Molecular Basis for Angiotensin II–Induced Increase of Chloride/Bicarbonate Exchange in the Myocardium

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Abstract—Plasma membrane anion exchangers (AEs) regulate myocardial intracellular pH (pHᵢ) by Na⁺/H⁺ exchange. Angiotensin II (Ang II) activates protein kinase C (PKC) and increases anion exchange activity in the myocardium. Elevated anion exchange activity has been proposed to contribute to the development of cardiac hypertrophy. Our Northern blots showed that adult rat heart expresses AE1, AE2, AE3fl, and AE3c. Activity of each AE isoform was individually measured by following changes of pHᵢ associated with bicarbonate transport, in transfected HEK293 cells. Exposure to the PKC activator, PMA (150 nmol/L), increased the transport activity of only the AE3fl isoform by 50±11% (P<0.05, n=6), consistent with the increase observed in intact myocardium. Cotransfection of HEK293 cells with AE3fl and AT₁ -Ang II receptors conferred sensitivity of anion transport to Ang II (500 nmol/L), increasing the transport activity by 39±3% (P<0.05, n=4). PKC inhibition by chelerythrine (10 μmol/L) blocked the PMA effect. To identify the PKC-responsive site, 7 consensus PKC phosphorylation sites of AE3fl were individually mutated to alanine. Mutation of serine 67 of AE3 prevented the PMA-induced increase of anion transport activity. Inhibition of MEK1/2 by PD98059 (50 μmol/L) blocked the PMA effect. To identify the PKC-responsive site, 7 consensus PKC phosphorylation sites of AE3fl were individually mutated to alanine. Mutation of serine 67 of AE3 prevented the PMA-induced increase of anion transport activity. Inhibition of MEK1/2 by PD98059 (50 μmol/L) did not affect the response of AE3fl to Ang II, indicating that PKC directly phosphorylates AE3fl. We conclude that following Ang II stimulation of cells, PKC phosphorylates serine 67 of the AE3 cytoplasmic domain, inducing the Ang II–induced increase in anion transport observed in the hypertrophic myocardium. (Circ Res. 2001;89:1246-1253.)

Key Words: hypertrophy ■ anion exchange ■ pH regulation ■ angiotensin II ■ protein kinase C

Intracellular pH (pHᵢ) regulates excitation-contraction coupling in cardiac cells through ionic conductances, 1 metabolic pathways, 2 Ca²⁺ homeostasis, 3 contractility, 4 and electrical conduction. 5 Four well-characterized membrane ion transporters regulate cardiomyocyte pHᵢ. Acid loads activate Na⁺/H⁺ exchange (NHE) and Na⁺/HCO₃⁻ symport (NBC), 6,7 whereas after an alkaline load, Na⁺/H⁺ independent Cl⁻/HCO₃⁻ exchangers (AEs) 8 and a second dual acid-loading mechanisms 9 are activated.

Anion exchange proteins, which facilitate the reversible electroneutral exchange of Cl⁻ for HCO₃⁻ across the plasma membrane, regulate pHᵢ, intracellular chloride concentration, bicarbonate metabolism and cell volume. The AE family comprises 3 members: AE1, AE2, and AE3. 10 Heart and retina coexpress 2 different isoforms of AE3, AE3 full length (AE3fl, the most abundant AE protein expressed in the brain) and AE3 cardiac (AE3c), which result from alternative promoter usage. 11,12 Rat AE3fl is 1227 amino acids long and AE3c contains 1034 amino acids. The C-terminal 957 amino acids of both polypeptides are identical, but the AE3c protein contains a unique N-terminal sequence of 73 amino acids, which replaces the unique first 270 amino acids of the AE3fl form. 11 A truncated form of AE1 was recently characterized in rat ventricular myocytes. 13 Although 2 forms of AE2 were found at the mRNA level in cardiac cells, low levels of the AE2 protein were detected only in neonatal myocytes. 13

Myocardial anion exchange activity is subject to regulation. Ang II, which activates a number of second messenger systems, including protein kinase C (PKC), elicits a positive inotropic effect in the heart, 14 stimulates Ca²⁺ mobilization, and induces cardiac hypertrophy. 15 Ang II and endothelin-1 both increase anion exchange activity in feline ventricular myocardium through a PKC-dependent regulatory pathway. 16,17 β-Adrenergic 18 and purinergic agonists 19 stimulate cardiac anion exchange activity. A role for tyrosine kinases is suggested by the observation that tyrosine kinase inhibitors inhibit purinergic activation of anion exchange activity in cardiomyocytes. 20

