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Liposomes with phosphatidylethanol as a carrier for oral delivery of insulin: studies in the rat

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Abstract

In this study, phosphatidylethanol formed by phospholipase D catalysed transphosphatidylation of phosphatidylcholine was employed as a component for preparation of liposomal carrier for oral delivery of insulin. Thermotropic behaviour of liposomes from mixtures of dipalmitoyl phosphatidylcholine and dipalmitoyl phosphatidylethanol, and their resistance to pancreatic phospholipase A_2 catalysed hydrolysis were studied. Three kinds of liposomes with insulin were prepared to examine the pharmacological availability of liposomes with phosphatidylethanol: (i) dipalmitoyl phosphatidylcholine/dipalmitoyl phosphatidylethanol (1:1 w/w) liposomes; (ii) dipalmitoyl phosphatidylcholine/dipalmitoyl phosphatidylethanol/palmitoyl–stearoyl sucrose (1:1:0.2) liposomes; and (iii) liposomes composed of natural phosphatidylcholine and phosphatidylinositol (1:1). The resultant liposomes were orally administrated to rats with blood glucose concentration of 270 mg/100 ml in a dose of 12 IU/kg body weight. Blood samples were collected 0.5, 1.5, 3, 5, and 24 h after treatment. Oral administration of all liposomal species resulted in hyperinsulinemia. Hyperinsulinemia induced by liposomes containing dipalmitoyl phosphatidylethanol was attended by a decrease of blood glucose concentration. No correlation between insulin level and glucose concentration in the rat blood after oral administration of phosphatidylinositol-containing liposomes was observed.

Keywords: Insulin; Liposomes; Phosphatidylethanol; Alloxan diabetes; Oral administration

1. Introduction

The enhancement of diabetes cases stimulates a search for new methods of insulinotherapy. Nowadays, the treatment of diabetes is healing predominantly by way of technical improvement of insulin injection. However, the classical procedure of insulin injection is in conflict with natural system for the maintenance of glucose homeostasys as on subcutaneous injecting the hormone does not arrive immediately at the liver. What is more, this procedure causes psychologic stress

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provoking the emission of contrainsulin hormones, which aggravate the pathological course of metabolism.

The most physiological way of insulin administration is oral mode because on adsorption in intestines insulin unambiguously lands in portal system. However, the oral mode of insulin administration is difficult to realise from the fact that protein molecules of hormone are destructed by proteases of alimentary canal. Previously a series of experiments has been undertaken to protect insulin against proteolytic action of proteases. The possibility of oral treatment by insulin incorporated into oil emulsions (Engel et al., 1968; Cho and Flynn, 1989; Matsuzawa et al., 1995), polymer capsules (Saffran et al., 1986; Damge et al., 1988; Saffran, 1991) and detergent micelles containing protease inhibitors (Kidron et al., 1989) has been studied.

In the past two decades the potential usefulness of liposomes as drug carriers has attracted considerable interest (Gregoriadis, 1980; Woodley, 1985; Gregoriadis, 1988; Rogers and Anderson, 1998). These phospholipid vesicles are capable of encapsulating both hydrophobic and hydrophilic drugs, they are biodegradable and are not toxic in vivo. The drugs encapsulated in liposomes are sufficiently protected from enzymatic attack and immune recognition. Various attempts to apply liposomes to the preparation of oral insulin have been reported (Dapergolas and Gregoriadis, 1976; Patel and Ryman, 1976; Arrieta-Molero et al., 1982; Axt et al., 1983; Spangler, 1990; Choudhari et al., 1994; Muramatsu et al., 1996; Iwanaga et al., 1999). The hypoglycaemic effect of liposomal insulin has been found to depend on the lipid composition, surface charge and physical state of phospholipid bilayer (Dapergolas et al., 1976; Dapergolas and Gregoriadis, 1976; Axt et al., 1983; Choudhari et al., 1994). A marked decrease in blood glucose concentration has been observed after intragastric administration of liposomes formed with high-melting dipalmitoyl phosphatidylcholine (Dapergolas and Gregoriadis, 1976) or negatively charged phosphatidylinositol (Dapergolas et al., 1976). The present communication concerns the development of liposomal insulin preparations using both high-melting and negatively charged dipalmitoyl phosphatidylethanol as a lipid component. Liposome-entrapped insulin was injected intragastrically to rats with alloxan diabetes and both glucose concentration and immunoreactive insulin level were evaluated in animal blood within 0.5, 1.5, 3, 5 and 24 h after administration.

