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# Lysophosphatidylcholine potentiates vascular contractile responses by enhancing vasoconstrictor-induced increase in cytosolic free $Ca^{2+}$ in rat aorta

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**Abstract**

We investigated the effects of palmitoyl-L-α-lysophosphatidylcholine on the contractile responses of the endothelium-denuded rat aorta to high $K^+$, noradrenaline, UK14,304 (5-bromo-6-[2-imidazolin-2-ylamino]-quinoxaline) (a selective $\alpha_2$ adrenoceptor agonist) and phorbol 12-myristate 13-acetate (PMA). Lysophosphatidylcholine at concentrations from $10^{-6}$ M to $10^{-4}$ M did not contract aortic strips. However, lysophosphatidylcholine strongly potentiated the UK14,304-induced contraction. High $K^+$- and PMA-induced contractions were also potentiated. In contrast, the noradrenaline-induced contraction was only slightly potentiated by $10^{-5}$ M lysophosphatidylcholine. In fura PE-3-loaded aortic strips, lysophosphatidylcholine ($10^{-5}$ M) markedly augmented the increase in both cytosolic free $Ca^{2+}$ ($[Ca^{2+}]_i$) and contractile tension induced by UK14,304, high $K^+$ and PMA. Nicardipine ($10^{-7}$ M) and $10^{-6}$ M Ro-31-8220 ({1-[3-(amidinothio)propyl-1H-indoyl-3-yl]-3-(1-methyl-1H-indoyl-3-yl)-maleimide-methane sulfate) strongly inhibited the increase in $[Ca^{2+}]_i$ and contractile tension induced by UK14,304 and in the presence of these inhibitors, the enhancing effects of lysophosphatidylcholine were attenuated. However, the enhancing effect on high $K^+$-induced contraction was not affected by Ro-31-8220. These results suggest that lysophosphatidylcholine may cause an augmentation of the increase in $[Ca^{2+}]_i$ induced by UK14,304 which response is depend on protein kinase C activation and in this way potentiate contractile responses in the rat aorta. Protein kinase C independent mechanisms may also be involved in the enhancing effect of lysophosphatidylcholine on smooth muscle contraction. 

*Keywords:* Lysophosphatidylcholine; $Ca^{2+}$ level, cytosolic; $Ca^{2+}$ channel, voltage-dependent ; α-Adrenoceptor agonist; Protein kinase C; Aorta, rat

## 1. Introduction

An elevated plasma concentration of low density lipoprotein (LDL) is well known to accelerate atherosclerosis, and it is also known that LDL may accumulate in the vessel wall in hypercholesterolemia (Goldstein and Brown, 1977; Hollander et al., 1979; Steinberg, 1983; Hoff and Morton, 1985; Daugherty et al., 1988). Various components of the vascular wall, including endothelial cells and macrophages, are involved in the oxidative modification of LDL (Henriksen et al., 1981). It has been reported that exogenous application of oxidized LDL, but not of native LDL, impairs endothelium-dependent relaxation in isolated arteries (Kugiyama et al., 1990, 1992; Rajavashisth et al., 1990; Witztum and Steinberg, 1991; Flavahan, 1992). It has also been reported that lysophosphatidylcholine is released from oxidized LDL after conversion from phosphatidylcholine, and that lysophosphatidylcholine also causes an endothelial dysfunction similar to that observed in hypercholesterolemia (Kugiyama et al., 1990; Flavahan, 1993; Sugiyama et al., 1994). Kugiyama et al. (1992) suggested that activation of protein kinase C by lysophosphatidylcholine was involved in the mechanism by which lysophosphatidylcholine caused an impairment of endothelium-dependent relaxation.

It is known that lysophosphatidylcholine also acts on vascular smooth muscle directly. Indeed, exposure to lysophosphatidylcholine causes $Ca^{2+}$ influx in cultured vascular smooth muscle cells (Locher et al., 1992; Stoll and Spector, 1993; Chen et al., 1995). Jensen et al. (1996) recently reported that in the permeabilized small mesen-

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teric artery, lysophosphatidylcholine increased $Ca^{2+}$ sensitivity via protein kinase C activation. As yet, the precise mechanism underlying these effects of lysophosphatidylcholine has not been identified.

The aim of the present study was to examine the effects of lysophosphatidylcholine on the vascular contractile responses to high $K^+$, noradrenaline, UK14,304 (5-bromo-6-[2-imidazolin-2-ylamino]-quinoxaline) and phorbol ester in the rat aorta.

## 2. Materials and methods

### 2.1. General

This study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals adopted by the Committee on the Care and Use of Laboratory Animals of Hoshi University (which is accredited by the Ministry of Education, Sciences, Sports and Culture, Japan).

### 2.2. Preparation of aortic strips

Male Wistar rats, 8–10 weeks old, were anaesthetized with sodium pentobarbitone (50 mg/kg, i.p.), then killed by decapitation. The thoracic aorta was rapidly dissected out and placed into modified, Krebs–Henseleit solution (KHS, composition in mM: NaCl 118; KCl 4.7; $CaCl_2$ 1.8; $NaHCO_3$ 25.0; $MgSO_4$ 1.2; $NaH_2PO_4$ 1.2; dextrose 11.0). The aorta was then cleaned of loosely adhering fat and connective tissue, and cut into helical strips 2 mm in width and 20 mm in length. The endothelium was removed from the aorta by rubbing the intimal surface with a cotton swab, and removal of the endothelium was functionally confirmed by the absence of relaxation to 10 μM acetylcholine.

### 2.3. Effects of lysophosphatidylcholine on vascular contraction

Each aortic strip was suspended in an organ bath containing 10 ml of well-oxygenated (95% $O_2$ + 5% $CO_2$) KHS at 37°C. The contractile responses were measured with the aid of a force-displacement transducer (Nihon Kohden, TB-611, Tokyo, Japan) and displayed on a pen recorder (Yokogawa, Model 3021, Tokyo, Japan). The resting tension in the aortic strip was adjusted to 1.0 g, which is the optimal tension for inducing a maximal contraction (preliminary experiments). The aortic strips were pretreated with lysophosphatidylcholine for 30 min, and then high $K^+$, noradrenaline, UK14,304 or PMA was cumulatively applied.

