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Biochemical and functional characterization of the rat liver glucose-transport system

Comparisons with the adipocyte glucose-transport system

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The properties of the glucose-transport systems in rat adipocytes and hepatocytes were compared in cells prepared from the same animals. Hormones and other agents which cause a large stimulation of 3-*O*-methylglucose transport in adipocytes were without acute effect in hepatocytes. Hepatocytes displayed a lower affinity for 3-*O*-methylglucose (20 mM) and alternative substrates than adipocytes (6 mM), whereas inhibitor affinities were similar in both cell types. The concentration and distribution of glucose transporters were determined by Scatchard analysis of D-glucose-inhibitable [^{3}H]cytochalasin B binding to subcellular fractions. In liver, most of the transporters were located in the plasma membrane (42 ± 5 pmol/mg of protein) with a small amount (4 ± 3 pmol/mg) in the low-density microsomal fraction ('microsomes'), the reverse of the situation in adipocytes. Glucose transporters were covalently labelled with [^{3}H]cytochalasin B by using the photochemical cross-linking agent hydroxysuccinimidyl-4-azidobenzoate and analysed by SDS/polyacrylamide-gel electrophoresis. A single D-glucose-inhibitable peak with a molecular mass of 40–50 kDa was seen in both plasma membrane and low-density microsomes. This peak was further characterized by isoelectric focusing and revealed a single peak of specific [^{3}H]cytochalasin B binding at pI 6.05 in both low-density microsomes and plasma membrane, compared with peaks at pI 6.4 and 5.6 in adipocyte membranes. In summary: (1) the glucose-transport system in hepatocytes has a lower affinity and higher capacity than that in adipocytes, and is also not accurately modulated by insulin; (2) the subcellular distribution of glucose transporters in the liver suggests that few intracellular transporters would be available for translocation; (3) the liver transporter has a molecular mass similar to that of the adipocyte transporter; (4) the liver glucose transporter exists as a single charged form (pI 6.05), compared with the multiple forms in adipocytes. This difference in charge could reflect a functionally important difference in molecular structure between the two cell types.

INTRODUCTION

Circulating serum glucose concentrations are maintained primarily by the balance between uptake of glucose into peripheral tissues and storage by or release from the liver [1]. The transport of glucose into the cell represents the first step at which glucose utilization can be controlled, and in peripheral tissues such as fat and muscle the activity of this system is acutely regulated by insulin [2]. Evidence obtained *in vivo* [3], in perfused liver [4] and in isolated hepatocytes [5] suggests that hepatic glucose transport is not influenced by insulin, though these cells also possess specific carriers [4,5]. In a sense, then, hepatocytes could be considered insulin-resistant with regard to glucose-transport stimulation. Earlier studies have suggested that uptake in liver also appears to differ from that seen in peripheral tissues, owing to its being a lower-affinity process [5–8]. These major differences in glucose transport between liver and the periphery may reflect the unique role that the former tissue fills with respect to regulating blood glucose concentrations.

It has been proposed that insulin acts to stimulate glucose transport in rat adipocytes [9,10], diaphragm [11] and cardiac muscle [12], primarily by causing translocation of glucose-transport proteins from an intracellular pool to the plasma membrane, where their activity is expressed as an increased rate of transport. Considering this, a possible mechanism for non-responsiveness of liver glucose transport could involve a concentration of glucose carriers in the plasma membrane, with few, if any, carriers in the intracellular pool available for translocation. Alternatively, the hepatocyte could be rich in intracellular glucose transporters, yet insulin would be unable to mediate their translocation.

With regard to the second possible explanation for lack of transport responsiveness, several of us have reported [13] that the intracellular pool of glucose transporters in rat adipocytes exists in two different forms, varying in the degree of post-translational modification by glycosylation. Only one of these forms is translocated by insulin, so it is possible that the liver may contain only the form that is resistant to insulin-induced translocation. The differences in covalent modification might also account for differences in functional activity. Differences in transporter structure would be best studied by covalently labelling the glucose transporters. We have employed [^{3}H]cytochalasin B in conjunction with a photochemical cross-linking agent, hydroxysuccinimidyl-4-azidobenzoate [14], to label

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transporters from adipocytes and liver so that their biochemical properties may be determined.

Comparing the functional properties of transport in intact cells, the subcellular distribution, and molecular properties of the transporter between insulin-responsive and non-responsive tissues may reveal which characteristics of the glucose-transport systems are regulated to fill the specific role of transport in each tissue.

