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Enalaprilat Attenuates Ischemic Rises in Intracellular Sodium in the Isolated Rat Heart Via the Bradykinin Receptor

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ABSTRACT

Purpose: *Angiotensin-converting enzyme (ACE) inhibitors have been shown to have beneficial effects on ischemic myocardium. We examined whether the ACE inhibitor, enalaprilat (EN), improves intracellular sodium homeostasis during myocardial ischemia and the relationship of this effect to bradykinin.* ***Methods:*** *EN (3.2 nM) was administered to isolated rat hearts that were subjected to ischemia and reperfusion. Intracellular sodium and pH were monitored using magnetic resonance spectroscopy (MRS). The specific bradykinin B2 receptor antagonist, HOE 140 (10 nM), was administered with EN in some hearts to determine the effect of bradykinin blockade on EN-mediated effects.* ***Results:*** *EN blunted the rise in ischemic intracellular sodium, measured using MRS. With reperfusion, EN-treated hearts recovered 80% of their preischemic ventricular function, compared with negligible recovery in controls. These beneficial effects of EN were blocked when the bradykinin receptor antagonist, HOE 140, was coadministered with EN. HOE 140 also blocked EN-mediated attenuation of ischemic intracellular acidosis.* ***Conclusions:*** *These results suggest that EN exerts beneficial effects on ischemic intracellular sodium and pH homeostasis via the bradykinin receptor. These effects of EN may provide a mechanism for the beneficial actions of this agent during ischemia.*

KEY WORDS: ACE inhibitors; Experimental; Heart; Ischemia; NMR; Reperfusion

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INTRODUCTION

Although recanalization therapy is the primary goal in the management of patients with acute myocardial infarction (AMI), adjunctive therapies, such as β-blockers or angiotensin-converting enzyme (ACE) inhibitors, remain important components of the management of ischemic heart disease. ACE inhibitor therapy early post-MI has been shown to improve postischemic cardiac function and to decrease subsequent left ventricular (LV) remodeling in both experimental and clinical studies (1–3).

One feature of ACE inhibitors not shared by other antihypertensive agents is a capacity to influence sodium in myocardium (4). This property might contribute to the beneficial effects of ACE inhibitors on ischemic myocardium and may be related to the ability of ACE inhibitors to increase bradykinin levels.

During myocardial ischemia, there is loss of cellular homeostasis, including reduction of the transmembrane sodium gradient. The normally low intracellular sodium concentration—maintained by net sodium efflux during normal perfusion conditions—rises as ischemia progresses, reflecting both increased influx and decreased extrusion of sodium. Many experimental studies have documented that reducing the ischemic rise in intracellular sodium improves postischemic performance. Thus, either lowering the sodium concentration in the perfusate (5) or treating ischemic hearts with sodium–hydrogen exchange inhibitors, which attenuate the rise in intracellular sodium (6–9), improves postischemic performance.

This study was designed to test the hypothesis that improvement in myocardial postischemic performance with ACE inhibitor therapy is associated with a reduction in the ischemic rise in intracellular sodium. We studied isolated rat hearts that were treated with the ACE inhibitor, enalaprilat (EN). Hearts were subjected to no-flow ischemia, followed by reperfusion. Postischemic heart function was monitored and ischemic intracellular sodium was measured using sodium magnetic resonance spectroscopy (MRS). The data demonstrate that the ACE inhibitor, EN, improves postischemic function, and that this improvement is related to an attenuation in the ischemic rise in intracellular sodium. In addition, the data suggest that the attenuation of this rise by EN is associated with a reduction in intracellular acidosis, which depends on the participation of functional bradykinin receptors.

METHODS

Isolated Heart Preparation

The investigation conformed with the Guide for the Care and Use of Laboratory Animals, published by the U.S. National Institutes of Health (NIH Publication No. 85-23, revised 1996). Hearts were rapidly isolated from anesthetized 350- to 400-g male Wistar rats (60 mg/kg ketamine and 20 mg/kg xylazine injected intraperitoneally). Retrograde perfusion at 37°C through the aorta was begun immediately. Hearts were perfused with a modified Tyrode's solution (pH 7.4) containing (in m*M*) NaCl 140, KCl 5, $MgCl_2$ 0.9, HEPES 6, $CaCl_2$ 1.5, and dextrose 15. The perfusate was vigorously bubbled with 95% O_2/5% CO_2 throughout each experiment. Perfusate reservoirs and lines were heated with a water-jacketed system to maintain perfusate temperature.

