

Losartan attenuates phospholipase C isozyme gene expression in hypertrophied hearts due to volume overload

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Abstract

Because the left ventricular (LV) hypertrophy due to volume overload induced by arteriovenous (AV) shunt was associated with an increase in phospholipase C (PLC) isozyme mRNA levels, PLC is considered to be involved in the development of cardiac hypertrophy. Since the renin-angiotensin system (RAS) is activated in cardiac hypertrophy, the role of RAS in the stimulation of PLC isozyme gene expression in hypertrophied heart was investigated by inducing AV shunt in Sprague-Dawley rats. The animals were treated with or without losartan (20 mg/kg, daily) for 3 days as well as 1, 2 and 4 weeks, and atria, right ventricle (RV) and LV were used for analysis. The increased muscle mass as well as the mRNA levels for PLC β_1 and β_3 in atria and RV, unlike PLC β_3 gene expression in LV, at 3 days of AV shunt were attenuated by losartan. The increased gene expression for PLC β_1 at 2 weeks in atria, at 1 and 4 weeks in RV, and at 2 and 4 weeks in LV was also depressed by losartan treatment. Likewise, the elevated mRNA levels for PLC β_3 in RV at 1 week and in LV at 4 weeks of cardiac hypertrophy were decreased by losartan. On the other hand, the increased levels of mRNA for PLC γ_1 in RV and LV at 2 and 4 weeks of inducing hypertrophy, unlike in atria at 4 weeks were not attenuated by losartan treatment. While the increased mRNA level for PLC δ_1 in LV was reduced by losartan, gene expression for PLC δ_1 was unaltered in atria and decreased in RV at 3 days of inducing AV shunt. These results suggest that changes in PLC isozyme gene expression were chamber specific and time-dependent upon inducing cardiac hypertrophy due to AV shunt. Furthermore, partial attenuation of the increased gene expression for some of the PLC isozymes and no effect of losartan on others indicate that both RAS dependent and independent mechanisms may be involved in hypertrophied hearts due to volume overload.

Keywords: volume overload • cardiac hypertrophy • phospholipase C • gene expression • angiotensin II type 1 receptor antagonist

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Introduction

The phosphoinositide-specific phospholipase C (PLC) isozymes are considered to play a central role in activating intracellular signal transduction pathways, during early key events in the regulation of various cell functions [1–4]. The most common sub-

strate, phosphatidylinositol 4, 5-bisphosphate is converted by the action of PLC isozymes into two messenger molecules, inositol 1,4,5-trisphosphate and 1,2-diacylglycerol, which have been shown to participate in many different physiological processes via downstream signaling mechanisms including the activation of protein kinase C (PKC) [5–10]. The role of PLC in the development of some types of cardiac hypertrophy has been well documented. In this regard, PLC activity was found to be increased in cardiac hypertrophy in cardiomyopathic (BIO 14.6) hamster [11]. In other studies the development of cardiac concentric hypertrophy in stroke-prone spontaneously hypertensive rats, was observed to involve an increase in the PLC signaling pathway [12, 13]. Also, the activation of PLC has been shown to be associated with the hypertrophic response of isolated cardiomyocytes exposed to different agents [14–16]. In an earlier study, we have reported an increase in PLC isozyme gene and protein expression as well as activities in the hypertrophied left ventricle (LV), due to volume overload induced by an arteriovenous (AV) shunt in the rat [17].

Although it is generally assumed that the mechanisms such as increased wall stress and stretch are involved in the development of hypertrophy in all chambers of the heart, we have recently shown that there are differential changes in the expression of PKC isozymes between the volume overloaded LV and right ventricle (RV) [18]. In addition, the β -adrenergic mediated signal transduction in the LV and RV was found to be differentially regulated in congestive heart failure due to myocardial infarction [19]. While these studies focused on the hypertrophied LV and RV; it is important to note that in the volume overload model the atria also undergo significant hypertrophy. Therefore, we sought to determine whether there are differential changes in PLC isozyme gene expression between the three cardiac chambers. Since the renin-angiotensin system (RAS) is activated in cardiac hypertrophy due to volume overload [20, 21], the role of RAS in the PLC isozyme gene expression was examined by testing if blockade of the angiotensin II (Ang II) type 1 receptor (AT₁) with losartan attenuates the changes in PLC isozyme gene expression due to volume overload induced by AV shunt. This study is the first to report the time-dependent increases in PLC isozyme expression in the atria and ventri-

cles of volume-overloaded hearts and that early initiation of losartan treatment partially attenuates alterations in gene expression for only some of the PLC isozymes.