### 2.4. Measurement of intracellular free $Ca^{2+}$ and tension

Tension and cytosolic free $Ca^{2+}$ ($[Ca^{2+}]_i$) were measured by the method of Sato et al. (1988). Smaller aortic strips (2 mm in width and 10 mm in length) were exposed to $10^{-5}$ M fura PE3-acetoxymethyl ester (fura PE3-AM) in the presence of 0.04% cremophor EL in the dark for 5 h at 26°C. The tissue was then rinsed with normal KHS and placed in the organ bath containing KHS at 37°C. The organ bath was part of a fluorometer (Japan Spectroscopic, CAF 110, Tokyo, Japan) and was designed to allow the simultaneous measurement of $[Ca^{2+}]_i$ and muscle contraction. The tissue was held in a horizontal configuration under a resting tension of 0.5 g (which is the optimal tension for the small size of aortic strip) and equilibrated for 30 min. One end of the tissue was connected to a force transducer for the measurement of isometric tension (Nihon Kohden, TB-611, Tokyo, Japan) and the other end was fixed. The intimal surface of the fura-PE3 loaded tissue was alternatively (frequency of 128 Hz) subjected to excitation wavelengths of 340 and 380 nm. The fluorescence emissions of 500 nm wavelength for each excitation light were separated by dichroic mirror and were measured with a photomultiplier. The ratio ($F_{340}/F_{380}$) of the emitted fluorescence signals was used to provide an index of $[Ca^{2+}]_i$. The contractile responses and the ratio ($F_{340}/F_{380}$) of the emitted fluorescence signals were displayed on a pen recorder (Yokogawa, Model 3021, Tokyo, Japan). After equilibration, each aortic tissue was firstly exposed to 80 mM $K^+$ (iso-osmotic substitution for NaCl). The fluorescence ratio ($F_{340}/F_{380}$) in the resting muscle and that in muscle depolarized by 80 mM $K^+$ were taken as 0% and 100%, respectively.

### 2.5. Drugs

Calphostin C, choline, cremophor EL, L-α-lysophosphatidylcholine (palmitoyl), nicardipine, noradrenaline, palmitic acid, L-α-glycerophosphorylcholine (from egg yolk) and PMA were purchased from Sigma Chemical (St. Louis, MO). UK14,304 was purchased from Funakoshi (Tokyo, Japan). Fura PE3-AM and Ro-31-8220 were purchased from Wako (Osaka, Japan). Fura PE3-AM was dissolved in dimethylsulfoxide (DMSO) and mixed with cremophor EL. The final concentrations of DMSO and cremophor EL were 1% and 0.04%, respectively. At these concentrations, DMSO and cremophor EL had no detectable effect on $[Ca^{2+}]_i$ or tension in the aortic strips. Calphostin C, nicardipine, PMA, Ro-31-8220 and UK14,304 were dissolved in DMSO and diluted in distilled water. All the other drugs were dissolved in distilled water.

### 2.6. Statistics

The results shown in the text and figures are expressed as the mean ± S.E. Statistical differences were assessed by Dunnett's multiple comparison test, following a one-way analysis of variance.

## 3. Results

### 3.1. Effects of lysophosphatidylcholine on contractile responses

Fig. 1 shows the effects of L-α-lysophosphatidylcholine (palmitoyl) on the contractile responses induced by 15 mM $K^+$ and $10^{-7}$ M UK14,304. The application of $10^{-5}$ M lysophosphatidylcholine alone did not affect the resting tension. However, lysophosphatidylcholine markedly potentiated the contractions induced by both 15 mM $K^+$ and $10^{-7}$ M UK14,304. Fig. 2 shows the effects of lysophosphatidylcholine on the concentration–response curves for high $K^+$, noradrenaline, UK14,304 and PMA. Lysophosphatidylcholine at a concentration of $3 \times 10^{-7}$ M did not affect the resting tension and did not potentiate the both high $K^+$-, UK14,304-induced contractions (data not shown). Treatment with $10^{-6}$ M to $3 \times 10^{-5}$ M lysophosphatidylcholine also did not affect the resting tension, but a higher concentration of lysophosphatidylcholine ($10^{-4}$ M) did slightly increase the tension (by $60.0 \pm 8.0$ mg). Lysophosphatidylcholine significantly potentiated the high $K^+$-induced contraction by an effect that was concentration-dependent at concentrations from $10^{-6}$ to $10^{-4}$ M. However, lysophosphatidylcholine did not affect the maximal response induced by 80 mM $K^+$ (the maximal contractions induced by 80 mM $K^+$ in the absence or presence of $10^{-4}$ M lysophosphatidylcholine were $1035.6 \pm 32.6$ mg and $983.2 \pm 49.33$ mg, respectively). Lysophosphatidylcholine at a concentration of $3 \times 10^{-4}$ M markedly produced vascular contraction (this contraction were $283.2 \pm 30.8$ mg) but significantly inhibited high $K^+$-induced contraction.

Lysophosphatidylcholine also strongly potentiated the UK14,304-induced contraction by an effect that was concentration-dependent at concentrations from $10^{-6}$ to $10^{-5}$ M (Fig. 2C), and effect of $3 \times 10^{-5}$ M lysophosphatidylcholine was equal to that of $10^{-5}$ M lysophosphatidylcholine. The magnitude of the $10^{-7}$ M UK14,304-induced contraction in the presence of $10^{-5}$ M lysophosphatidylcholine was 7.5-fold greater than that in the absence of lysophosphatidylcholine. Lysophosphatidylcholine was more effective on the UK14,304-induced response. Treatment with $10^{-4}$ M and $3 \times 10^{-4}$ M lysophosphatidylcholine significantly inhibited the maximal response induced by UK14,304.