LV pressure was measured using an intraventricular balloon (Hugo Sachs Elektronic, Germany) inserted into the LV cavity via the left atrium. Balloon volume was controlled using a Gilmont syringe. Heart rate, aortic perfusion pressure, LV developed pressure (LVDP), and LV end-diastolic pressure (LVEDP) were recorded on a multichannel Gould recorder (Gould Instruments, Ohio). Aortic pressure was maintained between 80 and 100 mm Hg. LVEDP was set during equilibration at 5–10 mm Hg.

Experimental Protocols

Control hearts were given 30 min of drug-free perfusion. Hearts treated with EN, 3.2 n*M* (Merck RL), were given 15 min of drug-free perfusion, followed by 15 min of perfusion with EN. Equilibration time was therefore 30 min for all hearts. EN was chosen as the ACE inhibitor, since it does not require metabolic activation and it does not have a sulfhydryl group—like captopril—that might scavenge free radicals. Zero-flow ischemia was then induced for 40 min, and reperfusion was subsequently instituted at the preischemic aortic pressure for 30 min. All hearts were paced at a rate of 240 bpm during the equilibrium phase of perfusion; pacing was stopped on the start of ischemia and not restarted.

ACE inhibitors suppress the catabolism of bradykinin. To study the role of the bradykinin receptor on the cardiac effects of EN, in some hearts, HOE 140 (10 n*M*, Research Biochemicals International), a specific bradykinin B2 receptor antagonist, was coadministered with EN. The experimental groups are depicted in Figure 1.

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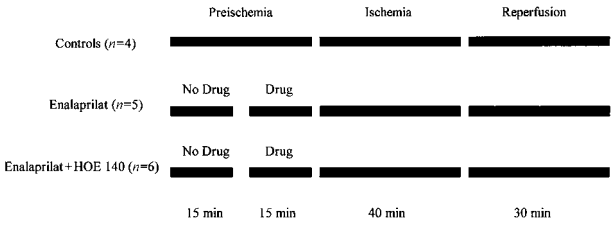

Figure 1. Experimental groups examined and the ischemia-reperfusion protocol used for each.

Magnetic Resonance Spectroscopy

All MRS data were acquired using a 7.1-T wide-bore magnet (Bruker, MA). Sodium spectra were acquired at a frequency of 79.4 MHz. After shimming and tuning the magnet, spectra were acquired every 2.2 min during baseline and ischemia. All intracellular sodium spectra were discriminated from extracellular sodium, using the shift reagent, thulium (DOTP) (4.5 mM). Calcium was added with thulium to compensate for the chelation of divalent cations by thulium. Triple-quantum filtered sodium spectra were acquired using the pulse sequence

$$90^\circ - \tau/2 - 180^\circ - \tau/2 - \theta - \delta - \theta - \mathrm{Acq}(t_1),$$

where τ represents the creation time, δ the evolution time, and t_1 the acquisition time (150 ms). The flip angle θ was set to 90°; the sweep width was 4 kHz, and the number of transients 384. Twelve-step phase cycling schemes were used to detect triple-quantum coherences. Processing of free induction decays was done with a line broadening of 10 Hz prior to Fourier transformation. Peak heights of the intracellular signal were measured and normalized to the average baseline value for each heart.

