PHYSIOLOGY

Influence of Lysophosphatidylcholine, Phosphatidylcholine, and Hen Egg Yolk on Contractile Effects of Acetylcholine on Smooth Muscles of Rat Stomach

A. A. Kunshin, V. I. Tsyrkin, and N. V. Prokazova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 143, No. 6, pp. 604-607, June, 2007
Original article submitted December 7, 2006

Experiments on smooth muscles of rat stomach showed that lysophosphatidylcholine in concentrations of 10^{-8} and 10^{-7} g/ml does not modulate the tonotropic effect of acetylcholine (10^{-6} g/ml), in a concentration of 10^{-6} g/ml potentiated this effect (similarly to phosphatidylcholine, 10^{-6} g/ml), and reduced it in concentrations of 10^{-5}-10^{-4} g/ml (similarly to hen egg yolk in dilutions of 1:500, 1:100, and 1:500). These data indicate that lysophosphatidylcholine modifies signal transduction from the receptor to G protein.

Key Words: *gastric myocytes; acetylcholine; muscarinic cholinergic receptors; lysophosphatidylcholine*

Lysophosphatidylcholine (lysoPC) is produced by various cells of the organism [5,10,12,15]. Phosphatidylcholine (PC), a precursor of lysoPC, is the major component of phospholipids in human and animal cells. This synthesis is activated by phospholipase A_2 and lecithin-cholesterol acyltransferase. In blood plasma of humans and animals, lysoPC exists in a free form (20-80 μM) or is bound to albumin and other proteins. The content of lysoPC reaches 20% of total phospholipid content in the blood [5]. Previous experiments studying the nature of muscarinic cholinoceptor inhibitor effect of animal plasma on the heart of frogs and rabbits showed that lysoPC (but not PC, lysoPC precursor) in relatively low concentrations (10 μM) potentiated the negative inotropic effect of acetylcholine (ACh), *i.e.* lysoPC increased the muscarinic cholinergic reactivity of the myocardium, while in high concentration (100 μM) lysoPC (similarly to blood plasma) decreased this parameter [5]. Further studies showed that human plasma in some dilutions (1:50, 1:100, 1:500, and 1:1000) decreases muscarinic cholinergic reactivity of rat myometrium [8], frog myocardium [9], and rat stomach [3]. This effect was associated with the presence of an endogenous muscarinic cholinoceptor antagonist in the blood. LysoPC probably serves as the major component of this compound.

Here we studied the ability of lysoPC, PC (lysoPC precursor), and hen egg yolk (EY, source for nonenzymatic formation of lysoPC) to modulate the ACh-induced activation of muscarinic cholinoceptors in smooth muscles of rat stomach.

MATERIALS AND METHODS

Experiments were performed on 64 strips (length 5-8 mm, width 2-3 mm). Circular strips were excised from the fundus of the stomach of 22 adult

Department of Normal Physiology, Kirov State Medical Academy; Department of Lipid Biochemistry, Institute of Experimental Cardiology, Russian Cardiology Research-and-Production Complex, Moscow. ***Address for correspondence:*** tsirkin@list.ru. V. I. Tsyrkin

0007-4888/07/1436-0663

female rats. The rats were euthanized without taking into consideration the phase of the estrous cycle. The study was performed according to regulations for the use of experimental animals.

Contractile activity of strips was recorded on a Miotsitograf 6-channel device [7] at 38°C. The working chamber was perfused with Krebs solution at a flow rate of 0.7 ml/min and passively aerated. The initial load was 500 mg (4.9 mN). A Miotsitograf device consisted of 6MKh1S mechanotrons, H-3020 self-recording units, and original spray batcher and thermostat.

Krebs solution (pH 7.4) contained 136 mM NaCl, 4.7 mM KCl, 2.52 mM $CaCl_2$, 1.2 mM $MgCl_2$, 0.6 mM KH_2PO_4, 4.7 mM $NaHCO_3$, and 11 mM $C_6H_{12}O_6$.

