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Differential regulation of diacylglycerol kinase isozymes in cardiac hypertrophy

Hirokazu Yahagi [a], Morihiko Takeda [a], Yasuhide Asaumi [a], Kenji Okumura [b], Ryotaro Takahashi [b], Jun Takahashi [a], Jun Ohta [a], Hiroko Tada [a], Yutaka Minatoya [a], Masahito Sakuma [a], Jun Watanabe [a], Kaoru Goto [c], Kunio Shirato [a], Yutaka Kagaya [a,*]

[a] *Department of Cardiovascular Medicine, Tohoku University Graduate School of Medicine, 1-1 Seiryo-machi, Aoba-ku, Sendai 980-8574, Japan*
[b] *Department of Cardiology, Nagoya University Graduate School of Medicine, Nagoya, Japan*
[c] *Department of Anatomy and Cell Biology, Yamagata University School of Medicine, Yamagata, Japan*

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Abstract

To examine the involvement of diacylglycerol kinase (DGK) and phosphatidic acid phosphatase (PAP) in pressure overloaded cardiac hypertrophy, rats were subjected to either ascending aortic banding for 3, 7, and 28 days or sham operation. In comparison with sham-operated rats, the left ventricular (LV) weight of the aortic-banded rats increased progressively. At 28 days after surgery, the expression of DGKε mRNA but not DGKζ or PAP2b mRNA in the LV myocardium significantly decreased in the aortic-banded rats compared with the sham-operated rats. DGKζ protein in the LV myocardium translocated from the particulate to the cytosolic compartment in the aortic-banded rats. Furthermore, the myocardial content of 1,2-diacylglycerol and PKCδ protein expression in the particulate fraction of the LV myocardium significantly increased in aortic-banded rats compared with sham-operated rats. These results suggest that DGKε and DGKζ play distinct roles in the development of pressure overloaded cardiac hypertrophy and that the two isozymes are differentially regulated.

Keywords: Diacylglycerol kinase; Phosphatidic acid phosphatase; Cardiac hypertrophy; Intracellular translocation; 1,2-Diacylglycerol; Protein kinase C

The signaling triggered by several neurohumoral factors such as angiotensin II [1] and endothelin-1 [2] is followed by the activation of phospholipase C (PLC) which results in the production of a lipid second messenger, 1,2-diacylglycerol (1,2-DG). 1,2-DG serves as an activator of phospholipid-dependent protein kinase C (PKC), which elicits several cellular responses in the heart, including cardiac hypertrophy [3] and protection of cardiomyocytes in ischemic preconditioning [4]. Therefore, DG signaling must be strictly regulated for the appropriate response. DG kinase (DGK) is an enzyme that is responsible for controlling the DG cellular level by converting it to phosphatidic acid (PA) [5], thus acting as a regulator of PKC. The molecular structures of nine DGK isozymes from mammalian species have been reported by other groups [6] and our group [7]. DGK represents a large gene family of isozymes that differ remarkably in their structures, modes of tissue expression, and enzymological properties [5,8,9]. We have reported [7,10,11] the detailed cellular expression of mRNAs for these isozymes and their functional implications. We have also reported [12] that among the mRNAs for these isozymes, those for DGKε and ζ were dominant in rat heart ventricles. Furthermore, we

* Corresponding author. Fax: +81 22 717 7156.
E-mail address: kagaya@cardio.med.tohoku.ac.jp (Y. Kagaya).

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demonstrated [12] that (1) DGKζ mRNA expression increased in infarcted and border areas at the early phase of myocardial infarction (MI), and the increased expression was mainly attributed to infiltrated granulocytes and macrophages, and (2) DGKε mRNA expression decreased in the viable myocardium 21 days after MI, and the decreased expression of DGKε was normalized by treatment with captopril. These data suggest that DGKε and ζ are involved in healing and left ventricular (LV) remodeling after MI and that these isozymes have distinct functional properties.

PA phosphatase (PAP) 2b converts PA to DG [13], which is the reverse of the reaction catalyzed by DGKs, and PA, the substrate of PAP2b as well as the product of DGK, may also serve as a second messenger [14,15]. In cardiomyocytes, PA has been shown to stimulate IP_3 production [16] and to increase intracellular free calcium [17]. Therefore, it is conceivable that PAP2b as well as DGK may have regulatory roles in pathological conditions in the heart by controlling the cellular DG and PA levels.

