Effect of ANG II on pH$_i$, [Ca$^{2+}$]$_i$, and contraction in rabbit ventricular myocytes from infarcted hearts

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Skolnick, Rita L., Sheldon E. Litwin, William H. Barry, and Kenneth W. Spitzer. Effect of ANG II on pH$_i$, [Ca$^{2+}$]$_i$, and contraction in adult rabbit ventricular myocytes from infarcted hearts. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H1788-H1797, 1998.—In this study we examined Na$^+$/H$^+$ exchange activity, Ca$^{2+}$ transients, and contractility in rabbit ventricular myocytes isolated from normal and chronically (8–12 wk) infarcted left ventricles. Myocytes from infarcted hearts (post-MI myocytes) were isolated from the peri-infarcted region of the left ventricle. Intracellular pH (pH$_i$) and Ca$^{2+}$ concentration ([Ca$^{2+}$]$_i$) were measured with the fluorescent pH indicators seminaphthorhodafluor 1 and fluo 3, respectively, and contractility was assessed from changes in cell shortening during field stimulation. Experiments were performed at extracellular pH 7.4 in the presence and absence (HEPES buffer) of CO$_2$ and HCO$_3$$. Our findings demonstrate that 1) myocytes after myocardial infarction (post-MI) were significantly larger than normal, 2) post-MI hypertrophy was not accompanied by changes in non-CO$_2$ intracellular buffering power, 3) post-MI hypertrophy did not significantly affect the ability of Na$^+$/H$^+$ exchange to mediate pH$_i$ recovery from intracellular acidosis, 4) the stimulatory effect of ANG II (100 nM) on Na$^+$/H$^+$ exchange was significantly reduced in post-MI myocytes, 5) in HCO$_3$$-buffered solutions, ANG II did not significantly stimulate pH$_i$ recovery from acidosis in post-MI myocytes, 6) the angiotensin AT$_1$ receptor mediates the stimulatory action of ANG II on Na$^+$/H$^+$ exchange in normal and post-MI myocytes, and 7) the stimulatory effect of ANG II on the Ca$^{2+}$ transient and contraction was blunted in post-MI myocytes bathed in HEPES-buffered solution. A suppressed ventricular responsiveness to ANG II may be beneficial in the intact myocardium by attenuating ATP consumption and by reducing intracellular Na$^+$ accumulation during ischemia-reperfusion.

acid extrusion; intracellular pH; contractility; sodium/hydrogen exchange

CARDIAC HYPERTROPHY and failure are accompanied by chronic changes in several sarcolemmal ion transport systems involved in intracellular Ca$^{2+}$ regulation, including Na$^+$/Ca$^{2+}$ exchange (30, 43) and Ca$^{2+}$ current (31, 32, 38). However, the extent to which cardiac intracellular pH (pH$_i$) regulatory systems are affected by cardiac overload is unresolved. Previous studies of pH$_i$ regulation in hypertrophied myocardium have focused on pressure overload-induced cardiac hypertrophy. For example, Na$^+$/H$^+$ exchange activity was enhanced in papillary muscles from spontaneously hypertensive rats (35), and aortic banding induced activation of mRNA for the NHE-1 isoform of Na$^+$/H$^+$ exchange in rabbit ventricular muscle (44). However, there is no information available concerning the effects of chronic infarct-induced remodeling on Na$^+$/H$^+$ exchange. Infarct-induced models of left ventricular dysfunction are useful in terms of their clinical significance. Although recent advances in the treatment of acute myocardial infarction (MI) have decreased overall cardiac mortality, many patients are left with chronic heart damage (22). Thus animal models that simulate postinfarction dysfunction may be of use in the clinical setting.

In cardiac muscle, Na$^+$/H$^+$ exchange is largely responsible for the recovery of pH$_i$ from intracellular acidosis (20) and is stimulated by ANG II (13, 23, 26). Activation of Na$^+$/H$^+$ also contributes to the myocardial damage and arrhythmias associated with ischemia-reperfusion (10, 19, 46). The cardiac renin-angiotensin system is activated during cardiac hypertrophy and failure, and enhanced intracardiac production of ANG II may cause adverse effects on ventricular function (15, 29, 37, 39). In this regard, numerous studies have shown that inhibition of angiotensin-converting enzyme attenuates the cardiac dysfunction accompanying MI and heart failure (8, 36, 45).

The major focus of this project was to obtain new insight into the contribution of Na$^+$/H$^+$ exchange to cellular events related to postinfarction (post-MI) remodeling in spared ventricular myocytes. Because ANG II significantly modulates cardiac Na$^+$/H$^+$ exchange in normal cells and may contribute to the pathophysiology of heart failure, we examined the effect of ANG II on Na$^+$/H$^+$ exchange in post-MI ventricular myocytes. We also evaluated the inotropic actions of ANG II on both cell types. All experiments were performed on single ventricular myocytes isolated from adult rabbit hearts using fluorescent indicators to measure pH$_i$ and Ca$^{2+}$ transients. The use of single cells avoids the possible complications associated with ANG II-induced changes in pH$_i$ and intracellular Ca$^{2+}$ concentration ([Ca$^{2+}$]$_i$) that may occur in noncardiac cells present in multicellular preparations and cell suspensions.

