The effect of membrane composition and alcohols on the insulin-sensitive reconstituted monosaccharide-transport system of rat adipocyte plasma membranes

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1. The monosaccharide transporter from the plasma membranes of rat adipocytes and insulin-stimulated adipocytes has been reconstituted in sonicated liposomes. 2. The stereospecific D-glucose uptake by liposomes made from a range of phospholipids and incorporating fatty acids has been investigated. 3. D-Glucose uptake is correlated with an increase in lipid fluidity as a consequence of the addition of fluidizing fatty acids, changes in phospholipid acyl chain length and temperature. 4. Benzyl alcohol and ethyl alcohol, which are generally considered to increase bilayer fluidity, decrease stereospecific D-glucose uptake in both whole adipocytes and reconstituted liposomes. 5. It is suggested that, although these alcohols may affect D-glucose transport by lipid-mediated fluidity changes, they also interact directly with the transporter resulting in inhibition of transport.

The insulin-sensitive glucose-transport system of adipocytes has been shown to be affected by temperature (Kono *et al.*, 1981; Ezaki & Kono, 1982), the lipid composition of the bilayer membrane (Czech, 1980; Pilch *et al.*, 1980) and by alcohols (Sauerheber *et al.*, 1982). There is substantial evidence that insulin-stimulated glucose transport occurs by a recruitment mechanism in which glucose transporters pass from intracellular storage sites into the plasma membrane (Kono *et al.*, 1981; Karnieli *et al.*, 1981; Wardzala & Jeanrenaud, 1981; Ezaki & Kono, 1982). Evidence has also been presented suggesting that insulin does not stimulate glucose transport solely by a recruitment mechanism (Carter-Su & Czech, 1980).

Effects of fatty acids (Pilch *et al.*, 1980) and alcohols (Sauerheber *et al.*, 1982) on glucose uptake by purified plasma-membrane vesicles or whole adipocytes have been discussed in terms of bilayer membrane fluidity. In the case of experiments with whole cells the effects of temperature on the basal level of glucose uptake have produced conflicting evidence. Sauerheber *et al.* (1982) report that D-glucose uptake increases with increase in tempera-

ture from 25 to 37°C in contrast with the uptake of 3-O-methyl-D-glucose, which is stimulated by reducing the temperature from 37 to 25°C (Vega & Kono, 1979; Ezaki & Kono, 1982). Since temperature influences more than membrane fluidity its effects cannot be interpreted without due consideration of metabolism in whole-cell systems.

Effects of changes in bilayer membrane fluidity on the glucose-transport system can be studied more directly in liposomal systems incorporating the monosaccharide transporter derived from adipocytes and insulin-stimulated adipocytes. In liposomal systems the bilayer membrane composition can be manipulated easily and effects of alcohols on the transport properties assessed. In the present paper we have reconstituted the transporter system of the adipocyte plasma membrane in liposomes of different lipid compositions using adaptations of established methods (Shanahan & Czech, 1977; Robinson *et al.*, 1982). The stereospecific uptake of D-glucose as a function of liposome composition together with the effects of ethyl alcohol and benzyl alcohol on transport have been studied.

Experimental

Materials

Labelled D-[U-¹⁴C]glucose (CFB-96) and [4(n)-³H]cytochalasin B (TRK-494) were obtained from Amersham International (Amersham, Bucks., U.K.),

Abbreviations used: Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid; DMPC, (dimyristoyl)-phosphatidylcholine (dimyristoylglycerophosphocholine); DPPC, (dipalmitoyl)phosphatidylcholine (dipalmitoylglycerophosphocholine); DSPC, (distearoyl)phosphatidylcholine (distearoylglycerophosphocholine).

L-[1-³H]glucose (NET 456) was obtained from New England Nuclear (Dreieich, Germany). Crude bovine serum albumin (fraction V), bovine insulin, cytochalasin B and all phospholipids were obtained from Sigma (London) Chemical Co. (Poole, Dorset, U.K.). Crude bacterial collagenase (batch no. 1162447) was obtained from Boehringer Corporation (London) Ltd. (Lewes, East Sussex, U.K.). Spectra/Por 1 dialysis membrane (molecular weight cut-off 6000–8000) was purchased from Spectrum Medical Industries Inc. (Los Angeles, CA, U.S.A.).

