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Lysophosphatidic Acids Induce Contraction of Rat Isolated Colon by Two Different Mechanisms

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Abstract—Lysophosphatidic acids (1-linoleoyl-, 1-linolenoyl, 1-arachidonoyl- and 1-*O*-hexadecyl-*sn*-glycero-3-phosphate) induced rapid contraction of rat isolated colon which was dependent on external Ca^{2+}, 1-linolenoyl-lysophosphatidic acid having the greatest effect. The contraction induced by 1-linolenoyl-lysophosphatidic acid was reduced considerably by nifedipine and verapamil, but not by atropine or indomethacin. Phosphatidic acids with two short-chain acyl groups induced a small, atropine-sensitive contraction at 100 μM, but phosphatidic acids with two long-chain acyl groups were inactive. These results suggest that unlike phosphatidic acids, lysophosphatidic acids act directly on extracellular sites of the plasma membrane of smooth muscle cells in rat colon, mainly through activation of voltage-sensitive Ca^{2+} channels.

Phosphatidic acid (PA) is formed in the degradation of inositol phospholipids by various Ca^{2+}-mobilizing agents (Abdel-Latif 1986). An alternative pathway for PA formation involving phospholipase D has recently attracted much attention (Exton 1990), and PA has been suggested to be a second messenger in a signal transducing system in a variety of animal cells, although little is known about its biological activity. In many types of animal cells, PA has been reported to be partly converted to diglycerides (Exton 1990), which are believed to be second messengers (Nishizuka 1984). Thus, some biological actions observed after addition of exogenous PA are believed to be ascribable, at least in part, to diglycerides (Ando et al 1989).

The platelet-aggregating and growth factor-like actions of PA have been found to be due to lysophosphatidic acid (LPA) contaminating commercial PAs (Benton et al 1982; Jalink et al 1990). In fact, LPA-induced platelet aggregation (Gerrard et al 1979; Schumacher et al 1979; Tokumura et al 1981; Simon et al 1982) and mitogen activity on fibroblasts (van Corven et al 1989) occurred at much lower concentrations than those of PA. These findings indicate that LPA may be an important mediator in signal transduction. LPA can contract isolated strips of rabbit duodenum (Vogt 1963), guinea-pig ileum (Tokumura et al 1982) and rat uterus (Tokumura et al 1980) at submicromolar concentrations, and PA weakly contracted smooth muscle cells cultured from guinea-pig ileum (Salmon & Honeyman 1980) and guinea-pig isolated taenia caeci (Dakhil & Vogt 1965; Ohta & Momose 1990). In this study, we have compared the actions of LPA and PA on rat isolated colon.

Materials and Methods

Materials

Polyunsaturated LPAs [1-linoleoyl (18:2)-, 1-linolenoyl (18:3)- and 1-arachidonoyl (20:4)-*sn*-glycero-3-phosphate as sodium salts] were gifts from Nippon Shoji Co. Osaka, Japan. An alkyl ether type LPA (1-*O*-hexadecyl-*sn*-glycero-3-phosphate) was prepared as reported previously (Tokumura et al 1985). Phosphatidic acids with dicaproyl (6:0), dioctanoyl (8:0) or didecanoyl (10:0) residues were obtained by hydrolysing the corresponding phosphatidylcholines (Sigma Chemical Co., St Louis, MO) with phospholipase D from cabbage (Sigma) in a mixture of ethyl ether/0·2 M sodium acetate buffer (pH 5·6) containing 1 mM Ca^{2+} (2:1, v/v) for several hours at room temperature with vigorous stirring. The PAs were then purified by preparative TLC (Merck Silica Gel 60 TLC plates) in benzene/pyridine/formic acid (60:40:11, v/v/v). 1-Stearoyl-2-arachidonoyl-*sn*-glycero-3-phosphate and phosphatidic acid from egg yolk lecithin were obtained from Sigma. CV-3988 and FR-900452, antagonists of platelet-activating factor (PAF), were generous gifts from Takeda Chemical Ind. Co. (Osaka, Japan) and Fujisawa Pharmaceutical Co. (Ibaraki, Japan). Acetylcholine chloride, atropine sulphate, nifedipine and verapamil hydrochloride were from Wako Pure Chemical Ind. Co. (Osaka, Japan). Indomethacin was from Sigma.

