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In Vitro Ethanol Effects on the Transport Properties of Isolated Renal Brush-Border Membrane Vesicles

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Summary. The *in vitro* effect of ethanol on membrane structure and transport properties was studied in isolated renal brush border membrane vesicles. ^{31}P-NMR studies showed a dose-dependent increase in the quantity of an isotropic, possibly inverted-micellar component of the renal brush-border membrane as a result of treatment with ethanol. Such structures have been shown to be instrumental in the translocation of material across membrane bilayers. A ^{23}Na-NMR study of Na^+ exchange in artificial phosphatidylcholine liposomes indicated that ethanol (0.1%) was capable of rending the otherwise inert vesicles permeable to sodium, supporting the idea that ethanol may exert its action via a direct effect on the structure of the phospholipid bilayer. In the isolated renal brush-border membrane vesicles, like in the artificial liposomes, amiloride-insensitive pathways of Na^+ transport were shown to be markedly activated by ethanol. These results were consistent with the inhibitory effect ethanol had on Na^+ gradient-dependent transport systems such as the Na^+ gradient-dependent D-glucose transport and Na^+/H^+ exchange. In conclusion, our results indicate that ethanol exerts its effect on the renal brush-border membrane by causing a structural change in the phospholipid bilayer which activates sodium intake. The inhibitory effect of ethanol on glucose uptake and Na^+/H^+ exchange is secondary, as a result of the dissipation of the energy-producing Na^+ gradient.

Key Words renal brush-border membrane · kidney · ethanol · transport · ^{31}P-NMR · ^{23}Na-NMR

Introduction

Several *in vivo* studies suggest that ethanol affects electrolyte handling in the kidney, both in humans (McDonald & Margen, 1979*a*,*b*) and in animal models (Beard, Barlow & Overman, 1965; Sargent, Simpson & Beard, 1974*a*,*b*, 1977; Morgan, 1982). The studies in animals have shown ethanol-induced retention of water, sodium, and chloride. This has been particularly manifest in animals that receive high doses of ethanol (Beard et al., 1965). Studies using clearance techniques have shown increased urinary losses of calcium and magnesium (Sargent et al., 1974*b*; Morgan, 1982), increased reabsorption of bicarbonate (Sargent et al., 1977), and increased potassium excretion following acute ethanol administration. Increased sodium reabsorption results from both acute and chronic ethanol administration (Sargent et al., 1974*a*). Such activation of mechanisms of Na^+ transport into the renal epithelial cells may be expected to reduce the Na^+ gradient between the cell and the tubular lumen if the Na^+/K^+ ATPase pump does not increase its activity to drive the additional Na^+ out. Such a reduction in the Na^+ gradient should result in an inhibition of the Na^+ gradient-dependent reabsorption processes, such as the reabsorption of D-glucose and amino acids. Evidence for such an inhibitory effect has recently been obtained in isolated rat intestinal brush-border membranes (Hunter et al., 1983).

Since it has been found that the potency of alkanol drugs depends on their lipid solubility, it is thought that ethanol exerts its biological effects by interacting with the lipid portion of biomembranes rather than by an interaction with a specific receptor molecule (Seeman, 1972; Chin & Goldstein, 1976; Goldstein & Chin, 1981). This interaction of alcohol with the membrane-lipid involves the incorporation of the alcohol molecules into the membrane as evident from the actual expansion of the membrane (Seeman, 1972), and from NMR (Metcalfe, Seeman & Burgen, 1968) and ESR (Goldstein & Chin, 1981) studies. In recent years, a ^{31}P-NMR methodology has been developed as a unique physical tool to probe into the structure of membranes. The ^{31}P-NMR spectrum of phospholipid headgroups strongly depends on the structural and motional conditions of a particular membrane system (McLaughlin et al., 1975; Cullis & McLaughlin, 1977). Therefore, it can provide invaluable information about the various lipid arrangements, such as liquid crystalline, gel, bilayer, or hexagonal (H_{II}). The ^{31}P nucleus is naturally found (100% isotopic abundance) in each phospholipid molecule and,