Baseline muscarinic cholinergic reactivity of strips was estimated as follows: Krebs solution→test concentration of ACh→Krebs solution. Each exposure lasted for at least 10 min. The effects of lysoPC, PC, and EY were studied by the scheme: Krebs solution→ACh (10^{-6} g/ml)→ACh+test substance→ACh→Krebs solution. We used ACh chloride (10^{-9}-10^{-5} g/ml), atropine sulfate (10^{-10}-10^{-7} g/ml), lysoPC (10^{-8}-10^{-4} g/ml), and PC (10^{-7}-10^{-4} g/ml). EY was dissolved with distilled water (1:1) and Krebs solution by 50, 100, 500, and 1000 times. Changes in the tone of strips were expressed in mN or percents of the basal level (1st test with ACh).

The results were analyzed by the parametric method. The differences were estimated by Tukey test and Newman—Keuls test [1]. The significance level was $p<0.05$.

RESULTS

Circular strips from the fundus of rat stomach had low basal tone during perfusion with Krebs solution; in some experiments low-amplitude phasic contractions were recorded. ACh in concentrations of 10^{-8}, 10^{-7}, 10^{-6}, and 10^{-5} g/ml induced a dose-dependent increase in the basal tone to 0.36±0.23 (n=4), 1.28±0.18 (n=8), 3.06±0.36 (n=8), and 4.41±1.05 mN (n=8), respectively (Fig. 1, *a*). The dissociation constant for ACh was 635±130 ng/ml. Atropine in concentrations of 10^{-10}-10^{-7} g/ml dose-dependently decreased the response of strips to ACh (10^{-6} g/ml; Fig. 1, *b*). These findings support the data that ACh increases contractile activity of myocytes in rat stomach due to activation of muscarinic cholinergic receptors, primarily of subtypes M_2 and M_3 [13,14]. Taking these data into account, the effects of lysoPC, PC, and EY were studied using ACh in a concentration of 10^{-6} g/ml.

LysoPC in concentrations of 10^{-8} and 10^{-7} g/ml did not modulate the tonotropic effect of ACh (10^{-6} g/ml, Table 1); in a concentration of 10^{-6} g/ml lysoPC had a muscarinic-sensitizing effect, *i.e.* potentiated the effect of ACh (Fig. 2, *a*) and in concentrations of 10^{-5} and 10^{-4} g/ml decreased the ACh-induced tone (Fig. 2, *b*; Table 1). This decrease was most pronounced after removal of lysoPC, but not during exposure. Hence, lysoPC in concentrations of 10^{-5} and 10^{-4} g/ml exhibited properties of muscarinic cholinergic antagonist. However, this effect was preceded by a 10-15 min latent period. In relatively low concentrations (10^{-6} g/ml) lysoPC increased muscarinic cholinergic reactivity of cells, *i.e.* acted as an endogenous sensitizer of muscarinic receptors (the presence of this sensitizer in blood plasma and liquor was previously hypothesized [6]), while in high concentrations (10^{-5} and, particularly, 10^{-4} g/ml) it decreased muscarinic cholinergic activity of cells, *i.e.* acted as an endogenous antagonist of muscarinic receptors.

PC in concentrations of 10^{-7}, 10^{-5}, and 10^{-4} g/ml did not modulate muscarinic receptors, while in a concentration of 10^{-6} g/ml potentiated the positive tonotropic effect of ACh (10^{-6} g/ml), *i.e.* demonstrated a muscarinic-sensitizing effect (Fig. 2, *c*, *d*; Table 1). Hence, PC (10^{-6} g/ml) similarly to lysoPC (10^{-6} g/ml) can be a component of endogenous sensitizers of muscarinic cholinergic receptors.