In contrast, while $10^{-5}$ M lysophosphatidylcholine slightly (though significantly at $10^{-9}$ M noradrenaline)

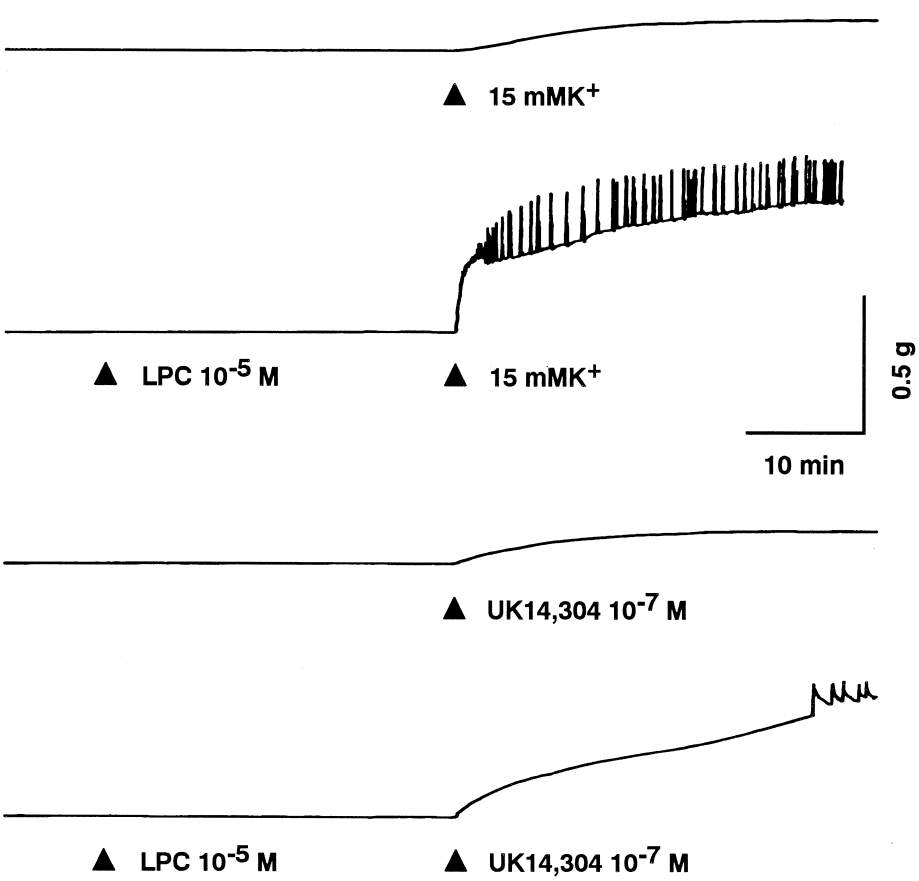


Fig. 1. Typical traces showing effect of lysophosphatidylcholine (LPC) on the contractile responses induced by 15 mM $K^+$ and $10^{-7}$ M UK14,304 in the isolated rat aorta.

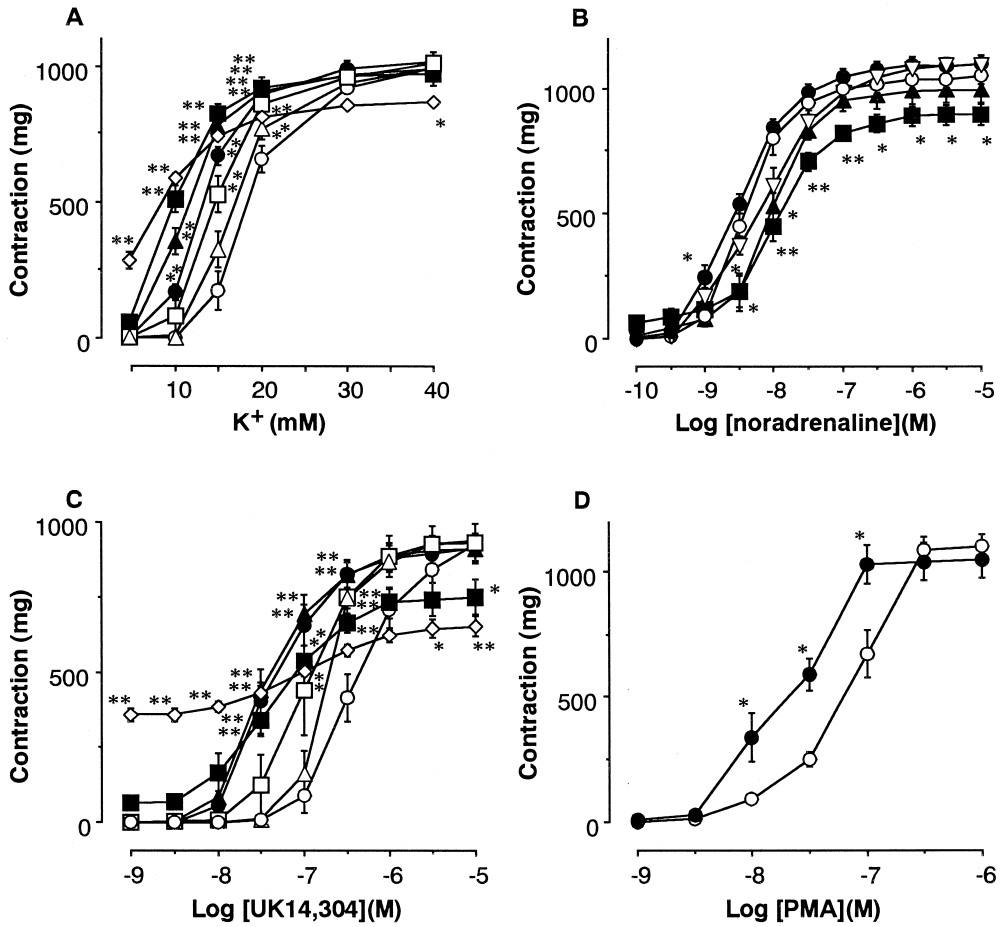


Fig. 2. Dose–response curves for high $K^+$ (A), noradrenaline (B), UK14,304 (C) and PMA (D) in isolated rat aorta. Aortic strips were incubated in the absence (open circles) or presence of $10^{-6}$ M (open triangles), $3 \times 10^{-6}$ M (open squares), $10^{-5}$ M (closed circles), $3 \times 10^{-5}$ M (closed triangles) $10^{-4}$ M (closed squares) or $3 \times 10^{-4}$ M (open diamonds) lysophosphatidylcholine. The reversed open triangles in (B) show the dose–response curves obtained form the tissue which had been treated with $10^{-4}$ M lysophosphatidylcholine for 30 min and then removed lysophosphatidylcholine for 1 h. Each point is the mean from 4–8 animals; vertical lines indicating ±S.E. are shown only when they exceed the dimensions of the symbol used. $*\ P < 0.05$, $**\ P < 0.01$ vs. control in the absence of lysophosphatidylcholine.

