Regulation of atrial contraction by PKA and PKC during development and regression of eccentric cardiac hypertrophy

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Haddad, Georges E., Bernell R. Coleman, Aiqiu Zhao, and Krista N. Blackwell. Regulation of atrial contraction by PKA and PKC during development and regression of eccentric cardiac hypertrophy. Am J Physiol Heart Circ Physiol 288: H695–H704, 2005. First published October 14, 2004; doi:10.1152/ajpheart.00783.2004.—ANG II plays a major role in development of cardiac hypertrophy through its AT1 receptor subtype, whereas angiotensin-converting enzyme (ACE) inhibitors are effective in reversing effects of ANG II on the heart. The objective of this study was to investigate the role of PKA and PKC in the contractile response of atrial tissue during development and ACE inhibitor-induced regression of eccentric hypertrophy induced by aortocaval shunt. At 1 wk after surgery, sham and shunt rats were divided into captopril-treated and untreated groups for 2 wk. Then isometric contraction was assessed by electrical stimulation of isolated rat left atrial preparations superfused with Tyrode solution in the presence or absence of specific inhibitors KT-5720 (for PKA) and Ro-32-0432 (for PKC) and high Ca2++. Peak tension developed was greater in shunt than in sham hearts. However, when expressed relative to tissue mass, hypertrophied muscle showed weaker contraction than muscle from sham rats. In shunt rats, peak tension developed was more affected by PKC than by PKA inhibition, whereas this differential effect was reduced in the hypertrophied heart. Treatment of shunt rats with captopril regressed left atrial hypertrophy by 67% and restored PKC-PKA differential responsiveness toward sham levels. In the hypertrophied left atria, there was an increase in the velocity of contraction and relaxation that was not evident when expressed in specific relative terms. Treatment with ACE inhibitor increased the specific velocity of contraction, as well as its PKC sensitivity, in shunt rats. We conclude that ACE inhibition during eccentric cardiac hypertrophy produces a negative trophic and a positive inotropic effect, mainly through a PKC-dependent mechanism.

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CARDIAC HYPERTROPHY is an adaptive process that enables the heart to meet excessive workload, whether pressure or volume overload. This hypertrophic response is mediated by hemodynamic, as well as humoral, factors that may play a synergistic role in modifying the cardiac contraction. Pressure and volume overload-induced cardiac hypertrophy may be related to such disease states as hypertension and valvular insufficiency, respectively. Eccentric hypertrophy is induced by volume overload and is mainly associated with diastolic hemodynamic changes, whereas systolic parameters are usually unchanged (15). In addition, during eccentric hypertrophy, there is mainly an elevation in local insulin-like growth factor I, with moderate increases in ANG II and no changes in endothelin-1 (15, 38). These growth factors, frequently associated with cardiac hypertrophy, possess inotropic properties and have been shown to modulate the contractile activity of the heart, irrespective of their hypertrophic effects. The pathological role of ANG II during eccentric cardiac hypertrophy has been determined through reversal of the associated hemodynamic changes by an AT1 receptor antagonist (37). Moreover, it seems that local ANG II plays a more important role than circulating ANG II in development of cardiac hypertrophy (20). Aortocaval shunt has been shown to produce substantial volume overload and to increase absolute and relative heart weights, with the greatest effect being in the atria (3, 17). These changes are paralleled by a corresponding elevation in the levels of cardiac angiotensin-converting enzyme (ACE) mRNA (25), atrial AT1 mRNA expression (3), and atrial collagen I and II levels (23). ACE inhibitors have proven to be potent and effective agents in preventing and regressing the cardiac hypertrophy during volume and pressure overload (14, 17, 21, 40).

On the other hand, AT1 receptor subtype is positively coupled to phospholipase C and negatively coupled to adenylate cyclase. Early during development of eccentric cardiac hypertrophy, PKA activity was elevated and correlated with the force-generating capacity of the heart (24), which is greatly influenced by intracellular Ca2+ homeostasis. Recently, it was reported that ANG II augmented cAMP formation by Ca2+-dependent activation of adenylate cyclase (28). On the other hand, binding of ANG II to AT1 receptors has been shown to activate the Ca2+-dependent PKC (for review see Ref. 39). It has been demonstrated that ANG II induces an increase in inositol phosphate production that was mediated through tyrosine phosphorylation (13). Accordingly, the expression of PKC-α mRNA was elevated with cardiac hypertrophy and suppressed after regression of cardiac hypertrophy with ACE inhibitor treatment (22). The PKA and PKC signaling pathways are Ca2+-dependent and have been shown to regulate Ca2+ homeostasis in the heart (16, 31, 35, 44), which would affect myocardial contractility.