Methods

Adipocyte and plasma-membrane preparation. Adipocytes were prepared by collagenase digestion of epididymal fat-pads from 20–25 male Wistar rats weighing approx. 250–300 g as described by Rodbell (1964). Isolated cells were washed twice with a modified Krebs–Henseleit buffer (Krebs & Henseleit, 1932) containing 2.5 mM-CaCl₂ and resuspended in the same buffer. These cell suspensions were either used directly for D-glucose uptake studies or incubated with gentle shaking for 30 min, at 37°C with 2 mM-D-glucose in the presence or in the absence of 100 nM-bovine insulin and used for preparation of a crude plasma-membrane fraction. The latter suspensions were washed once in 10 ml of 10 mM-Hepes/NaOH (pH 7.5) containing 0.25 M-sucrose and 2 mM-sodium EDTA, resuspended in 10 ml of the same buffer and homogenized at room temperature in a glass homogenizing tube fitted with a Teflon pestle (seven strokes). The homogenate was immediately centrifuged for 10 min at 5000 g; this and all subsequent centrifugations were carried out at 4°C. The pellet and fat-cake were discarded and the supernatant was centrifuged for 30 min at 42000 g to give a plasma-membrane pellet. This is a relatively crude plasma-membrane preparation that may contain microsomal contamination. The method, however, has been shown to give membranes with a 2.5–6-fold higher 5'-nucleotidase activity than the microsomal membranes (Carter-Su & Czech, 1980).

Reconstitution of transporter in liposomes. The crude plasma-membrane pellet was solubilized by a method similar to that described by Robinson *et al.* (1982). The pellet was resuspended in 1.0 ml of ice-cold 10 mM-Hepes (pH 7.5) containing 20 mM-sodium cholate. After incubation for 10 min at 0°C the mixture was briefly sonicated (10 s; Decon FS 100 bath sonicator) and rapidly frozen and stored in liquid N₂.

For reconstitution of the hexose transporter, samples were thawed in water at room temperature and centrifuged in an Eppendorf Microfuge for 10 min at 12000 g to sediment particulate material. Excess cholate was removed from the solubilized protein in the supernatant by elution of 0.2 ml samples

through a Sephadex G-50 column (4.5 cm × 1 cm) equilibrated and washed with 10 mM-Hepes buffer pH 7.5 at room temperature. The macromolecular fraction was collected between 0.1 and 0.7 ml of the void volume. The recovered protein fraction was used directly for reconstitution of transport activity by mixing 200 μl portions (containing 60–100 μg of protein) with 40 μl of a 150 mg/ml liposome suspension (see the Results and Discussion sections for details of the various liposome suspensions). These were prepared freshly by vigorous vortex-mixing of the phospholipids in N₂-saturated 10 mM-Hepes buffer, pH 7.5, followed by sonication of the mixture in Eppendorf tubes for various times (depending on the particular phospholipid) to yield a clear liposome suspension. Reconstitution was accomplished by a 10 s sonication of the mixture in the bath sonicator followed by rapid freezing in liquid N₂. Samples were thawed in water at 37°C and resonicated for an optimum time, which depended on the phospholipid: for crude egg lecithin and DMPC liposomes this was 6 s, whereas for DPPC and DSPC liposomes the sonication needed to be extended to 40 s. This freezing–thawing–sonication procedure has been shown to give liposomes with a large internal volume (Pick, 1981).

D-Glucose uptake measurements. The uptake of radioactive glucose into cells and reconstituted vesicles was measured using a rapid filtration technique (Shanahan & Czech, 1977; Kono *et al.*, 1981).

The stereospecific carrier-mediated uptake of D-glucose was taken to be the difference in the amounts of D- and L-glucose taken up by adipocytes or reconstituted liposomes and was assayed by mixing 45 μl of cells or 20 μl of liposome preparation with 5 μl of labelled glucose solution containing 5 mM-D-[U-¹⁴C]glucose (25 μCi/ml) and 5 mM-L-[1-³H]glucose (50 μCi/ml) in 10 mM-Hepes buffer, pH 7.5. Cells were maintained at 37°C, whereas liposomes were kept on ice and pre-incubated for 5 min at 37°C before the transport assay. Incubations were started by addition of the labelled glucose solution, agitated manually for the prescribed time and terminated by addition of 1 ml of ice-cold 2 mM-HgCl₂ in 10 mM-Hepes buffer, pH 7.5. In the case of intact cells this buffer also contained 0.25 M-sucrose. The effectiveness of HgCl₂ as an inhibitor of D-glucose transport for the sugar transport system of adipocytes has been demonstrated (Robinson *et al.*, 1982). A concentration of 2 mM-HgCl₂ gives over 90% inhibition. Mixtures were immediately filtered through 0.22 μm Millipore GSWP filters (25 mm diameter, shiny side down) held in a filtration assembly (Millipore; cat. no. SX 00 025 00). All assays were carried out in Eppendorf tubes, which were rinsed with three 1 ml portions of the stopping buffer; these washings were

