Attenuation of Lysophosphatidylcholine-Induced Suppression of ANP Release From Hypertrophied Atria

Jeong Hee Han, Chunhua Cao, Soo Mi Kim, Feng Lian Piao, Suhn Hee Kim

Abstract—Lysophosphatidylcholine (LPC) is an endogenous phospholipid released from the cell membrane during ischemia, and it has potent cardiac effects, including inhibition of atrial natriuretic peptide (ANP) release. The aim of this study was to investigate the effects of LPC on hemodynamics and ANP release in hypertrophied atria and to define its mechanism. Isolated, perfused, beating, hypertrophied atria from monocrotaline-treated rats were used. LPC (30 μmol/L), a mixture of stearoyl-LPC, palmitoyl-LPC, and oleoyl-LPC, caused suppression of ANP release, which was markedly attenuated in hypertrophied atria compared with nonhypertrophied atria. Suppression of ANP release by stearoyl-LPC, palmitoyl-LPC, or oleoyl-LPC was also attenuated in hypertrophied atria. The potency appeared to be dependent on the species of fatty acid residue of LPC. Changes in ANP release by LPC, palmitoyl-LPC, and oleoyl-LPC were positively correlated with the degree of cardiac hypertrophy, but that by stearoyl-LPC was not. Changes in ANP release by LPC also were negatively correlated with changes in pulse pressure. Stearoyl-LPC caused an increase in intracellular Ca²⁺ in single, atrial myocytes in a concentration-dependent manner, which was markedly attenuated in hypertrophied atrial myocytes. These results suggest that attenuation of LPC-induced suppression of ANP release from hypertrophied atria might partly be related to changes in pulse pressure in terms of cardiac hypertrophy and/or disturbance of intracellular Ca²⁺ regulation. (Hypertension. 2004;43:243-248.)

Key Words: calcium ▪ hypertrophy ▪ natriuretic peptides ▪ hypertension

Lysophosphatidylcholine (LPC) is naturally formed by phospholipase A₂-induced hydrolysis of a main membrane phospholipid, phosphatidylcholine. LPC is produced during normal phospholipid turnover and accumulates rapidly during myocardial ischemia. LPC produces potent, reversible, and localized cardiac effects, such as membrane depolarization, modulation of the cardiac Na⁺ current, and arrhythmogenesis. These cardiac effects are directly or indirectly related to an increase in intracellular Ca²⁺ ([Ca²⁺]). Recently, we reported LPC-induced suppression of atrial natriuretic peptide (ANP) release through the protein kinase C-Ca²⁺ and phosphoinositol 3-kinase pathway. Atrial cardiomyocytes are involved in both mechanical and endocrine functions of the heart, which are mainly mediated by [Ca²⁺]. Ca²⁺ might be one of the most important factors affecting ANP secretion, even though controversy still exists.

Abnormal [Ca²⁺], handling has been described in various cardiac diseases associated with hypertrophy and during ischemia. It has been reported that Ca²⁺ overload in hypertrophied ventricular myocytes might be related to an increased Ca²⁺ influx through Ca²⁺ channels as well as reduced Ca²⁺ reuptake by the sarcoplasmic reticulum. An increase in myocardial [Ca²⁺], in the hypertrophied heart has been proposed as a major mediator of the structural deterioration of the myocardium and has been implicated in the pathogenesis of contractile dysfunction and arrhythmia in the failing heart. Cardiac hypertrophy is the fundamental process of adaptation to an increased workload due to hemodynamic overload and is known to activate the cardiac ANP system, with a subsequent high plasma concentration as one of the cardiac compensatory mechanisms. Modifications of ANP release by endothelin-1 and C-type natriuretic peptide are reported in hypertrophied atria. However, it is not clear whether LPC-induced suppression of ANP release is modified by atrial hypertrophy. The aim of the present study was to investigate the effect of LPCs on atrial hemodynamics and ANP release in hypertrophied atria and to define its mechanisms.

