COMMUNICATIONS

Effects of Synthetic and Natural Lysophosphatidic Acids on the Arterial Blood Pressure of Different Animal Species

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ABSTRACT

Intravenous injection of lysophosphatidic acid was found to cause hypertension in rats and guinea pigs, but hypotension in cats and rabbits. The potencies of the pressor and depressor effects of synthetic lysophosphatidic acids in rats and cats depended on their chain length and the degree of unsaturation of their fatty acyl moieties.

INTRODUCTION

We previously observed a transient pressor response after intravenous injection of crude soybean lecithin into rats (1). The substance with pressor activity in the preparation was isolated and identified as lysophosphatidic acid (1-acyl-*sn*-glycero-3-phosphate) (2). It contained a high percentage of unsaturated fatty acids and about five times more pressor activity than synthetic 1-palmitoyl-*sn*-glycero-3-phosphate, suggesting that the fatty acid moiety in these molecules greatly influenced their pressor activity. Therefore, we examined nine lysophosphatidic acids for their effects on the systemic arterial blood pressure when injected intravenously into various experimental animals.

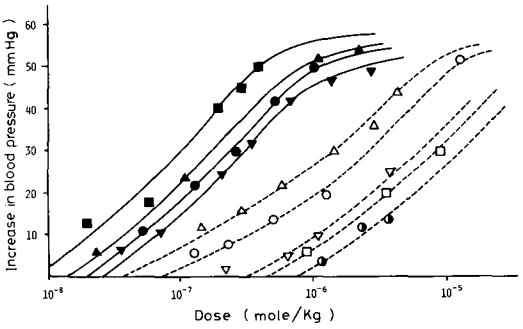

FIG. 1. Dose-response curves for the effects of synthetic LPA on the systemic arterial blood pressure of rats. Values are means of three observations. - - - ● - - - Decanoyl-LPA (10:0), - - - □ - - - Lauroyl-LPA (12:0), - - - ○ - - - Myristoyl-LPA (14:0), - - - △ - - - Palmitoyl-LPA (16:0), - - - ▽ - - - Stearoyl-LPA (18:0), —●— Oleoyl-LPA (18:1), —▼— Linoleoyl-LPA (18:2), —■— Linolenoyl-LPA (18:3), —▲— Arachidonyl-LPA (20:4).

MATERIALS AND METHODS

Lysophosphatidic acid (LPA) from crude soybean lecithin (Nakarai Kagaku, Kyoto, Japan) was prepared as described previously (1,2). Crude soybean lecithin was treated with cold acetone, and acetone-insoluble materials were chromatographed on a silicic acid column with chloroform-methanol mixtures increasing the polarity. The active fraction eluted with chloroform-methanol (4:6) was further purified by chromatography on Sephadex LH-20 with chloroform-methanol (1:1) and rechromatography with chloroform-methanol-water (60:35:8). Its fatty acid composition was as follows: palmitic acid, 24.7%; stearic acid, 7.5%; oleic acid, 8.7%; linoleic acid, 50.0%; and linolenic acid, 7.0%. Synthetic 1-palmitoyl-, 1-decanoyl-, and 1-oleoyl-LPA were purchased from Serdary Research Laboratories (London, Ontario, Canada) and purified by Sephadex LH-20 column chromatography with chloroform-methanol (1:1, v/v). The purified preparations migrated as single compounds by chromatography on silica gel plates (Merck, Silica gel plate 60, 250 μm-thick) using chloroform-methanol-water (60:35:5, v/v/v) or chloroform-methanol-28% ammonia (65:35:5, v/v/v) as developing solvents. Lauroyl-, myristoyl-, stearoyl-, linoleoyl-, linolenoyl-, and arachidonyl-LPA were prepared by the method of Long et al. (3,4) from the corresponding 1-acyl-*sn*-glycero-3-phosphocholines (Sigma Chemical Co., St. Louis, MO, and Serdary Research Laboratories) by hydrolysis with phospholipase D (phosphatidylcholine phosphatidohydrolase, EC 3.1.4.4: Sigma). 1-Acyl-*sn*-glycero-3-phosphocholine (25 mg) was sus-

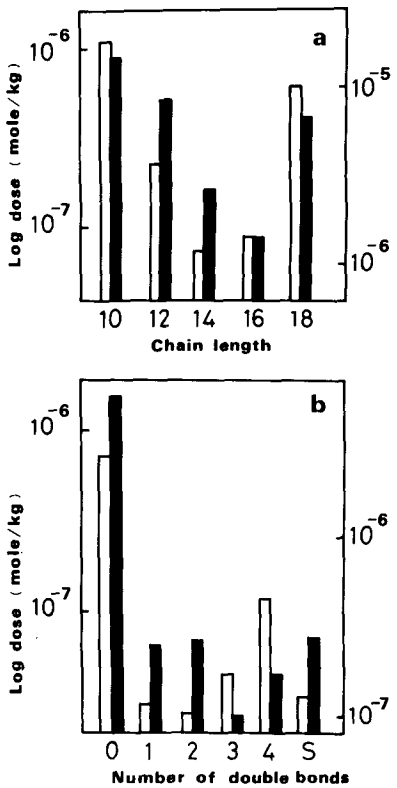

FIG. 2. The dependence of vasoactivity on the acyl chain length (a) and unsaturation of acyl chain (b) for lysophosphatidic acids. (a) Numbers represent the acyl chain length of saturated LPAs. (b) Numbers (0,1,2,3) represent degree of unsaturation of C_{18} acyl chain in LPAs. S: LPA from soybean lecithin, 4: arachidonyl-LPA. White bar shows the mean dose of LPA producing a half maximum fall in blood pressure of cats (left ordinates). Black bar shows the mean dose producing a half maximum rise in blood pressure of rats (right ordinates).