MATERIALS AND METHODS

Solutions and myocyte bathing system. Cell bathing solutions were held at 36.5 ± 0.5°C in glass reservoir bottles. Solutions were delivered by gravity from the bottles to the myocyte bath through thermally jacketed gas-impermeable tubing, thereby ensuring that the solution pH and temperature were the same in the bottles and the bath. The 1-ml Plexiglas bath had a clear glass bottom and was mounted on the stage of an inverted microscope (Diaphot, Nikon, Tokyo, Japan). The bottom of the bath was lightly coated with laminin (Collaborative Research, Bedford, MA) to improve myocyte adhesion. Solution flow and depth were held at 4–6 ml/min and 2 mm, respectively. The temperature of solutions...
in the bath was 36.5 ± 0.5°C. Complete replacement of a solution in the bath required ~4 s.

Because we were especially interested in studying Na+/H+ exchange, most experiments were performed in HEPES-buffered solution containing no added CO2 or H2O2. We have found that Na+-independent Cl-/HCO3 exchange is negligible under these conditions (48). Similarly, Na+-HCO3 symmetry is reported to be inactive in ventricular myocytes under these conditions (20). The normal HEPES-buffered bathing solution contained (in mM) 126.0 NaCl, 11.0 dextrose, 4.4 KCl, 1.0 MgCl2, 1.0 CaCl2, 24.0 HEPES, and 13.0 NaOH (pH 7.4). Na+-free solutions were prepared by equal molar replacement of NaCl and NaOH with N-methyl-D-glucamine, using 1.0 N HCl to adjust the pH to 7.4. HCO3-buffered solutions were used to examine the effects of ANG II under more physiological conditions. HCO3-buffered solutions contained (in mM) 126.0 NaCl, 11.0 dextrose, 4.4 KCl, 1.0 MgCl2, 1.0 CaCl2, and 18.0 NaHCO3 and were gassed with 5.0% CO2–95.0% O2 (pH 7.4). The gas mixture was made on-line with a precision mixing device (Utah Medical Products, Midvale, UT) using pure O2 and CO2. The barometric pressure in the laboratory was ~640 mmHg, yielding a PaCO2 of ~30 mmHg.

ANG II (human) was obtained from Calbiochem (La Jolla, CA). Losartan (DuP-753) was obtained as a generous gift from Merck Research Laboratories (West Point, PA). Semi-naphthorhodfluor (SNARF) 1-AM and fluo 3-AM were obtained from Molecular Probes (Eugene, OR).

Production of myocardial infarction. Rabbits were preanesthetized with acepromazine (16 mg/kg im) and xylazine (3 mg/kg im). Approximately 10–15 min later, they were anesthetized with isoflurane, orally intubated, and ventilated at 40 breaths/min. Under sterile conditions, a left lateral thoracotomy was made in the fifth intercostal space and a 6-0 polypropylene suture was used to create a pericardial incision. Under sterile conditions, a left lateral thoracotomy was made in the fifth intercostal space and a 6-0 polypropylene suture was used to create a pericardial incision. Under sterile conditions, a left lateral thoracotomy was made in the fifth intercostal space and a 6-0 polypropylene suture was used to create a pericardial incision. Under sterile conditions, a left lateral thoracotomy was made in the fifth intercostal space and a 6-0 polypropylene suture was used to create a pericardial incision.

Results were obtained from male rabbits (n = 25% of the total left ventricular circumference.

Hemodynamic measurements. To assess the extent of left ventricular dysfunction after MI, intracardiac pressures were recorded in a separate group of rabbits. Rabbits were anesthetized with ketamine (50 mg/kg im) and xylazine (3 mg/kg im). The right carotid artery was isolated by cut down and cannulated with a 1-mm micromanometer-tipped catheter (Millar Instrument, Houston, TX). The catheter was retrogradely passed across the aortic valve under constant pressure monitoring. Left ventricular systolic and end-diastolic pressures were recorded.

Myocyte isolation. Results were obtained from male rabbits (~2–4 kg) with normal (n = 37) and infarcted hearts (n = 42). The heart was retrogradely perfused via the aorta (60 mmHg pressure) with a Ca2+-free solution at 37°C for 5 min containing (in mM) 126.0 NaCl, 4.4 KCl, 22.0 dextrose, 5.0 MgCl2, 2.0 tauroine, 5.0 creatine, 5.0 sodium pyruvate, 1.0 NH4PO4, 24.0 HEPES, and 12.5 NaOH (pH 7.3). This was followed by 15–20 min of recirculation of the same solution (200 ml containing 1 mg/ml collagenase (class II, Worthington Biochemical, Freehold, NJ), 0.1 mg/ml protease (type XIV, Sigma Chemical), and 0.1 mM CaCl2. The heart was then perfused for 5 min with the same solution containing no enzymes. All cell isolation solutions were equilibrated with 100% O2. The left ventricle was separated from the right ventricle and the atria. For normal hearts the free wall of the left ventricle was minced. For infarcted hearts the scar was removed, and then a 2- to 3-mm rim of surviving tissue surrounding the scar was dissected free and minced. We chose to use tissue from the peri-infarcted region to minimize potential heterogeneity in cellular geometry or physiology in different regions of the infarcted heart. The minced tissue from normal and infarcted hearts was gently shaken in the 0.1 mM Ca2+ (enzyme-free) solution and strained, and the remaining myocytes were allowed to settle in a storage solution containing 1.0 mM Ca2+. The yield of Ca2+-tolerant cells using this procedure was >60%. All myocytes used in this study were rod shaped in appearance, had well-defined striations, and did not spontaneously contract. All cells were studied 2–5 h after isolation.

