Lysophosphatidic acid enhances contractility of isolated airway smooth muscle

M. L. TOEWS,1 E. E. USTINOVA,2 AND H. D. SCHULZ2

Departments of 1Pharmacology and 2Physiology and Biophysics, University of Nebraska Medical Center, Omaha, Nebraska 68198

Toews, M. L., E. E. Ustinova, and H. D. Schultz. Lysophosphatidic acid enhances contractility of isolated airway smooth muscle. J. Appl. Physiol. 83(4): 1216–1222, 1997.—The effects of the simple phospholipid mediator lysophosphatidic acid (LPA) on the contractile responsiveness of isolated tracheal rings from rabbits and cats were assessed. In both species, LPA increased the contractile response to the muscarinic agonist methacholine, but LPA did not induce contraction on its own. Conversely, LPA decreased the relaxation response to the β-adrenergic-agonist isoproterenol in both species. Concentrations of LPA as low as 10−8 M were effective, and the effects of LPA were rapidly reversed on washing. Phosphatidic acid was much less effective, requiring higher concentrations and producing only a minimal effect. Contractions induced by serotonin and by substance P also were enhanced by LPA, but KCl-induced contractions were unaffected. LPA inhibited the isoproterenol-induced relaxation of KCl-precontracted rings, similar to its effects on methacholine-precontracted rings, and relaxation induced by the direct adenyl cyclase activator forskolin was inhibited in a manner similar to that induced by isoproterenol. Epithelium removal did not alter the contraction-enhancing effect of LPA. The ability of LPA to both enhance contraction and inhibit relaxation of airway smooth muscle suggests that LPA could contribute to airway hypercontractility in asthma, airway inflammation, or other types of lung injury.

LYSOPHOSPHATIDIC ACID (LPA) is a simple bioactive lipid, the diverse physiological effects of which have come to be appreciated only recently. Effects of LPA include mitogenesis in fibroblasts and other cells; cell shape changes, including neurite retraction in cultured neurons, glial cell rounding, and changes in stress fibers, focal adhesion formation, and isometric contraction in fibroblasts; modulation of ion transport in epithelial cells; platelet activation; and tumor cell invasion. LPA effects have been observed in a wide variety of cell types, ranging from Dictyostelium amoebas to Xenopus oocytes to human neurons. These effects have been summarized in recent review articles (12, 13).

LPA is released from activated platelets and is present at relatively high concentrations in serum bound to albumin (4, 22). Several recent studies have presented evidence that LPA or an LPA-like compound is, in fact, the major factor responsible for several of the effects of serum on cells of various types. Thus LPA has been suggested to be the factor responsible for serum effects on stress fiber and focal adhesion formation in fibroblasts (17), on Xenopus oocyte chloride current activation (22), and on sensitization of adenosine 3′,5′-cyclic monophosphate (cAMP) accumulation (11) and shape changes (10) in glial cells. The release of LPA from activated platelets, together with its pronounced effects on fibroblast proliferation, contraction, and fibronectin assembly, suggests a likely role for LPA in wound repair (12, 13).

LPA has also been shown to modulate contraction of various types of smooth muscle. In fact, some of the earliest reports of physiological effects of LPA were on regulation of blood pressure, with either hypertension or hypotension observed depending on the species (24). A more recent study documented the ability of LPA to alter cerebrovascular reactivity in piglets and presented evidence for increased production of LPA in a model of cerebral hemorrhage (21). LPA has also been shown to stimulate contraction of gastrointestinal (25, 28) and uterine (26) smooth muscle.

During the course of our studies identifying LPA as the likely serum factor responsible for sensitization of cAMP accumulation in glial cells, our laboratory found that LPA could also induce sensitization of cAMP accumulation in cultured human airway smooth muscle cells (11). More recently, the complex effects of LPA on cAMP and phosphoinositide metabolism in human airway smooth muscle cells have been characterized in some detail (14). Our laboratory has also characterized the mitogenic effects of LPA in human airway smooth muscle cells in culture (2). The present studies were designed to further investigate the various physiological effects mediated by the LPA signaling pathways present in airway smooth muscle. In the present study we report the ability of LPA to potentiate contraction and to inhibit relaxation of isolated tracheal smooth muscle rings from both rabbits and cats. A preliminary report of some of these findings has been published previously (23).

