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Cyclic AMP-Dependent Phosphodiesterase Isozyme-Specific Potentiation by Protein Kinase C in Hypertrophic Cardiomyopathic Hamster Hearts

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SUMMARY

We recently reported that protein kinase C (PKC) potentiates cAMP-dependent phosphodiesterase (PDE) activity in Syrian hamster hearts with hypertrophic cardiomyopathy (HCM) but not in control hamster hearts. In this study, we examined the mechanism of this PKC/PDE interaction by identifying the PDE isozyme that is the target of PKC modulation. Using Mono-Q high performance liquid chromatography, both control and HCM hamster cardiac PDE could be partially purified into the calcium/calmodulin-dependent (I), the cGMP-stimulated (II), and the low K_M (III) isozyme fractions. The elution profiles of PDE isozyme fractions were similar to those in isolated hamster cardiac myocytes. The percentages of PDE isozymes activities (I/II/III) were 68.8:22.4:8.8% and 51.1:38.4:10.5% for HCM and control hearts, respectively ($n = 4$), suggesting a change in the quantitative expression of isozymes activities in HCM hearts with a significant increase in the calcium/calmodulin-dependent PDE isozyme activities ($p < 0.05$ compared with control). The addition of exogenous PKC (100 munits of rat brain) produced a 60% stimulation in the calcium/calmodulin-dependent PDE isozyme fraction but not in other PDE isozymes of HCM and in none of the isozymes in control hearts. This PKC-mediated potentiation of the calcium/calmodulin-dependent PDE activity was completely blocked by the PKC-specific peptide inhibitor PKC(19–31). Analysis of enzymatic kinetics showed that PKC enhanced the calcium/calmodulin-dependent PDE isozyme activity in HCM by increasing its V_{max} (from 350 pmol/mg/min at baseline to 758 pmol/min/mg with PKC) without changing its K_M (0.69 μM at baseline versus 0.89 μM with PKC). These results suggest that there are both quantitative and qualitative abnormalities in the expression of the calcium/calmodulin-dependent PDE isozyme in HCM hearts and that the PKC modulation of PDE activity in the HCM heart is isozyme specific.

We recently identified a novel interaction between PKC and PDE in Syrian hamsters with hereditary HCM (BIO 14.6 strain) (1). We demonstrated that HCM hamster hearts were deficient in cAMP, which could be due to increase in cAMP turnover by elevated PDE activity. The cardiac PKC activities were also significantly increased in HCM hamsters. Activation of PKC by PMA produced a >60% potentiation of the cardiac PDE activities in HCM hamsters, but such an effect of PMA was absent in control hamster hearts. This effect on PDE was completely inhibited by the PKC peptide inhibitor PKC(19–31). Also, after removal of PKC by immunoprecipitation with anti-PKC antibodies, PMA could no longer produce a stimulatory effect on PDE. Such cross-talk, or PKC/PDE interaction, may account for, at least in part, the deficiency of cAMP in cardiomyopathic hamster hearts. The purpose of this study was to characterize the alterations of cardiac PDE isozymes in cardiomyopathic hamsters and to determine the response of specific PDE isozyme to PKC stimulation.

Materials and Methods

Animals. Syrian cardiomyopathic hamsters (BIO 14.6; BioBreeders, Watertown, MA) and age-matched control (BIO RB) hamsters at 6 months of age were used for these experiments, and the BIO 14.6 hamster hearts were at the peak of cardiac hypertrophy. The animals were housed and maintained at the animal holding facilities at the University of Iowa. Handling and use of animals were in accordance with the National Institutes of Health's *Guideline for the Care and Use of Laboratory Animals* and approved by the Animal Care and Use Committee at the University of Iowa.

Preparation of cell fractions. Hamsters were anesthetized with ketamine (100 mg/kg intraperitoneally) and then killed with a

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ABBREVIATIONS: PKC, protein kinase C; PDE, cAMP-dependent phosphodiesterase; HCM, hypertrophic cardiomyopathy; HPLC, high performance liquid chromatography; PMA, phorbol-12-myristate-13-acetate; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid.

guillotine. The hearts were rapidly excised through a midline sternal dissection, and the ventricles were washed with an ice-cold buffer containing 0.25 M sucrose, 50 mM Tris·HCl, pH 7.4, 1 mM EDTA, 1 mM dithiothreitol, 1 mM EGTA, and protease inhibitors, including 10 μM phenylmethylsulfonyl fluoride, 2 μg/ml leupeptin, and 10 μg/ml soybean trypsin inhibitors. The ventricular myocardium was quickly rinsed and transferred to a 5-ml aliquot of the same buffer at 4° and homogenized with a Tissuemizer (Mark II, model T25-S1, IKA Labortechnik, (Staufen, Germany) at 70% full speed using three pulses of 5 sec each. The homogenate was then centrifuged at 1,000 × *g* at 4° for 15 min in a tabletop centrifuge (model TJ-6R, Beckman Instruments, Palo Alto, CA). The supernatant was then further centrifuged at 100,000 × *g* at 4° for 60 min in an ultracentrifuge (Beckman model L7-55). The supernatant was recovered and used for assay of PDE activities and for HPLC analysis.

