Inhibition of the Na\(^+\)/H\(^+\) exchanger attenuates the deterioration of ventricular function during pacing-induced heart failure in rabbits

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Abstract

Aims: Inhibition of the Na\(^+\)/H\(^+\)-exchanger (NHE) preserves myocardial morphology and function in rat and mouse models of hypertrophy and failure. The mechanism(s) involved in such cardioprotective effects remain(s) unclear, but might involve blockade of increased protein kinase activity as observed in untreated hearts. Methods and results: We investigated the functional, morphological and biochemical consequences of NHE-inhibition with BIIB722 in rabbits with pacing-induced heart failure (HF). In sham rabbits treated with placebo (n = 9) or BIIB722 (30 mg/kg/day po, n = 9), LV end-diastolic diameter (LVEDD) and systolic fractional shortening (FS, %) remained unchanged. In HF rabbits (n = 9), LVEDD increased and FS decreased from 31.5 ± 1.4 to 8.1 ± 0.9 (p < 0.05) at 3 weeks of LV pacing (400 bpm). Apoptosis, fibrosis and myocyte cross-sectional area as well as p38MAPK phosphorylation and iNOS protein expression were significantly increased in HF compared to sham rabbits. The activity of the 90 kDa NHE-kinase was greater in HF than in sham rabbits. In HF rabbits receiving BIIB722 prior to (18.1 ± 2.2, n = 9) or following 1 week (15.5 ± 1.6, n = 7) of pacing, FS at 3 weeks was better preserved than in untreated HF rabbits (p < 0.05). Apoptosis, fibrosis, myocyte cross-sectional area, p38MAPK phosphorylation and iNOS protein expression were significantly reduced in HF rabbits receiving BIIB722. Conclusion: NHE-inhibition attenuates the functional, morphological and biochemical derangements of pacing-induced HF in rabbits.

Keywords: Heart failure; Myocardium; Signal transduction

1. Introduction

The Na\(^+\)/H\(^+\) exchanger isoform-1 (NHE-1) is expressed in mammalian myocardium [1]. NHE-1 activity is regulated by muscle stretch [2] and a variety of protein kinases, including protein kinase C, protein tyrosine kinases and the extracellular signal regulated kinase (ERK) subfamily of mitogen activated protein kinases (MAPK), some of which can directly phosphorylate the exchanger’s regulatory domain [3–5]. Some putative NHE-regulatory protein kinases are activated in failing myocardium [6], and there is evidence that NHE-1 activity is increased in heart failure (HF) [7,8].

Long-term morphological alterations, such as fibrosis [8,9] and myocyte hypertrophy, secondary to hypertension [8–10], sustained β1-adrenoceptor stimulation [11], diabetes [12], myocardial infarction [13–15] or idiopathic dilated cardiomyopathy [16] are attenuated following NHE-1 inhibition. The mechanism(s) by which NHE-1 inhibition influences the development of cardiac hypertrophy and failure, however, are not clear in detail (for review, see Ref. [17]). In cardiomyocytes isolated from rabbits with HF secondary to pressure and volume overload, the intracellular sodium and diastolic calcium concentrations (via the sodium–calcium exchanger) are increased [8]. The calcium transient, however, is reduced due to impaired calcium handling of the sarcoplasmic reticulum. All of the above effects were partially restored by inhibition of NHE with cariporide [8], suggesting that apart from effects on left ventricular morphology activa-
tion of NHE contribute to the development of left ventricular dysfunction. However, most of the experimental studies—except one in mice post myocardial infarction [15]—describing a treatment benefit following NHE-1 blockade pretreated the animals. In the study in mice post myocardial infarction neither the effect of NHE-1 inhibition on the extent of apoptosis nor the effect on fibrosis were analyzed in failing hearts [15].

