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# In vitro effect of ethanol on sodium and glucose transport in rabbit renal brush border membrane vesicles

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**The effect of ethanol on sodium and glucose transport in rabbit renal brush border membrane vesicles was examined. When membrane vesicles were preincubated in the presence of ethanol the sodium-dependent D-glucose uptake was significantly inhibited. This effect, as suggested by O'Neill et al. (1986) FEBS Lett. 194, 183–188, may be due to a faster collapse of the $Na^+$ gradient. As a matter of fact, the amiloride-insensitive sodium pathway was increased by ethanol in our brush border membrane preparation. However, sodium/D-glucose cotransport was inhibited by ethanol, although to a lesser degree, also in the absence of a sodium gradient. In addition, ethanol inhibited glucose-dependent sodium uptake, suggesting that a direct interaction with the translocator was involved. This conclusion was also supported by kinetic measurements showing a decrease of $V_{max}$ and an increase in $K_m$ for glucose in membrane vesicles treated with ethanol. Moreover, ethanol influenced the interaction of phlorizin with the cotransporter: uptake experiments performed in the presence of the two inhibitors demonstrated that phlorizin and ethanol behave as not mutually exclusive inhibitors of D-glucose transport. These data indicate that in rabbit renal brush border membranes ethanol not only affects the 'passive pathway', i.e. the sodium permeability, but it also directly interferes with carrier functions.**

## Introduction

The in vitro exposure of cells or isolated membranes to different concentrations of ethanol leads to changes in the activities of membrane transport agencies such as $Na^+$-dependent solute cotransporters, ion channels, membrane enzymes and membrane signalling pathways [1–17]. The functional changes following ethanol interaction with biological membranes have been ascribed to alteration in the physical-chemical characteristics of the membrane: increase in membrane fluidity [4,5,9,12], increase in ion permeability [1,6], structural changes in the phospholipid bilayer [7], expansion of membrane volume [4] and direct interactions with membrane proteins [2,3,10,11,14,16,17].

The effect of ethanol on intestinal brush border membranes has been subject to many investigations [1–6]. With one exception [2], an increase in sodium permeability and/or in membrane fluidity have been proposed as primary causes for the observed inhibition of sodium-dependent transport systems by ethanol. Intestinal membrane transport functions were significantly modified after an exposure to 2% (v/v) ethanol, a concentration similar to that observed in the intestinal lumen of human subjects following acute ethanol ingestion [18]. In contrast, similar experiments performed on rat renal brush border membranes required higher ethanol concentrations, i.e. 4% (v/v), to reveal ethanol effects otherwise undetectable by the available experimental techniques [7].

Although a concentration of 4% ethanol is largely beyond blood plasma levels encountered *in vivo*, which normally do not exceed 0.14%, a 0.20% ethanol concentration producing intoxication [19], differences between transport proteins which perform the same func-

Abbreviations: BBMV, brush border membrane vesicles; FCCP, carbonylcyanide *p*-trifluoromethoxyphenyl hydrazone; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; Mes, 2-(*N*-morpholino)-ethansulfonic acid.

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tion in different cell types can be evidenced under non-physiological conditions, if appropriate control conditions were performed. In this work data on the effect of ethanol on D-glucose and sodium transport across BBMV from rabbit kidney cortex are presented and compared with those reported for intestinal BBMV.

## Materials and Methods

*Materials.* D-[U-$^{14}$C]Glucose 208 mC /mmol and $^{22}$NaCl (carrier free) 200 $\mu$Ci/ml were purchased from Amersham (U.K.), florizin from Sigma, St. Louis, MO (U.S.A.) and FCCP from Fluka, Buchs (Switzerland). All chemicals used were reagent grade and were obtained from Fluka or Merck, Darmstadt (F.R.G.).

