Right ventricular hypertrophy and apoptosis after pulmonary artery banding: regulation of PKC isozymes

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Abstract

Objective: Pressure overload induced by pulmonary artery banding (PAB) leads to right ventricular (RV) hypertrophy and cardiomyocyte apoptosis. The present study was performed to investigate whether protein kinase C isozymes (PKC-α, PKC-βI, PKC-βII, PKC-δ and PFC-ε), calcineurin and the renin–angiotensin system (RAS) contribute to PAB-induced cardiac remodeling. Methods and results: PAB in male Wistar rats for 3 weeks results in enhanced PKC activity (as determined by ELISA assay) in the cytosol and membrane fraction of the hypertrophied RV, which was accompanied by increased expression (as determined by Western blot analysis) of cytosolic PKC-δ (+72%), PKC-α (+49%), and PKC-βI (+39%), but not PKC-βII and PKC-ε. This differential regulation of cardiac PKC isozymes was limited to the strained ventricle and was not altered in response to chronic angiotensin-converting enzyme inhibition with ramiprilate. Furthermore, no significant changes in the expression of calcineurin α and β subunits were observed in RV pressure overload compared to controls. PAB-induced cardiac apoptosis was determined using Western blot analysis by a significantly increased expression of Bax protein and caspase-3 in the hypertrophied RV, which was diminished to almost control levels by chronic ramiprilate treatment. The myocardial expression of Bcl-2 was not significantly altered in the experimental groups. Conclusion: We have shown for the first time that PAB-induced RV hypertrophy is associated with a differential regulation of cardiac PKC isozymes independent of the RAS and further provide evidence for a pivotal role of the RAS in the development of PAB-induced cardiac apoptosis. © 2003 European Society of Cardiology. Published by Elsevier B.V. All rights reserved.

Keywords: Pulmonary artery bonding; Right ventricular hypertrophy; Protein kinase C; Apoptosis; Renin angiotensin system

1. Introduction

Mechanical overload has been reported to play a pivotal role in the development of cardiac hypertrophy [1]. In contrast to the abundance of studies on left ventricular (LV) hypertrophy, only very few investigations have evaluated the signaling mechanisms which transduce hemodynamic stress in right ventricular (RV) hypertrophy. Candidates include the renin angiotensin system (RAS) via stimulation of the angiotensin II type I receptor (AT1 receptor) or, independent from the RAS, mechanical stretch itself [2]. In vitro experiments have shown that stretch (which is one of the most potent hypertrophic stimuli) of cultured neonatal rat cardiomyocytes leads to the activation of several signaling pathways, including Ca2+-dependent calmodulin/calcineurin, and diverse protein kinases such as mitogen-activated protein kinase (MAPK) and protein kinase C (PKC) [3,4]. PKC is a family of growth-promoting isozymes that are known to induce cardiac hypertrophy in vivo and in vitro [1–3]. In adult rat heart the PKC isoforms PKC-α, PKC-βI, PKC-δ and PKC-ε predominate, whereas PKC-βII is less abundant [5]. We have shown previously that LV pressure overload due to banding of the ascending aorta results in a differential regulation of PKC isozymes with an upregulation of PKC-α and PKC-δ [6]. Thus, a major focus of the present study was to evaluate whether the expression pattern of cardiac PKC isozymes is altered in response to

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univentricular PAB-induced RV hypertrophy in a similar fashion as in LV hypertrophy and whether calcineurin might play a pivotal role in this remodeling process. Controversial data exist concerning the contribution of the local RAS in different experimental models of pressure-overload RV hypertrophy. Whereas RV hypertrophy induced by chronic hypoxia or monocrotaline (MCT) administration is associated with an activation of the local RAS [7] and can be attenuated by angiotensin-converting enzyme inhibitors (ACE inhibitors) or AT1 receptor antagonists [8,9], recent studies have shown that the RAS and its effector peptide angiotensin II (ANG II) are not crucially involved in the development of RV hypertrophy induced by pulmonary artery banding (PAB) [10,11]. These data imply that mechanical overload itself rather than the RAS may cause RV hypertrophy due to PAB, and that chronic hypoxia or MCT models may be confounded by additional factors such as tissue hypoxia or chronic inflammation. Clinically, ACE inhibitors and AT1 receptor antagonists are essential for the treatment of LV hypertrophy and failure, but have not been established in the therapy of right ventricular hypertrophy. Therefore, we investigated the influence of chronic ACE inhibition with ramiprilate on PAB-induced RV hypertrophy as well as on the expression of PKC isozymes and calcineurin as potential mediators involved in the molecular signaling responsible for RV myocardial growth.