Knowing that during eccentric cardiac hypertrophy the atria play an important role in ventricular filling and the renin-angiotensin system affects myocardial contractility through ANG II-dependent signaling, we hypothesize that PKA and PKC play a crucial role in defining the force of contraction of the atrium. Thus the present study was designed specifically to investigate the role of PKA and PKC in the contractile response of atrial tissue during the development and the ACE inhibitor-induced regression of eccentric hypertrophy.

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MATERIALS AND METHODS

Experimental Animals and Groups

All protocols and animal care were approved and performed according to the Institutional Animal Care and Use Committee at Howard University. Adult male Sprague-Dawley Rats (300 ± 85 g body wt; Charles Rivers Breeding Laboratories, Wilmington, MA) were housed in a temperature-controlled room with a 12:12-h light-dark cycle and fed a standard chow diet throughout their stay in the Animal Section of the College of Medicine.

Animals were separated into two groups: sham-operated rats [control (sham) group] and aorticaval shunt-induced eccentric hypertrophy rats [experimental (shunt) group]. At 1 wk after the surgery, each group was divided into captopril-treated and untreated groups to allow the development of eccentric cardiac hypertrophy. Captopril was administered to the treated groups in the drinking water at 500 mg/liter. The sham and shunt rats drank similar amounts of water during the 2-wk treatment period: 38.73 ± 5.31 ml/day and 5.31 ± 5.96 ml/day, respectively. With 500 mg/ml captopril, this breaks down to captopril intake of 19.80 and 19.36 mg/day, respectively.

Surgical Procedure

The aorticaval shunt was produced by the method described by Garcia and Diebold (12). Briefly, rats were anesthetized with pento-barbital sodium (50 mg/kg ip), and a midline incision was made to expose the abdominal aorta and vena cava. A bulldog vascular clamp was placed across the aorta, proximal to the area of the puncture. The aorta was punctured with a disposable 18-gauge needle, and the common wall between the aorta and inferior vena cava was grasped through the incision, creating a fistula between the two vessels below the renal arteries. The needle was withdrawn and sealed with a drop of cyanoacrylate glue. The abdominal incision was sutured with Biosyn monofilament synthetic absorbable sutures. The shunts were produced under sterile surgical conditions. Sham-operated rats, serving as the control, underwent the same surgical procedure, except the aorta and inferior vena cava were not punctured. Immediately after the procedure, the animals were given 1 ml of sterile saline to prevent dehydration from any blood loss and allowed to recover from surgery in warmed conditions.

Hemodynamic Measurements

Blood pressure measurements were recorded to rule out the development of hypertension and possible development of high-pressure concentric hypertrophy as well as the effects of captopril in the treated groups. Systolic blood pressure was measured by the tail-cuff method without anesthesia. Animals were placed in a specialized rat restrainer, and blood pressure was measured using a rat tail blood pressure system (model RTBP1001, Kent Scientific, Litchfield, CT) and recorded by a digital oscilloscope (model 310, Nicolet) for subsequent analysis. Blood pressures and body weights were recorded in sham and shunt rats 1, 5, 9, 13, 17, and 21 days after the surgery.

At the end of all experiments, wet weights of the heart chambers and total heart weight were recorded to assess the level of cardiac hypertrophy by measuring the heart weight-to-body weight ratio for the sham and shunt groups.

Multicellular Preparation Setup and Experimental Procedure

Tissue isolation and recording. Animals were killed by decapitation under pentobarbital sodium sedation (50 mg/kg ip). The left atrial muscle was isolated and mounted vertically in a Radnoti tissue organ bath system, with one end attached to the organ bearer with field stimulation electrodes and the other end attached to an isometric force transducer (model FTO3, Grass, Quincy, MA). The transducer was mounted on a micromanipulator, so that fine steps to adjust the resting tension could elongate the muscle. All experiments were carried out at 1 g of resting tension. The force transducer was connected to a amplifier (model 7P1G, Grass) and a data-recording system (model 79E, Grass) to record the tension developed. The signal was fed simultaneously into a differentiator amplifier (model 7P20C, Grass) to record the first derivatives of the active tension developed. The two signals were fed into a digital oscilloscope (model 310, Nicolet) and stored on disk for analysis.

