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EFFECTS OF LIPIDS ON THE TRANSPORT ACTIVITY OF THE RECONSTITUTED GLUCOSE TRANSPORT SYSTEM FROM RAT ADIPOCYTE

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The glucose transport system, isolated from rat adipocyte membrane fractions, was reconstituted into phospholipid vesicles. Vesicles composed of crude egg yolk phospholipids, containing primarily phosphatidylcholine (PC) and phosphatidylethanolamine (PE), demonstrated specific D-glucose uptake. Purified vesicles made of PC and PE also supported such activity but PC or PE by themselves did not. The modulation of this uptake activity has been studied by systematically altering the lipid composition of the reconstituted system with respect to: (1) polar headgroups; (2) acyl chains, and (3) charge. Addition of small amounts (20 mol%) of PS, phosphatidylinositol (PI), cholesterol, or sphingomyelin significantly reduced glucose transport activity. A similar effect was seen with the charged lipid, phosphatidic acid. In the case of PS, this effect was independent of the acyl chain composition. Polar headgroup modification of PE, however, did not appreciably affect transport activity. Free fatty acids, on the other hand, increased or decreased activity based on the degree of saturation and charge. These results indicate that glucose transport activity is sensitive to specific alterations in both the polar headgroup and acyl chain composition of the surrounding membrane lipids.

Introduction

The alteration of specific membrane phospholipids by liposome-adipocyte interaction has been shown to affect insulin-stimulated glucose uptake [1]. The general mechanisms by which membrane lipids affect specific membrane enzymes and transport functions have been extensively reviewed [2], and specific hypotheses [3,4] have emerged concerning the modulation of the glucose transport system by membrane lipids.

The hexose transport system of the rat adipocyte has served as a model system for insulin-sensitive facilitated diffusion, and has been isolated, partially purified, and reconstituted into phospholipid vesicles [5–7]. The results of such experiments have demonstrated time-dependent, cytochalasin B-sensitive glucose uptake. Experiments testing the effect of various lipid environments on the transport system [3,4,8,9] have suggested that this factor may play a key role in controlling the activity of the transport process.

Based on our results on the effects of phospholipid alteration in intact adipocytes, in this report we examine the effects of similar alterations of membrane lipid composition on activity of the glucose transport system in a reconstituted phospholipid environment. Our results indicate that glucose transport activity in reconstituted vesicles, as in intact cells, is significantly affected by changes in the phospholipid environment. Factors including the direct interaction of the glucose transport

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system with phospholipids are therefore possibly involved in modulating glucose uptake.

Materials and Methods

Preparation of isolated adipocytes and plasma membranes. Rat adipocytes were isolated according to the basic procedure of Rodbell [10]. The epididymal fat pads from Sprague-Dawley rats (150 g) were dissociated at 37°C for 45 min in Krebs-Ringer bicarbonate buffer pre-saturated in a 95% O_2/5% CO_2 gas mixture and containing 1% bovine serum albumin and 1 mg/ml collagenase. The cells were then washed three times and finally resuspended in Krebs-Ringer bicarbonate 1% albumin buffer, pH 7.4.

Cells were washed twice with 0.25 M sucrose in 10 mM Tris-HCl (pH 7.5) and 1 mM EDTA. The cells were homogenized in this buffer and centrifuged for 2 min at 7000 rpm. The supernatant was then centrifuged for 30 min at 14000 rpm. The pellet from this spin was resuspended and layered over a discontinuous 15%/32% sucrose gradient. After centrifuging at 13000 rpm for 30 min in an HB-4 rotor, the material at the gradient interface was collected at 20000 rpm for 30 min and pelleted by centrifugation.

Membrane solubilization. Glucose transport activity was solubilized according to Robinson et al. [9]. The membrane pellet was suspended in 0.6 ml of 20 mM sodium cholate in 10 mM Tris-HCl (pH 7.5). The suspension was kept at 0°C for 10 min, sonicated briefly, frozen for 15 min, thawed at room temperature, and centrifuged for 10 min at 10000 rpm. The macromolecular fraction of the supernatant was isolated by gel chromatography using Sephadex G-50. The protein concentration of this material was determined by the method of Bradford [11].

