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Review Article

Cardioprotective effects of Na^+/H^+ exchange inhibitors

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CONTENTS

Introduction 253
Pharmacology of NHE inhibitors 253
Cardioprotective effects of NHE-1 inhibition 254
Mechanistic basis for cardioprotection 254
Infarct size reduction 254
Comparison of NHE-1 inhibition and ischemic preconditioning 256
Remodeling and NHE-1 inhibition 257
Reduction in myocardial stunning 257
NHE-1 inhibition and apoptosis 258
Antiarrhythmic effects of NHE-1 inhibition 258
Clinical trials with NHE-1 inhibitors 258
Conclusions 258
Acknowledgements 259
References 259

Introduction

The sodium/hydrogen exchanger (NHE) is the major cardiac membrane transporter responsible for the control of intracellular pH in the myocyte under normal conditions and is thought to play an important role during pathophysiological states such as ischemia and reperfusion (1). Currently, 5 isoforms of NHE have been found in the plasma membrane of mammalian cells and a sixth has been found in the mitochondria (2). The predominant isoform in the heart is the type 1 (NHE-1) that is found in the sarcolemma and intercalated disks and this isoform regulates intracellular pH and cell volume via a 1:1 stoichiometric exchange of one proton for one sodium ion. Other homeostatic pH mechanisms also exist in the heart such as the Na^+/HCO_3^- symport, the Cl^-/HCO_3^- and Cl^-/OH^- exchangers which are stimulated by an excess acid load or intracellular alkalosis, respectively (2). Approximately 60% of the protons are removed from the cardiac cell by the NHE-1 isoform and its activity has been shown to be regulated by a number of factors via phosphorylation on the hydrophilic cytoplasmic domain (2) such as protein kinase C (PKC) and members of the mitogen-activated protein kinase superfamily (3). These reactions are thought to change the pH sensitivity of the H^+ sensor one way or the other. Phosphorylation-independent processes such as calmodulin binding have also been shown to activate NHE-1 activity and change the pH sensitivity of the H^+ sensor (2) when increases in intracellular calcium occur, such as during ischemia.

Pharmacology of NHE inhibitors

One of the first papers to suggest a cardioprotective role of inhibiting NHE was published by Karmazyn in 1988 (4) where it was shown that amiloride, a potassium-sparing diuretic with NHE inhibitory activity, produced an enhanced recovery of contractile function in isolated rat hearts subjected to global ischemia and reperfusion. Subsequently, several investigators used amiloride and its 5-amino substituted pyrazinoyl guanidine derivatives to demonstrate the cardioprotective potential of inhibiting NHE in the ischemic myocardium (2, 5). However, it was subsequently found that these amiloride derivatives interacted with other cation transporters and shared cardiodepressive activities independent of their NHE blocking activity.

Investigators at Hoechst (5) were the first to synthesize a new class of more selective NHE-1 inhibitors, the benzoylguanidine derivatives (Fig. 1). The first compound showing superior efficacy and selectivity over amiloride derivatives was Hoe-694. This compound showed marked antiarrhythmic and antiischemic activity in several animal models and had a low toxicity profile. To synthesize a superior compound to Hoe-694, investigators from Hoechst made Hoe-642, or cariporide mesilate, by substituting an isopropyl for a piperidine group. These changes enhanced water solubility, activity *in vitro* and NHE-1 selectivity over Hoe-694. Subsequently, other companies such as Merck KGaA and Boehringer also synthesized benzoylguanidine derivatives such as EMD-85131 (eniporide hydrochloride), EMD-96785 (eniporide mesilate) and BIIB-513 (6). All of these compounds have been shown to be cardioprotective in a number of ischemic animal models, including humans, and the results of ongoing and future clinical trials are eagerly anticipated.

