Biochimica et Biophysica Acta, 600 (1980) 870—881

BBA 78878

EFFECTS OF ANESTHETIC ALCOHOLS ON MEMBRANE TRANSPORT PROCESSES IN HUMAN ERYTHROCYTES

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(Received February 12th, 1980)

Key words: Anesthetic alcohol; Transport; (Human erythrocyte membrane)

Summary

1. Anesthetic alcohols (pentanol, hexanol and heptanol) were found to increase the fluidity of red cell membrane lipids as monitored by the fluorescence depolarization of diphenylhexatriene. The relative potency of the alcohols was found to be parallel to their relative membrane/water partition coefficients.

2. Hexanol had biphasic effect on erythritol uptake by simple diffusion by red cells. At concentrations less than 9 mM, hexanol had no significant effect. At concentrations greater than 9 mM, there was an approximately linear increase in erythritol permeability with increasing alcohol concentration.

3. The facilitated transport of uridine was markedly inhibited by hexanol. Hexanol at 6 mM produced a 65% inhibition of uridine (4 mM) uptake. Hexanol decreased both the apparent K_m and V values for the equilibrium exchange of uridine.

4. The facilitated transport of galactose was only slightly inhibited by hexanol.

5. Hexanol was without effect on the passive and active fluxes of Na^+ and K^+ in red cells with altered cation contents. Cells that were slightly depleted of K^+ and cells that were highly K^+-depleted were both insensitive to hexanol.

Introduction

The fluid mosaic model [1] is the most widely accepted working hypothesis of the structure of cell membranes. In this model the postulation that many membrane proteins are embedded in, but free to move in the plane of, the lipid bilayer part of the membrane has focused attention on the fluidity of the

bilayer as an important factor in membrane function. It now appears that the activity of certain membrane-bound enzymes, the rates of some membrane transport processes, and other membrane functions such as the capping of membrane antigens and the agglutination of cells by certain lectins, depend in various ways on the degree of fluidity of the lipid matrix of the membrane.

The lipid composition of a membrane appears to be a primary determinant of the fluidity of its lipid bilayer portion. In liposomes composed of cholesterol and naturally occuring phospholipids and in certain plasma membranes, an increase in the ratio of cholesterol-to-phospholipid has been found to dramatically increase the microviscosity as reported by lipophilic spin-label or fluorescent membrane probes [2—5]. More correctly stated, cholesterol appears to increase the disorder of lipid bilayers below their transition temperatures, but to decrease the disorder above the transition temperatures [6,7]. The latter effect is related to the well-known 'condensing effect' of cholesterol in phospholipid monolayers.

The degree of saturation of the fatty acyl side chains of membrane phospholipids is another important determinant of membrane fluidity. Increasing the degree of saturation of the fatty acyl chains of the phospholipids causes an increase in the viscosity of natural and artificial memrbanes [8].

Changes in membrane composition and fluidity have been discovered in certain disease states. The red cells of patients with hereditary spherocytosis have a markedly decreased membrane fluidity [9]. Chickens with hereditary muscular dystrophy show a 2-fold increase in the viscosity of lipids in isolated plasma membranes from skeletal muscle cells, liver cells and erythrocytes [10]. The erythrocytes of patients with progressive muscular dystrophy have been found to have markedly elevated levels of sphingomyelin and fully saturated fatty acyl chains, both of which should contribute to increased membrane viscosity [11]. Membranes from skeletal muscle of dystrophic mouse have reported to have almost twice the cholesterol content of controls [12]. One may speculate that the altered ion transport properties of dystrophic muscle are partly due to the decreased membrane fluidity. Malignant lymphoma cells have been reported to have a lower membrane viscosity than normal lymphocytes owing to the lymphoma cells having a much lower level of membrane cholesterol [5]. Increasing the cholesterol in the lymphoma cells to a level equal to that in lymphocytes made the lymphoma cells non-malignant [13]. It thus appears that altered membrane fluidity may play a role in the pathophysiology of some diseases.