Reconstitution of D-glucose transport activity. The transport activity was reconstituted by mixing and sonicating the protein solution with 0.2 vol. of the appropriate lipid suspension (150 mg/ml) in 10 mM Tris-HCl (pH 7.5) as detailed by Robinson et al. [9].

Glucose transport activity in the reconstituted liposomes was assessed essentially as previously described [9]. The amount of vesicle-associated D-[^{3}H]glucose and L-[^{14}C]glucose were determined after correcting for crossover efficiency and specific activity. The difference in these two values was taken as the carrier-mediated D-glucose transport activity.

Lipid analysis and purification. Complex mixtures of phospholipids were analyzed by high-pressure liquid chromatography according to the method of Kaduce et al. [12]. Lipid phosphorus was quantified by the procedure of Ames [13]. Crude egg yolk lecithin was fractionated by chromatography on silicic acid columns as previously described [14].

Materials. Defined acyl chain dioleoylphosphatidylcholine was obtained and purified as previously described [15]. Other phospholipids were purchased from Avanti Biochemical Co. All lipids exhibited single spots when analyzed by thin-layer chromatography using appropriate solvent systems [15] and were stored at −80°C under argon. Sodium cholate, bovine serum albumin, radioimmunoassay grade, fatty acids, cholesterol and egg yolk lecithin (Type IX-E) were obtained from Sigma Chemical Company and collagenase (Type 1) from Worthington Biochemical Company. D-[6-^{3}H]Glucose (41 Ci/mM) and L-[1-^{14}C]glucose (58 mCi/mM) were products of Amersham Corp.

Results

The crude phospholipid preparations derived from egg yolk [6,7] or soy fractions [5,8] which have been used to reconstitute adipocyte glucose transporter consists, in the case of the egg yolk material, of nearly equal amounts of PC and PE with trace levels of other phospholipids (Fig. 1B). When this material was reconstituted with adipocyte plasma membrane protein, specific D-glucose uptake is detected (Fig. 1A). Similar results were obtained using equimolar mixtures of purified PC and PE, although the kinetics of uptake were slower (Fig. 2), suggesting that minor components of the natural phospholipids play a role in transport activity.

The results of systematic addition of specific lipids to the purified PC + PE material before reconstitution indicated that alteration of the polar headgroup of PE with respect to the number of methyl groups did not affect transport activity (Table I). Addition of other major membrane lipid

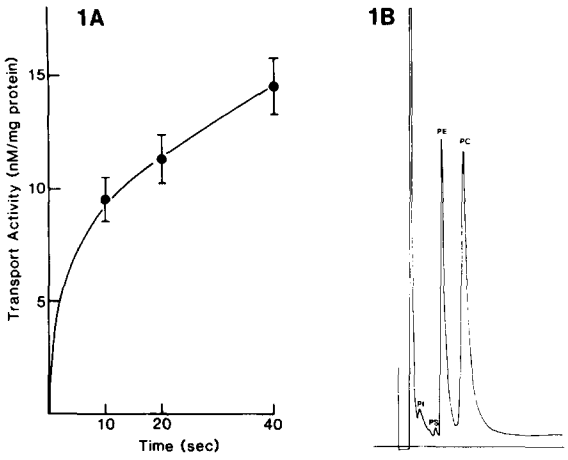

Fig. 1. (A) Time-course of D-glucose uptake in reconstituted vesicles composed of crude egg yolk lecithin. Experimental conditions are described in Materials and Methods. (B) The phospholipid composition of the lecithin material was determined by HPLC.

components to PC + PE resulted in a diminution of glucose transport activity. Lipids which exhibited this effect included cholesterol, sphingomyelin (SM), phosphatidylinositol (PI), phosphatidylserine (PS), and phosphatidic acid (PA). In order to determine whether the specific polar headgroup or the acyl chain composition modulates activity, PS composed of oleic acid,myristic acid, or a mixture of both oleic and myristic acid acyl chains, was introduced in the PE + PC vesicle. Regardless of the PS acyl chain compositions, an inhibition of transport activity was observed.

