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ALCOHOLS INHIBIT ADIPOCYTE BASAL AND INSULIN-STIMULATED GLUCOSE UPTAKE AND INCREASE THE MEMBRANE LIPID FLUIDITY

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Benzyl alcohol and ethanol, at aqueous concentrations that cause local anesthesia of rat sciatic nerve, affect structural and functional properties of rat adipocytes. The data strongly suggest that structurally-intact membrane lipids are required for the proper cellular uptake of glucose and for the physiologic response of adipocytes to insulin. The structure of adipocyte membrane lipids was examined with the spin label method. Isolated adipocyte 'ghost' membranes were labeled with the 5-nitroxide stearate spin probe I(12,3). Order parameters that are sensitive to the fluidity of the lipid environment of the incorporated probe were calculated from ESR spectra of labeled membranes. Benzyl alcohol and ethanol dramatically increased the fluidity of the adipocyte ghost membrane, as indicated by decreases in the polarity-corrected order parameter *S*. This concentration-dependent fluidization commenced at approx. 10 mM benzyl alcohol and progressively increased at all higher concentrations tested (up to 107 mM). *S* decreased approx. 5.7% at 40 mM benzyl alcohol, a change in *S* comparable in magnitude to that induced by a 6°C increase in the incubation temperature. Benzyl alcohol and ethanol inhibited basal glucose uptake in adipocytes and uptake maximally stimulated by insulin. Temperature-induced increases in membrane fluidity, detected with I(12,3), that closely paralleled the fluidity effects of alcohols were associated only with increases in basal and insulin-stimulated glucose uptake. The contention that the membrane lipid fluidity plays a role in insulin action needs further study.

Introduction

Numerous studies in recent years have been conducted to examine the mechanisms by which insulin influences cellular function. Specifically, insulin-sensitive D-glucose uptake* in isolated adipocytes has been an intensively studied experimental system. Although it is generally held that insulin, under normal conditions, increases glucose uptake subsequent to binding to extracellular plasma membrane receptors [1], the precise molecular mechanism by which insulin achieves its effects on adipocyte acitivites is not yet understood.

A wide variety of membrane-perturbing treatments has been employed to elucidate those structural features of the plasma membrane that are involved in glucose uptake and insulin action [2–6]. Several studies have focused on the role of the

* Uptake here denotes the transfer of exogenous substrate through the plasma membrane to the cell interior and is not limited to a particular mechanism or cellular process. Uptake may be measured with a number of experimental techniques that reflect to differing degrees such processes as facilitated diffusion by a saturable, selective membrane carrier (i.e. transport), passive membrane diffusion, and substrate metabolism.

lipid structure and the bilayer fluidity** of the adipocyte surface membrane in regulating these processes [3,5,6]. The lipid fluidity of biological membranes has been found to be an important parameter in regulating a wide variety of other membrane-associated functions [7–10], such as $(Na^+ + K^+)$-ATPase and glucagon-stimulated adenylyl cyclase activities.

The neutral, local anesthetic benzyl alcohol has been employed in many cases to examine the effects of increases in the lipid fluidity on plasma membrane activities. This agent readily partitions into membranes from aqueous solution and increases the fluidity of model [11] and biological [12] membranes. Being a neutral compound precludes any selective interaction with charged lipid species, and it is a suitable tool to study the relationship between bilayer fluidity and membrane-associated functions. We investigated the effects of benzyl alcohol, and also ethanol and temperature alterations, on adipocyte glucose uptake in the presence and in the absence of insulin. Furthermore, in parallel studies, changes in the lipid fluidity were determined through the use of electron spin resonance spectra of I(12,3)-labeled adipocyte ghost membranes.

Alcohols and temperature alterations influenced glucose uptake and insulin action. We cannot overrule the possibility that certain of the effects are mediated in part by increases in the membrane fluidity. Lastly, it is possible that anesthetic alcohols cause inhibition of glucose uptake, insulin action, and anesthesia all by similar membrane-mediated processes.

** The 'fluidity' of membrane lipids is a useful descriptive term but is potentially misleading. 'Fluidity' may refer to any of a number of molecular movements or motional processes that lipid molecules undergo in the membrane, contributing to the liquid-like state of the lipid matrix of the biomembrane. The membrane fluidity may be measured with a variety of biophysical techniques, but all such measurements in this report were made by calculating order parameters from ESR spectra of membranes labeled with 'magnetically-dilute' concentrations of the exogenous 5-nitroxide stearate lipid spin probe. The fluidity here logically reflects the flexibility of fatty acid chains of membrane lipid molecules sampled by the label.