The aim of the present study was to investigate the changes in the mRNA levels of DGK isozymes and PAP2b during the initiation and progression of pressure overloaded cardiac hypertrophy in rats. In addition, we also investigated the change in the intracellular localization of DGKζ in pressure overloaded hypertrophy, based on recent studies by Abramovici et al. [18], which demonstrated that DGKζ translocates in the skeletal muscle cells in some conditions. The present study provides important clues for revealing the putative roles of DGKs and PAP2b in pressure overloaded cardiac hypertrophy.

Materials and methods

Ascending aortic banding. LV pressure overload was induced in male Wistar rats ($n = 43$) aged 8 weeks by constriction of the ascending aorta as previously described [19]. Rats were anesthetized with sodium pentobarbital (40 mg/kg IP). The ascending aorta was constricted with 1-0 silk surgical thread by ligation of the aorta together with a 20-gauge needle, which was pulled out thereafter. Sham-operated animals ($n = 36$) underwent the same procedure without constriction. All rats were housed and fed according to the Guidelines for the Care and Use of Laboratory Animals of Tohoku University.

Echocardiography and hemodynamic measurements. Transthoracic echocardiography was performed (Aplio, Toshiba Medical Systems) with a 12 MHz imaging transducer. Rats were anesthetized with ketamine (25 mg/kg IP) and xylazine (5 mg/kg IP). All measurements were recorded by use of the leading edge-to-leading edge convention adopted by the American Society of Echocardiography [20]. After that, the chest was opened and a 22-gauge needle, which was connected to a pressure transducer with the polyethylene catheter (PE-50), was inserted into the LV cavity through the apex [19,21]. Data were recorded using a polygraph system (Nihon Kohden, Tokyo). After the measurement, hearts were excised and subjected to further analysis.

Northern blot analysis. Twenty micrograms of total RNA was separated on a 1% agarose/formaldehyde gel and blotted onto a nylon membrane (Hybond-N). The cDNA probes for ANP [22], DGKε [23], DGKζ [7], and PAP2b were labeled with ^{32}P using a Random Primed DNA Labeling Kit according to the manufacturer's instructions (Roche). The probe for rat PAP2b was cloned by RT-PCR using rat heart cDNAs as a template with the following primers (sense, 5′-CATCTGCCTCGACCTCTTCTGC-3′; antisense, 5′-GAACATG GAGAAGGAGGCGTGG-3′). Probes were hybridized at 42 °C in a hybridization solution (50% formamide, 5× Denhardt's solution, 5× SSPE, 0.1% SDS, and 200 μg/mL salmon sperm DNA). Blots were washed twice at room temperature in 2× SSC/0.1% SDS for 15 min. They were then washed once at 42 °C in 0.1× SSC/0.1% SDS for DGKε and PAP2b, and twice at 50 °C for DGKζ and ANP. All hybridization membranes were reprobed with synthetic ^{32}P-labeled oligonucleotide that is specific for 18S rRNA as an internal control [24]. Hybridizing bands were quantified with a FUJIX Imaging Analyzer BAS 1500 (Fuji Film).

Protein preparation. LV tissue was homogenized in buffer A [250 mM sucrose, 10 mM Tris–HCl (pH 7.4), 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 μM leupeptin, and 1 μM pepstatin A] following centrifugation (600*g*, 10 min). The supernatant was further centrifuged (100,000*g*, 60 min) to separate the cytosolic fraction. The pellet was resuspended in buffer A containing 1% Triton X, incubated for 2 h at 4 °C, and centrifuged (100,000*g*, 60 min). The final supernatant was separated as the particulate fraction. The purity of cytosol and particulate fractions was confirmed by the immunoblot analysis with antibody against triosephosphate isomerase (TPI, a cytosol marker, a generous gift from Dr. Ryoichi Yamaji) [25] and antibody against phospholamban (PLB, a membranous marker, Affinity BioReagents) [26], respectively. The protein concentration was determined by the method of BCA (Pierce).