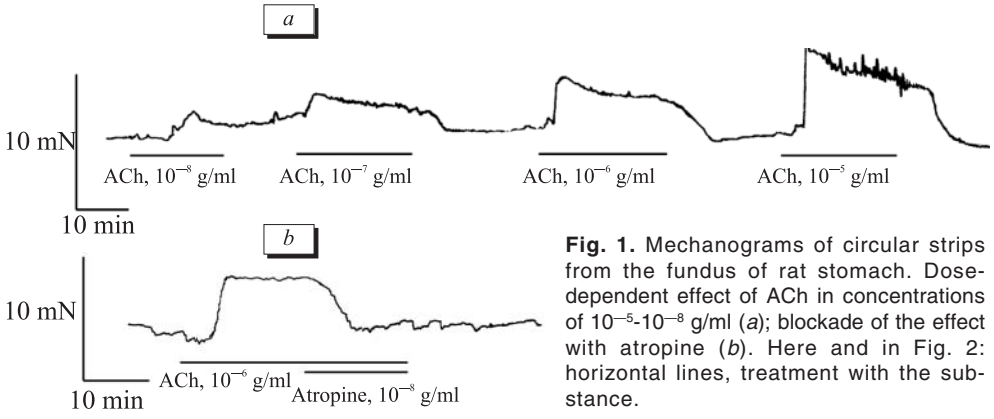

Fig. 1. Mechanograms of circular strips from the fundus of rat stomach. Dose-dependent effect of ACh in concentrations of 10^{-5}-10^{-8} g/ml (*a*); blockade of the effect with atropine (*b*). Here and in Fig. 2: horizontal lines, treatment with the substance.