Measurement of cell size. Cell length and width were measured in a large number of myocytes from control and infarcted hearts. To avoid bias, the microscope stage was randomly moved, and all the quiescent rod-shaped cells visible in the field were measured.

Measurement of pH, Ca2+ transients, and myocyte contractions. pH was measured in single myocytes according to previously described procedures (26, 41, 48). Myocytes were equilibrated at 37°C for 10 min in the normal solution containing 13 µM SNARF 1-AM. They were then placed in the bath, and normal solution containing no indicator was continuously directed through the bath. pH measurements were begun 30–40 min later. Excitation at 515 ± 5 nm was provided by a 200-W mercury-arc lamp and was directed to the myocyte bath via the objective lens (~40, oil, NA 1.3, Nikon). Myocytes were also illuminated from above with low-intensity blue light (410 ± 20 nm), which had no effects on the SNARF 1 emission signal. Optical signals from a myocyte were collected by the objective lens and sent out the microscope side port to an intensified charge-coupled device (ICCD) television camera (model IC-100, Photon Technology International, S. Brunswick, NJ) and a dichroic mirror (610 nm). The camera detected the cell image, and the mirror directed the fluorescence emission to two photomultiplier tubes equipped with band-pass filters centered at 640 ± 15 and 580 ± 15 nm. An adjustable rectangular window in the side port restricted the optical image and fluorescence to the cell of interest. The ratio of fluorescence emission (640 nm) to 580 nm) was then electronically subtracted from the loaded cell signal. All pH measurements were performed on resting myocytes, except those in which cell shortening and pH were simultaneously recorded.

The emission ratio from each myocyte was calibrated by exposing the resting myocyte to solutions of varying pH. Each solution contained 10 µM nigericin and 12.0 mM HEPES titrated with 1 M KOH, 140.0 mM KCl (KCl adjusted to keep K+ constant), 1.0 mM MgCl2, 11.0 mM dextrose, 2 mM EGTA, and 15 mM 2,3-butanedione monoxime. EGTA and 2,3-butanedione monoxime were used to prevent cell contracture during application of the calibrating solutions. Before application of the calibrating solutions, the myocyte was bathed in the normal solution containing 2 mM EGTA and no added Ca2+ for ~2 min to remove extracellular Ca2+.

Ca2+ transients were detected in single myocytes with the fluorescent indicator fluo 3, as previously described (49). Briefly, myocytes were incubated in the normal HEPES-buffered solution containing 10 µM fluo 3-AM and 0.5 mM probenecid at 30°C for 45 min. The cells were then continuously bathed in the same solution containing no indicator. Probenecid was used to help retard fluo 3 transport from the...
cells. Fluorescence emission (530 nm) was collected with a photomultiplier tube via the x40 objective during continuous excitation at 485 nm. Cell motion was simultaneously measured along with the Ca2+ transient.

The contractile activity (cell shortening) of field-stimulated myocytes was measured optically using the ICCD camera coupled to a video edge-detector device (42). Constant-current pulses (4-6 ms duration) were delivered at a cycle length of 3 s to the cells via a glass capillary tube (100-µm tip diameter) filled with normal bathing solution and positioned ~0.5 mm downstream from the cell. The other electrode was a silver wire positioned 0.5 mm from the capillary tube. pH and shortening or Ca2+ transients and shortening were simultaneously measured in the same myocyte.

Determination of intracellular buffering power and net flux of acid equivalents. To determine net H+ flux (J H+) it is necessary to measure intracellular buffering power. For experiments performed in the absence of CO2 and HCO3−, we assumed that HCO3− buffering was zero and that total intracellular buffering power (B T) was equal to intrinsic buffering power (B i). B i was experimentally estimated using step changes performed in the absence of NH4Cl. B i (mM/pH) was calculated as

\[ B_i = \frac{\Delta [NH_4^+] / \Delta pH_i}{[NH_4^+]_o} \]

where \( \Delta [NH_4^+] \) is the measured change in pH and \( \Delta [NH_4^+]_o \) is the change in intracellular concentration of NH4+ calculated as

\[ [NH_4^+] = [NH_4^+]_o \times 10^{PH_i-9.5} \]

where [NH4+]o is the total extracellular concentration of NH4Cl and pHo is extracellular pH (7.4), with the dissociation constant (pK) taken as 9.5. The relationship between Bi and pH was determined over the pH range of ~6.5-7.4 in normal and infarcted hearts. The best-fit equation of the data (Bi vs. pH) was used to estimate Bi at any given pH in other cells.