potentiated the noradrenaline-induced contraction, treatment with either $3 \times 10^{-5}$ M or $10^{-4}$ M lysophosphatidylcholine inhibited the noradrenaline-induced contraction (Fig. 2B). The inhibitory effect of $10^{-4}$ M lysophosphatidylcholine on noradrenaline-induced contraction was almost recovered by wash of lysophosphatidylcholine for 1 h (Fig. 2B). Fig. 1D shows the effects of lysophosphatidylcholine on the contractile responses induced by PMA. PMA-induced contraction was also potentiated by $10^{-5}$ M lysophosphatidylcholine treatment.

### *3.2. Effects of lysophosphatidylcholine metabolites on contractile responses*

It is possible that the effect of lysophosphatidylcholine could be due to its metabolite. Palmitoyl lysophosphatidylcholine could be degraded through several processes, and an active metabolite formed. However, palmitic acid, L-α-glycerophosphorylcholine and choline, at a concentration of $10^{-5}$ M did not potentiated UK14,304-induced contraction (Fig. 3).

### *3.3. Effects of lysophosphatidylcholine on contractile responses in nicardipine-treated aortic strips*

To clarify the mechanism underlying the selective effect of lysophosphatidylcholine on the contractions induced by UK14,304, high $K^+$ and PMA, and its lack of effect on that induced by noradrenaline, aortic strips were exposed to nicardipine, a blocker of voltage-dependent $Ca^{2+}$ channel. The high $K^+$-induced contraction was strongly inhibited by $10^{-7}$ M nicardipine and in the presence of nicardipine, $10^{-5}$ M lysophosphatidylcholine failed to potentiate the high $K^+$-induced contraction (Fig. 4). The noradrenaline-induced contraction was slightly inhibited by $10^{-7}$ M nicardipine. In the presence of nicardipine, $10^{-5}$ M lysophosphatidylcholine also failed to potentiate the noradrenaline-induced contraction. In contrast, the

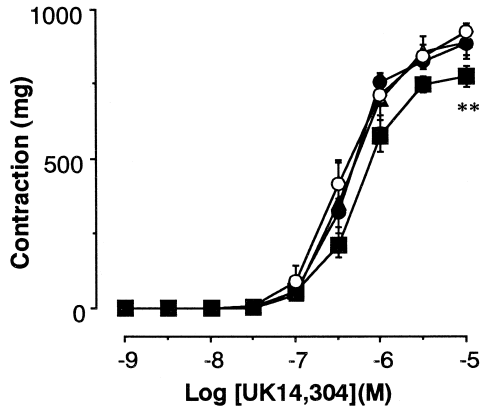


Fig. 3. Effects of palmitoyl-lysophosphatidylcholine metabolites on dose–response curves for UK14,304 in isolated rat aorta. Aortic strips were incubated in the absence (open circles) or presence of $10^{-5}$ M palmitic acid (closed circles), $10^{-5}$ M choline (closed triangles) or $10^{-5}$ M L-α-glycerophosphorylcholine (closed squares). Each point is the mean from 4–8 animals; vertical lines indicating ±S.E. are shown only when they exceed the dimensions of the symbol used. $** P < 0.01$ vs. control in the absence of lysophosphatidylcholine metabolites.

UK14,304-induced contraction was markedly inhibited by $10^{-7}$ M nicardipine. The enhancing effect of lysophosphatidylcholine on the UK14,304-induced contraction was strongly inhibited by nicardipine treatment (compare Fig. 4 with Fig. 2). The PMA-induced contraction was also inhibited by nicardipine and an effect of lysophosphatidylcholine on the PMA-induced contraction was barely detectable in nicardipine-treated aortic strips.

### *3.4. Effects of lysophosphatidylcholine on changes in cytosolic $Ca^{2+}$ during UK14,304-, high $K^+$- and PMA-induced contractions*

Fig. 5 shows typical changes evoked by lysophosphatidylcholine in muscle tension development and cytosolic free $Ca^{2+}$ ($[Ca^{2+}]_i$) in aortic strips loaded with fura PE-3. The application of $10^{-5}$ M lysophosphatidylcholine induced a small increase in $[Ca^{2+}]_i$ (6.12 ± 0.75% of the response to 80 mM $K^+$) with no detectable contraction.