METHODS

Preparation. Experiments were performed on 28 New Zealand rabbits and 4 cats. Animals were killed with pentobarbital (100 mg/kg iv). Tracheas were promptly removed and placed in Krebs-Henseleit solution at 4°C, oxygenated with a 95% O2-5% CO2 gas mixture. Tracheal segments were cleaned of loose connective tissue and cut into rings ~5 mm in length. In some experiments, epithelium was removed by gentle rubbing inside the tracheal ring with a cotton swab. Four tracheal segment rings (TSR) were used simultaneously from each animal. Each TSR was immersed in a 10-mL organ bath filled with oxygenated Krebs-Henseleit solution at 37°C. The TSRs were suspended longitudinally by stainless steel wire hook supports. The lower support was attached to the base of the organ bath, and the upper support was connected to an isometric force transducer and amplifier (models FT03C and 7P1A, respectively, Grass Instruments, Quincy, MA), from which isometric force was continuously monitored and recorded with a Macintosh SE computer by using a MacLab-8 software.
LYSOPHOSPHATIDIC ACID AND AIRWAY SMOOTH MUSCLE

data-acquisition system (AD Instruments, Milford, MA). Each transducer was mounted on a manipulator that was used to adjust the passive tension of the airway segment. The resting tensions of TSRs were individually set at 2.5–3.5 g. TSRs were equilibrated in the organ bath for 1 h and during this period were washed 2–3 times with fresh Krebs-Henseleit solution. The experimental treatments started when the baseline tension remained stable (± 0.10 g) for at least 20 min.

Protocols. In the first group of rabbit TSRs (n = 20) and in cat TSRs (n = 16), isometric tension was measured in response to cumulative addition of methacholine (from 10^{-8} to 10^{-5} M). Sufficient time (5–6 min) was allowed for the isometric tension to reach plateau during each treatment. Isoproterenol was added in cumulative doses (from 5 × 10^{-7} to 10^{-5} M) to the TSRs precontracted with the highest dose of methacholine (10^{-5} M). After thorough washout with fresh Krebs-Henseleit solution for 30 min, the TSRs were incubated with LPA (10^{-6} M) or vehicle (0.25% bovine serum albumin in Krebs-Henseleit solution) for 5 min, and the cumulative dose-response protocols to methacholine and isoproterenol were repeated.

In the second experimental group of rabbit TSRs (n = 20), changes in isometric tension in response to methacholine (10^{-6} M) were measured before and after incubation of TSRs with increasing doses of LPA (from 10^{-9} to 10^{-5} M) or phosphatidic acid (from 10^{-9} to 10^{-5} M).

In the third experimental group of rabbit TSRs (n = 16), isometric tension was measured in response to cumulative addition of KCl (10, 20, 40 and 50 mM). Prewarmed and gassed Krebs-Henseleit solution containing 100 mM KCl was added to the bath to obtain the indicated final concentrations. The tonicity of the buffer was not adjusted to compensate for the added KCl. Isoproterenol (10^{-5} M, n = 18) or forskolin (10^{-6} M, n = 8) was added to the TSRs precontracted with the highest dose of KCl.

In the fifth experimental group, cumulative dose-response protocols to methacholine and KCl were performed before and after incubation with LPA (10^{-6} M) in rabbit TSRs with intact epithelium (n = 8) or in rabbit TSRs with epithelium removed (n = 8).

In the sixth experimental group of rabbit TSRs (n = 10), the effect of LPA (10^{-6} M) on the isometric contraction caused by cumulative addition of serotonin (from 10^{-7} to 10^{-5} M) and substance P (from 10^{-7} to 10^{-5} M) was determined.

For each of these series of experiments, appropriate time controls were conducted, and the effects of all agonists were reproducible.

Data analysis. Responses of TSRs to the various agents tested were expressed as the change (Δ) in isometric tension (g). All data are presented as means ± SE. Differences among groups were determined by analysis of variance for repeated measures, and differences between means were isolated by the Bonferroni correction for multiple t-tests. Student’s paired and unpaired t-tests were used for single comparisons. Statistical significance was accepted at P < 0.05.