Polyunsaturated LPAs, atropine and acetylcholine were dissolved in 154 mM NaCl. An alkyl ether type of LPA was dissolved in saline containing 0·1% bovine serum albumin (BSA). PAs, CV-3988, FR-900452, verapamil and nifedipine were dissolved in ethanol. Indomethacin was dissolved freshly in 0·1 mM Na_2CO_3, and the pH of the solution was adjusted to 7·8. The final concentrations of BSA and ethanol in the bath fluid were below 0·001% and 0·1%, respectively. The vehicle controls did not affect tension of rat isolated colon.

Tissue preparation and measurement of contraction in rat isolated colon

The colons of male Wistar rats, 200–300 g, sectioned 2 cm from the ileocaecal junction, were removed and cut into segments of 1·5–2 cm length; these segments were named colon 1, colon 2 and colon 3, in order from the ileocaecal junction. The isolated segments were mounted in 10 mL of Tyrode's solution (mM: NaCl 137, KCl 2·7, $CaCl_2$ 2·5, $MgCl_2$ 1·0, $NaHCO_3$ 11·9, NaH_2PO_4 0·4 and glucose 5·5, pH 7·8) bubbled with air at 37°C for 1 h, as described previously (Tokumura et al 1988).

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Activity of the colon longitudinal muscle was recorded isotonically on a kymograph under 1 g load. The segments were induced to contract by cumulative addition of acetylcholine at intervals to allow a maximum in each contraction, washed with fresh Tyrode's solution and rested for 20 min before addition of a test material.

Statistics

Results are expressed as means ± s.e. The significance of differences among the group means were evaluated by 2-way analysis of variance followed by Student's *t*-test, and a *P* value of <0.05 was considered significant.

Results

18:3-LPA at 20 μM caused concentration-dependent contraction of isolated segments of different regions of rat colon (colon 1, colon 2 and colon 3); a large contraction was superimposed with rhythmical smaller contractions (Fig. 1A). Change of the bath solution 5 min after the addition of LPA, resulted in a rapid decrease in the tension to the baseline level ($t_{\frac{1}{2}} = 3.9 \pm 0.6$ min, n = 10). Subsequent addition of 18:3-LPA 15 min after washing, induced contractions of the same amplitude as those previously, indicating no tachyphylaxis.

18:3-LPA was the most potent of the LPAs tested; at 100 μM it induced contractions of about 73, 72 and 68% of the maximal contractions of colon 1, 2 and 3 induced by acetylcholine. At 20 μM 18:3-LPA induced greater contraction of colon 1 than of colon 2 or colon 3 ($P<0.01$, Table 1). Other polyunsaturated LPAs such as 18:2-LPA and 20:4-LPA, induced similar, but smaller contractions to those induced by 18:3-LPA ($P<0.01$, Table 1). An alkyl ether type of LPA, 16:0-LPA, also induced a large contraction on which were superimposed rhythmic small contractions, although it was less potent than 18:2-LPA ($P<0.01$, Table 1).

Next, we tested whether the lyso-structure of LPA was required for induction of contractions. We first examined the effects of PAs with two long-chain acyl groups. Neither 1-stearoyl-2-arachidonoyl-PA (n = 5), a physiological species, nor egg yolk PA (n = 7) induced contractions at concentrations of up to 100 μM. Next, we tested the effects of PAs with two short-chain acyl groups. Didecanoyl(10:0)-PA up to 100 μM was inactive, but 100 μM dioctanoyl(8:0)-PA induced small contractions (Table 1). A typical tracing for the contraction by 100 μM 8:0-PA is shown in Fig. 1B. Dicaproyl(6:0)-PA had a biphasic effect on colon 1, as shown in Fig. 1C. When 30, 70 or 100 μM 6:0-PA was added to the bath solution, the resting tone was rapidly decreased, but after replacing the bath solution, the tension increased to above the basal level in a concentration-dependent manner (Table 1). The sustained contraction was resistant to repeated washings of the colon with fresh Tyrode's solution, but atropine completely overcame the 6:0-PA-induced contraction. A typical result with 100 μM 6:0-PA is shown in Fig. 1C. The sustained contraction that appeared after washing was specific for colon 1. 6:0-PA induced relaxation of colon 2, like colon 1, but it did not raise the tone of the colon after washing the segments with fresh Tyrode's solution (Table 1).