Materials and Methods

Adipocytes. Adipocytes were isolated from the epididymal adipose tissue of male Sprague-Dawley rats (Simonsen Laboratories, Inc., Gilroy, CA) as described by Rodbell [13] (see Sauerheber et al. [14]). For each batch of intact cells, tissue was collected from 10 rats, individually weighing 130–160 g. The tissue was washed in saline and digested (in 4-g portions) with 3.2 mg collagenase (Lot No. 419CLS475239 from Worthington Chemicals, Freehold, NJ, or Lot No. 81F-6823 from Sigma Chemical Co., St. Louis, MO) in 2.1 ml Krebs-Ringer bicarbonate/3% albumin (Fraction V from Sigma) per g fat for approx. 30 min. Ghosts of adipocytes were prepared by lysing batches of approx. 4–6 ml of packed cells in hypotonic buffer [14] essentially as described by Rodbell [15]. The ghosts were washed by repeated centrifugation at $900 \times g$ for 15 s and finally suspended in either 50 mM Tris/8% sucrose (pH 7.4) or in Krebs-Ringer bicarbonate without albumin. Protein was determined by the method of Hartree [16].

Glucose uptake measurements. Following the procedure of Olefsky [17], washed adipocytes (10^6 cells, assuming that a 5 ml packed cell volume contains approx. $39 \cdot 10^6$ cells [18]) were incubated in plastic specimen vials (unless indicated otherwise) containing 2 ml Krebs-Ringer bicarbonate/3% albumin (pH 7.4) and 1 mM glucose (Mallinckrodt, St. Louis, MO). Dinonyl phthalate oil was added to the vials, and the cell suspensions were centrifuged at $900 \times g$ for 15 s to separate the cells from the buffer. The glucose concentration of the infranatants were determined in quadruplicate from each vial by the glucose oxidase-peroxidase method (kits were from Sigma Chemical Co. and were used as described previously [14]). Glucose uptake was defined as the difference between the average glucose concentration in three zero-time control vials and vials incubated for 2 h at 37°C. To examine the effects of insulin on glucose uptake, additional vials of cells were incubated at 37°C that also contained 5 μg/ml porcine insulin (Sigma).

The above basic procedure for measuring glucose uptake has been employed in several recent studies [14,17,19,20] and measures the overall abil-

ity of adipocytes to remove glucose from the incubation medium. Generally, the intracellular metabolism of glucose in intact adipocytes is rapid, and it is thus likely that the method (which utilizes a low substrate concentration) largely reflects the rate-limiting step in the cellular utilization of glucose, its passage through the surface membrane. At high substrate concentrations (e.g. 10 mM) or low temperatures (near 15°C), the membrane transport of sugar may not be rate-limiting, and these conditions were avoided.

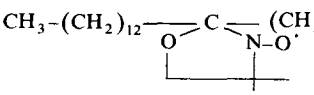
Alcohol effects on glucose uptake. To determine the influence of benzyl alcohol and ethanol on basal and insulin-stimulated glucose uptake, four or five concentrations of alcohol and a control addition of buffer were tested with a given preparation of cells. Triplicate vials of cells with and without insulin were incubated for each tested alcohol concentration.

The reversibility of the effects of benzyl alcohol on basal and insulin-stimulated glucose uptake was also tested. Here, adipocytes were preincubated for either 5 or 60 min with benzyl alcohol. The cells were subsequently washed with fresh buffer to remove alcohol [21], prior to assessing basal and insulin-stimulated glucose uptake as outlined above. As a control to test the effects of the washing step (e.g. the extra centrifugation [22]), additional cells were preincubated without benzyl alcohol. These cells were then washed, and basal and insulin-stimulated uptake were assessed both in the presence and in the absence of 40 mM alcohol.

Suitable controls were performed to test the possibility that benzyl alcohol might interfere with the determination of glucose by the glucose oxidase-peroxidase method. Benzyl alcohol, over a wide concentration range, was without detectable effect on the glucose concentration measurements under the experimental conditions employed (data not shown).

Temperature effects on glucose uptake. For experiments designed to test the effects of temperature reduction on insulin action and glucose uptake, cells from a given preparation were incubated at different temperatures in separate water baths. In each case, insulin was added only after a 5–10-min wait for equilibration of the cells to the listed incubation temperature. This avoids any influence that insulin exerts at a given temperature that might not be prevented or reversed by changing the incubation temperature to the desired value after hormone addition (see for example, Kono et al. [23]).