We have therefore now analyzed the effects of NHE blockade on LV systolic function and morphology in a pacing-induced HF model in rabbits. NHE-1 inhibition was started either prior to pacing or after 1 week of pacing, when first signs of LV dysfunction had developed. We also assessed the effects of NHE-1 inhibition on p38MAPK phosphorylation, since in the same animal model, functional and morphological alterations occurring during the progression of HF are closely correlated to p38MAPK phosphorylation [18]. Furthermore, we measured the myocardial expression of the inducible nitric oxide synthase (iNOS), since iNOS expression is increased in failing hearts [19–21] and pharmacological blockade of iNOS expression improves cardiac performance and attenuates cardiovascular remodelling in failing hearts of salt-sensitive hypertensive rats [21].

2. Methods

2.1. Experimental model

This study was approved by the bioethical committee of the district of Düsseldorf, Germany, and the investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996).

2.2. Experimental protocol

The experiments were performed in 43 male Chinchilla rabbits (Charles River, Kisslegg, Germany) weighing 3.4 ± 0.1 kg. When the rabbits had fully recovered from instrumentation, which has been described in detail previously [18,22], HF was induced in 25 rabbits by LV pacing (400 bpm) for 3 weeks. HF was evident from clinical signs, such as ascites and cachexia, and echocardiographic parameters, such as an increase in LV end-diastolic diameter (LVEDD) and a reduction of LV systolic fractional shortening (FS). Eighteen sham-operated rabbits served as controls.

Nine sham rabbits received placebo and additional nine rabbits received BIIB722 (sabiporide, Lot 8960040, Boehringer Ingelheim) at a concentration of 30 mg/kg/day po. In HF rabbits, nine animals received placebo, nine animals received BIIB722 prior to pacing while the remaining seven animals received BIIB722 after 1 week of pacing. BIIB722 inhibits NHE-1 by competing with sodium for its transport; the IC50 value for rat myocytes is 7 ± 1 nM [23].

After euthanasia of the rabbits, four to six tissue samples (50 mg each) were taken from the LV free wall; two to three samples each were frozen in liquid nitrogen and stored at −70 °C or were fixed in formalin and embedded in paraffin.

2.3. Echocardiography

LV function (Supervision 7000, Toshiba, Neuss, Germany) was measured in the short axis view at baseline, after 1, 2 and 3 weeks of pacing, with the rabbits in the conscious state and the pacemaker turned off for at least 60 min. FS was assessed by measurement of the end-diastolic and end-systolic diameter (end-diastolic diameter — end-systolic diameter/100) [18,22].

2.4. Histology

Apoptosis was determined using the TUNEL technique (In Situ Cell Death Detection Kit, La Roche Diagnostics, Mannheim, Germany), counterstaining with bisbenzimide (HOE-33342) and phalloidin (both Sigma, Taufkirchen, Germany). TUNEL-positive cardiomyocyte nuclei were counted using fluorescence microscopy (Leica DMLB, Bensheim, Germany) and calculated per mm2. The total area analyzed averaged 177 ± 5 mm2. The extent of myocardial fibrosis was determined by Masson-Goldner trichrome staining and expressed as percent of field of view (three fields of 0.075 mm2 each). Myocyte cross-sectional area was measured in hematoxylin and eosin stained tissue sections (μm2, two fields of 0.075 mm2 each) [18,22].

2.5. Analysis of the serum concentration of BIIB 722

Venous blood samples were taken from the ear vein 24 h after oral treatment, centrifuged and stored in liquid nitrogen. For analysis and quantification of the serum BIIB722 concentration and internal standards, BIIB722 was enriched by passing through a first HPLC column (Bondesil CH 40 mm, 10 × 2 mm, ammoniumformiate 0.025 M + 0.1% HCOOH) and thereafter separated using a second column (Nucleosil 100-5 C18 AB, 70 × 2 mm, ammoniumformiate 0.025 M + 0.1% HCOOH/MethOH). BIIB722 detection was obtained using electrospray injection (ESI) and mass spectrometry (MS/MS). The serum concentration of BIIB722 was expressed in μM.

