Effect of Lysophosphatidylcholine on α-Adrenoreactivity of Rat Aorta Smooth Muscles

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In experiments on rat aortic ring segments, lysophosphatidylcholine in concentrations of 2×10^{-6}, 2×10^{-5}, and 2×10^{-4} M did not suppress the tonotropic effect of phenylephrine (6×10^{-6} and 6×10^{-5} M) and in concentration of 2×10^{-5} M even potentiated it, which was noted for phenylephrine at a concentration of 6×10^{-6} M. It was concluded that the chemomodulating effect of lysophosphatidylcholine depends on the type of receptors and cells.

Key Words: α-*adrenoceptos; lysophosphatidylcholine; rat aorta*

It had been previously shown that lysophosphatidylcholine (LPC) synthesized in the body decreases the efficiency of activation of muscarinic cholinergic receptors in the myocardium [5,6] and smooth muscles of the stomach [3] and blood vessels [12-14]; therefore, LPC can be considered as a component of endogenous blocker of muscarinic cholinergic receptors, postulated to explain the antimuscarinic activity of blood serum [3,7,8]. It was shown that LPC attenuates the positive inotropic effect of epinephrine on frog and rat myocardium (*i.e.* reduced the efficiency of activation of cardiomyocyte β-adrenergic receptors (AR) [4]) and the stimulating effect of epinephrine on circular strips of the cow renal artery (*i.e.* reduced the efficiency of α-AR activation [2]). These data suggest that LPC can be regarded as a universal factor reducing the efficiency of signal transduction from G-protein coupled receptors into the cell. However, the data that LPC (10^{-5} M) does not attenuate, but even potentiates the effectiveness of α-AR activation by selective agonists contradict this hypothesis [9,14]. This was shown in experiments on rat aortic rings using selective α_2-AR agonist UK14,304 [9] and with segments of rat mesenteric artery using selective α_1-AR agonist phenylephrine (PE) [14]. The authors explained the potentiating effect of LPC by its influence on activity of tyrosine kinase [9] or cyclooxygenase leading to enhanced production of thromboxane in the endothelial cells [14]. Taking into account the fact that LPC is constantly produced in the body from phosphatidylcholine in the reaction catalyzed by phospholipase A_2 and lecithin-cholesterol acyltransferase [5], *i.e.* is a natural cell component, we studied the influence of LPC on the effects of selective α_1-AR agonist PE in experiments with rat aortic rings.

MATERIALS AND METHODS

Experiments were carried out on 63 aortic rings (3-4 mm wide) with intact endothelium isolated from the thoracic aorta of white outbred male rats weighing 200-250 g (n=16) and immediately used in experiments. The animals were sacrificed with ether in accordance to Rules for Work with Experimental Animals (1977). The baseline and evoked contractile activity was recorded as described previously [8] using a multichannel Myocytograph assembled on the basis of the 6MH1S mechanotrons at 38°C and 0.7 ml/min perfusion rate (Krebs solution, KS). The initial load in all experiments was 1 g. Six experimental series were performed: the effect of LPC in concentrations of 2×10^{-6}, 2×10^{-5}, and 2×10^{-4} M on vasoconstrictor effect of PE in concentrations of 6×10^{-6} M (series 1, 2,

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and 3) or 6×10^{-5} M (series 4, 5, and 6) was evaluated. Each series was carried out by the standard procedure: KS→PE→KS→LPC→LPC+PE→KS→PE. We used KS containing (in mM): 136 NaCl, 4.7 KCl, 2.52 $CaCl_2$, 1.2 $MgCl_2$, 0.6 KH_2PO_4, 4.7 $NaHCO_3$, and 11 $C_6H_{12}O_6$ (pH 7.4). We also used mezaton (PE, Experimental Plant, Research Center of Drugs, Ukraine) and LPC (Kharkov Plant of Bacterial Preparations).

The data were processed by methods of variation statistics ($M\pm m$). The differences were assessed by Student's t test and Wilcoxon's test and were significant at $p<0.05$ [1].

RESULTS

Rat aortic rings had a low basal tone during perfusion with KS. Preliminary experiments showed that PE in concentrations of $6\times(10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}$, and $10^{-4})$ M increased the basal tone to 1.6±0.3, 3.5±0.3, 4.8±0.5, 4.7±0.6, and 4.4±0.7 mN, respectively, *i.e.* produced vasoconstrictor effect of PE attained the maximum at a concentration of 6×10^{-6} M. Therefore, when studying the effect of LPC on PE-induced contration, we used PE in two concentrations, 6×10^{-6} and 6×10^{-5} M. It was previously shown that repeated applications of 6×10^{-6} M PE (alternating with KS washouts) increased the vascular tone by the same value: during the 2nd and 3rd applications it constituted 98.6±3.5 and 97.8±2.2% of the value observed during the first application, respectively (data of 10 experiments). This allowed us to study the effect of LPC on the effectiveness of α-AR activation using 3-fold PE application in the same concentration: before LPS addition, in the presence of LPS, and after its removal.