Spin labeling and spectral measurements. The spin probe 5-nitroxide stearate I(12,3), where nitroxide refers to the 4′,4′-dimethyl-*N*-oxyloxazolidine ring, was purchased from Syva Co., Palo Alto, CA.



I(12,3)

The label was dissolved in absolute ethanol (10^{-3} M) and stored at −15°C in liquid nitrogen storage tubes (Microbiological Assoc., Los Angeles, CA). Freshly-prepared adipocyte ghosts, or adipocyte ghosts stored at −70°C, were labeled with I(12,3) at room temperature as described earlier [14]. ESR spectra were recorded, after a 4–5-min period for temperature equilibration of the sample, with a Varian E-104A Century Series ESR spectrometer equipped with a variable temperature accessory [25]. All spectral data reported here were measured from adipocyte ghosts labeled with probe concentrations that may be considered 'magnetically-dilute' at 37°C as defined earlier [24].

ESR spectra recorded from the I(12,3)-labeled adipocyte ghost membranes indicate that the label undergoes rapid anisotropic motion about its long molecular axis in the membrane in an apparently homogeneous lipid environment; flexing or bending motions of the probe (i.e., the angular deviation of the hydrocarbon chain away from the preferred orientation perpendicular to the membrane surface) appear to be relatively restricted.

The fluidity of the membrane (or, more accurately, the flexibility of the incorporated label) was quantitated by first measuring the outer and inner hyperfine splittings $2T_{\parallel}$ and $2T_{\perp}$ (see Fig. 1 in Sauerheber et al. [14]), and then employing the following order parameter expressions [26]:

$$S(T_{\parallel}) = \tfrac{1}{2}\left[\frac{3(T_{\parallel} - T_{xx})}{(T_{zz} - T_{xx})} - 1\right] \quad (1)$$

$$S(T_{\perp}) = \tfrac{1}{2}\left[\frac{3[(T_{zz} + T_{xx}) - 2T_{\perp}]}{(T_{zz} - T_{xx})} - 1\right] \quad (2)$$

$$S = \frac{(T_{\parallel} - T_{\perp})(a_N)}{(T_{zz} - T_{xx})(a_N')} \quad (3)$$

T_{zz} and T_{xx} were previously determined by incorporating nitroxide derivatives into host crystals as substitutional impurities: $(T_{xx}, T_{zz}) = (6.1, 32.4)$ G [27]. a_N' and a_N are the isotropic hyperfine coupling constants for the probe in the membrane and crystal state, respectively ($a_N' = (T_{\parallel} + 2T_{\perp})/3$ and $a_N = (T_{zz} + 2T_{xx})/3$). a_N' is sensitive to the polarity of the membrane environment of the probe [26,28].

To examine the effects of alcohols, small aliquots of concentrated ethanol and well-mixed benzyl alcohol stock solutions were added to adipocyte ghosts labeled with a given I(12,3) probe concentration. Subsequent to addition of alcohol, samples were incubated for a 5-min period for temperature re-equilibration. Duplicate ESR spectra were recorded, both before and after addition of alcohol.

Results

The effects of benzyl alcohol on the order parameters calculated from ESR spectra of the I(12,3)-labeled adipocyte ghosts were first examined. Titrations of ghost membranes with benzyl alcohol from 2.5 to 40 mM were performed at 37°C (see Fig. 1A). 10 mM or higher concentrations of benzyl alcohol elicited decreases in the order parameters S, $S(T_{\parallel})$ and $S(T_{\perp})$. Progressive decreases in the order parameters occurred at all concentrations tested (up to 120 mM). The isotropic hyperfine coupling constant (a_N') value likewise was decreased by benzyl alcohol over the same concentration range. Benzyl alcohol additions to adipocyte ghosts at 25°C caused similar decreases in the calculated order parameters (Fig. 1B). A 5% decrease in S occurred at 36 and 36.5 mM alcohol at 37°C and 25°C, respectively. These experiments were conducted on adipocyte ghosts suspended in Tris buffer. Additional studies of the effects of benzyl alcohol on ghost membranes suspended in Krebs-Ringer bicarbonate buffer (without albumin) provided identical results; 25 mM benzyl alcohol decreased S by $5.1 \pm 2.0\%$ and $S(T_{\parallel})$ by $2.1 \pm 0.8\%$ at 37°C.

