brain natriuretic peptide and NHE-1 expression. Sildenafil blunted these effects. Neither a significant change in infarct size nor a change in arterial or left ventricular systolic pressure was detected after sildenafil. MI decreased fractional shortening and the ratio of the maximum rate of rise of LVP divided by the pressure at the moment such maximum occurs, effects that were prevented by sildenafil. Intracellular pH recovery after an acid load was faster in papillary muscles from post-MI hearts (versus sham), whereas sildenafil significantly inhibited NHE-1 activity in both post-MI and sildenafil-treated sham groups. We conclude that increased phosphoglycerate kinase-1 activity after acute phosphodiesterase 5A inhibition blunts NHE-1 activity and protects the heart against post-MI remodeling and dysfunction. (***Hypertension***. 2007;49:1095-1103.)

Key Words: basic science ■ membrane transport/ion channels ■ hypertrophy/remodeling ■ physiology/function
■ myocardial infarction ■ Na$^+$/H$^+$ exchanger ■ PDE5A inhibition

The Na$^+$/H$^+$ exchanger isoform 1 (NHE-1) is expressed at significant levels in heart tissue.[1] Several experimental studies performed after the seminal work by Karmazyn[2] have shown that NHE-1 activity plays a critical role in ischemia/ reperfusion injury and after myocardial infarction (MI).[1,3–5] Different compounds that inhibit the exchanger dramatically reduce infarct size (IS) and improve function and postinfarction hypertrophy.[1,5] NHE-1 inhibition seems to be beneficial even if it is started 2 or 4 weeks after MI,[5] suggesting that the protection is independent of IS. In a canine heart infarction model, NHE-1 inhibition was significantly more efficacious, protecting against prolonged ischemia than any other maneuver, including ischemic preconditioning.[6] These findings led to the clinical testing of selective NHE-1 inhibitors as potential interventions for cardiac protection in acute coronary syndromes. Although a small clinical trial with patients

with acute MI undergoing percutaneous-transluminal coronary angioplasty demonstrated beneficial effects,[7] 2 larger trials, The Guard During Ischemia Against Necrosis (GUARDIAN)[8] and the sodium-hydrogen EXchange inhibition to Prevent coronary Events in acute cardiac conDITIONs (EXPEDITION)[9] did not provide conclusive results. In GUARDIAN, only a subset of patients undergoing high-risk coronary artery bypass graft surgery showed beneficial effects after NHE-1 inhibition.[10] In the EXPEDITION trial, NHE-1 inhibition reduced the incidence of MI after coronary artery bypass graft surgery but increased the overall rate of cerebrovascular events, which resulted in early discontinuation of the study.[9]

A recent publication suggested that inhibition of the exchanger only after its activation by acidosis, but not during its basal function, may present therapeutic advantages.[11]

Although inhibition of the NHE-1 only after critical situations and not under basal conditions seems unrealistic, it was reported that inhibition of p90 ribosomal S6 kinase, which phosphorylates the cytosolic tail of the NHE-1, decreased agonist activated NHE-1 function without inhibiting its basal homeostatic function.[11] More interestingly, this inhibition was accompanied by beneficial effects.[11]

Because an increase in protein kinase G (PKG) activity in renal mesangial cells inhibited NHE activity only after an acidic load but not in basal conditions,[12] we hypothesized that the same pattern may be reproduced in myocardial tissue by chronic inhibition of the cGMP-catabolizing enzyme phosphodiesterase 5A (PDE5A). We will provide evidence that NHE-1 inhibition, after an acidic load but not under basal conditions, can be obtained by PDE5A inhibition and that this inhibition has beneficial effects on cardiac remodeling and function after experimental MI in the rat heart.

Methods

The investigation conforms with the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health Publication No. 85-23, revised 1996).

Experimental MI

MI was induced in 3- to 4 month-old male Wistar rats as described previously.[13] Briefly, the rats were anesthetized (50 mg/kg of sodium pentobarbital, IP), then intubated and ventilated with room air using a positive-pressure respirator (Model 680, Harvard). A left thoracotomy was performed via the fourth intercostal space, and the lungs retracted to expose the heart. The left anterior descending coronary artery was ligated with a 7-0 silk suture near its origin. Acute ischemia was deemed successful when the anterior wall of the left ventricle became cyanotic. The lungs were inflated by increasing positive end-expiratory pressure, and the thoracotomy site was closed in layers. Immediately after surgery, rats were randomized to receive placebo (MI group) or sildenafil (SIL) 100 mg kg^{-1} day^{-1} (MI+SIL group) orally through drinking water for 6 weeks. Another group of rats underwent sham ligation; the procedure was similar, but the suture was not tightened around the coronary artery. Shams were also randomly assigned to receive SIL 100 mg kg^{-1} day^{-1} (SHAM+SIL group) or placebo (SHAM group) for 6 weeks. SIL dosage was selected based on a previous report.[14]

Echocardiographic Examination

Rats were monitored echocardiographically under light anesthesia (35 mg/kg of pentobarbital sodium IP). 2D targeted M-mode tracings were recorded with a 7-MHz transducer. Left ventricular (LV) end diastolic and systolic internal diameters (LVEDd and LVESd, respectively) were measured using the American Society of Echocardiography leading-edge method from ≥3 cardiac cycles.[15] Endocardial fractional shortening was calculated as: (LVEDd−LVESd)/LVEDd×100.

In Vivo Determination of LV Pressure

Rats were lightly anesthetized (35 mg/kg of pentobarbital sodium IP), placed on a warm pad (37°C), and a 14-gauge catheter was placed in the trachea for room air ventilation. A fluid-filled catheter (Pebax 03) advanced to the left ventricle via the right carotid artery was used for in vivo LV pressure measurement, using a technique validated previously by Wang et al.[16] The ratio of the maximum rate of rise of LV pressure divided by the pressure at the moment such maximum occurs was determined as an index of inotropism.