Activation of NHE has been clearly implicated in development of cardiac hypertrophy, 21,22 but the link is less established for anion exchangers. NHE and AE activities increase in parallel in the myocardium of spontaneously hypertensive rats (SHR) compared with control rats. 23 Sustained hyperactivation of NHE is only possible in the presence of a parallel increase in an acidifying pathway, such as anion exchange, because sustained NHE activity will alkal-
ize the cell, which inactivates NHE through a cytosolic modifier site.24 Blockade of Ang II production by the angiotensin-converting enzyme inhibitor enalapril induced a regression of cardiac hypertrophy and reduced the activity of both NHE and AE in the same rat strain.25 In the SHR myocardium, NHE and AE activities did not rise above control levels when PKC was inhibited, suggesting that the increased ion exchange activities induced by Ang II are due to PKC-dependent mechanisms.

The goal of the present study was to identify the molecular basis for Ang II activation of anion exchange in the myocardium. We present evidence that Ang II through AT1a-Ang II receptors activates PKCε, which directly phosphorylates AE3fl at serine 67 (Ser67), inducing the Ang II–induced increase in anion transport observed in the rat hypertrophic myocardium.

Materials and Methods

Molecular Biology

Poly A mRNA was isolated from adult rat hearts using trizol reagent and poly (A`) tract mRNA isolation system (Promega). Samples were electrophoresed and blotted to Hybond N membranes.26 Blots were probed with isoform-specific probes and developed using a BASS 1000 phosphoimager and BASS III imaging plates (Fuji Medical Systems).

Anion exchanger cDNAs were cloned into the mammalian expression vector pRBG4,27 which placed them under control of a CMV promoter. Mutagenesis was performed using the megaprimer method.28

Cell Culture and Protein Expression

Neonatal rat ventricular cardiomyocytes were cultured as described previously.29 Anion exchangers were expressed by transient transfection of HEK293 cells using the calcium phosphate method, as previously described.30 Cells were grown at 37°C in an air/5% CO2 environment in complete Dulbecco’s modified Eagle’s medium (DMEM) containing penicillin/streptomycin/glutamine (Life Technologies), supplemented with 5% (v/v) fetal bovine serum and 5% (v/v) calf serum (Hyclone). Protein was quantified using the Bio-Rad Protein Assay.28

Electrophoresis and Immunoblot Analysis

Samples were resolved by SDS/PAGE on 7.5% (w/vol) acrylamide gels. Proteins were transferred to PVDF membranes (Millipore).31 Blots were probed with mouse monoclonal anti-HA epitope antibody 12 CA5 (Covance, Richmond, Calif), as described.32

Anion-Exchange-Activity Assay

HEK293 cells, grown on 6×11-mm glass poly-l-lysine–coated coverslips (Fisher Scientific Products) in 60-mm dishes, were transfected with cDNA encoding AEi. Cells were grown in complete DMEM without serum for 14 to 16 hours before the experiment. Two days after transfection, coverslips were incubated in serum-free DMEM containing 2 μmol/L BCECF-AM (Molecular Probes) at 37°C for 20 minutes. Coverslips were mounted in a fluorescence cuvette and perfused at 3.5 mL/min alternately with Ringer’s buffer (5 mmol/L glucose, 5 mmol/L potassium gluconate, 1 mmol/L calcium gluconate, 1 mmol/L MgSO4, 2.5 mmol/L NaH2PO4, 25 mmol/L NaHCO3, 10 mmol/L Hapes, pH 7.40) containing either 140 mmol/L NaCl (Cl– containing) or 140 mmol/L sodium gluconate (Cl– free). Both buffers were continuously bubbled with air/5% CO2.

Fluorescence changes were monitored in a Photon Technologies International RCR fluorimeter at excitation wavelengths 440 and 502 nm and emission wavelength 528 nm. All transport data were corrected for background activity of HEK293 cells transfected with pRBG4 vector alone. The intrinsic buffer capacity (β) was negligible at pHi values above 7.10,30 so that βtotal=βCO2, where 

\[ β_{CO2} = 2.3 \times [HCO_3^-] \]

Total flux of proton equivalents was calculated as

\[ J_{H^+} = \beta \text{total} \times \Delta pH_i \]

The online data supplement available at http://www.circresaha.org.