Isolation of ventricular myocytes. Ventricular myocytes from control hamster hearts were isolated by enzymatic dissociation using a modification of a previously described method (2). Briefly, hamster hearts were retrogradely perfused on a modified Langendorff apparatus with 0.017 mg/ml protease (type XXIV; Sigma Chemical, St. Louis, MO) for 10 min at 37°. Small pieces of myocardium (2 × 2 mm) were then removed from the ventricles and further digested with collagenase (0.6 mg/ml, Sigma type I) at 35° for 5 min in a nominally zero-Ca^{2+} solution containing 140 mM NaCl, 4.5 mM KCl, 1 mM $MgCl_2$, 10 mM HEPES, and 5.55 mM glucose, pH 7.35. After several rinses with the zero-Ca^{2+} solution to remove collagenase, single cells were dissociated by mild mechanical trituration and stored in the zero-Ca^{2+} solution at room temperature. More than 98% of the dissociated cells were cardiac myocytes, of which 40–50% were Ca^{2+} tolerant. These cardiac myocytes were electrically quiescent and had normal resting membrane potentials. Cell fractions of the isolated cardiac myocytes were prepared as described above.

Separation of PDE isozymes. Resolution of PDE isozymes from hamster isolated myocytes and from ventricular myocardium was performed using HPLC as described by Bode *et al.* (3). The cytosolic fractions (2–4 mg/ml) from HCM and control hamster hearts were filtered through a 0.22-μm syringe filter and then loaded onto a Mono-Q HPLC column (0.5 × 5 cm; bed volume, 1.0 ml; Pharmacia, Piscataway, NJ). Elution was performed with a 30-ml linear gradient of NaCl of 75–500 mM (in 25 mM bis-Tris, pH 6.5) at a flow rate of 1 ml/min at room temperature (21–23°). Fractions (0.4 ml) were collected, and 25 μl was used for assay of PDE activity.

Assay of PDE activity. PDE activity was assayed according to the two-step method of Thompson *et al.* (4). The PDE assay mixture contained 40 mM Tris·HCl, pH 7.5, 4 mM 2-mercaptoethanol, 0.5 mM EGTA, 5 mM $MgCl_2$, and 1 μM [8-^{3}H]cAMP (~1-2 × 10^5 cpm); 25 μl of HPLC eluent from each fraction; and the presence or absence of 0.5 mM 3-isobutyl-1-methylxanthine, with a total volume of 150 μl. The reaction was allowed to proceed for 20 min at 30° and was stopped by incubation at 100° for 45 sec followed by ice-cold incubation. After the addition of snake venom 5′-nucleotidase (100 μg) and incubation at 30° for 10 min, 10 μl of 10 mM adenosine was added as internal standard. The [^{3}H]adenosine formed was then isolated by chromatography with Dowex 1 × 8 resin (mesh 200–400), eluted with 1 ml of methanol. PDE-specific hydrolysis of cAMP was determined by the difference of [^{3}H]adenosine formed in the presence or absence of the PDE inhibitor 3-isobutyl-1-methylxanthine. The stock [8-^{3}H]cAMP was purified once every 2 weeks by HPLC (model 2360 Gradient Programmer, 2350 HPLC Pump; ISCO, Lincoln, NE) using a SAX column (4.6 × 250 mm, ISCO model 68–2207-085) eluted with a 20-ml linear gradient with 0–70% of 0.5 M ammonium formate at 1 ml/min. The 3-isobutyl-1-methylxanthine inhibited [^{3}H]adenosine formation by >95%. The data were corrected for recovery by monitoring the absorbance at 260 nm for adenosine standards. The PDE activities in the HPLC fractions from isolated myocytes and from ventricular myocardium were also measured in the presence of 1.5 mM $CaCl_2$/100 units of calmodulin or 3 μM cGMP. The HPLC elution profiles of PDE isozyme activities were then plotted, and the fractions corresponding to each isozyme were pooled and equilibrated with the PDE assay buffer.