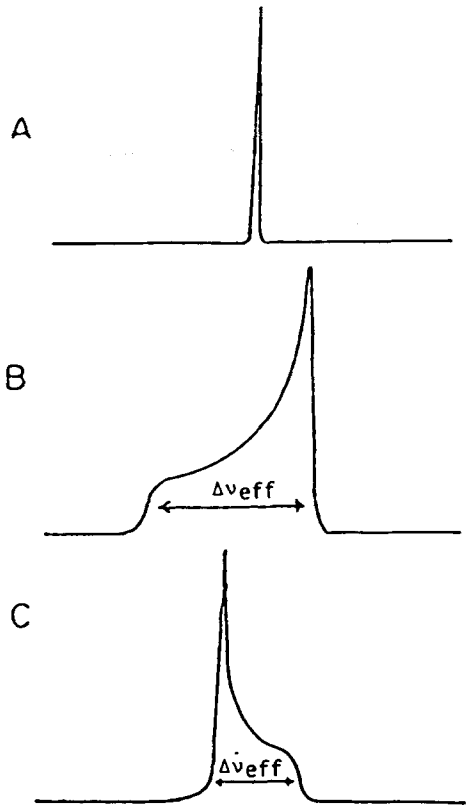

Fig. 1. The schematic ^{31}P-NMR spectra of various typical structures of phospholipid membranes. (*A*) Isotropic. (*B*) Bilayer. (*C*) Hexagonal (H_{II})

therefore, the need to introduce extrinsic probes, e.g., nitroxide labels in ESR studies, or deuterium labels in ^{2}H-NMR, does not exist. Also, ^{31}P is a relatively high-sensitivity NMR nucleus.

Therefore, we have used ^{31}P-NMR in conjunction with transport studies in an attempt to detect ethanol-induced structural changes in the renal brush-border membrane, under conditions identical to those that modify its transport properties.

Materials and Methods

Brush-Border Membranes

Brush-border membrane vesicles were isolated from the kidney cortex of Sprague-Dawley rats by a method described previously (Elgavish, Rifkind & Sacktor, 1983). After isolation, membranes used for Na^+ gradient-dependent D-glucose transport measurement or for ^{31}P-NMR studies of membrane structure were washed with a medium containing 100 mM sucrose, 100 mM KCl and 5 mM Tris/HEPES buffer, pH 7.5. Membrane vesicles used for Na^+ transport were washed with 150 mM KCl, 25 mM 2-(N-morpholino) ethanesulfonic acid (MES) and 4.6 mM Tris, pH 5.5.

Incubation of Brush-Border Membrane Vesicles with Ethanol

Membrane vesicles were incubated in a medium identical to that used for washing (vide supra) at 0°C, for 60 min, with varying concentrations of ethanol. The concentration range used, 1–4% vol/vol, is clearly beyond the blood plasma levels encountered *in vivo*, which rarely exceed 0.5%. Nevertheless, we decided to use these increased levels in our *in vitro* studies in order to enhance the transport and structural phenomena induced by ethanol. These phenomena are better observable at these levels. They are certain to exist at the lower "physiological" levels, although undetectable by available experimental techniques, and exert long-term deleterious effects. Magnification, in the short term experiments, by increasing concentrations is acceptable.

Transport Measurements

Na^+-gradient-dependent D-glucose uptake by brush-border membrane vesicles was measured by a Millipore filtration technique, using 0.45 μm filters, as described previously (Elgavish, Rifkind & Sacktor, 1983). The temperature of the uptake medium was 22°C. The extravesicular medium contained (in mM) 100 sucrose, 100 NaCl, 5 Tris/HEPES buffer, pH 7.5, and 50 μm [^{3}H]-D-glucose. The labeled sugar was obtained from New England Nuclear.

Na^+ transport was followed using the method described by Freiberg, Kinsella and Sacktor (1982). The extravesicular medium contained (in mM) 144 KCl, 5 MES, 13 Tris, 13 HEPES, pH 7.5, and 1 ^{22}NaCl (22°C). The labeled ^{22}NaCl was obtained from Amersham. Amiloride was kindly supplied by Merck, Sharp & Dohme Research Laboratories.

All transport experiments were carried out in duplicate or triplicate samples for each time point, and each experiment was repeated 3–4 times on different membrane preparations. Each result is presented as an average ± standard deviation (σ_{n-1}).

Assays

Protein was determined by the procedure of Lowry et al. (1951), using bovine serum albumin as standard. All assays were carried out in triplicate.