^{31}P MRS was performed in parallel experiments on isolated hearts to monitor changes in intracellular pH with and without treatment with drug(s). ^{31}P spectra were acquired at 121.5 MHz with 240–480 transients (total acquisition time, ~5 min), pulse width of 15 μs, acquisition time of 0.125 s, and a recycle time of 1.05 s. The intracellular pH (pH_1) of isolated hearts was calculated from the chemical shift (δ_O) of the inorganic phosphate (P_1) peak from the phosphocreatine (PCr) peak by the equation

$$pH_1 = pK - \log[(\delta_O - \delta_B)/(\delta_A - \delta_O)],$$

where $pK = 6.9$, $\delta_A = 3.290$, and $\delta_B = 5.805$ (10). In order to maintain the temperature of the isolated heart during no-flow ischemia, the bore of the magnet was warmed with temperature-controlled air.

Statistical Analysis

MRS-measured intracellular sodium and pH were compared between control hearts and hearts treated with drug(s), using repeated measures ANOVA. Each measure of intracellular sodium and intracellular pH was compared between controls and treated hearts, using a post hoc Tukey's procedure with Bonferroni correction. Rate-pressure products (RPP) were averaged for each heart during both preischemic perfusion and reperfusion. Values are expressed as means ± SD. RPP was compared between controls and drug-treated hearts, using ANOVA for repeated samples.

RESULTS

Effect of EN on Functional Recovery After Ischemia

The functional performance of control hearts and of hearts treated with EN is shown in Table 1. There were no significant differences in LVDP or the LVEDP during the preischemic phase of heart perfusion between control and EN-treated hearts. All hearts quickly lost all contractile function after 5 min of ischemia. On reperfusion, however, there was a significantly greater improvement in the rate-pressure product of EN-treated hearts as compared with controls ($p < 0.05$). EN-treated hearts recovered 80% of their baseline rate-pressure product whereas control hearts were either asystolic or in sustained ventricular fibrillation. While HOE 140 had no effect on cardiac dynamics during equilibration, HOE 140 treatment mitigated the beneficial effects of EN on reperfusion.

Effect of EN on the Ischemic Rise in Intracellular Sodium

Representative sodium spectra are shown in Figure 2. The administration of EN during the preischemic phase of perfusion did not significantly affect intracellular sodium as compared with controls (Fig. 3). As shown, the rise in intracellular sodium during ischemia was blunted by treatment with EN as compared with controls ($p < 0.0002$ by ANOVA for repeated measurements). From 10 min into ischemia until the end of ischemia, each individual measure of intracellular sodium in controls was significantly higher than the corresponding measure in EN-treated hearts ($p < 0.05$ by post hoc Tukey's procedure). While the co-administration of HOE 140 with EN had no

Table 1

Cardiac Function in Control, Enalaprilat (EN), and EN + HOE 140 Treated Isolated Rat Hearts During Equilibration and Reperfusion

	LVDP (mm Hg)		EDP (mm Hg)		RPP	
	Equilibration	Reperfusion	Equilibration	Reperfusion	Equilibration	Reperfusion
Controls ($n = 4$)	88.2 ± 9.9	[a]	7.7 ± 1.4	35.6 ± 2.6	$14{,}795 \pm 1{,}292$	[a]
Enalaprilat ($n = 5$)	71.2 ± 5.7	70 ± 40	4.8 ± 3.3	5 ± 10	$16{,}852 \pm 1{,}560$	$13{,}525 \pm 7{,}695$[b]
EN+HOE 140 ($n = 6$)	90.3 ± 15.8	[a]	7.8 ± 1.8	32.1 ± 5.6	$21{,}672 \pm 3{,}792$	[a]

LVDP—Left ventricular developed pressure; EDP—left ventricular end-diastolic pressure; RPP—Rate-pressure product. Hearts were paced at 240 bpm during equilibration.

[a] All hearts were either asystolic or in sustained ventricular fibrillation.

[b] EN-treated hearts recovered 80% of their preischemic rate-pressure product on reperfusion.

effect on intracellular sodium during baseline perfusion, blockade of the bradykinin receptor did attenuate the salutary effects of EN on the rise in intracellular sodium during ischemia (Fig. 3, $p < 0.0002$). From 10 min into ischemia until the end of ischemia, each individual measure of intracellular sodium in HOE 140 plus EN-treated hearts was significantly higher than the corresponding measure in hearts treated with EN alone ($p < 0.05$).