In the main 6 experimental series, we showed that the tonotropic effect of PE in concentrations of 6×10^{-6} and 6×10^{-5} M on ring segments was similar: 3.4±0.2 (n=30) and 3.2±0.3 mN (n=33), respectively (Fig. 1). PE removal was accompanied by recovery of the basal tone. Addition of LPC in concentrations of 2×10^{-6} and 2×10^{-5} M reduced the basal tone in 45 and 61% cases, respectively. The effect of LPC in a concentration of 2×10^{-5} M was more pronounced than in concentration of 2×10^{-6} M: 2.5±0.3 (n=14) *vs.* 1.5±0.3 mN (n=9). However, LPS in higher concentration (2×10^{-4} M), which is close to its cytolytic concentration [5], showed a negative tonotropic effect only in 20% cases. These results are consistent with published data [10] that LPC reduces the basal tone of myocytes in rabbit thoracic aorta. On the other hand it was shown that LPC does not reduce the tone of endothelium-free aortic segments [11]. This means that decreased tone of rat aortic myocytes under the influence of LPC can be a result of activation of NO production by endothelium.

Evaluation of the effect of LPC on tonotropic effects of PE in a concentration of 6×10^{-6} M showed (Table 1) that LPC in a concentration of 2×10^{-6} M did not affect vasoconstrictor effects of PE, in a concentration of 2×10^{-5} M enhanced it during the 2nd and 3rd applications (*i.e.* exhibits α-AR-sensitizing activity), and was ineffective in a nearly damaging concentration (2×10^{-4} M). At the same time, in all experiments LPC in concentrations of $2\times(10^{-6}, 10^{-5}, 10^{-4})$ M did

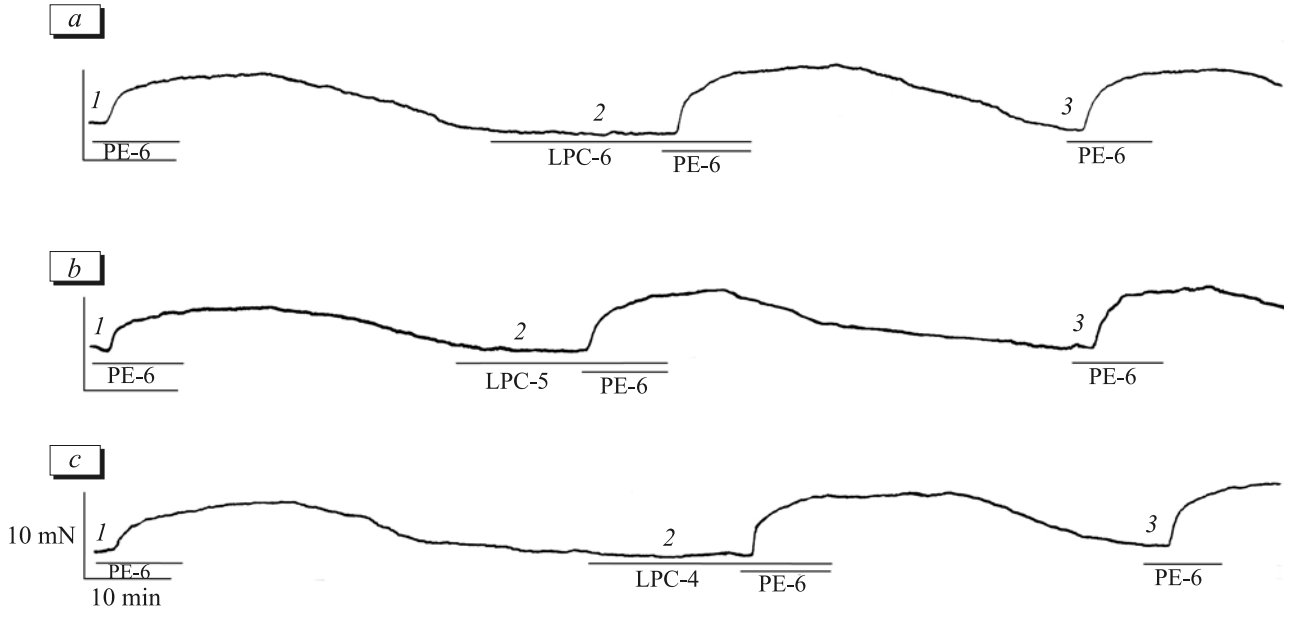

Fig. 1. Mechanograms of rat aortic rings demonstrating the tonotropic effect of PE in a concentration of 6×10^{-6} M (PE-6) before application of LPC (*1*), in the presence of LPS (*2*), and after removal (*3*) of LPC in concentrations of 2×10^{-6} M (LPC-6; *a*), 2×10^{-5} M (LPC-5; *b*) and 2×10^{-4} M (LPC-4; *c*). Horizontal lines under the mechanogram show the time of drug exposure.

