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SENSITIVITY OF ADIPOCYTE BASAL AND INSULIN-STIMULATED HEXOSE TRANSPORT TO THE MEMBRANE LIPID STRUCTURE

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Abstract—A series of anesthetic alcohols inhibited basal and insulin-stimulated 2-deoxy-D-[1-^{14}C]glucose transport in adipocytes over total alcohol concentration ranges that cause local anesthesia of rat sciatic nerve. The relative potencies of the inhibition caused by the alcohols increased in the following order: methanol < ethanol < propanol < butanol < benzyl alcohol < hexanol < octanol. The inhibition was reversible and correlated well with the known partitioning of the alcohols into lipids of bioligical membranes. Adipocyte membranes were labeled with the 5-nitroxide stearate spin probe to investigate the effects of the alcohols on the dynamic structure of membrane lipids of the adipocyte. The alcohols increased the membrane "fluidity", and the relative concentration dependence of the effects closely paralleled that noted from methanol to octanol in transport studies. Alcohols from methanol to hexanol caused inhibition of hexose transport at molar potencies comparable to that observed for membrane disordering. This suggests that hydrophobic regions of the transporter and its lipid environment are perturbed by a comparable mechanism for each alcohol. The cholesterol-complexing polyene antibiotic filipin inhibited hexose transport and influenced the mobility of lipid domains sampled with the nitroxide cholestane, cholesterol-like spin probe. The data are consistent with the concept that the membrane structural/functional effects are mediated by formation of 1:1 cholesterol:filipin complexes. Alcohols and filipin inhibited inherent transporter activity and perturbed the membrane lipid structure without dramatically diminishing transport stimulation by insulin above basal. The specific organization of membrane lipids (particularly cholesterol) may provide an essential environment for optimal transport system activity.

The D-glucose transport system appears to be fundamentally similar in a variety of cell types and has been characterized as a facilitated stereospecific diffusion process [1]. However, the detailed mechanism by which glucose is transported through the membrane, and the means by which transport is stimulated by insulin in intact cells are not understood in detail.

Several studies suggest that the lipid structure of the plasma membrane may play an important role in regulating the cellular uptake of glucose [2–10]. For example, the temperature dependence of hexose transport in rat adipocytes has been correlated with thermotropic lipid structural changes in the membrane [2, 4, 5]. In addition, the relative cholesterol content of the erythrocyte and fibroblast plasma membrane [6, 9] markedly influences hexose transport rates into the cells. Consistent with these observations, cholesterol complexing drugs markedly influence basal [7, 8] and insulin-stimulated [7] glucose uptake and metabolism by adipocytes. Finally, the anesthetic alcohols benzyl alcohol (*c.* 40 mM) and ethanol (*c.* 1 M) inhibit the net uptake of D-glucose by isolated adipocytes [4] and the activity of the purified, reconstituted adipocyte hexose transporter [10], at concentrations that cause increases in lipid disorder of adipocyte ghost membranes labeled with the 5-nitroxide stearate spin probe [4]. The correlation between the potency of various alcohols in producing anesthesia and membrane lipid disordering, measured with either cholesterol-like fatty acid spin [11, 12] or fluorescent labels [13], suggests that these agents elicit their widespread effects by partitioning into the membrane lipid.

To help characterize the structural features of the membrane that are required for optimal transporter activity, we here further elucidate the relationship between the disordering potencies of several alcohols, having membrane/buffer relative partitioning varying over a wide range, and the abilities of the alcohols to inhibit basal and insulin-stimulated glucose transport. We also examined the ability of the specific membrane cholesterol-complexing polyene antibiotic filipin to perturb regions of the adipocyte plasma membrane and the activity of the hexose transporter. We found that the alcohols and filipin were particularly useful tools to characterize functional properties of the protein transporter in its native lipid environment, and we suggest that changes in the organization of membrane lipids, particularly cholesterol, may mediate the inhibitory effects of these agents.