Effect of PKC on PDE isozymes. To study the effect of PKC on PDE isozyme activity, exogenous PKC (100 munits of rat brain; specific activity, 1.9 units/μg; Calbiochem, San Diego, CA) was added to each PDE isozyme fraction separated by HPLC. Activation of PKC was facilitated by the addition of 1 mM $CaCl_2$, 20 mM $MgCl_2$, 20 μM ATP, 0.28 mg/ml phosphatidylserine, and 10 μM PMA. The specificity of the PKC effects on PDE isozyme activity was determined by reversibility with the PKC pseudosubstrate peptide inhibitor PKC(19–31) (40 μM) (GIBCO BRL, Gaithersburg, MD). In PDE isozyme fractions that demonstrated significant modulation of activity by PKC, the effects of PKC on PDE isozyme kinetics were analyzed by Lineweaver-Burk plots, and V_{Max} and K_M values were determined by regression analysis using SigmaPlot (Jandel Scientific, San Rafael, CA).

PKC phosphorylation of partially purified calcium/calmodulin-dependent PDE. HPLC fractions of the hamster cardiac calcium/calmodulin-dependent PDE peak were pooled and concentrated using Centriprep microconcentrators (molecular mass cutoff, 10 kDa) (Amicon, Beverly, MA). The protein concentrations of the preparations from control and HCM hearts were measured and adjusted to 0.1 mg/ml. Of each preparation, 2.5 μg was used for PKC phosphorylation. The reaction mixture contained 2.5 μg of protein, 20 μM ATP, 20 mM $MgCl_2$, 1 mM $CaCl_2$, 0.28 mg/ml phosphatidylserine, 10 μM PMA, 100 munits of PKC (rat brain; Calbiochem), and 10^6 cpm of [γ-^{32}P]ATP (Amersham, Arlington Heights, IL) with a total reaction volume of 50 μl. The reaction was allowed to proceed at 30° for 20 min. A sample containing no cardiac preparation was also included to serve as the PKC autophosphorylation control. The phosphorylated protein bands were resolved electrophoretically on a vertical sodium dodecyl sulfate-polyacrylamide slab gel with 4% stacking gel and 12% running gel. The gel was then fixed, dried, and exposed to Kodak X-OMAT AR film (Eastman Kodak, Rochester, NY) in a cassette with intensifying screens overnight at −70°. The film was developed in a Kodak M35A X-OMAT processor (Cassling Diagnostic Imaging, Hillside, IL). Protein concentrations were measured according to the method of Bradford (5).

Statistical analysis. All enzyme assays were performed in duplicate immediately after isolation of cell fraction. Group data are expressed as mean ± standard error. Statistical significance was determined by Student's *t* test. A value of $p < 0.05$ was considered statistically significant.

Results

In these studies, HCM hamster hearts were studied at the peak of cardiac hypertrophy (1, 6, 7) and showed significant elevation in the PDE activities compared with hearts from age-matched controls (1709 ± 119 versus 1341 ± 113 pmol/mg/min in controls, $n = 12$, $p < 0.01$). Separation of the cardiac PDE isozymes could be accomplished by HPLC into three major fractions (Fig. 1). The first peak contained the calcium/calmodulin-dependent isozyme, the activity of which was dramatically augmented in both control and HCM preparations with the addition of calcium/calmodulin. The second peak contained the cGMP-stimulated isozyme, the activity of which was greatly enhanced in control and HCM hearts with the addition of 3 μM cGMP. The third peak contained the low K_M isozymes, which were not significantly modulated by the addition of calcium/calmodulin or cGMP. Quantitative analysis of the PDE isozyme activities by integration of the areas under the curves in the elution profiles showed that there were abnormalities associated with the development of HCM in BIO 14.6 hearts. Fig. 2 shows the percentage contribution of individual isozymes to total PDE activity; the contribu-

