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EFFECT OF LYSOLECITHIN AND LECITHIN OF BLOOD SERUM ON THE SENSITIVITY OF HEART TO ACETYLCHOLINE

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Abstract—The chemical nature of the blood serum lipid substance exerting the cholinolytic effect upon the myocard of different animals was determined by means of column, thin-layer and gas-liquid chromatography. The cholinolytic effect was due to lysolecithin (lysophosphatidylcholine) action. The protective action of lecithin upon the cholinolytic effect of lysolecithin was established. Cholinolytic activity of deoxylysolecithin and its analogues on the isolated frog heart ventricle was studied. This effect was shown to depend specifically on the chemical structure of these substances.

Isolated frog ventricle perfused with Ringer's solution, as well as rat or rabbit atrium increases their sensitivity to acetylcholine (ACh) for the first 30–60 min after isolation. The addition of serum to the perfusion medium of these animals, results in a decrease of cardiac sensitivity to ACh. This effect is apparently due to the presence of a cholinolytic factor (ChLF) in these sera [1–3], some properties of which have been previously examined [4]. Thus, the cholinolytic activity (ChLA) of the sera is almost doubled upon heating to 100° or treatment with ethanol, and it passes through a cellophane membrane, when the serum is dialysed against water. The level of ChLA varies depending on animal species. It increases in the series: frog, rat, rabbit. Extraction of serum being performed with chloroform–methanol, ChLA has been shown to pass into the lipid extract [4].

The aim of the present work was to elucidate the chemical nature of the rabbit ChLF and to obtain information about the molecular mechanism of its action on the cholinoreceptor membrane of the myocardium.

MATERIALS AND METHODS

Assay of ChLA. Isolated frog ventricles, perfused with Ringer's solution (6.5 g NaCl; 0.14 g KCl; 0.2 g $NaHCO_3$; 0.12 g anhydrous $CaCl_2$ per 1 l. of water) were contracted by an electric stimulator with rectangular stimuli of 5 V magnitude 5 msec duration and 0.5 Hz frequency. The stimulation was effected through unpolarized electrodes. The isotonic contraction amplitude of the isolated frog ventricle was recorded mechanographically on a kymograph.

Serum components to be examined for ChLA were dissolved or emulsified in Ringer's solution and administered to the frog ventricle through a cannula. The inhibitory effect of ACh on the amplitude of myocardiac contractions before and 10 min after treatment with the substances under investigation were compared.

The ChLA was observed qualitatively as a partial or complete neutralization of the inhibitory effect of ACh. For quantitative measurements the ChLF was regarded as an ACh-antagonist. We therefore determined the apparent dissociation constant of the complex formed by ACh and its receptor before (K_1) and after (K_2) the action of the tested substances from the following equations:

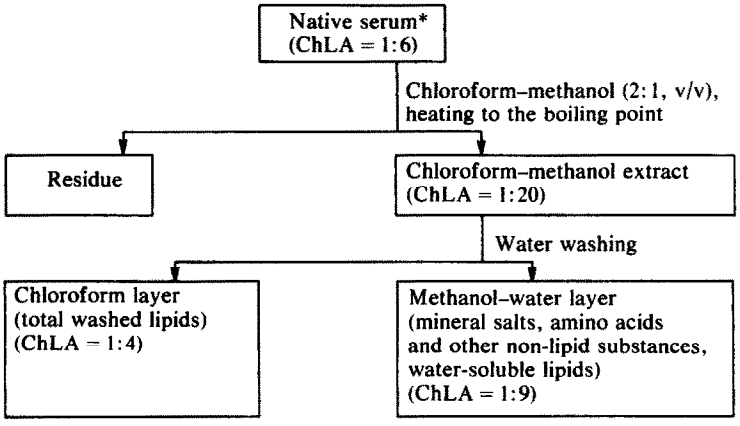
$$\frac{1}{e} = \frac{K}{E} \cdot \frac{1}{[\mathrm{ACh}]} + \frac{1}{E} \quad (1)$$

$$K_2 = K_1 \cdot \left(1 + \frac{[\mathrm{Ant}]}{K_{\mathrm{Ant}}}\right), \quad (2)$$

where e = the effect of a given concentration of ACh, E = the maximum effect of ACh, [ACh] = the concentration of ACh, K_{Ant} = the concentration of the antagonist of ACh, K_{Ant} = the dissociation constant of the complex formed by the antagonist of ACh and the cholinoreceptor.

Formally, the application of equations (1) and (2) to determine K_{Ant} was possible only on the basis of data determining the efficiency of ACh in the presence of its antagonist. Control experiments showed, however, that after treatment of the ventricle with ChLF-containing fractions for 10 min the cholinolytic effect lasted for at least 15–20 min. The cholinolytic effect of ACh on the ChLF-treated heart muscle was therefore measured 1–2 min after the removal of the ChLF from the myocardium. Resulting from equation (2) $K_2/K_1 = 2$ for [Ant] = K_{Ant}. K_{Ant} could thus be extrapolated graphically from plots of K_2/K_1 vs antagonist concentration, which for a K_2/K_1 ratio of 2 is equal to the value of K_{Ant}. In case the absolute concentration of the antagonist was unknown (native serum, total lipid fractions) K_{Ant} was expressed through the degree of serum delution or in μg of phosphorus lipid [2, 4].

