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.1 LPC is produced during normal phospholipid turnover and accumulates rapidly during myocardial ischemia.2–5 LPC produces potent, reversible, and localized cardiac effects, such as membrane depolarization, modulation of the cardiac Na+ current, and arrhythmogenesis.6–10 These cardiac effects are directly or indirectly related to an increase in intracellular Ca2+(Ca2+).11–13 Recently, we reported LPC-induced suppression of atrial natriuretic peptide (ANP) release through the protein kinase C-Ca2+ and phosphoinositol 3-kinase pathway.14 Atrial cardiomyocytes are involved in both mechanical and endocrine functions of the heart, which are mainly mediated by [Ca2+]i, Ca2+ might be one of the most important factors affecting ANP secretion, even though controversy still exists.15–18

Abnormal [Ca2+]i handling has been described in various cardiac diseases associated with hypertrophy and during ischemia.19,20 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.21,22 An increase in myocardial [Ca2+]i 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.23 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.24–26 Modifications of ANP release by endothelin-1 and C-type natriuretic peptide are reported in hypertrophied atria.27,28 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.27

Preparation of Perfused, Beating, Rat Atria
Isolated, perfused, beating atria were prepared by a previously described method.14 In brief, the right atrium was dissected from the

Received September 30, 2003; first decision October 15, 2003; revision accepted November 6, 2003.
From the Department of Physiology, Medical School, Institute for Medical Sciences, Chonbuk National University, Jeonju, Korea.
Correspondence to Suhn Hee Kim, MD, PhD, 2-20 Keum-Am-Dong-San, Department of Physiology, Chonbuk National University Medical School, Jeonju 561-180, Korea. e-mail shkim@moak.chonbuk.ac.kr
© 2004 American Heart Association, Inc.
Hypertension is available at http://www.hypertensionaha.org
DOI: 10.1161/01.HYP.0000107779.92645.89
heart after euthanasia by decapitation, and sinoatrial nodal tissue was removed. A cannula containing 3 small catheters sealed within it was inserted into the atrium and secured by ligatures. The cannulated atrium was transferred to an organ chamber, immediately perfused with oxygenated HEPES buffer solution at 36.5°C, and paced at 1.3 Hz (duration 0.3 ms, 40 V), as described previously.18 Intra-atrial pressure was recorded on a physiograph via a pressure transducer, and pulse pressure was obtained as the difference between systolic and diastolic pressure. The composition of the HEPES buffer solution was as follows: NaCl 118 mmol/L, KCl 4.7 mmol/L, CaCl₂ 2.5 mmol/L, MgSO₄·7H₂O 1.2 mmol/L, NaHCO₃, 25 mmol/L, HEPES 10 mmol/L, glucose 10 mmol/L, and bovine serum albumin 0.1%. The pericardial buffer solution, which contained [³H]inulin to measure translocation of the extracellular fluid (ECF), was also oxygenated by placing silicone-tubing coils inside the organ chamber. The atrium was perfused for 100 minutes to stabilize the secretion of ANP and to maintain a steady-state [³H]inulin level in the extracellular space. Experiments were performed with 4 groups described below. After the experiments were completed, the perfused atrium was cut below the ligature, and tissue weight was measured.

Experimental Protocols
Experiments were performed with 4 groups. Group 1 was the time control atria from control rats (n=6). Group 2 included the LPC-perfused control atria. LPC (type V from bovine brain, Sigma; 30 μmol/L, n=7) was introduced into the atrial lumen after a 10-minute control collection period, and perfusate was collected for 60 minutes. Three types of LPCs (each 30 μmol/L), stearoyl-LPC (n=7), palmitoyl-LPC (n=7), and oleoyl-LPC (n=7), were also used. Group 3 was the time control, hypertrophied atria from MCT-treated rats (n=6). Group 4 was the LPC-perfused, hypertrophied atria. LPC (n=12) was introduced into the atrial lumen after a 10-minute control collection period, and perfusate was collected for 60 minutes. Three types of LPCs (each 30 μmol/L), stearoyl-LPC (n=8), palmitoyl-LPC (n=8), and oleoyl-LPC (n=7), were also used.

Radioimmunoassay of ANP
The concentration of ANP in the perfusate was measured by a specific radioimmunoassay, as described previously.29

Measurement of ECF Translocation
The radioactivity of [³H]inulin in the atrial perfusate was measured with a liquid scintillation counter, and the amount of ECF translocated through the atrial wall was calculated, as described elsewhere.30

Measurement of [Ca²⁺], Concentration in Single, Atrial Myocytes
Single, atrial myocytes from the right atria of control and MCT-treated rats were isolated, and changes in [Ca²⁺], were measured with a fluorescence digital imaging microscopic system, as described previously.30 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 Dunnnett 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 hypertrophied 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 arterial 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 ≈60% (Figure 1A). In hypertrophied atria, however, LPC caused decreases in ANP secretion and concentration by only 25% (Figure 1B).