RESULTS

Enhancement by LPA of methacholine-induced contraction of rabbit TSRs. Figure 1 demonstrates dose-dependent contraction of isolated rabbit TSRs elicited by cumulative administration of methacholine. Incubation of TSRs with LPA alone at 10^{-6} M did not affect baseline tension, which was 3.0 ± 0.2 g before and 2.9 ± 0.2 g after incubation with LPA (n = 20, not significant). LPA clearly potentiated contraction of the TSRs in response to the lower doses of methacholine (10^{-8} to 10^{-6} M), with increases ranging from 27 ± 9 to 124 ± 34% greater than with methacholine alone (n = 20, P < 0.05).

LPA dose dependence and specificity of the enhancement of contraction. Figure 2 compares the effects of LPA and phosphatidic acid at doses from 10^{-9} to 10^{-5} M on the change in isometric tension induced by adminis-

Fig. 1. Methacholine (MCh)-induced contraction of rabbit tracheal rings (TSRs) in absence and presence of lysophosphatidic acid (LPA). Changes (Δ) in isometric tension of isolated rabbit TSRs induced by cumulative addition of MCh were measured both before (control; open bars) and after addition of 10^{-6} M LPA to bath (solid bars). *P < 0.05 vs. control.

Fig. 2. Dose-dependent effects of LPA and phosphatidic acid (PA) on MCh-induced contraction of rabbit TSRs. Changes in isometric tension of isolated rabbit TSRs in response to 10^{-6} M MCh were measured in absence (control) or presence of indicated concentrations of LPA (open bars) or PA (hatched bars). *P < 0.05 vs. control. †P < 0.05 vs. same concentration of LPA.
tation of methacholine at $10^{-6}$ M. LPA caused a dose-dependent increase in methacholine-induced contraction. The lowest effective dose of LPA was $10^{-8}$ M. LPA alone, even at the highest dose of $10^{-5}$ M, had no significant effect on baseline tension. Administration of phosphatidic acid had no significant effect on the methacholine-induced contraction. Changes in isometric tension in response to methacholine in rabbit TSRs incubated with phosphatidic acid were not significantly different from those in control TSRs and were significantly lower than those in TSRs incubated with the same doses of LPA.

Inhibition by LPA of isoproterenol-induced relaxation of rabbit TSRs. Figure 3 illustrates the ability of LPA to inhibit relaxation by isoproterenol of rabbit TSRs precontracted with methacholine at $10^{-5}$ M. The lowest dose of isoproterenol tested ($5 \times 10^{-7}$ M) caused only a slight relaxation that was not altered by LPA. Isoproterenol at $10^{-6}$ M caused a decrease in tension of $0.31 \pm 0.08$ g, and this response was inhibited by $45\% (0.17 \pm 0.06$ g; $n = 20$, $P < 0.05$) when $10^{-6}$ M LPA was included. Isoproterenol at $10^{-5}$ M decreased isometric tension by $0.69 \pm 0.11$ g, and LPA at $10^{-6}$ M inhibited this response by $29\% (0.49 \pm 0.08$ g; $n = 20$, $P < 0.05$).

Reversibility of the effects of LPA. Figure 4 demonstrates the changes in the isometric tension of rabbit TSRs in response to $10^{-6}$ M methacholine and $10^{-5}$ M isoproterenol before, during, and after incubation of the TSRs with $10^{-6}$ M LPA. The effect of LPA was completely reversible. By 15 min after the washout of LPA, both the contractile response to methacholine and the relaxation response to isoproterenol had returned to the control level.

Effects of LPA on responses to other contractile agonists. Figure 5A demonstrates dose-dependent contraction of the isolated rabbit TSRs elicited by cumulative administration of serotonin ($10^{-7}$ to $10^{-5}$ M). Incubation of TSRs with LPA ($10^{-6}$ M) increased the contraction of the TSRs in response to all doses of serotonin tested. Figure 5B demonstrates dose-dependent contraction of the isolated rabbit TSRs elicited by cumulative administration of substance P ($10^{-7}$ to $10^{-5}$ M). LPA ($10^{-6}$ M) significantly potentiated contraction of the TSRs in response to all doses of substance P, with increases ranging from $730 \pm 70\%$ at the dose of $10^{-7}$ M to $59 \pm 9\%$ at the dose of $10^{-5}$ M ($n = 8$, $P < 0.05$).