Scheme 1. Extraction of the cholinolytic factor from blood serum



* ChLA is expressed through the extent of serum dilution at which the apparent dissociation constant for the complex formed by ACh and the cholinoreceptor increases two times, i.e. $K_2/K_1 = 2$.

Extraction and chromatographic separation of lipids. Lipids were extracted by a modified Folch's procedure [5] from the serum of mature and healthy rabbits. Ten litres of chloroform–methanol (2:1, v/v) were added to 1 l. of serum. The mixture was shaken at room temperature for a few hours, heated to b.p., cooled and filtered. The filtrate was diluted by 2 l. of water, shaken and left overnight at 3°. The chloroform and the aqueous–methanol layers were separated, and the ChLA of each layer was determined (Scheme 1). For this purpose aliquots of each layer were evaporated to dryness, the residue was carefully emulsified in Ringer's solution and tested for ChLA as described above. The chloroform layer was evaporated, the residue, i.e. the washed total lipids [6], containing about 2500 μg of lipid phosphorus, were dissolved in a small volume of chloroform and chromatographed on a 35 × 120 mm column packed with 12 g of silica gel KSK (100–150 mesh) [7]. The adsorbent was pre-washed by 100 ml of methanol and 100 ml of chloroform–methanol (1:1, v/v). Neutral lipids were eluted by chloroform. Phospholipids were eluted by chloroform–methanol with an increasing methanol content and by methanol containing 4 per cent of water (Table 1). The rate of elution was 3–4 ml/min. Fractions of 20 ml each were collected and analysed by thin-layer chromatography (t.l.c.). The solution systems were used for development of the plates: chloroform–methanol–water (65:25:4, 65:35:6 and 65:35:6, by volume). Fractions, containing identical types of lipids were pooled and evaporated.

Lipids phosphorus was determined as described [8].

The aqueous phase of the serum extract was also evaporated to dryness. The residue consisting of

Table 1. Column chromatography of the washed total lipids extracted from 100 ml of rabbit serum and the distribution of ChLA between the fractions

Lipid fractions (t.l.c.)		Composition and volume of solvent system	Lipid P (μg)	Presence (+) or absence (−) ChLA	K_{Ant} (μg of P/ml)
	Total lipid extract (150 mg of lipids)		2700	+	1.7
1	Neutral lipids	chloroform, 150 ml	0	—	—
2	Neutral lipids, polyglycerophosphatides	chloroform–methanol (95:5, v/v), 50 ml	56	—	—
3	Phosphatidylethanolamine, phosphatidylserine, phosphatidyl inositol	chloroform–methanol (80:20, v/v), 100 ml	67	—	—
4	Phosphatidylethanolamine, phosphatidylcholine (lecithin)	chloroform–methanol (45:55, v/v), 150 ml	270	—	—
5	Lecithin	chloroform–methanol (30:70, v/v), 100 ml	600	—	—
6	Lecithin, sphingomyelin	chloroform–methanol (10:90, v/v), 200 ml	1210	—	—
7	Lecithin, sphingomyelin, lysolecithin	methanol–water (96:4, v/v), 100 ml	214	+	13.0
8	Lysolecithin	methanol–water (96:4, v/v), 100 ml	287	+	0.5

water-soluble lipids and nonlipid substances was re-dissolved in 25 ml of a water–methanol mixture (8:2, v/v) and extracted by chloroform (2 × 400 ml). The extract was concentrated by evaporation, and its lipid composition was studied by t.l.c.

Dialysis of serum. Extraction of lipids from dialysed serum and dialysate. One litre of rabbit serum was dialysed at 6–8° for 10–12 hr against 10–20 vols of distilled water or of saline solution, the dialysation bag being constantly rotated. The ChLA of the dialysate was determined. The dialysate was evaporated to dryness, the residue was dissolved in 50 ml of water–methanol (8:2, v/v), saturated by NaCl and extracted by chloroform (2 × 400 ml). The extract was analysed by silica gel t.l.c. After determining the ChLA of the dialysed serum, lipids were extracted from it as described above and analysed by t.l.c. The ChLA of each fraction obtained was determined.

Chemical study of lysolecithin fractions. The lysolecithin fractions of the isolated chloroform and methanol–water phases were subjected to a mild alkaline deacylation: 0.25 ml of 1 M KOH in 98% methanol was added to 0.5 ml methanolic solution of the lysolecithin fraction containing 250 μg of lipid P [8]. The reaction mixture was left at 40° for 60 min, and the excess of alkali was removed by heating the reaction products with 0.1 ml of ethyl formate to 40° for 5 min. The hydrolysate was cooled and mixed with chloroform–methanol–water (4:1.25:1.2, v/v). The lower layer was separated and concentrated by evaporation. The residue was treated with an excess of diasomethane in ether at 20° for 2 hr, evaporated to dryness and dissolved in 0.5 ml of ether. The obtained fatty acid methyl esters were analysed by gas-liquid chromatography (g.l.c.) on a 2000 mm × 5 mm column packed with 10% polyethyleneglycol succinate on Chromosorb W (60–80 mesh) at 160° using a 'Chrom-2' apparatus equipped with a flame-ionization detector (gas-carrier argon consumption = 60 ml/min, sample volume = 1 μl).

