Biochimica et Biophysica Acta 857 (1986) 131–138
Elsevier

BBA 73087

Effects of benzyl alcohol on enzyme activities and D-glucose transport in kidney brush-border membranes

Brigitte Carrière [a] and Christian Le Grimellec [b,*]

[a] *GRTM, Université de Montréal, Montréal, Quebec (Canada) and* [b] *INSERM U.251, Faculté de Médecine Xavier Bichat, 16, rue Henri Huchard, 75018 Paris (France)*

(Received December 6th, 1985)

Key words: Benzyl alcohol; Membrane fluidity; Alkaline phosphatase; γ-Glutamyltranspeptidase; Glucose transport; (Rat kidney brush border membrane)

Addition of increasing amounts of benzyl alcohol progressively reduced the steady-state anisotropies of diphenylhexatriene and trimethylammoniumdiphenylhexatriene in brush-border membranes from rat kidney. The decrease in order of membrane lipids, equivalent for 50 mM benzyl alcohol to that produced by a rise in temperature of approx. 6°C, had no effect on the activities of alkaline phosphatase or γ-glutamyltranspeptidase. On the other hand, benzyl alcohol markedly inhibited the D-glucose uptakes measured in the presence of a 100 mM sodium gradient. For concentrations less than 30 mM, benzyl alcohol reduced the J_{max} without significant effects on K_m, $^{22}Na^+$ uptake or the vesicular volume of brush-border preparations. Comparable results were obtained substituting octanol for benzyl alcohol. Our data strongly suggest that, at constant temperature, the D-glucose carrier present in renal brush-border membranes is extremely sensitive to variations in membrane physical state.

Introduction

Modifications of membrane lipid order and/or viscosity affect the activity of a wide variety of membrane-bound proteins. When corresponding to changes in membrane physical state, 'breaks' in Arrhenius plots of enzyme or transport activities strongly suggest that the protein under consideration is sensitive to its lipidc environment. Temperature-dependent experiments on intact brush-border membranes and on a reconstituted system [1–3] indicate that the Na^+-dependent D-glucose transport system present in the kidney may fall into this category of membrane proteins. Arrhenius plots of alkaline phosphatase activity suggest that this marker enzyme of brush-border membranes also may be affected by variations in membrane physical state [4,5]. From these plots, however, it is not possible to estimate to what extent a change in membrane fluidity at constant temperature will affect the activity of the protein. Modulation of membrane fluidity can be achieved by membrane-perturbing agents, the most popular being benzyl alcohol (for review, see Ref. 6). It is a local anesthetic which increases membrane fluidity. It readily partitions into membranes from aqueous solutions with its hydroxyl group aligned with the polar headgroup of the phospholipids, the aromatic residue being directed towards the bilayer interior [7]. Its being a neutral compound precludes any selective interaction with charged lipid species.

In the present paper, we investigated the effects of benzyl alcohol on rat kidney brush-border membranes with respect to: (1) the physical state

* To whom correspondence should be addressed.

Abbreviations: TMA-diphenylhexatriene, 1-(4'-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene.

of membranes, estimated from fluorescence polarization experiments using diphenylhexatriene and its trimethylammonium derivative as probes; (2) the Na^+-dependent D-glucose transport; (3) the activities of alkaline phosphatase and γ-glutamyltranspeptidase. γ-Glutamyltranspeptidase possesses a limited hydrophobic domain that is responsible for its membrane association (for review, see Ref. 8). Its catalytic activity, contained within a separate hydrophilic domain, is positioned away from the membrane surface. The effects of benzyl alcohol were compared to those produced by octanol, which has been shown to inhibit D-glucose transport in intestinal brush-border membranes [9].

Materials and Methods

Preparation of brush-border membrane vesicles

Brush-border membrane vesicles from rat kidney cortex (Sprague-Dawley; males, 250–300 g body weight) were prepared by the magnesium precipitation technique [10]. Enrichments in marker enzymes were, respectively, 12.7 ± 0.9 ($n = 15$) for alkaline phosphatase and 14.8 ± 1.2 ($n = 15$) for γ-glutamyltranspeptidase. Contamination by basolateral membranes was low (relative enrichment in $(Na^+ + K^+)$-ATPase activity $= 1.3 \pm 0.2$, $n = 11$). For transport experiments, vesicles were preloaded by resuspending and washing the pellet obtained from the first precipitation step (P_2 in Ref. 10) in medium A (10 mM Hepes-Tris/280 mM mannitol/50 mM KCl/0.1 mM $MgSO_4$ (pH 7.4); glucose transport) or in medium B (10 mM Tris/16 mM Hepes/300 mM mannitol/10 mM $MgSO_4$ (pH 7.4); sodium transport). The entire washing procedures were carried out in the corresponding media.

