EFFECT OF PHOSPHOLIPIDS OF VARIED COMPOSITION ON CORONARY VASCULAR TONE AND MYOCARDIAL CONTRACTILITY

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In several pathological states (ischemic heart disease, anoxia, etc.) changes take place in the phospholipid (PL) concentration in the circulating blood and the ratio between their fractions is disturbed [4, 5]. In experiments on animals to model certain diseases (ischemic shock, atherosclerosis) phospholipid vesicles, or liposomes, have been shown to have a therapeutic action [6, 15]. It is therefore necessary to study the effect of PL on functional parameters of the cardiovascular system, without which the little available information is contradictory. It has been shown that egg phosphatilethanolamine (PEA) lowers vascular tone of the isolated rabbit ear [2], and that soy and egg phosphatidylcholine (PC) have a hypertensive action when administered to rats but a vasoconstrictor effect on an isolated vascular strip [7, 12]. The effect of PL on coronary vascular tone and on myocardial contractility remains virtually unstudied.

The aim of this investigation was to study the effect of PL of varied composition on the coronary perfusion rate (CPR) and contractibility of the isolated rat heart and of a strip of frog myocardium.

EXPERIMENTAL METHOD

Experiments were carried out on Wistar rats and on frogs (Rana temporaria). The isolated rat heart was perfused (27 experiments) with oxygenated Krebs–Henseleit solution (KHS) by Langendorff's method, in Fallen's modification, under noncirculating conditions [9]. After the initial stabilizing perfusion (20-25 min) the heart was perfused for 10 min with KHS containing PL of varied composition. Next the heart was reperfused for 40 min with plain KHS (rinsing perfusion). The volume CPR was estimated by measuring the outflow from the pulmonary artery, and contractility of the heart was estimated from the maximal pressure in the left ventricle (P_{max}). Total PL (TPL) or a mixture of PEA and PC (5:1 by weight) were used in the experiments in a dose of 0.5 mg/ml perfusion fluid, and also PC alone in a concentration of 0.08 mg/ml, i.e., in a quantity equal to that contained in the mixture with PEA. TPL were isolated from egg yolk by precipitation, fractions of unpurified PEA and PC were obtained by column chromatography of TPL on alumina [11]. The PL were added to the perfusion fluid in the form of monolayer liposomes 40-50 nm in diameter, prepared by ultrasonic treatment of a suspension of lipids in KHS on a UZDN-1 disintegrator for 3-5 min at 4°C with a frequency of 22 kHz, until the optical density of the suspension at 400 nm was reduced to 0.2-0.4 unit, calculated relative to a lipid concentration of 2 mg/ml. The PEA–PC mixture also was used in the form of monolayer liposomes 0.1-20 μ in diameter [3]. The isolated strip of frog myocardium was perfused (10 experiments) under recirculating conditions with Ringer's solution at 20-22°C (pH 7.4) without additional oxygenation. A semicircular fragment of the transverse section through the ventricular myocardium 3-4 mm long and 1-1.5 mm in diameter was used as the preparation. Stabilizing perfusion was carried out for 1 h after which the strip was perfused for 2 h with a suspension of monolayer liposomes made from the PEA–PC mixture (5:1) in Ringer's solution in a concentration of 2.5 mg/ml. Contractibility of the strip was recorded by means of the 6MKh1S mechanotron under near-isometric conditions, under electrical stimulation with a frequency of 0.5 Hz. Parameters obtained at the end of the period of stabilizing perfusion were taken as the initial values in all experiments. In

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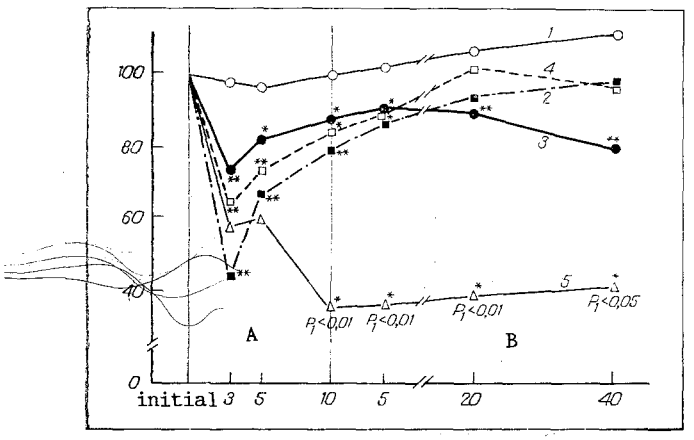