Material and methods

Experimental model

All experimental protocols for animal studies were approved by the Animal Care Committee of the University of Manitoba, following the guidelines established by the Canadian Council on Animal Care. An AV shunt was performed in male Sprague-Dawley rats (weighing 150–200 g) [17, 18, 20–22]. Briefly, the animals were anesthetized with 5% isoflurane with a flow rate of oxygen (2 l/min). After the abdominal fur was shaved, an abdominal laparotomy was performed. Following exposure of the abdominal aorta and inferior vena cava between the renal arteries and ileac bifurcation, the descending aorta and the ileac bifurcation was temporarily occluded proximal to the intended puncture site. An 18-gauge needle was inserted and withdrawn across the medial wall of the descending aorta three times to ensure the size and presence of the shunt and finally withdrawn. The puncture site was then immediately sealed with a drop of isocyanate (Krazy glue). The creation of the shunt was visualized by the pulsatile flow of oxygenated blood into the vena cava from the abdominal aorta. Throughout the operative procedure, the rats were maintained on 2.5% isoflurane in 2 l/min of oxygen. Age-matched, sham operated animals served as controls and were treated similarly, except that the puncture into the descending aorta was not performed. The animals were allowed to recover and were maintained on food and water *ad libitum*. The circulation system was only occluded for 25 s–1 min and the entire procedure was finished within 10 min. It is pointed out that the mortality rate of the control group was 0% and the mortality rate of the AV shunt animals operated on in this manner was less than 4% during 6 hrs after the surgery; thereafter no mortality was seen in either group as a result of the surgical procedure. The AT₁ receptor antagonist, losartan was administered (20 mg/kg body weight [20, 21], immediately after the surgery by oral gavage and was then given daily for the duration of the study) to some randomly chosen animals that had been induced with volume overload.

Table 1 General characteristics of sham control and AV shunt rats with or without losartan treatment for different time intervals.

	BW g	RVW mg	Atria wt mg	LVW mg	RVW/BW mg/100g	Atria/BW mg/100g	LVW/BW mg/100g
3 day							
Sham	263 ± 5	191 ± 8	123 ± 9	593 ± 12	73 ± 3	47 ± 3	225 ± 3
AV	258 ± 4	225 ± 10	159 ± 9*	655 ± 14*	87 ± 2*	62 ± 6*	253 ± 5*
AV + LOS	288 ± 4	225 ± 9	150 ± 13	631 ± 9	78 ± 3	52 ± 4	219 ± 3 [#]
1 wk							
Sham	267 ± 8	186 ± 10	169 ± 23	581 ± 12	70 ± 9	63 ± 16	218 ± 10
AV	251 ± 10	233 ± 14 *	218 ± 35 *	721 ± 13 *	93 ± 12 *	87 ± 23 *	287 ± 10 *
AV + LOS	242 ± 3	196 ± 9 [#]	121 ± 10 [#]	640 ± 15 [#]	81 ± 4	50 ± 4 [#]	264 ± 6 [#]
2 wks							
Sham	358 ± 12	191 ± 8	186 ± 12	694 ± 12	54 ± 10	52 ± 12	194 ± 10
AV	344 ± 20	283 ± 27 *	270 ± 10 *	1027 ± 14 *	83 ± 14 *	79 ± 15 *	299 ± 10 *
AV + LOS	339 ± 5	237 ± 9 [#]	166 ± 11 [#]	840 ± 13 [#]	74 ± 4	52 ± 4 [#]	248 ± 15 [#]
4 wks							
Sham	440 ± 21	249 ± 9	194 ± 18	875 ± 23	57 ± 15	44 ± 10	199 ± 10
AV	442 ± 11	360 ± 20 *	313 ± 23 *	1350 ± 31 *	82 ± 16 *	71 ± 17 *	305 ± 20 *
AV + LOS	436 ± 8	337 ± 11 [#]	185 ± 14 [#]	1065 ± 25 [#]	77 ± 5	42 ± 3 [#]	244 ± 5 [#]