Aside from its growth-promoting properties, PKC is also known to play a pivotal role in the regulation of cardiac apoptosis [12], which has been demonstrated to occur in the heart during aging [13] and in cardiac hypertrophy [14]. The relative abundance of proapoptotic (i.e. Bax, caspase-3) and antiapoptotic signaling factors (i.e. Bcl-2) determines the susceptibility to cell death [15]. Caspase-3 is a cysteine protease and exists as an inactive 32 kD precursor (procaspase-3) which is converted into the active 20 kD fragment upon activation with apoptosis-inducing stimuli. Again, little is known about the apoptosis signaling cascade in isolated RV hypertrophy.

The purpose of the present study was to determine (i) the expression pattern of distinct cardiac PKC isozymes in the development of PAB-induced RV hypertrophy, (ii) the expression of the calcineurin α and β subunits and (iii) the expression of the apoptosis-regulating proteins Bax (proapoptotic), Bcl-2 (antiapoptotic) and caspase-3 as effector enzymes in the apoptosis signaling pathway. The possible role of the RAS with respect to these features of the PAB-induced remodeling process was studied after chronic treatment with the ACE inhibitor ramiprilate.

2. Methods

2.1. Materials

Male Wistar rats (180–200 g) were purchased from Thomae, Biberach, the PKC ELISA kit from Mobitec, PKC isoform specific peptide antibodies from St. Cruz Biotechnology and the enhanced chemiluminescence (ECL) Western blotting detection reagents from Amersham International. Ramiprilate was kindly provided by Aventis Pharmaceuticals, all other reagents were of analytical grade obtained from Sigma.

2.2. Procedures

2.2.1. Banding of the main pulmonary artery

All animal procedures conformed to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health, 1996) and were approved by the local ethics committee. RV hypertrophy was induced by banding of the main pulmonary artery using sterile tantalum hemostatic clips (Edward Weck, Research Triangle Park, NC, USA) as previously reported [16]. Sham-operated animals underwent the same surgical procedure without insertion of the clip. Animals were separated into four groups: (1) sham-operated animals with saline administration (n=12), (2) rats subjected to pulmonary artery banding (PAB) with saline administration (n=12), (3) sham-operated rats with ramiprilate administration (n=12), and (4) rats subjected to PAB with ramiprilat administration (n=12). Ramiprilate was given twice daily by intraperitoneal injection (20 μg/kg×d), starting 1 day before intervention [17]. Hemodynamic and biochemical data were obtained 3 weeks after the operation. To determine the enddiastolic and systolic pressure in the left and right ventricle (LVEDP, LVSP, RVEDP, RVSP) the chest was opened and the values were measured using a 23 gauge needle inserted into the apex of the ventricles. The heart and lung were excised and placed in ice-cold saline. RV was dissected from the LV (plus interventricular septum). RV and LV weight as well as the lung wet weight were determined before the organs were shock-frozen in liquid nitrogen.

2.2.2. PKC enzyme activity and immunoblot analysis of cardiac PKC isozymes

Preparation of the cytosol and membrane fraction was performed as previously described [18]. PKC activity was measured in both fractions of the right and left ventricle using a commercially available ELISA-based assay (Mobitec, Germany) with a peptide substrate suitable for all isoforms as previously described in detail [17,18]. PKC activity was determined as the maximally stimulated enzyme activity after stimulation with calcium (1.25 mM), phosphatidylycerine (100 μg/ml) and diacylglycerol (20 μg/ml). Baseline activity was determined in parallel in the presence of 10 mM EDTA and 10 mM EGTA. For Western blot analysis, aliquots (20 and 40 μg protein) of the cytosol and the particulate fraction were separated on an 8% SDS–polyacrylamide gel, transferred to nitrocellulose and the membranes incubated with PKC-subtype-specific polyclonal antibodies (1:1000), washed three times in 100 ml TBS containing 0.3% Tween 20, incubated at room temperature for 45 min with a peroxidase-linked
secondary anti-IgG rabbit antibody (Amersham) and the immunocomplex visualized by ECL reaction. X-rays were evaluated by laser densitometry (Ultrorcan XL, LKB, Germany). To characterize the specificity of the labeled bands, aliquots were analysed in the presence of the antigenic peptide to abolish immunolabeling. Linearity of the detection system was confirmed in preliminary experiments by assaying serial dilutions of cytosolic and membrane protein in the range 10–100 µg protein sample loading for all PKC isozymes except PKC-βII. For significant PKC-βII immunolabeling the protein amount per sample was three times higher as compared to the other PKC isozymes. To ensure identical protein loading of the different lanes, Ponceau S staining of the blot was performed. The laser densitometric data of the Western blot analysis were corrected for the Ponceau S staining.