Effects of LPA on responses to KCl, isoproterenol, and forskolin. Figure 6 demonstrates dose-dependent contraction of the isolated rabbit TSRs elicited by cumulative administration of KCl (10 to 50 mM). Incubation of TSRs with LPA ($10^{-6}$ M) did not affect contraction of the TSRs in response to any of the doses of KCl. Administration of isoproterenol ($10^{-5}$ M) to the TSRs precontracted with $50$ mM KCl induced relaxation; the decrease in tension in response to isoproterenol before incubation with LPA was $1.04 \pm 0.21$ g, and this response was reduced to $0.63 \pm 0.18$ g after incubation with LPA ($10^{-6}$ M), an inhibition of $39 \pm 9\%$ ($n = 18$, $P < 0.05$). Administration of forskolin ($10^{-6}$ M) to the TSRs precontracted with $50$ mM KCl caused a decrease in tension of $1.30 \pm 0.19$ g before incubation with LPA and of $0.71 \pm 0.09$ g ($n = 8$, $P < 0.05$) after incubation with LPA ($10^{-6}$ M), an inhibition of $40 \pm 7\%$ ($n = 8$, $P < 0.05$).

Effects of epithelium removal on responses to methacholine, KCl, and LPA. Figure 7 illustrates the effect of LPA ($10^{-6}$ M) on the isometric contraction of rabbit TSRs with intact or removed epithelium to cumulative addition of methacholine (A) or KCl (B). Removal of epithelium did not change the contractile responses of TSRs to either agonist. Incubation with LPA similarly potentiated the contraction induced by methacholine in TSRs with intact or without epithelium. LPA had no effect on the contractile response to KCl in TSRs with intact or removed epithelium.

Effects of LPA on contraction and relaxation of cat TSRS. Figure 8 illustrates the effects of methacholine and LPA on contraction of isolated cat TSRS. Cumulative administration of methacholine elicited dose-dependent increases in isometric tension similar to those observed in rabbit TSRs. LPA alone at $10^{-6}$ M did not affect baseline tension, which was $2.98 \pm 0.25$ g before and $2.97 \pm 0.24$ g after incubation with LPA ($n = 16$, not significant). LPA significantly enhanced contrac-

---

**Fig. 3.** Isoproterenol-induced relaxation of rabbit TSRs in absence and presence of LPA. Changes in isometric tension in isolated rabbit TSRs precontracted with $10^{-5}$ M MCh were measured in response to indicated concentrations of isoproterenol in absence (control; open bars) or presence of $10^{-6}$ M LPA (solid bars). *P < 0.05 vs. control.

**Fig. 4.** Reversibility of effects of LPA on rabbit TSRs. Contractions of isolated rabbit TSRs induced by $10^{-6}$ M MCh and relaxations induced by $10^{-5}$ M isoproterenol were measured before exposure to LPA (open bars), during incubation with $10^{-6}$ M LPA (solid bars), and 15 min after washout of LPA (hatched bars). *P < 0.05 vs. control.
tion of the TSRs, ranging from 51 ± 15 to 136 ± 44% in response to all doses of methacholine tested (n = 16, P < 0.05).

Figure 9 illustrates the effects of isoproterenol and LPA on relaxation of cat TSRs. Cumulative administration of isoproterenol to cat TSRs precontracted with methacholine caused a dose-dependent relaxation, with marked relaxation at a concentration of isoproterenol of 5 × 10⁻³ M that was essentially ineffective in rabbit TSRs (Fig. 3). Inclusion of LPA at 10⁻⁶ M significantly attenuated the relaxation in response to 5 × 10⁻³ and 10⁻⁵ M isoproterenol by 61 ± 6 and 27 ± 8%, respectively (n = 16, P < 0.05) but did not have a statistically significant effect on the relaxation induced by 10⁻⁵ M isoproterenol.