Changes in Intracellular pH

Figure 4 shows the changes in tissue pH in control hearts and in hearts treated with EN. EN-treated hearts experienced less acidosis than control hearts during ischemia at each time point from 5 min into ischemia until reperfusion ($p < 0.02$). There was no effect of EN on tissue pH during baseline or postischemic perfusion. While

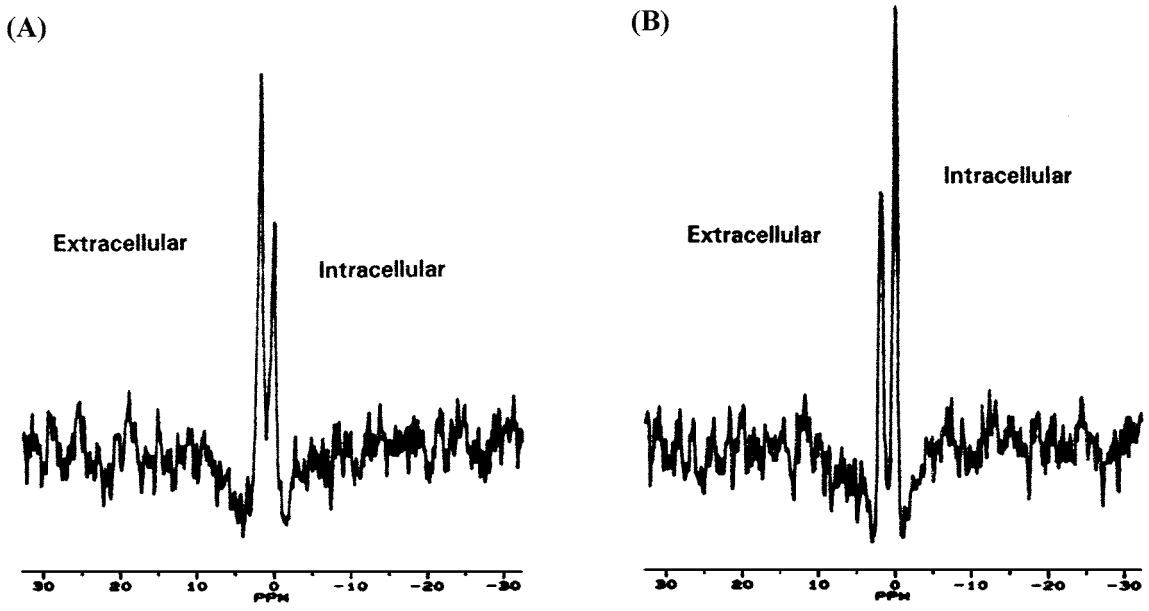

Figure 2. Representative triple-quantum sodium spectra from isolated perfused rat hearts during equilibration (A) and after 35 min of ischemia (B). Extracellular and intracellular represent extracellular and intracellular triple-quantum sodium signals, respectively.

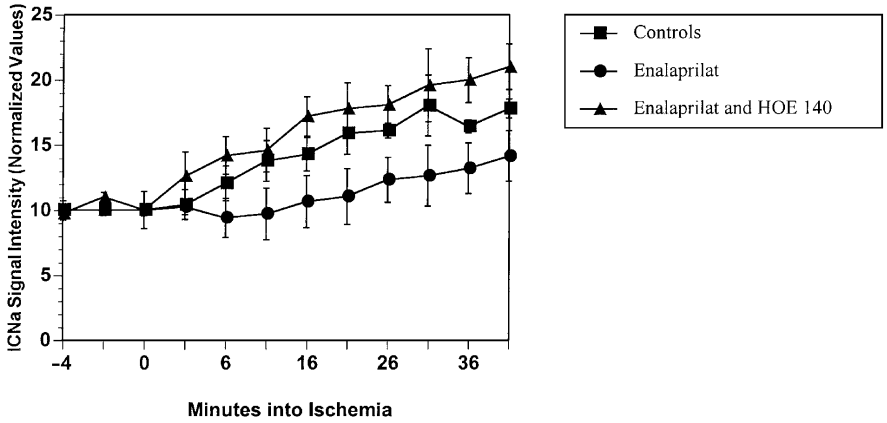

Figure 3. Intracellular sodium (ICNa) prior to and during ischemia in control hearts and in hearts treated with enalaprilat (3.2 n*M*) or EN+HOE 140 (10 n*M*). EN inhibited the ischemic rise in ICNa. This salutary effect was blocked when the bradykinin receptor was inhibited with HOE 140.