2. Materials and methods

2.1. Materials

Crystalline porcine insulin (27 U/mg) was purchased from Belmedpreparaty (Minsk, Belarus); pancreatic phospholipase A_2 was from Sigma. Tris, EDTA, and palmitoyl–stearoyl sucrose were from Serva (Germany). Phosphatidylcholine and phosphatidylinositol were isolated from egg yolk and *Sacharamyces cerevisia*, respectively, and purified by published methods (Bergelson, 1980). Dipalmitoyl phosphatidylcholine was synthesised by the procedure of Hermetter and Paltauf (1981). Phosphatidylethanol was obtained from phosphatidylcholine using the headgroup exchange reaction catalysed by phospholipase D (Omodeo-Sale et al., 1989). Each phospholipid gave only one spot on silica gel thin-layer chromatograms with solvent systems of chloroform/methanol/water (65:25:4 v/v/v) and chloroform–methanol–7N NH_4OH (13:5:1 v/v/v). Purified phospholipids were dissolved in chloroform and stored at −18°C until use. All other chemicals were of analytical grade and commercially available.

2.2. Preparation of liposomes

Liposomes were prepared by mixing phosphatidylcholine and phosphatidylinositol ('fluid', 1:1 w/w) or dipalmitoyl phosphatidylcholine and dipalmitoyl phosphatidylethanol ('solid', 1:1 w/w) or the latter supplemented with palmitoyl–stearoyl sucrose (1:1:0.2 w/w/w) in chloroform. Then, the solvent was evaporated under a stream of argon so that a thin film of lipid would be formed on the inner side of the flask. Insulin (2.0 mg/ml) dissolved in 10 mM Tris–HCl buffer (pH

8.0) containing 1 mM EDTA was added to the dried lipid, then the mixture was dispersed with a shaker under an argon atmosphere at 50°C (at 25°C for 'fluid' liposomes), yielding multilamellar liposomes. (A total 4 mg of insulin was used for each 50 mg of lipid.) After a 15-min swelling period, these multilamellar liposomes were sonicated 3 times for 1 min with 2-min intervals under a stream of argon at 50°C (at 4°C for 'fluid' liposomes) using titanium microtip in an UZDN sonicator (22 kHz, 20 mA). No titanium particles were observed after centrifugation at 3000 rpm for 10 min. The non-entrapped insulin was separated from liposome-encapsulated drug by gel filtration on Sephadex G-150. Liposomes were eluted immediately after the void volume. For studies on the ionic strength effect, liposomes were prepared likewise in the presence NaCl. For control experiments, liposomes containing no insulin were prepared in the absence of the hormone and this preparation was mixed with insulin just before using.

2.3. Differential scanning microcalorimetry

Samples for differential scanning calorimetry were prepared by dilution of liposomes composed of dipalmitoyl phosphatidylcholine and dipalmitoyl phosphatidylethanol to a total lipid concentration of 0.2 mg/ml. Experiments were performed with a high-sensitivity differential adiabatic scanning microcalorimeter DASM-1M (Privalov and Potekhin, 1986) at a heating rate of 1°C/min.