Immunoblot analysis. Each sample was subjected to 7.5% SDS–PAGE and transferred to PVDF membranes (Hybond-P) using a semi-dry transfer unit. Blots were blocked for 1 h with 5% nonfat dried milk in 0.1% Tween 20/Tris-buffered saline and then probed with anti-DGKζ polyclonal antibody, which was developed by ourselves [27], and anti-PKC isoform α, δ, and ε antibodies (Transduction Laboratories). This was followed by probing with horseradish peroxidase-conjugated anti-rabbit IgG (DGKζ) or anti-mouse IgG (PKCα, δ, and ε). The immunoreactive bands were detected by an ECL kit (Amersham).

Lipid analysis. Lipid analysis and measurement of the fatty acid composition of 1,2-DG were performed as described previously [28,29]. The 1,2-DG content was quantified by the TLC-FID (Iatroscan thin-layer chromatography with flame ionization detection) method. The fatty acyl moieties in 1,2-DG were transmethylated and methyl fatty acids were analyzed by gas chromatography. Peaks were identified by comparison to standards (Nu-Chek-Prep, Elysian), and the peak areas were calculated.

Statistical analysis. The results are expressed as means ± SEM. Values for aortic-banded rats and those for sham-operated rats were compared using the unpaired Student's t test. The time course of the LV weight/tibial length and the LV anterior wall thickness in end-diastole, measured by echocardiography, were analyzed by two-way ANOVA. A P value < 0.05 was considered significant.

Results

Characterization of pressure overloaded cardiac hypertrophy

Table 1 shows the heart weight, invasive LV hemodynamic measurements, and echocardiographic parame-

Table 1
Characterization of pressure overloaded cardiac hypertrophy

Number of animals	3 days		7 days		28 days	
	Sham 4	Banding 8	Sham 5	Banding 7	Sham 5	Banding 6
BW (mg)	162 ± 3	157 ± 2	192 ± 3	183 ± 3*	283 ± 3	278 ± 7
TL (mm)	30.3 ± 0.3	29.9 ± 0.2	30.8 ± 0.2	30.6 ± 0.2	35.0 ± 0.2	35.3 ± 0.4
LVW/TL (mg/mm)	11.9 ± 0.1	14.5 ± 0.7*	13.0 ± 0.1	17.4 ± 0.5**	15.1 ± 0.2	22.8 ± 0.8**
HW/TL (mg/mm)	16.0 ± 0.2	18.7 ± 0.8*	17.7 ± 0.1	21.9 ± 0.5**	20.1 ± 0.4	27.9 ± 0.9**
LVSP (mmHg)	136 ± 1	204 ± 7**	141 ± 7	213 ± 7**	129 ± 6	206 ± 12**
LVEDP (mmHg)	5.8 ± 0.9	14.3 ± 1.0**	7.4 ± 0.9	9.4 ± 0.5	7.0 ± 0.5	13.7 ± 0.9**
LVIDd (mm)	5.98 ± 0.4	5.83 ± 0.2	6.06 ± 0.1	5.95 ± 0.1	7.24 ± 0.1	6.15 ± 0.1**
LVIDs (mm)	3.89 ± 0.3	3.93 ± 0.2	3.66 ± 0.1	3.28 ± 0.1*	3.81 ± 0.2	3.22 ± 0.1*
LVAWd (mm)	1.28 ± 0.02	1.48 ± 0.06*	1.23 ± 0.04	1.63 ± 0.08**	1.24 ± 0.02	2.31 ± 0.09**
%FS (%)	35.0 ± 2.3	33.0 ± 1.4	39.2 ± 1.6	46.3 ± 1.0**	47.5 ± 2.4	47.7 ± 1.5
HR (beats/min)	351 ± 16	365 ± 16	358 ± 17	323 ± 10	374 ± 15	417 ± 31

Each parameter is shown for the sham-operated group (sham) and the ascending-aortic banding group (banding) at 3, 7, and 28 days after surgery. BW, body weight; TL, tibial length; HW, heart weight; LVW, left ventricular weight; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; LVIDd, left ventricular internal diameter at end-diastole; LVIDs, left ventricular internal diameter at end-systole; LVAWd, left ventricular anterior wall thickness; %FS, % fractional shortening; and HR, heart rate. Values are means ± SEM.

* $P < 0.05$ vs. sham-operated rats.