HOE 140 had no effect on pH during equilibration, when coadministered with EN, HOE 140 mitigated the effects of EN on ischemic decreases in pH.

DISCUSSION

This study confirms that ACE inhibitors—in this instance, EN—improve ventricular performance after an ischemic insult, and demonstrates that this improvement is associated with an attenuation in the ischemic rise of intracellular sodium. The mechanism by which EN improves sodium homeostasis appears to be dependent on bradykinin receptors.

Intracellular Sodium and Myocardial Ischemia

During ischemia, the rise in intracellular sodium is a consequence of net sodium influx. Sodium enters cells via the sodium–hydrogen exchanger, the sodium–potassium chloride cotransporter, and the lidocaine-sensitive Na channel. Loss of sodium–potassium ATPase activity

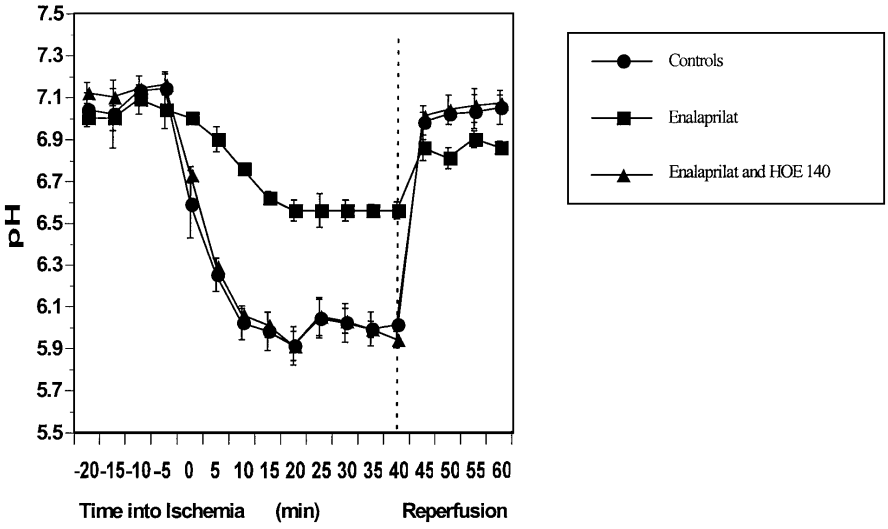

Figure 4. Intracellular pH during all phases of perfusion in control hearts and in hearts treated with either EN or EN+HOE 140. EN-treated hearts had a blunted decrease in pH during ischemia. HOE 140 attenuated this effect.

because of depletion of ATP stores during ischemia also reduces sodium efflux. Together, these changes in sodium homeostasis cause a rise in intracellular sodium. In this study, during ischemia, intracellular sodium rose approximately 50–70% above baseline in control hearts. Other studies, using single-quantum sodium NMR and bicarbonate-based buffers, have documented increases anywhere from two- to -four-fold for similar ischemic times (11,12). The less severe increase of intracellular sodium in this study is probably the result of both increased sodium invisibility when using triple-quantum filtered NMR pulse sequences and the use of a HEPES-buffered perfusate (13,14).

Considerable evidence supports the notion that the ischemic rise in intracellular sodium contributes to the calcium overload that occurs during reperfusion, a process mediated by the sodium–calcium exchanger. Elevated intracellular calcium activates proteases, such as calpains I and II, that degrade troponins, causing excitation-contraction uncoupling—one potential cellular basis of postischemic myocardial dysfunction (15,16). Blunting the ischemic rise in intracellular sodium reduces the driving force for the influx of calcium on reperfusion, lessens excitation-contraction uncoupling, and reduces postischemic myocardial contractile dysfunction (16–18). We observed better functional recovery during reperfusion in hearts that had a smaller rise in intracellular sodium during ischemia (Table 1).