Transport measurements

Uptakes of D-[^{3}H]glucose and $^{22}Na^+$ were measured at 25°C by a Millipore filtration technique [11] on freshly prepared brush-border membrane vesicles. In D-glucose uptake studies, membrane vesicles were resuspended (10–20 mg protein/ml) in medium A containing 10^{-6} M valinomycin and preincubated at 25°C for 2 min prior to incubation.

For glucose transport vs. time experiments, incubation was initiated by addition of 500 μl of uptake medium to 100 μl vesicle suspension in the absence (control) or presence of alcohol, to give (final concentration) 10 mM Hepes-Tris, 50 mM KCl, 88 mM mannitol, 100 mM NaCl, 0.1 mM $MgSO_4$, 0.1 mM L-glucose (containing 10 μCi D-[^{3}H]glucose) and 10^{-6} M valinomycin, adjusted to pH 7.4. Benzyl alcohol or octanol were added to the uptake medium to give final concentrations of 10–40 mM and 5 mM, respectively. At various times, from 6 s to 90 min, 50 μl of the suspension were collected, diluted in 1 ml ice-cold stopping solution (10 mM Hepes/50 mM KCl/120 mM NaCl/50 mM glucose/0.1 mM $MgSO_4$/0.2 mM phlorizin (pH 7.4)) filtered through 0.65 μm filters and washed with 6 ml of the cold stopping solution. For determinations of kinetic constants of D-glucose, transport uptakes were measured at 6 s and 90 min for D-glucose concentrations ranging from 0.48 to 24 mM. Uptake of $^{22}Na^+$ was measured at 5 s in medium B containing 2.5 mM NaCl (0.2–0.3 μCi $^{22}Na^+$ per assay). Uptakes were terminated by addition of 50 μl of the brush-border vesicles suspension to 1 ml of ice-cold solution containing 160 mM $MgSO_4$/1 mM Tris-Hepes (pH 7.4), filtration and washing with 6 ml of the cold stopping solution.

Kinetic parameters of D-glucose uptake were analysed as described by Gunther and Wright [12] and Peerce and Wright [13] with a computer program using an iterative non-linear regression technique (Dr. M. Gingold, SBPH, CEN Saclay). Briefly, glucose transfer across the membranes was resolved into two components: one saturable (Na^+-dependent D-glucose transport) and one diffusional, i.e.

$$J_{glucose} = \frac{J_{max}[S]}{[S] + K_m} + P_i[S]$$

where $J_{glucose}$ is the total glucose flux, J_{max} is the maximal rate of the saturable part of uptake, [S] is glucose concentration and P_i is the apparent permeability coefficient.

Fluorescence polarization studies

Fluorescence polarization measurements were performed on a SLM 4000 apparatus (SLM Inc., Urbana, IL) as previously described [4,14]. Lipid

soluble fluorophores 1,6-diphenyl-1,3,5-hexatriene and 1-(4'-trimethylammoniumphenyl)-6-phenyl-1,3,5-hexatriene (TMA-diphenylhexatriene) were added, from 2 mM stock solutions in tetrahydrofuran and dimethyl sulfoxide respectively, to membrane suspensions (50–100 μg protein/ml, final concentration of probes 10^{-6} M). For both probes membranes vesicles were incubated 90 min at 37°C before measurements. Such an incubation time is unnecessary for TMA-diphenylhexatriene, which readily partitions into membranes, but has been kept in order to maintain identical experimental conditions for both probes. Results of steady-state depolarization experiments are expressed in terms of fluorescence anisotropy, r, with $r = [I_{\parallel} - I_{\perp}/I_{\parallel} + 2I_{\perp}]$. $I_{\parallel}$ and $I_{\perp}$ are the fluorescence intensities observed with the analyzing polarizer parallel and perpendicular, respectively, to the polarized excitation beam.

Enzyme and protein determination

Alkaline phosphatase activity was determined at 21 and 37°C as previously described using *p*-nitrophenylphosphate as substrate [4] in the absence (control) or presence of alcohol. γ-Glutamyltranspeptidase activity was determined using γ-glutamyl-*p*-nitroanilide as substrate [15]. The activity of $(Na^+ + K^+)$-ATPase was assayed as described by Post and Sen [16]. Protein was determined according to the method of Bradford [17] using bovine serum albumin as standard.

Materials

$^{22}Na^+$ (carrier-free) and D-[^{3}H]glucose were purchased from New England Nuclear. Benzyl alcohol and octanol were obtained from Aldrich Chemicals. Diphenylhexatriene and TMA-diphenylhexatriene were purchased from Molecular Probes.