Comparison of Suppressive Effects of ANP Release by Different Types of LPCs in Hypertrophied Atria
The LPC used in the present study was a mixture of stearoyl-LPC, palmitoyl-LPC, and oleoyl-LPC. To compare the inhibitory effect of different forms of LPC on ANP secretion, normal and hypertrophied atria were perfused with stearoyl-LPC, palmitoyl-LPC, or oleoyl-LPC. Figure 2A shows the relative percentage changes from the mean of 5 control values and the last 5 experimental values of animals exposed to LPC (from Figure 1) and the different types of LPCs in nonhypertrophied atria from normal rats. Increases in pulse pressure and ECF translocation by stearoyl-LPC were significantly higher than those by LPC (Figure 2A). Palmitoyl-LPC and oleoyl-LPC caused changes in pulse pressure and ECF translocation similar to that of LPC. Decreases in ANP secretion by stearoyl-LPC and palmitoyl-LPC were similar to that by LPC (Figure 2A). Oleoyl-LPC caused a decrease in ANP secretion, which was lower than that by LPC.

In hypertrophied atria, LPC and the various constituents of LPC did not cause any significant change in pulse pressure (Figure 2B). Stearoyl-LPC caused an increase in ECF translocation compared with the LPC-infused group (Figure 2B). However, no significant differences in relative changes in ECF translocation between hypertrophied and nonhypertrophied atria were found. Interestingly, the decreases in ANP secretion and concentration by LPC, stearoyl-LPC, palmitoyl-LPC, and oleoyl-LPC were markedly attenuated in
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 ($r^2=0.14$). 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, $r^2=0.38$; palmitoyl-LPC, $r^2=0.18$; and stearoyl-LPC, $r^2=0.02$).

Effects of LPC on [Ca$^{2+}$]$_i$ in Single Myocytes From Hypertrophied Atria
Changes in [Ca$^{2+}$]$_i$, by LPC, palmitoyl-LPC, and stearoyl-LPC were measured in single, beating, atrial myocytes from hypertrophied and control atria. Basal [Ca$^{2+}$]$_i$, 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$^{2+}$]$_i$, which were greater than that by LPC. Palmitoyl-LPC caused a slight increase in [Ca$^{2+}$]$_i$, which was not different from that by LPC. An increase in [Ca$^{2+}$]$_i$, 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.3–5 LPC has been known to have various cardiac effects 6–10 and to be related to the development of hypertension and atherosclerosis.32 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$^{2+}$]$_i$.14 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,14 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 [Ca^{2+}], handling has been described in various cardiac diseases associated with hypertrophy, and the suppressive effect of LPC on ANP secretion has been reported to be partly related to [Ca^{2+}]. Therefore, we investigated the possible modification of LPC effects on atrial hemodynamics and ANP secretion in hypertrophied atria. Surprisingly, LPC caused an ~25% 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 [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–Ca^{2+} and phosphoinositol 3-kinase pathway) are also responsible for those effects.

LPC is known to alter cellular Ca^{2+} homeostasis. LPC causes an accumulation of [Ca^{2+}], in a dose-dependent manner in ventricular myocytes. LPC also causes Ca^{2+} efflux from isolated, rat ventricular myocytes through the Na^{+}–Ca^{2+} exchanger. We demonstrated previously that LPC slightly increases [Ca^{2+}], in single, atrial myocytes in a dose-dependent manner. However, the increase in [Ca^{2+}], in atrial myocytes by LPC was small compared with that in ventricular myocytes. Nevertheless, the suppression of ANP release by LPC was prominent. In atrial myocytes from hypertrophied hearts, increases in [Ca^{2+}] were significantly attenuated. Time indicates time control group. Values are mean±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<0.005 vs nonhypertrophied atria infused with the same type of LPC.
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 changes in pulse pressure and [Ca\(^{2+}\)] in terms of cardiac hypertrophy and/or a disturbance in [Ca\(^{2+}\)] 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\(^{2+}\)] regulation.

**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\(^{2+}\)] 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\(^{2+}\)] 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).

**References**


Attenuation of Lysophosphatidylcholine-Induced Suppression of ANP Release From Hypertrophied Atria
Jeong Hee Han, Chunhua Cao, Soo Mi Kim, Feng Lian Piao and Suhn Hee Kim

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
Copyright © 2003 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/43/2/243

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/