The first evidence that the degree of fluidity of membrane lipids influences membrane transport processes and the activity of membrane-bound enzymes came from studies of bacteria and of model membrane systems. The laboratories of Overath, Fox and van Deenen were among those to make important contributions. Much of this research has been well reviewed [14,15]. In animal cells the dependence of the kinetics of membrane transport processes on the fluidity of membrane lipids is apparently complex. Anesthetics under most conditons have the effect of expanding membrane area and this has the effects of making the membrane lipids more fluid [16,17]. Anesthetics have been reported to increase the fluidity of the lipid bilayer part of the red cell membrane [18,19]. Seeman [17] observed that, while facilitated transport systems

are invariably depressed by anesthetics, active transport fluxes and simple diffusion across membranes can either increase or decrease in response to anesthetics. If these generalizations are true, what do they mean in terms of the molecular mechanisms of membrane transport processes?

In this study we have determined the influence of increasing the fluidity of the membrane of the human erythrocyte with anesthetic alcohols on the transport of erythritol (simple diffusion), galactose and uridine (facilitated transport), and Na^+ and K^+ (active transport). The increase in membrane fluidity induced by the alcohols was quantified by measuring the polarization of the fluorescence of the lipophilic probe 1,6-diphenylhexa-1,3,5-triene.

Materials and Methods

Materials. Erythritol, D-galactose, uridine, 1,6-diphenylhexa-1,3,5-triene, Tris buffer, 1-hexanol, 1-heptanol, phloretin and trichloroacetic acid were purchased from Sigma Chemical Co. Tetrahydrofuran and D-glucose were obtained from J.T. Baker Chemical Co. 1-Pentanol was from Fisher Chemical Co. Dibutylphthalate was purchased from Aldrich Chemical Co. D-[1-^{3}H]-Galactose and [U-^{14}C]erythritol were purchased from Amersham/Searle. [G-^{3}H]Uridine was obtained from ICN, New England Nuclear, and Amersham/Searle. Hydroxynitrobenzyl-*S*-mercaptoguanosine was a gift of Dr. Hagai Ginsburg. All other chemicals were of reagent grade.

Assessment of the effects of alcohols on the fluidity of the red cell membrane. Hemoglobin-free red cell ghosts were prepared by using the method of Steck and Kant [20]. We added 0.3 ml of 1 mM diphenylhexatriene in tetrahydrofuran to 200 ml of 0.154 NaCl and mixed them thoroughly. This solution was then incubated at 37°C for 45 min with constant stirring. Red cell ghosts were then incubated in the saline solution of diphenylhexatriene using various concentrations of membrane and varying the diphenylhexatriene concentration to keep constant the value of mol diphenylhexatriene/mol membrane lipid. Various amounts of pentanol, hexanol, or heptanol were included in the incubation mixture. Incubation of the membranes with diphenylhexatriene for 20—30 min was sufficient for the probe to equilibrate with the membrane as indicated by a constant level of total fluorescence. Fluorescence measurements with the labeled ghosts were made with an Aminco fluorometer (American Instrument Co.) equipped with a 50 W mercury lamp. A 365 nm interference filter (Baird Atomic) was used in the excitation beam and the emitted light passed through a Wratten 2A filter. The excitation beam was polarized by passing through a Polacoat polarizing filter (Polacoat Division, 3M Corp.) and the emitted light passed through one of two film polarizers (Edmund Scientific), one of which was oriented parallel to the direction of polarization of the exciting light and the other oriented perpendicular to that. The fluorescence polarization (p) was computed from $p = (I_\parallel - I_\perp)/(I_\parallel + I_\perp)$, where $I_\parallel$ is the fluorescence intensity observed parallel to the direction of polarization of the exciting light and $I_\perp$ is the fluorescence intensity observed perpendicular to the exciting light.

The apparent intensity due to light scattering by the red cell ghosts was a significant fraction of the total fluorescence intensity and thus corrections

for this had to be made. The apparent intensities of $I_{\parallel}$ and $I_{\perp}$ due to scattering by a similar suspension of unlabeled ghosts were subtracted from $I_{\parallel}$ and $I_{\perp}$, respectively, which were obtained with the labeled membranes. The corrected values of $I_{\parallel}$ and $I_{\perp}$ were then used to compute the polarization of fluorescence [21].