Liposomes

Egg L-α-lecithin, purchased from Avanti Biochemicals, was used without further purification. Large unilamellar phosphatidylcholine vesicles were prepared by a dialysis method described by Mimms et al. (1981). Vesicles were loaded with 100 mM NaCl and 5 mM Tris/HEPES, pH 7.5, during the first three dialysis steps. Most of the extravesicular NaCl was then removed in a fourth dialysis step, against a solution that contained 100 mM LiCl and 5 mM Tris/HEPES, pH 7.5.

^{31}P-NMR Studies

^{31}P-NMR spectra were recorded at 162 MHz on a Bruker WH 400 NMR spectrometer of the UAB Comprehensive Cancer Center

NMR Core Facility (partially supported by NIH Core grant CA-13148). The detailed conditions for each experiment are given in the figure captions.

^{31}P-NMR has become well established as a major tool in investigating membrane polymorphism in biological membranes as well as in liposomes (Cullis & McLaughlin, 1977). The NMR spectrum of the phosphorus nucleus in the phospholipid headgroup is sensitive to the type of motion this headgroup may undergo. In the bilayer and the hexagonal-H_{II} phases the phospholipid molecules rapidly rotate about their long axis and diffuse laterally.

The extent of the motional restriction of the phospholipid headgroup can be characterized by an order parameter (McLaughlin et al., 1975). S, where S is the ratio of the observed resonance asymmetry and the theoretical one $S = \Delta\nu_{eff}/\Delta\nu_{CSA}$. S is zero for rapid isotropic motion and S equals one for "rigid lattice." These are the limiting situations while most biological membranes and unsonicated model liposomes will have intermediate values and will display ^{31}P-NMR powder spectra. Schematic spectra are shown in Fig. 1 for the isotropic (A), bilayer (B) and hexagonal (C) structures.

The bilayer-generated signal has its singular peak on the upfield side, and in the H_{II} phase the asymmetry is reversed. In other phases, like inverted-micellar, cubic or rhombic, conditions of fast lateral diffusion are able to create an effectively isotropic motion. Consequently, such structures are manifest in a relatively narrow, conspicuously symmetric phosphorus signal (Cullis & McLaughlin, 1977). In summary, the ^{31}P-NMR spectrum of membranes reflect the particular structures that may exist in these systems. Consequently, structural changes that different regions in the membranes may undergo may be observable in ^{31}P-NMR spectra.

^{23}Na-NMR Studies

^{23}Na-NMR spectra were recorded at 52.91 MHz on a Bruker CXP 200/300 spectrometer of the UAB Comprehensive Cancer Center NMR Core Facility, equipped with a broadband multinuclide probe. The detailed description of the experimental procedures is given with the results.

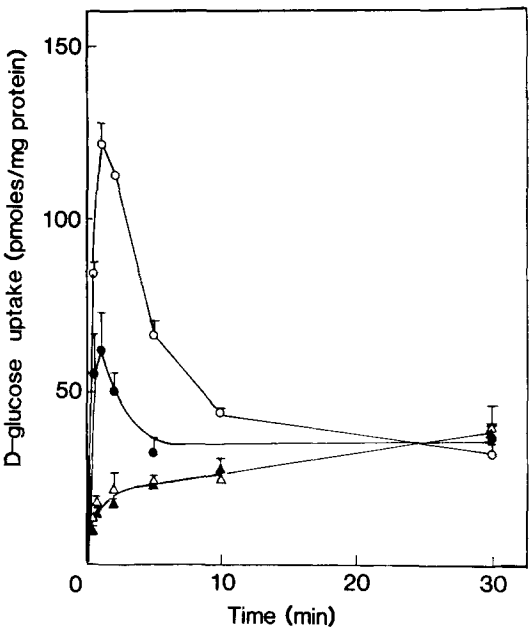

Fig. 2. The effect of ethanol on Na^+ gradient-dependent D-glucose transport in renal proximal-tubule brush-border membranes. Renal brush-border membrane vesicles were loaded with 100 mM sucrose, 100 mM KCl, 5 mM Tris/HEPES at pH 7.5. The membranes were incubated in the same medium, with or without 2% ethanol (60 min; 0°C). After incubation, the membranes were dispersed in samples of 20 μl volume (~200 μg protein), and uptake was initiated by adding 130 μl medium containing 100 mM sucrose, 100 mM KCl, 50 μM [^{3}H]-D-glucose, 5 mM Tris/HEPES at pH 7.5, with (▲) or without (△) 4% ethanol; or 100 mM sucrose; 100 mM NaCl, 50 μM [^{3}H]-D-glucose, 5 mM Tris/HEPES at pH 7.5, with (●) or without (○) 4% ethanol. Transport was carried out at 22°C, and stopped by adding 1 ml of cold solution (0°C) containing 100 mM sucrose, 100 mM NaCl, 5 mM Tris/HEPES at pH 7.5. The suspension was immediately filtered on a 0.45 μM Millipore filter. The filter was washed twice with 3.5 ml of the same solution (0°C), and the filter was then counted in an aqueous scintillation fluid