Methods

Animals

Male Sprague-Dawley rats (Daehan Biolink Co. Ltd, Korea) weighing 230 to 250 g were used. Rats were given a single subcutaneous injection of 50 mg/kg monocrotaline (MCT) or vehicle. Right atrial hypertrophy developed 4 to 5 weeks after MCT injection as a consequence of pulmonary hypertension.

Preparation of Perfused, Beating, Rat Atria

Isolated, perfused, beating atria were prepared by a previously described method. In brief, the right atrium was dissected from the...
Atrial Myocytes

Effects of LPC on ANP Secretion From Hypertrophied Atria

The concentration of ANP in the perfusate was measured by a radioimmunoassay, as described previously.[29] Measurement of [Ca2+]i, Concentration in Single, Atrial Myocytes

Single, atrial myocytes from the right atria of control and MCT-treated rats were isolated, and changes in [Ca2+]i were measured with a fluorescence digital imaging microscopic system, as described previously.[18,31] Statistical Analysis

Results are given as mean±SEM. Statistical significance of differences was assessed by repeated-measures ANOVA (Figure 1) or ANOVA (Figures 2 and 4), followed by Dunnett multiple-comparison test. Student unpaired t test was also used (Figures 2 and 4). The critical level of significance was set at P<0.05.

Results

Effects of LPC on ANP Secretion From Hypertrophied Atria

Tissue weights of hypertrophied atria averaged 41.94±3.06 mg (n=36), which was significantly higher than nonhypertrophied atria (24.6±0.27 mg; n=31, P<0.001). Basal ANP secretion, ECF translocation, and interstitial ANP concentration in hyper-trophied atria were 21.20±1.62 ng·min⁻¹·mg⁻¹, 52.71±4.66 μL·min⁻¹·mg⁻¹, and 0.15±0.01 μmol/L, respectively, which were significantly lower than for nonhypertrophied atria (32.9±1.58 ng·min⁻¹·mg⁻¹, P<0.001; 65.26±4.04 μL·min⁻¹·mg⁻¹, P<0.025; and 0.17±0.01 μmol/L, P<0.05, respectively). Basal ANP secretion and ECF translocation from hypertrophied atria were inversely correlated with atrial wet weight (y = −0.34x + 35.33, r² = 0.34, P<0.001; and y = −0.87x + 89.35, r² = 0.31, P<0.001). Pulse pressure, the difference between systolic and diastolic atrial pressure, was similar in both types of atria.

Figure 1 shows the effect of LPC on pulse pressure, ECF translocation, ANP secretion, and ANP concentration in hypertrophied atria from MCT rats compared with nonhypertrophied atria form normal rats. In both groups of atria, pulse pressure, ECF translocation, and ANP secretion were relatively constant throughout the experiment (Figure 1). After stabilization, the perfusate was collected 5 times every 2 minutes to serve as a control period, and then LPC was infused at a concentration of 30 μmol/L. During the period of LPC infusion, pulse pressure and ECF translocation did not change significantly (Figure 1A and 1B). In nonhypertrophied atria, ANP secretion and interstitial ANP concentration, which was calculated from the ANP secretion rate divided by ECF translocation and the molecular weight of ANP, were markedly decreased with time by </body>
hypertrophied atria compared with nonhypertrophied atria (Figure 2A and 2B).

Attenuation of LPC-induced suppression of ANP release appeared to be more prominent in the hypertrophied atria. Therefore, to determine whether attenuation of the LPC-induced suppressive effect on ANP release in hypertrophied atria was related to cardiac hypertrophy, the relative changes in ANP concentration by different types of LPCs were plotted against the degree of cardiac hypertrophy, as shown in Figure 3. The ratio of right ventricle to left ventricle and septum was positively correlated with changes in ANP concentration by LPC, palmitoyl-LPC, and oleoyl-LPC (Figure 3A). However, no significant correlation between changes in ANP concentration by stearoyl-LPC and cardiac hypertrophy was found ([LaTeX]r^2/[LaTeX] 0.14). A close negative correlation was found between the relative changes in ANP concentration and pulse pressure by LPC (Figure 3B) but not by other types of LPCs (oleoyl-LPC, [LaTeX]r^2/[LaTeX] 0.38; palmitoyl-LPC, [LaTeX]r^2/[LaTeX] 0.18; and stearoyl-LPC, [LaTeX]r^2/[LaTeX] 0.02).