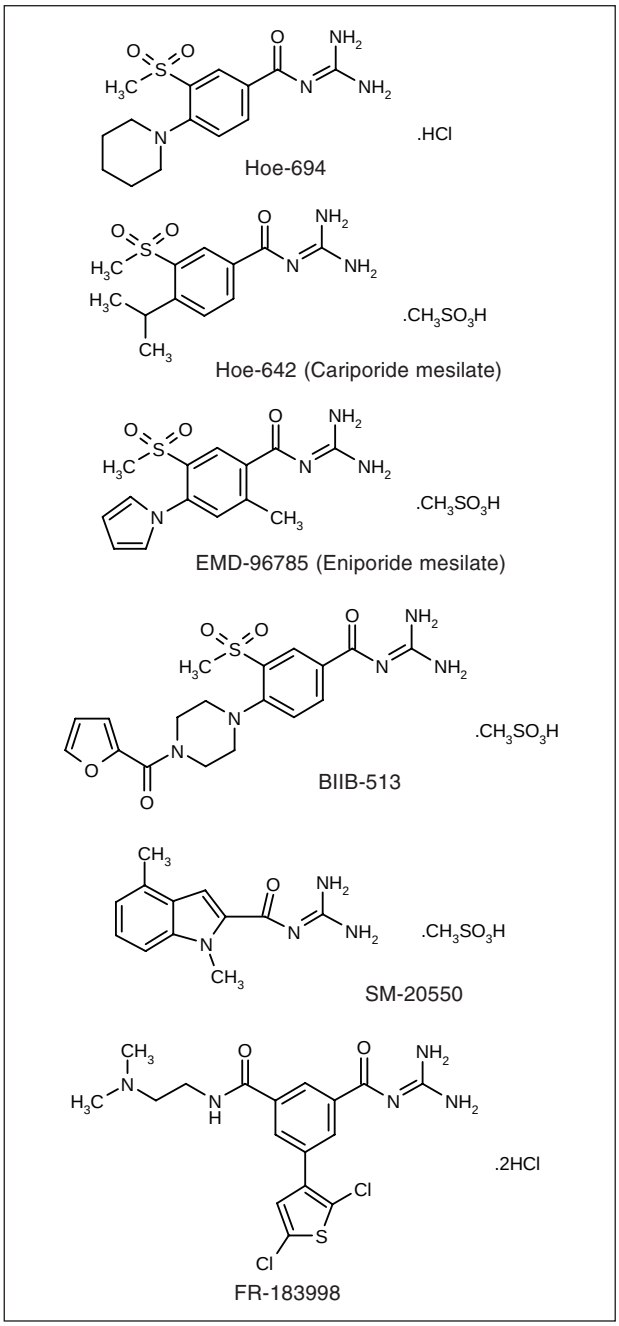

Fig. 1. Chemical structures of several benzoylguanidine NHE-1 inhibitors.

Cardioprotective effects of NHE-1 inhibition

Since the original study by Karmazyn (4), there have been a plethora of papers that uniformly support the idea that inhibition of NHE-1 results in a marked cardioprotective effect against infarction, hypercontracture, stunning and cardiac arrhythmias in a number of animal species and models (7-10). A summary of these findings will be the topic for the remainder of this review.