Results

Effect of benzyl alcohol on the brush-border physical state

Addition of increasing amounts of benzyl alcohol to rat kidney brush-border membrane vesicles progressively reduced the order of their membrane lipids (Fig. 1). Thus, the steady-state anisotropy, r, of diphenylhexatriene in vesicles maintained at 37°C decreased from 0.235 ± 0.003 in the control to 0.210 ± 0.005 in the presence of 50 mM benzyl alcohol (four different membrane preparations). Corresponding values at 21°C were 0.284 ± 0.003 and 0.267 ± 0.004, respectively. A similar pattern emerged from experiments using TMA-diphenylhexatriene. In accordance with the literature [5,18], steady-state anisotropies of TMA-diphenylhexatriene were significantly higher than those of diphenylhexatriene ($r = 0.264 \pm 0.004$ and 0.302 ± 0.003 at 37 and 21°C, respectively, control values).

Activities of membrane-bound enzymes

Addition to brush-border membranes of benzyl alcohol up to 50 mM had no significant effect on the activities of alkaline phosphatase and γ-glutamyltranspeptidase either at 21°C or at 37°C (Table I). Lowering the concentration of *p*-

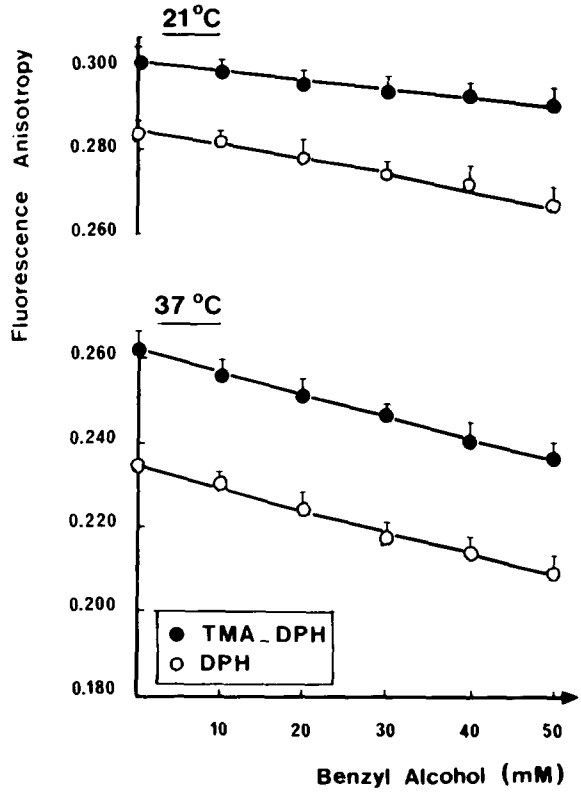

Fig. 1. Effect of benzyl alcohol on brush-border physical state. Brush-border membranes from four different membrane preparations were incubated with $1 \cdot 10^{-6}$ M diphenylhexatriene (○) or TMA-diphenylhexatriene (●). Data are means ± S.E.

TABLE I

ACTIVITIES OF ALKALINE PHOSPHATASE AND γ-GLUTAMYLTRANSPEPTIDASE IN PRESENCE OF BENZYL ALCOHOL

Values presented are means ± S.D. of three separate experiments.

[Benzyl alcohol](mM)	Alkaline phosphatase (μmol/g protein per min)		γ-Glutamyltranspeptidase (μmol/mg protein per min)	
	21°C	37°C	21°C	38°C
0	2613 ± 55	5471 ± 302	19.7 ± 2.2	55.8 ± 9.6
10	2571 ± 109	5533 ± 332	20.1 ± 2.5	58.1 ± 8.7
20	2570 ± 149	5414 ± 385	20.6 ± 2.8	58.6 ± 8.0
30	2642 ± 72	5472 ± 349	19.3 ± 2.1	53.5 ± 5.8
40	2520 ± 64	5419 ± 300	20.3 ± 2.6	53.3 ± 6.2
50	2549 ± 92	5557 ± 246	20.9 ± 2.2	54.9 ± 5.7

nitrophenylphosphate, the substrate for alkaline phosphatase, from 5 mM to 0.042 mM did not modify the response to benzyl alcohol. Similarly, for γ-glutamyltranspeptidase the kinetic constants obtained for membranes treated by 10–50 mM benzyl alcohol (K_m = 0.98–1.08 mM; V_{max} = 73–82 μmol/mg protein per min at 37°C) were comparable to those of control vesicles (K_m = 0.99 mM, V_{max} = 76 μmol/mg protein per min, 37°C).