2.2.3. Calcineurin expression
Calcineurin is a soluble Ca^{2+}/calmodulin-regulated enzyme consisting of a catalytic α subunit (61 kD, calcineurin A) and a Ca^{2+}-binding β subunit (18 kD, calcineurin B). Western blot analysis using a monoclonal calcineurin α subunit antibody and a monoclonal calcineurin β subunit antibody (Sigma) was performed to evaluate the expression of both calcineurin subunits in the cytosolic fraction. After the standard wash procedure, the nitrocellulose membrane was incubated with a peroxidase-linked secondary anti-mouse IgG and the immunocomplex visualized by enhanced chemiluminescence reaction.

2.2.4. Apoptosis
The expression of apoptosis-regulating proteins (Bax, Bcl-2) and caspase-3 was determined by Western blot analysis. For immunoblot assay of Bcl-2 and Bax proteins, aliquots of the cytosolic fraction from the left and right ventricle were separated on a 12% polyacrylamide minigel (Bio Rad), transferred to nitrocellulose and the membranes incubated with a rabbit polyclonal Bcl-2 antibody (St. Cruz and Bio Rad), transferred to nitrocellulose and the membranes incubated with a rabbit polyclonal Bax antibody (Oncogene). After washing, Bcl-2- and Bax-bound antibodies were detected by horseradish peroxidase-conjugated anti-rabbit IgG (Chemicon International) and the chemiluminescence detection system. A commercially available antibody (St. Cruz Biotechnology) was used for immunodetection of caspase-3, which recognizes the inactive 32 kD procaspase-3 and the 20 kD fragment of active caspase-3. Because procaspase-3 is a precursor and has to be proteolytically cleaved to become the activated protease caspase-3, the caspase-3/procaspase-3 ratio was used as an index of caspase-3 activation.

2.2.5. Statistical analysis
All data were analyzed by ANOVA with post hoc testing for statistical significance. Data are presented as mean±S.E.M. and P<0.05 was considered significant.

3. Results

3.1. Cardiac hypertrophy and hemodynamic parameters
PAB for 3 weeks significantly increased the RV index by 72%, RV systolic pressure by 84% and the pulmonary artery gradient (RVSP-PAP) as compared to sham-operated controls (Table 1). PAB-induced RV overload was reflected by an enhanced RVEDP, whereas overt signs of RV failure such as acites were absent. There were no changes in LVSP, LVEDP or the LV index, suggesting that LV function was not altered by PAB-induced RV hypertrophy. Furthermore, the RV parameters were not markedly altered by chronic treatment with the ACE inhibitor ramiprilate, providing evidence that ramiprilate did not influence RV hypertrophy in response to anatomically fixed afterload.

3.2. PKC enzyme activity after subcellular fractionation
PKC activity in the cytosol and membrane fraction of the right ventricle was significantly increased in the PAB group by 42 and 24% (Table 2) compared to sham controls and was not markedly attenuated in response to chronic treatment with ramiprilate (47 and 20%). In contrast to the right ventricle, PKC activity in the cytosol and membrane fraction of the left ventricle was not altered in the experimental groups. To exclude differences in protein

Table 1
Cardiac hypertrophy and hemodynamic parameters in PAB-induced pressure overload