If the concentration of diphenylhexatriene in the membrane is sufficiently high, depolarizing interactions occur among diphenylhexatriene molecules. It was thus necessary to determine that the concentration of diphenylhexatriene in our membrane suspensions was well below this level. The highest level of diphenylhexatriene we used was about 1 mol diphenylhexatriene/100 mol membrane lipid. Doubling or halving the ratio of diphenylhexatriene/membrane lipid caused no change in the polarization of fluorescence.

Measurement of erythritol uptake. We modified the method of Deuticke et al. [22] to suit our purposes. Freshly drawn human red cells were washed three times in isotonic saline, discarding the supernatant and the buffy layer after each centrifugation. Cells at about 5% hematocrit were incubated in Krebs-Ringer phosphate buffer containing 100 mM D-glucose and 100 mM erythritol for 180 min at 37° C. This allowed equilibration of the intracellular water with erythritol. The cells were then centrifuged at 6000 × *g* and the supernatant discarded. Equal volumes (2.5 ml) of the erythritol-loaded packed cells and Krebs-Ringer phosphate buffer containing [^{14}C]erythritol (100 mM, 2—4 mCi/nmol) were rapidly mixed in a 16 × 125 mm test-tube at room temperature. The cell suspension was kept at room temperature and was agitated by means of a Teflon-coated magnetic stirrer. Samples (0.2 ml) were taken 5, 10, 20, 30, 40, 50, 60, 120 and 130 min after mixing. Each sample was immediately mixed with 5 ml of ice-cold isotonic saline containing 100 mM glucose and 100 mM erythritol. The ice-cold suspension was layered on 4 ml of dibutylphthalate and centrifuged at 0° C at 16 000 × *g* for 5 min in the Sorvall RC-2B centrifuge. The supernatant was discarded and the cells were resuspended in another 5 ml of ice-cold saline with glucose and erythritol and the cells were again pelleted through dibutylphthalate. The supernatant was again discarded and the cells were lysed in 5 ml of 5 mM NaCl containing 5 mM Tris-HCl (pH 6.5). The lysate was centrifuged for 10 min at 48 000 × *g* and the membrane pellet discarded. Samples of the lysate (0.2 ml) were diluted in 5 ml of the Tris-HCl buffer and the hemoglobin concentration determined from the absorbance at 540 nm. To the hemolysate were added 2.5 ml of 20% (w/v) trichloroacetic acid and the precipitated protein removed by centrifugation. Triplicate samples (0.2 ml) of the supernatant were taken for determination of [^{14}C]erythritol by liquid scintillation counting. Control experiments showed that the back leak of accumulated erythritol at 0° C is negligible over the 15 min period required for the two centrifugations through dibutylphthalate. The data were analyzed by means of the following equation [23] for the transfer of label in a two-compartment system:

$$\ln[(p^*_\infty - p^*_t)/p^*_\infty] = -k_{12}[(V_1 + V_2)/V_1]t \qquad (1)$$

where t is time, V_1 and V_2 are, respectively, the extracellular fluid volume and the intracellular water volume available to solute, k_{12} is the rate constant for transfer of label from the extracellular to the intracellular compartment, and

p^* is the amount of radioactivity (dpm) per unit of hemoglobin absorbance at 540 nm at the time denoted by the subscript. The 130 min time point was taken to represent infinite time. A plot of the left-hand side of the equation vs. time was linear and k_{12} was calculated from the slope and values of V_1 and V_2 obtained from hematocrit determinations in microhematocrit tubes. The hematocrit determinations were corrected for trapped extracellular fluid in the pellet using determinations with [^{14}C]sucrose. The intracellular water available to erythritol was assumed to be equal to the 0.62 ml water/ml red cell which participates in osmotic water movements [24].