Glucose transport

The insensitivity of the two enzymes assayed contrasted with the marked effect of benzyl alcohol on D-glucose transport. Typical overshoot curves with peak height over equilibrium value ratios above height were obtained in control veciles. Addition of 20 or 40 mM benzyl alcohol decreased these ratios to values of about 6 and 3, respectively (Fig. 2). The volume/weight ratio of the vesicles (v = 1.40 ± 0.05 μl/mg protein), determined from D-[^{3}H]glucose equilibrium values, was unaffected by benzyl alcohol in that range of

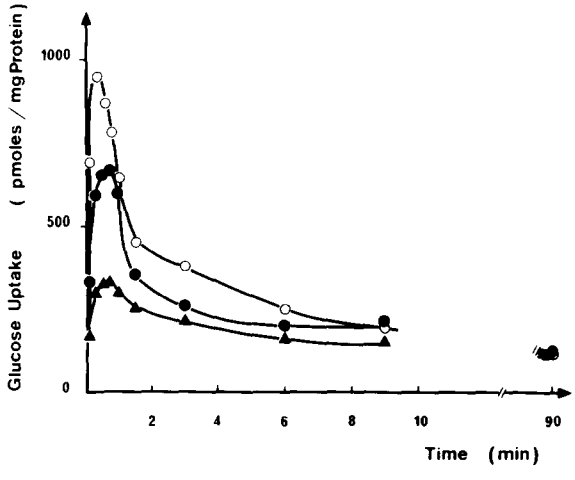

Fig. 2. D-Glucose utpake by renal brush-border vesicles. Transport was measured using 0.1 mmol/l D-[^{3}H]glucose under an inwardly directed gradient of 100 mmol/l NaCl in the absence (control, ○) or in presence of 20 (●) or 40 ▲) mM benzyl alcohol. Besides glucose, the uptake medium contained 10 mM Hepes-Tris, 50 mM KCl, 88 mM mannitol, 100 mM NaCl, 0.1 mM $MgSO_4$ and $1 \cdot 10^{-6}$ M valinomycin (pH 7.4). (Representative experiment.)

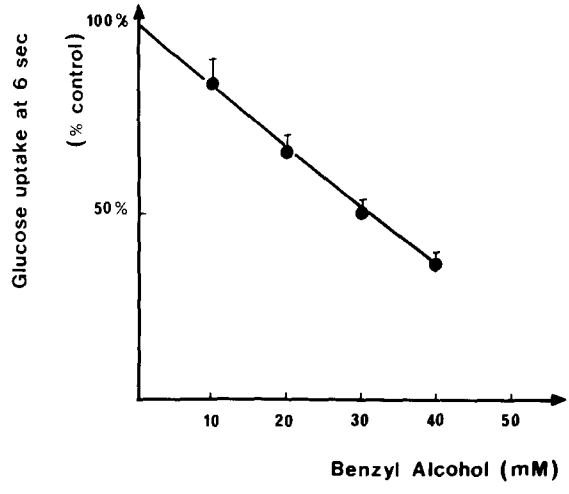

Fig. 3. Effect of benzyl alcohol on initial rates of D-glucose uptake. Uptakes at t = 6 s were measured using 0.5 mmol/l D-[^{3}H]glucose under an inwardly directed gradient of 100 mmol/l NaCl. Each data point is the mean of four determinations.

concentrations ($v = 1.46$, 1.45, 1.38 and 1.35 μl/mg protein for 10, 20, 30 and 40 mM benzyl alcohol respectively). On the other hand, initial rates of glucose uptake ($t = 6$ s) were inversely related to concentrations of benzyl alcohol between 10 and 40 mM (Fig. 3). Fig. 4 illustrates variations of these initial rates as function of D-glucose and benzyl alcohol concentrations. The kinetic constants obtained from these data are given in Table II. Addition of up to 20 mM benzyl alcohol decreased the J_{max} by 50%, leaving the K_m unchanged. Higher amounts of benzyl alcohol decreased both the K_m and the J_{max} of the transport system. The apparent permeability coefficient for glucose, P_i, corresponding to the diffusional pathways, i.e., Na^+-uncoupled glucose transport, was comparable to that determined in intestinal brush border (29.3 μl/mg per min, Ref. 13) and was not significantly affected by the addition of up to 40 mM benzyl alcohol. ^{22}Na uptake at 5 s was slightly, but not significantly, increased for 10–20 mM benzyl alcohol (Fig. 5). Addition of 30 mM and 40 mM alcohol resulted in a significant ($P \leqslant 0.05$) stimulation of Na uptake (22 and 38% respectively).