Preparations were stimulated via electrical field stimulation at a basic frequency of 0.75 Hz at 1.5 times threshold voltage and stimulus duration of 3.5 ms. At this frequency, preparations can be studied for long periods (3–4 h) without noticeable deterioration.

Multicellular contraction studies. The isolated preparation was superfused in the tissue bath with Tyrode solution (in mM: 137 NaCl, 5.4 KCl, 2.7 CaCl2, 1.5 MgCl2, 5.5 glucose) at 5 ml/min. The superfusate was continuously aerated with 95% O2-5% CO2, and pH was adjusted to 7.4 ± 0.03 with NaOH or HCl. Temperature was kept constant at 31 ± 0.5°C in the tissue bath by means of a thermostatically controlled water jacket surrounding the tissue bath.

At the start of the experimental procedure, the muscular preparations were left to equilibrate to a steady state for 60 min or until the resting tension remained constant. At the end of the equilibration period, control contractions were recorded. The superfusate was then changed sequentially to Tyrode solution supplemented with PKA inhibitor (KT-5720, 10−7 M), high Ca2+ (5.14 mM), normal Tyrode solution, PKC inhibitor (Ro-32-0432, 10−7 M), high Ca2+ (5.14 mM), and then normal Tyrode solution. The peak tension developed (PTD) and first derivatives of contraction and relaxation were recorded after 5, 10, and 15 min in each solution.

KT-5720 (Calbiochem, San Francisco, CA) is a potent specific cell-permeable inhibitor of PKA. It does not significantly affect the activity of other protein kinases, especially PKC, PKG, and myosin light chain kinase. Therefore, KT-5720 was chosen to study the effect of PKA inhibition in cardiac tissue. Ro-32-0432 (Calbiochem) is a selective cell-permeable PKC inhibitor. It is even highly selective for the Ca2+-dependent PKC isoforms (e.g., PKC-α and PKC-β) over the Ca2+-independent PKC isoforms (e.g., PKC-δ and PKC-ε). The PKC isoforms inhibited by Ro-32-0432 have been implicated in various models of cardiac hypertrophy, with the Ca2+-dependent isoforms showing a greater association with contractile function of the heart.
Thus this PKC inhibitor seems to be the best choice for determining PKC alterations of inotropic activity during cardiac hypertrophy.

**Statistical Methods**

Values are means ± SE. All statistical analysis was performed using SigmaStat software and verified using Microsoft Excel and Prism software, which gave the same result. Paired Student’s t-test was used to compare data before and after drug treatment of the same animal group. Because the inhibitors’ effects were not tested in a cumulative manner but, rather, an equilibrium control period (Tyrode) preceded the addition of each inhibitor, one-way ANOVA was used for significance when the effects of one drug were compared in two different animal groups among the four groups of animals: sham (control), shunt (hypertrophy), sham + captopril (experimental control), and shunt + captopril (regression). Furthermore, there were no statistical differences between the equilibrium periods tested in one protocol. Statistical significance was assumed for $P \leq 0.05$.

**RESULTS**

**Physiological Parameters Before and After ACE Inhibitor Treatment**

Figure 1 shows the blood pressure profile of sham and shunt groups during the 3 wk after surgery in the presence and absence of the ACE inhibitor captopril. At 3 wk after surgery, there was no significant difference in the blood pressure profile between the untreated and treated sham (106.0 ± 2.5 and 101.8 ± 3.1 mmHg, respectively) and shunt (109.2 ± 1.1 and 110.0 ± 4.3 mmHg, respectively) groups. Table 1 summarizes
the level of eccentric cardiac hypertrophy and its regression by captopril within 3 wk after surgery by measures of tissue weight relative to body weight. The body weight did not differ in all groups, whereas the absolute wet weights of the whole heart \((1.08 \pm 0.04 \text{ vs. } 1.43 \pm 0.04 \text{ g})\), left atria \((52 \pm 2 \text{ vs. } 98 \pm 4 \text{ mg})\), left ventricles \((684 \pm 22 \text{ vs. } 919 \pm 25 \text{ mg})\), and right ventricles \((146 \pm 14 \text{ vs. } 291 \pm 26 \text{ mg})\) were significantly lower in sham than in shunt rats. Thus, as shown in Table 1, this represents an increase of 34, 104, 90, 36, and 59% in the respective relative weights of the whole heart, left atria, right atria, left ventricles, and right ventricles.