*Brush border membrane preparation.* BBMV were obtained from rabbit kidney cortex according to the procedure described by Malathi et al. [20]. For preloading in the desired buffer, the pellet from the second centrifugation step and the final pellet were resuspended in an appropriate medium with the composition reported in the figure legends. Membrane vesicles were resuspended at 8–15 mg/ml of proteins. Enrichments in marker enzymes were, respectively, $12 \pm 1$ (mean $\pm$ S.E., $n = 3$) for alkaline phosphatase, $12 \pm 2$ for $\gamma$-glutamyl transpeptidase and $16 \pm 3$ for maltase. Contamination by non-brush-border membranes was low (relative enrichment in $(Na^+ + K^+)$-ATPase $0.35 \pm 0.10$, lactate dehydrogenase $0.25 \pm 0.12$ and cytochrome oxidase $0.11 \pm 0.09$).

*Transport measurements.* Transport experiments were performed in triplicate or quadruplicate at 24°C by the rapid filtration technique on freshly prepared BBMV [20]. Uptake was initiated by mixing one volume of membrane vesicles, preincubated for 10 min in the presence or in the absence of 4% ethanol, and one or four volumes, depending on experimental conditions, of a radioactive cocktail with the composition reported in the figure legends. At selected times, the incubations were stopped by 100-fold dilution in ice-cold 150 mM NaCl, 1 mM Hepes-Tris, pH 7.4 (stop solution), filtered through a Sartorius filter (0.6 $\mu$m pore-size) and rapidly rinsed with 10 ml of stop solution. Initial uptake rates were determined with a semi-automatic apparatus according to Kessler et al. [21] modified as previously described [22]. In our experimental conditions, the uptake determined after 5 s of incubation time represented a good estimate of the initial velocity in the range of glucose concentrations used (data not shown). Radioactivity was measured in a scintillation counter (Tri-Carb Packard, Model 3385) and referred to protein content of incubation mixture.

*Enzyme determination.* Maltase activity was measured according to Semenza and Von Balthazar [23]. $\gamma$-Glutamyltransferase and alkaline phosphatase were assayed with Boehringer kits, using, respectively L-$\gamma$-glutamyl-3-carboxy-4-*p*-nitroanilide and *p*-nitrophenyl phosphate as substrates. $(Na^+ + K^+)$-ATPase activity was determined according to Quigley and Gotterer [24], lactate dehydrogenase according to Bergmeyer and Bernt [25] and cytochrome oxidase according to Smith [26]. Protein were determined according to Bradford [27], using a Bio-Rad kit and bovin serum albumine as a standard.

*Calculations.* Kinetic parameters of D-glucose uptake were determined with a computer program using an iterative non-linear regression procedure (ENZ-FITTER, Biosoft). Data were fitted to the following equation:

$$v_0 = \frac{V_{max}[S]}{K_m + [S]} + K_d[S]$$

where $v_0$ is the initial uptake rate, [S] is the external glucose concentration, $V_{max}$ is the maximal initial uptake rate, i.e. when $[S] \gg K_m$, $K_m$ is the Michaelis-Menten constant and $K_d$ is the initial uptake rate due to non-carrier mediated processes.

## Results

The time course of D-glucose uptake into BBMV from rabbit kidney was significantly inhibited by ethanol at all times, except at the equilibrium value. The effect was evident when the transmembrane electrical potential was generated by an inwardly directed NaSCN gradient (Fig. 1) or by an outwardly directed proton gradient in the presence of the protonophore FCCP (Fig. 2). When sodium gradients were superimposed across the vesicles with different sodium salts, D-glucose uptake decreased according to the relative permeability of the sodium accompaining anion (Table I). Under these conditions ethanol affected sodium-driven D-glucose transport, giving a quite constant percent of inhibition, whereas no effects were obtained on passive glucose movement, i.e. in the absence of any salts. In contrast to data from guinea-pig intestinal BBMV [6], a smaller, but significant, inhibitory effect was present also in the absence of a sodium gradient (Table I).