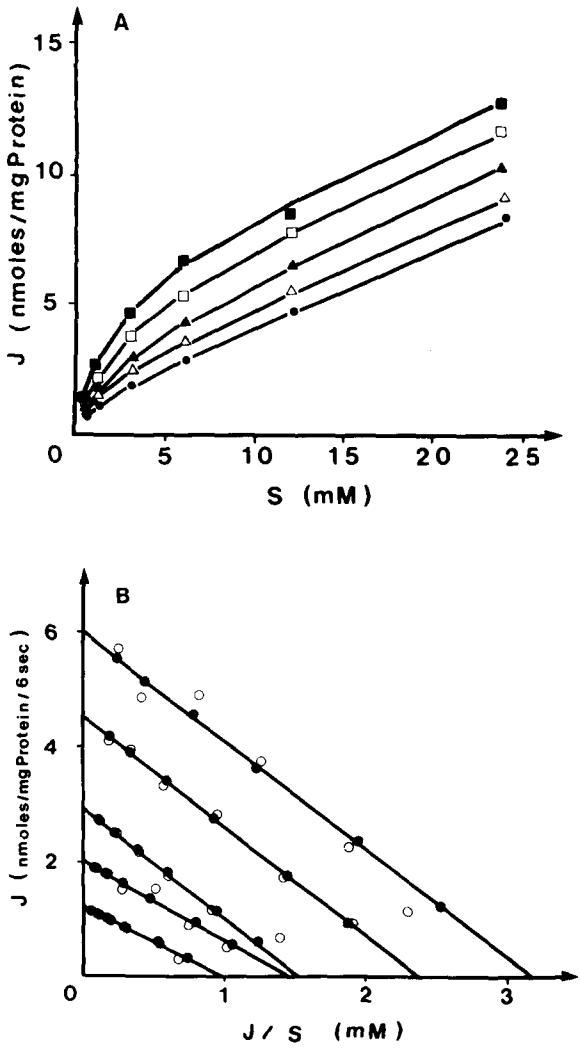

Fig. 4. Effects of benzyl alcohol on kinetic constants of D-glucose transport. Initial rates ($t = 6$ s) of D-glucose uptake were measured for D-glucose concentrations ranging from 0.48 to 24 mmol/l in control and benzyl alcohol treated vesicles. (a) Plots of $J_{glucose}$ against glucose concentrations: ■, control; □, ▲, △, ●, addition of 10, 20, 30, 40 mM benzyl alcohol, respectively. Curves were drawn by computer using a curve-fitting procedure. Each data point is the mean of four determinations. (b) Woolf-Augustinsson-Hofstee plot of glucose kinetics. $J_{glucose}$ values were corrected for contribution of the diffusional components determined from (a). ○, experimental values; ●, values calculated from curve fitting.

Effects of 5 mM octanol on brush-border membranes

Following addition of 5 mM octanol, the limiting solubility of this alcohol in buffer [19], anisotropies of diphenylhexatriene in brush border decreased from 0.284 ± 0.002 to 0.277 ± 0.002 ($P \leqslant 0.05$) at 21°C and from 0.235 ± 0.003 to 0.221 ± 0.004 ($P \leqslant 0.01$) at 37°C. These decreases were also observed with TMA-diphenylhexatriene, and compared with those produced by about 25 mM

TABLE II

EFFECT OF BENZYL ALCOHOL ON KINETIC PARAMETERS OF D-GLUCOSE TRANSPORT

Kinetic parameters were obtained from data presented in Fig. 5. Values ± S.E. P_i, apparent permeability coefficient of D-glucose. Temperature, 25°C.

[Benzyl alcohol] (mM)	K_m (mM)	J_{max} (nmol/mg protein per min)	P_i (μl/mg protein per min)
0	1.89 ± 0.21	60.0 ± 3.2	29.3 ± 1.9
10	1.94 ± 0.06	45.3 ± 0.6	30.6 ± 0.5
20	1.93 ± 0.05	29.2 ± 0.9	31.4 ± 0.5
30	1.38 ± 0.10	20.1 ± 1.8	29.5 ± 1.2
40	1.18 ± 0.03	11.9 ± 0.4	29.4 ± 0.3