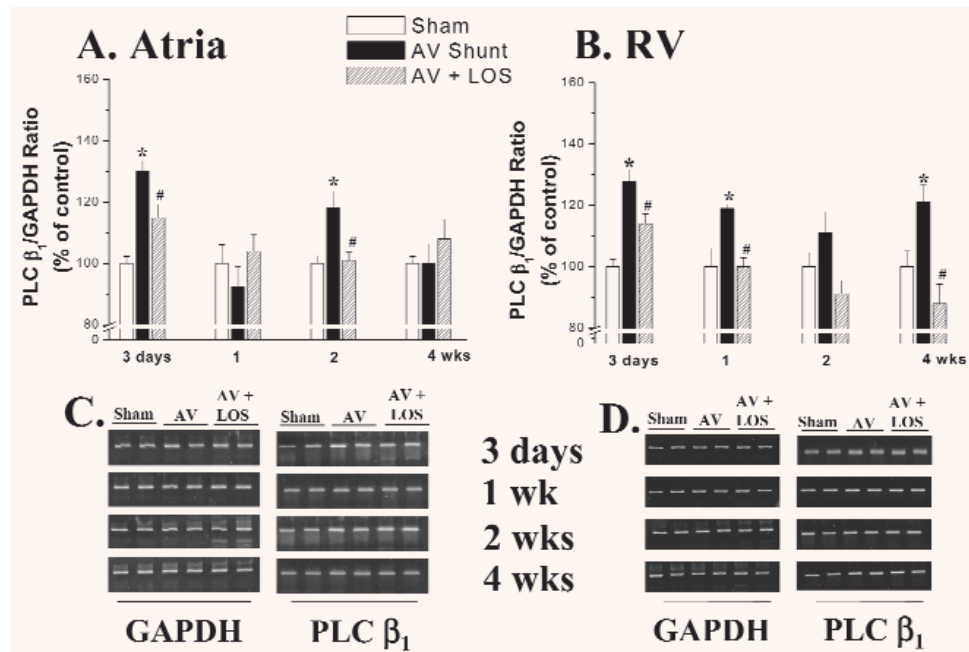
Data are mean ± S.E. of 12–14 animals for each group. BW, body weight; RVW, right ventricular weight; LVW, left ventricular weight; AV, arteriovenous; LOS, losartan. * P < 0.05 vs. sham control. [#] P < 0.05 vs. AV shunt.

RNA isolation and semi-quantitative PCR

Total RNA was isolated from atrial, RV and LV tissues using an RNA isolation kit (Life Technologies, ON, Canada) according to the manufacturer's procedures. Reverse transcription (RT) was conducted for 45 min at 48°C using the Superscript Preamplification System for First Strand cDNA Synthesis (Life Technology, ON, Canada) as previously described [17]. Primers used for amplification were synthesized as follows: PLC β_1 : 5'-AATAAGGAGACGGAGCTGTTAG-3' (forward) and 5'-ATGGAAGACAAGCCTCTAGCG-3' (reverse), PLC β_3 : 5'-TTGGAATCTTCGAGCGGTT-3' (forward) and 5'-AGGAACTGTTTGTTCGGCTCAT-3' (reverse), PLC γ_1 : 5'- CCTCTATGGAATGGAATTCCG-3' (forward) and 5'- CTAGGAGGACTCGCTGGAGAACT-3' (reverse) and PLC δ_1 : 5'- AGGATCGATGCTTCTCATTGT-3' (forward), 5'-TTATCAGCCTTCGCAAGCA-3' (reverse). Amplification of cDNAs of PLC isozyme genes was performed using specific primers and the Superscript Preamplification System (Life

Technology, ON, Canada). Temperatures used for PCR were as follows: denaturation at 94°C for 30 s, annealing at 62°C for 60 s, and extension at 68°C for 120 s, with a final extension for 7 min; 25 amplification cycles for each individual primer sets was carried out. For the purpose of normalization of the data, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers, 5'-TGAAGGTCGGTGTCAACGGATTTGGC-3' (forward) and 5'-GCATGTCAGATCCACAACGGATAC-3' (reverse) were used to amplify GAPDH gene as a multiplex with the target genes. It is pointed out that in previous studies [16, 17] we have established that 25 amplification cycles with the same amount of cDNA for each PLC isozyme and GAPDH primer is within the linearity range of RT-PCR. The RT-PCR products were analyzed by electrophoresis in 2% agarose gels. Staining of nucleic acids was performed with Vistra Green, which is up to 10 times more sensitive than ethidium bromide on a UV transilluminator with high signal-to-noise ratio and permits detection of < 20 pg/band. The intensity of the bands was photographed and quantified using a

Fig. 1 PLC β_1 gene expression in Atria (A) and RV (B). Representative blots of atrial (C) and RV (D) PLC β_1 isozyme mRNA levels in hearts of rats after induction of volume overload with or without treatment with losartan; LOS, losartan, * significantly different ($P < 0.05$) from sham control. # significantly different ($P < 0.05$) from AV shunt. Quantified data are means \pm SEM of 5 different experiments.