2.4. Phospholipase A_2 catalysed hydrolysis of insulin-containing liposomes

Hydrolysis was carried out in 0.05 M Tris–HCl (pH 8.0) at a lipid concentration of 15 mM in a volume of 1.0 ml. $CaCl_2$ was added to a final concentration of 2.0 mM. After preincubation at the 37°C the reaction was initiated by the addition of phospholipase (26 μg). The reaction was terminated by addition of EDTA to a final concentration of 15 mM and a double volume of a chloroform/methanol (2:1 v/v). After extraction the samples were centrifuged, the lower layer was evaporated, and the reaction products were analysed by thin-layer chromatography on plates with silica gel (Merck) in the solvent system chloroform/methanol/28% ammonia (13:5:1 v/v/v). After the phospholipids had been visualised with reagent of Vaskovsky et al. (1975), the spots corresponding to phospholipid substrate and its lyso-derivate were cut out and analysed for phosphorus content (Vaskovsky et al., 1975). The degree of hydrolysis was expressed in percent as the ratio of the lyso-products formed to the total amount of phospholipid.

2.5. Animals and model of experimental diabetes

Male Wistar rats (body weight, 180–250 g) were used in these experiments. Diabetes was induced by a single intraperitoneal injection of alloxan (150 mg/kg). The rats were used within 2 weeks after alloxan injection when acute disease phase was completed.

2.6. Therapeutic experiments

The rats were fasted for 24 h prior to the experiments. The liposome-entrapped insulin was administrated intragastrically using a hollow probe. The dose of insulin was fixed at 12 U/kg body weight. For control experiments, either buffer solution or mixture of empty liposomes with free insulin was similarly administrated. Blood samples were collected from the tail before and at 0.5, 1.5, 3, 5 and 24 h after dosing. Heparin was used for suppression of blood coagulation. Serum was separated by centrifugation at 3000 rpm for 10 min and kept frozen until analysis.

2.7. Analytical methods

The concentrations of insulin and phospholipid in the liposomes were determined by the methods of Lowry et al. (1951) and Vaskovsky et al. (1975), respectively. Radioimmunoassays of insulin were done using a commercial RIA kit according to the protocol provided. Plasma glucose concentrations were measured by a glucose oxidase method.

3. Results

3.1. Preparation and characterisation of liposome-entrapped insulin

Liposome-entrapped insulin was prepared by soft sonication of phospholipid dispersion in aqueous insulin solution. After sonication, the liposomes were fractionated by gel-filtration on a Sepharose 4B column, the separation being monitored by both protein and lipid phosphorus analysis. According to data of electron microscopy the fraction of insulin containing liposomes is heterogeneous and contains vesicles from 50 to 250 nm in size.

Fig. 1 shows the entrapment of insulin by liposomes composed of different phosphatidylcholine/phosphatidylethanol mixtures. As can be seen in Fig. 1, with a rise of proportion of negatively charged phosphatidylethanol in the mixture the amount of liposome-entrapped insulin is increased. Similar picture for liposomes involving yeast phosphatidylinositol in place of phosphatidylethanol was observed.

When used semisynthetic phospholipid mixtures, the encapsulation level was slightly below. Fig. 2 depicts the elution profiles of liposomes composed of dipalmitoyl phosphatidylcholine, dipalmitoyl phosphatidylethanol and insulin. As can be seen from Fig. 2, over 70% of insulin are eluted together with liposomes. Rechromatography of liposome-entrapped insulin did not cause any marked changes in the hormone/phospholipid ratio. Liposomes containing dipalmitoyl phosphatidylcholine alone entrapped not more than 10% of insulin (data not shown). In liposome fraction eluted from column a maximum of insulin content was offset to the right about lipid phosphorus maximum (Fig. 2). The shift would appear as insulin incorporation into smaller in size liposomes. On the other hand, it would come from desorption of insulin bound to liposome surface through electrostatic interactions when liposomes were moving along the column. If so, the entrapment level should depend on ionic strength of solution.