Treatment with captopril significantly reduced the increase in wet weight of the whole heart \((1.10 \pm 0.07 \text{ g})\), left atria \((22 \pm 5 \text{ g})\), right atria \((66 \pm 5 \text{ g})\), left ventricles \((772 \pm 18 \text{ g})\), and right ventricles \((207 \pm 18 \text{ g})\). As shown in Table 1, this represents a significant decrease of the relative weights to 7, 20, 32, 18, and 47% of the respective sham values.

**Left Atrial Contraction During Development of Eccentric Hypertrophy**

Modulation of left atrial PTD of sham and shunt rats by PKA, PKC, and high \(\text{Ca}^{2+}\) is presented in Fig. 2. In the left atrium of sham-operated rats, peak tension induced by electrical stimulation (Fig. 2A) was reduced from \(267.93 \pm 16.87 \text{ mg} \) to \(243.83 \pm 17.01 \text{ mg} \) \((P < 0.01)\) by the PKA inhibitor KT-5720 \((10^{-7} \text{ M})\). Increasing the extracellular \(\text{Ca}^{2+}\) concentration to \(5.14 \text{ mM}\) significantly augmented PTD to \(408.48 \pm 49.16 \text{ mg} \) \((P < 0.01)\). On the other hand, addition of the PKC inhibitor Ro-32-0432 \((10^{-7} \text{ M})\) reduced the peak tension to \(198.63 \pm 18.45 \text{ mg} \) \((P < 0.01)\), which was elevated to \(367.35 \pm 53.46 \text{ mg} \) \((P < 0.01)\) by high extracellular \(\text{Ca}^{2+}\) solution.

The left atrial PTD of shunt rats was reduced from \(357.95 \pm 31.85 \text{ mg} \) to \(332.31 \pm 36.21 \text{ mg} \) \((P < 0.05)\) by KT-5720 \((10^{-7} \text{ M})\). Elevated extracellular \(\text{Ca}^{2+}\) concentration increased PTD to \(524.24 \pm 32.10 \text{ mg} \) \((P < 0.01)\). Inhibition of PKC in shunt rats lowered the PTD from \(353.64 \pm 32.53 \text{ mg} \) to \(313.10 \pm 38.38 \text{ mg} \) \((P < 0.01)\), which was increased to \(476.48 \pm 34.04 \text{ mg} \) \((P < 0.01)\) by \(5.14 \text{ mM} \text{Ca}^{2+}\).

Relative left atrial weight is a measure of the level of left atrial hypertrophy. During cardiac hypertrophy, muscular dysfunction at the unit level may be mitigated by increased muscle mass, whereby normalization would definitely unveil its proper functional state. Thus, expressing the PTD in relation to the relative left atrial weight is a measurement of the specific PTD value, which is independent of the degree of tissue mass. In
that sense, specific PTD is an interesting approach when isotropy of shunt and sham rats is compared. Accordingly, in control conditions (Fig. 2B), the PTD was higher ($P < 0.05$), whereas the specific PTD was lower (38.96 ± 2.75 vs. 49.56 ± 3.84 mg/relative left atrial wt, $P < 0.05$) in shunt than in sham rats. High extracellular Ca\(^{2+}\) similarly increased PTD in sham and shunt rats (51.08 ± 0.13 and 49.24 ± 0.08%, respectively), with a tendency for higher specific PTD in shunt than in shunt atrial muscle that did not reach statistical significance (58.02 ± 4.84 vs. 77.05 ± 12.12 mg/relative left atrial wt, respectively).

Figure 2C shows the percent change in PTD of the left atrium due to PKA inhibitor (10\(^{-7}\) M) and PKC inhibitor (10\(^{-7}\) M) in sham and shunt rats. In the control sham group, PKC inhibition was 2.3-fold more effective than PKA inhibition in reducing PTD (7.94 ± 0.02 vs. 17.76 ± 0.04%, $P < 0.01$). On the other hand, in shunt rats, reductions of PTD induced by PKA and PKC inhibition were not significantly different. On the other hand, the percent change in PTD induced by elevation of extracellular Ca\(^{2+}\) (Fig. 2D) was not significantly different between sham and shunt rats.

