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Contractile actions of lysophosphatidic acids with a chemically-defined fatty acyl group on longitudinal muscle from guinea-pig ileum

A. TOKUMURA*, K. FUKUZAWA AND H. TSUKATANI

Faculty of Pharmaceutical Sciences, Tokushima University, Shomachi, Tokushima, 770, Japan

Various lysophosphatidic acids caused phasic contractions of longitudinal muscle from guinea-pig ileum, followed by rhythmical contractions of lower magnitude. Polyunsaturated lysophosphatidic acids were more potent than saturated lysophosphatidic acids. Among the lysophosphatidic acids tested, linolenoyl-lysophosphatidic acid was the most active, but its activity was about 3–5 times less than that of prostaglandin $F_{2\alpha}$. Contractile responses to various lysophosphatidic acids, were partially suppressed by tetrodotoxin, atropine and chlorpheniramine. In addition, lysophosphatidic acid-induced contractions were considerably reduced by pretreatment of the muscle with indomethacin.

Vogt (1949) originally reported the release of a smooth-muscle stimulating substance from frog isolated intestinal strips and named it 'Darmstoff'. Later, the active principle in Darmstoff was claimed to be acetal phosphatidic acid (Vogt 1957), but subsequent studies showed that this phospholipid was an artifact formed during acid-extraction of the intestinal tissues and that the principle in Darmstoff was actually prostaglandins (Vogt et al 1966). Lysophosphatidic acid (LPA), structurally related to acetal phosphatidic acid, was isolated from horse brain lipid extracts as a smooth-muscle stimulating factor (Kirschner & Vogt 1961; Vogt 1963). Vogt noted that these two active phospholipids had no significant effect on the cardiovascular system but she did not survey their pharmacological actions.

Recently, Schumacher et al (1979) reported that a vasodepressor substance named DAS in incubated feline plasma was LPA and that it induced aggregation of human and feline platelets. We have also reported that intravenous injection of LPA causes an immediate fall in blood pressure of cats and rabbits and transient hypertension in rats and guinea-pigs (Tokumura et al 1978), and that LPA contracts isolated uterine muscle of non-pregnant rats (Tokumura et al 1980) and aggregates human and feline platelets (Tokumura et al 1981). The chain length and degree of unsaturation of the *sn*-1-acyl moiety of the LPA molecule greatly affects the potency of the vasoactivity (Tokumura et al 1978), stimulatory action on rat isolated uterus (Tokumura et al 1980) and platelet aggregating activity (Tokumura et al 1981).

* Correspondence.

In the present study we examined the mechanism of the contractile action of LPA on isolated longitudinal strips of guinea-pig ileum and compared the potencies of LPAs with different chemically-defined fatty acyl groups in producing contrations of muscle strips.

METHODS

Male guinea-pigs, 300–380 g, were killed by a blow on the head and muscle segments were removed from the ileum 20–30 cm distal to the duodenum. The longitudinal strips (2–3 cm) were mounted in a 5 ml organ bath containing Tyrode solution maintained at 37 °C and bubbled with 95% O_2 + 5% CO_2. The Tyrode solution had the following composition (mM): NaCl, 137; KCl, 2·7; $NaHCO_3$, 11·9; NaH_2PO_4, 0·4; $MgCl_2$, 1·0; $CaCl_2$, 2·5 and glucose, 5·5 (pH 7·8). Contractions were measured with a isotonic lever with 7-fold magnification and 0·5 g load.

The muscle was incubated for 1–3 h with repeated washings until a constant response was attained. Contractions were evoked by additions of test compounds in a small volume of Tyrode solution. All LPAs and prostaglandin $F_{2\alpha}$ were dissolved in Tyrode solution. Arachidonic acid and indomethacin were dissolved in 0·1 M sodium bicarbonate and diluted with 0·9% NaCl. Because of the low solubility of saturated LPAs, high doses were dispersed in 1–3 ml of Tyrode solution, and the solution was sonicated in a Branson B-12 bath-type sonicator before addition to the organ bath.