Results

The Effect of *in Vitro* Incubation with Ethanol on the Transport Properties of the Renal Brush-Border Membrane Vesicles

Membrane vesicles loaded with 100 mM sucrose, 100 mM KCl, and 5 mM Tris/HEPES, pH 7.5, were incubated with 4% ethanol for 1 hr at 0°C. Control membranes were stored on ice for an identical period of time. D-glucose uptake was then measured in an uptake medium containing (in mM) 100 NaCl, 100 sucrose, 5 Tris/HEPES at pH 7.5, or 100 KCl, 100 sucrose, 5 Tris/HEPES at pH 7.5. As shown in Fig. 2, the characteristic overshoot of Na^+ gradient-dependent D-glucose transport was observed (Elgavish, Rifkind & Sacktor, 1983). Pretreatment of the membrane vesicles with 4% ethanol results in a significant inhibition of the D-glucose transport in the presence of a Na^+ gradient (Fig. 2). Ethanol had no effect on D-glucose transport in the absence of Na^+. Uptake of D-glucose in the presence of a Na^+ gradient was significantly inhibited at all times, except at 30 min, at which time equilibrium had already been established. Ethanol depressed the D-glucose uptake by the renal brush-border membrane vesicles in a dose-dependent manner (Fig. 3).

Using counter transport D-glucose transport studies in intestinal brush-border membrane vesicles Hunter et al. (1983) concluded that ethanol did not have a direct inhibitory effect on the putative D-glucose carrier. An alternative explanation for the inhibitory effect of ethanol on Na^+ gradient-dependent D-glucose could be that ethanol increases mem-

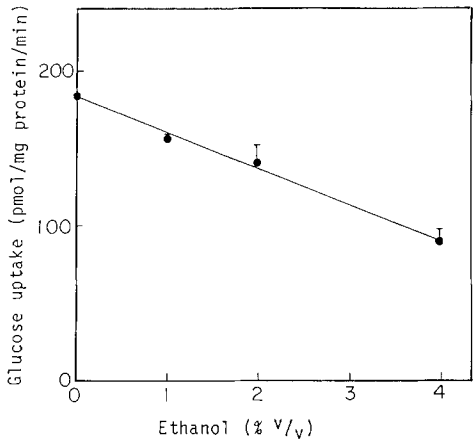

Fig. 3. The effect of varying the ethanol concentration on D-glucose uptake. Renal brush-border membrane vesicles were preincubated at 0°C for 60 min and incubated at 22°C for 1 min in the presence of 0, 1, 2, and 4% (vol/vol) ethanol. Except for ethanol concentrations, the conditions of the experiment were similar to those described in the legent of Fig. 2 in the presence of Na^+