**Left Atrial Velocity of Contraction During Development of Eccentric Hypertrophy**

Modulation of left atrial velocity of contraction (+\(dT/dt\)) of sham and shunt rats by PKA, PKC, and high Ca\(^{2+}\) is presented in Fig. 3. In sham rats, +\(dT/dt\) of the left atrium was not significantly affected by PKA inhibition (Fig. 3A). Elevation of extracellular Ca\(^{2+}\) resulted in an increase in +\(dT/dt\) to 597.06 ± 78.70 mg/s ($P < 0.01$). On the other hand, PKC inhibition reduced +\(dT/dt\) (429.05 ± 29.53 and 396.82 ± 41.13 mg/s with Tyrode solution and Ro-32-0432, respectively). Subsequently, switching to high Ca\(^{2+}\) increased +\(dT/dt\) to 582.55 ± 86.52 mg/s ($P < 0.05$). On the other hand, +\(dT/dt\) in the shunt group was reduced by PKC inhibition (715.45 ± 71.92 vs. 622.80 ± 83.49 mg/s, $P < 0.01$), with a slight effect of KT-5720. Specific +\(dT/dt\) (Fig. 3B) did not differ between sham and shunt groups (80.34 ± 9.70 and 74.06 ± 6.69 mg s\(^{-1}\) relative LA wt\(^{-1}\), respectively), although the absolute +\(dT/dt\) was higher in the shunt than in the sham group (425.35 ± 23.62 vs. 682.71 ± 76.76 mg/s, $P < 0.01$; Fig. 3A). High extracellular Ca\(^{2+}\) was more effective in...
increasing \(\text{+dT/dt}\) in the shunt than in the sham rats (963.97 ± 70.42 vs. 597.06 ± 78.70 mg·s\(^{-1}\)·relative LA wt\(^{-1}\), \(P < 0.01\)); however, specific \(\text{+dT/dt}\) was similar for both groups (114.34 ± 22.78 and 105.83 ± 8.45 mg·s\(^{-1}\)·relative LA wt\(^{-1}\)). This may indicate that factors that affect \(\text{+dT/dt}\) at the myofibrillar level may not be challenged in the shunt animals, although the eccentric hypertrophy of the heart is producing a higher \(\text{+dT/dt}\) at the tissue level.

Figure 3C shows the percent change in \(\text{+dT/dt}\) of the left atrium due to PKA inhibitor (10\(^{-7}\) M) and PKC inhibitor (10\(^{-7}\) M) in sham and shunt rats. PKA inhibition produced a similar percent reduction in both groups. However, PKC inhibition was significantly more effective than PKA inhibition in reducing \(\text{+dT/dt}\) in shunt rats (\(P < 0.05\)), but not in sham rats. The percent change in \(\text{+dT/dt}\) induced by elevation of extracellular Ca\(^{2+}\) (Fig. 3D) was not significantly different between sham and shunt rats.

**Left Atrial \(-dT/dt\) During Development of Eccentric Hypertrophy**

Modulation of left atrial velocity of relaxation \((\text{-dT/dt})\) of sham and shunt rats by PKA, PKC, and high Ca\(^{2+}\) is presented in Fig. 4. PKA inhibition reduced \(\text{-dT/dt}\) of the left atrium (Fig. 4A) only in the shunt group (from 581.40 ± 74.81 to 567.70 ± 75.60 mg/s, \(P < 0.05\)). High extracellular Ca\(^{2+}\) increased \(\text{-dT/dt}\) by 42.77 ± 0.15% \((P < 0.01)\) in sham rats and by 50.06 ± 0.08% \((P < 0.01)\) in shunt rats. On the other hand, PKC inhibition reduced \(\text{-dT/dt}\) to 346.31 ± 45.05 mg/s \((P < 0.01)\) in shunt rats and to 534.76 ± 78.62 mg/s \((P < 0.01)\) in sham rats. High-Ca\(^{2+}\) Tyrode solution increased \(\text{-dT/dt}\) to 517.88 ± 43.55 mg/s \((P < 0.01)\) in sham rats and to 796.06 ± 78.94 mg/s \((P < 0.01)\) in shunt rats.