Fig. 1

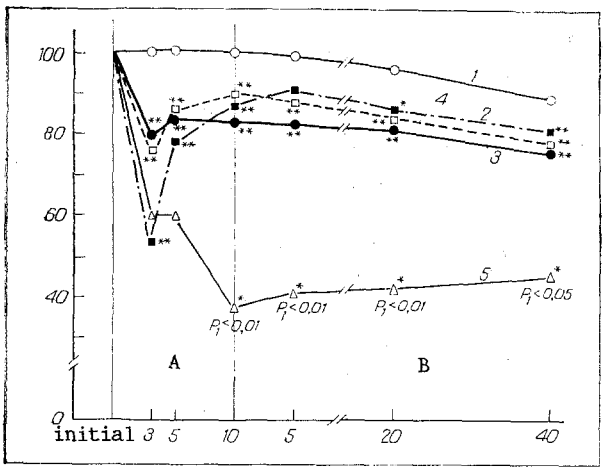

Fig. 2

Fig. 1. Effect of phospholipids of varied composition on CPR of isolated rat heart (in % of initial value). Abscissa, time (in min); ordinate, CPR. 1) Control; 2) TPL; 3) PEA + PC; 4) PC; 5) PEA + PC (multilayered liposomes). Remainder of legend as to Table 1.

Fig. 2. Effect of phospholipids on varied composition on contractility of the isolated rat heart (in % of initial value). Abscissa, time (in min); ordinate, P_{max}. Remainder of legend as to Fig. 1.

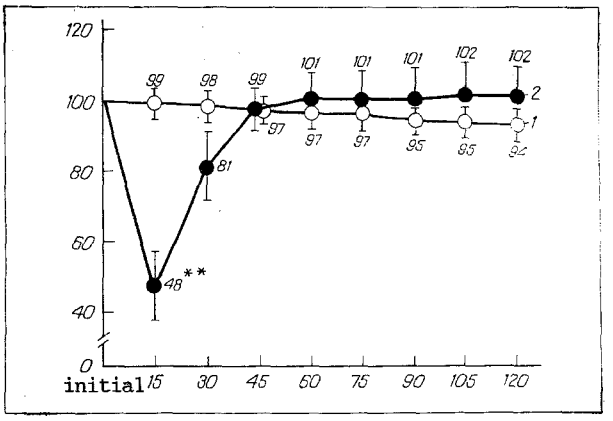

Fig. 3. Effect of phospholipids on contractility of isolated strip of frog myocardium (in %). Abscissa, time (in min); ordinate, amplitude of contractions. 1) Control; 2) PEA + PC. Remainder of legend as to Table 1.

the control experiments, perfusion was performed without the addition of PL. The significance of differences between experiments was estimated by Student's t test.

EXPERIMENTAL RESULTS

Estimation of the effect of PL on the isolated rat heart gave results which are summarized in Table 1 and Figs. 1 and 2. It will be clear from Table 1 and Fig. 1 that all the PL used caused a marked decrease in CPR of the rat heart. When PL were given in the form of monolayer liposomes (Fig. 1: 2-4) CPR was reduced to its lowest level after the first 3 min of perfusion, followed by the beginning of recovery despite continued administration of the liposomes. In all experiments (except when the PEA–PC mixture was used) rinsing perfusion restored CPR after only 20 min close to its original value. The degree of lowering of CPR after 3 min of perfusion was greatest when TPL were used; the effect of liposomes made from the PEA–PC mixture or from PC alone on CPR was about equal. Administration of the PEA–PC mixture in the form of multilayered liposomes caused a much more marked fall of CPR than administration of monolayer liposomes of the same composition (Fig. 1: 3, 5); CPR, moreover, continued to fall throughout the 10 min of PL administration and it remained low throughout the period of rinsing perfusion, i.e., the action of multilayered liposomes on coronary perfusion was irreversible in character, at least during the period of study.

Contractility of the rat heart (Fig. 2) in the control series did not change significantly but fell considerably on the addition of PL to the perfusion fluid; the effect of PL of different composition on P_{max} was similar to their effect on CPR, the only difference being that subsequent rinsing perfusion did not restore the normal values of P_{max}, which

TABLE 1. Effect of PL of Varied Composition on CPR and P_{max} of Isolated Perfused Rat Heart (M ± m)