RESULTS

For isolation of ChLF, rabbit serum was extracted by chloroform–methanol (2:1, v/v). The extract was washed by water, and the ChLA of the chloroform and methanol–water phases were determined. Scheme 1 presents the results of the biological testing. ChLA is expressed through the extent of serum dilution at which the apparent dissociation constant for the complex formed by ACh and the cholinoreceptor increases two times, i.e. $K_2/K_1 = 2$. The results obtained indicate that ChLA is present both in the washed chloroform layer containing the total lipids and in the methanol–water solution containing non-lipid substances and small amounts of polar lipids [6, 9].

As can be seen from Scheme 1, the ChLA level of the native serum was significantly lower than that of the chloroform–methanol extract. The ChLA was distributed between the chloroform and the aqueous–methanol layers.

The cholinolytic factor of the chloroform layer. The washed lipids from the chloroform layer were separated by silica gel column chromatography (see Table 1). The ChLA assay of each fraction was carried out on a frog ventricle. Table 1 presents the results of such an experiment. As can be seen from the data of Table 1 and from Fig. 1, only those fractions which contained lysolecithin possessed anti-ACh activity. At concentrations of 0.1–0.3 μg P/ml the chromatographically homogenous lysolecithin of serum had a prolonged positive inotropic effect on the heart muscle. Such an action of this phospholipid was earlier reported by Hajdu *et al.* [10]. At concentrations of 0.15–0.5 μg P/ml lysolecithin showed a definite anti-ACh action which increased with increasing concentrations of the phospholipid. This cholinolytic effect of lysolecithin was stable and prolonged. Even after washing of the heart muscle for 15–20 min its sensitivity to ACh did not change and its normal responsiveness to ACh could be restored after washing for a few hours. The antagonism of lysolecithin and ACh at the reported concentrations was of a competitive character during and after the lysolecithin treatment indicated by the 'dosage–effect' curve which was shifting to higher ACh concentrations. At still higher concentrations the ChLA of lysolecithin rose exceedingly fast, and the action of this phospholipid acquired an irreversible character (Fig. 2). It was impossible to overcome the inhibitory action of lysolecithin under such conditions even by using maximum concentrations of ACh (up to 1×10^{-3} M).

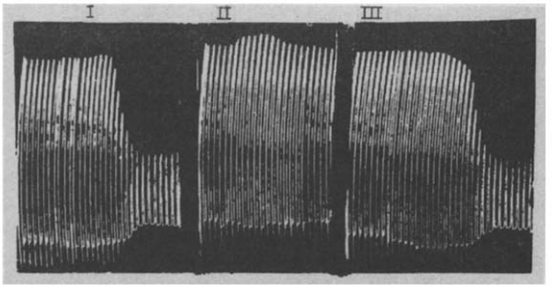


Fig. 1. Cholinolytic effect of the rabbit serum lysolecithin on an isolated frog ventricle. The effect of the successive addition of ACh at a concentration of 4.10^{-9} M. (1) Normal, (2) after 10 min perfusing with lysolecithin (0.6 μg P/ml), (3) after 2 hr washing out the ventricle with the Ringer's solution.

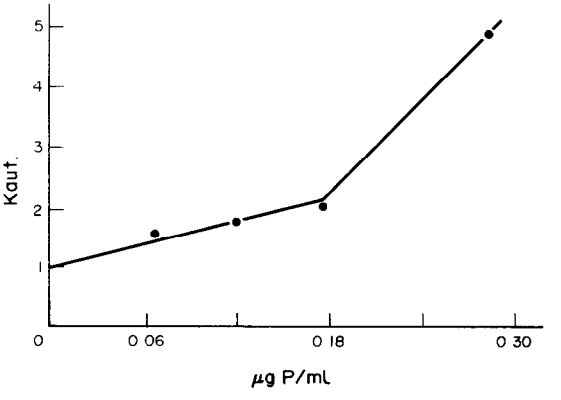


Fig. 2. Dependence of lysolecithin ChLA on lysolecithin concentration. Abscissa: lysolecithin concentrations (μg P/ml), ordinate: K_2/K_1 ratio (see text). In this experiment $K_{Am} = 0.17$ μg P/ml, i.e. about 3.10^{-6} M of lysolecithin.

Experiments carried out with lysolecithin obtained by the phospholipase A_2 hydrolysis of egg lecithin [11] showed this substance also to possess cholinolytic properties. However, some differences in the effective concentrations of the two lysolecithin samples have been found. Egg lysolecithin behaved like a competitive cholinolytic agent at concentrations of 0.05–0.2 μg P/ml, i.e. its ChLA was somewhat greater than that of the serum lysolecithin. These quantitative differences might be explained by difference in the fatty acid composition of the two samples tested.