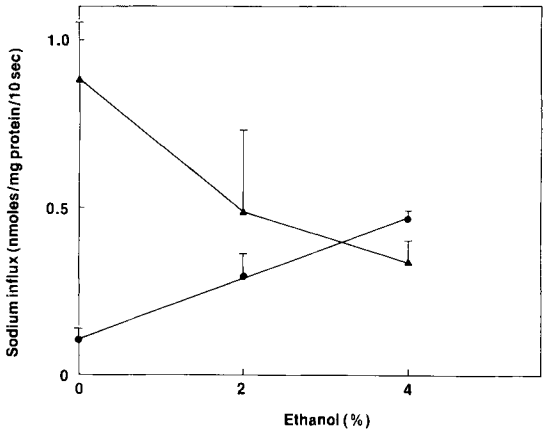

Fig. 4. The effect of ethanol on amiloride-sensitive and amiloride-insensitive pathways of Na^+transport. Renal brush-border membrane vesicles were loaded with 150 mM KCl, 25 mM 2-(N-morpholino) ethanesulfonic acid, 4.6 mM Tris at pH 5.5. The membranes were incubated in the same medium with various concentrations of ethanol (60 min; 0°C). After incubation, the membranes were dispersed in samples of 20 μl volume (~200 μg protein), and uptake was initiated by adding 130 μl medium containing 144 mM KCl, 5 mM 2-(N-morpholino) ethanesulfonic acid, 13 mM Tris, 13 mM HEPES, pH 7.5, 1 mM $^{22}NaCl$, various concentrations of ethanol in the presence or absence of 1 mM amiloride. Transport was carried out at 22°C and stopped by adding 1 ml cold solution containing 150 mM KCl, 16 mM HEPES, 10 mM Tris, pH 7.5. The suspension was immediately filtered on a 0.45 μM Millipore filter. Only results at 20 sec after the initiation of uptake (initial rate) are given. ●, Amiloride-insensitive Na^+ transport, Na^+ uptake in the presence of 1 mM amiloride. ▲, Amiloride-sensitive Na^+ transport, Na^+ uptake after subtraction of amiloride-insensitive Na^+ transport from total Na^+ transport in the absence of amiloride

brane permeability to sodium resulting in the rapid dissipation of the Na^+ gradient. Consequently, Na^+ gradient-dependent cotransport and antiport systems are inhibited. To test this possibility we measured the effect of ethanol on Na^+ transport in renal brush-border membrane vesicles. As evident from Fig. 4, ethanol had an inhibitory effect on the amiloride-sensitive Na^+/H^+ exchange and a marked stimulatory, dose-dependent effect on the amiloride-insensitive pathways. In Fig. 5, the time course of sodium uptake in ethanol-treated or untreated membrane vesicles, in the presence or absence of amiloride, shows that ethanol did not affect the steady-state level of intravesicular sodium (60 min) but rather its initial rate of uptake.

The Effect of Ethanol on Na^+ Uptake in Large Unilamellar Liposomes—^{23}Na-NMR Studies

The ^{23}Na-NMR study in large unilamellar phosphatidylcholine liposomes was initiated to find out whether ethanol may affect Na^+ permeability in this system, devoid of any active transporters, by a direct interaction with the lipid bilayer. A ^{23}Na-NMR spectrum of the liposome preparation is given in Fig. 6. Liposomes were loaded with 100 mM NaCl, 5 mM Tris/HEPES, pH 7.5. The concentration of NaCl in the extravesicular medium was lowered to ~0.5 mM by dialysis. The addition of 5 mM $Dy(EDTA)_2$ to the extravesicular medium caused a shift in the spectral position of the extravesicular sodium resonance, making possible the differentiation between the large peak of the intravesicular sodium at −0.2 ppm and a smaller peak of the extravesicular sodium at 1.7 ppm. The latter is ascribed to a residual ~0.5 mM Na^+ left from the dialysis against LiCl (*see* Materials and Methods above). The signal at 2.84 ppm is a reference line due to a NaCl solution in a separate coaxial tube. The liposome membrane is impermeable to sodium (Degani & Elgavish, 1978), and indeed no change with time in the intensity or shift in the position of these peaks was observed (results not shown). However, when 0.1% ethanol was added to this preparation a gradual decrease in the intensity of the intravesicular peak was observed accompanied by an increase in the intensity of the extravesicular peak, demonstrating a slow efflux of sodium through the phospholipid membrane. The kinetics of this slow efflux were followed and the corresponding spectra are shown in Fig. 6. No further decrease of the intravesicular Na^+ peak-intensity occurred beyond 30 min. At this time the ratio Na_{in}/Na_{out} was about 0.02, similar to the intracellular to

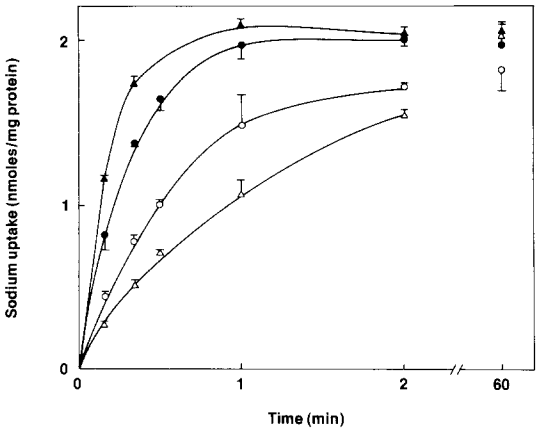

Fig. 5. The time course of sodium uptake in the presence or absence of ethanol. The experiment was carried out under conditions similar to those described in Fig. 4. ▲, Control: total sodium uptake in the absence of ethanol; ●, total sodium uptake in membrane vesicles pretreated with 4% ethanol; △, control: sodium uptake in the presence of 1 mM amiloride; ○, sodium uptake in the presence of 1 mM amiloride in membrane vesicles pretreated with 4% ethanol