To elucidate if the effect of ethanol can be ascribed to a faster collapse of the sodium gradient and/or to a direct inhibition of the carrier function, both sodium transport and kinetic parameters of the $Na^+$/D-glucose cotransporter were evaluated. Fig. 3 shows the 0.1 mM $^{22}Na^+$ uptake driven by an inwardly directed D-glucose gradient (0 $mM_{in}$/100 $mM_{out}$). Under these conditions an appreciable amount of sodium ions are forced through the $Na^+$/D-glucose cotransport. In fact, while sodium conductive pathways is still present, the other sodium pathway, i.e. the $Na^+/H^+$ antiporter, is reduced to a very low activity by the intravescicular

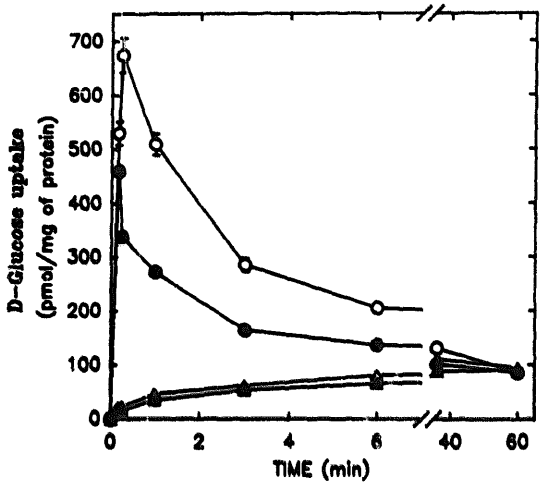


Fig. 1. Effect of ethanol on $Na^+$-dependent D-glucose uptake in BBMV from rabbit kidney cortex. BBMV were resuspended in 200 mM mannitol, 10 mM Hepes-Tris (pH 7.0). Uptake was initiated by mixing 70 $\mu$l of vesicles with 70 $\mu$l of a cocktail containing: 100 mM mannitol, 10 mM Hepes-Tris (pH 7.0), 0.2 mM D-[$^{14}$C]glucose, 200 mM NaSCN (circles) or 200 mM KSCN (triangles), with (closed symbols) or without (open symbols) 4% (v/v) ethanol. At the indicated times, 20-$\mu$l samples were withdrawn from the incubation mixture and rapidly filtered as reported in Materials and Methods. Typical experiment performed in triplicate. When not given S.E. bars were smaller than the symbol used.

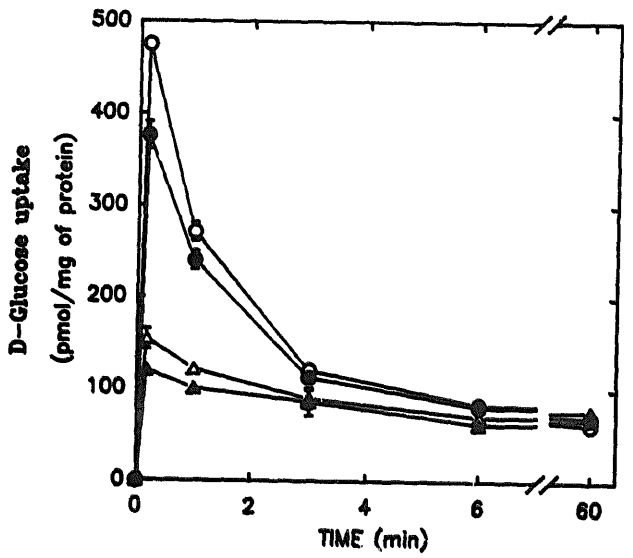


Fig. 2. Effect of ethanol on D-glucose uptake in the presence of a proton gradient in BBMV from rabbit kidney cortex. BBMV were resuspended in 193 mM mannitol, 90 mM MES-Tris (pH 5.5). Uptake was initiated by mixing 60 $\mu$l of vesicles with 240 $\mu$l of a cocktail containing: 160 mM mannitol, 90 mM Hepes-Tris (pH 7.5), 0.125 mM D-[$^{14}$C]glucose, 62.5 mM $Na_2SO_4$, with (circles) or without (triangles) 90 $\mu$M FCCP and in the presence (closed symbols) or in the absence (open symbols) of 4% (v/v) ethanol. The external pH of the incubation mixture was 7.35. At the indicated times 50-$\mu$l samples were withdrawn from the incubation mixture and rapidly filtered as described in Materials and Methods. Typical experiment performed in triplicate. When not given S.E. bars were smaller than the symbol used.

neutral pH and by the low external sodium concentration [28]. A net inhibition of $^{22}Na^+$ uptake by ethanol was observed, suggesting that an interaction of ethanol with the carrier has to be considered.