Relative decreases in the order parameters $S(T_{\parallel})$, $S(T_{\perp})$ and S, comparable to those achieved by benzyl alcohol, were also produced by the addition of ethanol to I(12,3)-labeled adipocyte ghosts at 37°C. Increases in the ethanol concentration between approx. 300 mM to 1.5 M caused progressive decreases in the order parameters (see Discussion) and in the isotropic hyperfine coupling constant a_N' (data not shown).

The temperature dependence of the order

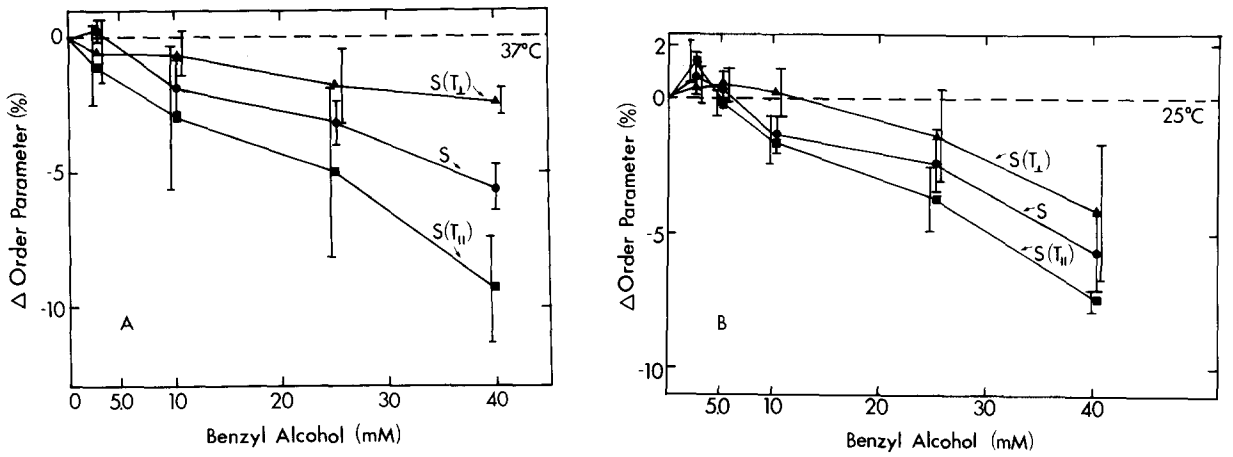


Fig. 1. Effects of benzyl alcohol on the order parameters of I(12,3)-labeled adipocyte ghost membranes. (A) At 37°C. (B) At 25°C. ΔS, $\Delta S(T_{\parallel})$ and $\Delta S(T_{\perp})$, the percentage changes in the order parameters from base-line values measured without benzyl alcohol, are plotted as a function of benzyl alcohol concentration. Each point and error bar represent the mean Δ(order parameter) ± 1 S.D. obtained from three determinations employing four separate ghost preparations. Ghosts were labeled with approx. 1.7 μg probe/403 μg protein, an experimentally determined low probe concentration at 37°C [14].

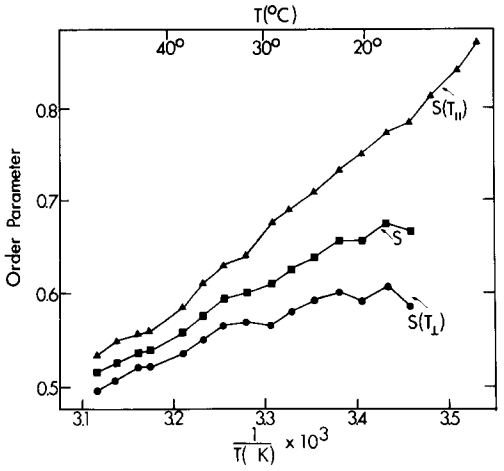


Fig. 2. Temperature-dependence plot of the order parameters of I(12,3)-labeled adipocyte ghost membranes. S, $S(T_{\parallel})$ and $S(T_{\perp})$ were calculated from the spectra of I(12,3)-labeled adipocyte ghosts as indicated in Materials and Methods. Samples were labeled with a probe/membrane protein ratio experimentally determined to be in the low range at 37°C [14]. The temperature range was 10°C to 48°C.

parameters determined from I(12,3)-labeled adipocyte ghosts is shown in Fig. 2. The membranes were labeled with a probe concentration considered to be low at 37°C [24]. The order parameters progressively increased with decreasing temperature (increasing $1/T$ K) over the range 10°C to 48°C. Although no clearly defined 'breaks' in S were apparent for the number of points examined, it is evident that at temperatures below approx. 30°C, the S and $S(T_{\perp})$ curves diverged considerably from the $S(T_{\parallel})$ line.