extracellular volume ratio, V_{in}/V_{out}. Thus, under the conditions of this experiment, equilibrium was reached in about 30 min. The results in artificial liposomes support the idea that ethanol may activate pathways of Na^+ transport via a direct interaction with the phospholipid component of biological membranes.

The Effect of Ethanol on the Brush-Border Membrane Structure— ^{31}P-NMR Studies

To corroborate the conclusions about ethanol-induced structural changes in the renal brush-border membrane suggested by the Na^+ transport results, the ^{31}P-NMR spectra of the brush-border system were obtained in the absence and in the presence of ethanol.

The main component in all these spectra (Fig. 7) is a broad ($\Delta\nu_{1/2} \sim 2000$ Hz), asymmetric ($\Delta\nu_{eff} = 36$ ppm) signal with a singularity on its upfield side. Such a signal is indicative of primarily a bilayer structure of the brush-border membrane in the absence, as well as in the presence of ethanol. The narrow ($\Delta\nu_{1/2} = 90$ Hz), isotropic signal at 0 ppm indicates, however, the existence, in this membrane, also of a small but significant population of "lipid particles" of a possibly inverted-micellar structure (Burnell et al., 1980). Electron micrographs done on similarly prepared renal brush-border membranes showed the range of vesicle sizes previously reported for this system (Sacktor, 1977),

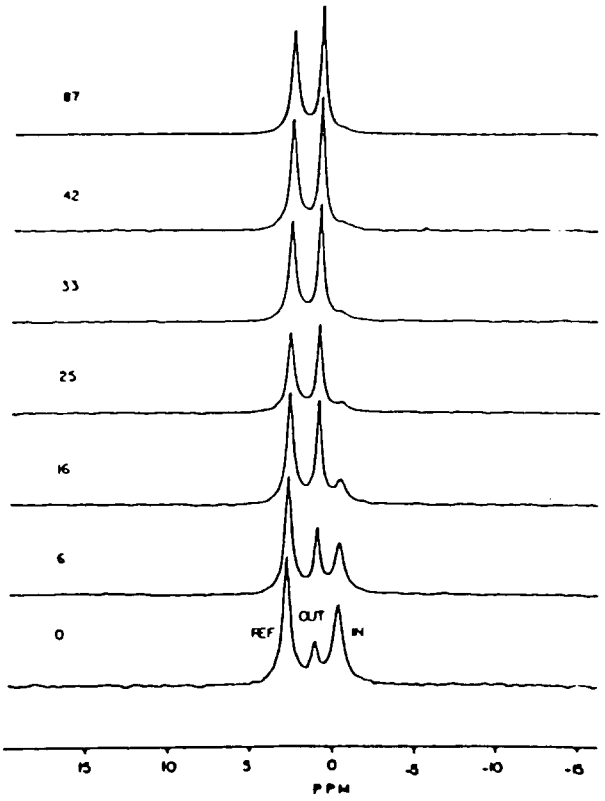

Fig. 6. The effect of ethanol on phosphatidylcholine liposome permeability to Na^+, measured by ^{23}Na-NMR. Two samples of large unilamellar phosphatidylcholine liposomes (LUV) were prepared by dialysis using octyl β-D-glucopyranoside (*cf.* Materials and Methods). In the first three dialysis steps, the detergent was removed and LUV's loaded with 100 mM NaCl, 5 mM Tris/HEPES, pH 7.5, were formed. In the last step, LUV's were dialysed against a large volume of 100 mM LiCl, 5 mM Tris/HEPES, pH 7.5. Therefore, after equilibration, LUV's suspended in a medium containing 100 mM LiCl, 5 mM Tris/HEPES, pH 7.5, and a residual amount of NaCl (approx. 0.5 mM) were obtained. In both samples, the addition of 5 mM $Dy(EDTA)_2$ separated between the intravesicular and the extravesicular Na^+ resonances. At time 0, ethanol was added to one of the samples to give a concentration of 0.1%. 52.91 MHz ^{23}Na-NMR spectra were monitored, both in the sample to which ethanol was added and in the one to which no ethanol was added, at various times afterwards. No change was observed at these times in the sample to which no ethanol was added (data not shown). Only time-dependent spectra of the sample to which 0.1% ethanol was added are shown. The spectra are the result of 200 scans accumulated with a repetition time of 0.2 sec, a 90° flip angle (60 μsec), 1 KHz spectral window and 2 K memory. *OUT* and *IN* denote the ^{23}Na-NMR resonances of the extravesicular and intravesicular Na^+, respectively. *REF* is a signal from a NaCl solution in a coaxial tube used for reference of the chemical shift scale

