Modulation of Organic Cation Transport and Lipid Fluidity by Benzyl Alcohol in Rat Renal Brush-border Membranes

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Purpose. Organic cations are actively transported in renal brush-border membranes (BBM) by the H^+/organic cation antiport system. In the present study, we investigated the relationship between membrane fluidity and organic cation transport in the BBM.
Methods. The effects of benzyl alcohol, a membrane fluidizing agent, on the organic cation tetraethylammonium (TEA) uptake were studied using renal BBM vesicles isolated from rat kidney. BBM fluidity was assessed by fluorescence polarization technique.
Results. H^+ gradient-dependent uptake of TEA in BBM vesicles was inhibited by benzyl alcohol in a dose-dependent manner, with an apparent half inhibitory concentration of 18mM. The decrease in fluorescence anisotropy of 1,6-diphenyl-1,3,5-hexatriene in BBM, which represents the increase in membrane fluidity, was correlated with the decrease in TEA transport activity. The dissipation rate of H^+ gradient, a driving force for organic cation transport in BBM, was increased by benzyl alcohol. In addition, H^+ gradient-independent TEA-TEA exchange was also inhibited by benzyl alcohol. These findings indicate that benzyl alcohol inhibits the uptake of TEA by affecting the intrinsic activity of the organic cation transporter and the H^+ gradient dissipation rate.
Conclusions. The membrane fluidity should be an important determinant for organic cation transport in renal BBM.

KEY WORDS: renal transport; organic cation; membrane fluidity; brush-border membrane.

INTRODUCTION

Many cationic drugs are actively secreted from blood to urine by the organic cation transport system in renal proximal tubules (1). H^+ gradient-dependent uphill transport of organic cations (H^+/organic cation antiport system) was demonstrated in brush-border membrane (BBM) vesicles from the kidneys of rats, rabbits, dogs and humans (1,2). Since the luminal pH is more acidic than the intracellular pH in proximal tubules (3), it is reasonable to assume that the inward H^+ gradient (from lumen to cell) can serve as a driving force for the secretion of organic cations.

Membrane fluidity is one of the important characteristics of biological membranes and it reflects dynamic properties of the membrane lipid bilayer. It is well known that membrane fluidity is altered by diet, aging and diseases, and changes in membrane fluidity affect the activities of various enzymes and transport systems (4). To date, however, there have been no reports regarding the relationship between organic cation transport activity and BBM fluidity. In the present study, we examined the effects of membrane fluidity changes on the transport of the organic cation tetraethylammonium (TEA) in renal BBM using benzyl alcohol, a membrane fluidizing agent. For comparison, we also studied the effects of benzyl alcohol on transport activities of choline, an endogenous organic cation, and D-glucose.

MATERIALS AND METHODS

Preparation of BBM Vesicles

BBM vesicles were isolated from the renal cortex of male Wistar rats (200–230 g) by the Mg/EGTA precipitation method as described previously (5). The membrane vesicles were used for transport and fluorescence polarization studies on the day which they were prepared. The animal experiments were performed in accordance with the *Guideline for Animal Experiments of Kyoto University.*

Transport and Fluorescence Polarization Studies

Uptake of labeled TEA, choline and D-glucose by BBM vesicles was measured by a rapid filtration technique as described previously (5,6). The fluidity of BBM was assessed by measuring the steady-state fluorescence anisotropy of 1,6-diphenyl-1,3,5-hexatriene (DPH), a known hydrophobic probe (4). BBM vesicles (300 μg protein) in phosphate-buffered saline were incubated for 30 min at 37°C with 1 μM DPH. The DPH was excited at 360 nm and the emission was observed at 430 nm at 25°C using a Shimadzu spectrofluorophotometer RF-5000 (Kyoto, Japan) equipped with a polarizer attachment. The fluorescence anisotropy was calculated using the equation, $r = (I_{\parallel} - I_{\perp}/I_{\parallel} + 2I_{\perp})$, where $I_{\parallel}$ and $I_{\perp}$ are the fluorescence intensities observed with the polarized light emitted parallel and perpendicular, respectively, to the excitation polarizer.

Analytical Methods

Protein was determined by the Bradford method using a Bio-Rad Protein Assay Kit with bovine γ-globulin as a standard. The dissipation rate of H^+ gradient across BBM was measured by monitoring the changes in fluorescence of acridine orange with time (6).