Specific \(\text{-dT/dt}\) did not vary between sham and shunt rats; however, \(\text{-dT/dt}\) was significantly higher in shunt than in sham rats (581.40 ± 74.81 vs. 392.64 ± 26.90 mg/s, \(P < 0.05\); Fig. 4B). Specific \(\text{-dT/dt}\) was not affected by PKA inhibition, but PKC inhibition significantly reduced \(\text{-dT/dt}\) in sham and shunt rats: from 72.57 ± 7.76 to 64.00 ± 8.73 mg·s\(^{-1}\)·relative left atrial wt\(^{-1}\) \((P < 0.01)\) and from 66.59 ± 8.42 to 58.39 ± 7.34 mg·s\(^{-1}\)·relative left atrial wt\(^{-1}\) \((P < 0.01)\), respectively.

Figure 4C represents the percent change in \(\text{-dT/dt}\) of the left atrium due to PKA inhibitor (10\(^{-7}\) M) and PKC inhibitor (10\(^{-7}\) M) in sham and shunt rats. PKA inhibition did not produce a significant reduction in either group. However, PKC inhibition
significantly reduced $-dT/dt$ in the shunt group ($P < 0.05$). In parallel, high extracellular Ca$^{2+}$ increased $-dT/dt$ more in the shunt than in the sham group (543.51 ± 42.60 vs. 854.57 ± 82.88 mg/s, $P < 0.05$), but specific $-dT/dt$ or percent change in $-dT/dt$ was increased to similar levels in both groups (Fig. 4D).

Changes in Left Atrial Contraction After Regression of Eccentric Hypertrophy by ACE Inhibitor

Inhibition of ACE reduces circulating as well as local tissue ANG II concentrations, which attenuates the activation of ANG II-dependent signaling pathways, such as those related to PKA and PKC. Thus modulation of the inotropic activity by these second messengers would depend on their relative contribution to muscular contractility. Figure 5 shows that treatment of shunt rats with ACE inhibitor improved their specific PTD to a level comparable to that of the untreated sham control rats (61.31 ± 9.91 and 49.56 ± 3.84 mg/relative left atrial wt, respectively), which is greater than that of the shunt rats (38.42 ± 5.82, $P < 0.05$). In addition and similar to the untreated sham rats, captopril-treated shunt rats showed an enhanced response to elevated extracellular Ca$^{2+}$ with respect to the untreated shunts ($P < 0.05$). Interestingly, this improved contractility in the captopril-treated hypertrophied hearts was associated with a restoration of the differential influence of PKA and PKC on PTD (2.78 ± 0.04 and 22.42 ± 0.07% for PKA and PKC, respectively, $P < 0.01$), as in control untreated sham rats. On the other hand, there was no significant difference in the effects of PKA and PKC inhibition or high extracellular Ca$^{2+}$ on PTD between treated and untreated sham rat hearts.

Changes in Left Atrial $+dT/dt$ and $-dT/dt$ After Regression of Eccentric Hypertrophy by ACE Inhibitor

With respect to the untreated shunt-operated rats, captopril treatment significantly improved the specific $+dT/dt$ by 37.41 ± 2.58% ($P < 0.01$) and its response to elevated extracellular Ca$^{2+}$ by 46.67% ± 2.88 ($P < 0.01$) in shunt rat hearts (Fig. 6). In addition, $-dT/dt$ was reduced by PKC inhibition (20.87 ± 0.04%, $P < 0.05$) but not by PKA inhibition in the captopril-treated shunt rat hearts.

On the other hand, captopril treatment increased the specific $-dT/dt$ by 63.64 ± 2.23% ($P < 0.01$) and its response to elevated extracellular Ca$^{2+}$ by 71.52 ± 2.22% ($P < 0.01$) of shunt rat hearts (Fig. 7). This increase in specific $-dT/dt$ was...
significantly reduced (41.34 ± 0.01%, P < 0.01) by PKC, but not PKA, inhibition.

In addition, there was no significant difference in the effects of PKA and PKC inhibitor or high extracellular Ca²⁺ on dT/dt or −dT/dt between treated and untreated sham rat hearts.