Molecular Dynamics STORM scanning system (Amersham Biosciences Corp., PQ, Canada) as a ratio of a target gene over GAPDH.

Statistical analysis

All values are expressed as mean \pm SEM. The differences between two groups were evaluated by Student's *t*-test. The data from more than two groups were evaluated by one-way analysis of variance (ANOVA) followed by Duncan's multiple comparison tests. A probability of 95% or more ($P < 0.05$) was considered significant.

Results

General characteristics

The time course of changes in the general characteristics of the control animals and animals with AV shunt treated with or without losartan are shown in Table 1. Although there was no significant difference in body weight among the sham, AV shunt and treatment groups at each time interval, both atrial and RV weights in the experimental group increased progressively during the 3 day to 4 weeks. Consistent with our ear-

lier study [17], LV mass was also observed to increase progressively during these time points. Hypertrophy of heart chambers was evident by an increase in the atrial, RV and LV weights to body weight ratios. Treatment with losartan (20 mg/kg body weight, daily) partially attenuated the atrial, RV and LV hypertrophy in this volume overload experimental model.

PLC isozyme mRNA levels in hypertrophied atria, RV and LV from volume-overloaded hearts

The data in Figs. 1 to 4 show differential and time-dependent changes in PLC β_1 , β_3 , γ_1 and δ_1 isozyme gene expression in atrial and RV hypertrophy due to volume overload. Biphasic changes in the expression of PLC β_1 isozyme were seen in the atria and RV. In this regard, while an early increase (3 day) in the PLC β_1 mRNA expression level was seen in both the atria and RV, a second peak of increased PLC β_1 mRNA level was seen at 2 weeks, post-AV shunt in the atria, whereas it was at 4 weeks in the RV. In contrast, increased PLC β_3 mRNA levels were seen in the atria and RV at 3 days after the induction of the AV shunt. While PLC β_3 gene expression was increased at this time point in the atria, the peak in the increase in the RV PLC

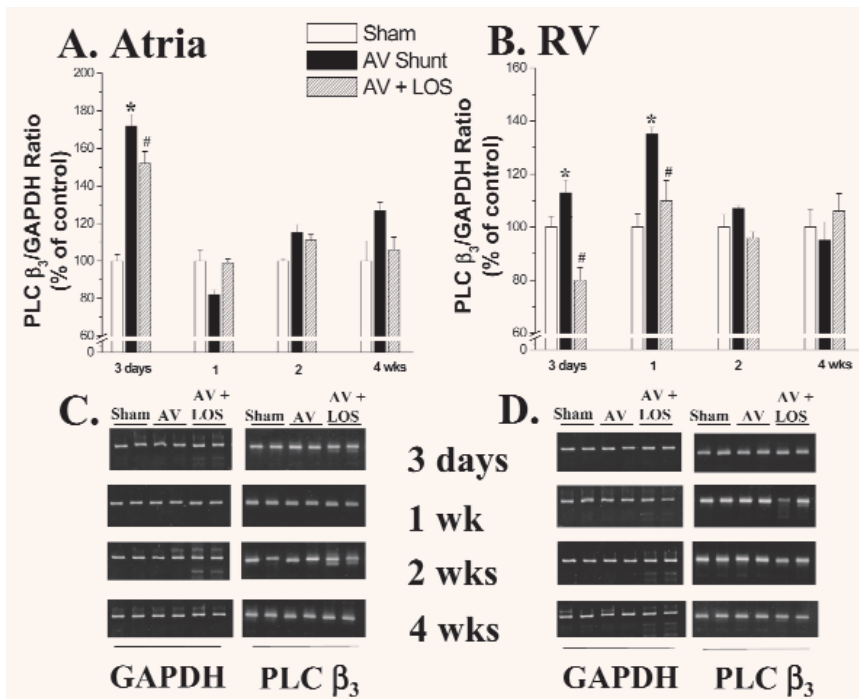


Fig. 2 PLC β_3 gene expression in Atria (A) and RV (B). Representative blots of atrial (C) and RV (D) PLC β_3 isozyme mRNA levels in hearts of rats after induction of volume overload with or without treatment with losartan; LOS, losartan, * significantly different ($P < 0.05$) from sham control. # significantly different ($P < 0.05$) from AV shunt. Quantified data are means \pm SEM of 5 different experiments.