Materials

[1-^{14}C]TEA bromide (0.19GBq/mmol) was obtained from Du Pont-New England Nuclear (Boston, MA) and [Methyl-^{3}H]choline chloride (3.18TBq/mmol) and [1-^{3}H]D-glucose (222GBq/mmol) were from Amersham International (Buckinghamshire, UK). All other chemicals used were of the highest purity available.

RESULTS

Effect of Benzyl Alcohol on TEA Uptake in the Presence of H^+ Gradient

Figure 1A shows the time course of TEA uptake by BBM vesicles in the presence of an outward H^+ gradient ([pH]in =

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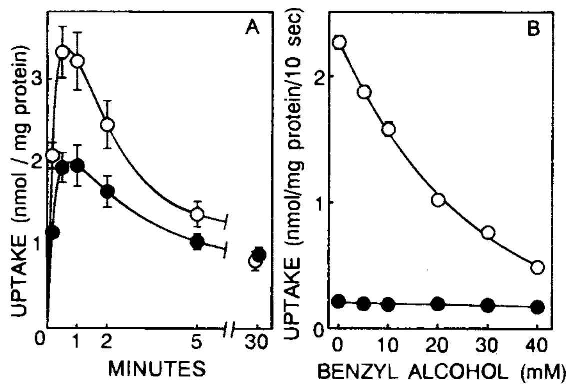

Fig. 1. (A) Effects of benzyl alcohol on TEA uptake by BBM vesicles (with H^+ gradient). Membrane vesicles (20 μl) suspended in 100 mM mannitol, 10 mM Mes (pH 6.0) and 100 mM KCl were incubated with substrate mixture (80 μl) comprised of 100 mM mannitol, 10mM HEPES (pH 7.5), 100 mM KCl and 0.3125 mM [^{14}C]TEA (final concentration, 0.25 mM) either in the absence or presence of benzyl alcohol: control (○); 20 mM benzyl alcohol (●). (B) Concentration dependence of the inhibition of TEA uptake by benzyl alcohol in BBM vesicles (with H^+ gradient). The uptake of [^{14}C]TEA for 10 sec was measured in the presence of various concentrations of benzyl alcohol either without (○) or with (●) 0.1mM cimetidine, which has high affinity to the H^+/organic cation antiporter. Each point represents the mean ± S.E. of six determinations from two experiments.

6.0, [pH]out = 7.5). In the absence of benzyl alcohol, TEA uptake was actively driven by the outward H^+ gradient, and showed marked overshoot. The initial rate and magnitude of the overshoot of TEA uptake were decreased to approximately 60% of control value by 20 mM benzyl alcohol. Equilibrium values of TEA uptake in the presence and absence of benzyl alcohol were similar, suggesting that the intravesicular volume and/or the integrity of membrane vesicles was not changed by benzyl alcohol. The inhibitory effect of benzyl alcohol on TEA uptake was reversible under our experimental conditions (data not shown).

The initial rate of TEA uptake was inhibited by benzyl alcohol in a dose-dependent manner (Fig. 1B). The apparent half inhibitory concentration (IC_{50}) was approximately 18.3 mM. The nonspecific uptake of TEA, which was measured in the presence of cimetidine, was not influenced by benzyl alcohol. Other membrane fluidizing agents, octanol, hexanol and butanol, also inhibited the H^+ gradient-dependent uptake of TEA dose-dependently, and their IC_{50}s were 0.8, 5.5 and 59.0 mM, respectively.

The relationship between fluorescence anisotropy and TEA uptake is plotted in Fig. 2. The results clearly showed that the increase in BBM fluidity was associated with the decrease in organic cation transport.

Effect of Benzyl Alcohol on the Dissipation Rate of H^+ Gradient

Effect of benzyl alcohol on the dissipation rate of the H^+ gradient, the driving force of the H^+/organic cation antiport system, was studied using acridine orange (Fig. 3), which has been used as a probe to estimate the ΔpH across membranes (6). As is evident from the plots of ln(F∞-Ft) against time, the dissipation rate of H^+ gradient was significantly increased by