Effects of LPC on [Ca^{2+}] in Single Myocytes From Hypertrophied Atria
Changes in [Ca^{2+}] by LPC, palmitoyl-LPC, and stearoyl-LPC were measured in single, beating, atrial myocytes from hypertrophied and control atria. Basal [Ca^{2+}] in atrial myocytes from control rats was 139.2±8.2 nmol/L (n=20) and that from hypertrophied atria was 148.2±11.4 nmol/L (n=25). As shown in Figure 4, stearoyl-LPC at doses of 10 and 30 μmol/L caused increases in [Ca^{2+}], which were greater than that by LPC. Palmitoyl-LPC caused a slight increase in [Ca^{2+}], which was not different from that by LPC. An increase in [Ca^{2+}] by stearoyl-LPC was attenuated in hypertrophied atrial myocytes (Figure 4).

Discussion
The present study clearly shows an attenuation of LPC-induced suppression of ANP release in hypertrophied atria, which is closely related to the degree of cardiac hypertrophy. LPC produced during normal phospholipid turnover accumulates rapidly in the coronary sinus or in effluents by 2-fold during myocardial ischemia. LPC has been known to have various cardiac effects and to be related to the development of hypertension and atherosclerosis. Recently, we found that LPC at a dose of 30 μmol/L caused an ~60% reduction in ANP release with a slight increase in intra-atrial pressure. The inhibitory effect of LPC on ANP secretion might be partially related to changes in [Ca^{2+}]. The LPC used in this study was a mixture of stearoyl-, palmitoyl-, and oleoyl-LPC. Therefore, we compared the potency of different types of LPCs. The suppression of ANP release was observed in stearoyl-LPC−, palmitoyl-LPC−, and oleoyl-LPC−-infused groups. Compared with a previous report, the potency of the LPC effect was similar to that of stearoyl-LPC and palmitoyl-LPC, whereas that of oleoyl-LPC was similar to the effect of lauroyl-LPC and myristoyl-LPC. The potency appears to be dependent on the species of fatty acid residue of LPC.
Abnormal \([\text{Ca}^{2+}]\), handling has been described in various cardiac diseases associated with hypertrophy,\(^{19}\) and the suppressive effect of LPC on ANP secretion has been reported to be partly related to \([\text{Ca}^{2+}]\).\(^{14}\) Therefore, we investigated the possible modification of LPC effects on atrial hemodynamics and ANP secretion in hypertrophied atria. Surprisingly, LPC caused an \(\approx25\%\) reduction in ANP release in hypertrophied atria, which was markedly attenuated compared with the 60% suppression of ANP secretion observed in control atria. The decreases in ANP secretion might be partially related to changes in \([\text{Ca}^{2+}]\). The relative changes in ANP concentration by LPC were positively correlated with the degree of cardiac hypertrophy and negatively with the changes in pulse pressure. In other words, the greater the degree of atrial hypertrophy, the greater the attenuation of the inhibitory effect of LPC on ANP secretion. The relative change in ANP concentration by palmitoyl-LPC or oleoyl-LPC was also positively correlated with the degree of cardiac hypertrophy but not with changes in pulse pressure. However, attenuation of the suppressive effect of ANP secretion by stearoyl-LPC showed a lack of correlation with either cardiac hypertrophy or changes in pulse pressure. Our results show that change in intra-atrial pressure is one of the important factors involved in the attenuation of LPC effects. Additionally, other undefined factors related to cardiac hypertrophy, such as receptor downregulation or disturbance of a signaling pathway (protein kinase C–\(\text{Ca}^{2+}\) and phosphoinositol 3-kinase pathway)\(^{14}\) are also responsible for those effects.