MATERIALS AND METHODS

Adipocytes were obtained from 180–200 g male Sprague–Dawley rats (Simonsen, Gilroy, CA) by

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digesting at 37° the minced epididymal adipose tissue in 25 mM HEPES (*N*-2-hydroxyethylpiperazine *N'*-2-ethanesulfonic acid) (Sigma, St. Louis, MO) buffer, pH 7.4 (physiologic HEPES), containing 4% albumin LOT (Sigma) and 1.5 mg collagenase (Worthington no. CLSF2B261) per ml [5, 14]. The cells were washed and filtered in HEPES buffer, 1% albumin, and were suspended at approximately 10^6 cells/ml buffer. Aliquots of cell suspension were incubated at constant ionic strength with or without insulin and other agents for 15 min at 37° in triplicate polypropylene culture tubes. D-[1-^{14}C]Glucose was then added (New England Nuclear) to each tube so that the final analog concentration was 0.1 mM (sp. act. 2.0 mCi/mole). The final assay volume was 250 μl. The samples were incubated for 10 min, and aliquots were transferred to microcentrifuge tubes containing 200 μl dinonyl phthalate oil. Cells were separated from the buffer by centrifugation in an Eppendorf microcentrifuge for 2 sec. The tubes were sliced at the cell–oil interface, and the cells were transferred to scintillation vials containing Aquasol (New England Nuclear) and counted for ^{14}C, with a counting efficiency greater than 90%. Net uptake of labeled hexose both in the presence and absence of insulin was linear for at least 20 min under the above conditions. Extracellular trapped label and label entering the cells via non-carrier mediated processes, were subtracted from the net uptake values by the inclusion of cytochalasin B (50 μM) (Calbiochem-Behring, La Jolla, CA) into triplicate assay tubes to completely inhibit hexose carrier activity [2]. Corrected hexose uptake as a function of assay time extrapolated to zero uptake at zero time and reflects the rate-limiting membrane transport process [14].

Aliquots of alcohols (Sigma) were directly added to duplicate incubation tubes both with and without insulin. Amounts of alcohols added are reported as molar aqueous concentrations, neglecting alcohol–water miscibility considerations. Equal ionic strength was maintained by adding alcohols, or aqueous controls, mixed with appropriate aliquots of concentrated physiologic buffer. Alcohols did not influence the morphology of intact adipocytes inspected visually at 100× under these conditions (not shown). Since all uptake rates were linear, alcohol evaporation from the tubes during the assay was considered negligible. Cell counts were determined microscopically by counting cells in small aliquots of a 1:10 dilution of cell suspension on a siliconized microscope slide at low magnification.

The effects of filipin (provided by either Dr. Anthony Norman or the Upjohn Co., Kalamazoo, MI) on hexose transport in adipocytes were conducted by mixing aliquots of filipin (dissolved in ethanol) to the cell suspension so that the final ethanol concentration was <50 mM [14].

Adipocyte "ghost" membranes (sacs depleted of intracellular of lipid [15]) were prepared from the intact cells as described earlier [3, 4, 15]. The membranes were labeled with experimentally determined "low" concentrations [16] of the 5-nitroxide stearate spin label I(12, 3) (Syva Co., Palo Alto, CA), where nitroxide refers to the 4'-4'-dimethyl-*N*-oxyloxazolidine ring (Fig. 1). ESR spectra were recorded with a Varian E-104A Century Series ESR spectrometer, equipped with a Varian variable temperature accessory, after 5-min waits for temperature equilibration. In all cases, duplicate unexpanded spectra with "magnified wings" [17] were recorded (see Ref. 17 for instrument settings).

The order parameter *S* [18] is sensitive to the membrane "fluidity" (or, more accurately the flexibility of the membrane-incorporated spin label) and may be measured from the outer ($2T_{\parallel}$) and inner ($2T_{\perp}$) hyperfine splittings observed in the ESR spectra of the labeled membranes [3–5].

