Enalapril Induces Regression of Cardiac Hypertrophy and Normalization of pH$_i$ Regulatory Mechanisms

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Abstract—Intracellular pH is under strict control in myocardium; H$^+$ are extruded from the cells by sodium-dependent mechanisms, mainly Na$^+$/H$^+$ exchanger and Na$^+$/HCO$_3^-$ symport, whereas Na$^+$-independent Cl$^-$/HCO$_3^-$ exchanger extrudes bases on intracellular alkalinization. Hypertrophic myocardium from spontaneously hypertensive rats (SHR) exhibits increased Na$^+$/H$^+$ exchange activity that is accompanied by enhanced extrusion of bases through Na$^+$-independent Cl$^-$/HCO$_3^-$ exchange. The present experiments were designed to investigate the effect of enalapril-induced regression of cardiac hypertrophy on the activity of these exchangers. Male SHR and normotensive Wistar-Kyoto rats (WKY) received enalapril maleate (20 mg/kg per day) in the drinking water for 5 weeks. Gender- and age-matched SHR and WKY were used as untreated controls. Enalapril treatment significantly reduced systolic blood pressure in SHR and completely regressed cardiac hypertrophy. Na$^+$/H$^+$ activity was estimated in terms of both steady pH$_i$ value in HEPES buffer and the rate of pH$_i$ recovery from CO$_2$-induced acid load. Na$^+$-independent Cl$^-$/HCO$_3^-$ activity was assessed by measuring the rate of pH$_i$ recovery from intracellular alkalinization produced by trimethylamine exposure. Regression of cardiac hypertrophy was accompanied by normalization of Na$^+$/H$^+$ and Na$^+$-independent Cl$^-$/HCO$_3^-$ exchange activities. Inhibition of protein kinase C (PKC) activity with chelerythrine (10 mmol/L) or calphostin C (50 nmol/L) returned both exchange activities to normal values. These results show that angiotensin-converting enzyme inhibition normalizes the enhanced activity of both exchangers while regressing cardiac hypertrophy. Because normalization of exchange activities could be also achieved by PKC inhibition, the data would suggest that PKC-dependent mechanisms play a significant role in the increased ion exchange activities of hypertrophic myocardium and in their normalization by angiotensin-converting enzyme inhibition. (***Hypertension***. 1998;31:961-967.)

Key Words: ion transport ■ hypertrophy, cardiac ■ angiotensin-converting enzyme inhibitors ■ protein kinase C ■ intracellular pH

We have recently reported that NHE activity is increased in the hypertrophic myocardium of SHR.[1] The blockade of angiotensin II production by ACE inhibitors is proven to be potent and effective for blood pressure reduction and cardiac hypertrophy regression.[2,3]

The enhancement of NHE activity has been described in various other cell types obtained both from hypertensive individuals and genetic animal models (for detailed review, see Reference 4), and it might result from an increased expression of exchanger protein units and/or an increased turnover rate of each unit. The question as to whether increased activity of NHE relates to increased turnover rate or increased mRNA and protein expression of the exchanger has been explored in different cell types, and it is still controversial.[5–9] However, experiments in membranes from SHR cardiac tissue suggested a posttranslational processing mechanism involving phosphorylation as responsible for NHE hyperactivity.[10] Activation of NHE by growth-promoting factors apparently involves an alkaline shift in the pH$_i$ dependency of NHE due to phosphorylation of the exchanger protein itself and/or of a putative regulatory protein.[11–13]

An increased activity of the AE in SHR myocardium has been reported by us.[1] The parallel increase of AE activity induces net bicarbonate efflux and blunts the increase in myocardial pH$_i$ that would be induced by the NHE. It was therefore tempting to investigate whether enalapril-induced regression of cardiac hypertrophy had an influence on NHE and AE activities in SHR myocardium. In an attempt to further examine possible underlying mechanism(s), the effect of PKC inhibition was also studied.