** $P < 0.01$ vs. sham-operated rats.

ters. The LV systolic pressure significantly increased in the aortic-banded rats at 3, 7, and 28 days after surgery in comparison with the sham-operated rats. The LV end-diastolic pressure significantly increased in aortic-banded rats at 3 and 28 days after surgery. It tended to increase in these rats at 7 days after surgery in comparison with the sham-operated rats. In comparison with the sham-operated rats, the LV weight/tibial length progressively increased by 22%, 34%, and 51% after 3, 7, and 28 days of aortic banding, respectively. Furthermore, in comparison with the sham-operated rats, the LV anterior wall thickness in end-diastole, which was measured by echocardiography, increased progressively by 16%, 33%, and 85% after 3, 7, and 28 days of aortic banding, respectively. The LV internal diameter in end-diastole had significantly decreased after 28 days of aortic banding in comparison with the sham-operated rats, and that in end-systole had decreased after 7 and 28 days of aortic banding. These data indicate that in the result of continuous pressure-overload to LV, the degree of LV hypertrophy progressively increased during the 28-day observation period in the present study. Increases in lung weight/tibial length and pleural effusion, which are considered as signs of heart failure, were not observed in our experimental model (data not shown).

Atrial natriuretic peptide (ANP) gene is one of the well-established markers for the development of cardiac hypertrophy. Northern blot analysis revealed that the ANP mRNA level in aortic-banded rats significantly increased by 14.6-, 8.6-, and 15.0-fold in comparison with sham-operated rats at 3, 7, and 28 days after surgery, respectively.

DGK isozyme and PAP2b gene expressions in pressure overloaded cardiac hypertrophy

Fig. 1 shows the results of the Northern blot analysis for DGKε, DGKζ, and PAP2b genes in LV myocardium from aortic-banded and sham-operated rats at 3, 7, and 28 days after surgery. No significant differences in the expression of DGKε, DGKζ, and PAP2b mRNAs were observed between the aortic-banded and sham-operated rats after 3 and 7 days of aortic banding. The DGKε mRNA level in LV myocardium from aortic-banded rats significantly decreased by 33% in comparison with the sham-operated rats after 28 days of aortic banding ($P < 0.01$), however, no significant difference was observed in the DGKζ and PAP2b mRNA levels between the two groups at this time point.

Intracellular localization of DGKζ in pressure overloaded cardiac hypertrophy

Recent studies [18,30] have reported the intracellular localization of DGKζ in skeletal muscle cells and COS-7 cells. Immunoblot analysis of DGKζ in LV myocardium was performed in aortic-banded and sham-operated rats in order to investigate whether LV pressure overload induces alterations in the intracellular localization of DGKζ. Immunoblot analysis of DGKε could not be performed due to the lack of a suitable antibody. The DGKζ protein was detected as an immunoreactive band at 115 kDa. Fig. 2 shows representative immunoblots of DGKζ from the total fraction, cytosolic fraction, and particulate fraction of the LV myocardium. No significant difference in the protein expression level of DGKζ