The time-course of the uptake of glucose by adipocytes was examined at 30-min intervals over a 2-h period. The basal and insulin-stimulated uptake progressed linearly during the incubation under the experimental conditions employed (see Materials and Methods). Although the glucose uptake measurements are necessarily sensitive to any 'nonspecific' passive diffusion through the membrane, the procedure apparently exhibits salient features that are comparable to other methods designed to more specifically measure the plasma membrane transport of hexose. The effects of insulin on glucose uptake, determined with the above method, were assessed over a wide insulin concentration range. The activation of glucose uptake by insulin was half maximal at $2.5 \cdot 10^{-11}$ M. Further, uptake is efficiently blocked by the glucose transport inhibitor, cytochalasin B (50 μM). Lastly, Arrhenius plots of the temperature dependence of basal and insulin-stimulated glucose uptake from 25 to 40°C (not shown) yielded apparent activation energies (8.8 kcal/mol) that were comparable in magnitude to those determined from transport studies employing nonmetabilizable glucose analogues [29–31].

At 37°C, glucose uptake in the presence of insulin was inhibited by benzyl alcohol (5–10 mM) (Table I). Further increases in the benzyl alcohol concentration (25–40 mM) dramatically decreased both basal and insulin-stimulated glucose uptake.

These inhibitory effects of benzyl alcohol were not likely due to generalized cell damage. The inhibition of basal glucose uptake appeared to be completely reversed (i.e., prevented) by washing benzyl alcohol pretreated cells with fresh medium prior to assessing uptake (Table II). No significant inhibition of basal uptake occurred, even when cells were pretreated with 40 mM benzyl alcohol for 60 min. However, the inhibitory effect of 40 mM benzyl alcohol on the insulin-stimulated uptake was not as effectively prevented by this washing of the cells.

The effects of benzyl alcohol on basal and insulin-stimulated glucose uptake examined at 25°C were similar to those described above at 37°C (Table I). However, at this lower incubation temperature, significant inhibitory effects of the alcohol on insulin-stimulated uptake ensued at slightly higher concentrations (approx. 10 mM). Moreover, the insulin-stimulated uptake was inhibited by 50% with 22 mM alcohol at 37°C and with 28–29 mM alcohol at 25°C.

Addition of ethanol at 37°C to adipocyte suspensions inhibited basal and insulin-stimulated glucose uptake in a fashion qualitatively similar to that for benzyl alcohol. However, the inhibitory effects occurred at much higher alcohol aqueous concentrations (approx. 300 mM) and required concentrations as high as 1.0 M to 1.5 M to achieve an inhibition equal in magnitude to that caused by 40 mM benzyl alcohol (see Discussion).

The effects of temperature alterations on basal and insulin-stimulated glucose uptake were next examined. Basal and hormone-activated uptake

TABLE I

BENZYL ALCOHOL AND TEMPERATURE EFFECTS ON ADIPOCYTE BASAL AND INSULIN-STIMULATED GLUCOSE UPTAKE

Adipocytes were incubated at 37°C or 25°C in Krebs-Ringer bicarbonate/3% albumin buffer (pH 7.4) containing 1 mM glucose, either with or without insulin, and various concentrations of benzyl alcohol (see Materials and Methods). Uptake values are averages of five (at 37°C) or four (at 25°C) different cell preparations. Each preparation represents an average of quadruplicate determinations of glucose from triplicate vials. Error bars are ±1 S.D.

Benzyl alcohol (mM)	Glucose uptake (μmol/2 h per 10^6 cells)			
	37°C		25°C	
	Basal	+Insulin	Basal	+Insulin
0.0	0.46±0.04	0.68±0.04	0.26±0.10	0.44±0.06
2.5	0.45±0.07	0.63±0.07	0.27±0.05	0.47±0.04
5.0	0.50±0.03	0.61±0.03	0.28±0.04	0.43±0.07
10.0	0.43±0.03	0.53±0.03	0.27±0.10	0.38±0.08
25.0	0.27±0.04	0.30±0.04	0.13±0.09	0.24±0.06
40.0	0.15±0.04	0.18±0.03	0.13±0.03	0.18±0.04

increased significantly with increases in temperature between 20°C and 40°C. The effects of increasing the incubation temperature from 25° to 37°C on glucose uptake are shown in Table I.