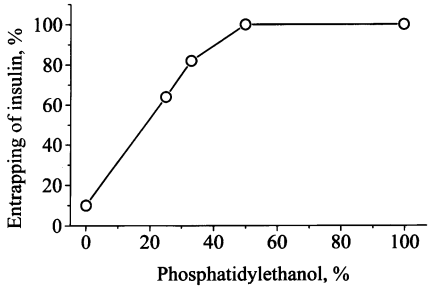

Fig. 1. Entrapping of insulin by liposomes composed of phosphatidylcholine and phosphatidylethanol: effect of phosphatidylethanol proportion (w/w). Twenty-five milligrams of phospholipids were dispersed in insulin solution (2 mg/ml) and sonicated as described in Section 2. Non-entrapped insulin was separated by gel-filtration on Sepharose 4B column.

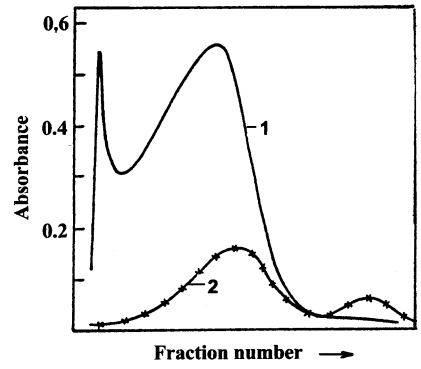

Fig. 2. Chromatographic separation of liposome-entrapped insulin (0.75 ml) on Sepharose 4B column (18×350 mm^2). Liposome-entrapped insulin was prepared from 25 mg of dipalmitoyl phosphatidylcholine, 25 mg of dipalmitoyl phosphatidylethanol and 4 mg of insulin in 2 ml of 10 mM Tris–HCl buffer solution (pH 8.0): (1) profile of phospholipid elution; (2) profile of insulin elution. Profiles were obtained from optical density of colorimetrical determination of phospholipid and protein (Section 2).

Fig. 3 shows the effect of NaCl in buffer solution on the liposome-entrapped insulin concentration. On addition of NaCl to liposomes prepared in 0.01 M Tris-buffered solution (pH 8.0), the proportion of encapsulated insulin tended to decrease with increase in salt concentration. When liposomes were formed in buffer solution containing NaCl, almost identical effect on the level of liposome-entrapped insulin was observed. These results suggest that electrostatic mechanism contributes significantly into binding of insulin with liposomes.

3.2. Thermotropic behaviour of liposomes composed of mixture of dipalmitoyl phosphatidylcholine and dipalmitoyl phosphatidylethanol

Dapergolas and Gregoriadis (1976) have shown that hypoglycaemic effect of insulin entrapped in dipalmitoyl phosphatidylcholine/cholesterol liposomes is superior to liposomal insulin based on egg yolk phosphatidylcholine. It is well known (Chapman, 1975) that bilayer structures of semisynthetic dipalmitoyl phosphatidylcholine exhibit the main phase transition (gel–liquid crystalline) at 41.7°C, whereas bilayer vesicles of natural phospholipid exist in liquid-crystalline state and do not undergo any endothermic transition above 0°C. At 37°C these liposomes are in different phase state. It is not unlikely that gel state of dipalmitoyl phosphatidylcholine liposomes may account for hypoglycaemic effect.

Thermotropic behaviour of our preparation composed of mixture of dipalmitoyl phosphatidylcholine and dipalmitoyl phosphatidylethanol (1:1 w/w) has been studied with using of differential scanning microcalorimetry method. The heating scans of lipid mixture are shown in Fig. 4. The main endothermic transition of empty liposomes occurred at 41°C. Only a single peak of similar or slightly greater width as dipalmitoyl phosphatidylcholine alone was observed. It may indicate that the two lipids are well miscible. When insulin was encapsulated the heating curve of the sample showed the main endothermic transition with a shoulder on the high-temperature side. The enthalpy was reduced somewhat and the main transition lowered in temperature from 41 to 40°C. These results let us suggest that a portion of insulin interacts with lipid bilayer through both hydrophobic and electrostatic mechanisms. As it takes place, lipid bilayer of liposomal preparation is in gel state at temperatures below 40°C.