**DISCUSSION**

The present study documents the ability of the simple phospholipid mediator LPA to both enhance contraction and inhibit relaxation of isolated tracheal smooth muscle rings from both rabbits and cats. Although effects of LPA on vascular, gastrointestinal, and uterine smooth muscle contraction and on blood pressure regulation have been reported previously (21, 24–26, 28), we believe this is the first report on the ability of LPA to modulate airway smooth muscle contraction.

LPA did not induce contraction on its own at any concentration. However, a significant enhancement of methacholine-induced contraction was observed with LPA concentrations as low as 10⁻⁶ M. The structurally similar lipid phosphatidic acid was markedly less effective. Both the enhancement of contraction and the inhibition of relaxation by LPA were rapidly reversed on removal of LPA. The potency, specificity, and reversibility of these effects on airway smooth muscle contraction and relaxation are typical of those for a variety of other responses to LPA in numerous cell types (12, 13). Considerable evidence indicates that the effects of LPA are likely to be mediated by one or more members of the G protein-coupled family of cell surface receptors (12, 13), and two recent reports have described the cloning of LPA receptors from *Xenopus* oocytes and from mammalian brain (6, 7).

Potential mechanisms for the effects of LPA on airway smooth muscle contraction were investigated. LPA enhanced the contraction of rabbit TSRs not only to methacholine but also to serotonin and to substance P. These results suggest that LPA modulates a step in the contractile signaling pathway that is shared by all of these agents rather than acting on receptors for specific contractile agonists. However, LPA did not alter the contractions induced by KCl, which bypasses receptor-signaling pathways and leads directly to depolarization-induced Ca²⁺ release. This result suggests that LPA does not alter the Ca²⁺ sensitivity or responsiveness of the contractile machinery and again is consistent with LPA modulation of more upstream signaling pathways leading to Ca²⁺ mobilization and contraction. The contraction-enhancing effects of LPA are apparently
mediated directly on the smooth muscle cells because removal of the epithelium did not alter either the contractions induced by methacholine or KCl or the enhancement of the methacholine contraction by LPA.

The mechanism by which LPA inhibits relaxation of rabbit TSRs was also investigated. LPA was able to inhibit isoproterenol-induced relaxation of TSRs contracted with KCl in a way similar to its inhibition of isoproterenol-induced relaxation of TSRs contracted with methacholine. Thus the inhibition of isoproterenol-induced relaxation by LPA is not simply an artifact related to its ability to enhance agonist-induced contraction because LPA did not enhance contraction induced by KCl as it did for methacholine. LPA also inhibited the relaxation induced by the direct adenylyl cyclase activator forskolin, indicating that the mechanism of inhibition of relaxation is not unique to the β-adrenergic receptor signaling pathway but is probably common to all activators of adenylyl cyclase.

LPA is known to activate a variety of signal transduction pathways in different cell types, including stimulation of phosphoinositide hydrolysis, inhibition of cAMP accumulation, and activation of tyrosine kinase-signaling cascades (12, 13). We have previously characterized some of the signal transduction pathways activated by LPA in cultured human airway smooth muscle cells (14). LPA stimulated phosphoinositide hydrolysis in these cells, and this seems a reasonable mechanism for the effects of LPA on contraction reported in this study. However, most agents that stimulate phosphoinositide hydrolysis in smooth muscle lead to contraction on...
their own. This is also true for LPA in previous studies in other types of smooth muscle (21, 24–26, 28). In contrast, LPA did not significantly affect tracheal smooth muscle contraction on its own but rather enhanced the responsiveness to methacholine and other contractile agonists.