MATERIALS AND METHODS

Materials

Male Sprague–Dawley rats (150–175 g) were obtained from Charles River Labs. Animals were fed on laboratory chow *ad libitum*. Pig monocomponent insulin was a gift from Dr. Ronald Chance of Eli Lilly Co. (Indianapolis, IN, U.S.A.). A^{14}-mono[^{125}I]-iodoinsulin was supplied by Dr. Bruce Frank, also of Eli Lilly. [4-^{3}H]Cytochalasin B (sp. radioactivity 10–15 Ci/mmol), [U-^{14}C]sucrose (sp. radioactivity 673 mCi/mmol), 3-*O*-methyl-D-[U-^{14}C]glucose (315 mCi/mmol) and L-[1-^{14}C]glucose (47 mCi/mmol) were purchased from New England Nuclear (Boston, MA, U.S.A.). [1-^{14}C]Leucine (54 mCi/mmol) was purchased from Amersham (Arlington Heights, IL, U.S.A.), collagenase (CLSII) was obtained from Worthington Biochemical Corp. (Freehold, NJ, U.S.A.), bovine serum albumin (Fraction V) from Boehringer Mannheim (Indianapolis, IN, U.S.A.), Minimal Essential Medium, pyruvate and Trypan Blue were from GIBCO (Grand Island, NY, U.S.A.), and phloretin was from ICN (Plainview, NY, U.S.A.). Cytochalasin B, 3-OMG, 2-deoxyglucose, D- and L-glucose, H_2O_2, glucagon, isoprenaline (isoproterenol) and phlorizin were from Sigma (St. Louis, MO, U.S.A.). Carrier ampholytes were from Pharmacia; other reagents for isoelectric focusing and electrophoresis were from Bio-Rad [19]. All other chemicals were reagent grade and from standard sources.

Adipocyte isolation

When both adipocytes and hepatocytes were prepared from the same animal, the rat was anaesthetized as described below and the epididymal fat-pads were removed at the start of the initial perfusion. Isolated fat-pads were prepared by a modification [15] of the method of Rodbell [16], in a Krebs–Ringer phosphate buffer containing 128 mM-NaCl, 5.2 mM-KCl, 1.2 mM-$CaCl_2$, 1.2 mM-$MgSO_4$, 1.29 mM-KH_2PO_4, 10 mM-Na_2HPO_4, pH 7.4, supplemented with 2 mg of collagenase/ml, 4% (w/v) albumin and glucose (3 mM). After digestion and filtration, cells were washed four times in Krebs–Ringer phosphate with 4% albumin (pH 7.4) and diluted to a concentration of 2×10^6 cells/ml.

Adipocyte counts were performed by a modification [17] of method III of Hirsch & Gallian [18]. Counting was done with a model ZM Coulter Counter (Coulter Electronics, Hialeah, FL, U.S.A.) with a 400 μm aperture tube.

Hepatocyte isolation

Hepatocytes were prepared by a modification of the technique of Seglen [19]. After Nembutal anaesthesia (8 mg/100 g body wt., intraperitoneally), the liver was perfused *in situ*, first with a Ca^{2+}-free EGTA (1 mM)-containing Hanks' buffer, pH 7.4, and then with Hanks' buffer containing 1.4 mM-$CaCl_2$ and collagenase (2 mg/ml). The liver was removed and gently dispersed into more of the collagenase buffer and incubated for a further 10 min. Cells were filtered twice through 60 μm nylon mesh, washed in Hanks' buffer (4 °C) and then washed twice more in the reaction buffer, Krebs–Ringer phosphate/2 mM-pyruvate/1% albumin, pH 7.4. Each of the final two washes were preceded by a 10 min incubation at 37 °C to permit cells to release glucose accumulated from the Hanks' buffer. Cells were resuspended in reaction buffer at a concentration of 1×10^6–2×10^6 cells/ml. All buffers and incubations were gassed with O_2/CO_2 (19:1) and aerobic conditions were maintained. Cells were stained with Trypan Blue to determine viability (> 92% in all studies). Cell number was determined by counting a sample of the suspension in a haemocytometer.

Subcellular fractionation of liver

Plasma membranes and microsomal membrane fractions were prepared from whole liver by a modification of the method of Fleischer & Kervina [20]. Plasma membranes were purified from a 1450 *g* pellet by the two-phase-polymer technique of Lesko *et al.* [21]. The initial supernatant was centrifuged at 25000 *g* for 10 min at 4 °C in a Beckman JA-20 rotor ($r_{av.}$ 7 cm). The pellet was discarded and the supernatant was centrifuged at 48000 *g* in a Beckman Ti 70 rotor ($r_{av.}$ 6.8 cm) for 30 min, yielding a pellet of high-density microsomal membranes. The supernatant was then centrifuged in the Ti 70 rotor at 430000 *g* for 90 min, yielding a pellet of low-density microsomal fraction ('microsomes'). All pellets were resuspended in Tes buffer, pH 7.4, and stored at −80 °C.

Fractionation of adipocytes

Isolated adipocytes were obtained by enzymic digestion of epididymal fat-pads from rats as described by Rodbell [16] and modified by Cushman & Wardzala [9]. Subcellular fractions from isolated adipocytes were prepared by differential ultracentrifugation as outlined by Karnielli *et al.* [22].

Protein and marker-enzyme assays

Protein was determined by the method of Lowry *et al.* [23], as modified by Peterson [24]. NaF-stimulated adenylate cyclase activity was measured by the method of Salomon *et al.* [25]. Rotenone-insensitive NADH cytochrome *c* reductase activity was determined by the method of Dallner *et al.* [26]. UDP-galactose: *N*-acetylglucosamine galactosyltransferase activity was determined by the method of Fleisher [27].

Glucose transport

Glucose transport was assayed by measuring initial rates of 3-*O*-methylglucose influx. All data presented are of measurement of zero-*trans* influx, which was assayed in fat-cells by a modification [15] of the method of Whitesell & Gliemann [28]. The portion of cellular uptake due to diffusion and trapping of the label in the extracellular space was measured by performing parallel reactions with L-[1-^{14}C]glucose as substrate. All results for transport are corrected for the L-glucose value.