Histological and Morphometric Analysis

For IS determinations, 3- to 4-mm-width cross-sections of each heart were cut from apex to base. The tissue slides were processed for paraffin embedding and stained with Masson trichrome. The IS was calculated as the mean percentage of LV epicardial and endocardial circumference occupied by the scar tissue averages for all of the ventricular levels.[17] LV cardiomyocyte cross-sectional area (CSA) was determined in hematoxylin/eosin-stained slides from 80 to 90 myocytes from each heart as described previously.[18,19] Cardiomyocytes were accepted for quantitative analysis if their cross-sections contain a centrally located nucleus and the cellular membrane was unbroken. The interstitial fibrosis was assessed in picrosirius red-stained tissue slides. The percentage of collagen was calculated as the sum of collagen areas divided by the total LV area (myocytes+collagen).[19]

Brain Natriuretic Peptide Expression

Brain natriuretic peptide (BNP) expression was assessed by real-time RT-PCR and normalized to GAPDH following a procedure described previously.[20] Briefly, total LV RNA was extracted using the RNeasy kit (Qiagen) and reverse transcribed using the Omniscript RT kit (Qiagen). A dilution of the resulting cDNA was used to quantify the relative mRNA content (iCycler iQ real-time PCR detection system, Bio-Rad) using appropriate primers and SYBR Green as the fluorescent probe. Primer sequences were: GAPDH forward primer 5′-GGGTGTGAACCACGAGAAAT-3′, reverse primer 5′-CCACAGTCTTCTGAGTGGCA-3′; BNP forward primer 5′-CCCAGATGATTCTGCTCCTG-3′, reverse primer 5′-TTCTGCATCGTGGATTGTTC-3′.

NHE-1 Protein Expression

NHE-1 expression was assessed as described previously[21] by immunoblot analysis of LV membrane–enriched fractions using a rabbit polyclonal antibody for NHE-1 (kindly donated by Dr M. Donowitz, Johns Hopkins University, Baltimore, Md). Immunoreactive protein detection was performed by chemiluminescence (Amersham), and signals were quantified by densitometric analysis.

Determination of PKG-1 Activity and Protein Expression

A previously described technique that uses phosphotransfer of [^{32}Pi] from [γ-^{32}P]ATP into a synthetic heptapeptide was used to determine PKG-1 activity.[22] The assay consisted of 50 μL of a mixture composed of 20 mmol/L of Tris, 200 μmol/L of ATP (30 000 cpm μL^{-1} assay mixture), 136 μg mL^{-1} of phosphoreceptor heptapeptide (RKRSRAE, Peninsula Laboratories Inc), 20 mmol/L of magnesium acetate, 100 μmol/L of 3-isobutyl-1-methylxanthine, and 1 μmol/L of synthetic peptide inhibitor of PKA (Peninsula Laboratories Inc; pH 7.4). To this mixture, 10 μL of cardiac homogenate fraction was added, and the reaction was incubated at 30°C for 10 minutes. The reaction was terminated by spotting 40 μL of the mixture on P81 phosphocellulose paper (Whatman). PKG-1 activity was expressed as femtomol of phosphate per minute per microgram of protein. PKG-1 protein expression was determined by Western blot immunoanalysis with rabbit polyclonal antibody (Santa Cruz Biotechnology) and mouse monoclonal antibody for actin (Sigma). Immunodetection of the primary antibody was performed with peroxidase-conjugated secondary antibodies and enhanced chemiluminescence reaction system (Amersham). Densitometric analysis of PKG-1 protein was referred to that of actin.

Measurement of Intracellular pH, Determination of NHE-1 Activity, and Intrinsic Buffer Capacity

Intracellular pH (pH_i) was measured in isolated LV papillary muscles following a previously described 2′-7′-bis(2 carboxyethyl)-5,6-carboxyfluorescein-epifluorescence technique.[23] The NHE-1 activity was assessed by the Na^+-dependent pH_i recovery from an ammonium prepulse-induced acute acid load.[24] The experiments were performed in the nominal absence of bicarbonate to assure that the pH_i recovery after Na^+ readdition was because of NHE-1 activation. Intrinsic buffer capacity (β_i) was determined as described previously.[24]

TABLE 1.   Maximal-Induced Intracellular Acidification After Ammonium Washout and β_i for All of the Experimental Groups

| Experimental Groups	pH_i	β_i, mmol/L
Control (n=6)	6.74±0.02	28.2±0.9
SIL (n=5)	6.76±0.04	27.3±1.6
Cariporide (n=3)	6.70±0.02	29.4±0.7
EMD360527/5 (n=4)	6.74±0.02	28.0±0.7
SIL+KT5823 (n=4)	6.72±0.03	28.6±0.9
KT5823 (n=4)	6.75±0.01	27.8±0.4

Statistical Analysis

Results are presented as mean±SEM. Statistical analysis was performed using the Student t test or 1-way ANOVA followed by the Student–Newman–Keuls test, when necessary. $P<0.05$ was considered significant.