**DISCUSSION**

In the present study, we have found that 2 wk of treatment with captopril prevented the development of eccentric cardiac hypertrophy by 80%, whereas the left atrial and ventricular hypertrophy regressed by 80 and 50%, respectively. This represents a regression from a moderate to a low level of cardiac hypertrophy (27). Similar findings were reported previously showing regression of eccentric hypertrophy by ACE inhibitors (17, 34), specifically in the atria. The aortocaval shunt did not induce a change in the systolic blood pressure, which was not affected by captopril treatment. In the sham rats, left atrial PTD was significantly more affected by PKC than by PKA inhibition. At the same concentration, PKC inhibition produced a greater response than PKA inhibition in the sham rats; this difference in the atrial response to protein kinase inhibitors was significantly reduced in rats with aortocaval shunt. In addition, the force of contraction produced by the hypertrophied left atrium was greater than that of the sham-operated controls. In contrast, the specific tension developed per relative left atrial weight was significantly weaker in the hypertrophied than in the sham controls. Therefore, these data may indicate a functional inotropic defect at the basic contractile unit (myofiber) level in shunt rats that is compensated for by the hypertrophy of the heart tissue to maintain a normal cardiac function (compensated phase). Treatment with ACE inhibitor effectively reduced the aortocaval shunt-induced structural changes and restored the differential in protein kinase influence on contractility. The tension developed in the captopril-treated shunt-operated rat hearts reverted to a predominantly PKC-dependent process, as in the sham hearts. Thus the ACE inhibitor showed

![Fig. 7. Changes in left atrial −dT/dt by PKA, PKC, and high Ca²⁺ after treatment with ACE inhibitor. A and B: effects of KT-5720 (10⁻⁷ M), high Ca²⁺ (5.14 mM), and Ro-32-0432 (10⁻⁷ M) on −dT/dt and specific −dT/dt of left atrial muscle from captopril-treated sham and shunt-operated rats. Effect of each protein kinase inhibitor was assessed after equilibration with Tyrode solution. Subsequent to each protein kinase inhibition, muscle perfusion was switched to high-Ca²⁺ solution. C and D: percent changes in −dT/dt of left atria from captopril-treated sham and shunt-operated rats after PKA and PKC inhibition and extracellular Ca²⁺ elevation. Values are means ± SE; n = 6. *P < 0.05 vs. preceding Tyrode in sham. †P < 0.05 vs. preceding Tyrode in shunt. ‡P < 0.05 vs. sham; §P < 0.05 Ro vs. KT.](http://ajpheart.physiology.org/doi/10.1152/ajpheart.00457.2004)
opposite, but disproportionate, effects on the PKA- and PKC-induced inotropic activity of the eccentric hypertrophied heart, with significant enhancement of the latter. Concurrently, captopril treatment restored the strength of the specific PTD in the shunt hearts to levels in the sham hearts, under normal as well as elevated extracellular Ca\(^{2+}\). This may suggest that treatment of eccentric cardiac hypertrophy with ACE inhibitor improves the inotropic activity of the left atrium mainly through a Ca\(^{2+}\)-dependent PKC mechanism. The effect of ACE inhibitors on PKC is still controversial because of differences in species or model used and various experimental designs (1, 4, 36). Nevertheless, it has been shown that chronic captopril treatment increases PKC activity in the rat (1, 45), which may improve myocardial contractility and intracellular Ca\(^{2+}\) transients (2, 30). On the other hand, it has been shown that treatment with ACE inhibitor does not significantly affect PKA level or activity in the heart (7, 19, 29), which is consistent with our findings.

In the hypertrophied left atria, there is an increase in the \(+\text{d}T/\text{d}t\) and \(-\text{d}T/\text{d}t\) that was not evident when expressed in specific relative terms. Treatment with ACE inhibitor increased the specific \(+\text{d}T/\text{d}t\) in the shunt-operated rat heart. In addition, our data show that, in contrast to the sham heart, the left atrial \(+\text{d}T/\text{d}t\) in the hypertrophied heart is mainly PKC sensitive. Captopril treatment of shunt-operated rats increased the atrial \(+\text{d}T/\text{d}t\) along with its PKC sensitivity. Therefore, the increase in \(+\text{d}T/\text{d}t\) in the hypertrophied left atrial muscle seems to be mediated mainly through a PKC-dependent mechanism. This observation is consistent with earlier findings that PKC improves myofilament Ca\(^{2+}\) sensitivity (41), decreases the Ca\(^{2+}\) requirement of the contractile machinery (31), and increases activation of the slow Ca\(^{2+}\) channels, which are main determinants of \(+\text{d}T/\text{d}t\) (2, 35). Furthermore, it has been reported that ACE inhibitor treatment increases junctural conductance through activation of PKC (10), but not PKA (9), suggesting an improved contractility due to enhanced synchronization and excitation-contraction coupling of the myocardial tissue. On the other hand, \(-\text{d}T/\text{d}t\) shows a stronger dependency on PKC than on PKA in both groups, which was more significant in the hypertrophied heart. ACE inhibitor treatment improved \(-\text{d}T/\text{d}t\) and its PKC, but not PKA, sensitivity. This is in agreement with the recent findings (43) showing an increase in the relaxation rate of the adult rat myocytes through a PKC-dependent phosphorylation of troponin I, which is conducive for an increase in the rate of Ca\(^{2+}\) dissociation from the myofilament.