Fig. 4 shows $^{22}Na$ uptake driven by an outwardly directed proton gradient, i.e. the $Na^+/H^+$ antiport activity. In the presence of ethanol the time course of sodium uptake differed from the control for a faster release of sodium from the vesicles after the overshoot value, which is consistent with an influence of ethanol on passive sodium permeability. To discriminate between the $Na^+/H^+$ exchange activity and the amiloride-insensitive pathway, the effect of ethanol on 10 mM $^{22}NaCl$ uptake in the presence of an outwardly directed proton gradient and with or without amiloride was evaluated. As shown in Table II ethanol significantly increased the amiloride-insensitive component of the sodium uptake in BBMV from rabbit kidney cortex. These data are in agreement with an inhibitory effect of ethanol on D-glucose uptake due to a faster collapse of the initial sodium gradient.

The initial uptake rates of D-glucose uptake as a function of D-glucose external concentration across BBMV from rabbit kidney cortex can be resolved into

TABLE I

*Effect of ethanol on D-glucose uptake in the presence of different sodium salts in BBMV from rabbit kidney cortex*

BBMV were resuspended in 200 mM mannitol, 10 mM Hepes-Tris (pH 7.0), with or without 100 mM NaCl. Uptake was initiated by mixing 20 $\mu$l of BBMV with 20 $\mu$l of a cocktail containing 200 mM mannitol, 10 mM Hepes-Tris (pH 7.0), 0.2 mM D-[$^{14}$C]glucose, 200 mM of the indicated sodium salt, 4% (v/v) ethanol. After 15 s of incubation 20 $\mu$l samples were withdrawn from the mixture and rapidly filtered as described in Materials and Methods. Data represent a typical experiment performed in quadruplicate and the means ± S.E. are reported. Comparisons were made with the *t*-test. N.S., not significant.

| Sodium salt | pmol/15 s per mg protein | | Inhibition (%) | *P* |
|---|---|---|---|---|
| | control | + ethanol | | |
| NaSCN (0 $mM_{in}$ / 100 $mM_{out}$) | 921 ± 32 | 690 ± 16 | 25 | < 0.01 |
| NaCl (0 $mM_{in}$ / 100 $mM_{out}$) | 528 ± 18 | 415 ± 26 | 22 | < 0.01 |
| $Na_2SO_4$ (0 $mM_{in}$ / 50 $mM_{out}$) | 260 ± 16 | 205 ± 13 | 21 | < 0.01 |
| NaCl (100 $mM_{in}$ / 100 $mM_{out}$) | 90 ± 4 | 75 ± 2 | 16 | < 0.02 |
| No salts (200 mM $mannitol_{in}$ / 200 mM $Mannitol_{out}$) | 31 ± 2 | 29 ± 1 | 6 | N.S. |

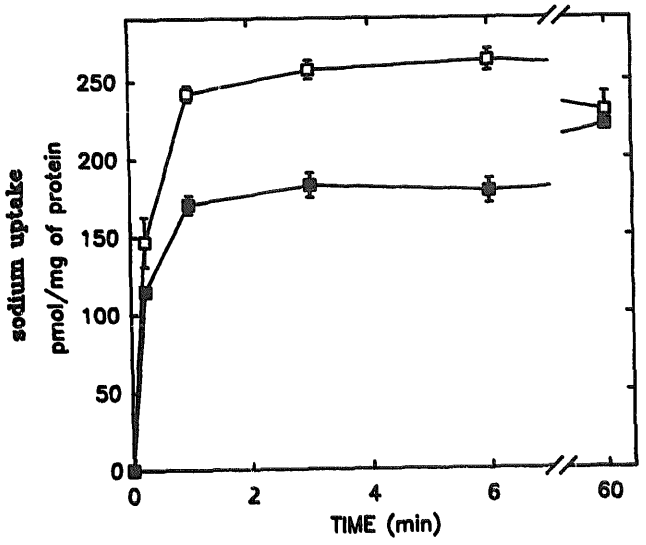


Fig. 3. Effect of ethanol on D-glucose-dependent sodium uptake in BBMV from rabbit kidney cortex. BBMV were resuspended in 200 mM mannitol, 10 mM Hepes-Tris (pH 7.0). Uptake was initiated by mixing 70 $\mu$l of vesicles with 70 $\mu$l of a cocktail containing: 200 mM mannitol, 10 mM Hepes-Tris (pH 7.0), 200 mM D-glucose, 0.2 mM NaCl, 16 $\mu$Ci/ml $^{22}$NaCl, with (closed symbols) or without (open symbols) 4% (v/v) ethanol. Typical experiment performed in triplicate. When not given S.E. bars were smaller than the symbol used.