Zero-*trans*-influx studies in hepatocytes were per-

formed under conditions similar to those for adipocytes. Substrate (20 μl) was placed in 1.9 ml polypropylene Microfuge tubes, to which 50 μl of hepatocyte suspension was added. Reactions were terminated by addition of 1.5 ml of chilled stopping solution and centrifuged at 1200 *g* in a Beckman Microfuge. The supernatant was removed by aspiration and the surface of the cell pellet washed twice with stopping solution. The tips of the tubes containing the cell pellet were cut off into scintillation vials for determination of radioactivity. The L-glucose-trapping control was also run.

Determination of glucose-transporter number

The number of D-glucose-inhibitable cytochalasin-B-binding sites in the subcellular fractions was determined as previously described [23]. Glucose transporters in liver plasma membranes were also measured by the Microfuge method of Axelrod & Pilch [29].

Photochemical cross-linking of membranes and SDS/polyacrylamide-gel electrophoresis

Membrane fractions prepared from rat liver and adipocytes were resuspended in 50 mM-sodium phosphate buffer (pH 7.4), to a final concentration of 1–5 mg of membrane protein/ml. After incubation for 10 min at 4 °C with [^{3}H]cytochalasin B (10 μM), the membranes were photochemically cross-linked with hydroxysuccinimidyl-4-azidobenzoate (400 μM) as previously described [14]. Experiments involving D- or L-glucose were carried out as described above, except that the sugar (500 mM) was incubated with the membranes for 30 min before the addition of cytochalasin B.

Membranes covalently cross-linked to [^{3}H]cytochalasin B were solubilized by boiling for 5 min in Laemmli [30] sample buffer and then processed for electrophoresis as previously described [14]. Prestained molecular-mass marker proteins (Bethesda Research Laboratories) were used to estimate the molecular mass of the ^{3}H-labelled membrane proteins. Proteins corresponding to a molecular mass of 40–55 kDa were excised from the slab gels and electrophoretically extracted as described by Hunkapiller *et al.* [31].

Isoelectric focusing

The extracted and concentrated SDS-solubilized glucose transporter was subjected to isoelectric focusing on cylindrical polyacrylamide gels. Gel composition and isoelectric-focusing conditions were as described previously [32]. Duplicate gels were run; one was processed for scintillation counting as above, and slices from the other gel were extracted for 1 h in 1 ml of water (four slices per tube) and the pH was determined [33].

RESULTS

Effect of anaesthesia

One of the goals of this project was to measure glucose transport in adipocytes and hepatocytes prepared from the same animals. However, since some forms of anaesthesia have been reported to modify adipocyte function [34], control studies were necessary to determine if the conditions of anaesthesia used in hepatocyte isolation were detrimental to fat-cells. Several aspects of adipocyte function were compared in cells prepared from rats killed by cervical dislocation (control) or anaesthesized with nembutal. Specific binding of a tracer concentration of insulin (at 16 °C) was similar in the two groups as was the degradation of the hormone (results not shown). The ability of cells to accumulate insulin in the presence of the lysosomotropic agent chloroquine was also not influenced by anaesthesia. Rates of 3-*O*-methylglucose transport, both basal and maximally stimulated, were comparable between the two groups, as was insulin-sensitivity. Thus there was no noticeable effect of anaesthesia on adipocyte function.

Table 1. Acute effects of hormones and H_2O_2 on initial rates of 3-*O*-methylglucose transport in isolated adipocytes and hepatocytes

Cells were incubated with agents for 30–120 min before measurement of 3-*O*-methyl[^{14}C]glucose transport. Results are means ± S.E.M. for three to six experiments, each done in triplicate.

	3-*O*-Methylglucose transport (pmol/s per 2×10^5 cells)	
Addition	Adipocytes	Hepatocytes
None	0.040 ± 0.006	1.24 ± 0.06
Insulin (100 ng/ml)	0.412 ± 0.029	1.18 ± 0.112
H_2O_2 (10 mM)	0.399 ± 0.128	1.28 ± 0.119
Glucagon (100 nM)	0.042 ± 0.019	1.27 ± 0.092
Isoprenaline (1 μM)	0.281 ± 0.070	1.06 ± 0.136

Table 2. Kinetic constants for 3-*O*-methylglucose transport in isolated cells

	Adipocytes	Hepatocytes
K_m (mM)		
Basal	6.4 ± 1.4	17.3 ± 4.3
Insulin (25 ng/ml)	6.2 ± 1.1	—
$V_{max.}$ (pmol/s per 2×10^5 cells)		
Basal	24 ± 6	1284 ± 131
Insulin	247 ± 49	—

Effects of hormones and mimickers

The next step was to confirm and characterize further the lack of insulin-responsiveness of glucose transport in hepatocytes. This was investigated by acutely treating fat-cells and liver cells with a variety of hormones and insulin mimickers. Results of these studies are given in Table 1. In adipocytes insulin caused an average increase of 13-fold in the transport rate. The data in Table 1 also illustrate the lack of insulin-responsiveness of glucose transport in liver cells. The mimicker H_2O_2, which acts at a step beyond the insulin receptor [15], causes a smaller but consistent increase in fat-cells, but is without effect in liver cells. The same is true for isoprenaline. Glucagon had no effect in either cell type. Although none of these treatments had any influence on hepatocyte transport, the rate of transport in hepatocytes was still severalfold greater than that in fully stimulated adipocytes.