Results

NHE-1 activity was assessed in isolated papillary muscles based on the Na^+-dependent initial rate of pH_i recovery from an acute and sustained acid load. Because the experiments were performed in the nominal absence of bicarbonate, the recovery of pH_i after the acidic load was exclusively because of NHE-1 activation. Given that the maximal-induced acidification and β_i were similar in all of the groups (Table 1), the maximal velocity of pH_i recovery ($_{max}dpH_i/dt$) was used as an index of NHE-1 activity (because $J_{H+}= dpH_i/dt \cdot \beta_i$; in the absence of changes in β_i among groups, dpH_i/dt directly reflects J_{H+}). NHE-1 activity was tested before and after PDE5A inhibition by SIL (1 μmol/L) added to the superfusate ≈40 minutes before the onset of the acidic challenge. Interestingly, SIL did not affect basal pH_i (7.22±0.03 versus 7.23±0.02, before and after SIL, respectively; n=5) but significantly blunted NHE-1 activity after the sustained acidic load (Figure 1). For comparative purposes, the results obtained with the well-known NHE-1 inhibitory compound cariporide are also shown (Figure 1). As expected, cariporide blunted the pH_i recovery significantly after the acidic load, but, in contrast to the PDE5A inhibitor, it also affected basal pH_i (7.24±0.03 versus 6.96±0.02, before and after cariporide, respectively; n=3; $P<0.05$). The results obtained with SIL can be reproduced by another PDE5A inhibitor, EMD360527/5 (0.1 μmol/L), as shown in Figure 1.

The fact that a similar decrease in the NHE-1 activity was obtained by using 2 structurally different PDE5A inhibitors suggests that increased PKG-1 activity might be responsible for this effect. This was confirmed by using the PKG-1 inhibitor KT5823 (1 μmol/L) in experiments in which the effect of this compound on NHE-1 activity after acute PDE5A inhibition by SIL was examined. Under these conditions, $_{max}dpH_i/dt$ was not found to be statistically different from controls (Figure 2), supporting the notion that PKG-1 inhibition blunted the inhibitory effect of SIL on NHE-1. KT5823 alone did not alter $_{max}dpH_i/dt$ significantly (Figure 2).
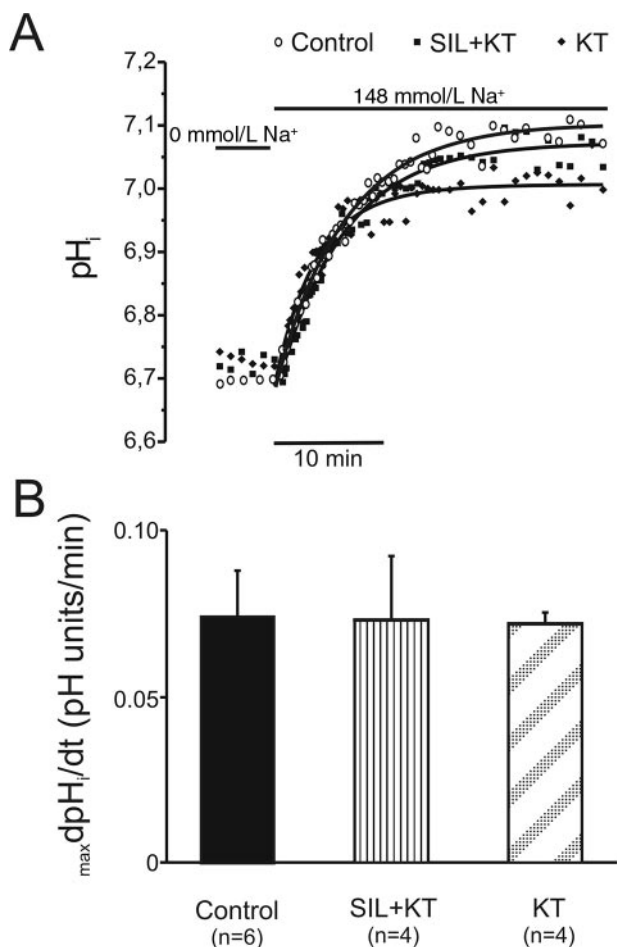
According to previous experimental evidence, the use of NHE-1 inhibitors, or even the genetic ablation of the exchanger, promotes protection not only on ischemia/reperfu-
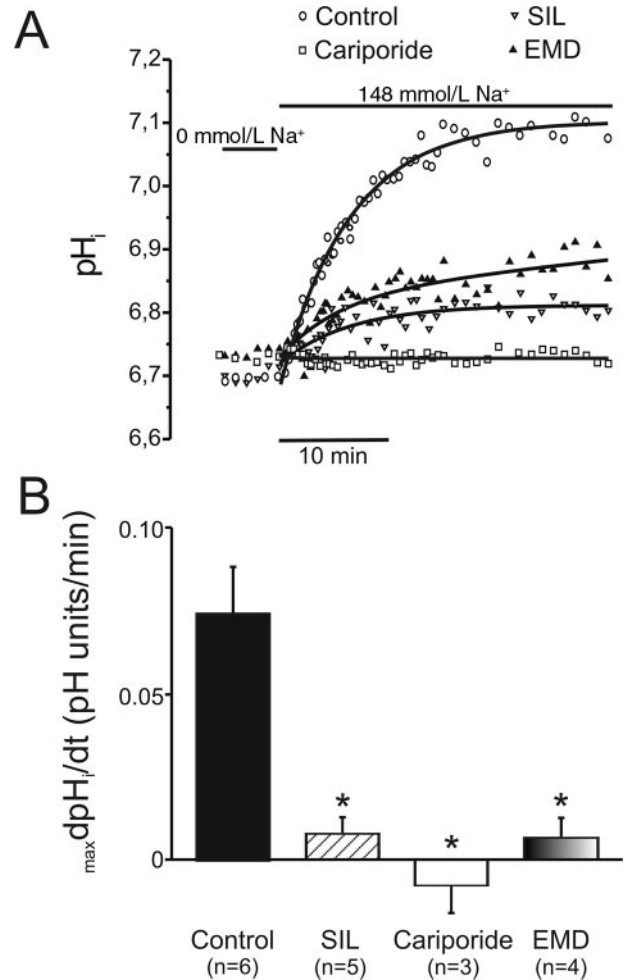
Figure 1. Acute PDE5A inhibition by SIL or EMD360527/5 (EMD) prevented NHE-1 activity. To appreciate the magnitude of NHE inhibition after PDE5A inhibition, results obtained with the widely known NHE-1 inhibitor cariporide are also shown. Typical experiments plus their best exponential fit for each group are displayed in A, and the corresponding pooled data for the $_{max}dpH_i/dt$ are displayed in B. *$P<0.05$ vs control.