In an HCO3−-buffered solution, intracellular buffering due to CO2 (B CO2) was calculated as

\[ B_{CO2} = 2.3 [HCO_3^-] \]

where [HCO3−] is intracellular HCO3− concentration and [HCO3−]o is extracellular HCO3− concentration, and in HCO3−-buffered solution, Bi = Bi + B CO2.

Net H+ efflux via Na+/H+ exchange was determined in HEPES-buffered solution from the rate of recovery of pH after a 10 mM NH4Cl prepulse. Application of NH4Cl initially increases pH, as basic NH3 rapidly enters the cell. pH subsequently falls as charged NH4+ enters the cell, mainly through K+ channels, and dissociates. On removal of NH4Cl an intracellular acid load is created as internal NH3 leaves the cell, causing intracellular retention of H+. J H+ via Na+/H+ exchange was calculated at successive values of pH during recovery according to

\[ J_{H^+} = (dPH_i/dt) \cdot B_i \]

where dPHi/dt is the rate of rise in pHi at each value of pHi. To calculate dPHi/dt, the pHi recovery was curve fit with a polynomial and dPHi/dt at successive values of pHi was determined.

In HCO3−-buffered solution, pHi recovery from an NH4Cl prepulse is mediated by Na+/H+ exchange and Na+/HCO3− cotransport (20). Under these conditions

\[ J_{H^+} = (dPH_i/dt) \cdot B_T \]

where J H+ represents net acid extrusion (or base influx) via both transporters.

Data acquisition. Analog signals (fluorescence ratio and cell shortening) were simultaneously recorded digitally with a computer using AxoScope and AxoTape software (Axon Instruments, Foster City, CA). pH signals were filtered at 1 Hz and digitized at a rate of 2 Hz. Cell shortening and fluo 3 signals were filtered at 500 Hz and digitized at 1 kHz.

Statistical analysis. Values are means ± SE. Statistical analysis was performed using paired and unpaired Student’s t-test. P < 0.05 was considered significant.

RESULTS

Dimensions of normal and post-MI remodeled myocytes. The mean length and width of normal myocytes were 132.2 ± 3.1 and 29.4 ± 0.7 µm, respectively (301 cells from 4 hearts). The corresponding values from post-MI cells were 142.9 ± 1.3 and 30.4 ± 0.4 µm, respectively (498 cells from 7 hearts). Cell length is significantly different in the two groups (P < 0.01, unpaired t-test). These changes in cell size suggest a predominant volume overload, which is typical of the infarcted ventricle (6). This characteristic pattern of cellular hypertrophy confirms that the single cells used in this project were subjected to the pathological processes that involve the surviving myocardium after transmural infarction. Hemodynamic measurements are summarized in Table 1.

B i in post-MI remodeled myocytes. To quantitatively assess J H+ via Na+/H+ exchange, it is necessary to determine B i. It is also important to directly measure B i, since it may be changed by cardiac hypertrophy. All Bi measurements were performed in Na+/H+ -free solution containing no added CO2 or HCO3−. An example of a Bi determination in a post-MI cell is shown in Fig. 1A, and the results from normal and post-MI cells are summarized in Fig. 1B. The Bi-pH relationship is given by Bi = 143.1 − 17.2pHi for normal cells (35 measurements in 15 cells) and Bi = 76.7 − 8.1pHi for post-MI cells (27 measurements in 13 cells). There was no significant difference in Bi between the two cell types.

Table 1. Hemodynamic parameters in rabbits with normal and infarcted left ventricles

<table>
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<th>Heart Rate, mmHg</th>
<th>LVSP, mmHg</th>
<th>LVEDP, mmHg</th>
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<tbody>
<tr>
<td>Normal</td>
<td>8</td>
<td>176 ± 8</td>
<td>77 ± 2</td>
<td>2.6 ± 0.4</td>
</tr>
<tr>
<td>Infarcted</td>
<td>13</td>
<td>159 ± 4</td>
<td>78 ± 2</td>
<td>8.3 ± 0.7</td>
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<td>P</td>
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Values are means ± SE. LVSP, left ventricular systolic blood pressure; LVEDP, left ventricular end-diastolic blood pressure.
Similarly, the mean steady-state pH$_i$ in post-MI myocytes (7.05 ± 0.05) was not significantly different (unpaired t-test) from the mean decrease in steady-state pH$_i$ in normal myocytes (7.09 ± 0.02, n = 29) at each pH$_i$ (unpaired t-test). However, in the post-MI myocytes the stimulatory effect was suppressed. The results are summarized in Fig. 4, with J$_{\text{H}^+}$ expressed as a function of pH$_i$. In contrast to post-MI myocytes, ANG II significantly increased J$_{\text{H}^+}$ in normal myocytes at pH 6.65, 6.70, and 6.75. This difference in ANG II responsiveness is further emphasized by comparing J$_{\text{H}^+}$ in the two groups at pH 6.85. In the presence of ANG II, J$_{\text{H}^+}$ was significantly less (unpaired t-test, P < 0.05) in post-MI myocytes (2.46 ± 0.84 mM/min, n = 7) than in normal cells (4.70 ± 0.69 mM/min, n = 8).