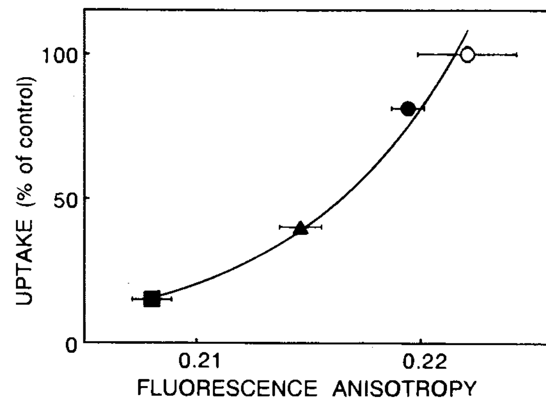

Fig. 2. Relationship between membrane fluidity and TEA transport in BBM. Fluorescence anisotropy was measured as described in "Materials and Methods" in the presence of various concentrations of benzyl alcohol: control (○); 5 mM (●), 20 mM (▲), and 40 mM benzyl alcohol (■). Error bars are ±S.E. of ten determinations from two experiments. TEA uptake by BBM vesicles was determined as described in Figure 1. Error bars are ±S.E. of six determinations from two experiments.

benzyl alcohol in a dose-dependent manner (Control, 0.027 ± 0.002; 5 mM benzyl alcohol, 0.029 ± 0.002; 20 mM benzyl alcohol, 0.041 ± 0.003; 40 mM benzyl alcohol, 0.047 ± 0.005 sec^{-1}, mean ± S.E. of six determinations from two experiments).

Effect of Benzyl Alcohol on TEA-TEA Exchange

Figure 4 shows the time course of TEA uptake at pH8.0 by BBM vesicles preloaded with unlabeled TEA. The uptake

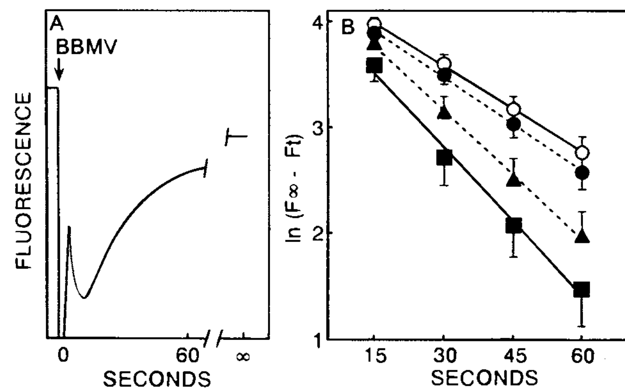

Fig. 3. Dissipation rate of the H^+ gradient in BBM vesicles. (A) Typical trace of acridine orange fluorescence with time. External buffer contained 100 mM mannitol, 10 mM HEPES (pH 7.5), 6 μM acridine orange and 100 mM KCl. At the arrow, BBM vesicles (50 μl) suspended in 100 mM mannitol, 10 mM Mes (pH 6.0) and 100 mM KCl were added to 3 ml of external buffer, and the rapid quenching of fluorescence was observed. Thereafter, the quenching of acridine orange recovered gradually with time, which reflects the dissipation phase of H^+ gradient across the BBM. (B) The plots of ln(F∞-Ft) against time, where F∞ is the final fluorescence level (10 min) and Ft is the fluorescence at time t, showing that the dissipation of the H^+ gradient follows first-order reaction kinetics. External buffer contained 100 mM mannitol, 10 mM HEPES (pH 7.5), 6 μM acridine orange, 100 mM KCl and various concentrations of benzyl alcohol: control (○); 5 mM (●), 20 mM (▲), and 40 mM benzyl alcohol (■). Each point represents the mean ± S.E. of six determinations from two experiments.

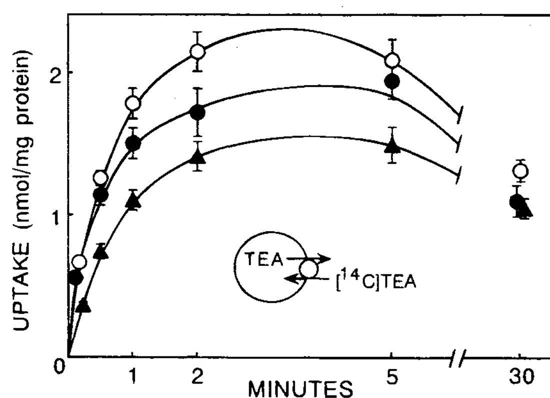

Fig. 4. Effect of benzyl alcohol on TEA-TEA exchange in BBM vesicles. Membrane vesicles (20 μl) suspended in 100 mM mannitol, 10 mM HEPES (pH 8.0), 100 mM KCl and 2.5 mM TEA were incubated with substrate mixture (180 μl) comprised of 100 mM mannitol, 10 mM HEPES (pH 8.0), 100 mM KCl and 0.278 mM [^{14}C]TEA (final concentration, 0.25 mM) in the presence of various concentrations of benzyl alcohol: control (○); 5 mM (●), and 20 mM benzyl alcohol (▲). Each point represents the mean ± S.E. of six determinations from two experiments.