sion injury but also after coronary artery occlusion influencing post-MI remodeling.[1,25,26] Therefore, we hypothesized that NHE-1 inhibition by PDE5A inhibitors should also protect the heart in a similar way to that described for NHE-1 inhibition by other compounds.[3,5] Thus, we decided to explore whether the protection described for NHE-1 inhibitors when treatment started after coronary occlusion[5] would be mimicked by chronic SIL treatment. Inhibition and reversal of MI-induced hypertrophy, fibrosis, and function have been reported for NHE-1 inhibitors even when the treatment started 2 or 4 weeks after coronary occlusion.[5] On the other hand, we have reported that NHE-1 inhibition was able to regress hypertensive hypertrophy and fibrosis in rats,[27–29] and Takimoto et al[14] reported that SIL was able to regress pressure overload–induced hypertrophy.

SIL treatment for 6 weeks did not modify blood pressure, LV systolic pressure, or LV end diastolic pressure significantly (which is also in line with the report of Takimoto et al[14]; Table 2). IS was not different between groups (MI: 40±10%, n=4, and MI+SIL: 31±7%, n=4). An increased

Figure 2. PKG-1 inhibition by KT5823 (KT) after SIL treatment restored normal NHE-1 activity. KT5823 alone did not alter $_{max}dpH_i/dt$. For the sake of comparison, the control experiments of Figure 1 are also shown here. As in Figure 1, typical experiments plus their best exponential fit for each group are displayed in A, and the corresponding pooled data for the $_{max}dpH_i/dt$ are displayed in B.

blunted significantly by SIL treatment (Figure 3). On the other hand, SIL treatment of sham rats did not affect cardiac fibrosis or myocyte CSA compared with nontreated shams (Figure 3).

Echocardiographic analysis 6 weeks after MI revealed a slight but nonsignificant increase in the LVEDd (Figure 4A) and a significant increase in LVESd (Figure 4B) compared with the SHAM and the SHAM+SIL groups. SIL treatment on MI animals did not modify LVEDd (Figure 4A) but prevented the increase in LVESd (Figure 4B). The functional correlation of these findings was a significant decrease in endocardial fractional shortening in the post-MI group that was prevented completely by SIL treatment (Figure 4C). Thus, MI decreased the ratio of the maximum rate of rise of LV pressure divided by the pressure at the moment that such maximum occurs, and SIL treatment restored it toward normal (Figure 4D). These parameters were not affected by SIL in the treated shams (Figure 4).

Myocardial expression of BNP, another index of cardiac hypertrophy, and NHE-1 were also analyzed after MI with and without SIL treatment. MI increased both BNP and NHE-1 expression, and these effects were blunted significantly by SIL treatment (Figure 5A and 5B). Hearts from SIL-treated shams did not show any significant change in BNP or NHE-1 expressions (Figure 5A and 5B).

The upregulation of NHE-1 expression after MI detected by us in the present study was also shown recently by Chen et al,[5] who reported that inhibition of the exchanger normalizes its expression and improves cardiac function and remodeling without affecting IS, in agreement with our results. Interestingly, our results show that SIL treatment after MI, similar to NHE-1 inhibition by EMD87580,[5] protected the heart against postinfarction hypertrophy and dysfunction and diminished the augmented BNP and NHE-1 expressions (Figures 3 to 5).

SIL treatment increased LV PKG-1 activity significantly after MI (Table 3). However, nonsignificant changes in PKG-1 activity were detected after SIL treatment in the absence of MI. Similar findings were reported by Takimoto et al.[14] LV PKG-1 expression was not significantly different among groups (Table 3).

To further explore the possible causal link between PDE5A inhibition–NHE-1 inhibition and post-MI protection, we evaluated NHE-1 activity in papillary muscles isolated from hearts explanted from all of the experimental groups after the 6-week follow up period. The Na^+-dependent pH_i recovery from an acute and sustained acid load induced by an ammonium prepulse was analyzed. Basal pH_i was similar in all of

heart weight/body weight ratio was detected 6 weeks after coronary artery ligation, which suggested that the ischemic insult promoted myocardial hypertrophy in the surviving ventricular zone; this effect was attenuated significantly by SIL treatment (in mg g^{-1}): SHAM, 2.52±0.07, n=7; MI, 3.30±0.20, n=5; MI+SIL, 2.85±0.04, n=5; and SHAM+SIL, 2.36±0.04, n=5 (P<0.05 MI versus all of the other groups and MI+SIL versus SHAM and SHAM+SIL). Histological analysis of the hearts lent support to these findings by showing a significant increase in the LV myocyte CSA and collagen content in the MI group; these effects were