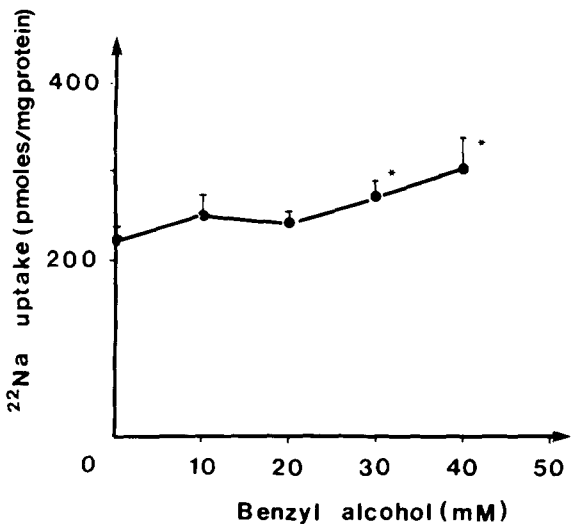

Fig. 5. ^{22}Na uptake in control and benzyl alcohol treated brush-border vesicles. Uptake at $t = 5$ s was measured using 2.5 mmol/l $^{22}Na^+$. Each data point is the mean ± S.E. of six determinations. *, different from control value ($P \leq 0.05$).

benzyl alcohol. Octanol had no significant effect on the activity of γ-glutamyltranspeptidase (treated/control ratio = 0.99), but induced a slight stimulation of alkaline phosphatase activity (treated/control ratio = 1.05). Using 0.5 mM D-glucose, D-glucose uptake at 6 s in presence of 100 mM sodium gradient was depressed by octanol to 35% of its control value (5.9 vs. 16.9 nmol/mg protein per min). The kinetic constants obtained in presence of 5 mM octanol were 1.84 ± 0.11 mM and 19.6 nmol/mg protein per min for K_m and J_{max}, respectively.

Discussion

As previously reported for liver plasma membranes [6,20], adipocytes [21], intestinal brush border [22], and more recently for kidney basolateral membranes [23], benzyl alcohol decreased the anisotropy of diphenylhexatriene, i.e., the order of lipids, in rat kidney brush-border membranes. Calculations of the lipid order parameters, S [24], indicated that addition of 50 mM benzyl alcohol to kidney brush-border membranes reduced by 4 (21°C) to 8% (37°C) the order of their lipids. In temperature-dependent experiments, this decrease, within the range of those obtained in the other biological membranes treated identically [20–22], was equivalent to that produced by an upward shift of approx. 6°C in the temperature of the brush borders. Experiments using TMA-diphenylhexatriene, which is essentially probing the glycerol backbone region and the fatty acyl chain regions down to C_8–C_{10} [25] confirmed the data obtained with diphenylhexatriene and were in agreement with the localization of benzyl alcohol in membranes.

Benzyl alcohol can regulate, by perturbing the lipid bilayer, the activity of membrane enzymes which have their functional globular domains embedded in the bilayer. On the other hand, those with their functional domains outside of the bilayer will not be perturbed by changes in membrane fluidity. Thus benzyl alcohol markedly stimulate the activity of various membrane-bound enzymes including adenylate cyclase (basal or hormone stimulated), 5′-nucleotidase, cAmP phosphodiesterase, $(Na^+ + K^+)$-ATPase and β-hydroxybutyrate dehydrogenase [20,23]. In contrast, the present data indicate that benzyl alcohol has, if any, a very limited effect on the activities of either γ-glutamyltranspeptidase or alkaline phosphatase present in brush-border membranes from rat kidney. This lack of effect on the activity of γ-glutamyltranspeptidase agrees with the observations that proteolytic treatment of membranes liberates an enzyme whose catalytic properties are unchanged [8]. Moreover, modifications of dietary essential fatty acids which markedly affect the activity of $(Na^+ + K^+)$-ATPase in rat kidney and salivary glands have no effect on the activity of γ-glutamyltranspeptidase [26]. On the other hand, the lack of a significant stimulating effect of benzyl alcohol on alkaline phosphatase activity contrasts with the 45% increase in activity reported by Brasitus and Dudeja [27] for rat colonic brush border membranes. One has to remember, however, that different genes code for kidney and intestinal alkaline phosphatase [28]. That interactions between these isoenzymes and their respective lipidic environment may also not be equivalent is shown by butanol treatment of brush-border membranes. Following butanol purification of the enzymes, the Arrhenius plot of the activity of the kidney enzyme is still biphasic [29] whereas the break is suppressed for the enzyme of intestinal origin [30]. Accordingly, differences in

the responses to benzyl alcohol addition could be due to the protein itself as well as to its relationships with membrane lipids. In fact, the behavior of renal alkaline phosphatase resembles that of phosphodiesterase I from rat liver plasma membranes: both enzymes are intrinsic proteins which show a break in their Arrhenius plot at a temperature which corresponds approximately to changes in membrane physical state [4,5,31]. For both proteins, the decrease in order of membrane lipids produced by 50 mM benzyl alcohol addition has, if any, a very limited effect on enzyme activity. Gordon et al. [20] have proposed that this insensitivity implies that the enzyme is not severely constrained in the membrane and that its functional portion may penetrate poorly into the bilayer. Such an explanation seems to hold for kidney alkaline phosphatase, which can be extracted, without damage to the transport properties of the vesicles [32,33], from brush borders by phosphatidylinositol-specific phospholipase C [34].