In normal myocytes a 5-min exposure to ANG II (100 nM) elicited a significant (P < 0.05, paired t-test) increase in steady-state pH$_i$, of 0.032 ± 0.014 (n = 8). In contrast, pH$_i$ was not significantly altered in the post-MI myocytes (mean ΔpH$_i$ = 0.001 ± 0.012, n = 6).

HEPES-buffered solution: effect of the AT$_1$-receptor antagonist losartan on ANG II-induced stimulation of Na$^+$/H$^+$ exchange. To identify the receptor subtype involved in the ANG II-induced changes in J$_{\text{H}^+}$, we used losartan, a specific nonpeptide blocker of AT$_1$ receptors (47). As shown in Fig. 5 (normal myocyte), ANG II elicited a fall in steady-state pH$_i$ when applied in the presence of 1 µM losartan. The mean decrease in steady-state pH$_i$ in normal myocytes was 0.011 ± 0.005 (n = 6) after 5 min in ANG II, which is significantly different (P < 0.05, unpaired t-test) from the mean increase in steady-state pH$_i$ (0.032 ± 0.014, n = 8) that occurs when ANG II is applied to normal myocytes in the absence of losartan.

In addition to affecting steady-state pH$_i$, losartan also completely blocked the stimulatory action of ANG II on pH$_i$ recovery from intracellular acidosis (Fig. 5). The results for normal and post-MI myocytes are summarized in Fig. 6. In normal and post-MI myocytes pretreated with losartan, ANG II did not stimulate J$_{\text{H}^+}$.

In normal cardiac cells (23, 26). In the present experiments we examined the effects of ANG II (100 nM) on Na$^+$/H$^+$ exchange in spared post-MI myocytes. Normal and post-MI myocytes displayed an ANG II-induced increase in the rate of recovery from acid loading that is indicative of stimulated Na$^+$/H$^+$ exchange (Fig. 3). However, in the post-MI myocytes the stimulatory effect was suppressed. The results are summarized in Fig. 4, with J$_{\text{H}^+}$ expressed as a function of pH$_i$. In contrast to post-MI myocytes, ANG II significantly increased J$_{\text{H}^+}$ in normal myocytes at pH 6.65, 6.70, and 6.75. This difference in ANG II responsiveness is further emphasized by comparing J$_{\text{H}^+}$ in the two groups at pH 6.85. In the presence of ANG II, J$_{\text{H}^+}$ was significantly less (unpaired t-test, P < 0.05) in post-MI myocytes (2.46 ± 0.84 mM/min, n = 7) than in normal cells (4.70 ± 0.69 mM/min, n = 8).

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HEPES-buffered solution: effect of post-MI remodeling on Na$^+$/H$^+$ exchange and steady-state pH$_i$. It seems possible that post-MI remodeling may affect expression and/or the ion transport capacity of Na$^+$/H$^+$ exchange. To test the latter hypothesis, we activated Na$^+$/H$^+$ exchange by inducing rapid intracellular acidosis using NH$_4$Cl pretreatments in normal and post-MI cells. In the absence of CO$_2$/HCO$_3^-$, pH$_i$ recovery from intracellular acidosis in normal ventricular myocytes is mediated by forward Na$^+$/H$^+$ exchange. The results are summarized in Fig. 2, in which J$_{\text{H}^+}$ is expressed as a function of pH$_i$ during recovery from acid loading. Although the mean values of J$_{\text{H}^+}$ in post-MI myocytes were less than normal, the differences at each pH$_i$ did not achieve statistical significance (P = 0.09–0.31, unpaired t-test). Similarly, the mean steady-state pH$_i$ in post-MI myocytes (7.09 ± 0.02, n = 29) was not significantly different from that in normal myocytes (7.05 ± 0.02, n = 23).

HEPES-buffered solution: effect of ANG II on Na$^+$/H$^+$ exchange and steady-state pH$_i$ in post-MI remodeled myocytes. Hypertrophied and failing hearts have an altered neurohormonal responsiveness (4) and an activated renin-angiotensin system (15, 37, 39). We previously showed that ANG II stimulates Na$^+$/H$^+$ exchange in normal myocytes (4.70 ± 0.69 mM/min, n = 8).

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In addition to affecting steady-state pH$_i$, losartan also completely blocked the stimulatory action of ANG II on pH$_i$ recovery from intracellular acidosis (Fig. 5). The results for normal and post-MI myocytes are summarized in Fig. 6. In normal and post-MI myocytes pretreated with losartan, ANG II did not stimulate J$_{\text{H}^+}$.
during acid load recovery. These results suggest that the AT1 receptor mediates the stimulatory action of ANG II on Na+/H+ exchange in normal and post-MI cells.

HCO3−-buffered solution: effect of ANG II on pHı recovery from acid loading. We also studied pHı recovery from 10 mM NH4Cl prepulses under more physiological conditions using an HCO3−-CO2 buffer system. In this setting all pHı regulatory systems are intact and JH+ reflects JH+ via Na+/H+ exchange and HCO3− influx via Na+-HCO3− cotransport (20). The results are summarized in Fig. 7. In normal myocytes, ANG II elicited significant (P < 0.05, paired t-test) increases in JH+ at pHı 6.95, 6.90, and 6.85. In contrast, no significant changes occurred in post-MI myocytes. The mean steady-state pHı of normal myocytes in HCO3−-buffered solution: effect of ANG II on pHı recovery from acid loading.