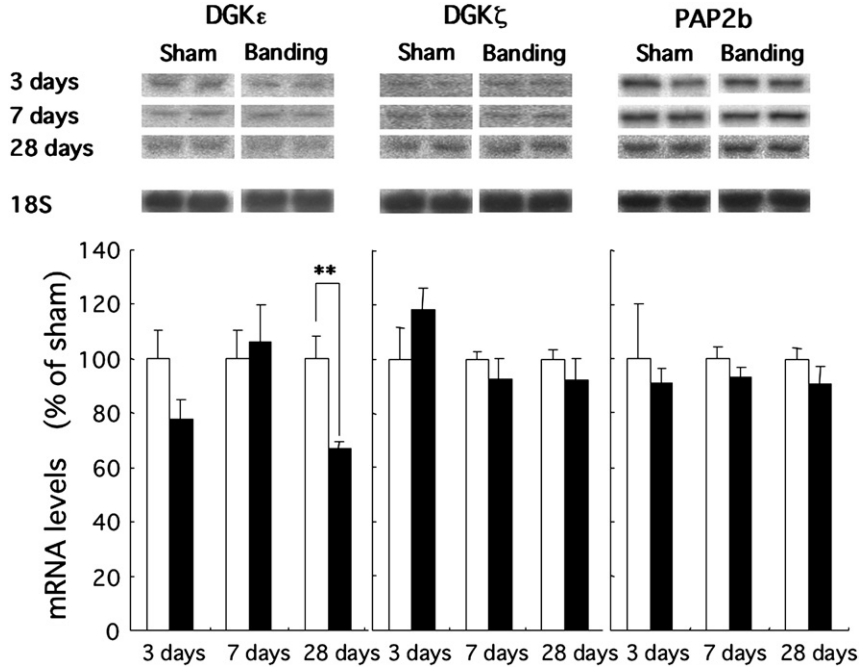

Fig. 1. Effects of pressure overloaded cardiac hypertrophy produced by ascending aortic banding on DGKε, DGKζ, and PAP2b mRNA expression levels. Upper panel, representative autoradiogram of Northern blot analysis of DGKε, DGKζ, and PAP2b in the left ventricle at 3, 7, and 28 days after surgery. Lower panel, the bar graphs represent the quantitative results of DGKε, DGKζ, and PAP2b mRNA expression levels in both groups. Sham indicates sham-operated rats; banding, ascending aortic-banded rats. The open bars represent Sham ($n = 4$, 5), and the solid bars represent Banding ($n = 6$–8). Values are means ± SEM. $^{**}P < 0.01$ vs. sham-operated rats.

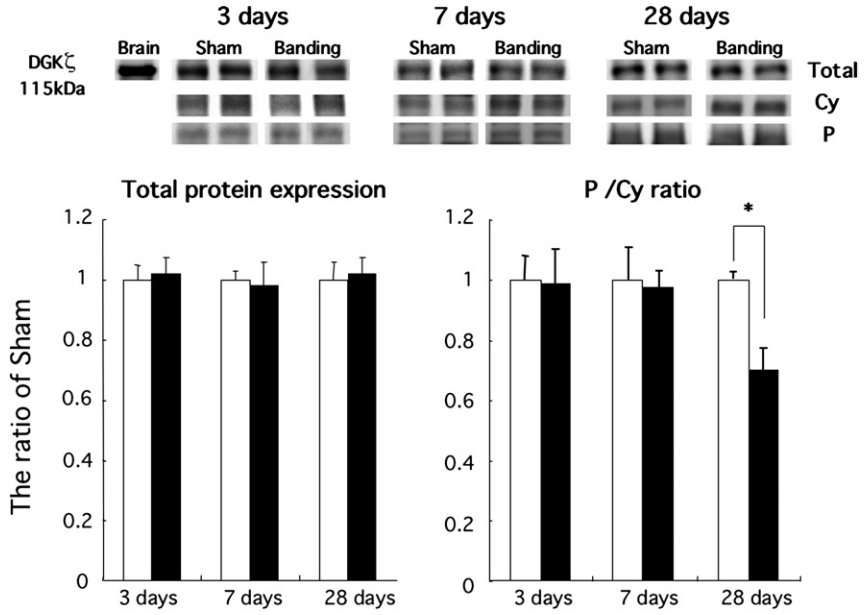

Fig. 2. Effects of pressure overloaded cardiac hypertrophy produced by ascending aortic banding on DGKζ protein expression level. Upper panel, representative immunoblot analysis of DGKζ in total protein, cytosolic (Cy), and particulate (P) fractions from the left ventricle at 3, 7, and 28 days after surgery. Lower panel, the bar graphs represent the quantitative results of the amount in total fraction (left), and particulate/cytosol ratio (P/Cy ratio, right) of DGKζ protein at 3, 7, and 28 days after aortic banding (solid bars, $n = 5$) and sham operation (open bars, $n = 5$). Values are means ± SEM. $^{*}P < 0.05$ vs. sham-operated rats.

was observed in the total LV myocardium between the aortic-banded and sham-operated rats at 3, 7, and 28 days after surgery. Interestingly, however, the particulate fraction/cytosolic fraction ratio of DGKζ protein expression significantly decreased in the LV myocardium from aortic-banded rats in comparison with that from sham-operated rats at 28 days after surgery ($P < 0.05$).

1,2-DG content, fatty acid composition of 1,2-DG, and PKC isozyme expression in severe pressure overloaded cardiac hypertrophy

It is well known that DGK regulates the 1,2-DG levels [31,32]. We performed TLC-FID in LV myocardium from both aortic-banded and sham-operated rats at 28 days after surgery. The myocardial 1,2-DG content in the aortic-banded rats significantly increased by 71% in comparison with the sham-operated rats (0.60 ± 0.04 μg/mg vs. 0.35 ± 0.03 μg/mg, $P < 0.001$, Fig. 3A). No significant difference was observed in the fatty acid profile of 1,2-DG in LV myocardium between the two groups (Fig. 3B).