TABLE 2.   In Vivo Determined Hemodynamic Parameters

Experimental Groups	LVEDP, mm Hg	LVSP, mm Hg	+dP/dt, mm Hg/s	−dP/dt, mm Hg/s	Aortic Pressure, mm Hg
SHAM (n=7)	4.7±1.0	99.4±7.3	6541±690	−4857±674	99±7/58±5
MI (n=7)	4.1±1.2	87.3±9.6	4575±987	−3521±609	87±10/50±8
MI+SIL (n=5)	7.6±0.8	88.2±6.8	5501±600	−3629±372	88±7/49±5
SHAM+SIL (n=5)	6.3±1.7	104.4±7.7	5045±748	−3651±465	104±8/46±6

LVEDP indicates left ventricular end diastolic pressure; LVSP, left ventricular systolic pressure, $_{max}$+dP/dt, maximum rate of LVP rise; −dP/dt, maximum rate of LVP decline; Aortic pressure, systolic/diastolic.
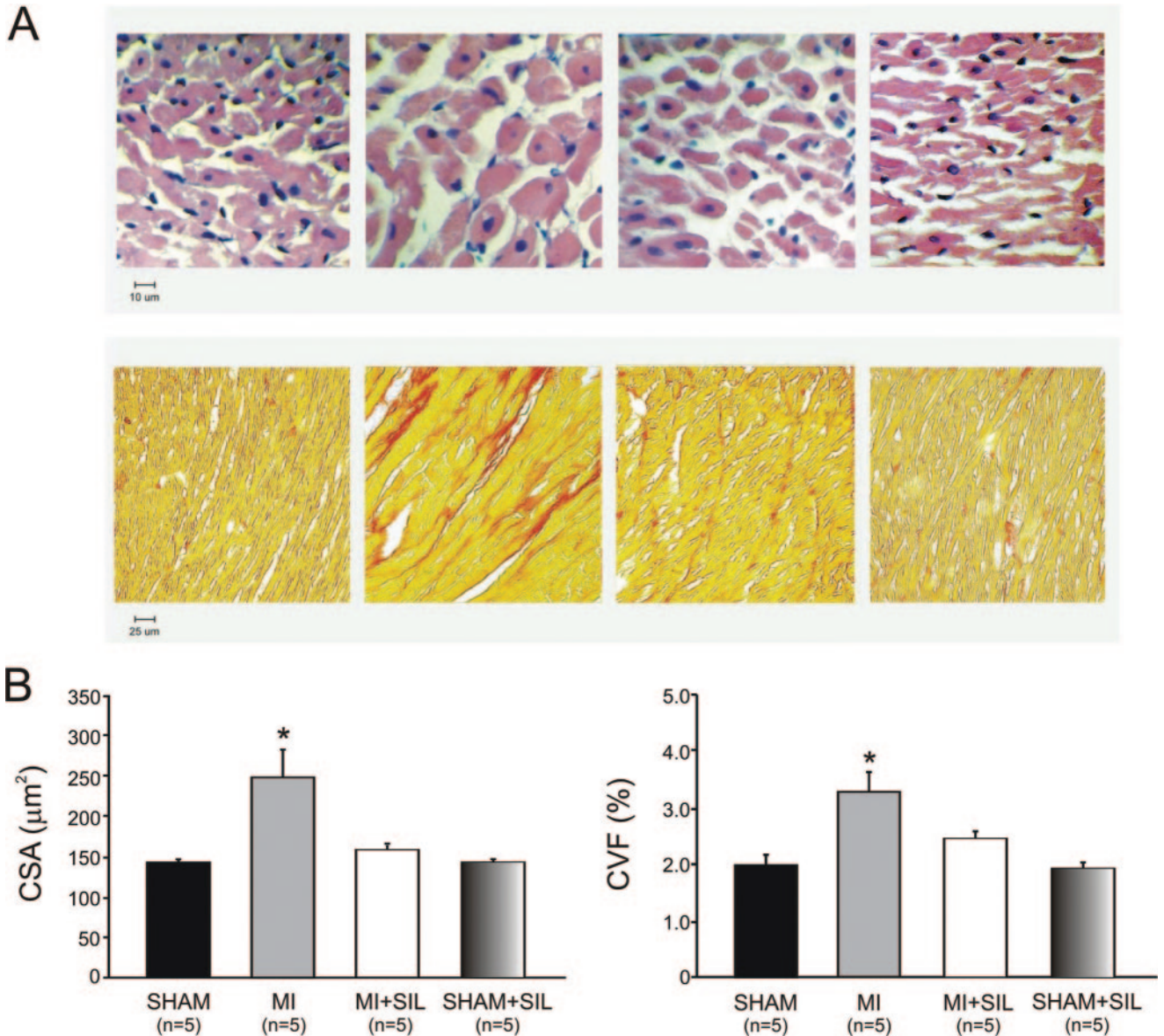
Figure 3. A, Original histological sections showing the CSA of LV myocytes (top) and the LV collagen volume fraction (CVF; bottom) for (from left to right): SHAM, MI, MI+SIL, and SHAM+SIL group. B, Overall results for CSA (left) and CVF (right) for the 4 experimental groups. MI increased myocyte CSA and CVF compared with SHAM and SIL treatment blunted these effects significantly. SIL alone (SHAM+SIL group) did not have any significant effect on either CSA or CVF. *$P<0.05$ vs all of the other groups.

the groups: SHAM, 7.24 ± 0.02, n=5; MI, 7.25 ± 0.01, n=5; MI+SIL, 7.21 ± 0.02, n=5; and SHAM+SIL, 7.25 ± 0.01, n=4. The maximal-induced acidification and β_i were also similar in all of the groups (Table 4). Papillary muscles from post-MI hearts showed enhanced NHE-1 activity detected by an ≈4-fold increase in $_{max}dpH_i/dt$ after the acidic load compared with SHAM (Figure 6). Interestingly, SIL treatment prevented the augmented NHE-1 activity in post-MI hearts and induced a dramatic inhibition of the exchanger (Figure 6), an effect comparable only to that obtained by other authors and ourselves with high doses of specific NHE-1 inhibitors[30] (Figure 1). On the other hand, and in agreement with the results obtained after acute PDE5A inhibition (Figure 1), muscles from chronically SIL-treated shams also showed profound NHE-1 inhibition (Figure 6).