Methods

Experiments were conducted in age-matched SHR and WKY male rats, which were originally derived from Charles River Breeding Farms, Wilmington, Mass. All animals were identically housed under controlled lighting and temperature conditions with free access to standard rat chow and tap water. All the experiments were conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (US Department of Health and Human Services). Beginning at 12 weeks of age, SBP was measured weekly in all animals by the standard tail-cuff method.[14] By 16 weeks of age, SBP was significantly elevated in SHR (overall mean, 175±3 mm Hg; n=42) compared with WKY (118±2 mm Hg;

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Selected Abbreviations and Acronyms

    β_i = intrinsic buffer capacity
  ACE = angiotensin-converting enzyme
   AE = Na⁺-independent Cl⁻/HCO₃⁻ exchanger
   BW = body weight
    -C = control
    -E = enalapril treated
   HW = heart weight
  J_H+ = net apparent acid equivalent efflux
  LVW = left ventricular weight
  NHE = Na+/H+ exchanger
  PKC = protein kinase C
  SBP = systolic blood pressure
  SHR = spontaneously hypertensive rat(s)
TMACl = trimethylamine hydrochloride
  WKY = Wistar-Kyoto rat(s)
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TABLE 1.   General Characteristics of Rat Groups

Rat Group	SBP, mm Hg	BW, g	HW/BW, mg/g	LVW/BW, mg/g	n
SHR-C	183±3*	318±8	3.5±.08*	3.0±.08*	23
SHR-E	131±4	299±11	2.8±.08	2.4±.06	14
WKY-C	126±2	303±7	2.7±.04	2.3±.04	15
WKY-E	106±5	301±16	2.8±.13	2.4±.12	12

Values are mean±SEM. n indicates number of animals.
*$P<.05$ vs every other group (ANOVA).

n=27). By this time, rats from each breed were respectively divided at random into two groups. One group of each rat strain was treated with enalapril maleate (SHR-E and WKY-E) by inclusion of the drug in the drinking water. Concentration was adjusted every 2 days to match the BW/consumption ratio to ensure a dosage of 20 mg/kg per day. The second group of each strain served as untreated controls. Treatment lasted 5 weeks, and at the end of this period animals were deeply anesthetized with ether and their hearts were removed. From each heart, a papillary muscle was dissected free and mounted, as previously described,[1] in an organ bath on the stage of an Olympus CK2 inverted microscope (Olympus Optical Co). Muscles were superfused with one of the following solutions: (1) HEPES- buffered solution containing (in mmol/L) 133.8 NaCl, 4.5 KCl, 1.35 CaCl$_2$, 1.05 MgSO$_4$, 11 glucose, and 25 HEPES (pH of the buffer solution was adjusted to 7.38±0.03 at 30°C with 3 N NaOH [total Na$^+$ amounted to 148.8], and the solution was gassed with 100% O$_2$) or (2) HCO$_3^-$/CO$_2$-buffered solution containing (in mmol/L): 128.3 NaCl, 4.5 KCl, 1.35 CaCl$_2$, 20.23 NaHCO$_3$, 0.35 NaH$_2$PO$_4$, 1.05 MgSO4, and 11 glucose. The solution was equilibrated with CO$_2$/O$_2$ gas mixture to ensure a PCO$_2$ value of 35 mm Hg at the chamber level, with the pH of CO$_2$/HCO$_3^-$-buffered solutions being 7.37±0.01 at 30°C. Chelerythrine chloride (Research Biochemicals) was used at 10 mmol/L as specific inhibitor of PKC activity[15] and calphostin C (Research Biochemicals) at 50 nmol/L.[16] Atria and all adjacent connective tissue were removed, and the remaining tissue was blotted and weighed to determine HW. The free wall of the right ventricle was excised to determine LVW separately. HW and LVW were expressed as ratios to BW to determine the degree of hypertrophy.

Measurements of pH$_i$

Measurements of pH$_i$ were made by epifluorescence as previously described.[1] Briefly, muscles were loaded with BCECF-AM (Molecular Probes). BCECF fluorescence was excited at 450 and 495 nm, and the fluorescence emission was monitored after passage through a 535±5-nm filter every 20 seconds. To limit photobleaching, a neutral-density filter (1% transmittance) was placed in the excitation light path. At the end of each experiment, fluorescence emission was calibrated by the high K$^+$-nigericin method.[17] The calibration solution contained (in mmol/L) KCl 150.0, MgCl$_2$ 1.0, CaCl$_2$ 1.0, HEPES 5.0, nigericin 0.01, sodium cyanide 4.0, and 2,3-butanedione monoxime 20.0. Buffer pH was adjusted to four different values ranging from 7.5 to 6.5. Such a calibration gave a linear relation between buffer pH values and the fluorescence ratio (F$_{495}$/F$_{450}$) as previously reported.[1] Values of autofluorescence at each wavelength were subtracted before the calculation of any F$_{495}$/F$_{450}$.