The effects of LPA on the cAMP signaling pathway in the human airway smooth muscle cells were complex (14). LPA inhibited cAMP accumulation stimulated by the direct adenyl cyclase activator forskolin, but it enhanced cAMP accumulation stimulated by the β-adrenergic receptor agonist isoproterenol. Pretreatment of these cells with LPA led to a "sensitization" of subsequent stimulation by both forskolin and isoproterenol. The enhancement of isoproterenol-stimulated cAMP accumulation by LPA would be predicted to enhance isoproterenol-induced relaxation as well, but in the present study an inhibition of relaxation was observed instead. The effects of LPA on cAMP accumulation may be different in the isolated rabbit and cat tracheal rings used in the present study than in the cultured human airway smooth muscle cells used in our previous study (14). Alternatively, the effects of LPA on contraction may not be mediated by a direct effect on cAMP. Other possibilities include LPA regulation of tyrosine kinase pathways or effects of G protein α- or βγ-subunits on various membrane ion channels. It seems likely that the effects of LPA on airway smooth muscle contractility may not be mediated by a single signaling pathway but rather by complex interactions among the multiple signaling pathways that can be activated by LPA. Clearly, additional studies will be required to understand the molecular basis for the observed effects of LPA on tracheal smooth muscle contraction.

LPA also stimulates DNA synthesis and cell growth in human airway smooth muscle cells in culture and exhibits a profound synergism with epidermal growth factor for these effects (2). Several agents classically thought of as contractile stimuli, such as histamine, endothelin, and thrombin, have recently been shown to also promote airway smooth muscle cell growth (8, 15, 19). Conversely, some agents classically thought of as growth factors, such as epidermal growth factor, have been shown to also exert contractile effects on various types of smooth muscle (9, including tracheal smooth muscle (16). LPA can now be added to this list of agents inducing both responses. Previous studies have documented effects of LPA on both contraction (21, 24) and growth (27) of vascular smooth muscle. Our studies indicate that LPA also induces both effects in airway smooth muscle.

The physiological significance of LPA for lung function is a question of obvious interest. The best-characterized source of LPA is from activated platelets; thus LPA is present in serum but not in carefully isolated plasma (4, 22). Local concentrations of LPA would presumably be high at a site of tissue damage, where platelet activation would occur. LPA has also been shown to be released from fibroblasts stimulated with platelet-derived growth factor (5). To our knowledge, LPA has not been quantitated or documented to be present in the lung. However, increased concentrations of phospholipase A2 and of various lysophospholipids that could serve as precursors for LPA have been documented in bronchoalveolar lavage fluids from allergic subjects challenged with antigen (3). We believe that LPA is likely to be present in the lung and to be of physiological relevance there because we have observed multiple effects of LPA on several different types of lung cells. In addition to the effects on airway smooth muscle, LPA also stimulates fibroblast release from airway epithelial cells (18) and fibroblasts (T. Mio, M. L. Toews, D. J. Romberger, and S. I. Rennard, unpublished observations), promotes filopodia extension by airway epithelial cells (1), and enhances lung fibroblast-mediated collagen gel contraction (T. Mio, M. L. Toews, D. J. Romberger, and S. I. Rennard, unpublished observations).

These diverse effects of LPA suggest several possible roles for LPA in both normal and pathological lung function. Increased smooth muscle mass and enhanced contractility are both characteristic features of asthma and other obstructive pulmonary diseases (20, 29). Thus the ability of LPA to enhance both contraction and proliferation of airway smooth muscle cells suggests the possible involvement of LPA in the pathology of these diseases. It seems likely that the normal physiological function of LPA in the lung may be to promote tissue repair in response to injury or inflammation, as proposed previously for other tissues (12, 13). The effects of LPA on release of fibronectin, extension of filopodia, contraction of collagen gel matrices, and promotion of cell growth are all consistent with a role in tissue repair in the lung. LPA could also play a role in lung fibrosis if these repair processes were not properly regulated.

In summary, the present study documents the ability of LPA to enhance contraction and inhibit relaxation of airway smooth muscle from two different species. These effects, together with additional responses documented in other studies, suggest that LPA may be an important regulator of lung cell function with both physiological and pathological significance. Further characterization of the receptors and signaling pathways by which LPA mediates these effects and of the factors controlling LPA release and metabolism in the lung may lead to novel approaches for the therapeutic modulation of lung cell function in various pulmonary diseases.

Address for reprint requests: M. L. Toews, Dept. of Pharmacology, Univ. of Nebraska Medical Center, 600 S. 42nd St., Omaha, NE 68198-6260.

Received 8 July 1996; accepted in final form 17 June 1997.

REFERENCES