Discussion

We report here a previously unknown effect of PDE5A inhibition by SIL on myocardial NHE-1 activity. This inhibition was obtained also by another structurally different and highly selective PDE5A inhibitor, EMD360527/5. Moreover, the inhibition of the NHE-1 activity after the sustained acidosis was blunted by specific PKG inhibition with KT5823, suggesting that increased PKG-1 activity might be responsible for the NHE-1 inhibition.

Although inhibition of cardiac NHE-1 by PKG was previously unknown, PKG-mediated NHE-1 inhibition has been already described in mesangial cells[12] and in ocular nonpigmented ciliary epithelium.[31] In addition, it has been reported that an increase in the cGMP-PKG pathway can decrease the activity of other NHE isoforms.[32,33]
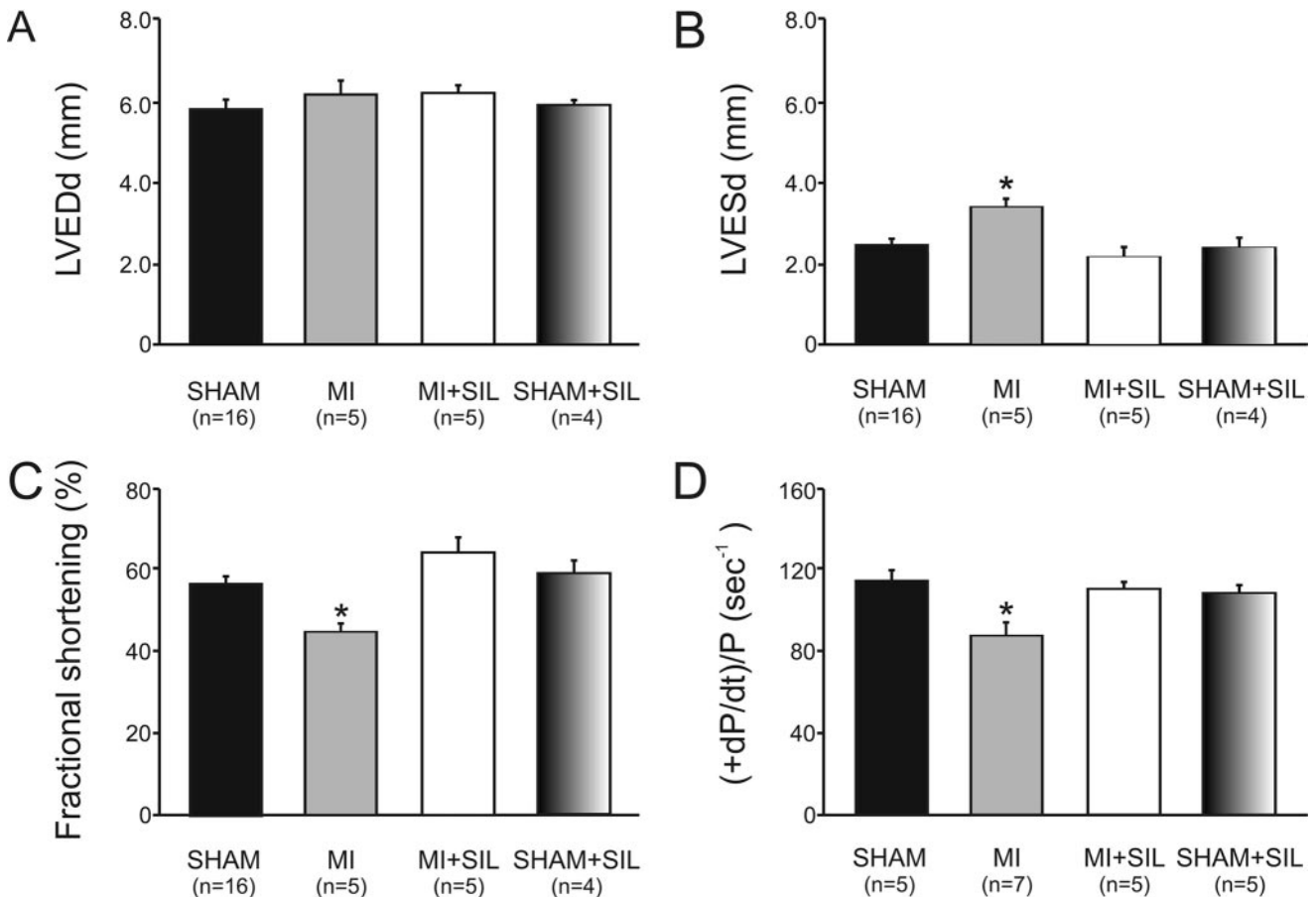
Figure 4. MI promoted a slight tendency to increase LVEDd and a significant increase in LVESd (A and B). SIL treatment did not modify LVEDd (A) but prevented the increase in LVESd (B). Thus, fractional shortening (FS) decreased after MI and recovered with SIL treatment (C). Accordingly, the ratio of the maximum rate of rise of LV pressure divided by the pressure at the moment such maximum occurs decreased after MI and recovered to control values after SIL treatment (D). None of these parameters was affected by SIL alone (SHAM+SIL group). *$P<0.05$ vs all other groups.