Assessment of NHE Activity

The activity of the antiporter was estimated in terms of both steady pH$_i$ values in the absence of external bicarbonate (HEPES buffer)

and J$_{H+}$ during the recovery from intracellular acidification. Acid loads were induced by switching from HEPES-buffered superfusate to CO$_2$/HCO$_3^-$ buffer. Recoveries of pH$_i$ after acid loads were analyzed by fitting the pH$_i$ versus time records to an exponential curve of the form $\Delta pH_{it}=\Delta pH_{i\infty}(1-e^{-k\Delta t})$, where ΔpH_{it} and $\Delta pH_{i\infty}$ are the changes in pH$_i$ from the initial value at time t and after steady state has been reached, respectively, and k is the rate coefficient. The rate of change of pH$_i$ at any selected pH$_i$ value was obtained by calculating the derivative of the exponential fit at that selected pH$_i$, and thus, intracellular buffer capacity×dpH$_i$/dt represents J$_{H+}$ (in mmol/L per minute) at that pH$_i$. Intracellular buffer capacity was calculated as the sum of β_i plus the buffering power due to intracellular CO$_2$/HCO$_3^-$. The latter was considered to be 2.3×[HCO$_3^-$]$_i$, assuming an open system for CO$_2$ and that its solubility and pK value are the same at either side of the cell membrane. [HCO$_3^-$]$_i$ at any given pH$_i$ was calculated from the Henderson-Hasselbach equation to be [HCO$_3^-$]$_i$=[HCO$_3^-$]$_o$×10$^{(pHi-pHo)}$. β_i was calculated as the ratio between Δ[HCO$_3^-$]$_i$/ΔpH$_i$ observed when the superfusing solution was switched from HEPES to CO$_2$/HCO$_3^-$ buffer.[18] Δ[HCO$_3^-$]$_i$ was considered to equal the value of [HCO$_3^-$]$_i$ immediately after CO$_2$/HCO$_3^-$ buffer introduction because in the absence of external CO$_2$ the value of [HCO$_3^-$]$_i$ is very low, ≈50 μmol/L.[19] The main problem for estimating β_i is that acid extrusion during the loading period may blunt the acidosis, thus leading to overestimation of β_i value (see Reference 18 for details). Back-extrapolation of pH$_i$ recovery to a point where it intersected the line defining the maximum initial rate of acid loading was used to reduce errors in β_i calculation, as previously shown.[1,20,21]

Assessment of AE Activity

The velocity of pH$_i$ recovery from imposed intracellular alkalinization was used to estimate AE activity. Exposure to TMACl (Sigma Chemical Co) has been previously demonstrated as a valid technique for investigating the activity of the AE based on the fact that no recovery from TMACl-induced intracellular alkalosis is detected in HCO$_3^-$-free solutions.[22,23] Ten-minute pulses of different TMACl concentrations (10, 20, or 30 mmol/L) were applied without osmotic compensation and pH$_i$ values recorded during the first minute after peak alkalosis were fitted to a straight line to estimate the initial velocity of pH$_i$ recovery (dpH$_i$/dt$_i$).[23]

Statistics

Data are expressed as mean±SEM. Statistical analysis of results was performed using either Student's t test or ANOVA followed by Bonferroni's test, as appropriate. Values of $P\leq.05$ were considered significant.

Results

General characteristics for the various rat groups at the time of death are shown in Table 1. SBP remained elevated at hypertensive levels in untreated SHR throughout the 5-week experimental period but significantly decreased in the SHR-E group. Enalapril treatment completely regressed cardiac hypertrophy as shown by HW/BW and LVW/BW ratios.