In contrast to the decrease in activity obtained with PS, PE, SM and cholesterol, a stimulation of transport activity was observed in the PC + PE reconstituted system with the addition of specific fatty acids. These included fatty acids of the *cis*-enoic series, oleic and vaccenic acid, as well as the charged fatty acid derivative, stearylamine. The

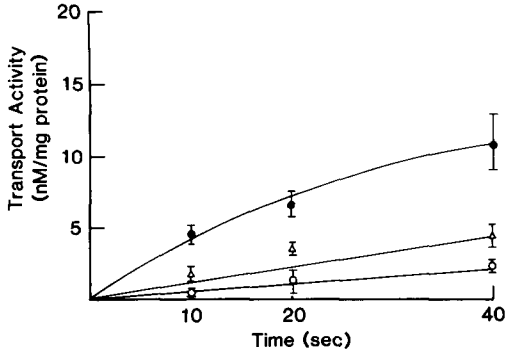

Fig. 2. Time-course of D-glucose uptake in reconstituted vesicles composed of PC + PE (50:50) purified from crude egg yolk lecithin (●); and PC (△) and PE (○) individually which were purified from the egg yolk lecithin. Experimental conditions were the same as those in Fig. 1.

TABLE I

EFFECTS OF THE PHOSPHOLIPID AND CHOLESTEROL COMPOSITION ON THE ACTIVITY OF THE RECONSTITUTED ADIPOCYTE GLUCOSE TRANSPORT SYSTEM

Lipid composition (mol%)	Transport activity (nmol/mg protein per 10 s)
PC + PE (50:50)	9.6 ± 0.4
+ 20 mol%:	
phosphatidyl methanol	9.0 ± 0.6
phosphatidyl *N*-monomethyl PE	9.1 ± 0.5
PS (natural)	5.2 ± 0.3
PS (oleic + myristic acid acyl chains)	4.9 ± 0.4
PS (oleic acid acyl chains)	5.5 ± 0.2
PS (myristic acid acyl chains)	5.6 ± 0.4
PI (natural)	4.6 ± 0.3
phosphatidic acid	5.8 ± 0.3
sphingomyelin	4.1 ± 0.2
cholesterol	3.5 ± 0.2

TABLE II

EFFECTS OF FATTY ACIDS AND DERIVATIVES ON THE ACTIVITY OF THE RECONSTITUTED ADIPOCYTE GLUCOSE TRANSPORT SYSTEM

Lipid composition	Transport activity (nmol/mg protein per 10 s)
PC + PE (50:50)	9.4 ± 0.3
+ 20 mol%:	
oleic acid	14.3 ± 0.5
cis-vaccenic acid	12.1 ± 0.5
stearic acid	4.5 ± 0.2
palmitic acid	5.3 ± 0.2
stearylamine	33.3 ± 1.2

saturated fatty acids stearic and palmitic acid, inhibited transport activity (Table II).

Discussion

The isolated adipocyte cell system has been used extensively to study the relationship between glucose transport and insulin action. In addition to the intact cell, the other fat cell derived systems which have been used for such studies include the adipocyte ghost [16] and membrane vesicle [17]. More recently, the adipocyte glucose transport system has been studied in solubilized fat cell membranes using methods similar to those originally developed for red blood cells [18]. These studies have demonstrated stereospecific, cytochalasin B-inhibitable glucose transport when membrane proteins were reconstituted into appropriate phospholipid vesicles.

Our data using this reconstitution system have shown that (1) glucose transport activity depends on the phospholipid composition, specifically on the polar head group composition of the reconstituted liposomes, and (2) the inhibition of uptake in vesicles containing PS bears similarities to the effect of similar lipid alterations in the intact adipocytes.