2.6. In-gel kinase assay

NHE-1 kinase activity in rabbit myocardial tissue was determined by an in-gel kinase assay, in which the substrate (GST or GST/NHE-1 fusion protein) was co-polymerized
within the sodium dodecyl sulfate (SDS)-polyacrylamide gel matrix [24]. Frozen ventricular tissue was powdered under liquid nitrogen and homogenized on ice in a 10 × volume of ice-cold lysis buffer (50 mM NaCl, 50 mM NaF, 50 mM Na2P2O7, 5 mM EDTA, 5 mM EGTA, 1 mM Na3VO4, 1 mM AEBSF, 10 μg/ml leupeptin, 10 μg/ml aprotonin, 0.1% Triton X-100 and 10 mM HEPES; pH 7.4), using a glass pestle and mortar (Wheaton). The homogenates were then centrifuged at 21,000 × g (10 min at 4 °C) and a Bradford protein assay (Bio-Rad) was performed on the supernatant. Equal amounts of protein (50 μg) from each sample were then separated by 9% SDS-PAGE, using gels containing 400 μg/ml GST (control for nonspecific phosphorylation) or the GST/NHE-1 fusion protein. A common internal standard sample was loaded on each gel, to allow normalization and pooling of data. The gels were then processed as described previously [24] and the dried gels were subjected to autoradiography, after which NHE-1 kinase signals were quantified by laser densitometry.

2.7. p38MAPK phosphorylation

The tissue samples were processed as described previously [18]. The blots were incubated for 2 h either with an antiserum recognizing total p38MAPK or with an antiserum specific for the dually phosphorylated form of p38MAPK (New England Biolabs, Beverly, MA). The resulting autoradiographs were analyzed by quantitative two-dimensional densitometry, using commercially available software (Herolab, Wiesloch, Germany). To quantify the extent of p38MAPK phosphorylation, the phosphorylated p38MAPK band intensity was expressed relative to the intensity of the total p38MAPK band.

2.8. iNOS protein expression

The tissue samples were weighed, diluted with sample buffer (1:50, 2% sodium dodecyl sulfate, 50 mM dithiothreitol, 10% glycerol, 0.1% bromphenol blue, 62.5 mM Tris, pH 6.8 at 25 °C) and homogenized on ice. The lysate protein from myocardial tissues (25 μg/lane) was separated by 10% PAGE-SDS gel and transferred to nitrocellulose membrane (Amersham, Illinois, USA). After incubation in blocking solution (TBS: 20 mM Tris, 120 mM NaCl at 25 °C) containing 5% nonfat milk for 90 min, membranes were incubated with 1:500 dilution primary antibody (mouse monoclonal iNOS antibody, BD Transduction Laboratories) overnight at 4 °C. Membranes were then washed and incubated with a 1:2000 dilution of the secondary anti-mouse antibody (Santa Cruz Biotechnology, California, USA) for 1 h. After four more washes, detection was performed by enhanced chemoluminescence. The resulting autoradiographs were analyzed by quantitative two-dimensional densitometry, using commercially available software (Herolab).

2.9. Statistical analysis

Values are expressed as mean ± S.E.M. Statistical comparison of hemodynamic data before and during the 3 weeks of pacing between the five groups was performed by two-way ANOVA. When a significant overall effect was detected, individual mean values were compared using Bonferroni’s method.

The number of TUNEL-positive cardiomyocytes, the extent of fibrosis, myocyte cross-sectional area, p38MAPK phosphorylation and iNOS expression data were compared between untreated and treated sham and HF rabbits using a one-way ANOVA.

For the determination of NHE-1 activity, ventricular samples from 19 hearts in the sham group (10 placebo, 9 BIIB722) and 16 hearts in the HF group (8 placebo, 8 BIIB722 with 3 weeks treatment) were assayed. The 35 samples were analyzed in four separate in-gel kinase assays, in a random and blinded manner. Densitometric data were normalized relative to the internal standard sample common to all assays, to allow the pooling of data. Intergroup comparisons were done by either ANOVA (when comparing four groups) or student’s t-test (when comparing two groups). Statistical significance was accepted for a p value <0.05.