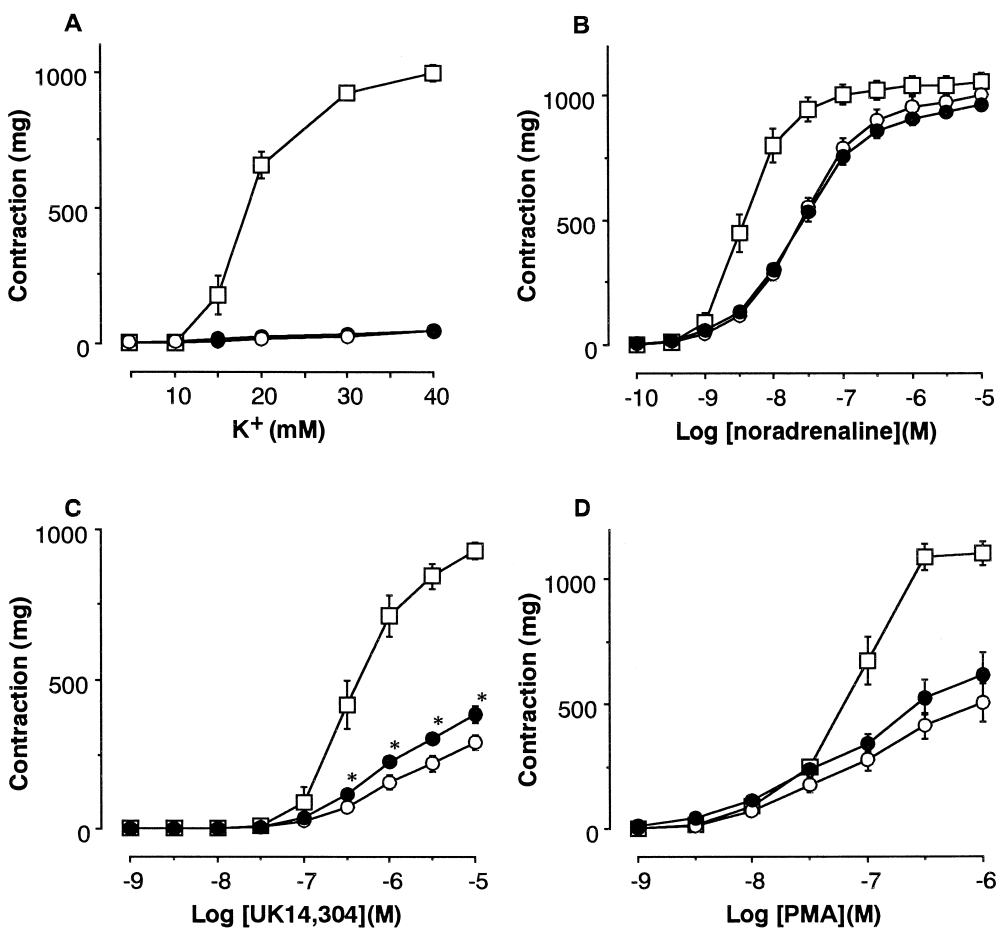


Fig. 4. Dose–response curves for high $K^+$ (A), noradrenaline (B), UK14,304 (C) and PMA (D) in the nicardipine ($10^{-7}$ M)-treated rat aorta. Aortic strips were incubated in the absence (open circles) or presence (closed circles) of $10^{-5}$ M lysophosphatidylcholine. For comparison, square symbols show the dose–response curves obtained in the absence of both nicardipine and lysophosphatidylcholine (control). Each point is the mean from 4–8 animals; vertical lines indicating ±S.E. are shown only when they exceed the dimensions of the symbol used. $* P < 0.05$ vs. value in the absence of lysophosphatidylcholine and presence of nicardipine.

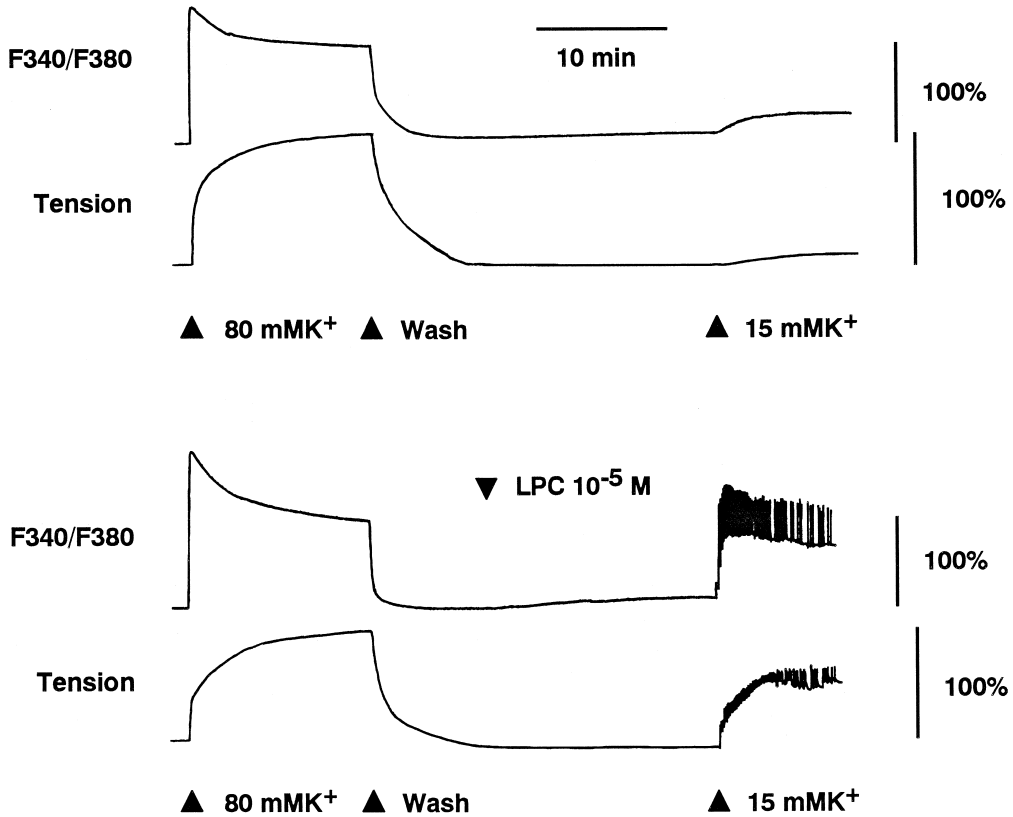


Fig. 5. Typical traces showing changes in the fluorescence ratio ($F_{340}/F_{380}$) and force development induced by 15 mM $K^+$ in the absence (upper) or presence (lower) of $10^{-5}$ M lysophosphatidylcholine (LPC) in the fura PE-3-loaded rat aorta. The resting level is taken as 0% and the 80 mM $K^+$-stimulated level as 100%.