The Cholinolytic factor of the aqueous–methanol layer. It is common to think that washing lipid extracts by water according to Folch's procedure does not lead to any appreciable transfer of lipid substances into the aqueous–methanol layer which usually contains no more than 2 per cent of the total lipids [6, 9]. Indeed, our attempts to extract lipids from the aqueous methanol layer by a repeated chloroform extraction proved unsuccessful (chloroform–methanol–water was taken in proportion 2:1:0.6). The characters of the ChLA of the chloroform and the aqueous–methanol layers were so similar, hence we concluded that they contained the same, or very similar cholinolytic substances.

To isolate ChLF from the aqueous–methanol layer, the latter was evaporated to dryness. The residue was dissolved in a small volume of 20% aqueous–methanol and extracted by chloroform. Thin-layer chromatography of the extract demonstrated that it contained all the main classes of serum lipid including lysolecithin (Fig. 3). Silica gel column chromatography of the lipids from the aqueous–methanol layer resulted in recovering of about 60 mg of lysolecithin per 1 l. of serum. This lysolecithin like that of the chloroform layer had a pronounced anti-Ach effect on the heart muscle. However, there was a difference: the recovery of the cardiac sensitivity to ACh occurred somewhat faster than in the case of equally effective doses of the lysolecithin obtained from the chloroform layer.

Thin-layer chromatography of the lysolecithins obtained from the chloroform and the aqueous–methanol layers revealed them to be homogenous and identical in different solvent systems, their R_f values coinciding with that of a standard lysolecithin sample.

Gas-liquid chromatography of the fatty acids methyl esters showed the lysolecithins of the chloroform and the aqueous methanol layers to contain respectively the following acids: linoleic (27 and 37 per cent), oleic (8 and 10 per cent), stearic (12 and 17 per cent), palmitic (42 and 29 per cent). The content of short-chain acids was some 10 per cent.

ChLF of dialysed serum and dialysate. The isolation of lysolecithin from the aqueous–methanol solution containing large amounts of mineral salts and low molecular non-lipid substances is accompanied by heavy losses of material. That is why we used dialysis in order to separate serum from mineral salts and non-lipid contaminants.

In contrast to the existing opinion that lysophosphatides do not pass through cellophane

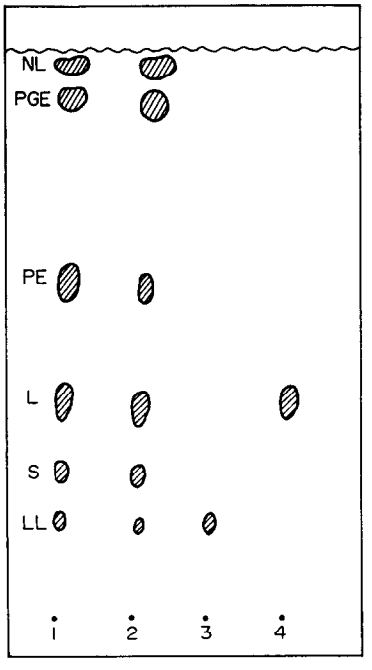


Fig. 3. Thin-layer chromatography of phospholipids extracted by chloroform from the concentrated methanol–water layer (obtained by water washing of the chloroform–methanol extract of the rabbit serum). Solvents: chloroform–methanol–water (65:25:4, v/v). Stains —phosphomolibdenic acid, Dragendorff's reagent; (1) the studied sample; (2) the total serum lipids after water washing; (3) standard of egg lysolecithin and (4) standard of egg lecithin. Lysolecithin—LL; sphyngomielin—S; lecithin—L; phosphatidylethanolamine—PE; polyglycerophosphatides—PGP; neutral lipids and fatty acids —NL.

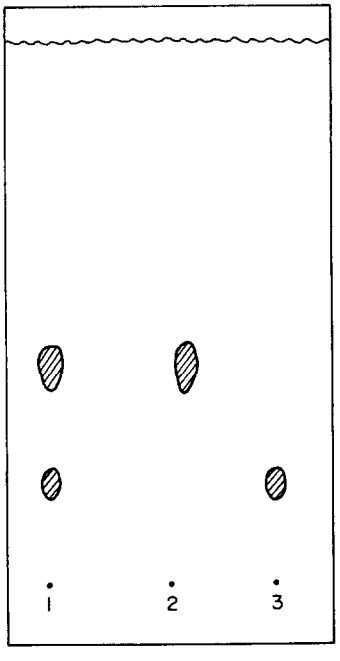


Fig. 4. Thin-layer chromatography of phospholipids extracted by chloroform from the rabbit serum dialysate (see the text). Solvents and stains as in Fig. 3; (1) chloroform extract of dialysate, (2) standard of egg lecithin and (3) standard of egg lysolecithin.

membrane [9] we found the dialysed serum to loose cholinolytic activity and the ChLF to be transferred to the dialysate. Extraction of dialysed serum according to Folch's procedure [6] results in the reappearance of ChLA in the chloroform extract. Thin-layer chromatography of the extract of the dialysate revealed the presence of lecithin and lysolecithin (Fig. 4).