<table>
<thead>
<tr>
<th></th>
<th>Sham/ saline (n=12)</th>
<th>PAB/ saline (n=12)</th>
<th>Sham/ ram (n=12)</th>
<th>PAB/ ram (n=12)</th>
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<tbody>
<tr>
<td>RV index (g/kg)</td>
<td>0.54±0.07</td>
<td>0.93±0.12**</td>
<td>0.51±0.04</td>
<td>0.87±0.1**</td>
</tr>
<tr>
<td>RVSP (mmHg)</td>
<td>24±3</td>
<td>44±6**</td>
<td>21±5</td>
<td>45±4**</td>
</tr>
<tr>
<td>PAP (mmHg)</td>
<td>23±4</td>
<td>24±5</td>
<td>19±6</td>
<td>21±3</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>3.6±0.3</td>
<td>5.4±0.7*</td>
<td>3.1±0.5</td>
<td>5.2±0.5*</td>
</tr>
<tr>
<td>LV index (g/kg)</td>
<td>2.6±0.1</td>
<td>2.7±0.2</td>
<td>2.5±0.1</td>
<td>2.6±0.1</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>4.1±0.5</td>
<td>3.7±0.8</td>
<td>4.4±0.6</td>
<td>4.1±0.1</td>
</tr>
<tr>
<td>Lung index (g/kg)</td>
<td>5.0±0.6</td>
<td>4.4±0.5</td>
<td>4.0±0.3</td>
<td>4.4±0.4</td>
</tr>
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RV index, right ventricular weight/body weight; LV index, left ventricular weight/body weight; RVSP, right ventricular systolic pressure; PAP, systolic pulmonary artery pressure; LVEDP/RVEDP, left/right ventricular enddiastolic pressure; lung-index, lung wet weight/body weight. Values are mean±S.E.M. *P<0.05, **P<0.01: PAB/saline vs. sham/saline; †P<0.05, ††P<0.01: PAB/ram (ramiprilate) vs. sham/ram.
3.3.2. Left ventricle

Representative immunoblot analyses of the PKC isoforms from the cytosol and membrane fraction of the LV are shown in Fig. 2 for all experimental groups in analogy to those shown for the RV (see Fig. 1). Quantification of the whole set of experiments by laser densitometry revealed that PAB did not significantly alter the expression pattern of LV cardiac PKC isoforms compared to sham-operated controls, which additionally was also not influenced by chronic ramiprilate treatment.

Taken together, these data show that the development of PAB-induced RV hypertrophy is accompanied by a differential upregulation of PKC-α, PKC-δ and PKC-β1 and suggest that the RAS is not essentially involved in this growth-regulating process.

3.4. Expression of calcineurin in PAB-induced RV hypertrophy

Western blot analysis was performed to investigate the expression of the calcineurin α subunit (61 kD, calcineurin A) and calcineurin β subunit (18 kD, calcineurin B) from the cytosol of the right and left ventricle (Fig. 3, upper part). The statistical analysis of the whole set of experiments shows that the expression of calcineurin A and calcineurin B was not markedly changed in the hypertrophied RV in response to PAB as well as in response to chronic ramiprilate treatment compared to sham-operated controls. Additionally, no significant differences in the immunolabeling of calcineurin A and calcineurin B were observed in LV for the evaluated groups (Fig. 3, lower part).

3.5. Apoptosis in PAB-induced RV hypertrophy

Apoptosis in PAB-induced cardiac hypertrophy was evaluated by Western blot analysis of Bax (proapoptotic), Bcl-2 (antiapoptotic) and caspase-3.

3.5.1. Expression of Bax and Bcl-2

Representative Bax and Bcl-2 protein immunoblot analyses are demonstrated in the upper part of Fig. 4. PAB-induced RV hypertrophy exhibited a 46% increase of Bax expression compared to sham-operated controls, which was significantly attenuated by chronic ramiprilate treatment. Immunolabeling of Bcl-2 failed to detect significant differences in the expression between the evaluated groups. Accordingly, the Bax/Bcl-2 ratio was significantly increased in PAB/saline by 42% compared to sham/saline.