However, treatment with $10^{-5}$ M lysophosphatidylcholine markedly augmented both the $[Ca^{2+}]_i$ and contractile tension responses induced by 15 mM $K^+$. The increases in $[Ca^{2+}]_i$ and contractile tension induced by 20 mM $K^+$ were also augmented by $10^{-5}$ M lysophosphatidylcholine (Fig. 6). The 20 mM $K^+$-induced increases in $[Ca^{2+}]_i$ and contractile tension were strongly inhibited by $10^{-7}$ M nicardipine. Nicardipine did not affect the increase in $[Ca^{2+}]_i$ induced by $10^{-5}$ M lysophosphatidylcholine but it strongly inhibited the enhancing effects of lysophosphatidylcholine on both $[Ca^{2+}]_i$ and contractile tension induced by 20 mM $K^+$. PMA ($3 \times 10^{-8}$ M) produced contraction with increase in $[Ca^{2+}]_i$. Treatment with $10^{-5}$ M lysophosphatidylcholine also markedly augmented the increase in both $[Ca^{2+}]_i$ and contractile tension responses induced by PMA. PMA-induced increase in $[Ca^{2+}]_i$ was strongly inhibited by nicardipine but the contraction was partly inhibited. In the presence of nicardipine, lysophosphatidylcholine did not augmented the increase in both $[Ca^{2+}]_i$ and contractile tension responses induced by PMA.

UK14,304 produced an increase in $[Ca^{2+}]_i$ and contraction in a dose dependently. Lysophosphatidylcholine ($10^{-5}$ M) markedly augmented the increase in $[Ca^{2+}]_i$ and contractile tension responses induced by UK14,304. Nicardipine ($10^{-7}$ M) abolished and strongly inhibited $10^{-7}$ M and $10^{-6}$ M UK14,304-induced responses, respectively. In the presence of nicardipine, enhancing effects of lysophosphatidylcholine on increase in $[Ca^{2+}]_i$ and contractile response induced by UK14,304 was strongly attenuated. UK14,304-induced increase in $[Ca^{2+}]_i$ and contractile response were also strongly inhibited by $10^{-6}$ M Ro-31-8220 ({1-[3-(amidinothio)propyl-1H-indoyl-3-yl]-3-(1-methyl-1H-indoyl-3-yl)-maleimide-methane sulfate), a protein kinase C inhibitor. In the presence of Ro-31-8220, enhancing effects of lysophosphatidylcholine on increase in $[Ca^{2+}]_i$ and contractile response induced by UK14,304 was strongly inhibited.

### *3.5. Effects of lysophosphatidylcholine on contractile responses in aortic strips treated with protein kinase C inhibitors*

To determine whether the enhancing effect of lysophosphatidylcholine on vascular contractions involves activation of protein kinase C, aortic strips were treated with calphostin C or Ro-31-8220, selective protein kinase C inhibitors. Calphostin C ($3 \times 10^{-7}$ M) did not affect the contractile response induced by high $K^+$. In calphostin C-treated aortic strips, lysophosphatidylcholine significantly augmented the contraction induced by high $K^+$ (Fig. 7). Ro-31-8220 ($10^{-6}$ M), another protein kinase C inhibitor, also did not affect the contractile response in-

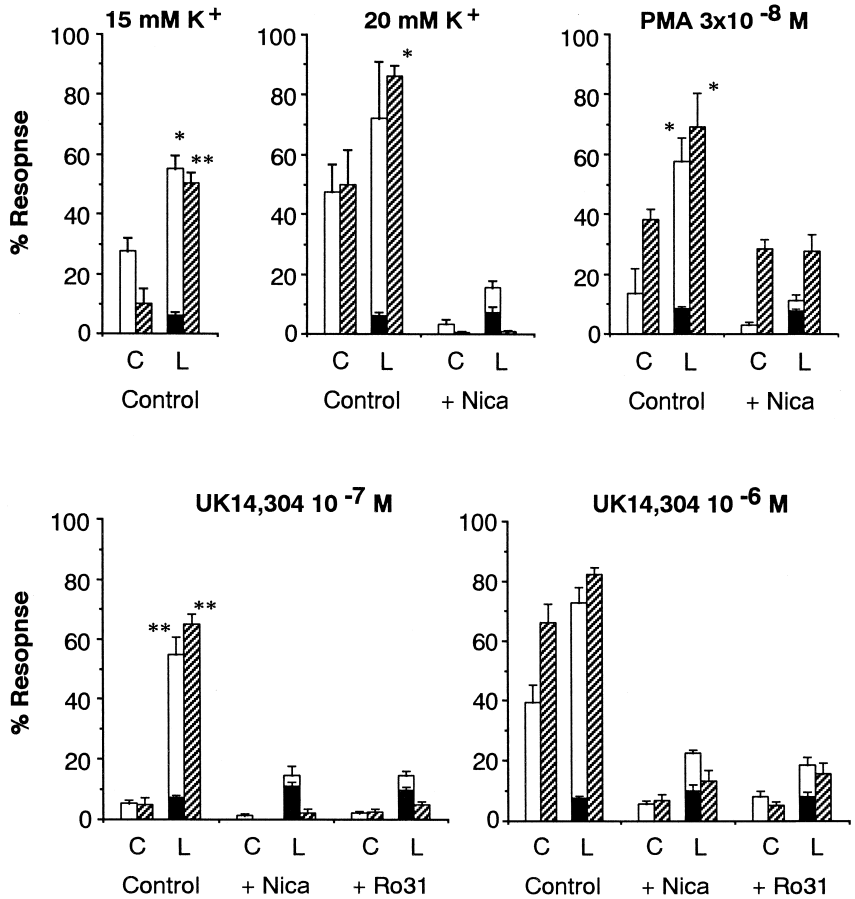


Fig. 6. Effects of lysophosphatidylcholine ($10^{-5}$ M), nicardipine ($10^{-7}$ M) and Ro-31-8220 ($10^{-6}$ M) on the changes in $[Ca^{2+}]_i$ level (open columns) and muscle tension (hatched columns) induced by high $K^+$, PMA and UK14,304 in the rat aorta. The closed columns show the changes in $[Ca^{2+}]_i$ level induced by lysophosphatidylcholine alone. The resting level is taken as 0% and the 80 mM $K^+$-stimulated level as 100%. Values represent the mean ± S.E. from 4–5 animals. $* P < 0.05$, $** P < 0.01$ vs. control in the absence of lysophosphatidylcholine. C: tissue was not treated with lysophosphatidylcholine (control), L: tissue was treated with $10^{-5}$ M lysophosphatidylcholine, +Nica: tissue was treated with $10^{-7}$ M nicardipine, +Ro31: tissue was treated with $10^{-6}$ M Ro-31-8220.