Adipocyte ghosts were also labeled with the cholesterol-like spin label nitroxide cholestane (Fig. 1), using Percoll (polyvinylpyrrolidine coated silica) (Sigma) to facilitate incorporation of label into the membranes [5, 19]. As indicated previously by Hubbell and McConnell [20], biological membrane dispersions labeled with steroid spin probes exhibit ESR spectra that indicate that the labels undergo rapid rotational motion about an axis essentially parellel to the fused ring of the steroid (the long molecular axis). Thus, for steroid spin labels, the outer (maximum) splitting in the ESR spectra of labeled membrane dispersions ($2T'_{\perp}$) corresponds roughly to the time-average splitting ($2T_{\perp}$) observed when the membrane surface is aligned parallel to the applied magnetic field H [21]. We here employ the polarity-uncorrected $2T'_{\perp}$ values as an index of the ordering (or "wobble") of the steroid long molecular axis about the normal to the membrane surface, by measuring the outer hyperfine splittings of the steroid-labeled membranes [5]. Increases in the

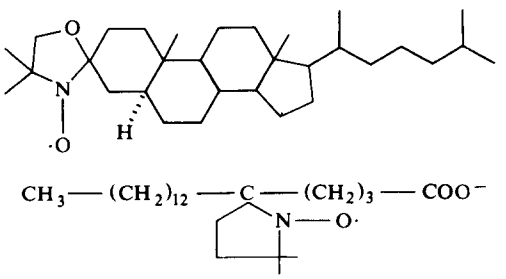

Fig. 1. Structures of exogenous spin labels used in the present study. I(12,3) is the 4',4'-dimethyl-*N*-oxyloxazolidine derivative of 5-ketostearic acid. CSL is the cholestane spin label, 4'-,4'-dimethylspiro[5α-cholestane-3,2'-oxozolidin]-3'-yloxyl.

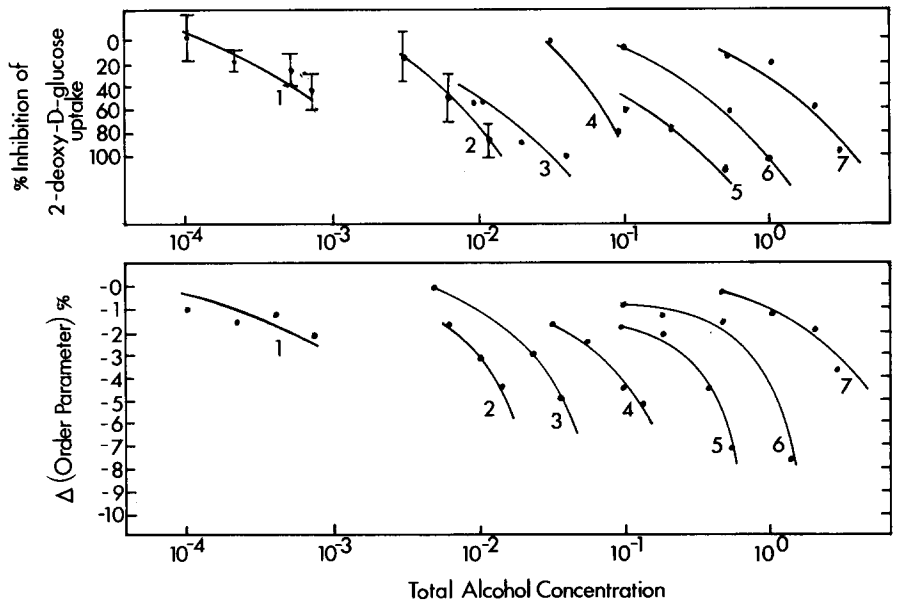

Fig. 2. Alcohol concentration dependence of the inhibition of 2-deoxy-D-[1-^{14}C]glucose transport and the decrease in order parameter S of I(12,3)-labeled membranes. Top frame: dose–response of the decrease in transport caused by alcohols over a wide total added concentration for intact adipocytes. Transport of hexose was measured as indicated in Materials and Methods. Data are averaged from three titrations of each alcohol with separate batches of adipocytes. Bottom frame: the percent decrease in the order parameter S is plotted as a function of added alcohol concentration. S was measured from I(12,3)-labeled adipocyte ghosts from duplicate ESR spectra before and after addition of alcohol, as indicated in text. Results are representative of at least four titrations with separate batches of ghosts for each agent at 37°. Key: (1) octanol, (2) hexanol, (3) benzyl alcohol, (4) butanol, (5) propanol, (6) ethanol, and (7) methanol. In corresponding plots, constructed using estimated aqueous (equilibrium) alcohol concentrations rather than total added (initial) concentrations, the line slopes are not changed significantly, but the octanol curves are shifted to the left comparably in both whole cell and ghost membrane plots. Comparable lipid/aqueous relative percentage volumes were used in both the structural and functional studies: 0.2×10^5 adipocytes/250 μl buffer and 300 μg membrane protein/70 μl buffer respectively.