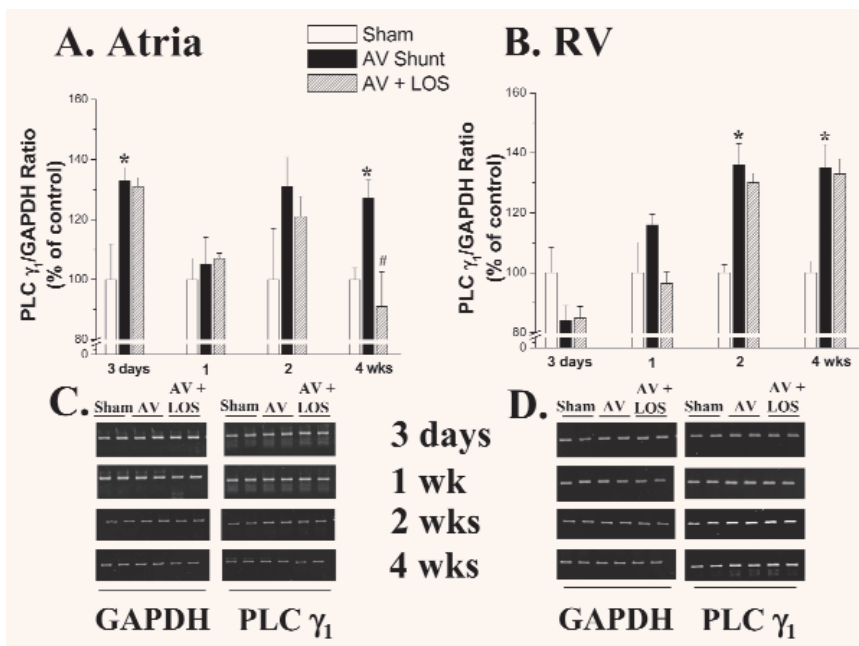
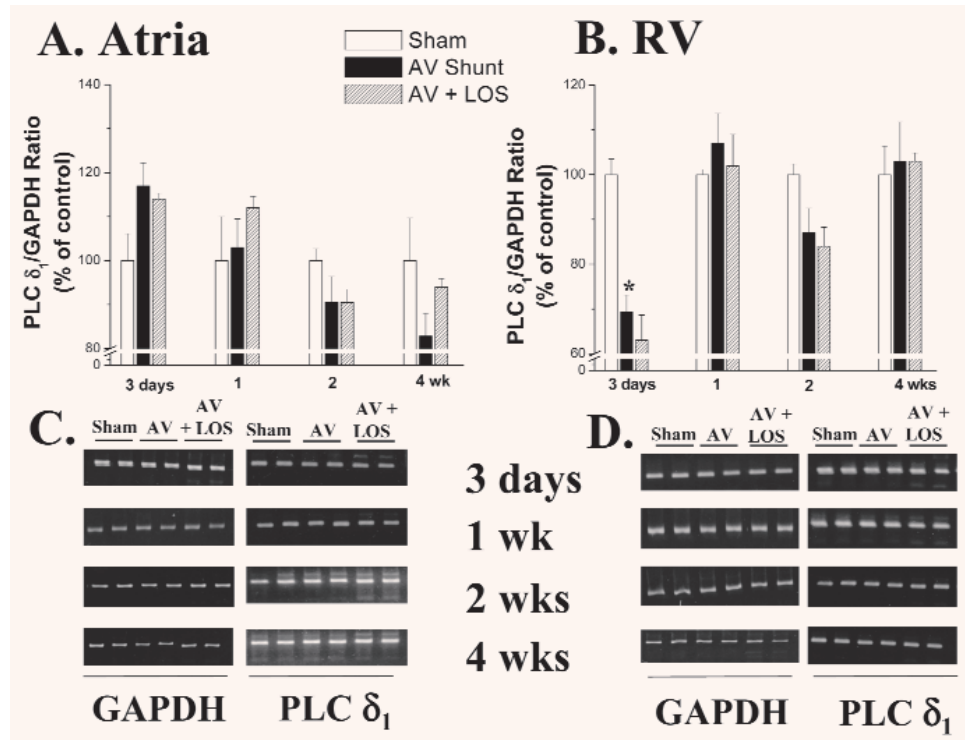