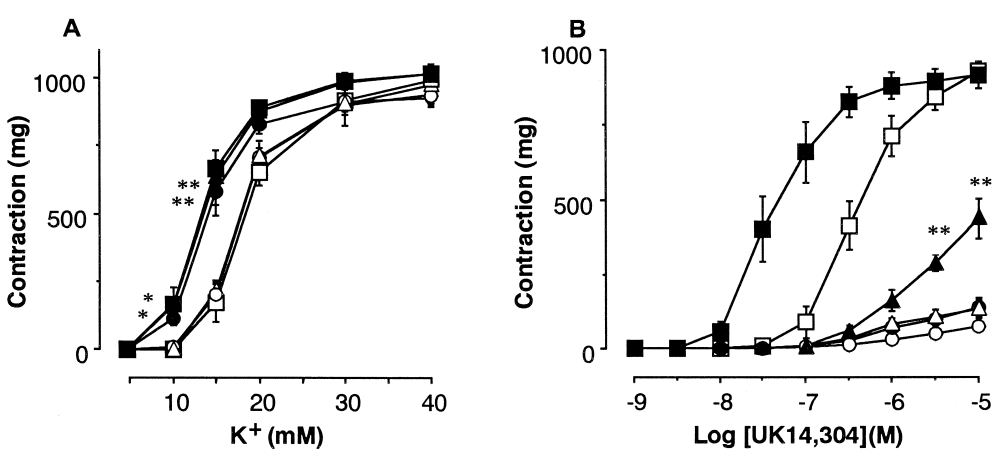


Fig. 7. Dose–response curves for high $K^+$ (A) and UK14,304 (B) in the rat aorta pretreated with $3 \times 10^{-7}$ M calphostin C (open and closed circles) or $10^{-6}$ M Ro-31-8220 (open and closed triangles). Aortic strips were incubated in the absence (open symbols) or presence (closed symbols) of $10^{-5}$ M lysophosphatidylcholine. For comparison, open squares show the dose–response curves obtained in the absence of both protein kinase C inhibitors (calphostin C and Ro-31-8220) and lysophosphatidylcholine (control), and closed squares show the dose–response curves obtained in the absence of protein kinase C inhibitors (calphostin C and Ro-31-8220) and in the presence of $10^{-5}$ M lysophosphatidylcholine. Each point is the mean from 5–8 animals; vertical lines indicating ±S.E. are shown only when they exceed the dimensions of the symbol used. $* P < 0.05$, $** P < 0.01$ vs. value in the absence of lysophosphatidylcholine and presence of calphostin C or Ro-31-8220.

duced by high $K^+$. In Ro-31-8220-treated aortic strips, lysophosphatidylcholine significantly augmented the contraction induced by high $K^+$. In contrast, the UK14,304-induced contraction was strongly inhibited by $3 \times 10^{-7}$ M calphostin C and $10^{-6}$ M Ro-31-8220 (Fig. 7). In the presence of calphostin C, lysophosphatidylcholine did not potentiate the UK14,304-induced contraction. The enhancing effect of lysophosphatidylcholine on UK14,304-induced contraction was also attenuated by $10^{-6}$ M Ro-31-8220.

## 4. Discussion

In the present study, on the endothelium-denuded rat aorta, we found that palmitoyl-L-α-lysophosphatidylcholine could selectively and markedly potentiate contractions induced by UK14,304. Lysophosphatidylcholine was also able to potentiate high $K^+$- and PMA-induced contractions, but slightly affected the noradrenaline-induced contraction. The mechanism underlying seems to involve lysophosphatidylcholine augmenting the increase in $[Ca^{2+}]_i$ induced by UK14,304, high $K^+$ and PMA.

It has often been reported that lysophosphatidylcholine impair endothelium-dependent relaxation in isolated arteries (Kugiyama et al., 1990, 1992; Flavahan, 1993; Sugiyama et al., 1994; Encabo et al., 1997). For example, Kugiyama et al. (1992) found that lysophosphatidylcholine inhibited thrombin-induced inositol trisphosphate ($IP_3$) production and attenuated $[Ca^{2+}]_i$ elevations in human umbilical vein endothelial cells, they also found that thrombin-induced endothelium-dependent relaxation was inhibited by lysophosphatidylcholine. In addition, Murohara et al. (1994) reported that lysophosphatidylcholine elicited a further contraction during the plateau contraction evoked by prostaglandin $F_{2\alpha}$ in the pig coronary artery; they suggested that this additional contraction was caused by an lysophosphatidylcholine-mediated inhibition of endothelium-derived nitric oxide release. These reports indicate that lysophosphatidylcholine is able to cause dysfunction of the endothelium and may consequently produce alterations in vascular tonus.

In the present study, lysophosphatidylcholine directly augmented the contractions induced by UK14,304, high $K^+$ and PMA in the endothelium-denuded rat aorta. The augmentation effects of lysophosphatidylcholine on both UK14,304- and high $K^+$-induced contractions were dose-dependently. These results parallel with the previous report that oxidative-LDL (which is involved in lysophosphatidylcholine) potentiates vasoconstriction in both endothelium-intact and endothelium-denuded rabbit femoral arteries (Galle et al., 1990). In the present study, palmitoyl lysophosphatidylcholine metabolites, palmitic acid, L-α-glycerophosphorylcholine and choline did not potentiated the UK14,304-induced contraction, suggesting that lysophosphatidylcholine itself may mediate the effect. Moreover, in the present study, the effect of lysophosphatidylcholine was selective insofar as it only slightly affected the noradrenaline-induced contraction, suggesting that lysophosphatidylcholine may not affect the contractile elements directly and that the augmentation of $[Ca^{2+}]_i$ by lysophosphatidylcholine may not due to the inhibition of $Ca^{2+}$ efflux.