Steady Myocardial pH_i

Fig 1 shows steady pH_i values determined in papillary muscles superfused with HEPES-buffered medium. In the absence of bicarbonate, steady pH_i is solely controlled by NHE activity. As a reflection of increased NHE activity in hypertrophic myocardium, steady pH_i value was more alkaline in SHR-C than in WKY-C. These data confirm previous results from our laboratory[1] and are probably the result of an alkaline shift of the antiporter "set point." In the same study, we also showed that the inhibition of NHE activity with the amiloride derivative EIPA normalized myocardial pH_i value in SHR.[1] Enalapril treatment returned myocardial pH_i of SHR to values not different from those found in myocardium of normotensive rats (Fig 1). No significant changes in pH_i values were detected after the treatment of normotensive rats with enalapril.

With HEPES used as extracellular buffer, NHE is the only mechanism regulating pH_i therefore, the steady pH_i values can be directly correlated to NHE activity. However, antihypertensive treatment could have changed the ability of cells to buffer protons. No significant difference between groups was detected when β_i values were determined (Table 2). Consequently, the reduction in steady myocardial pH_i value in the SHR-E group can be interpreted as the result of normalization of NHE activity.

When hypertrophic myocardium of SHR-C was exposed to a specific inhibitor of PKC activity, chelerythrine, pH_i value in HEPES buffer gradually decreased (Fig 2). By contrast, no significant effect of PKC inhibition on myocardial pH_i was observed in WKY-C. The different effect of PKC inhibition on SHR-C and WKY-C resulted in cancellation, after 25 minutes of drug administration, of the difference between pH_i values in hypertrophic and control myocardium. The best fit of chelerythrine-induced decrease in pH_i followed an exponential function that asymptotically approached a pH_i value of 7.07 ± 0.04 (n=6). Interestingly, this value was close to the steady pH_i determined in WKY control-matched experiments (7.03 ± 0.03, n=5) and not different from the overall mean value of steady pH_i in WKY rats (7.12 ± 0.03, n=20). The time constant of chelerythrine-induced pH_i decay was found to be 12.5 ± 2.01 minutes. Essentially the same results were obtained when a structurally and mechanistically different

PKC inhibitor, calphostin C, was used. In these experiments, pH_i in hypertrophic myocardium reached values not different from WKY-C (7.10 ± 0.02, n=4) with a time constant of 16.1 ± 4.1 minute after exposure to calphostin C. Therefore, the results suggest that the hyperactivity of NHE in hypertrophic myocardium is mediated by a PKC-dependent mechanism.

Fig 3 shows steady pH_i values in myocardium from control and enalapril-treated rats in CO_2/HCO_3^- buffer. In the presence of the physiological buffer, no significant differences in pH_i values were found between SHR-C and WKY-C or between enalapril-treated rats compared with their respective strain-matched untreated controls (ANOVA). We have previously reported the lack of pH_i difference between normal and hypertrophic myocardium under bicarbonate.[1] In the same article we also demonstrated that if anion exchangers were blocked by SITS, pH_i value increased in hypertrophic myocardium bathed with bicarbonate buffer but not in WKY.[1] The present results confirm therefore that NHE hyperactivity in hypertrophic myocardium is masked by a parallel enhancement of AE activity in the presence of bicarbonate.

Imposed Intracellular Acidification

When tissues are suddenly exposed to CO_2/HCO_3^--buffered media, CO_2 easily permeates the cell membrane, causing rapid and transitory intracellular acidification. During the pH_i recovery from CO_2-induced acid load, at least 50% of proton extrusion is carried by NHE in cardiac muscle.[24,25] The changes in pH_i brought about on switching from HEPES to CO_2/HCO_3^--buffered superfusate were analyzed to appreciate the effect of enalapril treatment (Fig 4). The initial fall in pH_i was similar in all groups (Table 2), a fact consistent with the lack of difference in β_i values. However (and due to more alkaline steady pH_i values in SHR superfused with HEPES buffer), pH_i values of peak acidosis were higher in this group than in the remaining ones. For this reason, the rates of pH_i recoveries (dpH_i/dt) at a common pH_i value of 6.98 were compared. Fig 5 shows that in SHR the rate of myocardial pH_i recovery was about three times faster than in any other group and that chronic treatment with enalapril decreased its value to normal.