mobility of the label cause decreases in $T'_\perp$. The motion of the label has been found to be sensitive to a variety of membrane structure-perturbing agents, including Ca^{2+}, cholesterol, anesthetic alcohols, and temperature [12, 21–24].

Alcohol effects on the spectra of I(12,3)-labeled adipocyte ghosts were determined by adding aliquots of alcohol stock solutions to the labeled suspensions, while maintaining ionic strength throughout, as above. Filipin was incorporated into the membranes as an ethanolic solution. The final concentration of ethanol (50 mM) had no significant effect on the spectra of either cholestane or I(12,3)-labeled membranes, and was included at 50 mM in control samples. Duplicate ESR spectra were recorded in the presence and absence of filipin at 37°. Percoll had no detectable effect at the employed doses on ESR spectra of either untreated or filipin-treated I(12,3)-labeled membranes.

Alcohol partitioning into cellular components. The alcohols will be expected to partition into aqueous, membrane and triglyceride compartments. A number of studies with anesthetic alcohols from methanol to octanol suggest that the relative order of partitioning is comparable between buffer and various biological plasma membranes, liposomes, and triolein [4, 24–28]. We utilized apparent partitioning values obtained by Seeman [24] and initial alcohol concentrations to estimate relative intramembrane concentrations after the added alcohols were partitioned into the lipid phase of the intact cell and membrane samples. Only in the case of octanol ($K_p > 150$) would we expect the estimated aqueous, equilibrium alcohol concentrations to differ considerably from the total initially added concentrations. However, we found in separate experiments that octanol added to adipocytes in excess volumes (20 ml octanol-containing buffer) did not cause functional effects significantly different from effects reported in this study for a given octanol dose without large volume preincubation. Further, the percent inhibition of uptake caused by octanol was independent of the cell density over the range of 0.2–0.8×10^5 cell/250 μl sample.

RESULTS

All alcohols inhibited the transport of 2-deoxy glucose in isolated adipocytes in a concentration-dependent fashion (Fig. 2). The total added concentration of alcohols required to elicit a 50% inhibition of transport was interpolated from the curves and placed in Table 1 for comparison. Incubation of cells with a dose of insulin sufficient to maximally stimulate hexose transport into adipocytes (10 ng/ml) [14] resulted in alcohol inhibition curves that

Table 1. Potencies of a series of alcohol anesthetics in perturbing adipocyte structure and function at 37°*

		I. Adipocyte ghost membrane disordering		II- 2-Deoxy-D-glucose transport inhibition	
	Membrane/buffer partition coefficient† (mole kg^1 membrane/mole l^{-1} H_2O)	Concentration‡ (M) causing a 2% decrease in order parameter S	Estimated membrane concentration§ (mmoles/kg membrane)	Concentration‡ (M) causing a 50% basal transport inhibition	Estimated membrane concentration§ (mmoles/kg membrane)
Methanol	0.045	2.0	95	2.0	95
Ethanol	0.14	6.25×10^{-1}	92	5.7×10^{-1}	85
Propanol	0.45	1.0×10^{-1}	45	1.0×10^{-1}	47
Butanol	1.5	4.0×10^{-2}	59	6.0×10^{-2}	87
Benzyl alcohol	4.0	1.5×10^{-2}	52	1.5×10^{-2}	50.3
Hexanol	13	6.5×10^{-3}	52	7.0×10^{-3}	52
Octanol	151	8.0×10^{-4}		8.0×10^{-4}	