Acetylcholine-induced contraction of colon 1 (ED50 = 0.24 ± 0.043 μM, n = 6) and colon 2 (ED50 = 0.27 ± 0.037 μM, n = 10) was inhibited by pretreatment of the segments with 100 μM 6:0-PA: the ED50 values in the presence of 6:0-PA were 0.88 ± 0.019 μM (n = 6) in colon 1 and 0.90 ± 0.012 μM (n = 10) in colon 2, respectively. The maximal contractions of colon 1 and colon 2 induced by acetylcholine were decreased by 20.9 ± 2.1% (n = 6) and 24.1 ± 2.7% (n = 10), respectively, in the presence of 100 μM 6:0-PA.

Verapamil or nifedipine (1 μM) for 15 min decreased the 18:3-LPA (20 μM)-induced contraction, but there was little or no inhibition by 1 μM atropine, 10 μM indomethacin, or PAF antagonists 10 μM CV-3988 and 10 μM FR-900452 (Table 2). A 10-fold higher concentration of nifedipine caused no further inhibition of the LPA-induced contraction.

Fig. 2A shows the concentration-response curve of 18:3-LPA-induced contractions of colon 1 in the presence and absence of nifedipine. The maximal contraction in the presence of nifedipine was observed at 2 μM 18:3-LPA, and corresponded to 20.8 ± 0.9% of the maximal concentration induced by acetylcholine in the absence of nifedipine. On increasing the LPA concentration to 100 μM, the nifedipine-sensitive component of the contraction induced by 18:3-LPA increased concentration-dependently to about 44% of the maximal contraction with acetylcholine, which was

Table 1. Potencies of various LPAs and PAs in inducing contraction of rat isolated colon.

LPA/PA	Dose (μM)	Contraction colon 1	colon 2	colon 3
18:2-LPA	20	20.3 ± 4.0*(3)	N.D.	N.D.
18:3-LPA	20	53.4 ± 2.8(18)	36.2 ± 4.6**(9)	30.0 ± 6.0**(8)
	100	73.1 ± 4.2(10)	71.6 ± 5.3(5)	67.5 ± 7.6(6)
20:4-LPA	20	38.3 ± 4.0*(10)	9.9 ± 3.9**(4)	11.3 ± 2.7**(6)
16:0-LPA	20	18.3 ± 5.0*(8)	N.D.	N.D.
6:0-PA	30	14.7 ± 2.3(3)	0	N.D.
	70	27.1 ± 1.1(7)	0	N.D.
	100	47.8 ± 2.0(5)	0	N.D.
8:0-PA	100	13.9 ± 2.1(9)	0	N.D.

Values are means ± s.e., expressed as percentages of the maximal contraction induced by acetylcholine. Numbers in parentheses are numbers of preparations examined. * $P<0.01$, compared with 20 μM 18:3-LPA; ** $P<0.01$, compared with colon 1. N.D. = not determined.