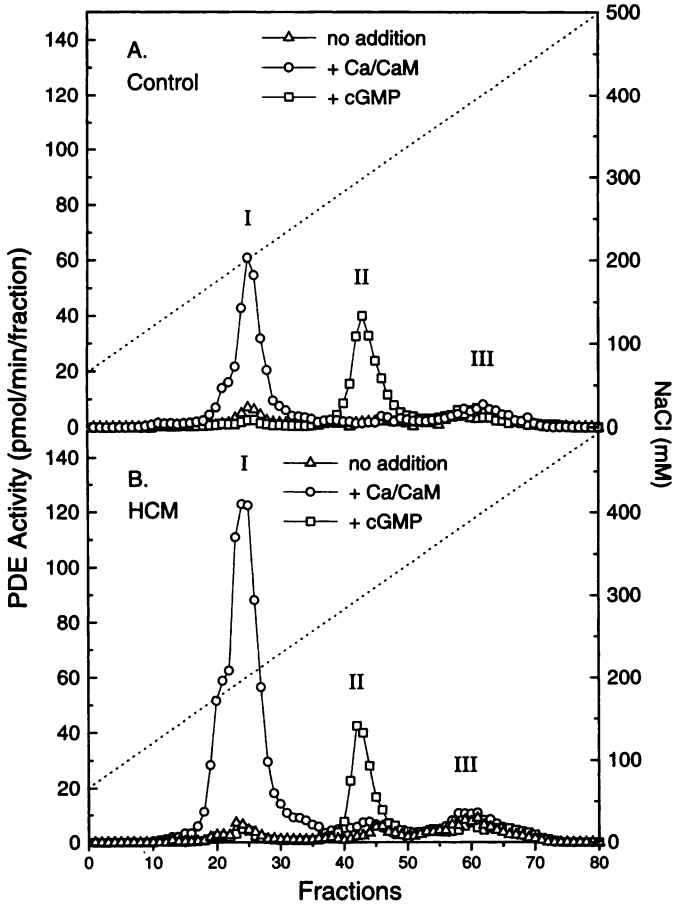

Fig. 1. HPLC elution profiles of PDE isozymes activities in control (A) and HCM (B) hamster hearts using a Mono-Q column. Samples were loaded onto a Mono-Q HPLC column and eluted with a 30-ml linear gradient of NaCl of 75–500 mM in 25 mM bis-Tris, pH 6.5, at a flow rate of 1 ml/min at room temperature (*dotted line*). Fractions (0.4 ml) were collected, and 25 μl of each fraction was used for assay of PDE activity. PDE assays were performed in the presence of 1.5 mM $CaCl_2$/100 units of calmodulin (○), 3 μM cGMP (□), or without any addition (△). *Peak I*, calcium/calmodulin-dependent PDE isozymes. *Peak II*, cGMP-stimulated PDE isozymes. *Peak III*, low K_M PDE isozymes.

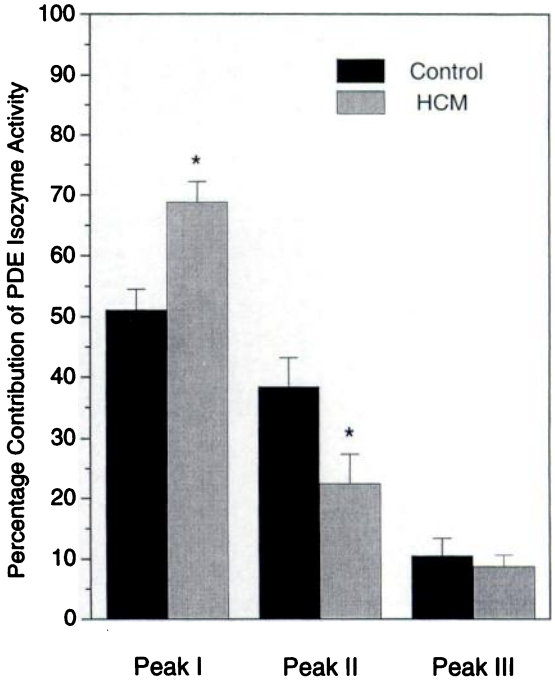

Fig. 2. Ratio of PDE isozymes activities in control and HCM hamster hearts. Results are expressed as percentage of total PDE activity (n = 4). *Peak I*, calcium/calmodulin-dependent PDE isozymes. *Peak II*, cGMP-stimulated PDE isozymes. *Peak III*, low K_M PDE isozymes.

tions of isozyme activities from peaks I/II/III (I, calcium/calmodulin dependent; II, cGMP stimulated; III, low K_M) were changed from 51.8 ± 3.4:38.4 ± 4.5:10.4 ± 0.5% in control to 68.8 ± 3.4:22.5 ± 4.6:9.8 ± 0.3% in HCM hearts (n = 4, $p < 0.05$ comparing peak I and peak II fractions between control and HCM hamsters). The calcium/calmodulin-dependent isozyme was significantly elevated in HCM hearts, as shown in the elution profiles (Fig. 1) and in the percentage expression of total PDE activities (Fig. 2). The percentage activity of the cGMP-stimulated PDE was reciprocally reduced, whereas the contribution of the low K_M isozymes remained unaltered in HCM hearts. These results suggested that there was a quantitative change in the expression of the PDE isozymes with increase in the calcium/calmodulin-dependent PDE isozyme in HCM hamster hearts.

To determine which PDE isozyme was the target of PKC modulation, exogenous PKC was added to the PDE isozyme fractions, and the effect on PDE activity was measured. The results are summarized in Fig. 3. The addition of exogenous PKC showed no demonstrable stimulation in any of the PDE isozyme fractions in control hamster hearts (Fig. 3A). In contrast, PKC produced a 60% stimulation in the activity of the calcium/calmodulin-dependent PDE isozyme but not those of the other isozymes in the HCM hearts (Fig. 3B). This PKC-mediated effect on the calcium/calmodulin-dependent PDE isozyme activity was completely suppressed by the PKC-specific inhibitor peptide PKC(19–31), suggesting that such effects were PKC specific. These results identified the calcium/calmodulin-dependent PDE isozyme as the target of PKC modulation in HCM hamster hearts.