Table 3. Specificity of glucose transport in isolated cells

Apparent IC_{50} is concentration of compound that gave 50% inhibition of initial rates of [^{14}C]3-*O*-methylglucose (9 μM) uptake in isolated adipocytes (+25 ng of insulin/ml) and hepatocytes. Substrates were present in the transport assay as competitive inhibitors; cells were pretreated with other inhibitors. Control transport values (pmol/s per 2×10^5 cells) were 1.22 ± 0.08 in liver cells and 0.50 ± 0.12 in fat cells. Results are means ± S.E.M. for three to five experiments for each compound. Abbreviation: NI, no inhibition.

Compound	Apparent IC_{50} (mM)	
	Adipocytes	Hepatocytes
Substrates		
2-Deoxyglucose	5.3 ± 1.2	18.6 ± 0.9
3-*O*-Methylglucose	6.8 ± 1.7	15.2 ± 0.2
D-Glucose	7.2 ± 1.6	21.7 ± 1.8
L-Glucose	NI	NI
Fructose	> 50	> 50
Inhibitors		
Cytochalasin B	0.06 ± 0.17 (μM)	0.99 ± 0.4 (μM)
Phloretin	0.03 ± 0.01	0.04 ± 0.01
Phlorizin	0.22 ± 0.15	0.26 ± 0.16

This failure to stimulate glucose uptake made it necessary to determine if the lack of insulin-responsiveness in hepatocytes was specific for glucose transport or was due to generalized cell damage. When protein synthesis, amino acid transport and glucose transport were measured in the same hepatocyte preparations, insulin (50 ng/ml) caused a 20% increase in leucine incorporation into protein, and a doubling of α-aminoisobutyric acid uptake, whereas glucose transport was unaffected. Hepatocytes thus have the ability to respond acutely to insulin with some effector systems, but not glucose transport.

Kinetic characterstics of transport

The results presented in Table 1 were obtained with studies using a single substrate concentration and single reaction time, leaving the possibility that an insulin effect might be present under other conditions. Time courses of 3-*O*-methylglucose uptake were performed over the concentration range 9 μM–50 mM, and under no circumstances was any insulin effect seen in hepatocytes (results not shown). The concentration-dependence of initial transport rates (v_i) was measured in both cell types, and the kinetic constants derived from a Lineweaver–Burk transformation of the data are given in Table 2. In confirmation of earlier studies [17,28], insulin stimulated glucose transport in adipocytes by increasing the $V_{max.}$ 8–9-fold without altering the affinity of the carrier for 3-*O*-methylglucose. The kinetic constants determined for hepatocytes differed greatly from those in adipocytes. The affinity of the liver-cell transport system was one-third of that in fat-cells (Table 2), yet, on a cell basis, the $V_{max.}$ was greater than 5 times the $V_{max.}$ in insulin-treated fat-cells. Preliminary results (not shown) revealed no influence of insulin on either K_m or $V_{max.}$ in hepatocytes.

Thus the liver cell has a high-capacity low-affinity system for taking up glucose. Studies on the zero-*trans* efflux of 3-*O*-methylglucose from preloaded hepatocytes gave results for the K_m and $V_{max.}$ that were similar to those determined above for influx. This finding is in quantitative and qualitative agreement with the work of Craik & Elliott [6], and suggests that the transport system is symmetrical.

Specificity of transport

The differing affinities of adipocytes and hepatocytes for 3-*O*-methylglucose do not completely exclude a common affinity for the natural substrate, DL-glucose. The affinities of different sugars for the transport systems were determined by adding the sugars to the transport assay and measuring their ability to block 3-*O*-[^{14}C]methylglucose transport. The results in Table 3 are reported as the apparent IC_{50}, as measurements were made only at a single 3-*O*-methylglucose concentration (9 μM). D-Glucose, 3-*O*-methylglucose and 2-deoxyglucose all had similar affinities within each cell type whereas the affinities in adipocytes were 3-fold higher than in hepatocytes. Both transport systems were stereospecific, as L-glucose did not compete for transport. Fructose, which is not taken up by the insulin-stimulated transport system in fat-cells [35], was equally potent in both cell types, causing 45% inhibition at 50 mM.

The order of potency of glucose-transport inhibitors can serve to characterize the transport system. Several such inhibitors were tested in fat-cells and liver cells (Table 3). The potency series cytochalasin B > phloretin > phlorizin was the same in both cell types. In contrast with the results of inhibition by sugars, the affinity of each inhibitor was the same for both cells, suggesting that the site or sites where inhibitors act might be common to adipocytes and hepatocytes, but the substrate-binding sites are distinct.