a saturable ($Na^+$/D-glucose cotransport) and a non-saturable component. The influence of ethanol on the saturable component is reported in Fig. 5. For convenience the data are presented according to the Eadie-Hofstee transformation. The figure clearly evidenced a

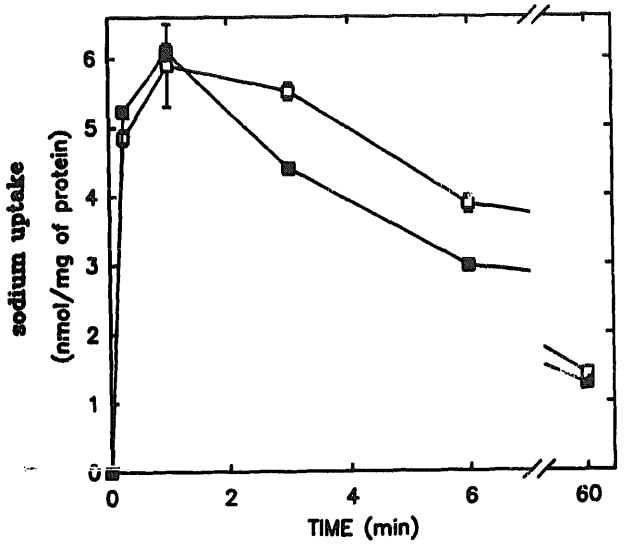


Fig. 4. Effect of ethanol on $Na^+/H^+$ exchange in BBMV from rabbit kidney cortex. BBMV were resuspended in 193 mM mannitol, 90 mM Mes-Tris (pH 5.5). Uptake was initiated by mixing 60 $\mu$l of vesicles with 240 $\mu$l of a cocktail containing: 160 mM mannitol, 90 mM Hepes-Tris (pH 7.5), 1.25 mM NaCl, 8 $\mu$Ci/ml $^{22}$NaCl with (closed symbols) or without (open symbols) 4% (v/v) ethanol. At the indicated times 50-$\mu$l samples were withdrawn from the incubation mixture and rapidly filtered as described in Materials and Methods. Typical experiment performed in triplicate. When not given S.E. bars were smaller than the symbol used.

TABLE II

*Effect of ethanol on pH-driven sodium uptake in BBMV from rabbit kidney cortex*

BBMV were resuspended in 172 mM mannitol, 90 mM Mes-Tris (pH 6.0). Uptake was initiated by mixing 60 $\mu$l of BBMV with 240 $\mu$l of a cocktail containing 156 mM mannitol, 88 mM Hepes-Tris (pH 7.5), 20 $\mu$Ci/ml $^{22}$NaCl, 12.5 mM NaCl, 4% (v/v) ethanol and, when present, 0.6 mM amiloride. After 10 s of incubation 50$\mu$l samples were withdrawn from the mixture and rapidly filtered as described in Materials and Methods. Data represent a typical experiment performed in triplicate and the means ± S.E. are reported. Comparisons were made with the *t*-test. N.S., not significant.

| Condition | pmol $^{22}$Na/10 s per mg protein | Comparisons | *P* |
|---|---|---|---|
| 1. Control | 12102 ± 1236 | 1 vs. 2 | < 0.01 |
| | | 1 vs. 3 | N.S. |
| 2. Amiloride | 2773 ± 207 | 2 vs. 4 | < 0.05 |
| 3. Ethanol | 11004 ± 250 | 3 vs. 4 | < 0.01 |
| 4. Amiloride and ethanol | 4829 ± 454 | | |

mixed-type inhibition pattern: both $V_{max}$ of the cotransporter and $K_m$ for D-glucose were altered by the presence of ethanol, suggesting that ethanol inhibition of D-glucose uptake can not be ascribed completely to the dissipation of the sodium gradient, but some inter-