PL	Number of experiments	CPR, ml/min/g						
		initial value	A, min			B, min		
			3	5	10	5	20	40
Control	6	15,9±0,4 (100)	15,6±0,4 (98)	15,5±0,5 (97)	15,9±0,7 (100)	16,5±0,8 (104)	17,2±0,5 (108)	18,0±0,5 (113)
TPL	6	17,0±0,8 (100)	7,4±0,6** (44)	10,4±1,0** (61)	13,5±0,7** (79)	15,7±1,3 (92)	16,2±1,2 (95)	16,7±1,2 (98)
PEA + PC	6	15,2±0,2 (100)	11,1±0,8** (73)	12,5±0,8* (82)	13,3±0,5* (88)	13,7±0,5* (90)	13,7±0,2** (90)	11,9±0,5** (78)
PC	5	15,5±0,5 (100)	9,8±0,9** (63)	11,3±0,7** (73)	13,5±0,6* (87)	14,1±0,5* (90)	16,0±0,6 (103)	15.1±1,1 (97)
PEA + PC (multilayered liposomes)	4	15,8±0,8 (100)	9,3±3,8 (58)	9,6±3,5 (61)	5,4±2,4* (34)	5,7±2,1* (36)	6,2±2,0* (39)	6,7±2,3* (42)
P_1					<0,01	<0,01	<0,01	<0,05

Table 1 continued

PL	Number of experiments	P_{max}, mm Hg						
		initial value	A, min			B, min		
			3	5	10	5	20	40
Control	6	140±3 (100)	140±4 (100)	140±4 (100)	140±4 (100)	139±3 (99)	134±3 (96)	125±4 (89)
TPL	6	139±6 (100)	75±12** (53)	108±12** (78)	121±9** (87)	127±11 (91)	120±11* (86)	114±5** (82)
PEA + PC	6	143±5 (100)	113±10** (79)	120±11** (84)	119±7** (83)	118±7** (83)	118±7** (83)	109±8** (76)
PC	5	152±4 (100)	114±7** (75)	131±5** (86)	137±6** (90)	134±10** (88)	130±7** (85)	119±7** (78)
PEA + PC (multilayered liposomes)	4	137±8 (100)	82±13 (60)	83±20 (60)	52±18* (38)	57±20* (42)	59±18* (43)	64±14* (47)
P_1					<0,01	<0,01	<0,01	<0,05

Legend. Percentage of initial value given in parentheses. Here and in Figs. 1 and 2: *p < 0.05, **p < 0.01 compared with initial value. A) Perfusion with medium containing PL, B) perfusion with plain medium. P_1) Significance of differences between experiments with monolayer and multilayered liposomes made from PEA–PC mixture (5:1).

remainded 18-24% below the initial level in the experiments with multilayered and by 53% in the experiments with monolayer liposomes.

Addition of PL to the medium during perfusion of the isolated rat heart thus depressed CPR and myocardial contractility. When PL were given in the form of multilayered liposomes the effect was considerably stronger and became irreversible in character, evidently due to mechanical disturbances of patency of the small vessels. When monolayer liposomes were used the decrease of CPR was caused by the temporary vasoconstrictor effect of PL on the coronary vessels, for the value of CPR began to recover even while PL administration was in progress.

As has already been stated, during perfusion of the heart with PL the time course of the change in P_{max} was similar to that of the change in CPR, i.e., worsening of contractility of the heart during this period may be the result of a decrease of coronary perfusion. To study the direct action of PL on myocardial contractility experiments were carried out on a model of perfusion of an isolated strip of frog myocardium, excluding the vasoactive effect of PL and enabling the duration of its action on the myocardium to be prolonged.

It will be clear from Fig. 3 that perfusion of the strip of frog myocardium with Ringer's solution for 2 h did not change the amplitude of myocardial contraction, whereas the addition of multilayered liposomes made from PEA–PC mixture to the perfusion fluid temporarily depressed it after 15 min of perfusion, i.e., it had a direct negative inotropic effect.

The mechanisms of the vasoconstrictor and negative inotropic effects of PL are still not quite clear. We know that unsaturated fatty acids have a vasoconstrictor action on coronary vessels and can also significantly worsen myocardial contractility [10, 14]. The effect of PL may therefore be due to residues of unsaturated fatty acids present in their composition. However, as was shown above, the use of a PEA–PC mixture (5:1) with a higher content of unsaturated fatty acids than TPL [1, 11] does not potentiate the negative inotropic effect and actually reduces the vasoconstrictor effect of PL. These results agree with data showing that PL, as a class of compounds, possess natural vasoactive properties, dependent to some degree on their fatty-acid composition [13]; moreover, egg PC has a significant effect on the isolated heart. This last observation is in agreement with existing data on strengthening of contractility of a strip of rat portal vein under the influence of chromatographically pure egg PC [7], and also with data showing that egg PC liposomes can inhibit spontaneous contractibility of myocardiocytes [8]. It must be pointed out that this inhibitory effect of PC is manifested when calcium or sodium ions are present inside the liposomes but absent when phospholipid vesicles loaded with potassium ions are used. Consequently, the effect of PC (liposomes) on the cardiovascular system evidently depends both on their composition and on the composition and concentration of ions in the internal water compartment of the liposomes. However, this problem requires further study.

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