Measurements of uridine transport. Freshly drawn red cells were washed as described above and made up to about 5% hematocrit in isotonic saline containing uridine at various concentrations. The cells were incubated for 45 min at 37°C and then pelleted at 6000 × *g* for 10 min. Packed cells (2.5 ml) were drawn into a 3 ml plastic syringe and another syringe filled with an equal volume of Krebs-Ringer phosphate buffer with [^{3}H]uridine at the same concentration used in the previous incubation and 1—4 mCi/mmol. The two syringes were connected to a three-port adapter (Becton-Dickinson) and the third port connected to a Hamilton dispensing syringe via a three-way stopcock. When the plungers of the two plastic syringes were pressed simultaneously, their contents were mixed and forced into the Hamilton dispensing syringe over a time of about 1 s or less. Samples (0.2 ml) were dispensed from the Hamilton syringe 5, 10, 15, 20, 25 and 30 s after mixing into 5 ml of ice-cold stopping solution. The stopping solution contained 5 μM hydroxynitrobenzyl-*S*-mercaptoguanosine in Krebs-Ringer phosphate buffer. Control experiments demonstrated the efficacy of the stopping solution under our conditions. The cells in ice-cold stopping solution were then layered on 4 ml of dibutylphthalate and centrifuged at 16 000 × *g* for 5 min. The supernatant was discarded and the cells resuspended in another 5 ml of stopping solution and again spun through dibutylphthalate. The cell pellet was lysed in 5 ml of 5 mM Tris-HCl (pH 6.5) in 5 mM NaCl. The hemoglobin concentration of the hemolysate was determined as described above. Proteins were precipitated with trichloroacetic acid and samples analyzed by liquid scintillation counting as described above. An 'infinite-time sample' was obtained from cells that were in contact with the labeled uridine for 30 min or more. The effective rate constant for transfer of isotope was computed from Eqn. 1.

Measurement of galactose transport. The procedure for measurement of galactose transport was essentially the same as that for uridine, but the stopping solution was 1 μM $HgCl_2$, 1.25 mM KI, 0.1 mM phloretin, 1% ethanol (the phloretin was dissolved in ethanol) and 190 mM NaCl.

Determination of the rates of Na^+ and K^+ transport. Red cells with altered intracellular concentrations of Na^+ and K^+ were prepared by using the method of Garrahan and Rega [25] as modified by Sachs [26]. Transport of Na^+ and K^+ was then determined by a method of similar to that described by Whittam and Ager [27]. Fresh venous blood was drawn for healthy volunteers into heparinized evacuated tubes (Vacutainers, Becton-Dickinson). The cells were spun down, plasma and buffy layer removed, and washed three times more in isotonic saline. The washed red cells were then used in the following procedures. All the media used were made up on the day of the experiment and titrated to pH 7.4.

Alteration of internal cation concentration. The packed, washed red cells were then suspended (at about 5% hematocrit) in either incubation medium 1A (containing 147 mM NaCl, 3.4 mM NaH_2PO_4, 5 mM glucose, 1 mM $MgCl_2$, and 0.1 mM *p*-chloromercuribenzenesulfonate) or incubation medium 1B (containing 37 mM NaCl, 110 mM KCl, 3.4 mM NaH_2PO_4, 5 mM glucose, 1 mM $MgCl_2$ and 0.1 mM *p*-chloromercuribenzenesulfonate and incubated at 3° C for 20 h.

Resealing the cells. The cells were then centrifuged and resuspended in 6 times their volume of either medium IIA (147 mM NaCl, 3.4 mM NaH_2PO_4, 5 mM glucose, 1 mM $MgCl_2$, 2 mM dithiothreitol, 3 mM adenine and 10 mM inosine) or medium IIB (same as IIA, but contains 37 mM NaCl and 110 mM KCl) and incubated at 37° C for 1 h. At the end of the incubation, the cells were washed three times in 4 vols. of medium IIIA (150 mM NaCl, 2 mM $MgCl_2$, 2.5 mM $CaCl_2$, 20 mM imidazole and 10 mM glucose) or medium IIIB (150 mM choline and 5 mM Tris-HCl).