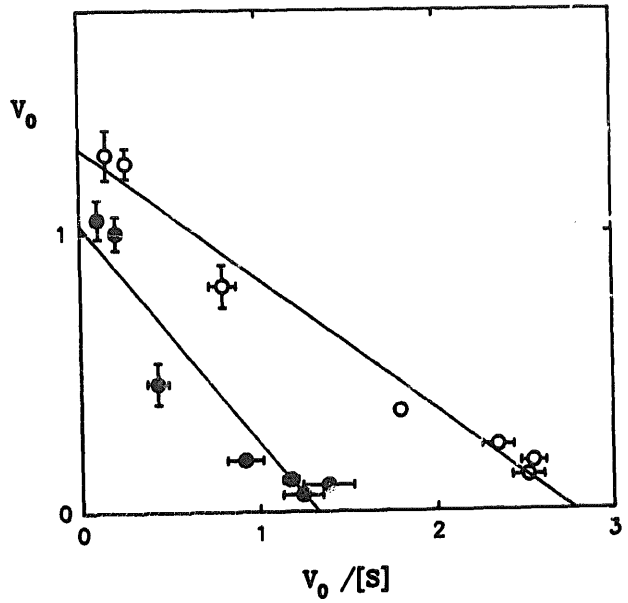


Fig. 5. Kinetics of inhibition of D-glucose uptake by ethanol in BBMV from rabbit kidney cortex. BBMV were resuspended in 200 mM mannitol, 10 mM Hepes-Tris (pH 7.0). Uptake was determined in a semiautomatic apparatus as described in Materials and Methods by mixing 5 $\mu$l of vesicles with 5 $\mu$l of different cocktails containing 200 mM mannitol, 10 mM Hepes-Tris (pH 7.0), 200 mM NaSCN, with (closed symbols) or without (open symbols) 4% (v/v) ethanol and variable amount of D-[$^{14}$C]glucose (final concentrations: from 0.05 to 10 mM). The amount taken up by the vesicles after 5 s is given. The Eadie-Hofstee plot of the initial uptake rates after subtraction of the linear component is reported. Each point represent the mean ± S.E. of quadruplicates. When not given S.E. bars were smaller than the symbol used.

TABLE III

*Kinetic parameters of D-glucose uptake in the presence and in the absence of ethanol*

Experimental conditions are reported in Fig. 4. The data from three different membrane preparations are presented. $K_m$ in mM, $V_{max}$ in pmol/5 s per mg protein. Comparisons were made by analysis of variance.

| | Experiment No. | Control | Ethanol | P |
|---|---|---|---|---|
| $K_m$ | 1. | 0.59 ± 0.05 | 0.89 ± 0.07 | |
| | 2. | 0.46 ± 0.03 | 0.76 ± 0.06 | < 0.02 |
| | 3. | 0.66 ± 0.03 | 0.79 ± 0.05 | |
| $V_{max}$ | 1. | 6275 ± 237 | 5698 ± 122 | |
| | 2. | 2405 ± 149 | 1952 ± 91 | < 0.05 |
| | 3. | 4820 ± 108 | 3628 ± 197 | |

action with the carrier must be considered. The kinetic parameters of the results presented in Fig. 5, calculated on the saturable component of D-glucose uptake are reported in Table III, where the data obtained from three different membrane preparations are shown. Although $V_{max}$ values are different within the same experimental condition, possibly as a results of variations in membrane protein recovery, the differences between ethanol-treated and control vesicles are reproducible.