Table 4. Distribution of marker enzyme activities of subcellular fractions of rat adipocytes and liver

	NaF-stimulated adenylate cyclase (pmol/min per mg)		NADH–cytochrome *c* reductase (μmol/min per mg)		Galactosyltransferase (nmol/2 h per mg)	
	Liver	Fat-cells	Liver	Fat-cells	Liver	Fat-cells
Homogenate	5.7	0.16	0.04	0.50	0.096	32.6
Plasma membranes	68.0	2.40	0.13	0.90	0.021	80.0
Low-density microsomes	6.8	0.09	0.30	1.10	0.128	170.0
High-density-microsomes	5.4	0.85	1.51	3.30	0.072	36.0

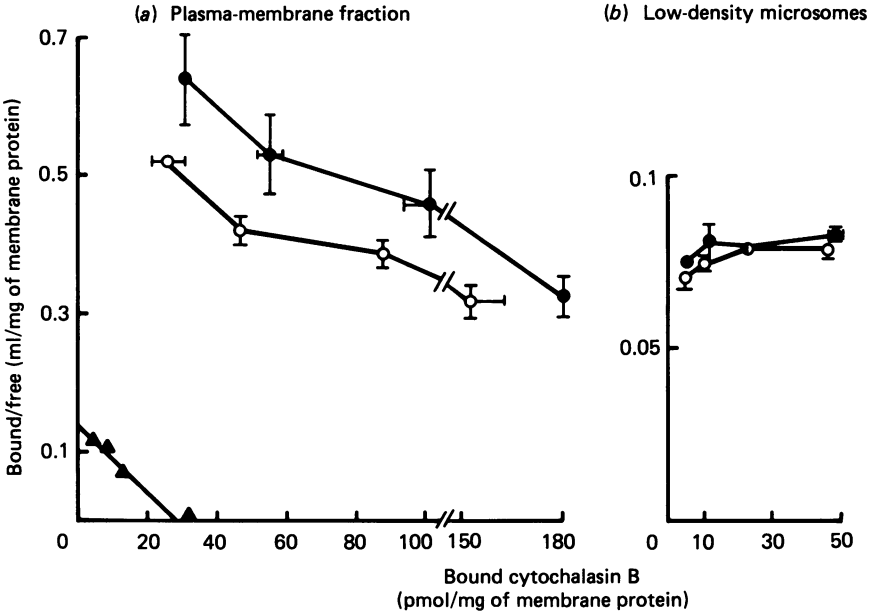

Fig. 1. Scatchard analysis of D-glucose-inhibitable [^{3}H]cytochalasin B binding to plasma membranes (*a*) and low-density microsomes (*b*) prepared from rat liver

Whole livers were subfractionated. Derived Scatchard plots (▲) were determined by subtracting the binding of each of four cytochalasin B concentrations measured in the presence of 500 mM-D-glucose (○) from that measured in the absence of D-glucose (●). The results are analysed by a simple linear regression. Results shown are means ± S.E.M. of triplicate determinations of a representative experiment. When error bars are not visible, they are contained in the symbol.

Table 5. Distribution of D-glucose-inhibitable [^{3}H]cytochalasin-binding sites

R_0, binding-site number (pmol/mg of membrane protein); K_d, dissociation constant (nM). Values were obtained by Scatchard analysis. Results are means ± S.E.M. for four separate preparations.

Fraction . . .	Plasma membrane		Low-density microsomes	
Tissue	R_0	K_d	R_0	K_d
Liver	42±5	719	4±3	990
Fat-cells: basal	8±2	150	82±5	140
Fat-cells: +insulin	32±3	150	36±4	140

Subcellular distribution of glucose transporters

We next investigated whether the inability of insulin to stimulate glucose transport in hepatocytes could be accounted for by differences in the subcellular distribution of glucose transporters in this tissue.

Subcellular membrane fractions from whole liver and isolated adipocytes were prepared as described in the Materials and methods section. The purity of the isolated subcellular membrane fractions was determined by measuring the distribution of specific marker enzymes in each fraction, and Table 4 shows the specific activities of various marker enzymes characteristic of different subcellular organelles. The plasma-membrane fractions were enriched 12–15-fold in adenylate cyclase activity. Rotenone-insensitive NADH–cytochrome *c* reductase, a marker enzyme characteristic of membranes of the endoplasmic reticulum, was most enriched in the high-density microsomal membrane fraction. UDP-galactose:*N*-acetylglucosamine galactosyltransferase, a marker enzyme characteristic of membranes of the Golgi apparatus, was increased most in the low-density microsomal membrane fraction of both tissues. The plasma membranes were relatively pure, with possible contamination by low-density microsomes limited to 4–10% and contamination by high-density microsomes ranging from 8% in liver to 35% in adipocytes. The contribution of plasma membranes to the low-density microsomal fraction did not exceed 16% in liver. Liver high-density microsomes were less pure, with a possible 20% contamination by low-density microsomes and 9% by plasma membranes.

The number of glucose transporters in the various membrane fractions was assessed by the D-glucose-inhibitable [^{3}H]cytochalasin-B-binding assay. Fig. 1 shows Scatchard plots of a representative study of binding to plasma membrane and low-density microsomal membrane fractions from rat liver. The results for binding to adipocyte fractions are similar to that reported by others [9] and are summarized in Table 5. The liver plasma membrane has a large number of D-glucose-inhibitable binding sites, which could represent glucose transporters. In the study shown in Fig. 1 there is a very small amount of specific binding to low-density microsomes, measured at the limits of resolution of the assay. All of the [^{3}H]cytochalasin-B-binding data for both tissues are presented in Table 5. In the adipocyte, the number of glucose transporters/mg of membrane protein (R_0) in the low-density microsomal membrane fraction in the basal state is approx. 10-fold that in the plasma membrane. In confirmation of earlier studies [9], insulin decreases the number of transporters in the