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Table 2

<table>
<thead>
<tr>
<th>PKC enzyme activity in PAB-induced pressure overload</th>
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<tr>
<td><strong>Sham/saline</strong></td>
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<tr>
<td><strong>(pmol/ mg protein×min)</strong></td>
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<tr>
<td><strong>Right ventricle</strong></td>
</tr>
<tr>
<td>Cytosol</td>
</tr>
<tr>
<td>453±81</td>
</tr>
<tr>
<td>398±92</td>
</tr>
<tr>
<td><strong>Left ventricle</strong></td>
</tr>
<tr>
<td>Cytosol</td>
</tr>
<tr>
<td>878±118</td>
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<td><strong>P</strong></td>
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</table>

PKC activity was determined in the cytosol and membrane fraction of the hypertrophied right and left ventricle. Values are presented as mean±S.E.M.; n=12 for each group. *P<0.05: PAB/saline vs. sham/saline; **P<0.05: PAB/ram (ramiprilate) vs. sham/ram.
PKC isozyme expression in the right ventricle after PAB

Fig. 1. PKC isozyme expression in the right ventricle after pulmonary artery banding. Representative Western blot analyzes of PKC-α, PKC-βI, PKC-βII, PKC-δ and PKC-ε from the cytosol (upper part) and membrane fraction (lower part) of the hypertrophied RV are shown at the top of each panel. The statistical analysis of the whole set of experiments (based on laser densitometric evaluation) is demonstrated in the bottom and reveals an increased immunolabeling for PKC-α, PKC-δ and PKC-βI in the P AB / saline group, which was not diminished by chronic administration of ramiprilate (ram). Values are mean ± S.E.M., n = 12; * P < 0.05: P AB / saline vs. sham / saline; # P < 0.05: P AB / ram vs. sham / ram.

and by 36% compared to PAB/ramiprilate (lower part of Fig. 4). The expression of Bax and Bcl-2 protein in the left ventricle did not show marked changes in any experimental group (Fig. 4, right panel).

3.5.2. Expression of caspase-3/procaspase-3

The expression of procaspase-3 (32 kD, inactive form) and caspase-3 (20 kD, active form) in the RV and LV was determined by Western blot analysis (Fig. 5, upper part). RV hypertrophy due to PAB for 3 weeks was associated with a significantly increased labeling of caspase-3 protein, which was diminished by chronic administration of ramiprilate. The expression of procaspase-3 was not altered in the experimental groups. A quantitative analysis of all experiments is illustrated at the bottom of Fig. 5 as a percentage of caspase-3 (hatched bars) or procaspase-3 (white bars) expression in PAB rats relative to the expression of sham-operated controls and shows a 52% increase in caspase-3 expression in PAB/saline vs. sham/saline. Chronic ACE inhibition with ramiprilate attenuates the PAB-induced upregulation of caspase-3 to almost control levels, suggesting that the RAS/ANG II system might play a pivotal role in PAB-induced caspase-3 upregulation. The expression of caspase-3 and procaspase-3 in the LV is demonstrated in the right part of Fig. 5, showing no significant alterations in the expression pattern.

4. Discussion

The present study demonstrates for the first time that PAB-induced RV hypertrophy is associated with enhanced total PKC enzyme activity and increased expression of PKC-α, PKC-βI and PKC-δ, whereas the amount of PKC-ε and PKC-βIII was unchanged. The degree of RV hypertrophy as well as the differential expression pattern of cardiac PKC isozymes in this model of anatomically fixed RV outflow tract obstruction were not significantly influenced by chronic treatment with the ACE inhibitor ramiprilate. These data are in good agreement with an earlier study of Zierhut et al., who showed that inhibition of the RAS in rats did not influence RV hypertrophy in response to PAB [16]. Similar results were found by Segar et al. in fetal sheep and by Rouleau et al. in rabbits, who demonstrated that PAB-induced RV hypertrophy was not attenuated by chronic ACE inhibition with captopril or...
Fig. 2. PKC isozyme expression in the left ventricle after pulmonary artery banding. Immunoblot analyses of PKC-α, PKC-βI, PKC-βII, PKC-δ and PKC-ε from the left ventricle are demonstrated for the indicated experimental groups in analogy to Fig. 1.

AT1 receptor blockade with losartan [10,11]. Controversial data exist concerning the influence of the RAS on pressure overload-induced LV hypertrophic growth. Whereas some authors report a significant contribution of the RAS to LV hypertrophic growth due to aortic stenosis [19] or systemic hypertension [15,20], others have questioned a major role of the RAS in the development of LV hypertrophy due to pressure overload [21,22]. The data of the present study support a limited impact of the RAS on pressure-overload RV hypertrophy, which might be explained by the anatomically fixed stenosis of the ventricular outflow tract, leading to a significant increase in ventricular load, which itself seems to be crucially involved in the development of cardiac hypertrophy.