TABLE II

REVERSIBILITY OF BENZYL ALCOHOL INHIBITION OF BASAL AND INSULIN-STIMULATED GLUCOSE UPTAKE

Adipocytes were prepared as described in Materials and Methods. Cells were preincubated for 5 min or 60 min at 37°C, either in the absence or presence of 40 mM benzyl alcohol (BeOH) in the buffer. All cells were then washed by centrifugation at 400×g and resuspended in fresh buffer either without or with 40 mM BeOH. Errors represent ±1 S.D. of the glucose measurements. The data are taken from one of three representative experiments.

Additions		Uptake at 37°C (μmol/2 h per 10^6 cells)	
Pretreatment	to assay media	Basal	+Insulin
None (5 min)	None	0.50±0.04	0.72±0.04
None (5 min)	BeOH	0.23±0.03	0.27±0.04
BeOH (5 min)	None	0.51±0.03	0.64±0.03
None (60 min)	None	0.53±0.04	0.67±0.04
None (60 min)	BeOH	0.21±0.02	0.22±0.03
BeOH (60 min)	None	0.54±0.04	0.59±0.04

Discussion

Benzyl alcohol and ethanol increased the lipid fluidity of the I(12,3)-labeled adipocyte ghost membrane, as evidenced by the concentration-dependent decreases in the order parameters S, $S(T_{\parallel})$ and $S(T_{\perp})$. The more dramatic decrease in $S(T_{\parallel})$ over that noted for S and $S(T_{\perp})$ for benzyl alcohol (Fig. 1A) and ethanol (not shown) is attributed to the concomitant effect of the alcohols in decreasing the polarity of the environment of the label. At 37°C, the isotropic hyperfine coupling constant values decreased from 15.22 ± 0.07 to 14.98 ± 0.05 due to addition of 40 mM benzyl alcohol.

From the plots of order parameter vs. $1/T$ (K) (Fig. 2), it is evident that the membrane fluidity increased dramatically with increases in temperature. Further, we suggest that the adipocyte membrane exhibits a thermotropic lipid phase separation having an onset temperature of approx. 30°C. The divergence of S and $S(T_{\perp})$ from the $S(T_{\parallel})$ curve at this temperature is analogous to that noted for I(12,3)-labeled rat liver and heart plasma membranes at 28°C and 32°C, respectively [25]. These results are consistent with an earlier study [31] in which a lipid phase separation was detected in I(12,3)-labeled adipocyte ghosts and plasma membrane fractions at approx. 30°C. A thermal structural transition was also noted by Korneeva

and coworkers [32] at 29–30°C in ANS (8-anilino-1-naphthalene sulfonate) fluorescence and light scattering studies of liposomes from adipocyte plasma membrane.

Houslay and coworkers [9,12,33–35] studied in particular detail the effects of temperature alterations, lipid substitution, and a variety of charged and uncharged anesthetics on the activity of glucagon stimulated adenylyl cyclase and on the liver plasma membrane lipid fluidity. These studies probably represent the most compelling evidence to data that membrane-associated activities may be modulated by the fluidity of surrounding bilayer lipid.

In our studies, basal and insulin stimulated glucose uptake were inhibited at aqueous benzyl alcohol concentrations that fluidize adipocyte ghost membrane lipid (Fig. 1 and Table I). One could thus suggest from the data that increases in membrane fluidity are involved in the alcohol-induced inhibition of adipocyte sugar uptake. An obvious difficulty in comparing the concentration dependence of the effects of lipid soluble drugs on adipocyte structural and functional membrane properties is that the actual concentration of drug in the membrane lipid phase is unknown. In view of the potential for benzyl alcohol to incorporate with differing avidity into membrane lipids of intact adipocytes compared to adipocyte ghost membrane, the concentration-dependence of the structural and functional effects of alcohol in ghosts and intact cells is viewed as being strikingly similar. The inability to interpret membrane spectra of I(12,3)-labeled intact adipocytes [31,14] and the relative insensitivity of isolated adipocyte ghosts to insulin prevent a detailed structural/functional comparison of either preparation separately.