It was previously suggested that the calcium/calmodulin-dependent PDE isozyme is not present in rat cardiac myocytes, and the presence of such isozyme in the rat heart was attributed to its location in nonmyocytes (3). To determine whether hamster cardiac myocytes contain the calcium/calmodulin-dependent PDE isozyme, myocytes were isolated by enzymatic dissociation as described in Materials and Methods. Such a procedure produced isolated heart cells with <2% of nonmyocytes. HPLC separation of the PDE isozyme fractions is shown in Fig. 4; it closely resembles the elution profile of PDE from whole heart, suggesting the calcium/calmodulin-dependent isozyme is present in hamster cardiac myocytes. This HPLC elution profile of PDE isozyme fraction from hamster heart myocytes was repeated in two preparations with the same results.

The effect of PKC on the enzymatic kinetic properties of the calcium/calmodulin-dependent PDE in HCM hearts was assayed using different cAMP substrate concentrations in the presence or absence of exogenous PKC (100 munits) (Fig. 5). Lineweaver-Burk plots showed that PKC increased the V_{max} of the calcium/calmodulin-dependent PDE isozyme activity from 350 to 758 pmol/mg/min without significantly changing the K_M of the enzyme (0.69 μM at base-line versus 0.89 μM in the presence of PKC). These results are in agreement with

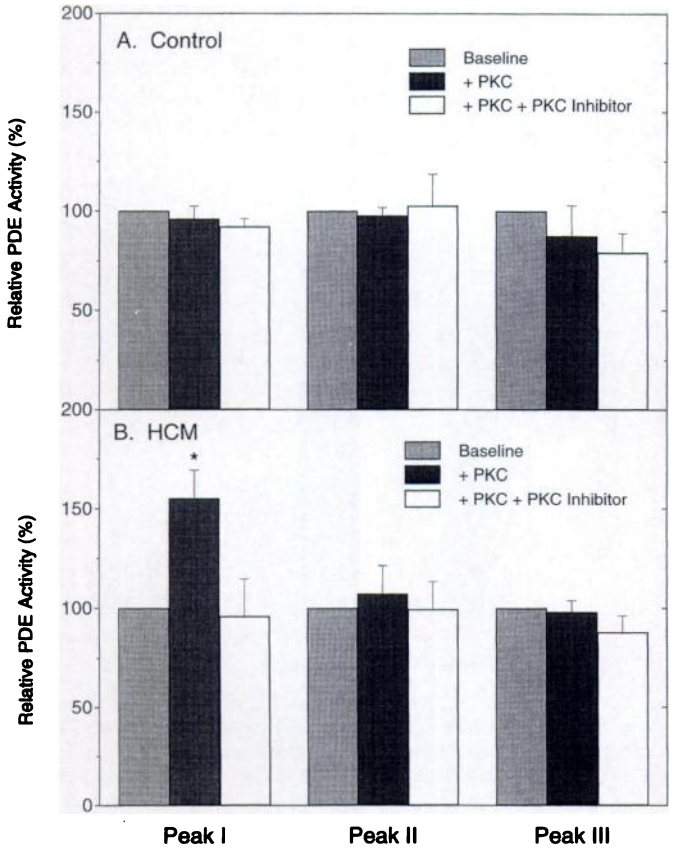

Fig. 3. Effect of exogenous PKC on the activities of PDE isozymes separated by HPLC in control (A) and HCM (B) hamster hearts. *Peak I*, calcium/calmodulin-dependent PDE isozymes. *Peak II*, cGMP-stimulated PDE isozymes. *Peak III*, low K_M PDE isozymes. The PDE activities in each fraction were assayed at base-line, in the presence of 100 munits of PKC, and in the presence of 100 munits of PKC plus 40 μM concentration of the PKC inhibitor peptide PKC(19–31). Results are expressed as percentage activity relative to base-line. *, $p < 0.05$ compared with base-line.