Fig. 3 PLC γ_1 gene expression in Atria (A) and RV (B). Representative blots of atrial (C) and RV (D) PLC γ_1 isozyme mRNA levels in hearts of rats after induction of volume overload with or without treatment with losartan; LOS, losartan, * significantly different ($P < 0.05$) from sham control. # significantly different ($P < 0.05$) from AV shunt. Quantified data are means \pm SEM of 5 different experiments.

β_3 gene expression was observed at 1 week. A biphasic increase in the PLC γ_1 mRNA levels was also detected in the atria, where an early increase occurred at 3 days followed by a second peak at 4 weeks post-surgery. On the other hand, a sustained increase in the PLC γ_1 gene expression at 2 and 4 weeks was seen in the RV. Interestingly, while no changes in PLC δ_1 mRNA levels were detected in the atria, a significant decrease in PLC δ_1 mRNA

levels was detected in the RV only at 3 days post-AV shunt. Consistent with our earlier findings [17], an increase in PLC β_1 mRNA levels was seen in the LV at 4 weeks after the induction of the AV shunt. In the present study, we also determined the PLC β_3 mRNA level in the LV, which was also found to be significantly elevated early at 3 days, and then again at 4 weeks after the induction of the AV shunt. On the other hand, while a delayed increase in the

Fig. 4 PLC δ_1 gene expression in Atria (A) and RV (B). Representative blots of atrial (C) and RV (D) PLC δ_1 isozyme mRNA levels in hearts of rats after induction of volume overload with or without treatment with losartan; LOS, losartan, * significantly different ($P < 0.05$) from sham control. # significantly different ($P < 0.05$) from AV shunt. Quantified data are means \pm SEM of 5 different experiments.



PLC γ_1 mRNA level occurred at 2 and 4 weeks, an early (3 day) upregulation of PLC δ_1 , gene expression was detected in the LV.

Although losartan either prevented or partially attenuated most of the alterations in PLC isozyme mRNA expression levels in the atria and ventricles, some anomalies were also observed. In this regard, while the early increase in atrial PLC γ_1 expression was unaffected by losartan treatment, the 4 week increase in PLC γ_1 was prevented. Furthermore, losartan did not prevent the increases in the RV PLC γ_1 mRNA levels (at 2 and 4 weeks) as well as the PLC γ_1 mRNA levels in the LV (4 weeks). Also, treatment with losartan did not correct the decrease in RV PLC δ_1 mRNA levels seen in the RV at 3 days after AV shunt.

Discussion

The needle technique used in this study to induce volume overload produces a reproducible animal model of eccentric cardiac hypertrophy of the LV [17, 18, 20–22], as well as significant atrial and RV hypertrophy resembling that occurring in humans during hyperthyroidism, anemia, and bundle branch

block [22]. Although cardiac hypertrophy may initially be a beneficial response, it can also be viewed as a stepping-stone or precursor to the development of heart failure [17, 23–26]. In the myocardium, PLC is considered to play a role in the development of myocardial hypertrophy [15, 17]. In fact, we have previously demonstrated that inhibition of PLC activities with U73122 attenuates norepinephrine-induced increases in atrial natriuretic factor gene expression and protein synthesis in isolated adult rat cardiomyocytes [16]. We have also shown earlier that hypertrophy of the LV due to volume overload is associated with increases in PLC isozyme gene and protein expression as well as activities [17]. Since the atria and RV also undergo significant hypertrophy in this model, it was decided to examine the time-dependent status of PLC isozyme gene expression in the atria and RV. These data provide information on whether the increases in PLC isozyme gene expression are generalized phenomena in all cardiac chambers during cardiac hypertrophy due to volume overload or if the increases in PLC isozyme mRNA levels are specific to the LV. Since RAS is activated in cardiac hypertrophy due to volume overload [20, 21], the role of RAS in PLC isozyme gene expression was examined by testing whether losartan attenuates the changes in PLC