Interestingly, and in contrast to other NHE-1 inhibitory compounds, both PDE5A inhibitors used here inhibited the NHE-1 only after an acidic load without modifying basal pH_i, thus preserving the important "housekeeping" function of the exchanger. A similar differential effect on basal and stimulated NHE-1 activity was reported recently after NHE-1 activation by phosphorylation through p90 ribosomal S6 kinase.[11] This report called attention to the potential therapeutic advantages of using NHE-1 inhibitory compounds that selectively blunt the exchanger activity only after an acidic challenge and not under basal conditions. The level of phosphorylation and activity of the exchanger probably results from a permanent equilibrium between kinase and phosphatase activities. The possible participation of the Ras/Raf/mitogen activated protein kinase kinase/extracellular signal regulated kinase/p90 ribosomal S6 kinase pathway and of protein phosphatases, such as PP1 and PP2A, in the PDE5A inhibition-induced decrease in NHE-1 activity deserves further investigation. We could speculate that an increased PKG-1 activity may decrease NHE-1 activity by a direct phosphorylation of the exchanger. However, we are not aware of studies demonstrating the existence of consensus sites for PKG-1 in the NHE-1. We can speculate also about possible changes in the activity of the exchanger independent

of the cytosolic tail phosphorylation. Deletion of the phosphorylation sites at the C-terminal seems to decrease only 50% of the growth factor–mediated increased activity of the exchanger.[34] In connection with this, the phosphorylation state of "accessory" proteins may regulate NHE-1 activity indirectly without affecting the NHE-1 phosphorylation itself. In this regard, 2 calcineurin-β homologue proteins associated with NHE-1 have been described recently, calcineurin-β homologue protein-1 and tescalcin,[34] both of which bind Ca^{2+} and appear to be crucial in regulating NHE-1 sensitivity to pH_i. In addition, calcineurin-β homologue protein-1 phosphorylation was shown to induce NHE-1 inhibition.[35] Whatever the case, we would like to highlight the surprising magnitude of the SIL effect on NHE-1 activity, because it almost abolished pH_i recovery from the acidic load, an action comparable only to that seen with high doses of specific NHE-1 inhibitors.[30]

The dosage used for chronic SIL treatment was 100 mg kg^{-1} day^{-1}, which may appear to be very high, at least when compared with that recommended for humans. However, it was the same dose used by Takimoto et al[14] in mice, and, as also shown by those authors, there was no evidence of a decrease in blood pressure that could have affected the conclusions (see Table 2). Takimoto et al[36]
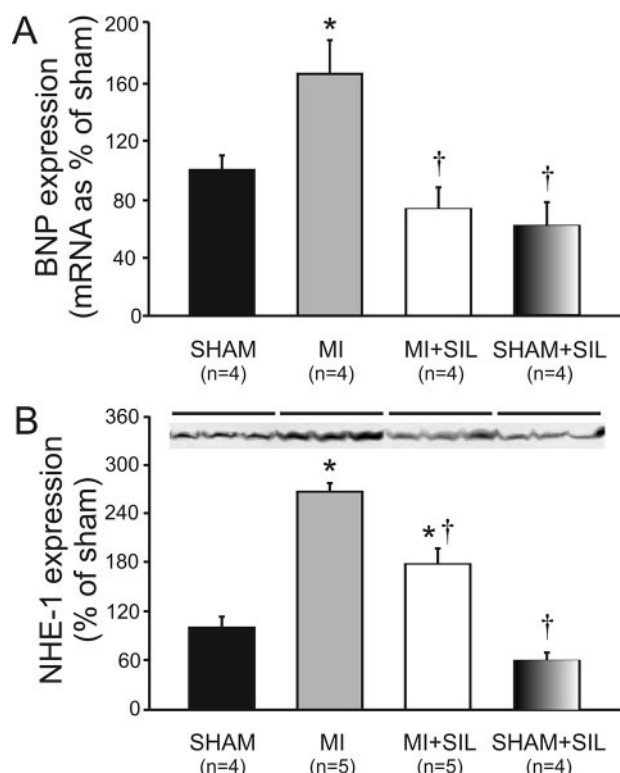
Figure 5. BNP mRNA (A) and NHE-1 protein (B) expression increased in the MI group. Both effects were inhibited significantly by SIL. SIL on shams did not affect BNP or NHE-1 expressions. A representative NHE-1 blot is displayed above the bar graph. *$P<0.05$ vs SHAM, †$P<0.05$ vs MI.

also used an even greater dose in a previous report in mice. In human beings, SIL promoted modest nitrate-like hemodynamic effects lowering systolic and diastolic blood pressure.[37,38]

The measurement of PKG-1 activity and expression performed by us revealed only an increase in PKG-1 activity after SIL treatment in the post-MI group, which is also in agreement to the results obtained by Takimoto et al[14] in a mice model of pressure overload–induced hypertrophy, where PKG-1 activity only increased in hypertrophic-treated hearts but not in treated shams. As mentioned in that work, cGMP-dependent signaling pathways seem generally more potent in hearts under stress, much like an automotive brake.[14] Larger effects of PDE5A inhibition can be seen in the presence of increased atrial natriuretic peptide stimulation or

TABLE 3. PKG-1 Activity and Expression After Chronic SIL Treatment

| Experimental Groups	PKG-1 Activity, fmol PO$_4$ min^{-1} (μg protein)$^{-1}$	PKG-1 Protein Expression
SHAM (n=4)	5.56±0.23	1.96±0.12
MI (n=5)	4.85±0.29	1.62±0.10
MI+SIL (n=5)	6.73±0.41*	1.86±0.30
SHAM+SIL (n=4)	4.90±0.28	2.15±0.31

PKG-1 protein expression indicates densitometric analysis by immunoblot referred to that of actine.