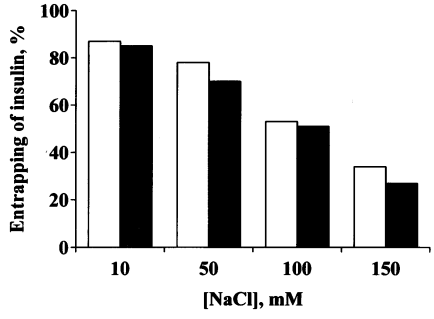

Fig. 3. Effect of NaCl on the concentration of insulin entrapped in liposomes composed of phosphatidylcholine and phosphatidylethanol (1:1 w/w). Liposomes were prepared in 10 mM Tris–HCl buffer solution and then NaCl was added (light bars); liposomes were prepared in 10 mM Tris–HCl buffer solution (pH 8.0) containing various concentration of NaCl (dark bars). Data represent an average of triplicate experiments.

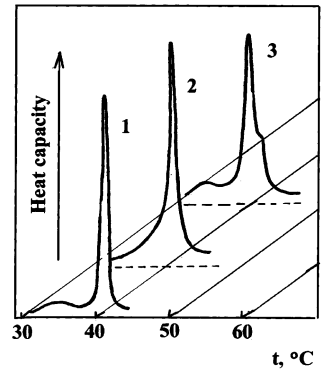

Fig. 4. Differential scanning calorimetry heating scans of liposomes composed of: (a) dipalmitoyl phosphatidylcholine; (b) dipalmitoyl phosphatidylcholine and dipalmitoyl phosphatidylethanol (1:1 w/w): (c) dipalmitoyl phosphatidylcholine, dipalmitoyl phosphatidylethanol and insulin (25:25:4 w/w/w). Liposomes were prepared in 10 mM Tris–HCl buffer solution containing 1 mM of EDTA (pH 8.0). Phospholipid concentration was 0.2 mg/ml. Heating rate was 1°C/min.

3.3. Phospholipase A_2 catalysed hydrolysis of phospholipids in liposomes containing insulin

Liposomal phospholipids are digested by phospholipase A_2 as they enter the alimentary canal. Phospholipase A_2 catalyses the hydrolysis of the *sn*-2 fatty acyl bond of phospholipids to liberate free fatty acids and lysophospholipids (Dennis, 1994). The hydrolysis rate depends on phospholipid composition (Akhrem et al., 1989) and may be modulated by soluble proteins (Naubatova et al., 1992). To make an estimate of rate of enzymatic disruption of liposomes, we studied the hydrolysis of phospholipids in both liquid crystal

and gel insulin-containing liposomes by pancreatic phospholipase A_2. The results, presented in Fig. 5, indicate that the rates of hydrolysis of 'fluid' phosphatidylethanol (obtained from egg yolk phosphatidylcholine) and 'solid' dipalmitoyl phosphatidylethanol are nearly equal. Towards phosphatidylcholines the phospholipase displays a different behaviour. Dipalmitoyl phosphatidylcholine is degraded by pancreatic phospholipase to a substantial degree, in contrast to egg phosphatidylcholine. A higher hydrolysis of dipalmitoyl phosphatidylcholine may be ascribed to structural irregularities in the packing of 'solid' substrate (Wilschut et al., 1978). Such irregularities are present in strongly curved bilayers and in bilayers containing structural defects. The latter can be caused by either drastic conditions of preparation of 'solid' liposomes (sonication at 50°C) or peculiar interaction of insulin with 'solid' liposome surface.