Our ethanol experiments and several other studies suggest a relationship between increases in the membrane lipid fluidity and decreased sugar uptake in agreement with the above findings with benzyl alcohol. Ethanol inhibited basal and insulin-stimulated glucose uptake in adipocytes at concentrations that increased the fluidity of spin-labeled adipocyte ghosts. The alcohol data are directly compared in a semi log plot of the percent inhibition of glucose uptake and the percent change in the order parameter S vs. the added concentrations of alcohols (Fig. 3). Earlier studies

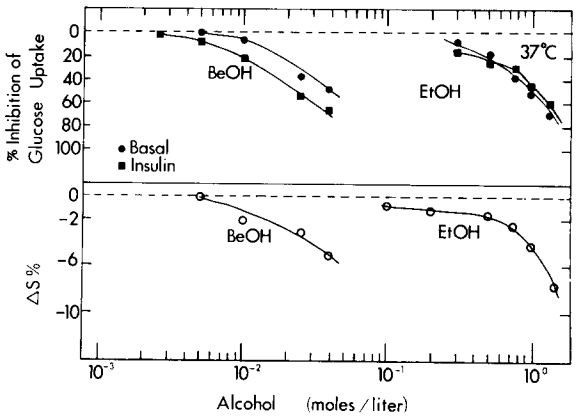


Fig. 3. Effects of benzyl alcohol (BeOH) and ethanol (EtOH) on the basal (●——●) and insulin-stimulated (■——■) uptake of glucose in intact adipocytes and on the fluidity of I(12,3)-labeled adipocyte ghosts. The influence of ethanol and benzyl alcohol on hexose uptake was determined as outlined in Materials and Methods, except that ethanol incubations were done in 17×100 mm polypropylene culture tubes in 1 ml Krebs-Ringer bicarbonate/3% albumin, in addition to polystyrene specimen vials. Each value represents the average percent inhibition from values measured in the absence of alcohol and were determined from at least five separate cell preparations. The average percent inhibition of basal and insulin-stimulated uptake by 0.5 M ethanol was 30 ± 13% and 34 ± 10%, respectively. Benzyl alcohol and ethanol effects on the fluidity were determined from adipocyte ghost membrane labeled with experimentally-determined low probe concentrations as described in Materials and Methods. The order parameter S was calculated from duplicate ESR spectra recorded at 37°C as described in text. $\Delta S\%$ is the average percentage change in S from base-line values measured in the absence of alcohol. At least three separate experiments were performed for each alcohol concentration tested. The ghost protein concentration was maintained at approx. 480 μg protein/70 μl buffer in all I(12,3)-labeled samples.

indicate that ethanol inhibits basal and insulin-stimulated oxidation of [^{14}C]glucose in intact rat adipocytes [36] and increases the fluidity of several other biological membranes labeled with exogenous lipid spin probes [37–41]. Yuli and coworkers [42] examined the influence of cholesterol and cholesterol esters on glucose transport and on the lipid fluidity of membranes of human erythrocytes and mouse 3T3 fibroblasts. Sterol-enriched cells exhibited alterations in glucose transport and the membrane lipid fluidity that was measured through the use of the fluorescence label 1,6-diphenyl-1,3,5-hexatriene. Decreases in membrane fluidity were associated with increases in glucose transport rates in both cell types, until a

peak was reached, beyond which further decreases in the fluidity were associated with progressively reduced transport rates.

Other evidence, however, supports the view that the alcohol effects on glucose uptake and insulin action may not be simply mediated by increases in the membrane lipid fluidity. For example, increases in incubation temperature that increase the lipid fluidity of the I(12,3)-labeled adipocyte membrane are associated only with increases in the basal and insulin-stimulated uptake of glucose (Table I). It is instructive to compare here the observed temperature and benzyl alcohol-induced fluidity effects in relation to glucose uptake. Increasing the temperature from 25°C to 37°C induced a decrease in the order parameter S comparable to addition of 40 mM benzyl alcohol at 25°C. Although 40 mM benzyl alcohol at 25°C inhibited basal glucose uptake by 40% and insulin-stimulated uptake by 60%, the corresponding effect of temperature increases from 25°C to 37°C gave rise to 77% and 60% increases in basal and insulin-stimulated uptake, respectively. It is thus difficult to reconcile the inhibition of the uptake process by benzyl alcohol in terms of an increase in membrane fluidity detected with the I(12,3)-label. Also, an insulin-like stimulation of glucose transport is induced by fatty acids that increase the membrane lipid fluidity [3]. Lastly, Czech [3] examined the temperature dependence of a reconstituted adipocyte membrane hexose transport system in phospholipid vesicles. Transporter activity increased markedly at or below the order-disorder transition temperature of the lipid, and Czech suggested the possibility that the glucose transport system may be more active in fluid membrane lipid domains.