* 2-Deoxy-D-[1-^{14}C]glucose transport was measured in isolated adipocytes as described in Materials and Methods. Alcohols were added over a range of concentrations prior to adding label, and amounts of alcohol causing 50% inhibition of transport were estimated from plots of the dose–response curves from at least three titrations for each alcohol. Adipocyte ghosts were prepared from intact cells by hypotonic lysis and were labeled with I(12, 3) at a low probe/protein ratio at 37° (see text). ESR spectra were recorded in duplicate both before and after addition of alcohol. Each agent was examined by titrating four to five separate ghost membrane samples suspended in physiologic HEPES buffer without albumin.

† Obtained from Seeman [24] for the partitioning of alcohols into red cell membrane by direct measurement or the calibrated partitioning in octanol/H_2O.

‡ Concentrations are reported as total alcohol added (see Discussion).

§ Aqueous concentration × membrane/buffer partition coefficient.

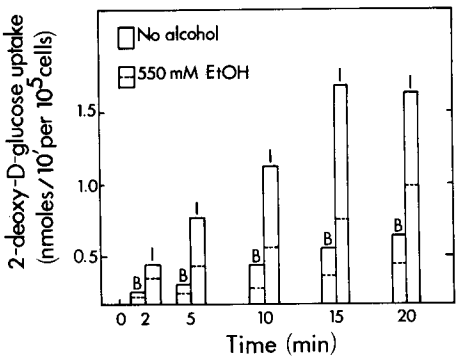

Fig. 3. Time dependence of basal and insulin-stimulated hexose uptake in the presence and absence of alcohol. Adipocytes were isolated and incubated for 2-deoxy-D-[1-^{14}C]glucose transport measurements as indicated in Materials and Methods. Reported data are from a representative experiment in which cells were incubated in the absence and presence (dotted bars) of 550 mM ethanol prior to addition of labeled substrate. Uptake was terminated by centrifugation of samples in oil at the indicated time points. Results comparable to the ethanol data were obtained for cells examined in the presence of 20 mM benzyl alcohol (data not shown).

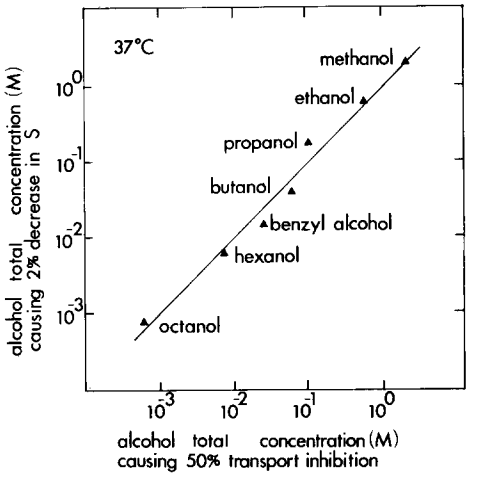

Fig. 4. Correlation diagram of the efficacy of alcohol inhibition of transport and perturbation of the adipocyte membrane. Hexose transport and order parameter S measurements were determined from adipocytes and isolated ghost membranes, as indicated in the legend to Fig. 1 (see Materials and Methods). A least squares line drawn through the points is shown ($r^2 = 0.99$) in the figure and has a slope of 1.0. Each point represents the alcohol-added concentration that caused 50% inhibition of basal transport of hexose and a 2% decrease in S; these were determined from the dose–response curves presented in Fig. 1. In a corresponding plot constructed using estimated aqueous (equilibrium) alcohol concentrations rather than total added (initial) concentrations, the slope of the line was not changed significantly (see legend to Fig. 2).

paralleled those obtained in Fig. 2 (data not shown). It was apparent that all the alcohols tested inhibited insulin-stimulated transporter activity to the same degree as basal transport, as was recently observed for ethanol [14]. Basal and insulin-stimulated uptake of hexose in the presence and absence of alcohols exhibited a linear time dependence as indicated in Fig. 3 for ethanol (550 mM).