1-Decanoyl-, 1-palmitoyl- and 1-oleoyl-LPA were purchased from Serdary Research Laboratories Inc.

(Canada) and purified as previously described (Tokumura et al 1978). 1-Linoleoyl- and 1-linolenoyl-LPA were generous gifts from Dr T. Nakajima of Nihon Shoji Co. (Japan). 1-Lauroyl- and 1-myristoyl-LPA were prepared by enzymatic degradation of the corresponding lysophosphatidylcholines (Sigma Chemical Co., U.S.A.) by the method of Long et al (1967). Other chemicals were obtained from the following sources: acetylcholine chloride (Daiichi Seiyaku Co., Japan), histamine dihydrochloride (Wako Pure Chemical Industries, Japan), prostaglandin $F_{2\alpha}$ (Sigma), atropine sulphate (Wako), chlorpheniramine maleate (Ono Pharmaceutical Co., Japan), tetrodotoxin (Sankyo Seiyaku Co., Japan), arachidonic acid (Sigma) and indomethacin (Sigma).

RESULTS AND DISCUSSION

Various LPAs caused phasic contractions of isolated longitudinal muscle strips from guinea-pig ileum, followed by rhythmical contractions of lower magnitude. A typical response of a strip to linolenoyl-LPA is shown in Fig. 1 together with those to arachidonic acid and prostaglandin $F_{2\alpha}$. Whereas arachidonic acid evoked only the initial phasic contraction, prostaglandin $F_{2\alpha}$ induced similar contractions to those produced by LPAs.

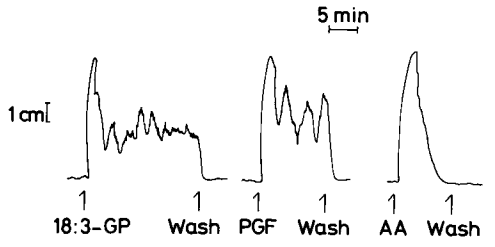

FIG. 1. Typical tracings of the responses of longitudinal muscle strips from guinea-pig ileum to various compounds. Equipotent doses of the following stimulants were used: linolenoyl-LPA (LPA, 7×10^{-6} M), prostaglandin $F_{2\alpha}$ (PGF, 2×10^{-6} M) and arachidonic acid (AA, 4×10^{-4} M). The vertical scale represents 1 cm, and the horizontal scale 5 min. Arrows indicate times of injection or washings.

Fig. 2 shows log dose-response curves of LPAs with a C_{18}-fatty acyl moiety. The curves were all parallel, and there was no differences in the maximal contractions to the polyunsaturated LPAs. The complete dose-response curve for stearoyl- and oleoyl-LPA could not be constructed because these compounds were poorly soluble in Tyrode solution and had less activity. Among the LPAs tested, linolenoyl-LPA was the most active, but its activity

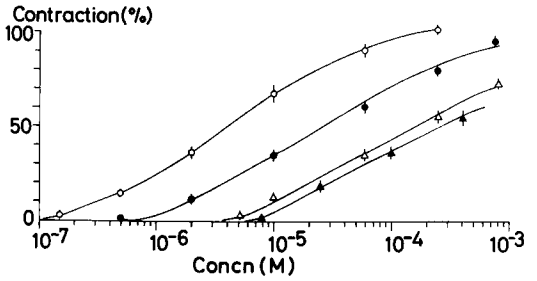

FIG. 2. Log dose-response relationships of contractile responses of longitudinal muscle strips to lysophoshatidic acids with a C_{18}-fatty acyl moiety. Contractions to LPAs are expressed as percentages of the maximal contractions produced by linolenoyl-LPA. Points represent means and vertical lines show s.e.m. O——O, linolenoyl-LPA; ●——●, linoleoyl-LPA; △——△, oleoyl-LPA; ▲——▲, stearoyl-LPA.

was about 3–5 times less than that of prostaglandin $F_{2\alpha}$. Saturated LPAs with a fatty acyl group (C_{12}–C_{16}) were all much less potent than the polyunsaturated LPAs.