The inhibition of the insulin effect on glucose uptake by benzyl alcohol is not likely to be due to inactivation of insulin by the anesthetic alcohol. Inhibition of insulin-stimulated uptake was only partially reversed by washing benzyl alcohol from pretreated cells (Table II).

Although we cannot overrule the possibility that ethanol or benzyl alcohol inhibit glucose uptake and insulin action, in part, by influencing intracellular activities, much evidence suggests that the alcohols exert the above effects at the adipocyte membrane level. For example, the approximately 30-fold difference in the aqueous concentrations of benzyl alcohol and ethanol (Fig. 3) required to produce effects on the membrane fluidity (and on basal and insulin-stimulated glucose uptake) is consistent with the known difference in partitioning of these alcohols in erythrocyte, nerve and model membranes [43–45] (see below). Moreover, we examined the effects of alcohols on the basal and insulin-stimulated adipocyte uptake of 2-deoxy-D-[1-^{14}C]glucose using an oil centrifugation technique essentially as described earlier [31]. 2-Deoxy-D-glucose is transported through the plasma membrane and phosphorylated by the same mechanisms as D-glucose, but is not further metabolized inside the cell [46]. Cytochalasin B (50 μM), which blocks the stereospecific D-glucose membrane transport system, was employed to correct for trapping and nonspecific diffusion of label. Benzyl alcohol and ethanol dramatically inhibit the basal and insulin-stimulated membrane transport of 2-deoxy-D-[1-^{14}C]glucose. Other studies indicate that benzyl alcohol (4–20 mM) and ethanol (200 mM) specifically inhibit the membrane glucose transport system of human erythrocytes [47,48] and that 1 M ethanol inhibits 2-deoxy-D-glucose transport in Novikoff hepatoma cells [49].

The alcohol-induced alterations in adipocyte function are not likely due to changes in the physicochemical properties of aqueous extracellular or cytoplasmic compartments. Any changes in dielectric constant [50], surface tension, viscosity, osmolarity, or other colligative properties would not be expected to require 30-fold differences in aqueous concentrations of the two alcohols. A logical conclusion is that a minimum alcohol concentration in the membrane lipid phase must be reached for the effects to occur. Thus, the respective alcohol effects would depend not only on the aqueous concentration, but also on the membrane/water partition coefficient.

In earlier studies [43–45,51] membrane/buffer partition coefficients for ethanol were found to be from 25- to 28-times lower than for benzyl alcohol in the human erythrocyte membrane (0.14 vs. 4.0) and in dimyristoyl phosphatidylcholine liposomes (0.55 vs. 13.9). The membrane fluidizing and glucose uptake inhibiting effects of benzyl alcohol (10–40 mM) and ethanol (300 mM–1.5-M) thus

correlate with the inverse of the membrane/buffer partition coefficients of these alcohols. This is in accordance with the classical Meyer-Overton rule of anesthesia, which states that narcosis is directly related to the concentration of the anesthetic in the cell membrane and to its lipid solubility [52].

It is tempting to suggest from the above discussion that these alcohols cause local anesthesia and inhibit adipocyte glucose uptake by similar mechanisms. The alcohols, for example, might inhibit adipocyte function by disrupting cholesterol-phospholipid adducts in the membrane [53,19] or by displacing annular lipid [12,34] from membrane protein components involved in glucose uptake and insulin action.

The lowered basal and insulin-stimulated glucose uptake rates at low temperatures may result in part from the associated alterations in the membrane lipid structure. Our studies of I(12,3)-labeled adipocyte membranes suggest that at 30°C a lipid phase separation occurs, distinct from and concomitant with a change in the flexibility of the lipid. Amatruda and Finch [31] correlated the membrane lipid structural change, detected in I(12,3)-labeled adipocyte plasma membranes, with transitions that occurred at the same temperature in the rate of uptake of D-glucose and the non-metabolizable analogs 2-deoxy-D-glucose and 3-*O*-methyl-D-glucose by intact adipocytes. Ludvigsen and Jarett [54] reported that D-glucose uptake in isolated adipocyte plasma membranes exhibits a break at approx. 33°C. These data taken together strongly suggest that the membrane lipid structure (e.g. the lipid phase separation at 30°C) is able to modulate the cellular uptake of glucose.

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