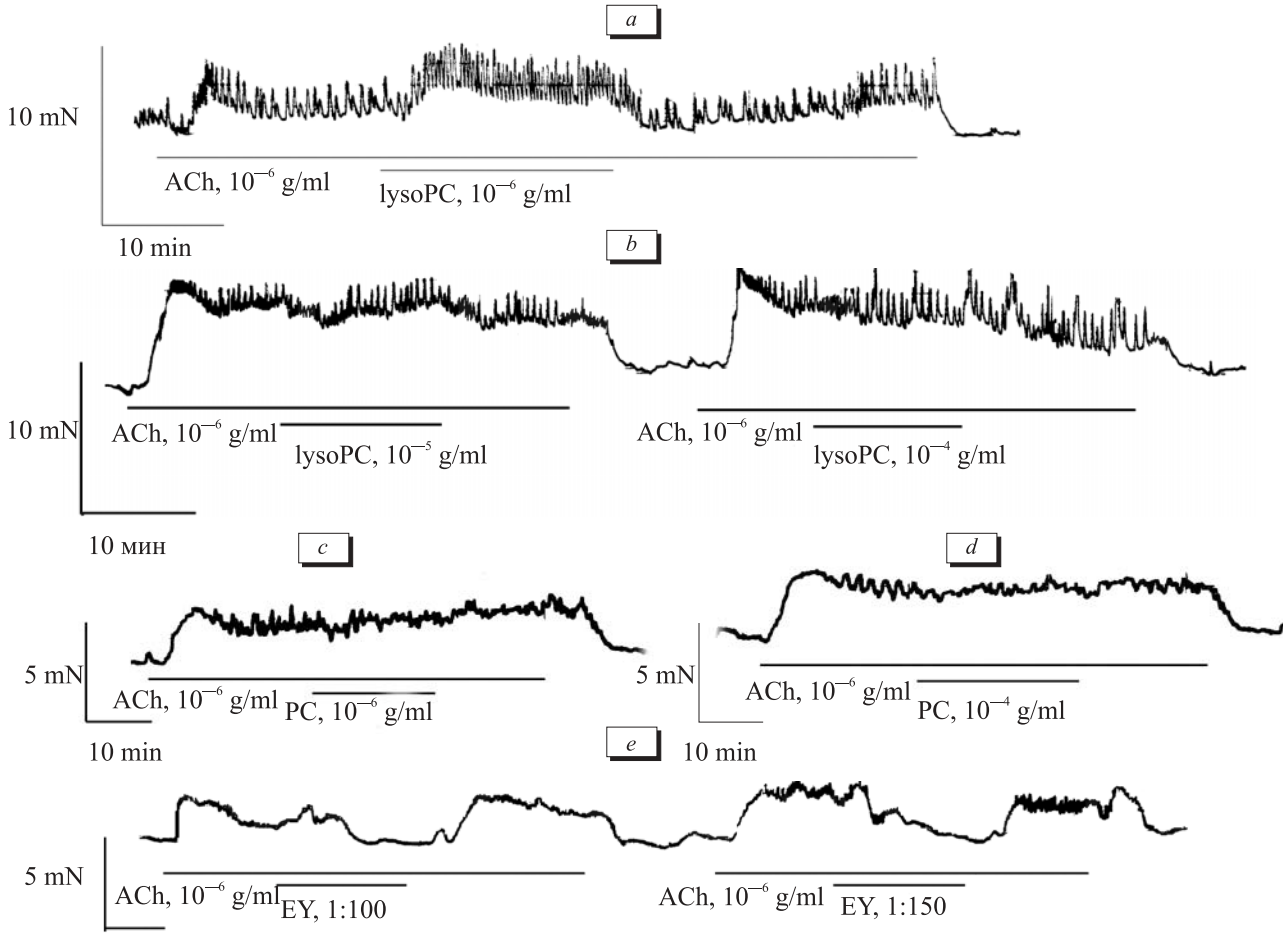

Fig. 2. Mechanograms of circular strips from the fundus of rat stomach. Muscarinic-sensitizing effect of lysoPC (*a*); muscarinic blockade after lysoPC treatment (*b*); muscarinic sensitization and no change after PC treatment (*c, d*); muscarinic cholinergic blockade after EY treatment (dilutions 1:100 and 1:50, *e*).

EY in a dilution of 1:1000 did not modulate the ACh-induced tone of muscle strips from rat stomach, but in dilutions of 1:500, 1:100, and 1:50 dose-dependently decreased it (Fig. 2, *e*; Table 1). Hence, EY similarly to lysoPC exhibited properties of a muscarinic cholinergic antagonist. These data are consistent with the results of previous experiments on longitudinal strips from rat uterine horn [6] demonstrating that EY in dilutions of 1:500, 1:100, and 1:50 induced a dose-dependent decrease in the stimulatory effect of ACh. It seems most likely that the antagonistic effect of EY on mus-

TABLE 1. Tone of Circular Strips from the Fundus of Rat Stomach Induced by ACh in a Concentration of 10^{-6} g/ml (% of the Basal Tone) under the Influence of lysoPC, PC, and EY (*M*±*m*)

Substance	Concentration of lysoPC and PC, g/ml				
	10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}
lysoPC	112.58±10.28 (*n*=6)	108.14±9.92 (*n*=7)	122.80±5.83* (*n*=10)	92.07±8.46 (*n*=13)	77.90±10.14* (*n*=11)
PC	—	96.62±2.92 (*n*=8)	113.11±5.48* (*n*=8)	110.88±7.24 (*n*=9)	96.66±9.17 (*n*=8)
EY Dilutions	1:1000 (*n*=9) 97.30±7.05	1:500 (*n*=9) 67.89±4.96*	1:100 (*n*=10) 45.50±8.94*	1:50 (*n*=10) 17.70±5.71*	— —

Note. *$p<0.05$ compared to the basal ACh-induced tone (Tukey test). Dash: not measured.

carinic cholinergic receptors in myocytes of the uterus and stomach is related to the presence of lysoPC. EY is enriched with PC. Nonenzymatic hydrolysis of PC results in the formation of lysoPC [5]. The results of experiments with EY support our hypothesis that lysoPC acts as a muscarinic cholinergic receptor antagonist. We conclude that lysoPC is the major constituent of endogenous antagonists of muscarinic cholinergic receptors. These data suggest that the modulatory effect of blood on the muscarinic cholinergic system depends on the type of nutrition.

