Thus DSIP acts in opposite ways on CES depending on its dose. This is in agreement with data in the literature: different doses of DSIP have opposite metabolic effects [3].

The antiedematous effect of drugs is known to take place through different mediator systems of the brain [6]. Meanwhile DSIP has well-marked neuromodulating properties [2, 4]. For that reason the mechanism of action of DSIP on CES is in all probability highly complex and multicomponent in nature. The possibility cannot be ruled out that the antiedematous effect of DSIP is realized through inhibition of the serotoninergic, adrenergic, and histaminergic systems and activation of GABA-ergic processes in the brain.

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EFFECT OF NEGATIVELY CHARGED LIPOSOMES ON ADP-INDUCED PLATELET AGGREGATION

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KEY WORDS: liposomes; platelets; aggregation; phosphatidylserine; phosphatidylinositol

Platelet activation is accompanied by intensive transfer of phosphatidylserine, phosphatidylethanolamine, and phosphatidylinositol from the inner to the outer surface of the membranes [6]. This process correlates with the intensity of aggregation and procoagulant activity of the cells [5, 7, 11]. The appearance of additional amounts of phospholipids in the blood stream in the composition of liposomes can alter the functional state of the platelets and of the hemostasis system as a whole [1, 2, 8]. Some workers [9] studied the effect of liposomes of different composition on platelet aggregation and found that while they themselves did not cause aggregation, they modulated the response of the platelets to ADP. The effect of the liposomes depended on the charge on the lipid membrane. Neutral vesicles had no effect, whereas positively charged liposomes significantly inhibited ADP-induced aggregation in platelet-enriched plasma (PEP). Negatively charged liposomes did not affect the PEP-ADP system but inhibited aggregation of washed platelets in response to thrombin. The authors cited used phosphatidylglycerol as a negatively charged phospholipid.

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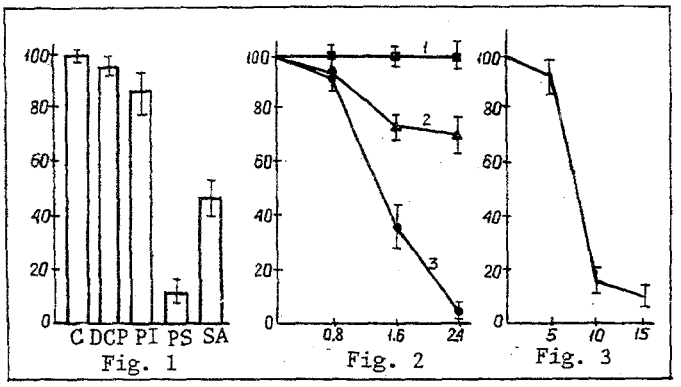

Fig. 1. Effect of lipid composition of liposomes on ADP-induced platelet aggregation. Platelets in plasma incubated for 5 min with 0.8 mg liposomal lipids in 1 ml of suspension. Aggregation induced by addition of ADP in a final concentration of $5 \cdot 10^{-?}$ (sic) M. C) control aggregation; DCP) liposomes containing dicetyl phosphate; PI) liposomes containing phosphatidylinositol; PS) liposomes containing phosphatidylserine; SA) liposomes containing stearylamine. Ordinate, aggregation (in percent of control).

Fig. 2. Effect of dose of lipids of liposomal preparations on platelet aggregation induced by $5 \cdot 10^{-?}$ (sic) ADP. Numbers indicate composition of liposomes: 1) OL:CSL:DCP = 7:2:1; OL:PI:CSL = 4:4:2; 3) OL:PS:CSL = 4:4:2. Abscissa, content of liposomal lipids (in mg/ml); ordinate, aggregation (in percent of control).

Fig. 3. Effect of duration of preincubation of phosphatidylserine liposomes on their ADP-induced aggregation. Abscissa, incubation time (in min); ordinate, aggregation (in percent).

In the investigation described below we studied the effect of liposomes containing these phospholipids on ADP-induced aggregation.

EXPERIMENTAL METHOD

Ovolecithin (OL) and phosphatidylinositol (PI) were obtained from Khar'kov Bacterial Preparations Factory, cholesterol (CSL), dicetyl phosphate (DCP), Tris, and sodium chloride from "Sigma" (USA), phosphatidylserine (PS) from "Serva" (Germany), stearylamine (SA) from "Aldrich" (USA), and ADP from "Chrono-log Corp." (USA). Small single-layered liposomes were prepared from 15 μmoles of a mixture of lipids in 1 ml of 10 mM Tris-HCl buffer, pH 7.5, with 0.15 M NaCl by ultrasonic treatment of a dried film of lipids in buffer at 25°C in an atmosphere of nitrogen until the suspension had cleared. Arterial blood was obtained from the abdominal aorta of mature male Wistar rats under ether anesthesia. The blood was stabilized with heparin in a concentration of 50 U/ml. PEP in a concentration of 50 cells/ml were prepared by the usual method [10]. Platelet aggregation was measured on a 2-channel "Labor APACT" aggregometer (Germany) at 37°C. PEP (0.23 ml) were introduced into the cuvette with 0.02 ml of a suspension of liposomes, and kept for 5-10 min with constant mixing (1000 rpm). Aggregation was induced by the addition of ADP to a final concentration of $5 \cdot 10^{-?}$ (sic) M. The maximal amplitude of aggregation was estimated as a percentage of the control. The results were subjected to statistical analysis in the usual way.