The importance of the polar headgroup in the reconstituted system has been previously observed [8] in the adipocyte system. These results indicate that an equimolar mixture of PC and PE yielded maximal transport activity, whereas PL, SM, gangliosides, or PS, either alone or in combination with PE proved ineffective. In the LM fibroblast cell system, the reconstituted glucose transport system showed maximal activity at PC/PE molar ratios of around 1:1 [19], suggesting that the transport system in LM cells is sensitive to the polar headgroup structure of phospholipids. Our studies with equimolar concentrations of PS and PC/PE have also shown that this combination inhibits transport activity. This is not apparently a result of poor vesicle formation, as evidenced by the fact that such vesicles entrap similar amounts of ^{3}[H]inulin, a non-permeable marker, as do vesicles composed of PC/PE. Furthermore, electron microscopic evidence suggests that the average size of the PS-containing vesicles is similar to that of the PC/PE vesicles (data not shown), which is consistent with our data that equilibrium glucose uptake levels are similar in these cases. Although such controls suggest differences in transport rate are not due to trivial factors like vesicle size or leakage, the method of reconstitution may itself confer lipid specifications in the reconstituted system.

Previous studies on the effects of altered lipid composition on glucose transport in the reconstituted system have primarily focused on changes in membrane fluidity by way of systematic alteration of phospholipid acyl chain composition [4] and alcohols [20,21]. The rate of hexose transport in a fluid bilayer is greater than in a crystalline bilayer and reaches a maximum value when the gel-liquid crystalline phase transition has been achieved. The observation that the transport system is totally inhibited before the complete crystalline state is reached suggests that the transport system may partition into rigid regions of the bilayer or become associated with specific membrane phospholipids. A positive correlation between membrane fluidity and D-glucose transport in isolated adipocyte plasma membranes has been made using fatty acid modified adipocyte membranes [3]. In these experiments, *cis*-vaccenic and oleic acid stimulated hexose transport whereas stearic and palmitic acid was ineffective. In our experiments, stearic or palmitic acid drastically inhibited uptake in the reconstituted system whereas *cis*-vaccenic, oleic acid, and stearylamine were stimulatory.

Our results are consistent with the hypothesis [4,22] that the control of hexose uptake may involve changes in the physical and/or chemical properties of lipid domains associated with the transport protein in reconstituted membranes. These changes may be mediated by a variety of mechanisms such as: (1) an alteration in the synthesis or turnover of specific membrane lipids. Recent work has indicated that insulin treatment of isolated rat adipocytes causes an acute increase in membrane PI levels [23]. In addition, changes in phospholipid composition in a variety of cells from diabetic animals have suggested a role for membrane lipids in the insulin-resistant state [24,25]; or (2) a modification of lipids already present in the cell membrane. Metabolic interconversion of phospholipids in situ has been documented [26] in a

number of normal hormone sensitive systems, including the isolated adipocytes-insulin system [27]. In either case, only minor overall changes in phospholipid composition are thought to have significant physiological consequences.

The interaction of PS containing phospholipid vesicles with intact adipocytes results in an inhibition of hexose uptake in the insulin-stimulated but not in the basal state [1]. These effects were evident at vesicle PS levels as low as 5 mol%. In the reconstituted system on the other hand, the inclusion of PS inhibits basal hexose uptake. These results suggest that PS may interact with an adipocyte membrane component in the intact cell which is altered or removed upon reconstitution. Other phospholipids such as PI and cholesterol, however, do not markedly inhibit hexose uptake in the intact cell [1]. Such considerations point out the complexities of extrapolating findings from reconstituted systems to the intact cell.

The differences between the effect of PS on the reconstituted vesicles and the intact cell also indicate a potential focus for further studies on the nature insulin-stimulated glucose uptake. The stimulation by insulin of hexose transport in ther econstituted system was evident when the starting material, the intact adipocyte, was first exposed to the hormone [28] whereas isolated membrane preparation or reconstituted liposomes do not respond to insulin. This observation has mechanistic implications for insulin action because it is consistent with the recruitment hypothesis [29,30]. In addition, insulin binding and subsequent receptor clustering [31] could alter the lipid environment of the transporter and hence its activity. Our results indicate that not only lipid fluidity but also charge plays an important role in glucose transport activity, thus the reconstituted insulin-stimulated glucose transport system appears to be an attractive approach to study the role of membrane lipids in insulin action.

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