Addition of alcohols to adipocyte ghosts labeled with I(12,3) caused decreases in the order parameter S in a concentration-dependent fashion. A plot of the total added alcohol concentration versus the percent decrease in calculated order parameter is shown in Fig. 2 for each alcohol tested. The total concentrations at which the alcohols caused a 2% decrease in S were interpolated from the curves and placed in Table 1 for comparison purposes. We chose a 2% decrease in S because for all alcohols tested, when hexose transport was inhibited by 50% into adipocytes, S was decreased in the vicinity of 2% in adipocyte ghosts by alcohol treatment. The relative order of effectiveness of all alcohols in decreasing S is identical to that observed for the inhibition of glucose transport; the initial alcohol concentrations required to elicit the structural/functional effects decreased progressively in the following order: methanol > ethanol > propanol > butanol > benzyl alcohol > hexanol > octanol. A plot was constructed of the total added alcohol concentrations causing 50% transport inhibition and causing 2% changes in S, and it is shown in Fig. 4. A least squares line through the points yielded a straight line with a slope of 1.00 ($r^2 = 0.99$). The intramembrane concentrations of alcohols required to inhibit transport by 50% and decrease S by 2% were estimated and are within a reasonably narrow range for the entire homologous series of alcohols.

Next we tested the cholesterol-complexing agent filipin for effects on the adipocyte uptake of 2-deoxy-D-[1-^{14}C]glucose in additional experiments. We found in four separately prepared batches of cells that both basal and insulin- (40 ng/ml) stimulated uptake of hexose were reduced markedly by the drug in the micromolar range (Table 2).

Filipin also exerted selective effects on adipocyte ghost membranes labeled with nitroxide cholestane, but not the I(12,3) label. The outer hyperfine splittings for the nitroxide cholestane-labeled membranes were decreased by filipin in a dose-dependent fashion between 1 and 3 mM. However, filipin exerted no detectable alterations over the same concentration range on the spectra of membranes labeled with the I(12,3) label (see Table 3).

Table 2. Effects of filipin on basal and insulin-stimulated hexose uptake in adipocytes at 37°*

	2-Deoxy-D-[1-^{14}C]glucose uptake (nmoles/10 min per 10^5 cells)	
Treatment	Basal	+ Insulin (40 ng/ml)
None	0.51 ± 0.12	1.45 ± 0.14
70 μM Filipin	0.38 ± 0.10	0.89 ± 0.37
100 μM Filipin	0.11 ± 0.03	0.25 ± 0.08

* Data for basal and insulin-stimulated uptake of hexose and uptake in the presence of filipin were averaged from four separately prepared batches of adipocytes, isolated as described in Materials and Methods. The values are averages (±1 S.D.). Drug concentration are total added (see Discussion).

Table 3. Effects of filipin on the outer hyperfine splittings of the I(12,3) and CSL-labeled adipocyte ghosts at 37°*

Label	Treatment	Outer hyperfine splitting (G)
I(12,3)	None	50.4 ± 0.4
	Filipin (1.8 mM)	50.8 ± 0.2
CSL	None	34.8 ± 0.2
	Filipin (0.3 mM)	34.9 ± 0.1
	(0.6 mM)	34.6 ± 0.1
	(1.0 mM)	33.7 ± 0.1
	1.3 mM)	33.4 ± 0.1

* Adipocyte ghosts were prepared and labeled with spin probes as indicated in Materials and Methods. A low probe/membrane protein ratio was employed for the I(12,3) label experiments [3, 17]. The hyperfine splittings were measured from duplicate spectra recorded with magnified wings both before and after addition of filipin, as indicated in text. Values (±1 S.D.) for the I(12,3) and CSL-labeled membranes are equivalent to $2T_{\parallel}$ and $2T'_{\perp}$ respectively (see Materials and Methods). Total added drug concentrations are reported (see Discussion).

Filipin does not complex directly with nitroxide cholestane. For example, the 3-β-hydroxyl of cholesterol is required for complex formation with filipin [29], and it is in this position that the cholestane is derivatized with the nitroxide ring. Further, filipin addition to membrane samples did not affect the incorporation of label into the ghost membranes, whereas exogenous cholesterol prevents incorporation of filipin into cholesterol-containing membranes [29].