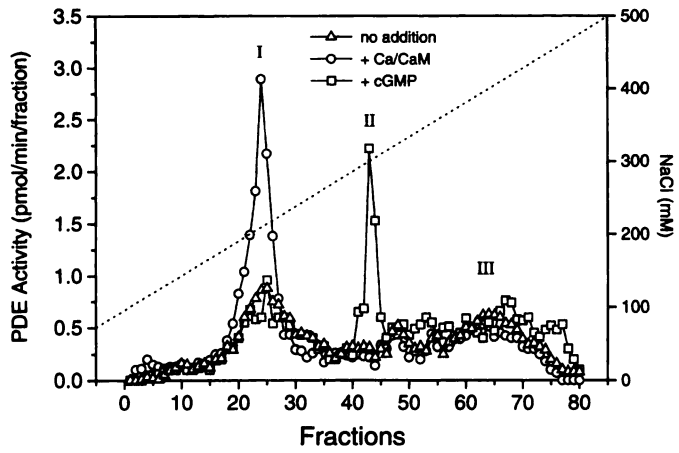

Fig. 4. HPLC elution profile of PDE isozymes in isolated hamster cardiac myocytes. Details of procedure as described in Methods and in Fig. 1. *Peak I*, calcium/calmodulin-dependent PDE isozymes. *Peak II*, cGMP-stimulated PDE isozymes. *Peak III*, low K_M PDE isozymes. PDE activities were measured in the presence of 1.5 mM $CaCl_2$/100 units of calmodulin, 3 μM cGMP, or without any addition.

the effect of PMA on unfractionated PDE activity in HCM hearts (1).

Fig. 6 shows the autoradiograph of PKC phosphorylation of

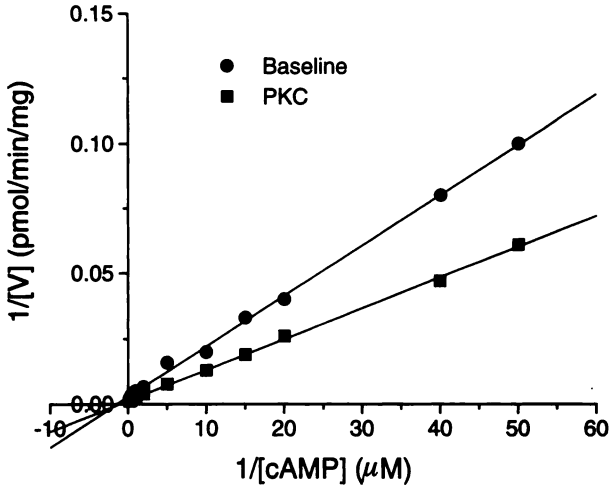

Fig. 5. Effect of PKC on the cardiac calcium/calmodulin-dependent PDE isozyme in the HCM hamster. Lineweaver-Burk plot demonstrating the kinetic properties of the isozyme at base-line and after the addition of exogenous PKC (100 munits), showing a 2-fold increase in V_{max} without significant change in K_M.

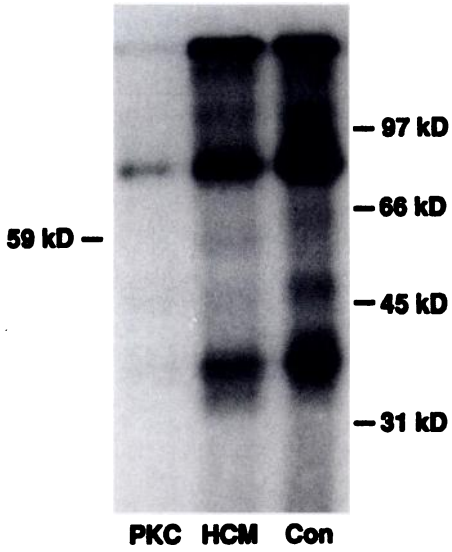

Fig. 6. Autoradiograph of PKC-mediated phosphorylation of the partially purified calcium/calmodulin-dependent PDE fractions. *PKC*, did not contain any cardiac samples; represents autophosphorylation of PKC with a band at 81 kDa. *Con*, control. *Left*, phosphorylated band at 59 kDa that is present in HCM but not in control. *Right*, positions of the molecular mass standards.

the partially purified calcium/calmodulin-dependent PDE fractions from control and HCM hearts. The phosphorylation patterns were not identical between the HCM and control preparations. There was a broad band at 50 kDa that was more densely phosphorylated in control. Of particular interest is the area close to the size of the calcium/calmodulin-dependent PDE. The HCM preparation contained a PKC phosphorylated band with a molecular mass of 59–60 kDa that was absent in the control hamster preparation.

These results suggest that in HCM hearts, the calcium/calmodulin-dependent PDE isozyme has become a target of PKC modulation and that such cross-talk may significantly augment the activity of the isozyme, resulting in enhanced cAMP hydrolysis and leading to deficiency of the second messenger.