also put through the filter. Filters were air-dried at room temperature and placed in plastic scintillation mini-vials, which were filled with 4.6 ml of scintillation fluid (Scintran cocktail T, BDH Chemicals, Poole, Dorset, U.K.). Radioactivity retained on the filters was determined using a Beckman LS 9800 liquid-scintillation counter at counting efficiencies of 35% for ^3H and 75% for ^{14}C .

The protein concentration was determined by the method of Wang & Smith (1975) with bovine serum albumin as standard; due allowance was made for the interference of Hepes buffer by use of appropriate blanks.

Polyacrylamide-gel electrophoresis. The protein composition of the adipocyte plasma membrane and plasma-membrane extracts was assessed by electrophoresis on 7.5% polyacrylamide rod gels by the method of Neville & Glossman (1971). The gels were stained with Coomassie Blue and scanned at 550 nm on a Beckman 35 spectrophotometer.

Cytochalasin B binding. Cytochalasin B binding was estimated using equilibrium dialysis by an adaptation of a semi-micro method devised by Dr. A. G. Lowe (Department of Biochemistry, University of Manchester). The suspension of reconstituted liposomes (0.1 ml) was held in a small dialysis unit, which consisted of a piece of dialysis membrane, fitted over the wide end of a disposable 1 ml pipette tip and held in place by means of two rubber 'O' rings. The unit was immersed in 0.3 ml of [^3H]cytochalasin B (0.1 μM , 0.05 $\mu\text{Ci}/\text{ml}$) in 10 mM-Hepes, pH 7.5, containing benzyl alcohol in the concentration range 0–4% (v/v). Equilibrium was attained after 24 h incubation at 37°C in an orbital shaker (100 rev./min). After this, 50 μl portions were withdrawn from both sides of the dialysis membrane and counted as described previously.

Results

The dose-response curve for insulin stimulation of stereospecific D-glucose uptake by isolated adipocytes is shown in Fig. 1(a). In these experiments the basal level of D-glucose uptake was similar to that found by McCaleb & Donner (1982). Maximum stimulation occurred after exposure to 100 nM-insulin for 10 min. Fig. 1(b) shows the D-glucose uptake as a function of incubation time with 100 nM-insulin and confirms that stimulation reaches a constant level after a period in excess of 5 min. From these observations a protocol for insulin stimulation was established in which cells were exposed to 100 nM-insulin for 20 min.

Typical time courses for stereospecific D-glucose uptake in adipocytes and egg-lecithin liposomes incorporating transporters are shown in Fig. 2. Routinely a 3–7-fold stimulation was observed in whole cells, but in reconstituted liposomes stimu-

Fig. 1. Stimulation of D-glucose uptake in rat adipocytes in response to insulin at 37°C

(a) Rat adipocytes were exposed to insulin at the specified concentrations for a period of 10 min, and the stereospecific uptake of D-glucose was measured after incubation with 1 mM-glucose for 60 s as described in the text. (b) Rat adipocytes were incubated with 100 nM-insulin for the specified times and the stereospecific uptake of D-glucose was measured as in (a).

lation was less than this, although the D-glucose uptake per mg of protein was much larger than in whole cells as a consequence of transporter purification. It should be noted that in these experiments the transporter was reconstituted from relatively crude plasma-membrane fractions (Carter-Su & Czech, 1980), which may contain some microsomal contamination. This would have the effect of reducing the apparent extent of stimulation in liposomes derived from insulin-treated cells. The observed stimulation can be improved when plasma membranes are purified by flotation in a sucrose gradient (J. E. More & M. N. Jones, unpublished work). The polyacrylamide-gel pattern of the protein extracts in the reconstituted liposomes shows fewer peaks than for the adipocyte plasma mem-

Fig. 2. Time course of stereospecific D-glucose uptake in rat adipocytes and egg lecithin liposomes incorporating the monosaccharide transporter at 37°C

(a) ○, Basal level of D-glucose uptake in adipocytes; □, basal level in the presence of 25 μM-cytochalasin B; ●, D-glucose uptake after insulin stimulation (100 nM for 20 min); ■, D-glucose uptake of insulin stimulated cells in the presence of 25 μM-cytochalasin B. (b) ○, Basal level of D-glucose uptake in liposomes; □, basal level in the presence of 25 μM-cytochalasin B; ●, D-glucose uptake of liposomes incorporating monosaccharide transporter from insulin-stimulated cells (100 nM for 20 min); ■, D-glucose uptake by liposomes incor-

porating monosaccharide transporter from insulin-stimulated cells in the presence of 25 μM-cytochalasin B. The insets in (a) and (b) show the polyacrylamide-gel electrophoretograms of adipocyte plasma membranes and plasma-membrane fraction containing the monosaccharide transporter respectively. Abbreviation used: R_m , relative mobility.