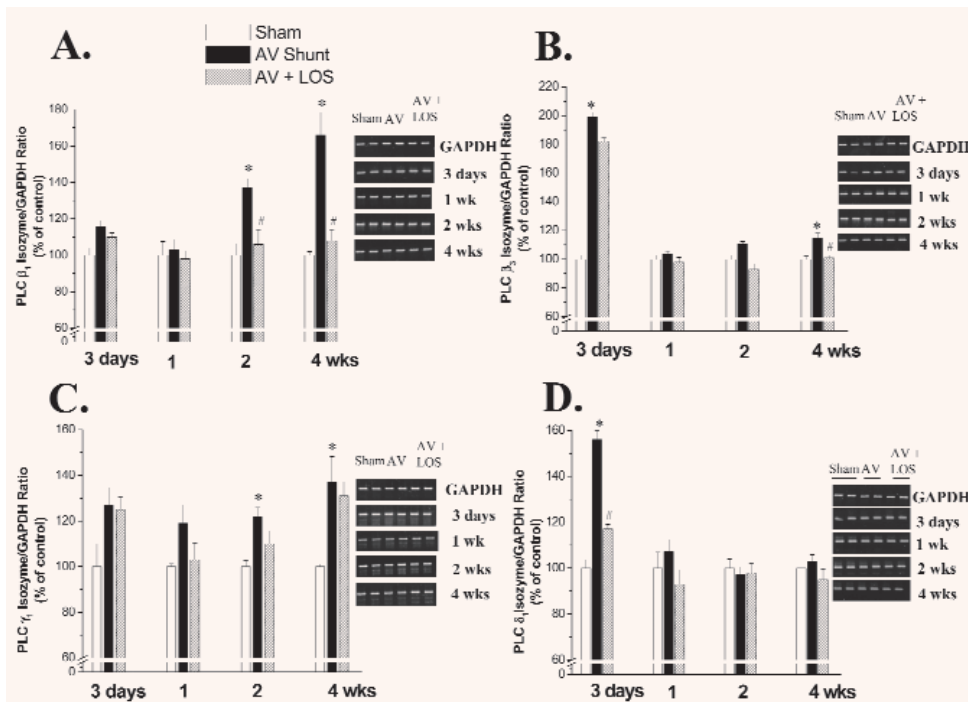


Fig. 5 Respective LV PLC isozyme gene expression after the induction of volume overload with or without treatment with losartan (A-D). LOS, losartan, * significantly different ($P < 0.05$) from sham control. # significantly different ($P < 0.05$) from AV shunt. Quantified data are means \pm SEM of 5 different experiments.

isozyme gene expression with a resultant attenuation in cardiac hypertrophy due to volume overload induced by AV shunt. This is the first study to show that an AT_1 receptor antagonist, losartan, attenuates PLC isozyme gene expression and is correlated to regression of cardiac hypertrophy. Furthermore, this study has shown that PLC isozymes may have a significant role in hypertrophy of the atria and ventricles due to volume overload.

PLC β_1 , β_3 , γ_1 and δ_1 isozyme gene expression was examined at 3 days, 1, 2 and 4 weeks post-AV shunt to determine whether there is a time-dependent effect on PLC isozyme gene expression after the induction of volume overload. It is conceived that there are specific alterations in the PLC isozyme gene expression depending on the exposure time to volume overload. For example, PLC isozyme gene expression may be increased or decreased at the initiation (3 day) of cardiac hypertrophy and/or increased or decreased during the development (1–4 weeks) of cardiac hypertrophy. In addition to examining the time-dependent effect on PLC gene expression in the atria, RV and LV, we also sought to determine whether there are differences in PLC isozyme expression between the cardiac chambers. Our findings demonstrate an early (3 days after induction of AV shunt) increase in PLC β_1 and β_3 isozyme mRNA levels in the atria