Final incubation and measurement of transport. The packed, resealed cells were then suspended at about 15% hematocrit in medium IVA (130 mM NaCl, 20 mM KCl, 2 mM $MgCl_2$, 2.5 mM $CaCl_2$, 20 mM imidazole and 10 mM glucose) or medium IVB (same as IVA, but contains 20 mM NaCl and 130 mM KCl). The final incubations were carried out in the presence and absence of 0.1 mM ouabain and in the presence of various concentrations of hexanol. The cells were divided at the beginning of the incubation into four separate flasks: (1) no ouabain, no hexanol; (2) with ouabain, no hexanol; (3) no ouabain, with hexanol; (4) with both ouabain and hexanol. Aliquots from each flaks were removed at various time points from 0 to 6 hrs of incubation. The cells were centrifuged and washed twice in ice-cold isotonic choline chloride. The cell pellet was lysed in 14.4 mM Li_2SO_4 and the hemoglobin concentration in the lysate determined by absorption at 540 nm as described above. The intracellular concentrations of Na^+ and K^+ were computed from the Na^+ and K^+ concentrations of the lysate, which were determined by flame photometry.

Results and Discussion

Effects of alcohols on the fluidity of red cell membrane lipids

Fig. 1 shows the changes in the polarization of the fluorescence of diphenylhexatriene as a function of alcohol concentration for pentanol, hexanol and heptanol. The relative potencies of the three *n*-alkanols are approximately directly proportional to their relative partition coefficients between red cell membrane lipids and aqueous solution [28,29]. The changes in the 'microviscosity' of the membrane lipids induced by the alcohols are considerable, with about a 20% change in microviscosity corresponding to a 10% change in fluorescence polarization (the behavior of diphenylhexatriene in White American Oil of known viscosity is used as the calibration standard for computing microviscosity [21]). While absolute values of microviscosity should be viewed skeptically [30—33], a sense of the relative changes in micoviscosity that occur due to the presence of the alcohols is heuristically useful. In the studies below we have used hexanol to increase the fluidity of red cell mem-

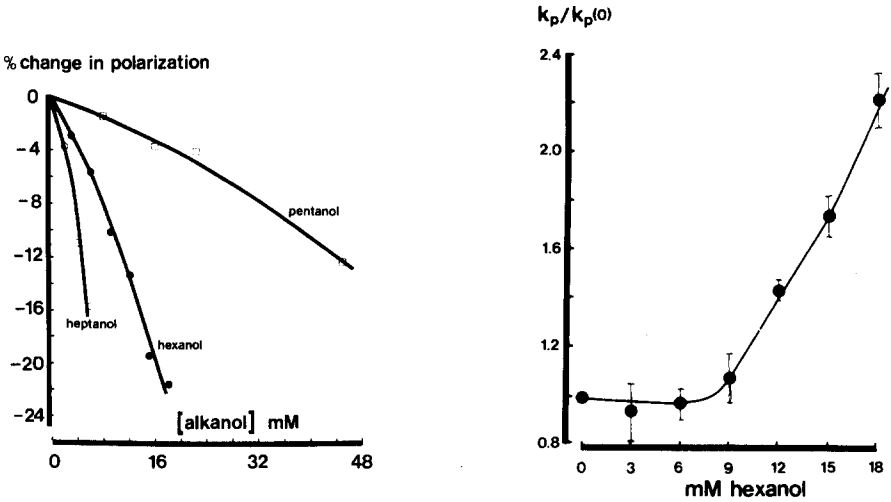

Fig. 1. The effect of pentanol, hexanol and heptanol on the polarization of fluorescence of diphenylhexatriene in hemoglobin-free red cell ghost membranes at 23° C.

Fig. 2. The effect of hexanol on the rate of entry of erythritol (100 mM) into human red cells at 23 ± 1° C. The ratio of the rate constant for erythritol entry to that in the absence of hexanol is plotted vs. hexanol concentration.

brane lipids. but we believe that similar results would be obtained with levels of pentanol or heptanol that decrease the polarization of diphenylhexatriene to the same extent [29].

Erythritol uptake

The effect of hexanol on erythritol uptake is shown in Fig. 2. There is no significant effect of hexanol at concentrations less than 9 mM. For concentrations of hexanol above 9 mM there is an approximately linear increase in the apparent permeability of the red cell membrane to erythritol; the permeability approximately doubles between 9 and 17 mM hexanol.