The effects of increasing ethyl alcohol and benzyl alcohol on basal and stimulated stereospecific D-glucose uptake in whole cells are given in Fig. 3(a) and on liposomes in Fig. 3(b). In both systems the alcohols progressively suppress stereospecific uptake, benzyl alcohol being considerably more effective than ethyl alcohol. Sauerheber *et al.* (1982) observed similar effects for whole cells.

The effects of liposomal bilayer composition on stereospecific D-glucose uptake are illustrated in Table 2. The uptake by egg lecithin liposomes was taken as the basal level. Increasing amounts of vaccenic acid [(Z)-octadec-11-enoic acid] increase both basal and stimulated uptake levels, as do oleic [(Z)octadec-9-enoic] and linoleic [(Z,Z)octadeca-9,12-dienoic] acids. The effect of cytochalasin B on the inhibition of D-glucose uptake by egg lecithin liposomes incorporating vaccenic acid is given in Table 3. These results show that cytochalasin B inhibition is comparable in the presence and in the absence of vaccenic acid, indicating that vaccenic acid is having a direct effect on the transporter. If the effect of vaccenic acid was to cause the bilayer to become 'leaky' to D-glucose then cytochalasin B inhibition would have been less than approx. 40%.

Liposomes made from pure DMPC, DPPC and DSPC display lower levels of uptake relative to egg

porating monosaccharide transporter from insulin-stimulated cells in the presence of 25 μM-cytochalasin B. The insets in (a) and (b) show the polyacrylamide-gel electrophoretograms of adipocyte plasma membranes and plasma-membrane fraction containing the monosaccharide transporter respectively. Abbreviation used: R_m , relative mobility.

Table 1. *The effects of cytochalasin B, colchicine and vinblastine on the insulin stimulation of D-glucose transport in rat adipocytes at 37°C*

Adipocytes were pre-incubated with the agents for 30min followed by a 5min incubation with 100nM-insulin. D-Glucose uptake was measured after 2min.

	Glucose uptake (nmol/10 ⁶ cells)		Insulin stimulation (fold)	Inhibition of uptake (%)	
	–Insulin	+Insulin		–Insulin	+Insulin
Control	0.104	0.648	6.2		
Ethanol (1%, v/v)*	0.113	0.781	6.9		
Cytochalasin B (25 μM)	0.006	0.012	—	94	98
Colchicine (10 μM)	0.098	0.610	6.2	14	22
Colchicine (100 μM)	0.079	0.473	6.0	30	40
Vinblastine (10 μM)	0.115	0.741	6.4	0	5
Vinblastine (50 μM)	0.109	0.626	5.7	4	20

* Ethanol at this concentration was the solvent for cytochalasin B, colchicine and vinblastine.

Fig. 3. *Effects of benzyl alcohol and ethyl alcohol on the stereospecific uptake of D-glucose by adipocytes and egg lecithin liposomes incorporating monosaccharide transporter at 37°C*

(a) Shows D-glucose uptake by adipocytes and insulin-stimulated adipocytes (100nM for 20 min) as

Table 2. *Effect of membrane composition on stereospecific D-glucose transport activity in liposomes incorporating the adipocyte monosaccharide transporter from basal and insulin-stimulated cells*

Liposomes were pre-incubated for 5 min at 37°C. D-Glucose uptake was measured after 2min. Values are means ± S.D. Values in parentheses refer to the basal level of D-glucose uptake (nmol/mg of protein).