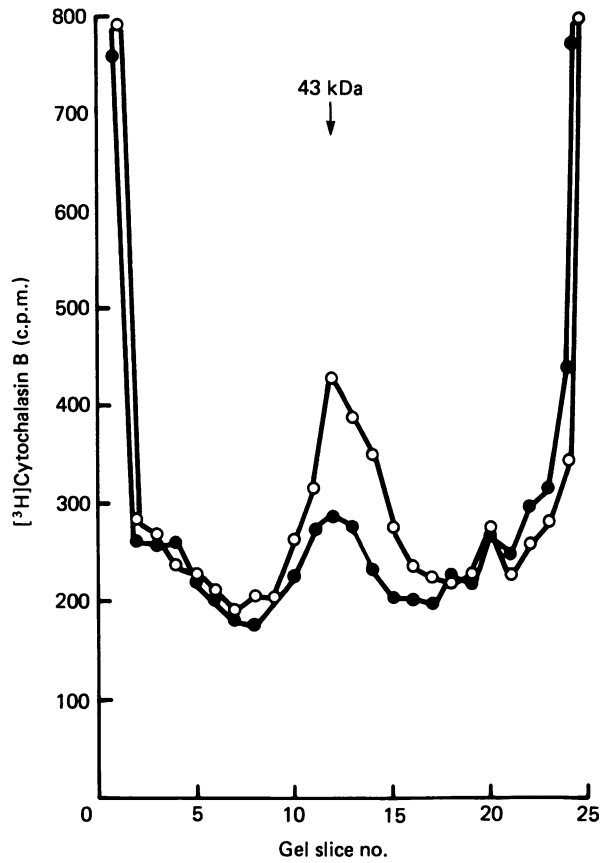

Fig. 2 Electrophoretic profile of [3H]cytochalasin-B-labelled liver plasma membranes

Liver plasma membranes were prepared as described in the Materials and methods section, and preincubated with 500 mM-D-glucose (●) or -L-glucose (○) before addition of [3H]cytochalasin B for 10 min at 4 °C. Hydroxysuccinimidyl-4-azidobenzoate was then added and, after incubation in the dark for 5 min, the membranes were irradiated with u.v. light at 254 nm for 2 min. Membrane protein (200–300 μg) was applied to 9%-acrylamide gels and analysed by SDS/polyacrylamide-gel electrophoresis. Prestained marker proteins were used as standards; arrow indicates the molecular mass of ovalbumin (43 kDa). Experiments were done four times; the data shown represent a typical experiment.

low-density microsomes and stimulates a comparable increase in the plasma-membrane fraction. The situation in the liver is the exact reverse of that in fat, with the concentration of transporters in the plasma membranes being 10-fold that in the low-density microsomes. No glucose transporters could be detected in high-density microsomes prepared from liver. The affinity of the cytochalasin-B binding sites in liver membrane fractions (719 and 990 nM) is in good agreement with the K_i for transport inhibition measured in whole cells (Table 3), but is higher than that measured in adipocyte membranes. It is clear that, in the absence of insulin, liver plasma membranes have a far greater concentration of glucose transporters than adipocyte plasma membranes. In contrast, the low-density microsomal pool of transporters from liver is very small, and suggests that few, if any, intracellular transporters are available for translocation in response to insulin. Incubation of hepatocytes with insulin had no significant effect on the number or distribution of glucose transporters in subcellular fractions measured by the cytochalasin-B-binding assay (results not shown). These data are fully consistent with our finding that there are relatively few intracellular glucose transporters.

Biochemical properties of liver glucose transporters

The next step was to characterize the glucose transporters of liver plasma membranes to see how they compared with those in adipocytes. Analysis of the [3H]cytochalasin-B-labelled liver plasma-membrane proteins by SDS/polyacrylamide-gel electrophoresis is shown in Fig. 2. The liver plasma-membrane proteins were incubated with either D- or L-glucose (500 mM) and then photochemically labelled with [3H]cytochalasin B by using the cross-linking agent hydroxysuccinimidyl-4-azidobenzoate as described in the Materials and methods section. Analysis of the [3H]cytochalasin-B-labelled liver plasma-membrane proteins by SDS/polyacrylamide-gel electrophoresis revealed a single D-glucose-inhibitable (66%) peak, migrating with a molecular mass of 40–50 kDa. Previous studies [13] assigned a similar value to the adipocyte transporter. A similar 40–50 kDa D-glucose-inhibitable peak was also observed when liver low-density microsomes were labelled with [3H]cytochalasin B (results not shown), but the low incorporation of the cytochalasin B achieved reflected the sparsity of glucose transporters in this membrane fraction.

The molecular properties of the liver glucose transporters were further characterized by subjecting the SDS/polyacrylamide-gel-electrophoresis-purified 40–55 kDa membrane proteins to isoelectric focusing. In rat adipocyte membranes analysed in this manner, we previously observed a heterogeneity of glucose transporters in the intracellular pool, whereas the plasma-membrane transporters were more uniform in charge properties [13]. The adipocyte plasma membrane contained a single specific band focusing at pI 5.6, whereas the low-density microsomes contained bands focusing at pI 5.6 and 6.4. Here we show the two-dimensional separation of liver subcellular membrane fractions (Fig. 3). In liver plasma membranes a single major D-glucose-inhibitable peak focusing at pI 6.05 was observed. When the same experiment was repeated with low-density microsomes the same pI 6.05 D-glucose-inhibitable [3H]cytochalasin B binding profile was also observed.