Discussion

In this study, wee demonstrated that there are qualitative and quantitative abnormalities associated with the expression of PDE isozymes in HCM hearts. First, PDE activity is increased in HCM hearts, with major contribution from increased calcium/calmodulin-dependent isozyme activity. Second, the calcium/calmodulin-dependent PDE isozyme has become a target for PKC cross-talk in HCM hearts. Third, PKC increases the V_{max} of the calcium/calmodulin-dependent PDE isozyme without significantly changing its affinity for cAMP. These findings have important pathophysiological implications. cAMP is a major determinant of cardiac excitation/contraction coupling/ with important regulatory effects on the sarcolemmal ion channels (6), sarcoplasmic reticulum function (7), and myofilament sensitivity to Ca^{2+} (8). These cAMP effects play a central role in governing the cardiac inotropic, chronotropic, and lusitropic properties (9). The anomalous interaction between PKC and the cAMP regulatory pathways in the HCM hearts may be pertinent in determining the intracellular levels of cAMP. This is especially relevant because we previously reported that cAMP levels are indeed significantly diminished and that PKC activities are elevated in HCM hearts (1).

PDE is a diverse group of enzymes that hydrolyze cAMPs into their 5′ nucleotides (10–12). PDE and adenylyl cyclase are key regulators of cellular cAMP. In heart, four distinct PDE isozymes have been identified that differ from each other in kinetic characteristics, sensitivity to calmodulin, and substrate specificity (10, 12). PDE I is stimulated by calcium/calmodulin and has high or low K_M values for cAMP depending on the species. PDE II has high K_M values for cAMP, is stimulated by cGMP, and is insensitive to calmodulin. The V_{max} for PDE II is ~10-fold greater than that for PDE I or III. PDE III has low K_M values for cAMP and is insensitive to calmodulin but is sensitive to cardiotonic agents such as milrinone, amrinone, and enoximone. PDE III is also inhibited by cGMP. PDE IV, which often coelutes with PDE III, has been identified in some cardiac preparations and is known as a cAMP-specific isozyme. PDE IV is less sensitive to cardiotonic agents but is more sensitive to PDE inhibitors such as rolipram (13). Our results with the hamster heart are in agreement with this general scheme, and the PDE isozyme profile and elution positions are similar to those reported by others and in other species (3). In our study, the PDE isozymes in hamster hearts can be separated into three major fractions. The first fraction was greatly stimulated by calcium/calmodulin; the second was stimulated by cGMP; and the third fraction was sensitive to neither calcium/calmodulin nor cGMP and was consistent with the low K_M isozymes. It is quite possible that peak III may contain both the cGMP-inhibited and the cAMP-specific isozymes that were not resolved by HPLC. We did not further analyze the isozyme composition of peak III because its contribution to total PDE activity was minor, apparently was not altered in HCM, and was not the substrate of PKC modulation.

The finding that the calcium/calmodulin-dependent PDE isozyme is modulated by PKC has not been previously reported. This PDE isozyme family encompasses several different gene products as well as various splice variants and may exhibit distinct enzymatic characteristics and regulatory properties depending on the tissue of origin (11, 14). The properties of the calcium/calmodulin-dependent PDE isozyme is subject to post-translational modification and has been shown to be a substrate of cAMP-dependent protein kinase phosphorylation (15, 16) at sites near the calmodulin-binding domain resulting in a 20-fold decrease in sensitivity to activation by calmodulin. The 63-kDa calcium/calmodulin-dependent PDE from bovine brain is also known to be a substrate for calmodulin-dependent protein kinase II (16–18), and phosphorylation increases the Ca^{2+} concentration required for the PDE activation by calmodulin. Whether the calcium/calmodulin-dependent PDE in control and HCM hamster hearts is modulated by the cAMP-dependent and the calmodulin-dependent protein kinases is unknown. Examination of the primary sequences of the calcium/calmodulin-dependent PDE isozymes available from published literature or from GeneBank (19–24) does not reveal a typical consensus sequence for PKC phosphorylation (25), nor does it contain the sequence motif with highly selective substrate specificity for PKC (26). However, the absence of these recognition motifs does not necessarily indicate the absence of calcium/calmodulin-dependent PDE phosphorylation by PKC because it is known that PKC would also phosphorylate other sequences based on studies in synthetic peptides, albeit with less efficiency (26). In addition, the primary structure of the calcium/calmodulin-dependent PDE in the HCM hamster heart is unknown. A potential mechanism for the PKC/PDE cross-talk in the HCM hamster could involve a mutation on the calcium/calmodulin-dependent PDE gene that would render the isozyme a substrate for PKC phosphorylation. Proof of this hypothesis must await elucidation of the primary structure of the HCM calcium/calmodulin-dependent PDE and direct demonstration of PKC-mediated phosphorylation of the isozyme. However, our results, which showed enhancement of partially purified calcium/calmodulin-dependent PDE activity by exogenous PKC, should be considered as strongly suggestive that the calcium/calmodulin-dependent PDE isozyme in HCM hearts is a substrate of PKC phosphorylation. It is possible that PKC may mediate its effect on the PDE isozyme through a protein that is present in the same HPLC peak. However, we found that exogenous PKC potentiates the PDE activities in all of the fractions of the calcium/calmodulin-dependent PDE peak to a similar extent (data not shown), suggesting that PKC probably exerts its modulatory effects directly on the PDE isozyme rather than via another protein. We also found different PKC phosphorylation patterns of the partially purified calcium/calmodulin-dependent PDE preparations from HCM and control. In particular, there is phosphorylation of a 59-kDa protein band by PKC in the HCM preparation that is absent in the control preparation. This could be PKC-mediated phosphorylation of the PDE isozyme in HCM, but confirmation of this mechanism will require identification of the 59-kDa protein, possibly using isozyme-specific antibodies.