3. Results

3.1. Hemodynamics

Heart rate tended to decrease from baseline to 3 weeks in sham rabbits receiving placebo (from 259 ± 5 to 232 ± 10 bpm, NS) or BIIB722 (from 252 ± 12 to 229 ± 12 bpm, NS). In HF rabbits receiving placebo or BIIB722 prior to the initiation of pacing, heart rate increased from 251 ± 14 to 268 ± 13 bpm or from 247 ± 9 to 268 ± 6 bpm, respectively (both p <0.05 vs. baseline). In HF rabbits receiving BIIB722 after 1 week of pacing, heart rate at 3 weeks of pacing (250 ± 5 bpm) was not different from baseline (241 ± 15 bpm).

In sham rabbits receiving placebo or BIIB722, LVEDD (15.3 ± 0.7 to 15.6 ± 0.4 vs. 15.5 ± 0.3 to 16.0 ± 0.5 mm; both NS) and FS (Fig. 1) remained unchanged over 3 weeks. HF was characterized by an increase in LVEDD from 15.1 ± 0.4 to 18.3 ± 0.6 mm (p <0.05) and a reduction of FS (Fig. 1). In HF rabbits receiving BIIB722 prior to or 1 week following the initiation of pacing, LVEDD increased from 15.5 ± 0.4 to 18.0 ± 0.3 mm or 15.7 ± 0.4 to 17.9 ± 0.4 mm, respectively (both p <0.05). The relative increase in the LVEDD significantly differed between untreated HF rabbits (122 ± 3% of baseline) and BIIB722 treated rabbits, independent of rabbits were pretreated (115 ± 4% of baseline, p < 0.05 vs. untreated HF rabbits) or treated after 1 week of pacing (113 ± 2% of baseline, p < 0.05 vs. untreated HF rabbits).
FS decreased in both groups, but remained significantly higher than in HF rabbits receiving placebo (Fig. 1). There was no difference in LVEDD and FS in HF rabbits receiving BIIB722 prior to the initiation of pacing or after 1 week of pacing.

3.2. Serum concentration of BIIB722

The serum concentration of BIIB722 24 h following oral treatment averaged $0.94 \pm 0.40 \mu M$ ($n = 9$) in sham rabbits and $1.01 \pm 0.25 \mu M$ ($n = 9$) in heart failure rabbits.

3.3. Morphology

BIIB722, when administered to sham rabbits, did not alter the number of TUNEL-positive cardiomyocytes, the extent of fibrosis or the myocyte cross-sectional area (Figs. 2–4). In HF rabbits receiving placebo, the number of TUNEL-positive cardiomyocytes, the extent of fibrosis and myocyte cross-sectional area were increased compared to sham rabbits receiving placebo (Figs. 2–4). In HF rabbits receiving BIIB722 prior to the initiation of pacing or following 1 week of pacing, the number of TUNEL-positive cardiomyocytes and the extent of fibrosis were greater than in sham rabbits receiving BIIB722, but significantly smaller than in HF rabbits receiving placebo. Myocyte cross-sectional area was increased in HF rabbits receiving BIIB722 prior to the initiation of pacing compared to sham rabbits receiving BIIB722, but significantly decreased compared to HF rabbits receiving placebo. In HF rabbits receiving BIIB722 after 1 week of pacing, myocyte cross-sectional area was significantly decreased compared to HF rabbits receiving placebo and also significantly decreased compared to HF rabbits receiving BIIB722 prior to the initiation of pacing (Figs. 2–4).
3.4. NHE-kinase activity