Fig. 3. Effect of 100 nM ANG II on pHı recovery from intracellular acidosis in normal and spared post-MI myocytes. All solutions were buffered with HEPES. Stimulatory effect of ANG II on pHı recovery from intracellular acidosis is blunted in post-MI myocyte.

Fig. 4. Summary of effect of 100 nM ANG II on relationship between JH+ via Na+/H+ exchange and pHı in normal (A; ○, control; ●, ANG II) and spared post-MI myocytes (B; ○, control; ●, ANG II). Experimental protocol is shown in Fig. 3. Each myocyte served as its own control for ANG II application. n = 3–7. *Significantly increased compared with control (P < 0.05, paired t-test).

Fig. 5. AT1-receptor inhibitor losartan blocks stimulatory effect of 100 nM ANG II on pHı recovery from intracellular acidosis (A, normal myocyte). B: overlay of pHı recoveries shown in A. All solutions were buffered with HEPES.
solution without ANG II (7.03 ± 0.02, n = 5) was not significantly different from that in post-MI myocytes (7.02 ± 0.02, n = 10).

HEPES-buffered solution: effect of ANG II on pHi and myocyte shortening during pacing. We and others previously showed that the positive inotropic action of ANG II in normal rabbit ventricular myocytes is mediated, in part, by stimulation of Na\(^{+}/H^{+}\) exchange (3, 16, 26). To examine the acute inotropic effects of ANG II (100 nM) on post-MI myocytes, we simultaneously measured pHi and cell shortening in field-stimulated myocytes (cycle length = 3 s). Cell shortening in post-MI myocytes displayed reduced responsiveness to ANG II compared with normal cells (Fig. 8). The accompanying changes in pHi also differed in the two cell types, with alkalosis in the normal cell and acidosis in the post-MI cells. The mean increase in cell shortening measured 5 min after ANG II application was 91.5 ± 21.4% (n = 8) in normal myocytes compared with 30.0 ± 11.9% in post-MI myocytes (n = 6, P < 0.05, unpaired t-test). The corresponding changes in pHi in the beating myocytes were 0.06 ± 0.02 and −0.04 ± 0.02 in normal and post-MI myocytes, respectively (P < 0.01, unpaired t-test). In addition to demonstrating that post-MI hypertrophy is associated with a diminished inotropic sensitivity to ANG II, these results also show that the positive inotropic action of ANG II can occur without an accompanying increase in pHi.

HEPES-buffered solution: effect of ANG II on the Ca\(^{2+}\) transient and myocyte shortening during pacing. To determine whether the ANG II-induced changes in pHi and contraction described above (Fig. 8) were accompanied by alterations in the Ca\(^{2+}\) transient, we repeated the pacing protocol in fluo 3-loaded cells under identical conditions. In both cell types, ANG II increased the amplitude of the Ca\(^{2+}\) transient and the simultaneously measured cell shortening (Fig. 9). The results are summarized in Fig. 10 and demonstrate that the ANG II-induced increase in both parameters was significantly less in post-MI myocytes.

**DISCUSSION**

In this study we examined Na\(^{+}/H^{+}\) exchange activity, Ca\(^{2+}\) transients, and cell shortening in rabbit ventricular myocytes isolated from normal and chronically (8–12 wk) infarcted left ventricles. Experiments were performed at pH\(_{o}\) 7.4 in solutions that contained no added CO\(_{2}\) or HCO\(_{3}\) (HEPES buffered) or were buffered with 18.0 mM HCO\(_{3}^{-}\)-5.0% CO\(_{2}\). The post-MI myocytes were isolated from the peri-infarcted region of the left ventricles. Given the high incidence of heart failure...
induced by ischemic heart disease, this model has clinical relevance.

Our results demonstrate for the first time that 1) post-MI hypertrophy is not associated with changes in Bi (Fig. 1), 2) post-MI hypertrophy does not significantly affect the ability of Na\(^+\)/H\(^+\) exchange to mediate pHi recovery from intracellular acidosis (Fig. 2), 3) the stimulatory effect of ANG II on Na\(^+\)/H\(^+\) exchange is significantly reduced in post-MI myocytes (Figs. 3 and 4), 4) in HCO\(_3\)-buffered solutions, ANG II does not significantly stimulate pHi, recovery from acidosis in post-MI cells (Fig. 7), 5) the AT\(_1\) receptor mediates the stimulatory action of ANG II on Na\(^+\)/H\(^+\) exchange in post-MI myocytes (Fig. 6), and 6) in HEPES-buffered solution, post-MI myocytes have a reduced positive inotropic response to ANG II (Figs. 8–10) that correlates with a blunted increase in the amplitude of the Ca\(^{2+}\) transient (Figs. 9 and 10).