Figure 1. Effect of Ang II on anion exchange activity in rat neonatal cardiomyocytes and expression profile of AEs in the adult rat myocardium. A, Rat neonatal cardiomyocytes were cultured for 72 hours. Anion exchange assays were performed on these cells before and 20 minutes after exposure to Ang II (500 nmol/L). Initial rates of change of pH during the first minute were estimated from the slope of the line fitted by least squares method (dashed line). B, Northern blots of rat heart tissue. Poly (A`) mRNA from adult rat hearts (3 μg per lane) was electrophoresed, blotted onto Hybond N membranes, and probed for AE1 (lane 1), AE2 (lane 2), and AE3 (lanes 3 and 4). Blots were imaged together for 24 hours on an imaging plate using a Fuji Medical Systems phosphoimager. Lanes 1 to 3 were imaged under identical conditions to emphasize the relative expression levels of transcripts. Arrows indicate identified bands. Lane 4 is the same sample as lane 3 but underexposed to emphasize the presence of 2 separate bands.

Statistics

Data are expressed as mean±SEM. Statistical analysis was performed by Student paired t test or one-way ANOVA, as appropriate. Probability of null hypothesis of P<0.05 was considered significant.

An expanded Materials and Methods section can be found in the online data supplement available at http://www.circresaha.org.

Results

Changes in anion exchange activity on Ang II stimulation have been observed in ventricular myocardium.16,24 When cultured cardiomyocytes were subjected to the removal of extracellular Cl–, pHi rose due to the influx of HCO3–, mediated by AE (Figure 1A). After Cl– readdition, pHi reached steady state values due to anion exchange activity, as described previously.29 In all anion exchange assays, transport rates have been expressed as the average of absolute rates of alkalization and acidification.8,30 Treatment of cultured
myocytes with Ang II (500 nmol/L) increased the initial rate of pH change in anion exchange assays (6.1±1.3 mmol/L · min⁻¹ and 8.0±1.4 mmol/L · min⁻¹ before and after treatment with Ang II respectively) by 34%, without change of steady-state pH.

Northern Blot Analysis of Adult Rat Heart
To examine which AE isoform could be responsible for the effect of Ang II on anion exchange activity, Northern blots of normal Sprague-Dawley adult rat heart were probed with AE isoform-specific probes. Lane 1 shows three different mRNA species present when probed for AE1. AE1 mRNA has previously been shown to be alternatively spliced, generating three alternative transcripts.13 Blots probed with a probe specific for the 5' end of the AE1 transcript found in erythrocytes indicated that the major transcript (top band) corresponds to the erythroid isoform, while the lower bands are truncated at the N-terminus (data not shown). Lane 2 shows the presence of a single AE2 species in adult rat heart. Two different species of AE3 were detected, which have previously been identified as AE3 full length (AE3fl, upper band) and AE3 cardiac (AE3c, lower band).34 This identification was confirmed with a probe specific for the cardiac isoform (data not shown). Because different probes, which differed slightly in their specific activity, were used for each AE isoform, accurate comparisons of AE expression levels are not possible. However, the relative mRNA levels were estimated as AE3c>AE3fl>AE2>AE1.

Identification of the PKC-Sensitive AE Isoform
Ang II stimulates anion exchange in myocardial preparations (Figure 1A).16,17 However, the isoform responsible is unclear because Northern blots (Figure 1B) indicated that multiple AE isoforms are expressed in heart tissue. To identify which isoform is Ang II–sensitive, AE1, AE2, and AE3 were individually expressed in HEK293 cells. HEK293 cells have been extensively used to study AE because they do not express measurable levels of endogenous AE.30 Because Ang II activates PKC,35 we examined the effect on anion exchange activity of the PKC activator, PMA. To optimize experiments with PMA, a dose-response curve of the phorbol 12-myristate 13-acetate esters (PMA) effect on anion exchange for AE3fl was prepared (Figure 2A). On the basis of this data, experiments were performed at 150 nmol/L PMA. Figure 2B shows HEK293 cells subjected to the Cl⁻ removal-readdition protocol. Treatment of AE3fl with PMA increased the initial rate of both alkalization and acidification in anion exchange assays (2.9±0.3 mmol/L · min⁻¹ in the absence of PMA and 4.5±0.5 mmol/L · min⁻¹ following treatment with PMA); baseline pH did not change following PMA treatment. In other experiments (not shown), 2 successive transport assays, under control conditions, did not differ from each other (2.4±0.2 and 2.2±0.2 mmol/L · min⁻¹, n=3). The transport activity measured for AE1 and AE2 was not affected by PMA treatment, as shown in Figure 2C. PMA (150 nmol/L) had no effect on background activity of sham-transfected cells (not shown). We conclude that AE3 is the PKC-sensitive AE of the heart.