Mechanistic basis for cardioprotection

Before discussing the evidence for the cardioprotective effect of NHE-1 inhibition, it is necessary to provide a general mechanistic basis that is thought to be responsible for the beneficial effect of inhibiting NHE-1 in the ischemic/reperfused myocardium. During myocardial ischemia the intracellular concentration of hydrogen ions (H^+) increases and activates several pH regulatory systems, including the NHE-1 which is dominant in the heart (2). Activation of NHE-1 results in the extrusion of one H^+ in exchange for one Na^+ in an attempt to maintain intracellular pH. Since ischemia is known to inhibit the Na^+/K^+-ATPase, intracellular Na^+ would be expected to accumulate followed by a concomitant increase in intracellular Ca^{2+} as a result of a decrease in the activity of or reversal of the Na^+/Ca^{2+} exchanger (11). This would result in intracellular calcium overload which is detrimental to cell survival due to protease activation and rigor contracture unless the ischemia is relieved either by coronary artery reperfusion or by pharmacological intervention. NHE-1 inhibitors would be expected to decrease calcium overload produced during the ischemic interval by inhibiting the activation of this key pH regulatory exchanger. However, a recent study by Park *et al.* (12) in rat hearts is at odds with this hypothesis. These investigators were unable to demonstrate a direct increase in intracellular sodium during 10 min of ischemia or anoxia. In fact, they noted a decrease in NHE activity during these interventions. A rapid rise in intracellular Na^+ occurred upon reperfusion and this was inhibited by the NHE-1 inhibitor methylisobutylamiloride, which suggested that the major activation of NHE-1 occurs at reperfusion and not during ischemia. Nevertheless, it is well accepted that following reperfusion of the previously ischemic area, the rapid washout of extracellular H^+ will result in a large intracellular to extracellular gradient for H^+ diffusion out of the cell by reactivation of NHE-1 which would be expected to produce a marked influx of Na^+ and Ca^{2+}. Again, inhibition of NHE-1 at reperfusion has been shown to attenuate the massive Ca^{2+} influx which is known to occur at this time. Thus, although the function of NHE-1 during ischemia is still open to question, it appears that there is an overall increase in intracellular sodium and calcium during ischemia and/or at reperfusion which is detrimental to cell viability and cardiac function due to cell swelling, rigor contracture, cell necrosis, apoptosis and the generation of severe cardiac arrhythmias. Inhibition of NHE-1 will theoretically reduce this sequelae of events and has been experimentally shown to reduce infarct size, hypercontracture, stunning and the incidence of severe ventricular arrhythmias (2).

Infarct size reduction

Rohmann *et al.* (7) and Klein *et al.* (13) both showed the marked protective effect of the benzoylguanidine NHE-1 inhibitor, Hoe-694, to reduce myocardial infarct size in pigs. Rohmann *et al.* (7) administered Hoe-694

either 15 min prior to a 60-min coronary artery occlusion period or 15 min prior to reperfusion. These investigators demonstrated that Hoe-694 produced a marked reduction in infarct size when given either before occlusion or at reperfusion; however, the pretreatment regimen produced a significantly greater effect. Similarly, Klein *et al.* (13) administered Hoe-694 either 10 min prior to a 45-min occlusion period or 10 min prior to reperfusion. Pretreatment with Hoe-694 produced a marked reduction in infarct size assessed histologically or histochemically 24 h postreperfusion, whereas treatment 10 min prior to reperfusion produced only a marginal reduction in infarct size. Pretreatment also resulted in less contracture development and an improvement in regional systolic shortening at 24 h of reperfusion. This same group (14), using cariporide, calculated that inhibition of NHE-1 in pigs increased the time window of tolerance to ischemia/reperfusion by 20-25 min. Similarly, Garcia-Dorado *et al.* (15) found that pretreatment with cariporide in pigs reduced rigor contracture during occlusion and markedly reduced infarct size and arrhythmia development on reperfusion. When intracoronary cariporide was given just prior to reperfusion, it only reduced the incidence of arrhythmias but had no effect on infarct size. In rabbits, Bugge *et al.* (16) also found that pretreatment with ethylisopropylamiloride markedly reduced infarct size but had no effect when administered during the first part of the reperfusion period.