DISCUSSION

All the alcohols tested in this study caused increases in the membrane lipid fluidity of the I(12,3)-labeled adipocyte ghost membrane, as evidenced by the concentration-dependent decreases in the order parameter S. In an earlier study, we found that benzyl alcohol and ethanol increase the membrane lipid fluidity and decrease the polarity of the environment of the label [4]. The present data extend these findings to various straight chain alcohols having widely differing lipid solubility. These structural data may be compared with an earlier study in which alcohols from methanol to octanol disordered lipid sampled by the cholesterol-like nitroxide cholestane spin label [15].

In contrast to the general disordering effects elicited by alcohols, we found that filipin exerted more specific effects. This agent over the tested doses did not cause alterations in the fluidity of adipocyte membranes labeled with I(12,3), but significantly reduced the outer hyperfine splittings ($2T'_{\perp}$) calculated for membrane lipid regions sampled by the nitroxide cholestane probe. Although $2T'_{\perp}$ is not a polarity corrected value, the observed changes likely reflect an increased mobility of the probe and a disordering of these domains by filipin (see Materials and Methods). Consistent with this, the interaction of this drug with cholesterol indeed may reverse the observed specific condensing effect of cholesterol on cholesterol-phospholipid dispersions [30]. We suggest that filipin structurally alters cholesterol-rich membrane regions, or cholesterol-phospholipid adducts in the bilayer. For example, the I(12,3) and nitroxide cholestane labels are believed to partition selectively into cholesterol-poor [31–33] and -rich [21] domains, respectively. Further, filipin is routinely used as a tool to microscopically identify cholesterol-rich clusters in a wide variety of cell types [34, 35]. An earlier study also reported selective structural effects of filipin on lipid vesicles labeled with perylene that were not sensed by 8-anilino-1-naphthalene sulfonate [36].

The alcohol effects on basal transport were completely reversible upon washing cells with alcohol-free buffer and, at the tested doses, were not associated with any observed changes in cell size or breakage measured microscopically. That the alcohols inhibited inherent membrane transporter activity and did not indirectly inhibit hexose uptake by inhibiting phosphorylation of transported 2-deoxy-D-glucose is supported by the observations of More and Jones [10] who recently found that the activity of extracted adipocyte hexose transporters into liposomes is inhibited by benzyl alcohol and ethanol at relative doses comparable to those employed here. Also, the effects of the alcohols were mediated by dissolution into the membrane lipid phase (see below) which would not likely alter the activity of generally water-soluble hexokinase. Finally, the phosphorylation of hexoses is not directly stimulated by insulin, whereas we note that the stimulation by insulin of transport occurred in the presence of inhibitory doses of alcohols.

In an earlier study [10], a direct correlation between the ability of an alcohol to produce intoxication in higher animals and its ability to disorder neuronal membranes was reported. A linear relationship was found between the log of the general anesthetic potency and the log of the membrane disordering potency for several straight chain and branched chain alcohols. A comparable relationship between the log of the transport inhibiting and adipocyte membrane disordering potencies was noted in our study (Fig. 3). Since submission of this work, a study of the effects of alcohols on Na^+-coupled D-glucose uptake and the membrane fluidity in intestinal cells appeared, and our results on the adipocyte membrane fluidity and on hexose transport in the absence of insulin [37] are remarkably similar.

The possibility that the general membrane lipid fluidity effects of alcohols, as measured with exogenous spin labels, modulates glucose transport or insulin action has been considered earlier [4]. Our results do not establish whether a cause–effect relationship occurs. However, the alcohol-induced alterations in adipocyte structure/function depend not only on the added concentration but also on the membrane/buffer partition coefficients [4]. Thus, the respective alcohol effects correlate with the inverse of the membrane/buffer partitioning in accordance with the classical Meyer–Overton rule of anesthesia.