DISCUSSION

Insulin acts to lower blood sugar concentrations by increasing glucose uptake into the periphery and by decreasing glucose output from the liver [1]. The current study had two objectives: first, to study the kinetic properties of glucose transport in isolated cells, to determine how they might vary to satisfy the different roles that transport would fill in liver and a peripheral-tissue model, the isolated adipocyte. The second goal was to determine the possible mechanism of non-responsiveness to insulin in liver in an attempt to shed light on the process coupling receptor binding to transport stimulation in responsive cells. Previous investigators have addressed certain aspects of these questions [4–8,32], but it was hoped that a direct comparison of glucose transport at the level of the lowest functional unit, cells

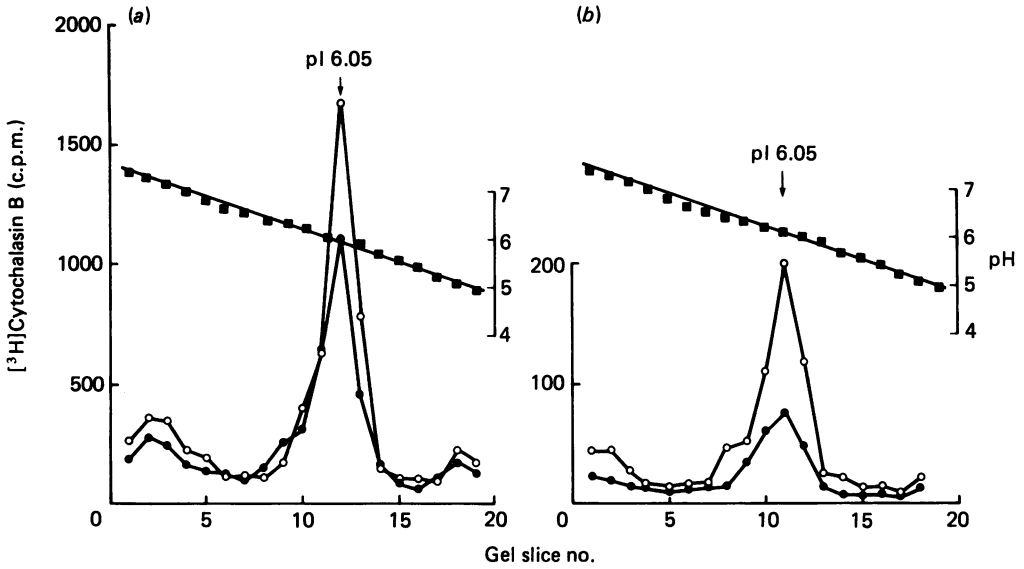

Fig. 3. Isoelectric focusing of the SDS/polyacrylamide-gel-electrophoresis-purified photoaffinity-labelled rat liver glucose transporter from plasma membranes (*a*) and low-density microsomes (*b*)

Membranes were preincubated with 500 mM-D-glucose (●) or -L-glucose (○) and applied to SDS/9%-polyacrylamide gels. The [^{3}H]cytochalasin-B-labelled proteins migrating at 40–55 kDa were excised from the gel and, after elution from the gel, were subjected to isoelectric focusing. Arrows indicate the pI values of the focused peaks. Experiments were done three times. Data shown above represent a typical experiment.

isolated from the same animal, would lessen biological and assay variations.

Glucose transport in fat-cells and liver cells shares the following properties; it is rapid, non-concentrative, and stereospecific (Table 2). Insulin increases the maximal velocity of transport in adipocytes by approx. 10-fold (Table 1), whereas hepatocyte transport is not accelerated further by insulin.

The maximal velocity, a measure of maximum transport capacity, in hepatocytes is 45 and 5 times the $V_{max.}$ in basal and insulin-treated adipocytes, respectively, when determined on a per-cell basis (Table 2). The affinity of the transporter in fat-cells for sugars is 5–7 mM (Tables 2 and 3), a value near the blood sugar concentration in starvation. The K_m of the hepatocyte transport system (15–20 mM) is 3–4-fold higher than in adipocytes for all sugars tested but fructose. This result suggests that the substrate-binding site of the glucose transporter in liver is different from that in fat.

The data in Table 1 demonstrate that the liver glucose-transport system is not acutely regulated by a variety of hormones or mimickers. However, the same cells responded to insulin with increases in total protein synthesis and amino acid transport. Thus the lack of responsiveness is not a generalized one, but at present it is not known if it is limited to the glucose-transport system.

The significance of the differences in glucose transport at the hepatocyte/adipocyte level is best considered in light of the different roles that glucose transport plays in the periphery and the liver. The adipose cell, which uses glucose as its primary fuel, has an affinity near the blood sugar concentration in starvation. Glucose entry into fat-cells is thus regulated by insulin's ability to increase $V_{max.}$, at which point processes beyond transport become rate-limiting for glucose metabolism [36]. Since the primary role of the liver is glucose storage and production [1], glucose uptake by the liver would increase in significance as the blood sugar concentration rose, approaching the K_m of liver transport. The higher K_m of the liver also ensures that, at physiological glucose concentrations, uptake into the periphery, and not liver, will be the primary avenue of glucose disposal. Hormonal sensitivity is usually limited to rate-limiting control processes [36] and, since liver glucose transport is not rate-limiting for hepatic glucose utilization, it is not surprising that the transport system does not appear to be under short-term hormonal regulation.