3.4. Therapeutical effect of liposome-entrapped insulin

The effectiveness of liposome-entrapped insulin was studied in rats, which were made diabetic by a single injection of alloxan. Within 2 weeks after alloxan administration the rats with blood-glucose level of 250 mg/100 ml of blood and more were selected. We did not use the rats in acute disease phase because under complex homeostasis disruption the insulin action might be distorted by pathologic process independent of diabetes.

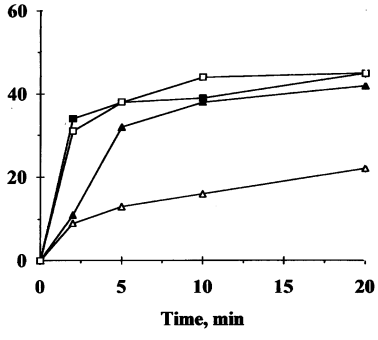

Fig. 5. Hydrolysis of 'solid' liposomes composed of dipalmitoyl phosphatidylcholine, dipalmitoyl phosphatidylethanol and insulin (closed symbols) and 'fluid' liposomes composed of egg phosphatidylcholine, egg phosphatidylethanol and insulin (open symbols) by pancreatic phospholipase A_2: triangles – hydrolysis of choline phospholipids; squares – hydrolysis of ethanol phospholipids. Phosphatidylcholine/phospatidylethanol/insulin ratio is 25:25:4 w/w/w. Percentage of hydrolysis and experimental conditions are described in Section 2.

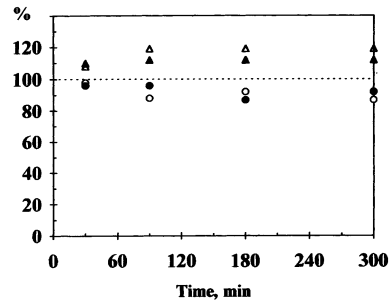

Fig. 6. The effect of blood-letting procedure on the plasma glucose levels (circles) and plasma insulin concentrations (triangles) in diabetic (closed symbols) or healthy (open symbols) rats. Glucose level and insulin concentration before blood-letting are taken as 100%. Each value represents the mean for seven animals.

As on trials the blood samples were taken from the tail vein 5 times at short intervals, the stress state of rats might result in change of biochemical index of blood. Because in the initial stage we tried to elucidate the effect of blood-letting procedure on the plasma glucose levels and plasma insulin ones in both diabetic and healthy rats. As can be seen in Fig. 6, stress in response to blood-letting procedure and blood loss resulted in both modest decrease of glucose concentration (about 10%) and insignificant increase of insulin (15–20%) in plasma. As it took place, the responses of diabetic and healthy rats were in line.

Oral administration of insulin entrapped in liposomes of composition dipalmitoyl phosphatidylcholine and dipalmitoyl phosphatidylethanol (1:1) to diabetic rats produced a rise in plasma immunoreactive insulin which reached a peak at 1.5 h. A high insulin concentration was retained within 3.5 h. The fall in blood glucose levels from 270 mg/100 ml to 140 mg/100 ml followed the rise in plasma immunoreactive insulin was observed (Fig. 7). Oral administration to diabetic rats of the same dose of free insulin with empty liposomes produced change neither in

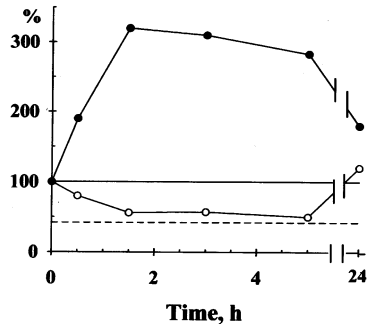

Fig. 7. The effect of orally administrated insulin-containing liposomes composed of dipalmitoyl phosphatidylcholine and dipalmitoyl phosphatidylethanol (1:1 w/w) on the plasma glucose levels (open circles) and plasma insulin concentrations (closed circles) in diabetic rats. Glucose level (270 mg/100 ml) and insulin concentration before administration are taken as 100%. Dotted line means glucose level in healthy rats (115 mg/100 ml). Each value represents the mean for seven animals.

blood glucose levels nor in immunoreactive insulin levels. The incorporation of synthetic lipid, palmitoyl–stearoyl sucrose, into liposomes composed of dipalmitoyl phosphatidylcholine and dipalmitoyl phosphatidylethanol reduced the hypoglycaemic effect of liposome-entrapped insulin (Fig. 8).