PMA stimulates sarcolemmal NHE of isolated ventricular myocytes.36 To rule out an effect of NHE in the observed PMA-induced increase of AE3fl activity, anion exchange assays were repeated under conditions where NHE was fully blocked (amiloride, 1 mmol/L). In the presence of amiloride, steady-state pH decreased slightly after PMA addition (7.29±0.03 and 7.23±0.01 before and after PMA, respectively, n=3), but NHE blockade did not affect the PMA-induced increase of AE3fl activity, 2.6±0.1 and 3.8±0.2 mmol/L · min⁻¹ before and after PMA, respectively (48±3% of increase, n=3, P<0.05).

Transport experiments were performed in the presence of the PKC inhibitor, chelerythrine (CHE, 10 μmol/L). Figure 3A shows that no increase of AE3fl activity was observed on treatment with PMA if cells were also treated with CHE. Figure 3B shows that CHE treatment abolished the PMA stimulation of AE3fl activity. This indicates that the increase of AE3fl activity on treatment with PMA is mediated through PKC.

Because the heart expresses both AE3c and AE3fl isoforms, we also examined the effect of PMA on AE3c.
Surprisingly, PMA treatment reduced AE3c activity (22±7% decrease in transport following PMA) (Figure 4A). This contrasts sharply with the increase of transport activity by AE3fl following PMA treatment (Figure 2C). The negative regulation of AE3c by PMA was also blocked by CHE, indicating that the effect is PKC-dependent. AE3c mRNA, which contains an N-terminus distinct from that of AE3fl (Figure 4B), results from alternative promoter usage of a single gene.37 To identify the protein region responsible for the difference in response to PMA, we examined the properties of AE3 truncated (AE3tr). AE3tr, which does not occur naturally, corresponds to the amino acid sequence common to AE3fl and AE3c. Figure 4A shows that AE3tr activity is inhibited by PMA similarly to AE3c. Taken together these data suggest that the region shared by AE3c and AE3fl mediates negative regulation of anion exchange by PKC, while the unique region of AE3fl mediates positive regulation by PKC.

Identification of the Phosphorylation Sites Responsible for PKC Activation of AE3fl
PKC mediates its effects through phosphorylation of known consensus sequences (S/TXK/R, K/RXXS/T, or K/RXS/T).38 The common region of AE3 and the unique region of AE3fl contain 6 and 7 consensus PKC phosphorylation sites, respectively (Figure 4B). The enhanced AE3fl activity after PMA treatment and decreased AE3c and AE3tr activity after PMA treatment implicates one or more PKC-phosphorylation sites in the unique AE3fl region in the activation of AE3fl by PMA.

Each serine or threonine residue of the 7 consensus PKC sites in the unique region of AE3fl was replaced by alanine by mutagenesis of the cDNA. To facilitate the detection of these proteins, a hemagglutinin epitope tag (HA tag, amino acid sequence YPYDVPDYA) was introduced at the extreme cytoplasmic C-terminus of AE3fl and phosphorylation site mutants. Figure 5A shows the level of expression of AE3fl mutants in transfected HEK293 cells. There was no significant difference in the expression level of AE3fl and its mutants on the basis of quantification of expression levels (bottom, Figure 5A).

Basal activity of AE3fl phosphorylation site mutants was indistinguishable from AE3fl (Figure 5B). Similarly, there was no significant difference in transport rates between AE3fl and the HA-tagged version; the 2 AE3fl proteins also had the same response to PMA (Figure 5C). Addition of the HA tag therefore did not affect the protein for purposes of the present study.

The activity of each AE3fl phosphorylation site mutant was measured before and after PMA treatment. Figure 5C shows that all mutants, except S67A, had the same increase in transport activity following PMA treatment. This suggests that PMA treatment resulting in phosphorylation of Ser67 of

![Figure 4. Effect of PMA and chelerythrine on transport activity of AE3c and AE3tr.](http://circres.ahajournals.org/)

A. Representative experiment in which HEK293 cells transfected with AE3fl were treated with PMA (150 nmol/L) in the presence of chelerythrine (10 μmol/L). B. Mean values of relative anion exchange activity following PMA treatment in the presence (n=3) or absence (n=5) of chelerythrine. *P<0.05.
AE3fl mediates the PMA-induced increase of AE3fl transport activity. The decreased transport activity elicited by S67A mutant after PMA treatment reinforces the hypothesis of a negative regulatory site(s) present in the AE3c/AE3fl common region (Figure 4B).