LPC is known to alter cellular \(\text{Ca}^{2+}\) homeostasis. LPC causes an accumulation of \([\text{Ca}^{2+}]\), in a dose-dependent manner in ventricular myocytes.\(^{12,13}\) LPC also causes \(\text{Ca}^{2+}\) efflux from isolated, rat ventricular myocytes through the \(\text{Na}^{+}–\text{Ca}^{2+}\) exchanger.\(^{33}\) We demonstrated previously that LPC slightly increases \([\text{Ca}^{2+}]\), in single, atrial myocytes in a dose-dependent manner.\(^{14}\) However, the increase in \([\text{Ca}^{2+}]\), in atrial myocytes by LPC was small compared with that in ventricular myocytes.\(^{12,13}\) Nevertheless, the suppression of ANP release by LPC was prominent. In atrial myocytes from hypertrophied hearts, increases in \([\text{Ca}^{2+}]\) were significantly attenuated. Time indicates time control group. Values are mean\(\pm\)SEM. Other abbreviations are the same as in Figure 1. *\(P<0.05\), **\(P<0.01\) vs group infused with LPC. #\(P<0.05\), ###\(P<0.001\), ####\(P<0.005\) vs nonhypertrophied atria infused with the same type of LPC.

What is the physiologic significance of the attenuation of LPC-induced suppression of ANP secretion by atrial hypertrophy? We have already reported the modification of ANP secretion from hypertrophied atria characterized by accentuation of the stimulatory effect of endothelin-1 and attenuation of the inhibitory effect of C-type natriuretic peptide.\(^{27,28}\) In this study, we found an attenuation of the inhibitory effect of LPC on ANP secretion. Taken together with the aforementioned data, ANP secretion from hypertrophied atria appears to be activated by attenuating the inhibitory effects and accentuating the stimulatory effects on ANP secretion. It has been reported that disruption of the ANP receptor causes high blood pressure and cardiac hypertrophy as well as fibrosis.\(^{34,35}\) and ANP inhibits cardiomyocyte hypertrophy.\(^{36}\) Therefore, we speculate that activation of ANP secretion as well as synthesis\(^{24}\) and downregulation of a clearance receptor\(^{17}\) might be explained as a cardiac compensatory response.
to reduce overload in established pulmonary hypertension via
dilation of pulmonary arterioles and diuresis. The activated ANP system might be involved in the regulation of
cardiac hypertrophy or fibrosis to reduce energy consumption of the heart, even though more studies for its exact mechanisms are needed.

In conclusion, we suggest that attenuation of the LPC-induced suppression of ANP release by atrial hypertrophy might partially be related to alterations in the responsiveness of pulse pressure and \( [Ca^{2+}]_i \) to LPCs and other as yet undefined factors.

**Perspectives**

LPC is an endogenous phospholipid released from the cell membrane during ischemia. LPC has potent and localized cardiac effects and is related to the development of hypertension. In this study, LPC caused suppression of ANP release, and the potency appeared to be dependent on the species of fatty acid residue of LPC. The suppressive effects of LPC on ANP release are markedly attenuated in hypertrophied atria. The greater the degree of atrial hypertrophy, the greater the attenuation of the inhibitory effect of LPC on ANP release. This effect might partially be related to changes in pulse pressure in terms of cardiac hypertrophy and/or a disturbance in \( [Ca^{2+}]_i \) regulation. We speculate that the activation of ANP secretion might be explained as a cardiac compensatory response to reduce overload in established pulmonary hypertension via dilation of pulmonary arterioles and diuresis. In addition, the activated ANP system might also be involved in the regulation of cardiac hypertrophy or fibrosis to reduce energy consumption of the heart. If there were no activation of the ANP system in these conditions, pulmonary hypertension might be aggravated. However, more studies are needed to search for the undefined factor(s) related to cardiac hypertrophy other than the disturbance in \( [Ca^{2+}]_i \) regulation.

**Acknowledgments**

This work was supported by the Korea Health 21 R&D Project, Ministry of Health and Welfare (01-PJ1-PG1-01CH06-0003) and (02-PJ1-PG10-21401-0004).

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Hypertension. 2004;43:243-248; originally published online December 8, 2003;
doi: 10.1161/01.HYP.0000107779.92645.89
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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