Our results are consistent with published data that lysoPC acts as a muscarinic cholinergic receptor blocker [5]. However, this activity of lysoPC is nonspecific, because lysoPC attenuates the effect on target cells of not only ACh, but also endothelium-dependent vasodilators thrombin and serotonin [11], epinephrine as a positive inotropic factor in the myocardium of frogs and rats [4] and as vasoconstrictor of the renal artery in cows [2]. Receptors for these compounds, similarly to muscarinic cholinergic receptors, are coupled with G protein [6]. Therefore it can be hypothesized that the blocking effect of lysoPC (including blockade of muscarinic cholinergic receptors) is associated with uncoupling of signal transduction from receptors to G protein. The unique ability of lysoPC to isolate the cells from exogenous signals confirms the important role of this molecule under normal and pathological conditions. Moreover, lysoPC production undergoes significant changes under the influence of various factors [5,10,12,15]. It should be emphasized that lysoPC not only modulates transmembrane signal transduction, but also serves as a secondary messenger, contributes to phosphorylation of p38, transcription factors GREB and ATF-1, and protein tyrosine kinase [12], and activates atherogenesis due to the interaction with specific receptors [10,12]. Further studies of chemomodulatory activity of lysoPC hold much promise for clinical practice.

REFERENCES

1. S. Glants, *Medical and Biological Statistics* [in Russian], Moscow (1999).
2. R. Yu. Kashin, V. I. Tsirkin, and N. V. Prokazova, *Usp. Sovrem. Estestvoz.*, No. 11, 48-49 (2006).
3. A. A. Kunshin, *Ros. Fiziol. Zh.*, **90**, No. 8, Pt. 2, 15 (2004).
4. Yu. A. Penkina, I. Tsirkin, and N. V. Prokazova, *Usp. Sovrem. Estestvoz.*, No. 12, 70-71 (2006).
5. N. V. Prokazova, N. D. Zvezdina, and A. A. Korotaeva, *Biokhimiya*, **63**, No. 1, 38-46 (1998).
6. E. N. Sizova and V. I. Tsirkin, *Physiological Characteristics of Endogenous Modulators of b-Adrenergic and Muscarinic Cholinergic Activity* [in Russian], Kirov (2006).
7. V. I. Tsirkin, S. A. Dvoryanskii, A. D. Nozdrachev, *et al.*, *Dokl. Ros. Akad. Nauk*, **352**, No. 1, 124-126 (1997).
8. V. I. Tsirkin, A. D. Nozdrachev, E. N. Sizova, *et al.*, *Byull. Eksp. Biol. Med.*, **136**, No. 7, 18-22 (2003).
9. V. I. Tsirkin, A. N. Trukhin, E. N. Sizova, *et al.*, *Ros. Kardiol. Zh.*, No. 2, 64-69 (2004).
10. J. H. Kabarowski, K. Zhu, L. Q. Le, *et al.*, *Science*, **293**, No. 5530, 618-619 (2001).
11. Y. Miwa, K. Hirata, S. Kawashima, *et al.*, *Arterioscler. Thromb. Vasc. Biol.*, **17**, No. 8, 1561-1567 (1997).
12. Y. Rikitake, K. Hirata, S. Kawashima, *et al.*, *Biochem. Biophys. Res. Commun.*, **281**, No. 5, 1291-1297 (2001).
13. B. Wang and F. Cheng, *Zhongguo Bingli Shengli Zazhi*, **17**, No. 11, 1093-1096 (2001).
14. L. Xue, H. Fukuta, Y. Yamamoto, and H. Suzuki, *Jpn. J. Physiol.*, **46**, No. 2, 123-130 (1996).
15. T. Yamakawa, S. Tanaka, Y. Yamakawa, *et al.*, *Arterioscler. Thromb. Vasc. Biol.*, **22**, No. 5, 752-758 (2002).