Regulation of Anion Exchange by Angiotensin II

We were interested in the regulation of myocardial anion exchangers by PKC because the hypertrophic hormone, Ang II, has been shown to elevate anion exchange activity in the myocardium and Ang II works, in part, by activation of PKC.16 To determine whether Ang II could regulate anion exchange, HEK293 cells transfected with AE cDNA were treated with Ang II. To assay the Ang II effect in HEK293 cells, an Ang II concentration of 500 nmol/L was used on the basis of studies that showed this was sufficient to stimulate the Na\(^{+}\)/H\(^{+}\) exchange activity in AT1 murine atrial tumor cell line,39 to produce the maximal rise in pH, in feline papillary muscles,16 and to elicit the maximal positive inotropic effect in cardiomyocytes.14 Neither 500 nmol/L nor 10 μmol/L of Ang II, a concentration that is higher than reported values in heart extracellular fluids,40 significantly altered the relative anion exchange rates of HEK293 cells transfected with AE3fl (Figure 6A). The low response to Ang II may be explained by the low expression of the AT1, Ang II receptor (AT1aR) in HEK293 cells.41 Consistent with this interpretation, when HEK293 cells were cotransfected with AE3fl and rat AT1aR cDNAs, both 500 nmol/L and 10 μmol/L of Ang II elicited increased anion exchange activity (Figure 6A). Other experiments (not shown) showed that maximum response to Ang II was observed within 10 minutes of treatment and that the same level of activation was found 20 minutes after Ang II treatment. The PKC inhibitor CHE (10 μmol/L) blocked

Figure 6. Effect of Ang II on anion exchange activity. HEK293 cells were transfected with AE3fl cDNA, with or without cotransfection with AT1aR cDNA as indicated at the bottom of the figure. A, Anion exchange rates were measured before and after cells were treated with Ang II for 20 minutes (n=3). B, Transport activity of AE3fl proteins. No significant difference was measured between rates (one-way ANOVA). C, Relative anion exchange activity of HEK293 cells transfected with AE3fl cDNAs following treatment with 150 nmol/L of PMA (n=3).
responsiveness of AE3fl to Ang II treatment in cells expressing AT1aR (Figure 6B). This result confirms that Ang II activates AE3fl by a PKC-dependent regulatory pathway linked to the AT1aR. AE3c activity decreased in response to 500 nmol/L Ang II (Figure 6B), consistent with the effect of PMA on AE3c activity. CHE blocked the Ang II–induced decrease of AE3c activity. The response of HEK293 cells cotransfected with AE3fl, S67A, or S207A mutants and AT1aR to treatment with 500 nmol/L Ang II (Figure 6C) was similar to PMA treatment (Figure 5C). Taken together, Ang II increases the activity of AE3fl in a manner that is dependent on Ser67 and PKC.

Isoform selective inhibitors were used to identify the PKC isoform that modulates AE3fl activity. Activation of AE3fl by Ang II was not affected by Rottlerin at a concentration that is selective for PKCδ, but the PKCε-specific inhibitor, Myr-PKCε V1-2 blocked the Ang II effect (Figure 7). We conclude that Ang II stimulates AE3fl through activation of PKCε.

However, AE3fl Ser67 may not be a direct target of PKC because PKC can also activate the mitogen-activated protein (MAP) kinase cascade through MEK1/2. To determine whether the effect of Ang II on AE3fl activity is mediated through kinases of the MAP kinase pathway, HEK 293 cells over-expressing AT1aR were treated with either the PKC inhibitor Rottlerin (10 μmol/L) or the Myr-PKCε V1-2 (6 μmol/L) for 20 minutes. Subsequently transfected cells were treated with Ang II (500 nmol/L, 10 minutes). Anion exchange rates were measured before and after this protocol, and data represent the rate after the protocol compared with the rate before. *P<0.05.