The change in protein kinase dependence of \(+\text{d}T/\text{d}t\) is consistent with a major role of the PKC-dependent signaling transduction during eccentric hypertrophy. In addition, it has been shown that the ventricular myosin light chain subunit isoforms VLC1 and VLC2 are ectopically reexpressed and α-myosin heavy chain becomes largely replaced by β-myosin heavy chain in atrial tissue from volume-overloaded hearts. PKC, but not PKA, phosphorylates VLC to increase the Ca\(^{2+}\) sensitivity of force development and ATPase activity (32). This may translate into an increased \(+\text{d}T/\text{d}t\) and \(-\text{d}T/\text{d}t\) of the hypertrophied heart.

The aim of this study is to assess the contribution of PKA and PKC, and not the different subtypes, to atrial contraction; however, recent work by others lends additional support to our data. Accordingly, one PKC isoform has been shown to be involved in regulating cardiac contractility. Adenoviral-mediated expression of wild-type PKC-α, but not PKC-β, PKC-δ, PKC-ε, or PKC-ζ, induced cardiac hypertrophy characterized by increased cell surface area, increased protein synthesis, and increased expression of atrial natriuretic factor. In agreement with our data using Ro-32-0432 as a selective Ca\(^{2+}\)-dependent PKC inhibitor, experiments with dominant-negative forms of PKC isoforms revealed a necessary role for PKC-α as a mediator of agonist-induced cardiac hypertrophy. More recently, observations with PKC-α knockout and transgenic mice suggest that PKC-α is a fundamental regulator of cardiac contractility and Ca\(^{2+}\) handling. Modulation of PKC-α activity affects dephosphorylation of phospholamban and alters sarcoplasmic reticulum Ca\(^{2+}\) loading and the Ca\(^{2+}\) transient. Mechanistically, PKC-α directly phosphorylates protein phosphatase inhibitor-1, altering the activity of protein phosphatase-1, which may account for the effects of PKC-α on phospholamban phosphorylation (5). This is in agreement with a recent report that heart PKC-α-deficient mice are hypercontractile, whereas mice overexpressing PKC-α are hypcontractile (5).

On the other hand, the specific rates of contraction and relaxation in hypertrophied and normal hearts were not different at normal or elevated extracellular Ca\(^{2+}\); nevertheless, they were increased by captopril treatment. Because the slow Ca\(^{2+}\) channels are the main mediators of extracellular Ca\(^{2+}\) transport to the cytoplasm during the atrial action potential, we may speculate that the slow Ca\(^{2+}\) channels are not directly affected during the compensated phase of eccentric cardiac hypertrophy; however, they may be activated by ACE inhibitor treatment through a PKC-dependent mechanism (35).

In summary, we have shown that, in the aortocaval shunt-operated rat atrium, there is a decrease in the specific PTD that is overcome by the hypertrophy of the heart tissue to sustain normal cardiac function in the presence of a volume overload (compensated phase). ACE inhibitor treatment induces regression of eccentric cardiac hypertrophy. In addition, captopril improved peak tension, \(+\text{d}T/\text{d}t\), and \(-\text{d}T/\text{d}t\) and increased PKC responsiveness of the contractile machinery in the shunt-operated rat heart. We suggest that ACE inhibition during eccentric cardiac hypertrophy produces a negative trophic and a positive inotropic effect through a PKC-dependent mechanism, inasmuch as PKC isoforms may have different and/or opposing effects in the heart (6).

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REFERENCES

ROSE OF PKA AND PKC IN CARDIAC HYPERTROPHY