pended in 1.2 ml of water. Acetate buffer, pH 5.8 (0.2 M; 3.1 ml) and 1 M $CaCl_2$ (0.75 ml) were added, followed by 1.25 ml of enzyme solution (25 mg). The reaction mixture was incubated at 38 C for 24 hr. The products were extracted with a chloroform-methanol mixture from the reaction solution adjusted to pH 3 with 1 M citrate. Lysophosphatidic acid was purified by column chromatography on Sephadex LH-20 with chloroform-methanol (1:1) to remove a by-product (cyclic phosphatidic acid). The purified fractions appeared homogeneous on thin layer plates coated with silica gel and developed with the two solvent systems described above. The purified LPA preparations were assayed biologically as reported in detail previously (1). In brief, systemic arterial blood pressure was measured manometrically

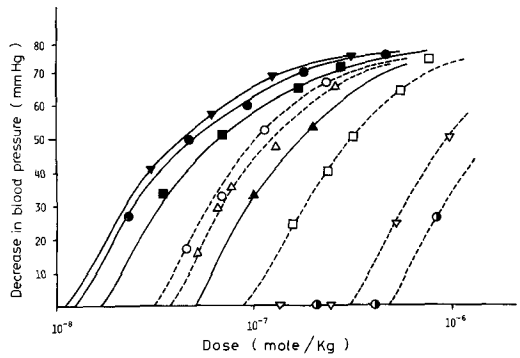

FIG. 3. Dose-response curves for the effects of synthetic LPA on the systemic arterial blood pressure of cats. Values are means of two observations. Symbols are the same as those in Figure 1.

via a cannula inserted into the carotid artery. The test materials as suspension in 200 μl of saline were injected from a microsyringe into the cannulated jugular veins of the anesthetized rats (200-250 g, urethane; 1.8 g/kg, i.p.) and guinea pigs (350-400 g, sodium pentobarbital; 40 mg/kg, i.p.), and femoral veins of the anesthetized male cats (2.8-3.2 kg, sodium pentobarbital; 40 mg/kg, i.p.) and rabbits (3.0-3.5 kg, urethane; 1.5 g/kg, i.p.).

RESULTS AND DISCUSSION

In 1960, McQuarrie and Anderson (5) detected a substance in commercial soybean lecithin with depressor activity on cats, but they did not examine it further either chemically or pharmacologically. Lysophosphatidic acid isolated from soybean lecithin was found to be hypertensive to rats and guinea pigs but hypotensive to cats and rabbits. This species specificity is the same as that of the F-series of prostaglandins (6-8) but different from that of histamine (9,10) which is hypertensive to rabbits and guinea pigs but hypotensive to cats and dogs. The present findings on the species specificity of LPA suggest that their depressor substance may have been LPA.

Cats were the most sensitive of the animals tested to isolated LPA (threshold doses, 2-3 μg/kg), rabbits being much less sensitive (threshold doses, 400-600 μg/kg. Rats were about three times more sensitive than guinea pigs, the threshold doses of LPA for the two being 10-15 μg/kg and 30-40 μg/kg, respectively. These values were in the same order as those of prostaglandin $F_{2\alpha}$ except those for rabbits. The qualitative and quantitative similarity in the vasoactive effects of LPA and prostaglandin $F_{2\alpha}$ may be indicative of similar mechanisms of

action of LPA.

Nine molecular species of LPA were assayed on cats and rats, which were used as representatives of animals showing depressor and pressor reactions, respectively. Figure 1 shows the log dose-pressor response relationships of these nine compounds on rats. The curves were similar in shape and horizontally parallel. Palmitoyl-LPA was the most active of the saturated LPA. The activity decreased progressively with increase or decrease from 16 in the fatty acid hydrocarbon chain length. Incorporation of *cis*-double bonds into the hydrocarbon chain of LPA resulted in increase in the activity in the order, 18:3>18:2=18:1>16:0≫18:0 (Fig. 2). Arachidonyl-LPA had a slightly higher pressor activity than did oleoyl-LPA.

As shown in Figure 3, similar log dose-depressor response relationships of LPA preparations were observed in cats, but myristoyl-LPA was slightly more active than palmitoyl-LPA. The most active molecular species was linoleoyl-LPA, and arachidonyl-LPA had low depressor activity relative to its pressor activity in rats (Fig. 2).

In the previous paper (1), we suggested from data obtained with different pharmacological blockers that LPA might exert a direct effect on cell membranes of cardiovascular smooth muscles, but not influence afferent and efferent sympathetic nervous systems. The present results suggest that certain limited hydrophobic regions in the molecules are required for acute, potent vasoactive effects. The presence of at least one *cis* double bond in the hydrocarbon chain resulted in high pressor and depressor activites, possibly by the optimal stereospecific interactions with some receptor sites. The requirement for hydrophobic regions may reflect the important hydrophobic interactions of LPA with the cell membranes of smooth muscles, causing activation of intracellular contraction systems.

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