PKC potentiated the activity of the PDE isozyme by increasing its V_{max} without changing its K_M for cAMP. These results are similar to the enzyme kinetics effects of phorbol esters on unfractionated PDE activities in HCM hearts (1), and the PKC/PDE interaction can be accounted for by modulation of a single PDE isozyme. It is also interesting to note that the amino acid sequence of the cGMP-stimulated PDE from bovine heart contains a consensus sequence for PKC phosphorylation (27), but neither the HCM nor the control

hamster cardiac cGMP-stimulated PDE isozyme activities were affected by PKC.

In the normal rat heart, the calcium/calmodulin-dependent PDE is thought to be present only in nonmyocyte cell types (3), but this PDE isozyme has been demonstrated in cardiac myocytes in other species (28). We found that the PDE isozyme elution profiles from HPLC purification are similar for myocardium and isolated myocytes from hamster hearts, suggesting that the calcium/calmodulin-dependent PDE isozyme is present in the hamster cardiac myocytes. Therefore, the cardiac myocyte distribution of this PDE isozyme seems to be species dependent: present in myocytes from hamsters and guinea pigs but absent in rats. The functional role of this PDE isozyme may be particularly important in HCM hamster hearts, which have been shown to have elevated intracellular Ca^{2+} levels as well as derangements in Ca^{2+} regulation (29).

The calcium/calmodulin-dependent PDE has assumed increasing physiological and pathophysiological importance as more is known about this PDE isozyme. The calcium/calmodulin-dependent PDE is a major PDE isozyme in the human heart; together with the cGMP-inhibited PDE, they constitute >90% of the PDE activity (30). It is also an important PDE isozyme that contributes to the regulation of intracellular cAMP in smooth muscle cells (31) and hepatocytes (32). Abnormalities of the calcium/calmodulin-dependent PDE have been implicated in the pathogenesis of certain cardiac and skeletal disorders. The cardiac cAMP levels are elevated in hyperthyroid rats and reduced in hypothyroid rats. These changes were found to result from alterations in the cardiac calcium/calmodulin-dependent PDE activities, which are significantly decreased in hyperthyroid rats and markedly increased in hypothyroid animals (33). PDE activities in skeletal muscles are also known to be elevated in several neuromuscular disorders, including myotonic dystrophy, Duchenne's muscular dystrophy, and amyotrophic lateral sclerosis (34). Interestingly, in the case of Duchenne's muscular dystrophy, the concentrations of Ca^{2+} and calmodulin are also significantly increased, thus implicating the role of the calcium/calmodulin-dependent isozyme in the abnormal second-messenger regulation and the development of muscular dystrophy in this condition (34). All of these underscore the significance and relevance of the calcium/calmodulin-dependent PDE isozyme in the regulation of cellular and tissue physiology.

PKC is known to exist in multiple isozymes that may phosphorylate different physiological targets (35). Recently, we reported the identity of the PKC isozyme responsible for modulation of the calcium/calmodulin-dependent PDE in HCM hearts as PKCα (36). The observation that exogenous PKC could reproduce the phenomenon of PKC/PDE cross-talk suggests that the underlying abnormality resides in the PDE isozyme rather than in PKC. The finding that a specific PDE isozyme is modulated by the activation of a specific PKC isozyme has not been previously appreciated in mammalian hearts. The consequence of such cross-talk may promote acceleration of cAMP turnover, leading to derangements in excitation/contraction coupling in the cardiomyopathic hamster hearts. We do not know whether inhibition of the calcium/calmodulin-dependent PDE isozyme would prevent the development of cardiomyopathy in HCM hamsters. However, it is interesting that treatment with verapamil has been shown to preserve the contractile function in HCM hearts (37). One of the effects of the calcium channel antagonist is to lower the intracellular Ca^{2+} concentration, which may suppress the activities of both PKCa and the calcium/calmodulin-dependent PDE, thereby preventing or minimizing the functional consequence of the PKC/PDE cross-talk.

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