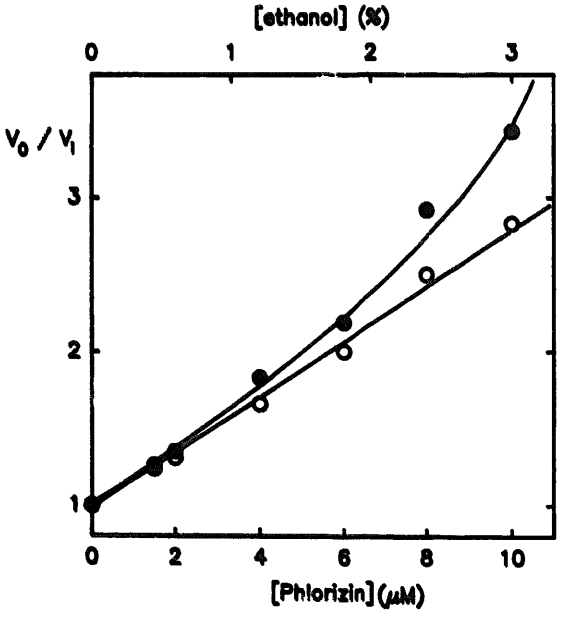


Fig. 6. Inhibition of D-glucose uptake by phlorizin and ethanol in BBMV from rabbit kidney cortex. Experimental conditions are those reported in Fig. 1 in the presence of a sodium gradient. Uptake was performed after 5 s of incubation in the presence of variable amounts of phlorizin (open symbols) or of a mixture of phlorizin and ethanol in a constant concentration ratio (Yagi-Ozawa plot; closed symbols). $v_0$ = uninhibited initial uptake rate; $v_i$ = initial uptake rate in the presence of inhibitor(s).

Additional informations about an interaction of ethanol with the $Na^+$/D-glucose cotransporter can be obtained from transport experiments performed in the presence of both phlorizin, a well known competitive inhibitor of D-glucose carrier [29], and ethanol. A diagnostic plot for the mutual exclusivity of two inhibitors has been suggested by Yagi and Ozawa [30]. The plot is constructed by measuring the initial uptake rate at different concentration of the two inhibitors, maintaining them to a constant concentration ratio. The observed uptakes is then referred to the uninhibited uptake rate. The plot gives a straight line or a parabolic line for mutually or non-mutually exclusive inhibitors, respectively. Fig. 6 reports the Yagi-Ozawa plot of D-glucose uptake in the presence of phlorizin and ethanol according to the following concentration ratio: [phlorizin]/[ethanol] = 0.02. The inhibition of D-glucose uptake by phlorizin alone is also reported. The calculated $K_i$ for phlorizin was 6.25 ± 0.33 μM, in agreement with that previously reported [29]. The presence of ethanol increased the $K_i'$ for phlorizin and the Yagi-Ozawa plot is concave upward, indicating that the two inhibitors are not mutually exclusive. These data are consistent with the mixed-type inhibition of D-glucose uptake given by ethanol.

## Discussion

Several studies indicate that the $Na^+$-stimulated D-glucose transport across brush border membranes is influenced by the pretreatment with ethanol [1–7]. A dissipation of the sodium gradient, due to an increase in sodium permeability induced by ethanol, has been proposed by some authors [1,6] for the observed decrease of D-glucose uptake. Studies with electron paramagnetic resonance indicate that ethanol has a significant fluidizing effect on rat intestinal brush border membranes [4]. These data are in contrast with a preliminary report by Tillotson et al. [1] who found that ethanol does not change membrane fluidity as measured by fluorescence polarization experiments. Changes in the fluidity of the lipid bilayer was ruled out also by O'Neill et al. [6]. However, $^{31}$P-NMR studies indicate that ethanol causes structural changes of the phospholipid bilayer of rat kidney brush border membranes [7].

Recently, Hoek and Taraschi [15] did not exclude that ethanol may interfere with specific lipid–protein or protein–protein interactions in the membrane, bind to hydrophobic sites on a protein or disrupt the hydrogen-bond network that has been proposed to extent across the surface of the membrane [31]. A direct interaction of ethanol with membrane transport proteins [10,14,17] or membrane receptors [16] has been described and changes of ionic or electrostatic surface properties of membrane lipids which in turn should

affect enzyme activity have been suggested by others [11,32]. Although the experimental results reported by many authors agree with a relatively unspecific effect of ethanol on membrane transport protein, alternative hypothesis, concerning a partial interaction of ethanol with carrier proteins have not been completely explored. Only recently a direct inhibition by ethanol and other *n*-alkanols of the translocation step of choline carrier in human erythrocytes has been clearly demonstrated [17]. In different intestinal brush border membrane preparations, ethanol, in a concentration range between 0.5 and 3 %, have been reported to affect significantly D-glucose uptake [1–6], whereas a higher dose of ethanol (4%) has been used to magnify the effect on rat renal BBMV [7]. In order to present additional informations on the effect of ethanol on renal membranes, BBMV from rabbit kidney cortex were examined. As reported for rat kidney a 4% ethanol has been used to magnify some ethanol effects otherwise undetectable at lower concentrations.