Several investigators have studied the intracellular translocation of PKC isozymes, which are activated by 1,2-DG as a result of various hypertrophic stimuli in the heart [33,34]. Immunoblot analysis of PKCα, δ, and ε in the cytosolic and the particulate fractions of LV myocardium from aortic-banded and sham-operated

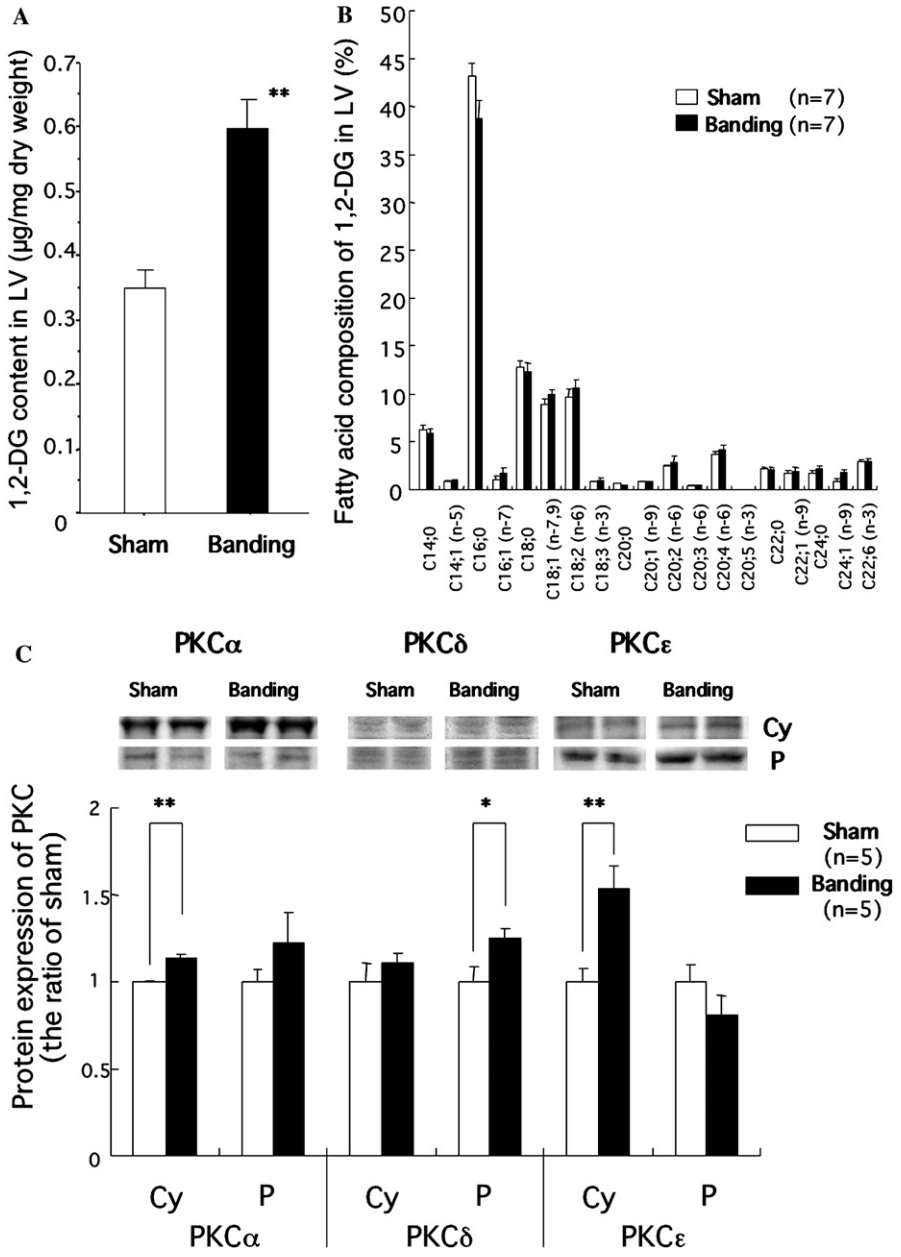

Fig. 3. (A) Myocardial 1,2-diacylglycerol (DG) content in the left ventricle (LV) at 28 days after surgery. 1,2-DG content in LV of aortic-banded rats ($n = 7$) significantly increased in comparison with sham-operated rats ($n = 7$). (B) Fatty acid composition of 1,2-DG in LV at 28 days after surgery. Sham indicates sham-operated rats; banding, ascending aortic-banded rats. The data are means ± SEM. (C) Upper panel, representative immunoblot analysis of PKCα, δ, and ε in cytosolic (Cy) and particulate (P) fractions from the left ventricle at 28 days after surgery. Lower panel, the bar graphs represent the quantitative results of PKCα, δ, and ε protein expression levels in both groups. Sham indicates sham-operated rats; banding, ascending aortic-banded rats. Values are means ± SEM. $^{*}P < 0.05$, $^{**}P < 0.01$ vs. sham-operated rat.

rats was performed 28 days after surgery in order to verify the translocation of PKC isozymes in our experimental cardiac hypertrophy. In comparison with sham-operated rats, the PKCα and PKCε protein levels in aortic-banded rats significantly increased by 13% ($P < 0.01$) and 54% ($P < 0.01$), respectively, in the cytosolic fraction but not in the particulate fraction (Fig. 3C). In contrast, when compared with sham-operated rats, the PKCδ protein level in aortic-banded rats significantly increased by 25% ($P < 0.05$) in the particulate fraction; however, it did not increase in the cytosolic fraction. These data suggest that LV pressure overload resulted in the translocation of PKCδ but not of PKCα or PKCε from the cytosolic fraction to the particulate fraction in our experimental model.

Discussion