The partition coefficients for alcohols into erythrocyte plasma membranes obtained by Seeman [24] were utilized to estimate apparent intramembrane concentrations of alcohols in both the transport and ESR studies (Table 1). From the data obtained from methanol to hexanol, a mean effective concentration

of the summed alcohol data required to inhibit transport was estimated to be 65 mmoles/kg membrane, while that required to reduce S by 2% was 69 mmoles/kg membrane. The partition coefficients for alcohols from ethanol to hexanol were measured in several independent studies for partitioning into dipalmitoyl-phosphatidylcholine (DPPC) [38–40] and are in good agreement with the values orginally found by Seeman. A significant discrepancy was found (4-fold) for octanol partitioning among the reported studies, which was omitted from the calculations here.

We suggest that a region of the transporter environment is capable of being perturbed by all the alcohols in a manner which does not depend strongly on their hydrophobicity or Van der Waals volume. It is possible that it is the interfacial region of the membrane that is perturbed to a similar degree by long and short chain alcohols, since NMR studies [41] indicate that a series of benzyl alcohol homologues position in the bilayer such that their hydroxyl groups resided in the phospholipid polar head group region of the membrane, while the phenolic groups position in the interior of the bilayer.

Filipin interacts specifically and with high affinity with membrane cholesterol (1:1) [29], and it is reasonable to suggest that both structural and functional effects of filipin are mediated, at least in part, by formation of cholesterol: filipin complexes within the membrane matrix. Indeed, the total number of cholesterol molecules estimated for each assay system (hexose transport into whole cells: 0.15×10^{16} molecules/sample; and adipocyte ghosts ESR studies: 3×10^{16} molecules/sample [42] compares favorably with the number of filipin molecules required in each sample to observe the structural and functional effects (hexose transport assay: 10^{16} molecules; ESR studies: 4×10^{16} molecules).*

Since filipin and the alcohols all reduce the outer hyperfine splittings obtained from the ESR spectra of the nitroxide cholestane-labeled membrane lipid (Ref. 15 and Table 3) and fluidize cholesterol-rich membrane lipid, it is likely that the inhibitory effects of these agents on hexose transport might in part be mediated by perturbation of cholesterol-rich regions of the membrane. Although some intrinsic membrane proteins may be excluded from cholesterol-rich lipid domains [31, 32], recent evidence indeed suggests that certain proteins may be preferentially associated within such domains in the membrane [34] or may be in close contact with the sterol [43]. Further, studies with intact cells [9] and transporters reconstituted in liposomes [44] demonstrated that modulation of the cholesterol content of the membrane can indeed affect hexose transport rates. An optimum cholesterol content in the membrane is apparently required for full activity of the transport system [6, 9]. If the activity of the adipocyte hexose transporter *in vivo* is sensitive to the organization of the cholesterol-phospholipid lattice in which it is embedded, it is conceivable that the above agents could influence hexose transport by perturbing this organization.

It has been proposed that insulin stimulates glucose transport activity in adipocytes subsequent to binding to the membrane insulin receptor, by either increasing the number of transporters in the membrane [45, 46] or by changing the intrinsic activity of available transport systems [1]. We found that pretreatment of adipocytes with insulin did not affect the magnitude of the alcohol or filipin-induced inhibition of basal or insulin-stimulated transport, as reported earlier for ethanol [14]. This suggests that these agents under our conditions do not affect the process by which insulin stimulates transporter activity. Also, varying the cholesterol content of the fibroblast does not apparently interfere with the stimulation of transport activity by insulin under conditions causing dramatic alterations in inherent transporter activity in the cell [4]. The above data are consistent with the view that basal and insulin-sensitive transporters are structurally identical and possibly reside in the same membrane lipid environment [14]. Although the fluid structure of the membrane bilayer may influence the interaction of the insulin receptor with transport proteins in the bilayer [47], our data suggest that increases in the "fluidity" measured under our conditions, do not cause alterations in the responsiveness of the cell to maximally-stimulating levels of insulin.

* Previous studies of adipocytes from 150 to 200 g rats reported 0.1 mg cholesterol/g lipid in isolated intact cells [42], 12 mg lipid/0.3 ml adipocytes [2], and 2.3×10^6 cells/0.3 ml packed cell volume [3].

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