It was shown that the aqueous solution of lysolecithin was dialysed against water, lysolecithin proved to pass easily through the membrane. These results enabled us to explain the increase of ChLA during the extraction of serum by organic solvents as follows: this procedure results in liberating certain amounts of lysolecithin which was in a bound (physiologically inactive) state, the ChLA of the native serum was due only to free lysolecithin which passed through the cellophane membrane into the dialysate.

Protective effect of lecithin against cholinolytic action of lysolecithin. The data presented in the previous sections demonstrates that the ChLA of serum was due to lysolecithin. However, when the total ChLA of pure lysolecithin isolated from the lipid extract of serum was compared with that of the extract itself we found the ChLA of lysolecithin to increase after isolation. This fact gave rise to the assumption that some serum lipids decreased the ChLA of lysolecithin.

To test this hypothesis we compared the ChLA of chromatographically homogenous lysolecithin and that of its mixture with other lipids obtained by column chromatography of serum extracts (Table 1). Lecithin was proved to decrease or even completely to remove the cholinolytic action of lysolecithin, whereas other serum phospholipids did not alter the ChLA of lysolecithin.

It can be seen from Fig. 5 that the ChLA level of lecithin–lysolecithin mixtures was considerably lower than that of pure lysolecithin taken in the same concentration. At the same time lecithin was found not to modify the cardiac sensitivity to ACh (Fig. 6) at doses up to 40 μg P/ml. Such a protective action

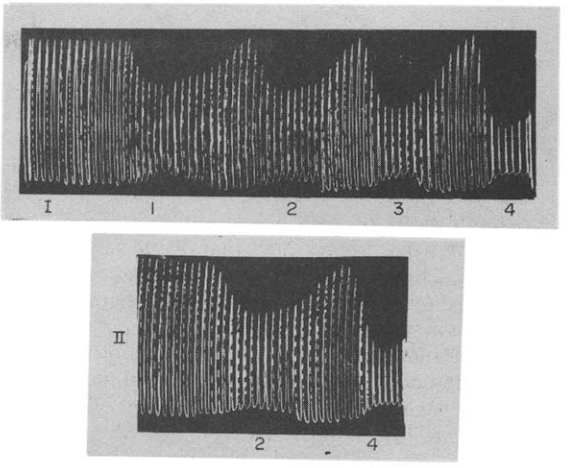


Fig. 6. Absence of the lecithin action on cardiac sensitivity to ACh. The successive effects of a number of ACh concentrations on an isolated frog ventricle: (I) normal (concentrations 1–4 are 1.5×10^{-9} M; 2×10^{-9} M; 3.5×10^{-9} M; 7×10^{-9} M respectively) and (II) after 10 min perfusing with serum lecithin (40 μg P/ml; ACh concentrations 2 and 4 are 2×10^{-9} M and 7×10^{-9} M respectively).

was characteristic both for blood serum lecithin and egg lecithin. Lecithin showed its protective effect both against the lysolecithin obtained from the washed total lipids or from the aqueous–methanol layer and against the lysolecithin obtained from egg yolk by phospholipase A_2 digestion.

If the frog ventricle was perfused with lecithin solution prior to the application of lysolecithin the anti-ACh effect of the latter was suppressed, even if a 60 min period of washing was included between lecithin and lysolecithin addition. Lecithin alone had no effect at all on the cardiac sensitivity to ACh (Fig. 7). When lecithin is added after the treatment with lysolecithin, it also does not interfere with the cardiac sensitivity to ACh.

Thus, lysolecithin acts in the native serum as a cholinolytic substance in the presence of its antagonist, lecithin, which considerably decreases the