Cardiac hypertrophy is a physiological adaptation process that occurs in response to increased ventricular wall stress. We have characterized the differential regulation of cardiac DGK isozyme expression in order to determine whether the expression of DGK isozymes, which are key enzymes considered to be attenuators of PKC activity, may be modulated during the initiation and progression of cardiac hypertrophy induced by pressure overload due to aortic banding. The major findings of this study were as follows: in the pressure overloaded cardiac hypertrophy model (1) the expression of DGKε mRNA decreased after 28 days of aortic banding, (2) the expression of DGKζ and PAP2b mRNA remained unchanged, (3) the particulate/cytosolic ratio of DGKζ protein expression decreased after 28 days of aortic banding although the expression in the total LV myocardium remained unchanged, and (4) the 1,2-DG content in the hypertrophied LV myocardium increased after 28 days of aortic banding.

In the present study, Northern blot analysis revealed significant downregulation of DGKε expression in hypertrophied LV myocardium after 28 days of banding although its expression remained unchanged after 3 and 7 days of aortic banding. It is conceivable that the decreased DGKε level in hypertrophied LV myocardium increases the PKC activity through the elevated 1,2-DG level. The result of the present study is consistent with that of our previous study [12] in which we demonstrated that DGKε mRNA expression in the viable LV myocardium significantly decreased in comparison with sham-operated rats after 21 days of left coronary artery ligation but not after 3 or 7 days of coronary artery ligation. In that study, the LV weight had already increased 3 days after the onset of myocardial infarction, suggesting that a hypertrophic response in the viable myocardium had been initiated. A discrepancy between the downregulation of DGKε and the degree of cardiac hypertrophy was also demonstrated in the present study. The result of the present study as well as that of our previous study therefore suggests that DGKε might be involved in the progression or maintenance of cardiac hypertrophy but is probably not involved in its initiation. Further studies using mice with cardiac-specific overexpression of DGKε might be helpful for elucidating the individual role of DGKε in the initiation and progression of cardiac hypertrophy.