Acid-extruding mechanisms are modulated by pH_i; therefore, values of J_{H+} during the recovery from CO_2-induced intracellular acid load were estimated as a function of pH_i in each experimental group. Fig 6 shows that a rather linear
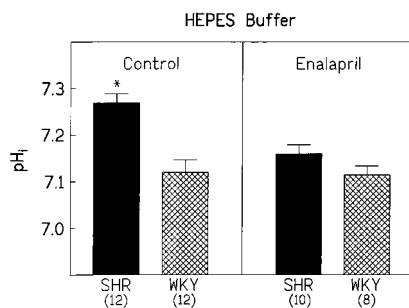

Figure 1. Myocardial steady pH_i values in HEPES buffer. Note that after enalapril treatment, myocardial pH_i in SHR was decreased to values similar to those observed in myocardium from normotensive rats. Enalapril did not change pH_i significantly in WKY. Values in parentheses indicate the number of determinations. *$P<.05$ compared with every other group (ANOVA).

TABLE 2. Cellular Buffer Capacity

	Experimental Group			
	SHR-C	SHR-E	WKY-C	WKY-E
β_i	20.7 ± 5.9	23.2 ± 5.1	22.9 ± 4.2	23.1 ± 5.2
ΔpH_i	$0.35\pm.05$	$0.35\pm.05$	$0.27\pm.06$	$0.29\pm.04$
n	6	10	6	8

Values are mean$\pm$SEM. β_i (mmol/L per pH unit) was calculated as the ratio between $\Delta[HCO_3^-]/\Delta pH_i$ observed after switching from HEPES to CO_2/HCO_3^- buffer. ΔpH_i was estimated from pH_i immediately before the switch, and the lowest pH_i value was determined by back-extrapolation, as explained in "Methods." n indicates number of determinations. No significant difference between groups was detected (ANOVA).
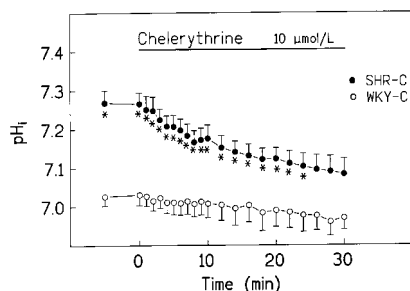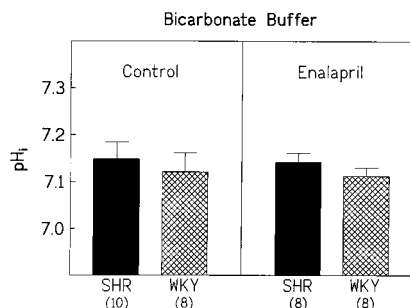
Figure 2. Effect of PKC activity inhibition on steady pH$_i$ of papillary muscles superfused with HEPES buffer. Specific inhibition of PKC by chelerythrine produced a significant decrease of pH$_i$ in SHR (n=6) without appreciable effect in WKY (n=5). *$P<.05$ (Student's t test).
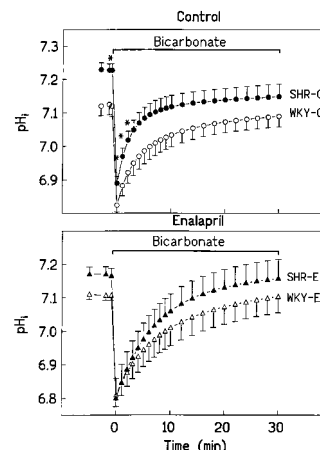

Figure 4. Transient intracellular acidification induced on switching from HEPES- to CO_2/HCO_3^--buffered superfusate. Top, Data from untreated control SHR (n=6) and WKY (n=6); bottom, data from enalapril-treated SHR (n=10) and WKY (n=8). *$P<.05$ (ANOVA).

relationship between J_{H+} and pH$_i$ was obtained in every case. However, for any given pH$_i$ value, J_{H+} values were larger in SHR compared with any other group, with the difference being larger for more acidic pH$_i$ values. The enhanced acid extrusion in SHR was reduced to values close to those seen in normotensive rats after enalapril-induced regression of cardiac hypertrophy. No significant difference was detected between WKY-C and SHR-E groups or between WKY-C and WKY-E. Thus, the enhancement of NHE activity detected after acid load in SHR was blunted by chronic treatment with enalapril. No significant difference was detected between the intercepts with the x axes among groups. Values of intercepts were 7.17±0.02, 7.14±0.02, 7.10±0.01, and 7.13±0.03 in SHR-C, SHR-E, WKY-C, and WKY-E, respectively (NS, ANOVA). The intercepts usually reflect the value of the "set point" for the NHE when the experiments are performed in the absence of bicarbonate. However, we should keep in mind that in our experiments the acid load was induced by introducing CO_2/HCO_3^- buffer. The contribution of bicarbonate-dependent mechanisms to pH$_i$ regulation takes place along with the recovery in pH$_i$.