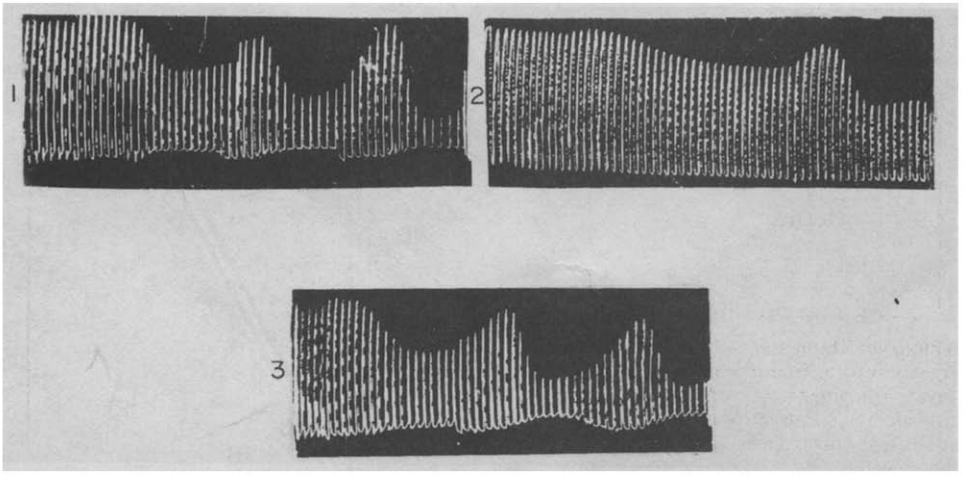


Fig. 5. Comparison of the cholinolytic effect of pure lysolecithin obtained from rabbit serum to that of a lysolecithin–lecithin mixture. Successive effect of a number of ACh concentrations on an isolated frog ventricle: (1) normal, (2) after 10 min perfusing with lysolecithin (0.3 μg P/ml). Cardiac sensitivity to ACh was completely restored by washing with Ringer's solution, and (3) after 10 min perfusing with lysolecithin–lecithin mixture (1.5 μg P/ml and 40 μg P/ml respectively).

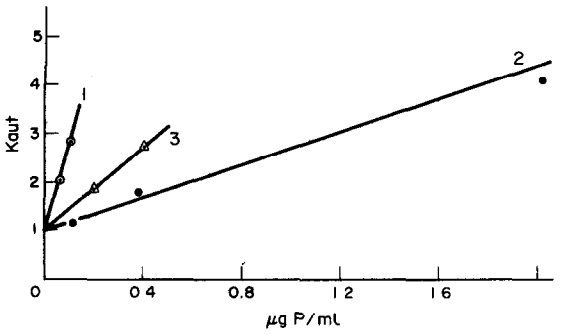


Fig. 7. Relationship between ChLA of egg lysolecithin and its concentration. Determination carried out on the same isolated frog ventricle in the presence or in the absence of egg lecithin: (1) after perfusion with pure lysolecithin (K_{Ant} = 0.08 μg P/ml); (2) after perfusion with lysolecithin–lecithin mixture (concentrations 0.08 μg P/ml and 30 μg P/ml respectively) and (3) after repetitive perfusion with pure lysolecithin (the ventricle was pre-washed for 60 min with the Ringer's solution; K_{Ant} = 22 μg P/ml).

anti-ACh effect of lysolecithin and makes it more reversible.

Comparative study of ChLA of various phospholipids and detergents. It was of interest to study the structural specificity of the cholinolytic action of lysolecithin and to elucidate which chemical group was responsible for this effect. For this purpose a comparative study of the ChLA of the following substances was undertaken (see Chart 1): lysolecithin (a) isolated from rabbit serum or obtained from egg lecithin by phospholypase A_2 digestion, bovine heart plasmalogen–lysolecithin (b), synthetic analogues of lysolecithin (c), and of phosphatidyl ethanolamine (d) [12]. Besides these substances glyceryl phosphorylcholine (e), ethyleneglycol phosphorylcholine (f), ethylene glycol phosphate (g) and a number of surface-active agents (potassium oleate, bile acids and non-ionic detergent Triton X-100) were also tested.

Table 2 presents the results of this investigation and shows the K_{Ant} values of the substances studied.

The character of the ChLA of lysolecithin and of its diol analogues (c) was similar, their effect rose relatively quickly and was prolonged. All the analogues of lysolecithin tested showed ChLA at concentrations of 1×10^{-6}–5×10^{-6} M, the decrease of the cardiac sensitivity to ACh keeping for a long time, after supplying the substances to the heart muscle (experiments 5–12). The initial cardiac sensitivity to ACh was lowered three to four times on adding the synthetic lysolecithin analogues and was not recovered, after washing the heart muscle by Ringer's solution for 12 hr.

The effect of lysolecithin and its analogues seems to be connected with the presence of a quarternary ammonium group in their molecules. This is supported by the absence of ChLA in the case of a diol analogue of phosphatidyl ethanolamine (d) (experiment *N*4). At the same time this compound is surface active and causes lysis of erythrocytes at relatively small concentration [13].

Besides the trimethylammonium group the presence of an aliphatic chain seems to be a necessary prerequisite for ChLA in lysolecithin-like compounds. This is supported by the fact that neither L-glyceryl phosphorylcholine (e) nor ethylene glycol phosphorylcholine (f) exhibit any ChLA (experiments *Nn*14 and 15).

Lysolecithin analogues having an unsaturated fatty acid chain (c, $R = C_{17}H_{33}$) are more active than the corresponding derivates with a saturated chain (c, $R = C_{17}H_{35}$) (experiments *NN*5–7 and 8–12). Lysolecithin and its propanediol analogue with an oleic acid chain (c, $n = 1$) have approximately equal ChLA (experiments *NN*1, 2 and 9). It follows that the hydroxyl group of lysolecithin may be not essential for the manifestation of ChLA. Since the ChLA of plasmalogen–lysolecithin (b) only slightly differs from that of blood serum or egg yolk lyso-