Mechanical overload has been reported to represent one of the most potent stimuli of cardiomyocyte hypertrophy, leading to increased protein synthesis, the induction of immediate early genes and the re-expression of fetal gene programs [2,3]. Several growth factors, including endothelin-1 (ET-1), as well as plasma membrane associated mechanosensitive ion channels have been proposed as the transduction mechanism between pressure overload and protein synthesis in cardiomyocyte hypertrophy [23]. Among intracellular signaling molecules, the calcineurin/calmodulin pathway and several protein kinases such as MAPK and PKC have been reported to regulate the development of stretch-induced cardiac hypertrophy [3,24–26]. Whereas Molkentin et al. and others recently reported that the Ca^{2+}-regulated phosphatase calcineurin plays a pivotal role in the development of cardiac hypertrophy [25,26], several groups reported controversial results [25,27]. It should be pointed out that the above in vivo and in vitro data were predominantly obtained from the left ventricle, which differs in its biochemical and physiological properties from the right ventricle [28]. In contrast to LV hypertrophy, no data are available on calcineurin regulation of pressure-overload-induced RV hypertrophy. To our knowledge the present study shows for the first time that the expression of both calcineurin α and β subunits was not changed in PAB-induced RV hypertrophy, suggesting that calcineurin is not involved in PAB-induced RV myocardial growth.

Selective increased expression of PKC-α, PKC-βI and PKC-δ in PAB-induced RV hypertrophy was observed in the cytosol and to a lesser extent in the membrane fraction. Whereas acute stimulation of PKC has been reported to be associated with intracellular translocation from the cytosol to the particulate fraction [18], permanent activation of PKC might not lead to chronic translocation. Several studies provide evidence for a redistribution of activated...
PKC isozymes from the plasma membrane back to the cytosol. Huang et al. demonstrated in arachidonic acid-treated isolated adult rat cardiomyocytes a transient increase of activated PKC-ε in the membrane fraction within 1 min, whereas after 5 min most of the enzyme was found in the cytoskeletal filaments and nuclear region, indicating a re-translocation of activated PKC-ε to cytosol compartments [29]. In this respect, PKC isozymes have been shown to play an important role in nuclear signaling with the induction of immediate early response genes (c-fos, c-myc) and fetal gene programs (ANF, skeletal α-actin), both of which are known to play an important role in the development of myocardial growth [30]. Regulation of gene expression by PKC is mediated by activation of distinct transcription factors such as serum response element (SRE) and AP-1 [31]. Shoh et al. and Page et al. recently reported that PKC-α and PKC-δ are able to upregulate these and other transcription factors (SRE, CREB), which are known to induce gene expression responsible for myocardial growth [32,33]. However, the molecular mechanism responsible for the upregulation of distinct cytosolic PKC isozymes is not known. In this respect, we have observed in rat heart that global myocardial ischemia (Langendorff model) increased the expression of PKC-δ in the cytosol, which can be prevented by pretreatment with the PKC inhibitor bisindolylmaleimide (data not shown). These results implicate an autoregulatory process and suggest that PKC activation might play a role in the upregulation of PKC isozyme protein expression.

Differential regulation of PKC isozymes has also been reported in other models of cardiac hypertrophy. We have previously shown that banding of the ascending aorta leads to an increased expression of PKC-α and PKC-δ in LV, both at the protein and the mRNA level, whereas PKC-ε remained unchanged [6]. Furthermore, an enhanced expression of PKC-β in this model of LV hypertrophy has been reported by Gu and Bishop [34], suggesting a pivotal role of PKC-α, PKC-δ and PKC-β in the development of cardiomyocyte growth due to pressure overload. A recent study by Mende et al. supports our data showing that overexpression of a constitutively active mutant of the Gq subunit in the heart of transgenic mice is sufficient to induce cardiac hypertrophy, which is accompanied by an upregulation of PKC-α and PKC-δ isozymes without enhanced expression of PKC-ε [35]. Similar results have been reported by Wettschureck et al., who showed that cardiomyocyte-specific overexpression of the α1-adrenergic or the AT1 receptor as well as the Gq/11 signaling pathway in mediat-
Fig. 4. Expression of Bax and Bcl-2 protein in PAB-induced RV hypertrophy. Bax and Bcl-2 protein expression was determined in the right and left ventricle by Western blot analysis. The statistical analysis of the whole set of experiments (n=12) is presented as the ratio of Bax/Bcl-2 expression (an index of cell susceptibility to apoptosis). Values are mean±S.E.M.; *P<0.05: PAB/saline vs. sham/saline; †P<0.05: PAB/ram (ramiprilate) vs. PAB/saline. Molecular masses of the proteins (kD) are indicated.