Imposed Intracellular Alkalinization

To explore AE activity, and due to the anion exchanger's sensitivity to increases in pH$_i$, papillary muscles isolated from enalapril-treated and from untreated rat hearts were exposed to TMACl. TMACl-induced intracellular alkalinization has been previously demonstrated to be a valid technique for investigating the activity of the AE based on the fact that no
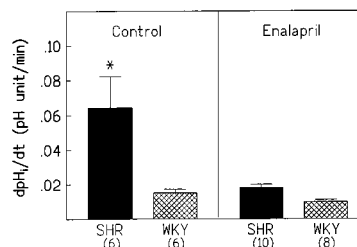
pH$_i$ recovery is detected in HCO_3^--free solutions.[22,23] Fig 7 (top) shows the results of representative experiments in which similar peak pH$_i$ values were attained during TMACl pulses carried out on papillary muscles obtained from SHR-C and SHR-E superfused with CO_2/HCO_3^- buffer. The initial velocity of pH$_i$ recovery was used as indicative of AE activity, and it was estimated from the linear fits of pH$_i$ records during the first minute after peak TMACl-induced alkalinization.[23] Using the same experimental approach, we have previously shown that recovery from alkaline loads was faster in SHR than in WKY and that lower pH$_i$ values were necessary to drive AE activity in hypertrophic myocardium.[1] It can be appreciated now that enalapril treatment reduced the velocity of pH$_i$ recovery as well as it regressed cardiac hypertrophy (Fig 7, top). Bars in Fig 7 (bottom) depict the mean initial rate of pH$_i$ recovery in the overall experiments of both groups. A significant decrease in the rate of pH$_i$ recovery was observed after chronic treatment with enalapril. The initial rate of pH$_i$ recovery in SHR-E (0.019±.005 pH unit/min, n=10) was not different from the values measured in WKY-E and WKY-C (0.024±.01, n=8, and 0.021±.009, n=10, respectively).

The rate of pH$_i$ recovery from TMACl-induced intracellular alkalinization was also measured after the inhibition of PKC activity in hypertrophic myocardium. Two structurally different PKC inhibitors, chelerythrine and calphostin C,

Figure 3. Myocardial steady pH$_i$ values in CO_2/HCO_3^- buffer. In the presence of the physiological buffer, no difference between resting pH$_i$ value in SHR compared with WKY was detected. Values in parentheses indicate the number of determinations.

Figure 5. Rate of pH$_i$ recovery from CO_2-induced intracellular acidification. Mean±SEM values of dpH$_i$/dt calculated at a common pH$_i$ value of 6.98 are shown. Values in parentheses indicate the number of determinations. *Significant difference from any other group (ANOVA).
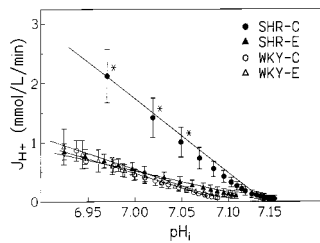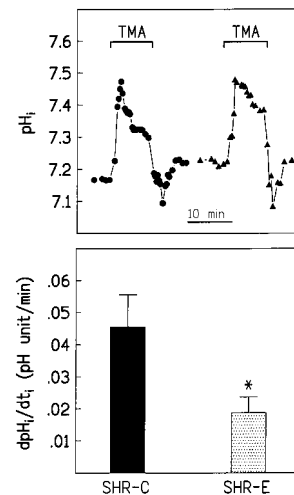
Figure 6. J_{H+} as a function of pH_i. J_{H+} was estimated to be equal to intracellular buffer capacity×dpH_i/dt. Note that the enhanced acid extrusion in SHR was reduced close to values seen in normotensive rats after enalapril-induced regression of cardiac hypertrophy. *Significant difference from any other group (ANOVA).