Hyperinsulinemia was also observed when insulin entrapped in liposomes composed of mixture of phosphatidylcholine and phosphatidylinositol was administrated (Fig. 9). But in this case no fall

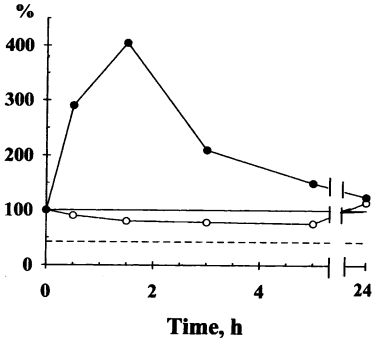

Fig. 8. The effect of orally administrated insulin-containing liposomes composed of dipalmitoyl phosphatidylcholine, dipalmitoyl phosphatidylethanol and palmitoyl–stearoyl sucrose (1:1:0.2 w/w/w) on the plasma glucose levels (open circles) and plasma insulin concentrations (closed circles) in diabetic rats ($n = 7$). The lines are the same as that of Fig. 7.

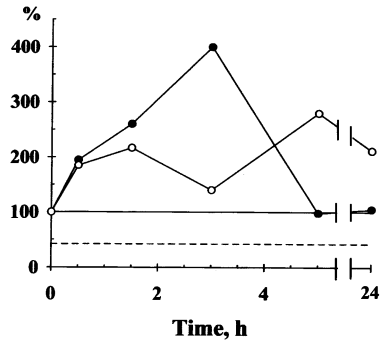

Fig. 9. The effect of orally administrated insulin-containing liposomes composed of phosphatidylcholine and phosphatidylinositol (1:1 w/w) on the plasma glucose levels (open circles) and plasma insulin concentrations (closed circles) in diabetic rats ($n = 6$). The lines are the same as that of Fig. 7.

in plasma glucose was detected. On the contrary, this liposomal preparation, when given orally into rats increased blood glucose levels by more than 100% in 1.5 h. Glucose concentration was slightly reduced by 3 h after liposomal insulin administration (when blood insulin level had a maximum) and thereafter rose back. Within 24 h blood glucose level was considerably above the initial value.

4. Discussion

The results obtained demonstrate that after encapsulation in liposomes of definite type, insulin became an active therapeutic agent when administrated orally. By this is not meant that insulin finds its way to receptors and interacts with them uniquely in liposomal form. The manifestation of hypoglycaemic effect in rat blood when orally administrated large doses of insulin (12 IU/kg weight) is circumstantial evidence that under hard conditions of gastrointestinal tract and blood circulation insulin-containing liposomes go through a series of transformations and a part of hormone is inactivated. A fraction of non-active insulin may contain the transformated protein molecules or their fragments detected by radioimmunoassay. Active hormone appears to be delivered to receptors by specific pool of liposomes or in the form of lipid-protein complex. It is not practical to assess such pool quantitatively and to measure the

proportion of insulin in it. The factors controlling the process for penetration of a portion of orally administrated liposomes into bloodstream from gastrointestinal tract are still not fully understood. From our data it follows that liposomes containing phosphatidylethanol show hypoglycaemic effect by oral administration as they are sensitive to salt concentration and pancreatic phospholipase A_2 action.