4.1. PAB-induced RV apoptosis

Another salient finding of the present study was the occurrence of myocardial apoptosis in PAB-induced RV hypertrophy, which was demonstrated by an increased expression of Bax protein and caspase-3, whereas the myocardial expression of Bcl-2 was unchanged compared to sham-operated controls. In contrast to PAB-induced RV hypertrophy, cardiac apoptosis in RV pressure overload was significantly attenuated by chronic treatment with ramiprilate.

Cardiac apoptosis in response to pressure overload has been reported to occur in LV hypertrophy induced by aortic banding or systemic hypertension [14,15]. Using spontaneous hypertensive rats (SHR) Fortuno et al. and Diez et al. have shown that SHR-induced cardiomyocyte apoptosis is not only related to enhanced afterload, but is dependent on humoral factors such as activation of the local/systemic RAS [20,37]. Long-term administration of the ACE inhibitor enalapril or the AT1 receptor antagonist losartan was accompanied by a “normalization” of myocardial apoptosis [37]. Only very few studies are available which evaluate myocardial apoptosis in pressure-overload RV hypertrophy. Ikeda et al. reported increased cardiomyocyte apoptosis after experimental banding of the main pulmonary artery in Sprague–Dawley rats, which was accompanied by increased mRNA levels of p53 and Bax protein, both of which are known to be important effector enzymes in apoptosis [38]. It should be mentioned that the signaling pathway representing the linkage between pressure overload and apoptosis in RV hypertrophy is not known and remains to be evaluated. Several recent studies have reported a pivotal role of PKC isozymes in the signaling cascade of ANG II-induced apoptosis [12,15] and the potential role of PKC-δ [39]. These data were predominantly obtained from cardiomyocytes isolated from the left ventricle or the whole heart. However, in the present study, the expression pattern of cardiac PKC isozymes in PAB rats was not changed in response to ACE.
Expression of Procaspsase-3 and Caspase-3 in PAB

Fig. 5. Expression of procaspsase-3 and caspase-3 in PAB-induced RV hypertrophy. Representative Western blot analyses of procaspsase-3 (32 kD, inactive precursor) and caspase-3 (20 kD, active enzyme) are shown in the upper part of the figure. PAB results in an enhanced labeling of caspase-3 protein vs. sham-operated controls. Laser densitometric analysis of the whole set of experiments is presented in the bottom of the figure as a percentage of caspase-3 (hatched bars) or procaspsase-3 (white bars) expression in PAB rats relative to the expression in sham-operated controls. Values are mean±S.E.M.; */P<0.05: PAB/saline vs. sham/saline.

Inhibition with ramiprilate, implying that PKC isozyme upregulation might not play a major role in PAB-induced RV apoptosis.

A limitation of the present study is that the cell types responsible for the upregulation of the PKC isozymes have not been characterized. Both PKC-α and PKC-δ have been shown to be expressed in neonatal and adult rat cardiomyocytes [2,5,12,24]. Since cardiomyocytes are the predominant cell type in the heart, it is proposed that those changes in PKC isozyme regulation likely occur in this cell type.

In summary, PAB-induced RV hypertrophy results in an increased expression of PKC-α, PKC-βI and PKC-δ in the cytosol and the membrane fraction, whereas the amount of PKC-ζ and PKC-βII was unchanged. This differential expression pattern of cardiac PKC isozymes in PAB rats was not influenced by chronic ACE inhibition with ramiprilate, suggesting a mechanism which is independent of the local/systemic RAS. Furthermore, PAB-induced RV hypertrophy was accompanied by an increased RV myocardial apoptosis (as determined by an increased expression of Bax protein and caspase-3), which could be diminished by chronic ACE inhibitor treatment with ramiprilate.

Acknowledgements

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References