Contrasting with this insensitivity, D-glucose transport was markedly reduced by benzyl alcohol. A similar effect on glucose uptake by adipocyte was previously reported by Sauerherber et al. [21]. The present experiments further demonstrate that, at least for concentrations of benzyl alcohol less than 30 mM, the decrease in glucose uptake was not attributable either to a change in brush-border vesicular volume or to an enhanced permeability towards sodium ions resulting in a more rapid dissipation of the Na^+ gradient. In addition, for the experimental conditions chosen ($[K_i] = [K_0]$, $1 \cdot 10^{-6}$ M valinomycin), the decrease in Na^+-coupled D-glucose uptake cannot be accounted for by a possible modification of the overall conductivity of brush border. Accordingly, the inhibition of glucose transport is likely to result from an effect of benzyl alcohol on the glucose carrier itself either direct or via modifications of its environment. The reversibility of the effects of benzyl alcohol both in isolated membranes and in intact cells [20,21] strongly argues against an inhibition due to a partial denaturation of the carrier. The repeated observations that potency of alcohols to inhibit glucose transport in various systems, and in particular in intestinal brush border [9], is function of their solubility in membranes also argue against a direct effect on the hydrophilic part of the carrier bearing the binding site for the substrate. Moreover, for benzyl alcohol concentrations less than 30 mM, affinity of the carrier for its substrate is unchanged, whereas the V_{max} is depressed by 50%. Thus inhibition of D-glucose uptake can be due either to the alcohol competing for site(s) on the protein that are buried in the bilayer and previously occupied by lipids [20] or to modifications of the lipid order of the carrier environment. Although our experiments do not allow us to exclude the former possibility, several lines of evidence strongly argue for a major role of membrane lipid physical state as a modulator of D-glucose transport. Thus (a) breaks in Arrhenius plots of Na^+-coupled D-glucose transport corresponding to changes in membrane physical state have been described for both intestinal and renal brush borders [1,2,30]; (b) in reconstituted systems, the temperature of the break corresponds to the gel-to-liquid-crystal transition of the lipid used for the reconstitution [3]; (c) Using a series of *n*-aliphatic alcohols, Fernandez et al. [9] have shown that, in intestinal brush border, a 3% increase in membrane fluidity was associated with an 80% inhibition of D-glucose uptake whatever the alcohol used. In accordance with their data, addition of octanol to renal brush-border membranes which resulted in a 2% decrease in lipid order was associated with a 65% inhibition of D-glucose uptake. It is noteworthy that amounts of benzyl alcohol producing a similar decrease in lipid order led to a comparable inhibition of glucose transport, in spite of a strongly different molecular structure; (d) the physical state of kidney brush-border membranes has been modified by incorporation of vitamin D-3 [35]. Following this treatment, the order of membrane lipids decreased by 2–3% and the Na^+-coupled D-glucose uptake by membrane vesicles was inhibited by 40%. Taken together, these data strongly suggest that, for a constant temperature, the glucose carrier is extremely sensitive to variations in membrane physical state. In their work on glucose transport in erythrocyte and 3T3 mouse fibroblasts, Yuli et al. [36] have provided a theoretical analysis to account for an inhibition of a transport activity secondary to an increase in membrane fluidity. Briefly, an increase in membrane fluidity could produce two opposite

effects, i.e., a decrease in the number of carriers in an operating form associated with an increase in the translocation step. The over transport rate can therefore decrease if the relative weight of the former effect is predominant. Our data suggest that such a situation could prevail, at constant temperature, for kidney brush-border membranes. On the other hand, increasing membrane fluidity by raising the temperature enhances D-glucose uptake. Under these conditions, one has to suppose that the stimulating effect of temperature on the translocation process overcomes the inhibitory effect of membrane fluidity on the number of operative carriers.

Acknowledgments

This work was supported in part by the Medical Research Council of Canada and the Kidney Foundation of Canada. We thank Marguerite Bizien and Françoise Carlier for their assistance in preparing this manuscript.