Liposome composition	D-Glucose uptake (% of basal control)	
	Basal	Insulin-stimulated
Egg lecithin	100 ± 1 (2.91)	138 ± 1
Egg lecithin + vaccenic acid (3%, w/w)	116 ± 3	164 ± 15
Egg lecithin + vaccenic acid (6%, w/w)	153 ± 4	237 ± 8
DMPC	48 ± 6	89 ± 6
DPPC	16 ± 2	33 ± 3
DSPC	9 ± 1	15 ± 1
Egg lecithin	100 ± 11 (2.80)	200 ± 8
Egg lecithin + vaccenic acid (6%, w/w)	154 ± 27	243 ± 38
Egg lecithin + oleic acid (6%, w/w)	191 ± 22	313 ± 3
Egg lecithin + linoleic acid (6%, w/w)	176 ± 17	280 ± 2

a function of benzyl alcohol concentration (■ and □) and ethyl alcohol concentration (● and ○). The basal uptake was 0.145nmol/10⁶ cells. (b) shows D-glucose uptake by egg lecithin liposomes incorporating transporter from insulin-stimulated adipocytes as a function of benzyl alcohol concentration (■) and ethyl alcohol concentration (●). The basal uptake was 4.46nmol/mg of protein. Other conditions were as for Table 2.

Table 3. Influence of cytochalasin B on glucose transport activity of egg lecithin liposomes incorporating rat adipocyte transporters from basal (–I) and insulin-stimulated (+I) cells in the presence or in the absence of cytochalasin B. Conditions were as for Table 2.

Liposomes	D-Glucose uptake (nmol/mg of protein)					
	Without cytochalasin		+ 25 μ M-Cytochalasin B		Inhibition (%)	
	–I	+I	–I	+I	–I	+I
Egg lecithin	6.17 $\pm$ 0.53	9.81 $\pm$ 0.65	0.82 $\pm$ 0.11	1.26 $\pm$ 0.01	87 $\pm$ 2	87 $\pm$ 1
Egg lecithin + vaccenic acid (6%, w/w)	16.7 $\pm$ 0.79	20.8 $\pm$ 1.0	4.57 $\pm$ 1.0	4.34 $\pm$ 1.0	73 $\pm$ 5	79 $\pm$ 4

Fig. 4. Influence of temperature on the stereospecific D-glucose uptake by DMPC liposomes incorporating the adipocyte monosaccharide transporter from insulin-stimulated cells

●, DMPC incorporating the transporter. At 37°C the D-glucose uptake was 6.6 ± 0.2 nmol/mg of protein. ■, DMPC liposomes. The error bars indicate standard deviations. Incubation times were as for Table 2.

lecithin, the uptake falling as the diacyl chain length increases. Of the pure phospholipids DMPC liposomes gave the largest uptake levels and the effect of increasing temperature on this system reconstituted from plasma-membrane extracts from insulin-stimulated adipocytes is shown in Fig. 4. Interestingly pure DMPC liposomes show a low level of stereospecific D-glucose uptake.

Discussion

The results in Fig. 2 show that the mono-saccharide-transport system of the adipocyte plasma membrane displays similar kinetic characteristics when reconstituted in egg lecithin liposomal membranes and is readily inhibited by cytochalasin B. The stereospecific transport activity (nmol of uptake/mg of protein) of the reconstituted system is comparable with that reported by Kono *et al.* (1981). However, it should be stressed that the level of L-glucose uptake by the liposomes is also large, as was found by Robinson *et al.* (1982) (see below), so

that stereospecific D-glucose uptake, although reproducible, is obtained from the difference between two relatively large quantities.

The effects of varying the liposome composition (Table 2) on stereospecific uptake superficially correlate with expected changes in the fluidity of the bilayer membrane. Addition of the unsaturated fatty acids enhances both the basal level of uptake and uptake in liposomes reconstituted from insulin-stimulated cells. The data on vaccenic acid, however, indicate that the percentage stimulation increases with increasing vaccenic acid concentration in the liposomes. The control level of stimulation was 38%; incorporation of 3% (w/w) and 6% (w/w) vaccenic acid resulted in 41% and 55% stimulation respectively. Liposomes prepared from insulin-stimulated cells contain more transporters; if this was the only factor affecting transport activity then the percentage stimulation would be constant. Thus not only are there more transporters but they are also more active in a more fluid membrane.