but no evidence for the presence of small vesicular structures (results not shown). The latter type, fast-tumbling small vesicles have been previously suggested as an alternative source, in some systems, of the narrow, isotropic signal (Cullis, DeKruijff &

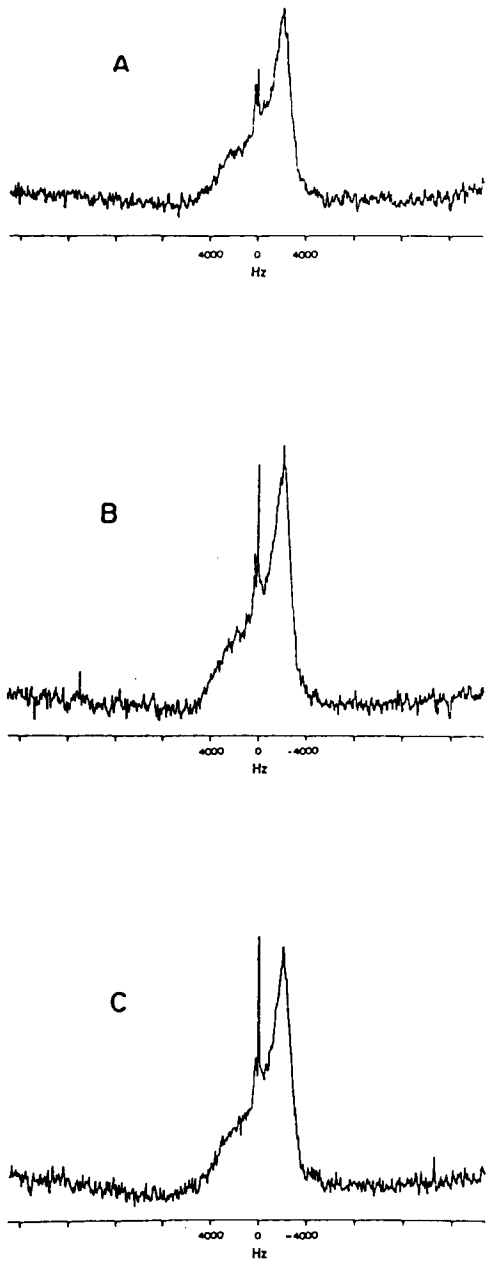

Fig. 7. ^{31}P-NMR spectra, at 162 MHz, of untreated and ethanol-treated renal brush-border membrane vesicles. A 2 ml sample (containing 30 mg brush-border membrane protein) in a 10-mm tube was run with 0.17 sec repetition time, 50 KHz spectral window, 10 μsec pulse width (45° flip angle), and 16 K memory. No proton decoupling was employed. (*A*) Control, no ethanol added (40,000 scans). (*B*) 1% ethanol added (54,000 scans). (*C*) 4% ethanol added (52,000 scans)

Richards, 1976). Their absence in our preparations strengthens our conclusion about "lipid-particle" being the source of the isotropic signal. Upon the addition of 1% (vol/vol) ethanol there is a significant increase in the intensity of the isotropic signal (Fig. 7*B*). There is further enhancement of this component when the concentration of ethanol is brought to 4% (Fig. 7*C*). No other spectral changes take place upon ethanol treatment. The additional, relatively sharp peak at 1.8 ppm, on the downfield side of the isotropic signal, does not seem to increase in intensity. It may be the downfield singularity of an H_{II} signal superimposed on the more intense bilayer component. The $\Delta\nu_{eff}$ of 18.5 ppm measured between this peak and the upfield shoulder would indeed be consistent with a hexagonal asymmetry reduced by a factor of 2 relative to the bilayer component.