It is reported that lysophosphatidylcholine has a cytotoxic effect on vascular smooth muscle (Chen et al., 1995). In the present study, $3 \times 10^{-4}$ M lysophosphatidylcholine produced vascular contraction and treatment with $3 \times 10^{-4}$ M lysophosphatidylcholine attenuated agonists-induced contractions, suggesting that $3 \times 10^{-4}$ M lysophosphatidylcholine may be act as a cytotoxic agent in rat aorta. Treatment with $10^{-4}$ M lysophosphatidylcholine also inhibited maximal contractions induced by both UK14,304 and noradrenaline. However, we consider that the $10^{-4}$ M lysophosphatidylcholine has a little or no cytotoxic effect on the rat aorta. Since $10^{-4}$ M lysophosphatidylcholine did not affect the maximal contraction induced by high $K^+$ and the inhibitory effect of $10^{-4}$ M lysophosphatidylcholine on noradrenaline-induced contraction was almost recovered by removal of lysophosphatidylcholine for 1 h.

It is well known that the application of KCl depolarizes the plasma membrane and produces a contraction that depends mainly on increased $Ca^{2+}$ influx through voltage-dependent L-type $Ca^{2+}$ channels (Godfraind et al., 1986). It is also known that the elevation of $[Ca^{2+}]_i$ induced by noradrenaline is due both to $Ca^{2+}$ release from the intracellular $Ca^{2+}$ stores and to $Ca^{2+}$ influx through both L-type and non-L-type $Ca^{2+}$ channels (Minneman, 1988; Karaki et al., 1997). It has been reported that, in the rat aorta, the noradrenaline-induced contraction can be partially inhibited by $Ca^{2+}$ channel blockers (Godfraind et al., 1982; Beckeringh et al., 1984; Vinet et al., 1991). By comparison with the noradrenaline-induced contractile response, contractions induced by $\alpha_2$-adrenoceptor agonists are known to be more strongly dependent on $Ca^{2+}$ influx through voltage-dependent $Ca^{2+}$ channels in the rat tail artery (Medgett and Rajanayagam, 1984; Hicks et al., 1985; Su et al., 1986), rabbit saphenous vein (Schümann and Lues, 1983) and rat aorta (Godfraind et al., 1982; Beckeringh et al., 1984; Vinet et al., 1991). In the present study, the contractile response induced by UK14,304, a selective $\alpha_2$-adrenoceptor agonist, was strongly inhibited by nicardipine, whereas the noradrenaline-induced contraction was only slightly inhibited by this $Ca^{2+}$ channel blocker. In the presence of nicardipine, the UK14,304-, noradrenaline- and high $K^+$-induced contractile responses were hardly potentiated by lysophosphatidylcholine. These results suggest that lysophosphatidylcholine may principally potentiate contractions of the rat aorta that depend on $Ca^{2+}$ influx through voltage-dependent L-type $Ca^{2+}$ channels.

Protein kinase C activation by phorbol ester is known to produce sustained contraction in isolated arteries. It has

been reported that phorbol ester-induced contractions are dependent on the extracellular $Ca^{2+}$ concentration and can be inhibited by $Ca^{2+}$ channel blockers (Rasmussen et al., 1984; Baraban et al., 1985). However, contractions due to phorbol 12, 13-dibutyrate in the rabbit aorta are apparently not sensitive to zero calcium or to $Ca^{2+}$ channel blockers (Sybertz et al., 1986). It is likely, therefore, that two distinct mechanisms are involved in mediating phorbor ester-induced contractile responses, one that involves protein kinase C activation and is not dependent on extracellular $Ca^{2+}$, another that is due to $Ca^{2+}$ influx through voltage-dependent $Ca^{2+}$ channels. We have previously reported that PMA produces biphasic contractions of the rat aorta by activation of $Ca^{2+}$-independent and $Ca^{2+}$-dependent contractile mechanisms (Suenaga et al., 1993). In the present study, the PMA-induced contraction and increase in $[Ca^{2+}]_i$ were markedly potentiated by lysophosphatidylcholine, but lysophosphatidylcholine hardly potentiated the PMA-induced contraction in the nicardipine-treated aorta. Our results suggest that, in the rat aorta, the elevation of $[Ca^{2+}]_i$ induced by protein kinase C activation was potentiated by lysophosphatidylcholine.

Previous studies have suggested that lysophosphatidylcholine activates protein kinase C and that the endothelial dysfunction induced by lysophosphatidylcholine can be inhibited by protein kinase C inhibitors (Kugiyama et al., 1992; Sugiyama et al., 1994). Recently, Jensen et al. (1996), using the α-toxin-permeabilized rat small mesenteric artery, found that lysophosphatidylcholine increased $Ca^{2+}$ sensitivity through a protein kinase C-dependent mechanism. In the present study, lysophosphatidylcholine more effectively potentiated UK14,304-induced contraction and UK14,304-induced response was strongly inhibited by calphostin C and Ro-31-8220. In Ro-31-8220 treated tissue, the augmentation effects of lysophosphatidylcholine on the increase in $[Ca^{2+}]_i$ and contractile tension induced by UK14,304 was strongly inhibited. Moreover, PMA-induced increase in $[Ca^{2+}]_i$ and contractile response were also potentiated by lysophosphatidylcholine. These results suggest that lysophosphatidylcholine may selectively potentiated α adrenoceptor stimulated contraction which was induced by protein kinase C activation. However, in the present study, lysophosphatidylcholine was still able to potentiate the high $K^+$-induced contraction in calphostin C and Ro-31-8220 treated tissues, suggesting that protein kinase C independent mechanisms may also involved in the enhancing effect of lysophosphatidylcholine on smooth muscle contraction in the rat aorta.

In conclusion, lysophosphatidylcholine selectively potentiate $\alpha_2$ adrenoceptor agonist, UK14,304-induced contraction which was produced by both $Ca^{2+}$ influx through voltage-dependent $Ca^{2+}$ channels and protein kinase C activation in the rat aorta. Lysophosphatidylcholine may cause an augmentation of the increase in $[Ca^{2+}]_i$ induced by UK14,304 and in this way potentiate contractile responses in the rat aorta. Protein kinase C independent mechanisms may also be involved in the enhancing effect of lysophosphatidylcholine on smooth muscle contraction.

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