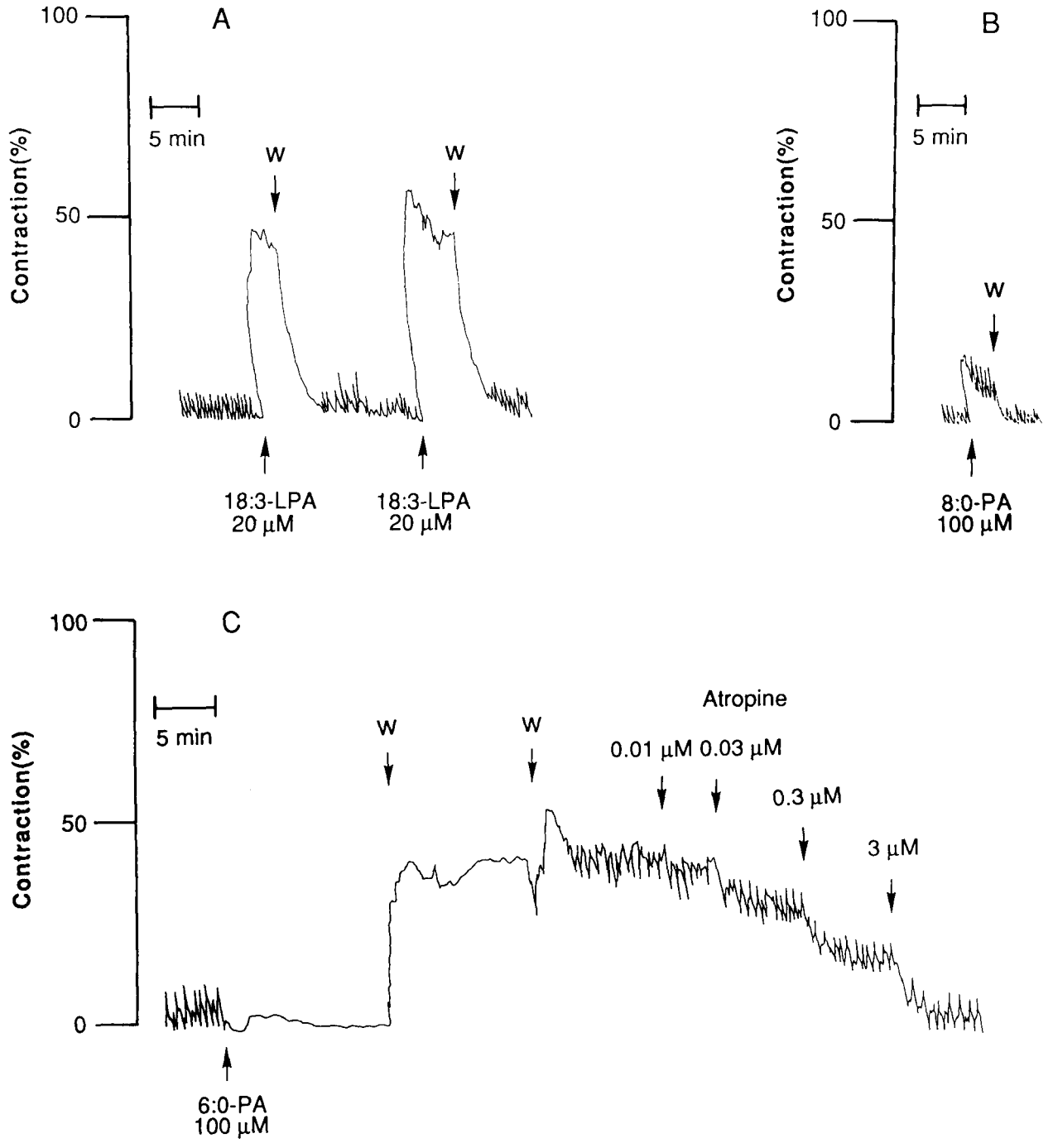

FIG. 1. Contractions of rat isolated colon (colon 1) induced by 20 μM 18:3-LPA(A), 100 μM 8:0-PA(B) and 100 μM 6:0-PA(C).

almost the same as the maximal contraction attained with high K^+ (Fig. 2B).

Discussion

Previously we reported that the potencies of LPAs with a C_{18}-fatty acid to induce contraction of guinea-pig isolated ileum (Tokumura et al 1982) and rat uterus (Tokumura et al 1980), increased with increase in the number of *cis*-double bonds in the fatty acyl moiety. Consistent with these previous findings, in this study, 18:3-LPA had a greater contractor effect on rat isolated colon than 18:2-LPA. Alkyl ether-type LPAs were more effective than the acyl type of LPAs in aggregating human (Simon et al 1982) and feline (Tokumura et al 1987) platelets, but the reverse occurred with contraction of rat isolated colon. Thus, the present result confirmed that the structure-activity relationships of LPAs on smooth muscle contraction are different from those on platelet aggregation.

Table 2. Effects of various chemicals on 18:3-LPA-induced contraction of rat isolated colon.