Since pigs and rabbits are known to possess a very limited collateral blood flow for delivering drug when administered during ischemia just prior to reperfusion, we undertook a study in dogs, which are known to possess a significant collateral circulation, to test the efficacy of the new NHE-1 inhibitor, EMD-85131, to reduce infarct size when administered prior to a 60-min coronary occlusion or 15 min before reperfusion (8). Two doses of EMD-85131 were used under each condition. Pretreatment with either 0.75 or 3.0 mg/kg of drug produced a marked reduction in infarct size from 24.3% to 9.3 and 6.4% (Fig. 2), respectively. When these same two doses were administered 15 min prior to reperfusion, infarct size was again significantly reduced from 24.3% to 12.2 and 13.0% (Fig. 3), respectively. Although there was a slightly better effect of EMD-85131 when the dogs were pretreated, the effect of EMD-85131 when given at reperfusion was still substantial. In agreement, Ito *et al.* (17), using SM-20550, a new NHE-1 inhibitor, also found that this compound significantly reduced infarct size either when it was given before occlusion or after reperfusion in dogs. These authors also found that NHE-1 blockade protected against microvasculature damage normally seen after reperfusion. Taken together, these results uniformly suggest that pretreatment with NHE-1 inhibitors is markedly cardioprotective in all species studied; however, there appears to be less or little protection observed when these compounds are administered just prior to reperfusion in animals with a sparse collateral blood supply, which would limit drug delivery to the ischemic myocardium. However, in species such as the dog, which has a substantial collateral circulation, NHE-1 inhibitors are effective against myocardial infarction and contracture when administered just prior to reperfusion although a recent study by Ohara *et al.* (18) using FR-183998, a new NHE-1 inhibitor, in rats, a species with a minimal

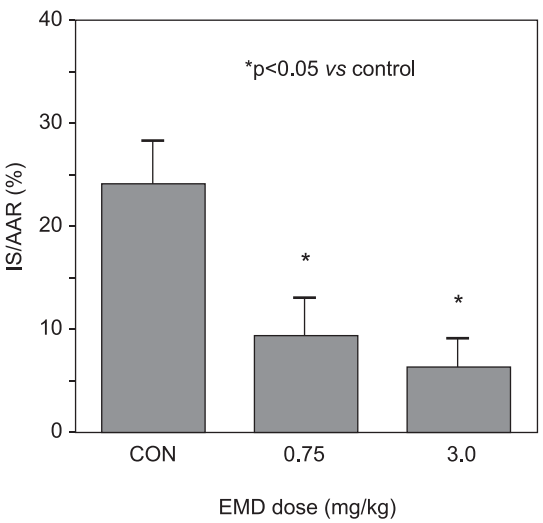

Fig. 2. Effect of 2 doses of EMD-85131 (0.75 and 3.0 mg/kg i.v.) on myocardial infarct size expressed as percent of the area at risk (IS/AAR) in dogs. Drug was administered 15 min prior to a 60-min occlusion of the left anterior descending (LAD) coronary artery. All values are the mean ± SEM (n = 7/8 dogs/group). **p* <0.05 *vs.* the control (CON) group. (From Gumina, R.J. et al. J Pharmacol Exp Ther 1998, 286 (1): 175-83. Reprinted by permission of the American Society of Pharmacology and Experimental Therapeutics).

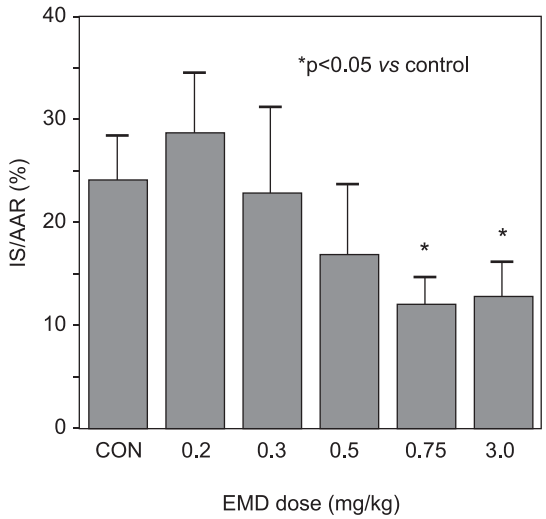

Fig. 3. Effect of 5 doses of EMD-85131 (0.2, 0.3, 0.5, 0.75 and 3.0 mg/kg i.v.) on myocardial infarct size expressed as percent of the area at risk (IS/AAR) in dogs. Drug was administered 15 min prior to reperfusion after a 60-min LAD occlusion. All values are the mean ± SEM (n = 7/8 dogs/group). **p* <0.05 *vs.* the control (CON) group. (From Gumina, R.J. et al. J Pharmacol Exp Ther 1998, 286 (1): 175-83. Reprinted by permission of the American Society of Pharmacology and Experimental Therapeutics.)

collateral circulation, suggested that this compound had cardioprotective effects when administered prior to or after ischemia. Again, the effect of FR-183998 was greater in pretreated rats. Obviously, more studies are needed with multiple doses and times of drug administration in different species to resolve the question as to the efficacy of these compounds when given near or at reperfusion.