In BBMV isolated from rabbit kidney ethanol affected the $Na^+$-gradient dependent D-glucose transport, by decreasing the overshoot phenomenon in the presence of a sodium electrochemical gradient or in the presence of a sodium plus a proton gradient with the addition of the protonophore FCCP (Figs. 1 and 2, respectively). However, these data can not distinguish between alterations of membrane sodium permeability and interferences with the carrier protein. To investigate these two possibilities, D-glucose and sodium transport have been studied in more details. The data reported in Tables I and III indicate that (i) ethanol decreased the $Na^+$-dependent D-glucose transport both in the presence and in the absence of a sodium gradient; (ii) both $K_m$ and $V_{max}$ of the cotransporter were modified in vesicles pretreated by ethanol. Our data are consistent with the hypothesis that ethanol interacts with the carrier. This conclusion is also confirmed by the experiments performed in the presence of phlorizin. In fact, the initial rate of D-glucose uptake, measured in the presence of both ethanol and phlorizin and plotted according to the analysis proposed by Yagi and Ozawa (Fig. 6), indicated that the two compounds behave as not mutually exclusive inhibitors of D-glucose carrier, i.e. ethanol does not directly interact with the binding site of phlorizin on the carrier. It has been demonstrated that phlorizin increases sodium permeability in hamster intestinal BBMV [33]; however, a similar effect seems unlikely under our experimental conditions, since the highest phlorizin concentration is 10-fold lower than the lowest dose used in the intestinal membranes. The overall inhibition of D-glucose across the vesicles fits with a mixed-type inhibition pattern, since a modification of $K_m$ may be explained by the collapse of membrane potential [34,35], whereas a decrease of $V_{max}$ is compatible with an uncompetitive inihibition of the protein. This implies that ethanol does not interfere with the binding of the substrate and therefore modify the translocation step. A similar conclusion has been proposed for the interaction of ethanol with the choline transport system in erythrocyte [17]. The data reported in Fig. 3 are also in agreement with these observations. As a matter of fact, sodium uptake measured in the presence of a gradient of D-glucose was significantly inhibited by ethanol. By contrast an increase in the initial sodium uptake should be obtained if ethanol would act only on the sodium permeability. A speculative explanation of all these results could be the perturbation of the interaction between the D-glucose carrier and its domain surrounding membrane lipids. A decrease of the vesicular volume, as a cause of the observed affects (suggested by Hunter et al. [4]) is unlikely since, under all experimental conditions, the amount of D-glucose or sodium associated with the membrane vesicles at the equilibrium value was not decreased by ethanol.

However, ethanol seems to increase sodium permeability as judged by the stimulation of the amiloride-insensitive sodium transport obtained when vesicles are preincubated in the presence of ethanol (Table II). In the absence of amiloride no significant effect of ethanol can be detected on the $Na^+/H^+$ exchanger. The modification of the overshoot curve, when 1 mM sodium uptake was stimulated by an outwardly directed proton gradient (Fig. 4), can be explained by a higher permeability to sodium of the BBMV. The different sensitivity to ethanol of our membrane preparation in comparison of that reported for other BBMV preparations [1–6] suggests that a general increase in sodium permeability may not be the sole effect of ethanol on membrane transport proteins, so a different composition in proteins and lipids may explain quantitatively different effects.

In conclusion, the $Na^+$/D-glucose transport in BBMV from rabbit kidney cortex is affected by ethanol. The observed effect cannot be completely accounted for by an increase in sodium permeability, but an interaction with the carrier protein and/or with surrounding lipid should also be taken into account. The different sensitivity to ethanol of the renal glucose cotransporters as compared to the intestinal ones may be the result of different interactions between membrane proteins and lipid bilayer in relation to differences in the structure of membrane components. How these interactions modify transport properties remains open to investigation.

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