In the rabbit ventricular samples, the in-gel kinase assay utilizing the GST/NHE-1 fusion protein as substrate detected four major NHE-kinase activities, with approximate molecular masses of 40, 50, 55 and 90 kDa (Fig. 5A). There was no discernible kinase activity detected when GST alone was used as substrate (data not shown), indicating specific phosphorylation of the 178 amino acid segment from the regulatory carboxyl terminus of NHE-1 (amino
acids 637–815). NHE-kinase activities of comparable molecular mass have been reported previously in neonatal rat ventricular myocytes [25,26] and adult rat ventricular myocardium [25], using similar in-gel kinase assays. Treatment of the rabbits with the NHE inhibitor BIIB722 had no significant effect on myocardial NHE-kinase activity, either in the sham or in the HF rabbits. Data from the placebo and BIIB722 subgroups were therefore combined, to allow the determination of differences in NHE-kinase activity between sham \((n = 19)\) and HF rabbits \((n = 16)\). As illustrated in Fig. 5B, there was no significant difference in the activities of the 40, 50 and 55 kDa NHE-kinases between sham and HF rabbits. In contrast, the activity of the 90 kDa NHE-kinase was significantly greater in HF rabbits than in sham rabbits (Fig. 5B).

3.5. p38MAPK phosphorylation

In HF rabbits receiving placebo, the p38MAPK phosphorylation was increased compared to sham rabbits receiving placebo (Fig. 6). In HF rabbits receiving BIIB722 prior to the initiation or after 1 week of pacing, the p38MAPK phosphorylation was significantly increased compared to sham rabbits receiving BIIB722, but significantly reduced compared to HF rabbits receiving placebo (Fig. 6). Pooling all data points, p38MAPK phosphorylation correlated to the number of TUNEL-positive cardiomyocytes \((r = 0.58, p < 0.05)\), the extent of fibrosis \((r = 0.70, p < 0.05)\), the myocyte cross-sectional area \((r = 0.60, p < 0.05)\), and also to the iNOS protein expression \((r = 0.67)\).

3.6. iNOS protein expression

In heart failure rabbits receiving placebo, the iNOS protein expression was increased compared to sham rabbits receiving placebo (Fig. 7). Also in heart failure rabbits receiving BIIB722 prior to the initiation or at 1 week of pacing the iNOS protein expression was increased compared to sham rabbits receiving BIIB722, but still reduced compared to heart failure rabbits receiving placebo. There was no difference between heart failure rabbits receiving BIIB722 prior to the initiation or at 1 week of pacing.

4. Discussion

The present study demonstrates a protective effect of the NHE-1 inhibitor BIIB722 on LV systolic function and morphology in a non-ischemic HF model in rabbits. The cardioprotective effects were apparent even when treatment started after the first signs of LV dysfunction had developed.

A limitation of the present study is that cardiomyocyte NHE activity isolated from sham and failing rabbit hearts with and without BIIB722 administration was not directly measured. Nevertheless, our data from the in-gel kinase assays revealed a significant increase (by approximately 45%) in the activity of the 90 kDa NHE-kinase in the HF group (Fig. 5B). Previous data indicate that such NHE-kinase activity is likely to reflect the activity of the 90 kDa ribosomal S6 kinase \((p90^{RSK} \text{ or } RSK) [25]\), an enzyme which is activated downstream of ERK MAPK and has been shown to stimulate NHE-1 activity in fibroblasts through the direct phosphorylation of Ser703 in the exchanger’s regulatory carboxyl terminal domain [5]. Also, in adult rat ventricular myocytes, an ERK-mediated increase in RSK
activity has been causally linked to the stimulation of sarcolemmal NHE activity in response to diverse stimuli such as adrenergic agonists and oxidative stress [27,28]. Furthermore, human HF is associated with not only an increased ERK MAPK and RSK activity [29] but also an increased sarcolemmal NHE activity, the latter most likely through post-translational mechanisms [7]. Although a causal link between increased RSK activity (Takeishi et al. [29] and present study) and increased sarcolemmal NHE activity [7] remains to be established, the present model of HF in the rabbit—for the first time—recapitulates the increased RSK activity described in human failing hearts [29]. Based on the known functional consequences of RSK-mediated NHE phosphorylation, it is likely that increased RSK activity does indeed result in increased NHE activity in this setting.