The percentage of the left ventricle affected by coronary artery ligation in our experiments varied from 20 to 30% of the total mass of the ventricle. Myocytes from post-MI rabbits showed a predominant increase in cell length, suggesting that chronic infarction caused changes in myocyte structure similar to chronic volume overload. Similar changes have been noted in a rat model of post-MI heart failure (33). The main hemodynamic effects of infarction induced by coronary artery ligation in the rabbit are increased left ventricular end-diastolic pressure, slowed left ventricular relaxation, and decreased rate of left ventricular pressure development (25). These effects are also very similar to those reported for the more extensively characterized rat model of postinfarction heart failure (22).

Despite their increased size, post-MI myocytes did not display significant changes in their ability to buffer intracellular H\(^+\) in the absence of CO\(_2\)-HCO\(_3\). Pressure overload hypertrophy in rat (17) and ferret (11) ventricular muscle is also not accompanied by changes in Bi. Similarly, normal Bi values are reported for ventricular muscle from spontaneously hypertensive rats (35). Interestingly, these results contrast with exercise-induced hypertrophy, in which Bi is significantly increased (9).

The J\(_{\text{H}^+}-\text{pHi}\) relationship for Na\(^+\)/H\(^+\) exchange in normal myocytes was not significantly different from that of post-MI myocytes (Fig. 2). This contrasts with recent findings that the activity and gene expression of ventricular Na\(^+\)/H\(^+\) exchange are increased in spontaneously hypertensive rats (35) and pressure-overloaded rabbit hearts (44), respectively. The molecular basis for this difference is unclear. However, chronic pressure and volume overload are known to induce morphologically different forms of myocardial hypertrophy (1) and to elicit distinctly different molecular phenotypes and patterns of peptide growth factor induction (5).

We previously showed that ANG II stimulates Na\(^+\)/H\(^+\) exchange in normal rabbit atrial (23) and ventricular (26) myocytes. Although the exact mechanism of this effect is unresolved, it seems likely that protein kinase C activation and phosphorylation of the transporter are involved (26). In the present study we demonstrate that the stimulatory action of ANG II on Na\(^+\)/H\(^+\) exchange is attenuated in post-MI myocytes. A suppressed responsiveness of Na\(^+\)/H\(^+\) exchange to ANG II and endothelin 1 has also recently been observed in rat ventricular myocytes subjected to pressure-induced hypertrophy (17). This similarity suggests that, despite their differences (1, 5), pressure- and volume-induced left ventricular hypertrophies may share similar mechanisms for inducing impaired sensitivity to vasoactive peptides.

Acid load recovery in HCO\(_3\)-buffered solutions is mediated by Na\(^+\)/H\(^+\) exchange and Na\(^+\)-HCO\(_3\) cotrans-
port (20). In adult rat and guinea pig ventricular myocytes, the relative contribution of Na\textsuperscript{+}/HCO\textsubscript{3}\textsuperscript{−} co-transport to this process is 33% (21) and 40% (20), respectively. Values for rabbit ventricular muscle are apparently not available. However, a comparison of our normal J\textsubscript{H}\textsuperscript{+} values (without ANG II) in the absence (Fig. 4) and presence of HCO\textsubscript{3}\textsuperscript{−} (Fig. 7) suggests that Na\textsuperscript{+}/HCO\textsubscript{3}\textsuperscript{−} may contribute, 30% at pHi 6.75. The acid loader, Na\textsuperscript{+}-independent Cl\textsuperscript{−}/HCO\textsubscript{3}\textsuperscript{−} exchange, mediates HCO\textsubscript{3}\textsuperscript{−} efflux at pHi above 6.7 and thus does not contribute to pH\textsubscript{i} recovery from intracellular acidosis (20, 48). In contrast, AT\textsubscript{1}-receptor density is decreased in hypertrophied rat ventricle subjected to 8–9 wk of pressure overload (24). Similarly, AT\textsubscript{1} receptor density (2) and expression (14) are reduced in human heart failure. AT\textsubscript{2} density (2) or expression (14) was unchanged. Although the rabbits used in our experiments (8–12 wk post-MI) had not progressed to overt left ventricular failure, these earlier studies raise the possibility that a similar reduction in AT\textsubscript{1}-receptor density and expression may contribute, in part, to the decreased ANG II responsiveness we observed. Regarding intracellular signaling, Ito et al. (17) recently reported that the stimulatory effect of phorbol esters on Na\textsuperscript{+}/H\textsuperscript{+} exchange was markedly suppressed in hypertrophied rat ventricle, suggesting that the coupling between protein kinase C activation and Na\textsuperscript{+}/H\textsuperscript{+} exchange was impaired. A similar mechanism may also be operational in the post-MI myocytes.

Our results do not reveal the mechanism of reduced sensitivity of Na\textsuperscript{+}/H\textsuperscript{+} exchange to ANG II in post-MI myocytes. However, there are several possibilities, including 1) reduction in the density and/or sensitivity of ANG II receptors, 2) suppression of the intracellular signaling pathway leading to protein kinase C activation, and 3) reduction in Na\textsuperscript{+}/H\textsuperscript{+} exchanger density and/or the H\textsuperscript{+} sensitivity of the transporter’s proton modifier site.