Chart 1. Substances tested for cholinolitic activity

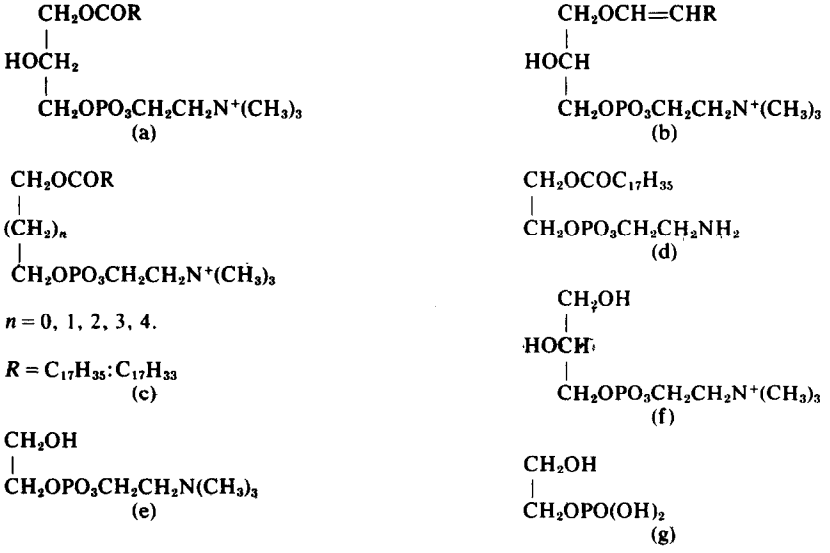


Table 2. ChLA of phospholipids, polyol phosphates and various detergents

Experiments	Substances tested	Presence or absence of surface activity (+ or −)	Effect on the amplitude of myocardiac contraction	Presence or absence of ChLA (+ or −)	K_{Ant} (M)	Duration of physiological effect on myocardium
1	Blood serum lysolecithin (a)	+	Increase of amplitude	+	1.3×10^{-5}	10–12 hr
2	*Egg lysolecithin (b)	+	,,	+	5.0×10^{-5}	,,
3	Bovine heart plasmalogen lysolecithin (c)	+	,,	+	1.0×10^{-5}	,,
4	2-Aminoethyl-2′-stearoyl-hydroxyethyl phosphate (d)	−	,,	−	—	—
5	2-Trimethylaminoethyl-2′-stearoyl hydroxyethyl phosphate (c), ($n = 0$, $R = C_{17}H_{35}$)	+	,,	+	3.3×10^{-5}	12 hr and more
6	2-Trimethylaminoethyl-3′-stearoyl hydroxyethyl phosphate (c), ($n = 1$, $R = C_{17}H_{35}$)	+	,,	+	3.2×10^{-5}	,,
7	2-Trimethylaminoethyl-4′-stearoyl hydroxyethyl phosphate (c), ($n = 2$, $R = C_{17}H_{35}$)	+	,,	+	5.0×10^{-5}	,,
8	2-Trimethylaminoethyl-2′-oleoyl hydroxyethyl phosphate (c), ($n = 0$, $R = C_{17}H_{33}$)	+	Slight increase of amplitude	+	1.2×10^{-6}	,,
9	2-Trimethylaminoethyl-3′-oleoyl hydroxyethyl phosphate (c), ($n = 1$, $R = C_{17}H_{33}$)	+	,,	+	6.4×10^{-6}	,,
10	2-Trimethylaminoethyl-4′-oleoyl hydroxybutyl phosphate (c), ($n = 2$, $R = C_{17}H_{33}$)	+	,,	+	1.2×10^{-5}	,,
11	2-Trimethylaminoethyl-5′-oleoyl hydroxypenthyl phosphate (c), ($n = 3$, $R = C_{17}H_{33}$)	+	,,	+	1.1×10^{-5}	,,
12	2-Trimethylaminoethyl-6′-oleoyl hydroxyhexyl phosphate (c), ($n = 4$, $R = C_{17}H_{33}$)	+	,,	+	3.0×10^{-6}	,,
13	Potassium salt of ethyleneglycol phosphate (g)	−	—	−	—	—
14	Glycerylphosphorylcholine (e)	−	—	−	—	—
15	Ethyleneglycolphosphorylcholine (f)	−	—	−	—	—
16	Triton X-100	+	Slight decrease of amplitude	−	—	—
17	Sodium desoxycholate	+	,,	−	—	—
18	Taurocholic acid	+	Slight increase of amplitude	−	—	—
19	Glycocholic acid	+	,,	−	—	—
20	Potassium oleate	+	Increase of amplitude	+	1.4×10^{-5}	70–80 min

* Obtained by phospholipase A_2 catalysed hydrolysis of egg lecithin.

† The K_{Ant} values for each of the substances studied are given as means of five to six experiments, the variance not exceeding 5 per cent.

lecithin (experiments *NN*1–3), the cholinolytic effect appears not to depend on the type of bond between the aliphatic chain and the glycerol residue.

It should be noted that the ChLA of the diol analogues of lysolecithin (c) is dependent on the chain length of the diol. Thus, the ethanediol (c, $n = 0$) and propanediol (c, $n = 1$) analogues with unsaturated fatty acids and lysolecithin itself are nearly equally active (experiments *NN*1, 2, 6, 9). The distance between an ester and a trimethylammonium group in the propanediol analogue is similar to that in lysolecithin. When this distance is increased by one or two methylene groups (c, $n = 2, 3$) ChLA decreases (experiments *NN*7, 10, 11). However, when the chain length of the diol increases further (c, $n = 4$) ChLA goes up again (experiment *N*12).