A potential negative regulation of NHE-1 activity by p38 MAPK, either by direct phosphorylation or indirectly through attenuated ERK activity, was originally reported by Kusuhara et al. [30] in vascular smooth muscle cells. However, this observation has not been confirmed in other cell types. Indeed, more recent evidence suggests that p38 MAPK may stimulate NHE-1 activity in some settings [31]. Furthermore, in rat cardiomyocytes, increased p38 MAPK activity secondary to increased oxidative stress did not affect NHE-1 activity [28]. Therefore, the question of whether p38 MAPK regulates NHE-1 activity in myocardium in a variety of settings requires more extensive investigation.

Fig. 6. Percentage of phosphorylated p38MAPK in the five different groups of rabbits measured at the end of the 3 weeks protocol.

Fig. 7. Expression of inducible nitric oxide synthase (iNOS) in the five different groups of rabbits measured at the end of the 3 weeks protocol.
Any increase in NHE activity in failing hearts would of course be negated in the presence of an NHE-inhibitory concentration of BIIB722. We are confident that effective inhibition of NHE activity was achieved, since the serum BIIB722 concentration measured 24 h after oral administration of BIIB722 averaged 0.94 ± 0.40 μM (n = 9) in sham rabbits and 1.01 ± 0.25 μM (n = 9) in heart failure rabbits. In isolated rat cardiomyocytes, which like rabbit cardiomyocytes express mainly NHE-1 [32,33], 1 μM BIIB722 was as effective as 10 μM cariporide in inhibiting NHE activity [23], and 10 μM cariporide completely abolished the increased NHE activity in myocytes isolated from failing as compared to sham rabbit hearts [8].

In the present study, LV systolic function was better preserved during the development of HF in rabbits receiving BIIB722 compared to rabbits receiving placebo. This increase in LV systolic function was not related to a direct positive inotropic effect of BIIB722, since in sham animals LV systolic function remained unchanged following BIIB722 and was also not different from placebo treated animals. Although we did not assess LV pressure in the present study, a reduction in afterload following BIIB722 as the underlying cause for the increased LV systolic function is unlikely, since acute NHE-1 blockade did not decrease LV pressure in normal hearts [34,35], and in failing hearts secondary to myocardial infarction [13–15] or sustained β1-adrenoceptor stimulation [11] NHE-1 blockade even preserved LV pressure and LVdP/dtmax. Also chronic blockade of NHE-1 in spontaneously hypertensive rats did not affect LV pressure [12].

Part of the improved systolic function following blockade of NHE-1 might relate to improved calcium handling, as demonstrated in cardiomyocytes isolated from rabbits with HF secondary to pressure and volume overload. In this study, increases in the intracellular sodium and diastolic calcium concentrations as well as a decrease in the calcium transient measured in cardiomyocytes from failing hearts were partially prevented by inhibition of NHE with cariporide [8]. Improved LV systolic function following BIIB722 administration in failing hearts could also reflect less morphological derangement, especially myocardial fibrosis, and indeed FS at 3 weeks of pacing was inversely related to the extent of myocardial fibrosis (r = 0.81, p < 0.05).

In the present study with pacing-induced HF, the findings of reduced myocyte apoptosis, fibrosis and hypertrophy following NHE-1 blockade are in agreement with previous results obtained in HF models secondary to hypertension [9,10,12], sustained β1-adrenoceptor stimulation [11], diabetes [36], myocardial infarction [13–15] or idiopathic dilated cardiomyopathy [16]. Thus, the beneficial effects of NHE-1 blockade on LV morphology appear to be independent from the underlying cause of HF. The reduction of myocyte hypertrophy following NHE-1 blockade was more pronounced with treatment starting after 1 week of pacing compared to treatment starting prior to the initiation of pacing. While this was a consistent finding, we have no explanation for this.