The abrupt transition at 9 mM hexanol is noteworthy. A similarly abrupt transition was observed by Grunze and Deuticke [34] in the effect of cholesterol depletion on the transport of erythritol (and other solutes) in mammalian erythrocytes. Up to 35% of the cholesterol could be removed from the red cell membrane with no significant effect on erythritol transport. Removal of more than 35% caused a marked increase in erythritol transport that was roughly linearly related to the degree of cholesterol depletion. Similar behavior was noted for porcine, bovine and human red cells. Cooper et al. [35] found that removal of 35% of the cholesterol from the human red cell membrane resulted in an approx. 20% decrease in membrane microviscosity at 37° C as monitored using diphenylhexatriene. We found that 9 mM hexanol causes an approx. 18% decrease in membrane viscosity at 37° C as measured with diphenylhexatriene. Thus, in terms of membrane fluidity, the transition in erythritol transport we observed by adding hexanol corresponds closely to that observed [34] when cholesterol was removed. Membrane viscosity (as monitored using diphenylhexatriene) is approximately linearly related to both hexanol concentration

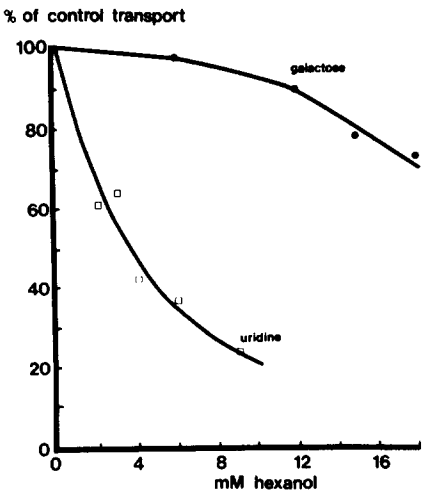

Fig. 3. The effect of hexanol on the transport of galactose (10 mM) and uridine (4 mM) across the red cell membrane at 23 ± 1°C. The apparent rate constant (expressed as a percent of control) is plotted vs. hexanol concentration.

(see Fig. 1) and membrane cholesterol content [35]. It is not clear why erythritol permeability should be such a nonlinear function of membrane fluidity. Cooper et al. [36] found a similar sort of transition point in Na^+ influx into human red cells: removal of less than 40% of membrane cholesterol was without effect, but removal of more than 40% caused an abrupt increase in Na^+ influx.

Galactose and uridine transport

Galactose is a substrate of the monosaccharide transport system of the red cell and uridine is transported by a well characterized nucleoside transport system. Both of these transport systems display the characteristic properties of facilitated transport. As shown in Fig. 3, we found both galactose and uridine transport to be inhibited by hexanol. The much greater sensitivity of the uridine system is noteworthy: 6 mM hexanol has only an insignificant effect on galactose transport, but inhibits uridine transport by about 61%. The effects of hexanol on the apparent values of K_m and V for uridine transport are shown in Table I. Hexanol at 6 mM (this concentration causes an approx. 12% decrease in membrane microviscosity as monitored using diphe-

TABLE I

EFFECT OF HEXANOL (6 mM) ON THE APPARENT KINETIC PARAMETERS OF URIDINE TRANSPORT UNDER EQUILIBRIUM EXCHANGE CONDITIONS AT 23 ± 1°C

[hexanol] (mM)	Apparent K_m (mM)	Apparent V (μmol/s per ml cell water)
0	1.24	0.353
6	0.659	0.131

nylhexatriene) causes significant changes in the kinetic parameters with K_m being depressed by 48% and V being decreased by 63%. Hexanol thus increases the apparent affinity of the system for uridine, but decreases the maximum rate of transport to a somewhat greater extent.

Galactose transport is rather refractory to hexanol inhibition. As was the case with erythritol diffusion, concentrations of hexanol below 10 mM or so have no effect on galactose transport. Higher concentrations of hexanol do depress galactose transport, but the responsiveness of the system to hexanol is quite small.