*$P<0.05$ vs other groups.

TABLE 4. Maximal-Induced Intracellular Acidification After Ammonium Washout and β_i for the 4 Experimental Groups

Experimental Groups	pH$_i$	β_i, mmol/L
SHAM (n=5)	6.73±0.02	28.3±0.8
MI (n=5)	6.76±0.02	27.4±0.7
MI+SIL (n=5)	6.77±0.03	26.9±0.9
SHAM+SIL (n=4)	6.74±0.04	28.5±0.7

NO donors.[39] We should keep in mind that the experimental group subjected to MI showed increased myocardial BNP levels (Figure 5). However, we cannot explain why chronic PDE5A inhibition decreased NHE-1 activity even in the experimental group in which no significant change in PKG-1 activity was detected. In this regard, 2 alternative explanations of these apparently contradictory results should be examined. First, the PDE5A inhibitor could be acting through a different mechanism than the increase in PKG-1 activity. This possibility seems unlikely, given that the PKG-1 inhib-
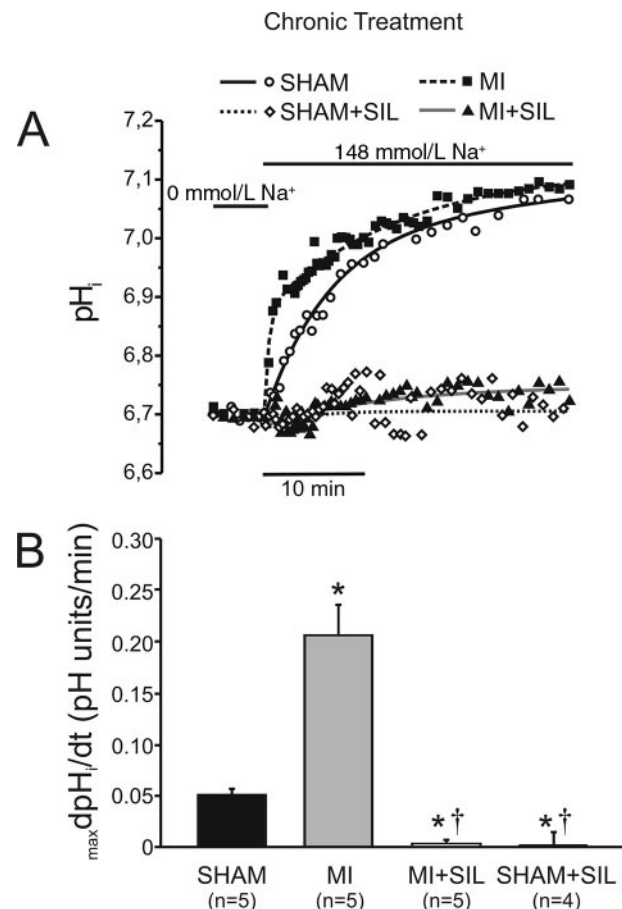
Figure 6. The increased NHE-1 expression in post-MI hearts correlated with enhanced NHE-1 activity detected by the higher $_{max}$dpH$_i$/dt after an acidic load vs SHAM. As detected in the acute experiments (Figure 1), SIL treatment abolished the NHE-1 activity almost completely in post-MI hearts, as well as in the treated shams (SHAM+SIL group). As in Figures 1 and 2, typical experiments plus their best exponential fit for each group are displayed in A, and the corresponding pooled data for the $_{max}$dpH$_i$/dt are displayed in B. *$P<0.05$ vs SHAM, †$P<0.05$ vs MI.

itor (KT5823) cancelled the inhibitory effect on NHE-1. Second, under certain conditions of sustained stress, the PDE5A inhibitor may increase PKG-1 activity in a subcellular compartment that is sensed by NHE-1. In this regard, Castro et al[39] reported that cGMP is highly compartmentalized in rat cardiomyocytes and that the level of PDEs may be quite different depending on which intracellular compartment is measured. In any case, PDE5A inhibition seems to inhibit NHE-1 and exerts beneficial effects on post-MI remodeling, similar to what has been reported for NHE-1 inhibitors[1,3–7] and for SIL after aortic constriction.[14] In line with our findings are the reports showing that PDE5A inhibition, as well as the specific NHE-1 blockade, both induced regression of hypertrophy in pressure-overloaded myocardium.[14,27–29]

Some limitations of this study deserve to be considered. First, the IS in our model seems to be relatively small ($\approx$30%), and the animals were followed up for only 6 weeks after the coronary occlusion. This is probably the reason why cardiac function was only mildly depressed. Whether, with larger IS or longer follow-up, SIL treatment will show similar cardioprotective effects and/or prevention of heart failure similar to the NHE-1 inhibitors[5,40] cannot be ascertained from the present results. Also, although we do know that PDE5A inhibition by another structurally different compound (EMD360527/5) also inhibited NHE-1 (see Figure 1), probably by increasing PKG-1 activity, we do not know whether other interventions leading to PKG-1 activation by PDE inhibition or by pathways different to PDE inhibition will induce similar beneficial effects on myocardial remodeling to those described here.

Perspectives

The beneficial effect of blocking PDE5A after MI appears to operate by inhibiting NHE-1 and is manifested by a decrease in post-MI hypertrophy and remodeling and improvement in function. Interestingly, the pharmacological intervention did not alter the steady-state pH_i but prevented the increase in NHE-1 activity after the acidic load, which may result in a better therapeutic approach than NHE-1 inhibitors. Whether or not increasing PKG-1 after PDE5A inhibition might represent an alternate pathway to inhibit NHE-1 that would not have the adverse effects observed with other NHE inhibitors needs to be investigated.

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Disclosures

None.

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