Lipid composition and physical state of liposomes were important determinants of therapeutic effect. Insulin-containing liposomes composed of dipalmitoyl phosphatidylcholine and dipalmitoyl phosphatidylethanol significantly decreased the plasma glucose level in rats by oral administration. According to the microcalorimetric data, these liposomes are 'solid' at 37°C (body temperature). Another peculiarity of this type of liposomes is that they bear negative surface charge due to acidic phosphatidylethanol.

Phosphatidylethanol was found in tissues from ethanol-treated rats (Alling et al., 1984). This finding lets us fit phosphatidylethanol into a family of endogenous phospholipids. Thus, phosphatidylethanol would be expected to be non-toxic chemical as it involves into common metabolic pathways. Indeed, the subcutaneous injection of phosphatidylethanol/phosphatidylcholine liposomes to mice did not result in any visible damage to animals. Using ELISA-plates test we obtained experimental evidence that phosphatidylethanol liposome-entrapped insulin injected intraperitoneally to mice did not induce the production of antibodies to insulin as opposed to free or glutharic aldehide cross-linked insulin injected in Freund's adjuvant. This suggests that the lipid bilayer based on the negatively charged phosphatidylethanol prevents the immune response to insulin. Preliminary immunological study of liposome-entrapped insulin is extremely encouraging in the context of use of phosphatidylethanol for administration of proteins into living organism. In addition, the advantages of phosphatidylethanol over other negatively charged phospholipids are its easier preparation and high capacity for insulin.

Liposomes consisting of phosphatidylcholine and negatively charged phosphatidylinositol also had the properties of entrapping high amounts of insulin and delivering it in bloodstream from gastrointestinal tract. In contrast to liposomes based on phosphatidylethanol, the oral administration of phosphatidylinositol liposomes to diabetic rats caused the blood glucose concentration to increase. This effect may be thus explained. Firstly, 'fluid' liposomes based on phosphatidylinositol may be destructed by lipolytic enzymes to a greater extent than liposomes with phosphatidylethanol. Under these conditions insulin becomes accessible to protease action with the result that it will lose activity, but in so doing will hold the ability for interaction with receptors. Other authors reported that tryptic digestion of insulin was accelerated by negatively charged liposomes containing phosphatidylinositol (Kato et al., 1993). If such insulin enters the bloodstream, it will be competitive in receptor binding with hormone molecules produced by unkilled pancreatic islet cells. Secondly, inositol residues in phosphatidylinositol liposomes may interfere with insulin binding to receptor and subsequent internalisation of hormone by receptor-mediated endocytosis. It is known that the liposome association with islet cell suspensions decreases in the presence of glycolipids (Welsh et al., 1988) which like phosphatidylinositol contain the hydroxyl groups in polar moiety of molecules. Finally, phosphatidylinositol may differently than phosphatidylethanol activate insulin receptor kinase function, which is crucial for the cellular actions of insulin (Kahn and White, 1988).

Our data on hydrolysis of insulin-containing liposomes by pancreatic phospholipase A_2 show that phospholipids of 'solid' liposomes are preferential substrate for this enzyme. It is not improbable that the hypoglycaemic effect of liposome-entrapped insulin is governed by the degree of advancement of the phospholipase A_2-dependent lysis of liposomes. It will suffice to suppose that the realisation of hormone action is made possible when insulin is freed of phospholipids, which interfere with interaction of hormone with receptor. A marked increase in glucose concentration over 24 h after oral administration of insulin in

phosphatidylinositol liposomes as well as an excess of starting level of hyperglycaemia within 24 h after phosphatidylethanol-based liposomes treatment may be a consequence of interlocking of receptors with highly resistant lipid–insulin complexes.

When palmitoyl–stearoyl sucrose in lipid composition of 'solid' liposomes is incorporated, the hypoglycaemic effect of liposome-entrapped insulin is decreased. Hence the results of these experiments bear witness to an important role of liposome surface properties in therapeutic effect of liposome-entrapped insulin. Such properties are largely given by phosphatidylethanol as a lipid component of liposome-entrapped insulin.

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