Our results are qualitatively similar in several respects to those of Read and McElhaney [37] who studied the effects of ether and of exchanging membrane cholesterol for cholest-4-en-3-one (a noncondensing steroid) on glucose and uridine transport in human red cells. Both of these treatments are known to increase fluidity of membrane lipids, but since fluidity was not quantified in this study, quantitative comparisons with our results are difficult. Read and McElhaney also found the red cell monosaccharide transport system to be relatively insensitive to increased membrane fluidity. Red cells treated with 100 mM ether and red cells in which a fraction of membrane cholesterol was replaced by cholestenone showed only modest (15—20%) decreases in the V value for glucose transport. The uridine transport system responded to replacement of cholesterol with cholestenone in a similar fashion to our cells treated with hexanol: decreases in both K_m (17%) and V (52%) were observed. One significantly different finding was that Read and McElhaney found that 100 mM ether was without significant effect on uridine transport. Thus, it appears that the monosaccharide and nucleoside transport systems are affected quite differently by changes in membrane fluidity, uridine being sensitive and galactose being relatively insensitive, but in both cases transport appears to change in similar ways when the membrane lipids are made more fluid by hexanol and when they are made more fluid by exchanging cholesterol for cholestenone.

Na^+ and K^+ transport

Incubation of red cells in *p*-chloromercuribenzenesulfonate in medium IA (high $[Na^+]$, low $[K^+]$) gave typical concentrations in the resealed ghosts of Na^+ of about 110 mequir./l red cells and of K^+ about 15 mequir./l red cells. Incubation with medium IB (low $[Na^+]$, high $[K^+]$) led to resealed ghosts with $[Na^+]$ about 36 mequir. red cells and $[K^+]$ about 88 mequir./l red cells. Hexanol, at concentrations from 3 to 15 mM, was without significant effect on the pump-mediated (ouabain-inhibited) or passive (ouabain-insensitive) fluxes of Na^+ and K^+. We found that 15 mM hexanol produces an approx. 30% decrease in the microviscosity of membrane lipids as monitored using diphenylhexatriene. Fig. 4 shows the results of a typical experiment with high $[Na^+]$, low $[K^+]$ red cells: 9 mM hexanol has no discernable effects on ion fluxes. Similar results were obtained with the low $[Na^+]$, high $[K^+]$ red cells. We did find, on the other hand, that 12 mM hexanol produces a 40% inhibition of the K^+-dependent *p*-nitrophenylphosphatase activity that is believed to be associated with the $(Na^+ + K^+)$-stimulated ATPase (Geddis, L.M. and Kutchai, H., unpublished results).

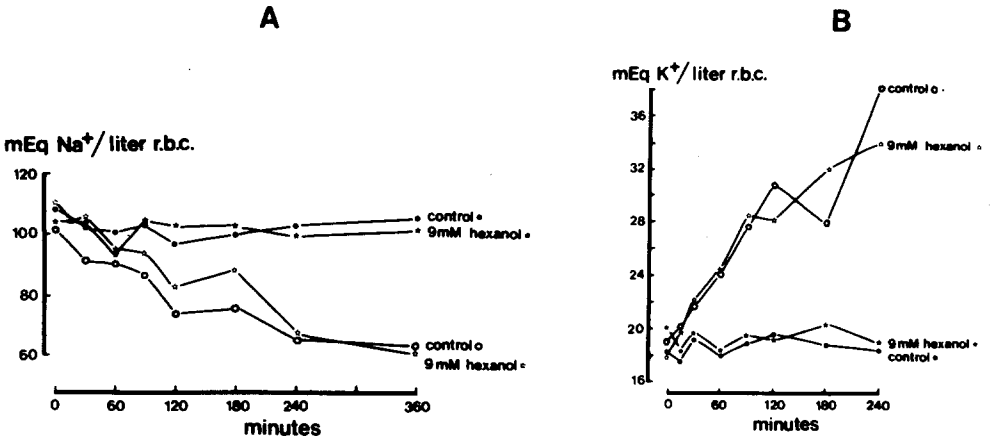

Fig. 4. The effects of 9 mM hexanol on the passive and active fluxes of Na^+ (A) and K^+ (B) in red blood cells with altered cation concentrations. Data are shown for the presence of 0.1 mM ouabain (solid symbols) and in its absence (open symbols).