Comparison of NHE-1 inhibition and ischemic preconditioning

Ischemic preconditioning (IPC), the phenomenon in which brief periods of ischemia render the heart resistant to more prolonged periods of ischemia (19), has been demonstrated to occur in all species studied, including man, and has been touted as the gold standard by which other interventions should be compared when evaluating their efficacy to reduce myocardial infarct size. During brief periods of myocardial ischemia accompanying IPC, it has been shown that intracellular pH decreases and intracellular sodium increases which might imply a role for NHE-1 in IPC (20). Moreover, it has been demonstrated that rat hearts subjected to IPC recover from acidosis more quickly than nonpreconditioned hearts, which suggests that NHE-1 may be turned on by IPC (20). Therefore, several questions come to mind and will be addressed in this review. Do NHE and IPC protect the heart by similar mechanisms? Do NHE inhibition and IPC have an antagonistic effect, no effect or an additive or synergistic effect when combined? Which intervention is more efficacious when considering the intensity or length of the ischemic insult?

A recent publication by Xiao and Allen (21) in isolated perfused rat hearts suggests that both IPC and NHE inhibition produced by 5-(*N*-methyl-*N*-isobutyl)amiloride (MIA) slow the rate of pH recovery during early reperfusion by inhibiting NHE at that time. These authors suggest that during prolonged ischemia NHE-1 is actually inhibited and that this inhibition persists during the early reperfusion period thereby mimicking the effect of a pharmacological inhibitor such as MIA. These authors did not combine the two interventions to determine if their effects were additive or not.

Shipolini *et al.* (22) investigated the interaction between IPC and NHE-1 inhibition with cariporide in isolated rat hearts subjected to either 40 or 60 min of global ischemia followed by reperfusion. In the 40-min model, both interventions separately produced nearly equivalent effects on the recovery of left ventricular developed pressure (LVDP). When both interventions were combined, the recovery of LVDP was slightly higher than when each were given separately. However, when the ischemic period was extended to 60 min, each intervention produced nearly equivalent protective effects but the combination produced an additive effect. In a separate protocol, cariporide was only administered during the preconditioning period and then washed out during a 40-min ischemic period. In this situation, cariporide did not affect the beneficial effect of IPC. Based upon these results, these investigators concluded that NHE-1 inhibition does not contribute to the protective effects of IPC and may even add benefit to IPC when the ischemic period is prolonged. In agreement, Sato *et al.* (23), Miura *et al.* (24) and Munch-Ellingson *et al.* (25) all showed in rabbit models of infarction that IPC and NHE-1 inhibition were not additive and did not appear to act through similar mechanisms. In particular, Sato *et al.* (23) and Miura *et al.* (24) demonstrated that a PKC inhibitor was able to antagonize the protective effects of IPC but not NHE-1 inhibition.

More recent evidence reported by Aye *et. al.* (10) in intact rat hearts also showed that both cariporide and IPC were very effective in reducing infarct size and the incidence of ventricular arrhythmias. However, since NHE-1 inhibition and a subthreshold period of IPC had additive effects in their model, these investigators concluded that changes in NHE-1 activity did not contribute to the cardioprotective effects of IPC. Similarly, Gumina *et al.* (26) showed in dogs that blockade of the ATP-sensitive potassium channel (K_{ATP} channel) with glibenclamide or