TABLE 1. Tone in Rat Aortic Rings during Three Applications of PE (before LPC Application, in the Presence of LPC, and after Its Removal; *M*±*m*)

LPC concentration, M		*n*	Test					
			1 (before LPC)		2 (simultaneously with LPC)		3 (after LPC)	
			mN	%	mN	%	mN	%
PE, 6×10^{-6} M	2×10^{-6}	10	3.1±0.2	100	3.9±0.5	129.6±16.6	4.2±0.5	137.9±19.9
	2×10^{-5}	10	2.9±0.3	100	4.9±0.6*+	171.7±12.0*+	5.3±0.6*+	189.9±19.8*+
	2×10^{-4}	10	4.2±0.6	100	4.7±1.0	105.3±16.7	5.5±0.8	127.3±21.5
PE, 6×10^{-5} M	2×10^{-6}	10	3.3±0.7	100	3.9±0.6	126.5±15.0	3.2±0.6	107.7±31.3
	2×10^{-5}	13	3.9±0.5	100	3.2±0.4	83.8±8.7	2.2±0.4*+	73.0±7.4*+
	2×10^{-4}	10	2.1±0.2	100	2.2±0.3	114.1±19.0	2.0±0.3	87.9±9.6

Note. $p<0.05$ in comparison with test 1 (*Student's *t* test, +Wilcoxon's test).

not abolish the vasoconstrictor effect of PE (6×10^{-6} M) even after its removal.

Evaluation of the effect of LPC on tonotropic effects of PE in a concentration of 6×10^{-5} M showed (Table 1) that LPC in a concentration of 2×10^{-6} M does not affect the effects of PE. LPC in a concentration of 2×10^{-5} M did not potentiate the tonotropic effects, as it was observed for PE in a concentration of 6×10^{-6} M, but even significantly attenuated this effect; it should be noted that this attenuation was observed during the 3rd, but not the 2nd testing, *i.e.* after LPS removal. In a higher concentration (2×10^{-4} M) LPC attenuated the tonotropic effect of PE neither during the 2nd, nor during the 3rd testing. If LPC really blocked PE effects, it obviously would have produced this blockade at higher concentration. Thus, we can conclude that LPC in doses of $2\times(10^{-6}, 10^{-5}, 10^{-4})$ M most likely does not reduce the tonotropic effect of PE in concentrations of 6×10^{-6} and 6×10^{-5} M. Moreover, LPC increased PE tonotropic effect in a concentration of 6×10^{-6} M

Thus, our results confirm previous observations [9,14] that LPC does not reduce the effectiveness of α-AR activation in rat aortic myocytes, and under certain conditions even increases it. We observed this phenomenon during combined application of 2×10^{-5} M LPC with 6×10^{-6} M PE. According to published data, the α-AR-sensitizing effect of LPC is determined by modulation of tyrosine kinase activity [9] or cyclooxygenase activity [14]. We did not investigate the mechanisms underlying these effects of LPC, but we hypothesized that LPC can not only reduce, but also increase the efficiency of activation of G-protein coupled receptors. This assumption is indirectly confirmed by experiments [3], where LPC improves the efficiency of activation of muscarinic cholinergic receptors in smooth muscles of the stomach in a concentration of 2×10^{-6} M, but reduces it in higher concentrations (2×10^{-5} and 2×10^{-4} M). From this standpoint it is clear that LPC can be considered as a natural factor modulating the effect of hormones on cell functioning and the direction of this modulating effect depends on LPC concentration in the medium. At the same time, our findings and published data [9,14] differ significantly from those obtained in experiments on cow renal artery [2], according to which LPC in concentrations of 2×10^{-7}-2×10^{-4} M dose-dependently and reversibly reduced the tonotropic effect of epinephrine. These findings suggest that myocytes of rat aorta are highly resistant to LPC. On the other hand, LPC was shown to reversibly reduce the effectiveness of β-AR activation in cardiomyocytes of rat and frog [4] and muscarinic receptors in frog and rabbit myocardium [5,6], myocytes of rat stomach [3] and blood vessels [12,13], including the aorta [14]. This suggests that LPC can reduce the effectiveness of transmembrane signal transduction from the receptors into the cell depends not only on the type of cells, but also on the type of G-protein coupled receptors.

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