The inhibitory effects of atropine (1×10^{-7} M), tetrodotoxin (1×10^{-6} M) and chlorpheniramine (1×10^{-6} M) were examined on half-maximal contractions of the strips in response to LPAs with a C_{18}-fatty acyl moiety. These concentrations of atropine and chlorpheniramine completely blocked the half-maximal contractions to acetylcholine and histamine, respectively. The contractile responses to various LPAs were only partially suppressed by tetrodotoxin and atropine, suggesting that stimulation of cholinergic nerves contributes in part in the contractile actions of various LPAs. In addition, chlorpheniramine partially antagonized the contractile action of LPAs, indicating the involvement of the release of histamine in the stimulating effects of LPA. There were no significant differences in the percent inhibitions by these antagonists of the contractile actions of various LPAs. Table 1 shows representative experimental data for linolenoyl-LPA.

Table 1. Effects of various antagonists on the contractile action of linolenoyl-LPA.

Antagonist	Dose(M)	Inhibition
Atropine	1×10^{-7}	19 ± 5 (11)*
Tetrodotoxin	1×10^{-6}	23 ± 5 (9)*
Chlorpheniramine	1×10^{-6}	11 ± 3 (10)*
Indomethacin	$5{\cdot}6 \times 10^{-6}$	56 ± 8 (8)**

Values are means ± s.e.m. Numbers in brackets are numbers of experiments. * and ** indicate significant differences from the control at $P < 0{\cdot}05$ and $P < 0{\cdot}01$, respectively.

To examine the contribution of endogenous prostaglandins, the muscle strips were pretreated with $5{\cdot}6 \times 10^{-6}$ M indomethacin for 30 min. Contractions were then induced by adding LPA in the presence of indomethacin and after washing it out. The inhibitory effect of indomethacin on prostaglandin synthesis was confirmed by the fact that it abolished arachidonic acid-induced half-maximal contractions. As shown in Table 1, LPA-induced contractions were much reduced by indomethacin. One possible explanation for this inhibition is that it is due to non-specific and reversible inhibition in the activity of guinea-pig ileal muscle by high doses of indomethacin, which was demonstrated as previously (Famey et al 1977, 1978; Lembeck & Juan 1974; Sorrentino et al 1972). However, the concentration of indomethacin used in this study was relatively low, and LPA-induced contractions were suppressed to similar degrees before and after washing out the indomethacin. In addition, the half-maximal contractions produced by acetylcholine and histamine were not significantly reduced by $5{\cdot}6 \times 10^{-6}$ M indomethacin (percent inhibition: acetylcholine, 2 ± 1, $n = 6$; histamine, 3 ± 1, $n = 6$). These results indicate that the above possibility is unlikely. Another possibility is that trace amounts of endogenous prostaglandins were released during contractions produced by LPAs, and potentiated the contractile action of exogenously added LPA, as reported in the case of the contractile action produced by angiotensin II (Chong & Dowing 1973; Aboulafia et al 1976), bradykinin (Aboulafia et al 1976) and cholecystokinin (Zséli et al 1979).

Little attention has been paid to the action of LPA, except for its intermediary role in *de novo* synthesis of glycerophospholipids, because there is no measurable accumulation of LPA in animal tissues. Although LPA has been isolated as an active principle from horse brain lipid extracts (Kirschner & Vogt 1961; Vogt 1963) and from feline incubated plasma (Schumacher et al 1979), it is unknown whether its synthesis has any physiological significance and whether it is released by stumuli. The diverse biological activities of LPA suggest that LPA has some physiological significance in animal cells.

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