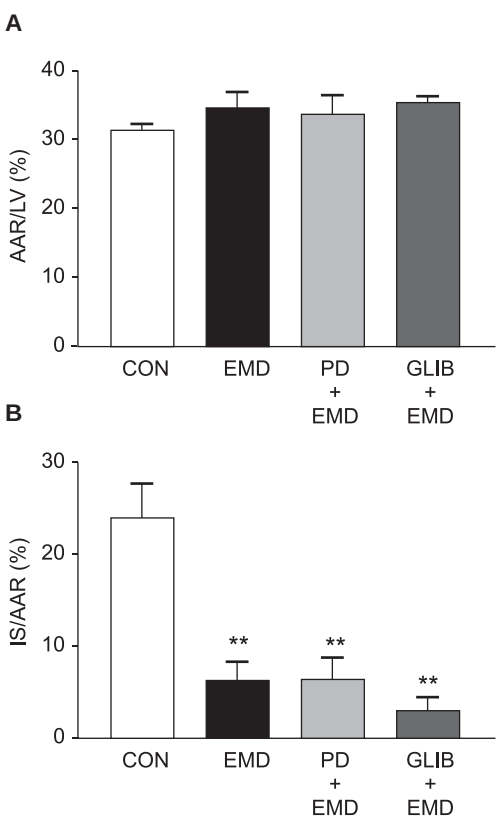

Fig. 4. Effect of inhibitors of ischemic preconditioning (IPC) on NHE-1 inhibition-mediated cardioprotection. Glibenclamide (GLIB; 0.3 mg/kg) or PD-115199 (PD; 3.0 mg/kg) was infused 15 min prior to EMD-85131 (EMD; 3.0 mg/kg). The LAD was occluded for 60 min followed by 180 min of reperfusion. The area at risk was expressed as percent of left ventricular weight (AAR/LV) (A) and infarct size as percent of the area at risk (IS/AAR) (B). All values are expressed as the mean ± SEM (n = 8 dogs/group). *p <0.05 *vs.* the control (CON) group. (Reprinted by permission of Lippincott, Williams and Wilkens Company.)

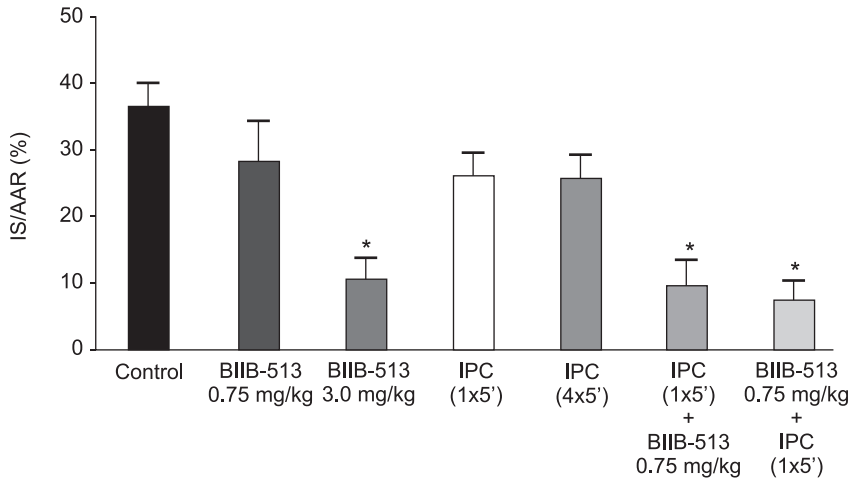

Fig. 5. Effect of NHE-1 inhibition and IPC on infarct size following 90 min of LAD occlusion. BIIB-513 (0.75 or 3.0 mg/kg) was administered 15 min prior to occlusion or before IPC (1 x 5) or (4 x 5) was conducted. Infarct size was expressed as percent of the area at risk (IS/AAR). All values are the mean ± SEM (n = 6-16 dogs/group). *p <0.05 *vs.* the control group.

adenosine receptors with a nonselective antagonist, PD-115199, did not antagonize the infarct size reducing effect of the NHE-1 inhibitor, EMD-85131 (Fig. 4). Since the K_{ATP} channel and adenosine receptor blockade have been clearly shown to block IPC in most species, these results further suggest that IPC and NHE-1 inhibition do not act via similar mechanisms.