Effect of EN on Intracellular pH During Ischemia

We observed that EN-treated hearts maintained a higher pH during ischemia than in controls. This finding parallels that in another study in which lisinopril also attenuated acidosis during ischemia in isolated rat hearts (19). Since the observed intracellular pH during ischemia is a net result of changes in proton production (from anaerobic glycolysis, ATP hydrolysis) and proton efflux (as well as buffering), it is not possible to determine the precise reasons by which acidosis is attenuated in EN-treated hearts. Nevertheless, by reducing acidosis, EN may improve sodium homeostasis by reducing pH-driven intracellular sodium loading (via the sodium–hydrogen exchanger) during ischemia. Reducing acidosis may be another mechanism by which EN improves postischemic function.

The Role of Bradykinin

Since ACE inhibitors not only prevent the formation of angiotensin II (AII) but also prevent the degradation of bradykinin, either reduced production of AII or increased bradykinin levels, or both, could explain the beneficial effects of ACE inhibition. Two observations, however, point to bradykinin as the mediator of cardioprotection. First, in the present study, treatment of hearts made ischemic and then reperfused with EN was associated with markedly improved functional recovery, a decreased rise of intracellular sodium during ischemia, and reduced acidosis. Blockade of the bradykinin B2 receptor with HOE 140 prevented each of these effects. These observations have also been made in other settings. The administration of a bradykinin B2 receptor antagonist completely abolished any beneficial effects of ACE inhibitors on ischemic myocardium (20,21). This would not be expected if beneficial effects were mediated via direct inhibition of AII. Second, any beneficial effects of AT_1 receptor antagonism by an agent such as losartan were blocked when the bradykinin B2 receptor was simultaneously antagonized (22). The bradykinin receptor antagonist used, HOE 140, was found to be devoid of effects on cardiac function and was thus chosen for the present study (22). Finally, modulation of pH by EN through bradykinin, not AII inhibition, is consistent with another study in which administration of losartan did not affect ischemic pH in rat hearts (23). These previous observations, as well as the results of the present study, strongly suggest that the participation of functional bradykinin B2 receptors is crucial to the cardioprotection afforded by ACE inhibitors in this setting.

ACE inhibitors, by promoting bradykinin, reduce tissue levels of endothelin (24), which is one of the most potent stimulators of sodium–hydrogen exchange (25). By inhibiting sodium–hydrogen exchange, ACE inhibitors may reduce sodium loading during ischemia. Improved sodium homeostasis might account not only for the better postischemic function that we observed, but also for the reduction of reperfusion arrhythmias such as ventricular fibrillation (26,27).

LIMITATIONS

There are several limitations to this study. First, we used a HEPES-based buffer, which might exaggerate the contribution of the sodium–hydrogen exchanger to sodium extrusion during ischemia (13,14). Second, we also did not perform a control experiment to examine the effect of HOE 140 alone on myocardial performance and sodium homeostasis. A prior study, however, documented that HOE 140 alone has no effect on myocardial function during or after ischemia (22). Third, the rate-pressure product for our rat hearts was lower than that achieved using a

bicarbonate-based crystalloid buffer. Finally, we did not use angiotensin receptor antagonists to fully elucidate the effect of AII on myocardial performance. As noted above, however, a previous study documented that angiotensin receptor antagonists confer no benefit to myocardial performance during ischemia and reperfusion when the bradykinin receptor is simultaneously antagonized (22).

CONCLUSIONS

This study demonstrates that EN improves postischemic myocardial performance in an isolated rat heart model, which is consistent with prior experimental and clinical data using other ACE inhibitors. This improvement in performance appears linked to an improvement in sodium and pH homeostasis during ischemia. Both the improvement in postischemic function and the enhanced sodium and pH regulation during ischemia showed dependence on the bradykinin receptor. This suggests that a possible mechanism by which ACE inhibitor therapy is beneficial in patients may be related to a bradykinin-mediated pathway, perhaps via reduction in tissue levels of endothelin.

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