Figure 7. Effect of enalapril treatment on pH_i recovery from TMACl-induced intracellular alkalinization in SHR. Papillary muscles were superfused with CO_2/HCO_3^--buffered media. Top, Data from representative experiments carried out on myocardium from untreated control SHR (●) and enalapril-treated SHR (▲) exposed to 30 mmol/L TMACl. In both experiments, a similar peak pH_i value was attained, but the rate of pH_i recovery was reduced in SHR-E. Initial velocity of pH_i recovery (dpH_i/dt_i) was estimated by fitting the pH_i values recorded during the first minute after peak alkalosis to a straight line. Bottom, Bars show mean±SEM values of dpH_i/dt_i in the overall experiments (SHR-C, n=16; SHR-E, n=10). *$P<.05$ (Student's t test).

were used. Exposure to TMACl increased pH_i to a similar value in both the absence and presence of PKC inhibitors. However, the rate of pH_i recovery was significantly reduced by both PKC inhibitors (Fig 8). After PKC inhibition with either chelerythrine or calphostin C, the rate of pH_i recovery in hypertrophic myocardium was not different from the value measured in myocardium from normotensive rats at a comparable pH_i value. The results therefore suggest that enhanced AE activity in hypertrophic myocardium from SHR is mediated by a PKC-dependent mechanism(s).

Discussion

Increased NHE activity is one of the most common phenotypic differences found in hypertension. NHE is a member of a multigene family, and four NHE isoforms (NHE-1 through NHE-4) have recently been cloned.[11] The NHE-1 isoform is expressed in virtually all tissues and species; it controls cytosolic pH and may also participate in cell growth.[26] Evidence of enhanced NHE activity in hypertension is provided by observations in skeletal muscle of SHR[27] and of hypertensive patients[28]; in circulating blood cells such as platelets, leukocytes, erythrocytes[5]; and in immortalized lymphoblasts[29] derived from individuals with essential hypertension. The vascular smooth muscle from SHR also seems to exhibit enhanced antiport activity.[30] In addition, we have reported an increased NHE activity in hypertrophic myocardium of SHR.[1] However, the data presented here and our previous results show that no difference in myocardial pH_i between hypertrophic and normal myocardium can be detected in the presence of bicarbonate, despite enhanced NHE activity. This is due to the simultaneous hyperactivity of an acidifying (AE) and an alkalinizing (NHE) mechanism.

The question of whether antihypertensive therapy could affect the enhanced NHE activity was investigated before by Rosskopf et al[31] in experiments conducted on platelets of hypertensive patients. These authors were unable to detect any normalization of NHE activity after 6 weeks of antihypertensive treatment with enalapril. They claimed that NHE hyperactivity was refractory to antihypertensive treatment and therefore appeared to be a relatively fixed parameter. Whether these contradictory results are a matter of difference in tissues, species, or the relative duration of treatments is not apparent at this time and requires further study. While this manuscript was in preparation, Sánchez et al[32] reported that Na^+/Li^+ coundertransport (a mode of operation of NHE) in

erythrocytes from hypertensive individuals was normalized after 6 months of enalapril treatment.

The major finding of this study was that the enhancement of NHE and AE activities in hypertrophic myocardium of SHR normalized after PKC inhibition. The C terminal of the rat NHE-1 possesses a putative PKC phosphorylation site,[33] and many agonists promote phosphoinositide hydrolysis generating inositol triphosphate and diacylglycerol, the latter then stimulating PKC activity. In connection with this, it was demonstrated that inhibitors of PKC were able to relax aortic tone in vitro and lower blood pressure of SHR in vivo.[34] Recent experiments from our laboratory showed that PKC inhibition decreased NHE activity in platelets from SHR but not in WKY.[35] These present and previous data are consistent with a "PKC syndrome" that was suggested to play a central pathogenic role in hypertension.[36] Kimura et al[37] and Aviv et al[38,39] have also presented several lines of evidence supporting a connection between $[Ca^{2+}]_i$, PKC, and NHE in the increased peripheral vascular resistance, cardiovascular hypertrophy, salt sensitivity, and insulin resistance of established hypertension. Moreover, stimulation of cell hypertrophy and activation of PKC by stretching isolated cardiomyocytes have been described[40] and seem to be linked to autocrine-paracrine secretion of angiotensin II and/or endothelin-1.[41,42] Significantly, both of these agonists are well known to stimulate PKC activity in the myocardium.