Finally, in a comprehensive study from our laboratory, Gumina *et al.* (27) compared the efficacy of IPC and NHE-1 inhibition to reduce infarct size induced by a 60- or 90-min occlusion period followed by 3 h of reperfusion in dogs and the possible interaction between these two cardioprotective strategies. BIIB-513, a new NHE-1 inhibitor, was used as a comparison to either one 5-min period or four 5-min periods of preconditioning ischemia. Both IPC and BIIB-513 produced equivalent reductions in infarct size in the 60-min model; however, only BIIB-513 produced a significant reduction in the 90-min model. When a subthreshold dose of BIIB-513 and IPC were combined in the 90-min model, a greater than additive effect to reduce infarct size was observed. It was concluded from these results that NHE-1 inhibition is more efficacious than IPC in limiting infarct size/area at risk in dogs subjected to a prolonged ischemic insult and that their combination may result in a greater than additive effect (Fig. 5). These results also suggest that enhanced NHE-1 activity may limit the efficacy of IPC or that these two interventions act via different mechanisms. In this regard, a recent study of Gan *et al.* (28), in isolated rat hearts, indicated that NHE-1 mRNA is markedly increased in response to ischemia with or without reperfusion and in response to hydrogen peroxide or lysophosphatidyl choline; however, in preconditioned hearts NHE-1 message was markedly inhibited. These data support the notion that these two interventions may be acting in concert to produce cardioprotection.

Remodeling and NHE-1 inhibition

It has been well established that one of the most detrimental sequelae following an acute myocardial infarction is remodeling of the heart and the eventual development of left ventricular hypertrophy (LVH) and congestive heart failure. A number of factors such as myocardial stretch, angiotensin II and endothelin (29) may be contributing factors. Some evidence exists that these factors may all result in the downstream activation of NHE-1 by phosphorylation- and nonphosphorylation-dependent mechanisms (29). Hasegawa *et al.* (30) showed that rats with a ligation of the left anterior descending coronary artery developed LVH in 4 weeks and the presence of the NHE-1 blocker amiloride in their drinking water for 4 weeks prevented the development of LVH. More recently, Ruzicka *et al.* (31) found that blockade of angiotensin I receptors by losartan or NHE-1 by amiloride had beneficial effects on cardiac remodeling when administered alone; however, when combined their effects were much more pronounced. These results suggested that in addition to angiotensin II, other factors must also activate NHE-1 following a myocardial infarction and contribute to the remodeling response. In a more recent study, Yoshida and Karmazyn (32) found that cariporide, in the absence of changes in hemodynamics or infarct size, was still able to prevent remodeling 1 week postinfarction in rats. These data suggest that NHE-1 inhibition might be an effective therapeutic means of preventing ventricular remodeling in patients following a myocardial infarction.

Reduction in myocardial stunning

A number of studies in whole animals, isolated hearts and ventricular myocytes (33-38) have clearly shown that NHE-1 inhibitors produce an increase in the recovery of

global or regional function, prevent Ca^{2+} overload, prevent rigor contracture and improve the energy status of the heart following an ischemic insult. In contrast to the findings of Park *et al.* (12), these studies all present evidence that inhibiting NHE-1 during ischemia will prevent Na^+ overload during this period and by prolonging the period of acidosis during reperfusion attenuate Ca^{2+} influx. Taken together, all these actions should produce cardioprotection which has been clearly demonstrated to occur.

With a clinical perspective in mind, Karmazyn's group (39, 40) recently studied the interaction between anesthetics and NHE-1 inhibitors. These investigators found that isoflurane, sevoflurane, propofol and cariporide all produced an enhanced recovery of ventricular function following ischemia and reperfusion in isolated rat hearts and cariporide also reduced ischemic contracture and preserved ATP. Combining the volatile anesthetics with cariporide provided superior protection to either drug regimen alone. These data suggested that both volatile anesthetics and NHE-1 inhibition are cardioprotective; however, they appear to act via different mechanisms and when combined may provide a marked cardioprotective effect during surgical interventions.