Chemical	Dose(μM)	Inhibition(%)
CV-3988	10	0·4 ± 5·0(5)
FR-900452	10	7·1 ± 6·1(5)
Indomethacin	10	14·3 ± 3·9(5)
Atropine	1	2·6 ± 8·8(6)
Verapamil	1	59·0 ± 4·8*(4)
Nifedipine	1	61·5 ± 5·8*(7)
	10	60·5 ± 6·9*(5)

The preparation was contracted with 20 μM 18:3-LPA. Values are means ± s.e. Numbers in parentheses are numbers of specimens tested. * $P < 0.01$.

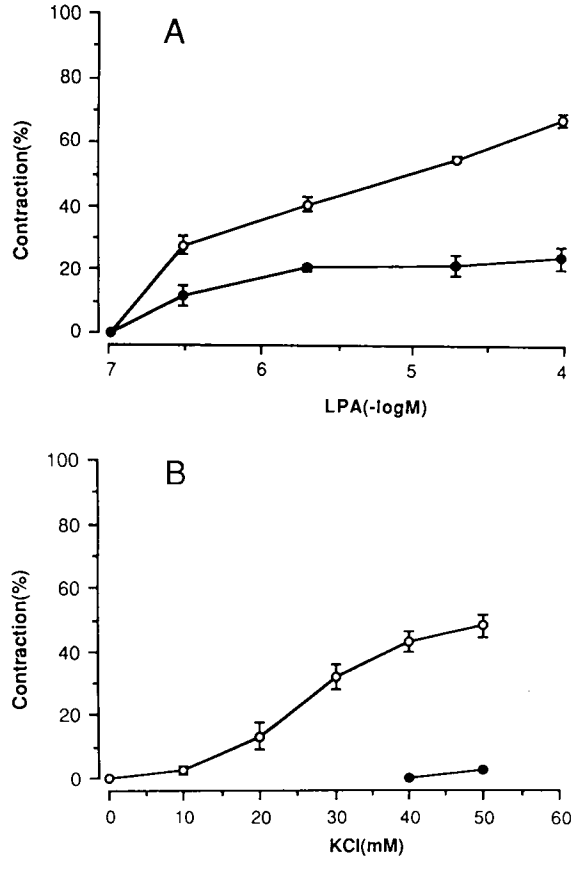

FIG. 2. Concentration-response curves of (A) 18:3-LPA- and (B) K^+-induced contraction of rat isolated colon (colon 1) in the presence (●) and absence (○) of 1 μM nifedipine.

PA is formed in many animal cells in response to various stimuli, and has been suggested to be a second messenger (Exton 1990), but little is known about its biological activity. This is partly because PA is poorly soluble in aqueous solution and has high affinity for bivalent cations (e.g. Ca^{2+}), so the biological activity of exogenous PA is difficult to determine. On the other hand, polyunsaturated LPAs are sparingly soluble in water, and so may be able to bind to the plasma membranes of target cells and penetrate into the cells. Thus exogenous LPA might mimic the potential functional activities of PA. However, our findings that PAs with two long-chain acyl fatty acids had no effect on rat isolated colon, and that PAs with two short-chain fatty acids induced a slight contraction of the colon possibly through cholinergic stimulation, do not support the above idea.

Previously, we suggested that contraction of longitudinal muscle from guinea-pig ileum by polyunsaturated LPA was, in part, mediated by endogenous prostaglandins. However, prostaglandins seem unlikely to contribute significantly to LPA-induced contraction of rat colon. LPA presumably acted on an extracellular site on rat isolated colon, because the contraction occurred rapidly and was easily removed by changing the bath fluid. LPA action may be induced by nifedipine-sensitive and insensitive mechanisms. The nifedipine-sensitive contraction of the colon induced by 100 μM 18:3-LPA was almost equal to maximal contraction induced by K^+, so LPA at this concentration seemed to evoke full depolarization of the plasma membrane, stimulating voltage-sensitive Ca^{2+} channels. The mechanism of nifedipine-insensitive contraction of the colon was induced maximally by a lower concentration of 18:3-LPA than that required for maximal nifedipine-sensitive contraction. This mechanism remains to be clarified.

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