References

1 Kinne, R., Murer, H., Kinne-Saffran, E., Threes. M. and Sachs, G. (1975) J. Membrane Biol. 27, 375–395
2 Kippen, I., Hirayama, B., Klinenberg, J.R. and Wright, E.M. (1979) Biochim. Biophys. Acta 556, 161–174
3 Da Cruz, M.E.M., Kinne, R. and Lin, T.J. (1983) Biochim. Biophys. Acta 732, 691–698
4 Le Grimellec, C., Giocondi, M.-C., Carrière, B., Carrière, S. and Cardinal, J. (1982) Am. J. Physiol. 242, F246–F253
5 Molitoris, B.A., Alfrey, A.C., Harris, R.A. and Simon, F.R. (1985) Am. J. Physiol. 249, F12–F19
6 Houslay, M.D. and Gordon, L.M. (1983) in Current Topics in Membranes and Transport (Kleinzeller, A. and Martin, B.R., eds.), Vol. 18, pp. 179–231, Academic Press, New York
7 Colley, C.M. and Metcalfe, J.C. (1972) FEBS Lett. 24, 241–246.
8 Curthoys, N.P. and Hughey, R.P. (1979) Enzyme 24, 383–403
9 Fernandez, Y., Goigegrain, R.A.M., Cambon-Gros, C.D. and Mitjavila, S.E. (1984) Biochim. Biophys. Acta 770, 171–177
10 Booth, A.G. and Kenny, A.J. (1974) Biochem. J. 142, 575–581
11 Leblanc, G. and Le Grimellec, C. (1979) Biochim. Biophys. Acta 554, 156–167
12 Gunther, R.D. and Wright, E.M. (1983) J. Membrane Biol. 74, 85–94
13 Peerce, B.E. and Wright, E.M. (1984) Proc. Natl. Acad. Sci. USA 81, 2223–2226
14 Carmel, G., Rodrigue, F., Carrière, S. and Le Grimellec, C. (1985) Biochim. Biophys. Acta 818, 149–157
15 Glossmann, H. and Neville, D.M., Jr. (1972) FEBS Lett. 19, 340–344
16 Post, R.A. and Sen, A.K. (1967) Methods Enzymol. 10, 762–768
17 Bradford, M.M. (1976) Anal. Biochem. 72, 248–254
18 Prendergast, F.G., Haugland, R.P. and Callahan, P.J. (1981) Biochemistry 20, 7333–7338
19 Hill, M.W. (1974) Biochim. Biophys. Acta 356, 117–124
20 Gordon, L.M., Sauerheber, R.D., Esgate, J.A., Dipple, I., Marchmont, R.J. and Houslay, M.D. (1980) J. Biol. Chem. 255, 4519–4527
21 Sauerheber, R.D., Esgate, J.A. and Kuhn, C.E. (1982) Biochim. Biophys. Acta 691, 115–124
22 Brasitus, T.A. and Schachter, D. (1980) Biochemistry 19, 2763–2769
23 Martin, K.J., McConkey, C.L., Jr. and Stokes, T.J., Jr. (1985) Am. J. Physiol. 248, E31–E35
24 Blitterswijk, K.J., Van Hoeven, R.P. and Van der Meer, B.W. (1981) Biochim. Biophys. Acta 644, 323–332
25 Engel, L.W. and Prendergast, F.G. (1981) Biochemistry 20, 7338–7345
26 Alam, S.Q. and Alam, B.S. (1983) Biochim. Biophys. Acta 819, 10–17
27 Brasitus, T.A. and Dudeja, P.K. (1985) Biochim. Biophys. Acta 819, 10–17
28 McKenna, M.J., Hamilton, T.A. and Sussman, H.H. (1979) Biochem. J. 181, 67–73
29 Hiwada, K. and Wachsmuth, E.D. (1974) Biochem. J. 141, 283–291
30 Brasitus, T.A., Schachter, D. and Mamouneas, T.G. (1979) Biochemistry 18, 4136–4144
31 Housley, M.D. (1979) Biochem. Soc. Trans. 7, 843–846
32 Yusufi, A.N.K., Low, M.G., Turner, S.T. and Dousa, T.P. (1983) J. Biol. Chem. 258, 5695–5701
33 Schäli, C., Vaughn, D.A. and Fanestil, D.D. (1984) Biochim. Biophys. Acta 769, 277–283
34 Low, M.G. and Zilvermit, D.B. (1980) Biochemistry 19, 3913–3918, Acta 769, 277–283
35 Elgavish, A., Rifkind, J. and Sacktor, B. (1983) J. Membrane Biol. 72, 85–91
36 Yuli, I., Wilbrandt, W. and Shinitzky, M. (1981) Biochemistry 20, 4250–4256