The regulation of the AE has been less investigated than that of NHE, but evidence for PKC involvement in its regulation in Vero cells was presented by Ludt et al.[43] The amino acid sequences of the cardiac-specific AE3 isoform has been recently examined in rats and compared with that of mice and humans.[44,45] In all three species, potential consensus
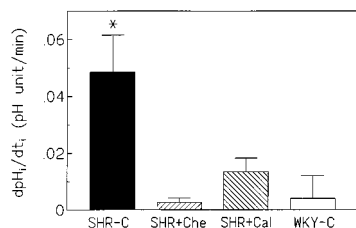
Figure 8. Effect of PKC inhibition on AE activity in SHR myocardium. Data are mean±SEM of the initial rate of pH_i recovery after TMACl-imposed alkali load on papillary muscles from hypertrophic hearts (n=5) under control conditions (SHR-C) and after inhibition of PKC activity with either chelerythrine (SHR+Che, 10 mmol/L chelerythrine, n=5) or calphostin C (SHR+Cal, 50 nmol/L, n=4). For comparison, data from untreated normotensive rats (WKY-C, n=3) are shown. Similar values of peak intracellular alkalosis during TMACl pulses were attained in each group (7.45±.03 in SHR-C, 7.45±.03 in SHR+Che, 7.42±.07 in SHR+Cal, and 7.47±.01 in WKY-C). Notice that PKC inhibition significantly decreased the rate of pH_i recovery of hypertrophic myocardium. *$P<.05$ compared with the other groups (ANOVA).

phosphorylation sites for protein kinases A and C were identified.

An increase in PKC activity would therefore explain the increased activity of both exchangers in hypertrophic myocardium. The normalization of their activities by enalapril treatment, as well as by PKC inhibition with chelerythrine and calphostin C, would suggest that the pharmacological intervention is mediated through a decrease in PKC activation. No measurements of PKC activity were performed in our study, but increases in $PKC-\beta_{1,2}$ and $PKC-\epsilon$ isozymes were detected in left ventricular hypertrophy induced by aortic banding in the rat.[46]

We would like to emphasize that in the presence of the physiological bicarbonate buffer, the steady-state pH_i value of the hypertrophic myocardium results from the interplay between the increased alkalinizing NHE activity and the enhanced acidifying activity of AE. Parallel hyperactivity of both exchangers will normalize pH_i, but it will not prevent the increase in $[Na^+]_i$ caused by NHE activity. Under these circumstances, an increase in $[Ca^{2+}]_i$ would take place through the Na^+/Ca^{2+} exchange. Increased $[Ca^{2+}]_i$ is known to be an important signal for cellular growth[47] and to activate conventional α, β, and γ PKC isoforms.[48,49] However, novel $PKC-\delta$ has been also reported to be activated after Ca^{2+} infusion, and its activation was attributed to a Ca^{2+}-dependent production of diacylglycerol through phospholipase C.[50] In addition, by stimulating both acid loading and extrusion systems in parallel, the cell's ability to recover from acid and alkaline loads is improved despite no significant change in pH_i.[51]

Another possibility to be considered is a "primary" increase in $[Ca^{2+}]_i$, which could activate NHE through different mechanisms either directly[37,52] or via PKC activation and/or a Ca^{2+}-calmodulin complex.[53] The increase in $[Ca^{2+}]_i$ in hypertension as the result of different mechanisms has been reported by several authors.[54–56]

In summary, our data show that chronic treatment with enalapril induced normalization of enhanced NHE and AE activities while regressing cardiac hypertrophy in SHR and

that its pharmacological effect was linked to a common PKC-related pathway. Whereas the link between PKC and NHE activity in hypertension is not new,[5,34–39] the participation of the AE and its link with PKC in hypertension is novel and significant. Many questions related to the data presented herein need to be addressed. For example, which does enalapril treatment change first: hypertrophy, NHE activity, or AE activity? What is the temporal relationship between structural changes and exchanger activities? Is the increase in intracellular Ca^{2+} level found in hypertension the result or the cause of the hyperactivity of the exchangers? And finally, which PKC isoform is involved in determining the hyperactivity of the exchangers? All these questions should be addressed in future investigations.

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