The causes of the differences in glucose transport between fat-cells and liver cells could reside at several levels. Firstly, the primary structure of the transport protein(s) in each tissue could differ. Alternatively, post-translational processing of the transporter could vary, with significant functional consequences. Also, the membrane environment in which the transporters are situated could vary, with large effects on activity.

The lack of insulin-responsivenes of glucose transport in liver could be due to a defect in the mechanism that couples insulin-receptor occupancy to transport stimulation in other tissues, or to the nature of the glucose-transport system itself. Current thought suggests that insulin acts to stimulate glucose transport by increasing the translocation of transporters from an intracellular pool to the plasma membrane [9,10]. The high transport activity seen in hepatocytes could be evidence that most of the transporters are currently active in the cell surface.

The D-glucose-inhibitable portion of [^{3}H]cytochalasin B binding has been accepted as a means of identifying and quantifying glucose transporters [9]. In an early report Riordan & Alon [37] studied cytochalasin B binding to liver fractions and stated that a portion of the binding to the plasma membrane was inhibited by D-glucose, but did not mention if this was the case in the other membranes. We found that D-glucose-inhibitable

cytochalasin-B-binding sites were present in both the plasma membrane and low-density microsomal fractions, but not in the high-density microsomes (Fig. 1). Although the concentration of transporters in the plasma membrane is 10-fold that in low-density microsomes (Table 5), the fact that the low-density microsomal fraction contains significantly more protein per cell than the plasma membranes creates the possibility that low-density microsomes could contain an appreciable proportion of total cellular transporter. It should be noted, however, that the measurement of transporters in low-density microsomes occurred at the lower limit of resolution of the assay.

Cytochalasin B binding was also measured in the liver plasma membrane by Axelrod & Pilch [29], who found a concentration of binding sites of 130 pmol/mg of protein. Using their assay technique we obtained the following values: $R_0 = 50.9 \pm 2.9$ pmol/mg, $K_d = 1.05 \pm 0.13$ μM, with 50% inhibition by D-glucose at 20 mM ($n = 3$). These values are similar to those obtained by us with the ultracentrifugation method, but R_0 is less than that reported by Axelrod & Pilch [29]. This discrepancy is most likely due to differences in the method of membrane preparation.

The data in Fig. 1 suggest that: (1) the high transport activity in liver cells is due to the large number of carriers already on the cell surface and (2) the lack of insulin-responsiveness of liver transport could be due in large part to the small pool of intracellular transporters for translocation. Depletion of intracellular transporters has already been proposed to explain the decrease in insulin-responsiveness in adipocytes for obese rat and guinea pig [14], and could be a general mechanism for regulation of responsiveness.

Although the $V_{max.}$ of transport in liver cells is more than 5-fold greater than in insulin-treated adipocytes, the plasma-membrane concentrations of transporters (R_0) are similar under these conditions. However, several factors make it difficult to make quantitative comparisons between the systems studied here. Transport activities were measured in whole cells, especially hepatocytes, whereas plasma membranes where prepared from whole liver, which includes many cell types.

Axelrod & Pilch [29] have previously reported that they were unable to photolabel the liver plasma-membrane glucose transporter by using direct activation of [^{3}H]cytochalasin B, an approach which has been successfully employed by other investigators in different cell types [25,26]. However, using the photochemical cross-linking agent hydroxysuccinimidyl-4-azidobenzoate we were able to cross-link [^{3}H]cytochalasin B to the liver plasma-membrane glucose transporter. The specifically labelled protein shown in Fig. 2 migrates at a molecular-mass range of 40–50 kDa. Thus the liver plasma-membrane glucose transporter is of the same general size as that of other mammalian cells [36,38–40], with the major exception of the kidney transporter, which migrates as a 75 kDa protein on SDS/polyacrylamide-gel electrophoresis [41].

The next step in characterizing the liver glucose transporter was to study the charge properties of the molecule by isoelectric focusing. Only a single peak of DL-glucose-inhibitable [^{3}H]cytochalasin B binding, focusing at pI 6.05, was seen in both plasma membrane and low-density microsomes from liver (Fig. 3). In contrast, in adipocyte plasma membranes the glucose transporter focuses as a single peak, at pI 5.6, whereas the low-density microsomes display two transporter isoforms, focusing at pI 5.6 and 6.4. We have reported that insulin mediates the translocation of the pI 5.6 isoform to the cell surface, whereas the pI 6.4 isoform is unaffected by the hormone [13]. The lack of the translocated pI 5.6 isoform in liver could also explain non-responsiveness of liver transport.

The reason for the differences in charge properties of glucose transporters from liver and adipocytes could derive from differences in post-translational modifications such as glycosylation and/or phosphorylation. Evidence for such a mechanism is contained in the observation that neuraminidase treatment of the adipocyte plasma-membrane pI-5.6 transporter isoform converts it into a form that focuses at pI 6 [13]. Such a control mechanism would involve synthesis of the glucose transporter from a common gene, with post-translational processing altering charge properties and functional characteristics.

In conclusion, these studies have revealed important differences in the functional properties, subcellular distribution and biochemical characteristics of rat liver glucose transporters compared with adipocyte transport proteins. The depletion of intracellular transporters in liver could be an example of a common mechanism for regulation of insulin-responsiveness of transport, and post-translational modifications would be reflected in such functional properties as substrate affinity.

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