We previously reported that DGKα, ε, and ζ mRNAs are expressed in normal rat LV myocardium and that DGKζ showed the highest intensity among these [12]. Northern blot analysis in the present study revealed that the expression of DGKζ mRNA remained unchanged in the progression of cardiac hypertrophy induced by pressure overload. This finding is consistent with our previous observations in the viable LV myocardium from rats with myocardial infarction [12]. In the present study, we demonstrated for the first time that the particulate fraction/cytosolic fraction ratio of DGKζ protein significantly decreased after 28 days of aortic banding although the total expression in LV myocardium was unchanged. Several studies have reported that DGKζ translocates in vitro. Santos et al. [35] reported that DGKζ translocated from the cytosol to the plasma membrane in response to muscarinic type I receptor stimulation. Davidson et al. [36] showed that gonadotropin-releasing hormone receptor stimulation induced membranous translocation of DGKζ. But there is no report on whether DGKζ translocates in cardiomyocytes by some exoteric stimulation. The results of the present study suggest that DGKζ translocates from the membranous fraction to the cytosolic fraction in the progression of cardiac hypertrophy due to a sustained stimulation of pressure overload. Since we did not assess the protein expression level or the intracellular localization of DGKζ in the viable LV myocardium from infarcted hearts in the previous study [12], further studies using several different experimental models are required to clarify whether the decrease in the particulate fraction/cytosolic fraction ratio of DGKζ protein is a general phenomenon in response to myocardial hypertrophic stimuli or is unique in pressure overload-induced cardiac hypertrophy.

PAP2b converts PA to DG, which is the reverse of the reaction catalyzed by DGKs. We observed that the expression of PAP2b mRNA was not altered in the initiation and progression of cardiac hypertrophy induced by pressure overload. Yu et al. [37] recently reported that sarcolemmal PAP2 activity increased in failing LV myocardium of rats with left coronary artery ligation. They also demonstrated that the increased PAP2 activity was associated with an increased sarcolemmal DG content. As described in Results, our model of LV hypertrophy was not accompanied by LV systolic dysfunction or LV chamber dilatation. The difference in

the pathological conditions, i.e., compensated LV hypertrophy induced by pressure overload and failing LV myocardium following myocardial infarction, might explain the discrepant results. Since we assessed the PAP2b mRNA expression but not its activity, further study is required to draw a more definite conclusion.

Since we found that the DGKε mRNA level and the particulate/cytosol ratio of DGKζ protein level decreased in the LV myocardium after 28 days of aortic banding, we assessed the myocardial content of 1,2-DG, which is a substrate for DGK. We observed that the myocardial 1,2-DG content significantly increased by 1.7-fold in the hypertrophied LV myocardium in comparison with that from sham-operated rats. The result of the present study is consistent with that reported by Pettitt and Wakelam [38] who demonstrated that overexpression of DGKε and DGKζ in porcine aortic endothelial cells resulted in a decrease in the DG content. Therefore, it is highly conceivable that the downregulated DGKε mRNA expression and/or decreased particulate/cytosol ratio of the DGKζ protein level revealed by the present study might result in an elevated 1,2-DG content.

We also demonstrated increased PKCδ protein expression in the particulate fraction of the hypertrophied LV tissues after 28 days of aortic banding. This is consistent with an earlier report by Braun et al. [39] and suggests that PKCδ plays an important role in the development of cardiac hypertrophy induced by pressure overload. It is well known that the process of PKC activation includes its translocation from the cytosolic fraction to the membranous fraction and its binding to 1,2-DG [40]. In the present study, the 1,2-DG content significantly increased in the hypertrophied LV myocardium from rats with aortic banding in comparison with that from sham-operated rats. It is possible, therefore, that the downregulated DGKε mRNA expression and/or decreased particulate/cytosol ratio of the DGKζ protein level demonstrated in the present study elicited an elevated 1,2-DG content and subsequent PKCδ activation in pressure overloaded cardiac hypertrophy; however, further studies using transgenic mice with cardiac-specific overexpression of DGKε and DGKζ are required to clarify this issue. We also demonstrated that PKCα and ε expression in the cytosolic fraction also increased in the hypertrophied LV myocardium in comparison with the non-hypertrophied LV myocardium from sham-operated rats. Increased PKCα expression in the cytosolic fraction of hypertrophied LV tissue was also reported by Braun et al. [39]. However, its contribution to the hypertrophic response remains to be elucidated.

As a limitation, we could not determine the protein expression levels of both PAP2b and DGKε in the LV myocardium because we could not obtain suitable antibodies of good quality against these enzymes.

In conclusion, the results of the present study suggest that both DGKε and ζ are involved in the progression of pressure overload-induced cardiac hypertrophy in rats and that DGKε and ζ are differentially regulated at the levels of transcription and translocation, respectively.

Acknowledgments

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