In a conscious pig model of repetitive stunning produced by 25 cycles of coronary occlusion (2 min each) interspersed with 8 min of reperfusion, Symons *et al.* (38) demonstrated that cariporide delayed the onset of regional dysfunction and reduced the degree of dysfunction. Preliminary studies from our laboratory in dogs also showed that NHE-1 inhibition enhanced the recovery of systolic shortening following 15 min of coronary artery occlusion and 2 h of reperfusion (data not shown).

NHE-1 inhibition and apoptosis

Apoptosis is another mechanism by which myocardial cell death may occur and the effect of NHE-1 inhibition on this process has recently been addressed by Chakrabarti *et al.* (41) in isolated rat hearts subjected to varying periods of ischemia and 30 min of reperfusion. Evidence for apoptosis first appeared at 10 min of ischemia and was maximal at 30 min. Pretreatment of hearts for 15 min prior to ischemia with cariporide markedly reduced the number of apoptotic cells from 31 ± 3 to 2 ± 1 during ischemia and enhanced the early recovery of contractile function following reperfusion. These results suggest another potential cardioprotective mechanism by which NHE-1 inhibition may be of benefit to the ischemic heart.

Antiarrhythmic effects of NHE-1 inhibition

Several recent studies have shown that NHE-1 inhibition also results in a potent antiarrhythmic and antifibrillatory effect (10, 42). Aye *et al.* (10) found that cariporide significantly suppressed the incidence of fatal ventricular fibrillation in canine hearts without any notable effects on the incidence of ventricular tachycardia or premature ventricular contractions. In agreement, Gumina *et al.* (43) also found a reduction in the incidence of ventricular fibrillation in dogs upon reperfusion and in addition demonstrated a decrease in Type II arrhythmias which commonly occur in dogs at 20-30 min following a coronary artery occlusion. In rat hearts, Aye *et al.* (42) found that cariporide produced a dose-dependent reduction in the duration and incidence of ventricular tachycardia during ischemia and/or reperfusion and reduced the incidence and mortality of reperfusion-induced ventricular fibrillation. Cariporide also attenuated ouabain-induced arrhythmias in rats. These antiarrhythmic effects are most likely the result of a reduction in intracellular Na^+ and Ca^{2+} overload produced by NHE-1 inhibition during ischemia and during the early period of reperfusion.

Clinical trials with NHE-1 inhibitors

The efficacy of cariporide was recently evaluated in a large multicenter clinical trial, the GUARDIAN trial, with 12,000 patients (44). The patients enrolled were a heterogenous group with unstable angina, a non-Q-wave myocardial infarction or patients undergoing angioplasty or coronary artery bypass graft surgery (CABG). Cariporide had a favorable safety profile; however, its overall efficacy to reduce several indices of ischemia was not statistically significant compared to the control group. Nevertheless, when a subset of patients undergoing CABG surgery was evaluated, a significant beneficial effect was observed in the presence of high-dose cariporide. In support of these results, a recent study published by Rupprecht *et al.* (45) showed that a single dose of cariporide given before reperfusion in patients undergoing percutaneous transluminal coronary angioplasty (PTCA) improved functional and biochemical indices of ischemia when evaluated 21 days after PTCA. The results of these two studies are encouraging and suggest that NHE-1 inhibition may represent a novel and important new approach for the treatment of acute myocardial ischemia and reperfusion in patients with coronary artery disease (46).

Conclusions

The evidence which has accumulated over the past 10-15 years in a variety of experimental models of ischemia clearly suggests that inhibition of the NHE-1 demonstrates a marked cardioprotective effect against myocardial infarction, myocardial stunning and the incidence of severe ventricular arrhythmias. The results of several clinical trials using cariporide are also encouraging and suggest that other NHE-1 inhibitors should also be tested for their efficacy and safety in well-controlled studies in patients undergoing CABG surgery or PTCA (47).

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