and RV, whereas an increase in PLC β_3 and δ_1 was seen in the LV at the same time point. Also, the increase in PLC β_1 expression in the atria and RV were biphasic in nature, which was different to the profile of the expression of PLC β_1 in LV, which occurred at 2 and 4 weeks. On the other hand, a specific early activation of PLC γ_1 was seen only in the atria which, coupled to the increase in PLC β isozyme mRNA expression, may reflect the greater degree of hemodynamic overload and thus stretch encountered by the atria. Another notable difference is that while the atrial PLC δ_1 expression was unaltered, its expression was significantly decreased in the RV and increased in the LV at 3 days after AV shunt. It is pointed out that while the RV expression of PLC δ_1 was decreased only at 3 days after the induction of AV shunt, it was elevated in the LV at the same time point; however, the significance of this change is presently unclear. Although the profile of the increases in PLC isozyme gene expression are chamber specific and time-dependent when inducing cardiac hypertrophy due to AV shunt, it appears that the increase in PLC isozyme mRNA levels may indeed be a common feature occurring in atrial, RV and LV hypertrophy due to volume overload. A limitation of our work is that the PLC isozyme protein contents have not been measured in the hypertrophied RV and atria, however; in our

earlier study with this model [17] the changes in LV PLC isozyme mRNA level did not always correlate with their encoding protein levels. In view of the small changes in PLC isozyme mRNA levels in the ventricles and atria, it could be suggested that the synthesis or stability of PLC proteins may play a predominant role in controlling the AV shunt-induced regulation of PLC activities. While this is beyond the scope of the present study, it does warrant future investigation.

In this study, treatment of animals with losartan was found to partially prevent cardiac hypertrophy, which is in agreement with other investigators as well as with other AT₁ receptor antagonists [27–32] using this model. While some of these investigators [27, 28] also reported attenuation of the increased expression of a number of cardiac genes including atrial natriuretic peptide and collagen types I and III in volume overloaded hearts, the attenuation of cardiac hypertrophy may be related to partial attenuation of some of the PLC isozyme gene expression by the action of losartan. This indicates that RAS is partially involved in inducing cardiac hypertrophy and increases in specific PLC isozyme gene expression. It should be noted, however, that losartan had no effect on the early and late increases in atrial and RV PLC γ_1 as well as the early increase in LV PLC β_3 expression levels suggesting that the sympathetic nervous system and/or other hypertrophic stimuli may be involved in regulating PLC isozymes gene expression.

Since changes in some of the PLC isozymes were affected partially while others were unaffected by losartan treatment in hypertrophied hearts, it appears that both RAS dependent and independent mechanisms may be involved in inducing changes in PLC isozyme gene expression in this experiment model. Although the exact mechanism for changes in cardiac PLC isozyme gene expression remains to be defined, increased RAS and sympathetic nervous system activities and subsequent alterations in signal transduction mechanisms have been suggested to play an important role in the genesis of these molecular changes [33, 34]. An early increase in angiotensinogen mRNA levels as well as that Ang II is the first growth factor produced in volume overloaded hearts has been reported [35]. In addition, both growth hormone and insulin-like growth factor-1 (IGF-1) both have

been shown to induce a substantial increase in PLC β_3 mRNA expression [15]. Interestingly, growth hormone and IGF-1 are both increased in the volume overloaded heart [35, 36] and therefore may also be involved in the increased expression of PLC β isozymes seen in the present study. Because PLC is documented to play a role in cardiac hypertrophy [11–17], this study has shown that the chamber specific and time-dependent increases in PLC isozyme gene expression may be part of the amplification of the PLC mediated signal transduction processes involved in cardiac hypertrophy. It should be noted that the regression of cardiac hypertrophy in experimental animals may indicate a causal relationship between PLC function and losartan therapy. However, it remains to be established if PLC correction is due to an attenuation of the effects of Ang II or to the hemodynamic effects following losartan treatment. In view of the fact that the AT₁ receptor is coupled to the α -subunit of the heterotrimeric Gq family and known to transduce its signal to PLC β isozymes as well as the fact that Ang II can activate PLC β and γ isozymes [4, 37–39], it is conceivable that PLC isozymes could constitute a mechanism of action of losartan. Furthermore, it is important to mention that we have recently reported that PLC activities may regulate their own gene expression through a PKC and ERK 1/2 –dependent pathway [40], which could represent a cycle that perpetuates the hypertrophic response.

Conclusion

Although it is known that LV PLC isozyme mRNA levels are increased in the initiation of volume overload induced hypertrophy of the LV, the present study has demonstrated that there is also an increased expression of PLC β and γ isozymes in the initiation of atrial and RV hypertrophy. Since the increases in PLC isozyme mRNA levels occurs, in part, in response to the activation of RAS and were partially attenuated by losartan, it can be suggested that the pharmacological modulation of the PLC signal transduction pathway may constitute novel therapeutic targets for the prevention of cardiac hypertrophy and subsequent progression to heart failure.

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