The activity of the NHE-1 in normal hearts is low and therefore its blockade with BIIB722 did not alter any of the measured parameters in sham animals. With progression of LV dysfunction the renin–angiotensin system and the sympathetic nervous system become activated [37,38] and the increased concentrations of angiotensin II and catecholamines subsequently activate the NHE-1 [3,4]. Therefore, with increasing severity of LV dysfunction the activity and thus the contribution of NHE-1 to the progression of the disease probably increases. In support of this hypothesis, pretreatment with BIIB722 did not affect LV systolic function during the first 2 weeks of pacing, but clearly preserved LV systolic function thereafter. Treatment with BIIB722 starting after 1 week of pacing—at a time when first signs of LV dysfunction had developed—was as protective as pretreatment with BIIB722, again supporting the notion that, in this model, NHE-1 activity plays a causal role in the later stage of HF development. This finding is in agreement with a recent study in mice [15], in which NHE-1 inhibition starting prior to or 4 weeks after coronary artery occlusion improved cardiac function and attenuated left ventricular hypertrophy.

Overexpression of p38MAPK alpha in mice cardiomyocytes increases apoptosis, and overexpression of p38MAPK beta induces hypertrophy [39]. Similarly, in mice in vivo which overexpress MKK 3b and 6b [40], subsequently leading to activation of p38MAPK, the extent of myocardial fibrosis is increased and—over time—LV ejection fraction is decreased. In contrast, targeted inhibition of p38MAPK alpha in mice also reduces LV fractional shortening and promotes cardiomyocyte hypertrophy secondary to aortic banding, angiotensin II, isoproterenol or phenylephrine infusion [41]. Thus, activated p38MAPK might influence the development and progression of HF by inducing cardiomyocyte apoptosis and isoform specifically contribute to or inhibit cardiomyocyte hypertrophy; the most important determinant for the development of HF, however, appears to be the extent of cardiomyocyte apoptosis [42]. In the same pacing-induced HF model as in the present study, FS as well as the extent of apoptosis and fibrosis at 3 weeks of pacing closely correlated to p38MAPK phosphorylation [18]. In the present study, blockade of NHE-1 with BIIB722 completely abolished the increase in p38MAPK phosphorylation seen in placebo treated failing hearts and attenuated cardiomyocyte apoptosis and preserved LV systolic function. The mechanism by which NHE-1 inhibition could have possibly attenuated the increase in p38MAPK phosphorylation during the progression of HF involves a reduced accumulation of intracellular calcium [43] which then subsequently would result in less activation of protein kinase C and other MAPK.

The expression and activity of the inducible nitric oxide synthase (iNOS) is increased after stimulation with lipopolysaccharide, cytokines, ischemia/reperfusion, and hypoxia and may contribute to myocardial dysfunction [44]. In failing myocardium of salt-sensitive hypertensive rats [21]
as well as in myocardium from patients with chronic ischemia and LV dysfunction [19] or end-stage heart failure [20] iNOS expression is increased. Also in the present study, iNOS expression was increased in failing myocardium, but such increase was attenuated in rabbits receiving BIIB722 (prior to or after the initiation of LV dysfunction). Also decreased iNOS expression was associated with preserved left ventricular function and less cardiomyocyte hypertrophy in failing hearts of salt-sensitive hypertensive rats [21].

However, whether or not the decreased p38MAPK activity and/or iNOS expression in failing hearts are required for the protection obtained by NHE-1 blockade or simply reflect epiphenomena requires further studies.

In conclusion, NHE-1 inhibition attenuates the functional, morphological and biochemical derangements in pacing-induced HF in rabbits, even when treatment is started after the first signs of LV dysfunction have already developed.

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