DISCUSSION

The results of the present investigation have shown the ChLA of blood serum to depend on the presence of two serum phospholipids—lysolecithin and lecithin. The former decreases the cardiac sensitivity to ACh while the second lowers the ChLA of lysolecithin. In the absence of lysolecithin lecithin does not change the cardiac sensitivity to ACh.

Various authors [14, 15] have shown lecithin and

lysolecithin to be present in the blood serum of homoiothermal animals in marked concentrations. According to our results, the content of lysolecithin in the rabbit blood serum amounts to 2.9 μg P/ml. Our experiments have shown that serum lysolecithin even at a concentration of 0.5 μg P/ml markedly decreases the cardiac sensitivity to ACh. This effect becomes irreversible at higher concentrations.

How is the toxic action of serum lysolecithin on the cholinoreceptor of the heart prevented in the organism?

The results of our study present evidence for two factors decreasing the ChLA of lysolecithin in the blood. It is known that part of the serum lysolecithin is bound to albumin [16, 17]. This may result in forming physiologically inactive complex. Such a possibility has been confirmed by our data on the ChLA of the dialysed serum and of the dialysate. Our experiments have shown that the total ChLA of the native serum passes into the dialysate and after dialysing the serum becomes inactive. However, when the inactive dialysed serum is extracted by chloroform–methanol the extract shows ChLA. Thus, dialysis results in separating two fractions of the blood serum lysolecithin: a 'free', dialysable lysolecithin which is the carrier of ChLA in the native serum and a physiologically inactive, non-dialysable lysolecithin, bound to macromolecular components of the serum. When the serum is extracted by chloroform–methanol the bound lysolecithin is liberated and passes into the extract causing the ChLA of the latter. The second factor decreasing the ChLA of the blood lysolecithin is the protective effect of lecithin which is present in serum in a high concentration. The action of serum lysolecithin may also be partially inhibited by cholesterol which is known to form a complex with lysolecithin [18–20]. It is further known that the myocardium can convert serum lysolecithin into lecithin [22]. It seems thus probable that a certain balance of the levels of lysolecithin and lecithin in the blood is maintained. This balance is of utmost importance for regulating the cardiac sensitivity to ACh.

The molecular mechanism of the action of lysolecithin and lecithin on the cardiac cholinoreception remains obscure. Lysolecithin is known to possess a strong detergent action [23] and to change markedly the properties of artificial [24–31] and natural membranes [32–41]. It seems therefore possible that lysolecithin may interact as a detergent with the cholinoreceptive membrane of the myocardium, affecting the interaction of the membrane with ACh. We have shown, however, that other surface-active substances (bile acids and their salts, Triton X-100, 2-aminoethyl-2 stearoyl-hydroxythyl phosphate, etc.) practically do not affect the cardiac sensitivity to ACh at all. On the other hand, potassium oleate which shows marked detergent and anti-ACh activity does not cause such a prolonged and strong decrease of the cardiac sensitivity to ACh as do lysolecithin and its analogues (see Table 2).

It should be also emphasized that the detergent action of lysophosphatides leading to an impairment of artificial membranes was observed only at relatively high concentrations of these compounds. Barsukov *et al.* [31] have studied the effect of low (1×10^{-6}–1×10^{-5} M) concentrations of lysolecithin and its diol analogues on the potassium permeability of liposomes. They have found that these substances taken in low concentrations augment the efflux of K^+ without impairing the integrity and vesicularity of the liposomal membrane.

Thus, moderate concentrations of lysolecithin seem to induce some structural rearrangement of the membrane without producing large pores. The ChLA of lysolecithin may be a consequence of such a rearrangement of the cholinoreceptor membrane.

It is known that alkyl trimethylammonium compounds and acylcholines with long chains act as antagonists of ACh. The cholinolytic effect of these substances is related to the interaction of their fatty chains with the hydrophobic surrounding of the cholinoreceptor [42, 43].

By contrast the ChLA of lysolecithin may be due to a high affinity of its choline moiety to an active site of the cholinoreceptor. This suggestion is supported by our results demonstrating the presence both of an aliphatic chain and a trimethylammonium group to be necessary for manifesting ChLA in the lysophosphatide series.

The effect of lysolecithin taken in low concentrations is quite similar to that of the competitive antagonists of ACh. However, when the lysolecithin concentration is increased the ChLA grows extremely fast and the effect becomes non-competitive and irreversible. This second phase may be caused by a non-specific detergent action of lysolecithin on the myocardiac membrane. As to the 'anti-lysolecithin' effect of lecithin it seems to be connected with the ability of lecithin to form mixed micelles with lysolecithin [26, 29, 44]. The introduction of exogenous lecithin in the perfusion solution protects the membrane against the action of lysolecithin. However, if the interaction between the membrane and lysolecithin has already occurred the exogenous lecithin is unable to restore normal cholinoreception.

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