Discussion

A change in the physical state of lipids following acute ethanol exposure has been shown in the intestine (Hunter et al., 1983), erythrocytes, synaptosomes and mitochondria (Chin & Goldstein, 1977; Goldstein & Chin, 1981). However, the mechanism by which this alteration is translated into a change in ion permeability is not clear (Hunter et al., 1983). ^{31}P-NMR studies may provide the desired link.

The ability of the ^{31}P-NMR spectra to differentiate among the various polymorphic phases of membranes has enabled observations that, alongside with freeze-fracture experiments, have led to the proposal that the bilayer structure of biomembranes may be in dynamic equilibrium with other phases (Cullis & DeKruijff, 1978). Such an equilibrium may be of functional importance. A 'flip-flop' mechanism, for example, could redistribute 'non-bilayer' lipids from one monolayer to the other via an inverted-micelle intermediate (Cullis & DeKruijff, 1978). In a number of liposomal systems a narrow, isotropic signal has indeed emerged as an intermediate phase whenever, by various means, transition from the bilayer to the H_{II} phase is induced. Such a transition has been induced by the introduction of either bilayer-stabilizing or H_{II}-stabilizing lipids, Ca^{2+} ions (Nayar et al., 1982), or various detergents (Madden & Cullis, 1982). It has been suggested that this isotropic intermediate phase is in fact local lipid particles with inverted-micellar structure. These lipid particles may be instrumental in translocation of material across membrane bilayers (Burnell et al., 1980), like in processes of endocytosis, exocytosis and transport of calcium or of other metal-ions (Boni et al., 1981). Since in such inverted micelles the hydrophilic phospholipid headgroups point inwards, they may maintain an aqueous internal milieu, particularly suitable for the transport of ions from one side of the bilayer to the other. Results from our ^{31}P-NMR studies in renal brush-border

membrane vesicles show a dose-dependent increase in the quantity of an isotropic, possibly inverted-micellar component of the renal brush-border membrane after treatment with ethanol.

The ^{23}Na-NMR study of Na^+ exchange in large unilamellar phosphatidylcholine liposomes indicates that ethanol (0.1%) is capable of inducing permeability to sodium in a system devoid of any active transporters. These results support the idea that ethanol may exert its action via a direct effect on the structure of the membrane phospholipids. Ethanol induced increase in the concentration of the above described lipid particles may increase the rate of Na^+ translocation across the membrane.

In the isolated renal brush-border membrane vesicles, like in the artificial liposomes, amiloride-insensitive pathways of Na^+ transport are shown to be markedly activated by ethanol. We suggest that sodium translocation by "lipid-particles" is such a pathway. However, ethanol inhibits the amiloride-sensitive Na^+/H^+ exchange. It is interesting that such a dramatic effect of ethanol on the amiloride-insensitive pathways should be measurable in the conditions under which *in vitro* Na^+ uptake experiments can be performed in isolated renal brush-border membrane vesicles ($[Na^+]$ = 1 mM). At physiological concentrations of sodium ($[Na^+]$ = 150 mM), due to the saturability of the Na^+/H^+ exchange mechanisms, the relative contribution of amiloride-insensitive pathways may be much larger. Therefore, at high Na^+ concentrations ethanol is expected to have a much larger relative effect on the amiloride-insensitive pathways than on the amiloride-sensitive Na^+/H^+ exchange. Hence, at physiological concentrations of Na^+, the stimulatory effect of ethanol is expected to be prevalent, causing a fast dissipation of the sodium gradient. This conclusion may explain the reason for the fact that in our studies in renal brush-border membranes as well as in intestinal vesicles (Hunter et al., 1983), ethanol inhibits D-glucose uptake which depends on the presence of a Na^+ gradient. We find that Na^+/H^+ exchange, another transport system which depends on the presence of a Na^+ gradient, is also inhibited by ethanol. It is unlikely that ethanol interacts specifically with two distinct carrier proteins. It would rather seem that the effect of ethanol on the Na^+ gradient-dependent transport processes is secondary to the activation of Na^+ uptake which dissipates the energy producing Na^+ gradient.

We propose the following sequence of events for the *in vitro* ethanol effect on the renal brush-border membrane. (1) Ethanol causes a change in the membrane phospholipid structure. (2) Fast, amiloride-insensitive pathways of sodium transport are activated. (3) As a result of increased Na^+ permeability, the Na^+ gradient-dependent mechanisms of transport are inhibited.

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