The transport activity of liposomes made from pure phosphatidylcholines appears to correlate with the diacyl chain lengths and bilayer fluidity. DMPC liposomes are the most active. The chain-melting temperatures of DMPC, DPPC and DSPC are 23.9°C, 41.4°C and 54.9°C respectively (Mabrey & Sturtevant, 1976), so that at 37°C DSPC liposomes are in the gel state, whereas the shorter-chain-length phospholipids will be in the liquid-crystalline state. It is possible that these results are to some degree a reflection of the relationship between the effectiveness of reconstitution and phospholipid chemistry. Robinson *et al.* (1982) found that pure phospholipids, including DPPC, were not as effective as egg lecithin for reconstitution. The results in Fig. 4 show, however, that D-glucose uptake is markedly dependent on temperature and increases steeply in the region of the chain-melting temperature. These data reflect the temperature-dependence of transport activity and not variations in reconstitution conditions, since the latter were identical. It is reasonable to suppose, therefore, that the lower transport activities of DPPC and DSPC at 37°C largely reflect the fact

that they are approx. 4°C and 18°C below their chain-melting temperatures.

The effect of phospholipid composition and temperature on D-glucose transport are qualitatively consistent with a carrier diffusion model in which transport rate increases with increase in the bilayer membrane fluidity (Janacek & Kotyk, 1969). The effects of benzyl and ethyl alcohol are clearly at variance with such a simple correlation, as the data of Sauerheber *et al.* (1982) also show. The relationship between glucose transport in erythrocytes and lipid fluidity was investigated by Yuli *et al.* (1981). Here transport rate was found to pass through a maximum with increasing viscosity. This observation was interpreted in terms of a decreasing rate of carrier diffusion with increasing viscosity, together with a concomitant increase in the number of operative carriers. Such a model requires the carrier to be distributed between dormant and operative states. The more fluid the membrane the larger the 'free volume', which favours dormant carrier conformations. On the basis of this model the decrease in transport activity found for reconstitution in saturated phospholipid liposomes relative to egg lecithin liposomes could arise as a consequence of the reduced diffusion rates, whereas the decrease in viscosity brought about by the alcohols while increasing carrier diffusion would increase the proportion of carriers in dormant conformations so that the net effect might be a decrease in transport activity.

Further insight into the effects of alcohols is obtained from an analysis of the separate transport measurements for D- and L-glucose (Fig. 5a).

Fig. 5. D- and L-glucose uptake by egg lecithin liposomes incorporating the adipocyte monosaccharide transporter from insulin-stimulated cells as a function of benzyl alcohol concentration for low (a) and high (b) concentration ranges at 37°C

●, D-Glucose uptake; ■, L-glucose uptake. The error bars indicate standard deviations. Conditions were as for Table 2.

Stereospecific transport is measured as the difference between the uptake rates of the two conformers. As the benzyl alcohol concentration increases the uptake of D-glucose converges with that of L-glucose, so that stereospecific carrier-mediated uptake is lost. This implies that there is a direct action of benzyl alcohol on the carrier inhibiting transport. Above an alcohol concentration of 10 mM uptake of both D- and L-glucose increases markedly (Fig. 5b) as a result of the bilayer membranes becoming 'leaky', and at very high concentrations (>100 mM) the membranes are disrupted and uptake falls off markedly.

To investigate further whether there is a direct action of benzyl alcohol on the transporter the binding of cytochalasin B to reconstituted egg lecithin liposomes was measured as a function of alcohol concentration. The binding of cytochalasin B to liposomes incorporating transporters was found to be 20% greater than for control liposomes in the absence of transporters. This enhanced binding was maintained up to a benzyl alcohol concentration of 1 mM. Between 1 mM- and 10 mM-benzyl alcohol concentrations, cytochalasin binding fell to within experimental error of that for control liposomes. These data also support the view that benzyl alcohol inhibits cytochalasin B binding (and hence D-glucose binding) by direct interaction with the transporter. Benzyl alcohol has been shown to have both stimulatory and inhibitory effects on other membrane-associated enzymes (Gordon *et al.*, 1980). Stimulation of activity at low benzyl alcohol concentrations has been observed for adenylate cyclase, 5'-nucleotidase and cyclic AMP phosphodiesterase, whereas Mg^{2+} -dependent ATPase was inhibited at comparable concentrations. However, at higher benzyl alcohol (>100 mM) concentrations all these enzymes were inhibited. Inhibition at low alcohol concentrations in the case of Mg^{2+} -dependent ATPase was attributed to a direct interaction between the alcohol and the enzyme.

Although there seems little doubt that the primary mechanism of insulin stimulation is a recruitment process it is clear from this and other work that sugar transport activity can be significantly influenced by membrane fluidity. The relevance of the fluidity factor *in vivo* has yet to be fully determined. However, the recruitment mechanism may well be modulated by the fluidity of the membranes involved and it is not difficult to also envisage insulin receptor clustering, insulin internalization and transporter recruitment not only depending on the fluidity of the lipid environment but possibly having an indirect effect on it.

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