Results reported in the literature on the effects of increased membrane fluidity on the (Na^+ + K^+)-stimulated ATPase are complex. General anesthetics are effective agents in increasing membrane fluidity at clinically effective levels [17]. Halsey et al. [38] and Hale et al. [39] reported that ether and chloroform at clinically used levels were without effect on the red cell (Na^+ + K^+)-stimulated ATPase, but at levels 5 to 10 times above clinical levels ether and chloroform both stimulated the pump-mediated fluxes of Na^+ and K^+. Levitt [40] found that halothane and enflurane were also without effect on the (Na^+,K^+)pump in rat brain synaptosomes at clinically used levels, but (in contrast to the results of Hale et al.) found that supra-clinical levels inhibited the pump fluxes of Na^+ and K^+. Hegyvary [41] reported that anesthetic alcohols and ether inhibited the (Na^+ + K^+)-stimulated ATPase of guinea-pig kidney, but found that the effects of these agents were complex in that they decreased the rate of hydrolysis of phosphoenzyme intermediate, but increased the rate of rephosphorylation of dephosphoenzyme.

The effects of fluidity increases by cholesterol depletion or substitution by a noncondensing steroid are similarly complex. Poznansky et al. [42] found that cholesterol depletion of human red cells led to a decrease in ouabain-sensitive K^+ efflux. Fiehn and Seiler [43] and Seiler and Fiehn [44] found that partial replacement of cholesterol by desmosterol in red blood cells and heart plasma membranes led to an increase in the activity of the (Na^+ + K^+)-stimulated ATPase. Claret et al. [45] reported that depletion of membrane cholesterol had no effect on pump fluxes in low [K^+], high [Na^+] red cells, but increased active K^+ influx in high [K^+], low [Na^+] cells. Our experiments were conducted with similar ion concentrations and under similar conditions to those of Claret et al. Our failure to find any effect of hexanol in the high [K^+], low [Na^+] cells suggests that increasing the fluidity of the membrane by using alcohols may produce a somewhat different effect on the (Na^+, K^+)-pump than increasing the fluidity of the membrane by using cholesterol removal. The effects of altered membrane fluidity on the (Na^+ + K^+)-stimulated

ATPase are sufficiently complex that we cannot place any detailed interpretation in our results or those of others.

Conclusions

One purpose for this study is to ask whether increasing the fluidity of red cell membrane lipids with anesthetic alcohols has the same effect on membrane transport processes as increasing the fluidity of the membrane to a similar extent by removing cholesterol. The answer appears to be that it occurs sometimes.

The simple diffusion of erythritol, as has been described above, shows a sort of threshold effect when hexanol is added or when cholesterol is removed [34]. The threshold occurs when the fluidity of membrane lipids (as monitored using diphenylhexatriene) has increased by approx. 20%.

Galactose transport is relatively insensitive to increase in membrane fluidity either by hexanol addition or cholesterol removal. Uridine transport is quite sensitive to both treatments and in both cases the inhibition of uridine transport involves decreases in the apparent K_m and V values, with V being decreased to a greater extent.

With the $(Na^+ + K^+)$-stimulated ATPase, on the other hand, there appears to be a discrepancy between the response to hexanol and to cholesterol removal. We found no effect of hexanol at concentrations up to 15 mM on passive or active fluxes of Na^+ and K^+ under conditions in which other workers [45] find that cholesterol removal stimulates active pump fluxes.

Water transport in red cells also responds differently to alcohols and to cholesterol removal [46]. Alcohols decrease the osmotic water permeability of the red cell membrane, while cholesterol removal has no effect.

A more complete understanding of the effects of alterations of membrane lipid fluidity on transport processes will probably depend on more precise ways to define, alter, and measure membrane fluidity and on a more detailed understanding of the molecular mechanisms of membrane transport.

Acknowledgements

This work was supported by grants R01 GM24168 and K04 HL00014 from the National Institutes of health.

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