EXPERIMENTAL RESULTS

Introduction of liposomes of different composition (OL:CSL:DCP = 4:4:2; OL:PS:CSL = 4:4:2; OL:SA = 9:1) into the PEP did not induce platelet aggregation, confirming observations in [9] according to which, irrespective of their charge, liposomes are unable to induce aggregation. Meanwhile, preliminary incubation of PEP with liposomes modifies the response of platelets to ADP in a final concentration of $5 \cdot 10^{-?}$ (sic) M (Fig. 1). Addition of liposomal preparations containing the different charged lipids to PEP showed that the effect depends not on the charge, but on the structure and properties of the concrete phospholipid. Liposomes containing DCP, for instance, did not affect platelet aggregation in PEP. The presence of PI in the liposomal membrane led to some decrease in aggregation. Incorporation of PS into the lipid membrane caused a sharp decrease in platelet aggregation. Incubation of PEP for 10 min with liposomes containing PS led to inhibition on average by 72% compared with the control. Liposomes (OL:SA = 9:1) inhibited aggregation on average by 40%. A similar effect was described in the literature [9] and was evidently due to electrostatic interaction of positively charged liposomes with negatively charged plasma proteins (including fibrinogen) and the surface of the platelets.

The inhibitory effect of acid phospholipids was particularly demonstrable in the presence of a small dose of the inducer ($5 \cdot 10^{-?}$ (sic) M ADP), causing reversible aggregation. Under these conditions dependence of the inhibitory action of the liposomes on dose was studied (Fig. 2). Phosphatidylinositol liposomes affected activity of the platelets by a much lesser degree. In a dose of 2.4 mg/ml these liposomes reduced platelet aggregation by 30% compared with initially. Liposomes containing dicetyl phosphate did not affect ADP-induced aggregation in any of the doses used. Addition of liposomes with PS in the proportion of 0.8 mg lipids to 1 ml PEP caused a small decrease (not significant) in platelet aggregation. The liposomes, in a dose three times greater, caused almost complete inhibition of aggregation. The dose dependence was virtually linear in the range from 0.8 to 2.4 mg lipids in 1 ml of reaction mixture. The effect of phosphatidylserine liposomes depended not only on their quantity, but also on the duration of contact with PEP. An increase in the duration of preliminary incubation of PEP with 0.8 mg/ml of liposomes from 5 to 15 min reduced the level of aggregation by an order of magnitude. Moreover, the strongest inhibition took place between the 5th and 10th minutes of incubation (Fig. 3).

The results are evidence that in order to demonstrate the inhibitory action of PS-liposomes, a definite duration of contact with PEP is essential, and this duration can be reduced by increasing the dose of liposomes. Bornikov and co-workers [2] observed an inhibitory effect of liposomes containing PS on ADP-induced aggregation in blood plasma from healthy individuals and patients with ischemic heart disease. These workers explained the phenomenon by the formation of a prothrombin-phosphatidylserine complex with low activity. Activation of platelets is accompanied by an increase in the quantity of PS on the outer side of the membrane, which coincides with activation of thrombin with the participation of factor Xa on the platelet surface [5, 7]. Meanwhile the appearance of phosphatidylserine liposomes in the plasma leads to binding of prothrombin and other coagulation factors by them [3, 4, 12].

The effect of liposomes on ADP-induced platelet aggregation in PEP is thus determined more by the presence of particular types of phospholipids in their membrane, taking an active part in hemostasis, than by their charge.

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EFFECT OF PENTACTASTRIN AND SUBSTANCE P ON PARIETAL GASTRIC GLANDULOCYTES

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KEY WORDS: pentagastrin; substance P; gastric secretion; glandulocytes; acetylcholinesterase

Endogenous and exogenous peptides, circulating in the blood stream, have both direct and indirect action on effector cells. In the latter case the effect of the peptides is linked with their action on the sympathetic and parasympathetic nervous system [9, 10], while their direct action is due to interaction of their molecules with specific receptors [1, 6-8, 11]; primary binding of peptides with the lipid matrix of the membranes has been postulated [3, 4]. There is evidence in the literature of a mediator role of peptides for the metasympathetic nervous system [2], on the effect of peptides on effector cells through cholinergic pathways (acetylcholine receptors), and on the leading role of peptides in adrenergic mechanisms of regulation of the functions of the body under physiological and pathological conditions [5].

This polarity of views on the ways of interaction of peptides with effector cells motivated the study of the effect of the peptides pentagastrin and substance P on the secretory cells of the stomach. The aim of this investigation was to study the effect of these peptides on secretion of gastric juice and acid production by the parietal glandulocytes of the dog's stomach.

EXPERIMENTAL METHOD

The effect of pentagastrin (6 μg/kg), substance P (2.5, 5, and 10 g/kg), and the acetylcholinesterase blocker calimin (0.2 μg/kg), injected subcutaneously, on the secretion of gastric juice was studied in chronic experiments on dogs with a gastric fistula [1]. Total acidity, free hydrochloric acid, and the rate of its production, were determined in the gastric juice.

EXPERIMENTAL RESULTS

As Fig. 1a, b shows, subcutaneous injection of pentagastrin stimulates the secretion of gastric juice and of free hydrochloric acid by the parietal glandulocytes of the stomach, which was recorded reliably during the first 10 min after injection of pentagastrin. The volume of juice and concentration of free hydrochloric acid in it increased during the next 40 min (the peak of secretion), after which these parameters of gastric secretion declined until 90 min (Fig. 1a). During acetylcholinesterase blockade by calimin, against the background of pentagastrin-induced gastric secretion, potentiation of the secretion of gastric juice and of free HCl production was observed (Fig. 1b). The total volume of gastric juice and the duration of gastric secretion in this case both were increased by 2-2.5 times, and gastric secretion and free HCl production reached their peak values 10-15 min earlier than in the absence of calimin.

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