The extent to which mechanism 3 contributes to our findings requires further investigation. However, previous studies of ANG II receptors and intracellular signaling pathways provide possible explanations for our findings. Our losartan experiments indicate that the AT\textsubscript{1} receptor mediates the stimulatory effect of ANG II on Na\textsuperscript{+}/H\textsuperscript{+} exchange, in accord with previous findings in normal perfused ferret hearts (13). AT\textsubscript{1}-receptor density is reported to increase in rat ventricular myocytes after a relatively brief period (1 wk) of coronary artery ligation (28). In contrast, AT\textsubscript{1}-receptor density is decreased in hypertrophied rat ventricle subjected to 8–9 wk of pressure overload (24). Similarly, AT\textsubscript{1} receptor density (2) and expression (14) are reduced in human heart failure. AT\textsubscript{2} density (2) or expression (14) was unchanged. Although the rabbits used in our experiments (8–12 wk post-MI) had not progressed to overt left ventricular failure, these earlier studies raise the possibility that a similar reduction in AT\textsubscript{1}-receptor density and expression may contribute, in part, to the decreased ANG II responsiveness we observed. Regarding intracellular signaling, Ito et al. (17) recently reported that the stimulatory effect of phorbol esters on Na\textsuperscript{+}/H\textsuperscript{+} exchange was markedly suppressed in hypertrophied rat ventricle, suggesting that the coupling between protein kinase C activation and Na\textsuperscript{+}/H\textsuperscript{+} exchange was impaired. A similar mechanism may also be operational in the post-MI myocytes.
We also found that the stimulatory effects of ANG II on contraction and the Ca\(^{2+}\) transient were suppressed in post-MI myocytes bathed in HEPE5-buffered solution (Figs. 8–10). Recent work suggests that the positive inotropic action of ANG II in normal rabbit ventricular myocytes is mediated, in part, by stimulation of Na\(^+/H^+\) exchange (16, 26). The resulting intracellular alkalosis may increase L-type Ca\(^{2+}\) current (18) and myofilament Ca\(^{2+}\) sensitivity (16). Our finding of reduced ANG II-induced activation of Na\(^+/H^+\) exchange may account, in part, for the suppressed contractile response of post-MI myocytes to ANG II. However, factors other than intracellular alkalosis (e.g., intracellular Na\(^+\) accumulation) must be involved, since, in contrast to normal cells in which pH, and cell shortening were increased by ANG II, in post-MI myocytes shortening increased but pH fell (Fig. 8). A dissociation between pHi and contractility during exposure to ANG II has also been reported for normal paced cat papillary muscles and was attributed to activation of Cl\(^--/HCO_3^-\) exchange (27). Intracellular acidosis reduces ventricular contractility (34, 41) and thus by itself cannot account for the positive inotropic effect of ANG II we observed in post-MI myocytes. However, we previously showed in normal ventricular myocytes bathed in HEPE5-buffered solution (no added CO\(_2\) or HCO\(_3^-\)) that ANG II has a dual effect on pH (26): one mechanism acts to stimulate forward Na\(^+/H^+\) exchange and increase pH; the other, which appears to be metabolic in origin, decreases pH. The final effect of ANG II on steady-state pH depends on the relative magnitudes of acid extrusion via Na\(^+/H^+\) exchange and acid production via the metabolic pathway. Thus the action of ANG II to decrease steady-state pH in post-MI myocytes bathed in HEPE5-buffered solution may reflect predominance of the acid production pathway. However, the accompanying activation of Na\(^+/H^+\) exchange would increase intracellular Na\(^+\) and, because of Na\(^+\)/Ca\(^{2+}\) exchange, would raise cytosolic Ca\(^{2+}\) and thus increase Ca\(^{2+}\) loading of the sarcoplasmic reticulum. The resulting increase in sarcoplasmic reticulum Ca\(^{2+}\) release would act to increase cell shortening, albeit to a smaller extent in post-MI myocytes. Consistent with this hypothesis is our finding that ANG II increased the amplitude of the Ca\(^{2+}\) transient in normal and post-MI myocytes (Figs. 9 and 10).

We did not examine the effect of ANG II on contraction and the Ca\(^{2+}\) transient in an HCO\(_3^-\)/CO\(_2\) buffer system. However, the suppressed stimulatory effect of ANG II on Na\(^+/H^+\) exchange in post-MI myocytes will also be present in this system and by itself would act to reduce ANG II-induced increases in both parameters. The lack of a significant stimulatory effect of ANG II on J\(_{\text{H^+}}\) in post-MI myocytes in an HCO\(_3^-\)/CO\(_2\) buffer supports this hypothesis.

The physiological significance of a reduced ANG II sensitivity of Na\(^+/H^+\) exchange, the Ca\(^{2+}\) transient, and contractility in spared post-MI myocytes is unclear. However, suppression of the positive inotropic action of ANG II may help preserve intracellular ATP. Similarly, suppression of ANG II-induced increases in Na\(^+/H^+\) exchange and the Ca\(^{2+}\) transient may help attenuate the myocardial damage and arrhythmias associated with intracellular Ca\(^{2+}\) overload during ischemia-reperfusion (19).

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Preliminary results have been presented as an abstract (40).

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Na\textsuperscript{+}/H\textsuperscript{+} EXCHANGE IN INFARCTED HEARTS