**Discussion**

Angiotensin II has profound effects on the heart, including a positive inotrophic effect and induction of hypertrophy. The mechanism by which Ang II induces hypertrophy is not established, but coactivation of AE and NHE may contribute. In this study, we have examined the mechanism of regulation of myocardial anion exchange by Ang II. We found that Ang II activated anion exchange in cultured cardiomyocytes. The only Ang II–responsive AE isoform was AE3fl. Analysis of phosphorylation site mutants showed that Ser67 of AE3fl alone is responsible for the Ang II–induced stimulation of AE3fl. Because the stimulation of AE3fl activity was insensitive to the MEK1/2 inhibitor, PD98059, but sensitive to the PKC inhibitors, CHE, and the selective PKCε inhibitor, Myr-PKCε V1-2 peptide, we conclude that Ang II stimulates myocardial anion exchange activity by activation of PKCε, which phosphorylates Ser67 in the cytoplasmic domain of AE3fl. The mechanism of transport stimulation by phosphorylation of Ser67 is not obvious because the site is distant from the membrane transport domain of AE3. However, the regulatory phosphorylation sites of NHE1 are similarly remote from the membrane domain, near the protein’s cytoplasmic C-terminus.

In isolated rat neonatal cardiomyocytes, Ang II stimulated anion exchange by 34%, representing the total effect of Ang II on all sensitive AE isoforms. Northern blots showed that the relative expression level of AE isoforms is AE3c>AE3fl>AE2>AE1. Our data showed that Ang II stimulates AE3fl by 39% and suppresses AE3c activity by 15%. This suggests that in cardiomyocytes AE3fl activity predominates, in spite of the higher mRNA level of AE3c. Interestingly, in the myocardium of SHR the expression of AE3fl mRNA was elevated, AE3c expression was reduced, and expression of other AE isoforms was unchanged. In addition, the myocardium of SHR is marked by elevated anion exchange and hypertrophy, both of which are reversed by inhibition of angiotensin-converting enzyme (ACE). A link between elevation of anion exchange and hypertrophy is therefore apparent.

We have shown that Ang II simulates AE3fl activity. Ang II activates PKC-dependent and PKC-independent kinase pathways. That PKC directly stimulates AE3fl is supported by our observations that Ang II stimulation of AE3fl could be blocked by (1) inhibition of PKC and (2) mutation of a single consensus PKC phosphorylation site (Ser67) on AE3fl. Our data showed that Rottlerin, which inhibits PKCδ but not PKCε, did not affect Ang II–stimulation of AE3fl. Conversely, the PKCε selective inhibitor, Myr-PKCε V1-2, blocked Ang II stimulation of AE3fl. We conclude that PKCε mediates the Ang II effect on AE3fl. PKCε, the major isoform of the myocardium, has been proposed as responsible for induction of hypertrophy.

How does Ang II activation of AE3fl relate to development of cardiac hypertrophy? Increased NHE and AE activities in hypertrophic myocardium of SHR were reduced to control levels after PKC inhibition. A link between hypertrophy and Ang II activation of these transporters is suggested by reversal of hypertrophy, following inhibition of Ang II signaling by blockade of ACE or AT1 receptors. The importance of NHE in cardiac hypertrophy is underscored by the reduction in hypertrophy induced by NHE inhibition. Importantly, the activity of NHE and AE is increased in the...
pHi but will result in net cell loading with NaCl. Elevated [Na+] in animals to 12 mmol/L in hypertrophic animals has been shown to activate PKC, releasing diacylglycerol, activating PKC and inhibiting the plasma membrane Na+/H+ exchanger (NCX). The resulting elevation of [Ca2+]i could be the hypertrophic signal.

The hypertrophic myocardium of SHR, but no difference in the pH, between hypertrophic and normal myocardium was detected. This is likely due to the parallel activation of chloride/bicarbonate and sodium/proton exchangers, which results in no net change of pH, but will result in net cell loading with NaCl. Elevated [Na+]i will activate PKC and inhibit the plasma membrane Na+/Ca2+ exchanger (NCX). The resulting elevation of [Ca2+]i could be the hypertrophic signal.

In the present study, we established the molecular mechanism by which Ang II stimulates cardiac chloride/bicarbonate exchange activity as follows: Ang II through AT1R activates phospholipase C, releasing diacylglycerol, activating PKC, which activates AE3fl by phosphorylation of Ser67. We demonstrate that phosphorylation of Ser67 of AE3fl is an early event following Ang II stimulation of cardiomyocytes. Increased myocardial acid loading by AE3fl sets the stage for the hypertrophic effects of a hyperactive NHE. PKCε activation of myocardial anion exchange through Ser67 of AE3fl likely contributes to Ang II–stimulated hypertropy, but to what extent?

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