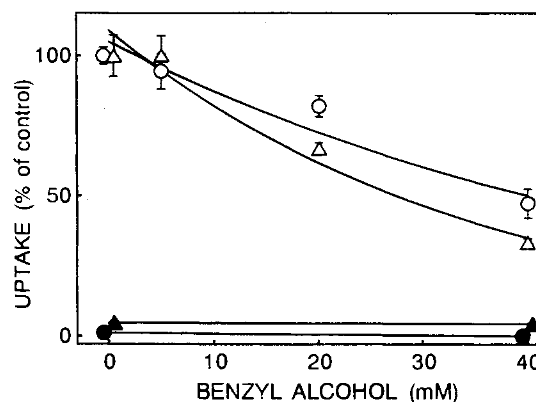

Fig. 5. Effects of benzyl alcohol on choline and D-glucose uptake by BBM vesicles. The uptake of [^{3}H]choline was measured by incubation of membrane vesicles (20 μl) suspended in 100mM mannitol, 10 mM HEPES (pH 7.5) and 100 mM Kgluconate with substrate mixture (180 μl) comprised of 100 mM mannitol, 10 mM HEPES (pH 7.5), 7 μM valinomycin and 55.6 μM [^{3}H]choline (final concentration, 50 μM) in the presence of various concentrations of benzyl alcohol either without (○) or with (●) 40 mM unlabelled choline for 10 sec. The uptake of [^{3}H]D-glucose was measured by incubation of membrane vesicles (20 μl) suspended in 300 mM mannitol and 10 mM HEPES (pH 7.5) with substrate mixture (20 μl) comprised of 100 mM mannitol, 10 mM HEPES (pH 7.5), 200 mM NaCl and 0.1 mM [^{3}H]D-glucose (final concentration, 0.05 mM) in the presence of various concentrations of benzyl alcohol either without (△) or with (▲) 0.1 mM phlorizin, an inhibitor of the Na^+/glucose cotransporter, for 10 sec. Each point represents the mean ± S.E. of six determinations from two experiments.

of [^{14}C]TEA was stimulated by preloading the vesicles with unlabeled TEA and showed apparent overshoot. Under these experimental conditions, the uptake is stimulated by direct exchange of unlabeled TEA and [^{14}C]TEA, but not by the generation of a H^+ gradient (6). Benzyl alcohol inhibited the H^+ gradient-independent TEA-TEA exchange in a dose-dependent manner.

Effect of Benzyl Alcohol on Choline and D-glucose Uptake

The initial rate of choline uptake driven by an interior-negative membrane potential was decreased by benzyl alcohol in a dose-dependent manner with an IC_{50} of 37.4 mM (Fig. 5). The initial rate of D-glucose uptake driven by an inward Na^+ gradient was also decreased by benzyl alcohol with an IC_{50} of 24.3 mM. The nonspecific uptake of choline and D-glucose, which was measured in the presence of unlabeled choline or phlorizin, respectively, was not affected by benzyl alcohol. The uptake of choline and D-glucose in the absence of an interior-negative membrane potential and an inward Na^+ gradient, respectively, was also inhibited by benzyl alcohol (data not shown).

DISCUSSION

The present study demonstrated that the H^+ gradient-dependent uptake of TEA was inhibited by benzyl alcohol in a dose-dependent manner (Fig. 1). Benzyl alcohol facilitated the dissipation rate of the H^+ gradient, the driving force for TEA uptake in BBM (Fig. 3). In addition, benzyl alcohol inhibited H^+ gradient-independent transport of TEA ([^{14}C]TEA-TEA exchange) (Fig. 4). These findings suggest that benzyl alcohol inhibits TEA transport, partly by facilitating the dissipation rate of H^+ gradient and partly by decreasing the intrinsic activity of the transporter.

In this study, we showed that the decrease in organic cation transport is well correlated with the increase in BBM fluidity by benzyl alcohol (Fig. 2). In addition, other membrane fluidizing agents, such as octanol, hexanol and butanol, also decreased the organic cation transport. Therefore, it seems likely that decrease in organic cation transport by benzyl alcohol is due to the increased BBM fluidity. However, a possibility of direct interaction between benzyl alcohol and the organic cation transporter protein can not be excluded.

It is reported that benzyl alcohol differentially affects the activities of various enzymes and transport systems (4,7). Therefore, to compare the effect of benzyl alcohol on the organic cation transport system with those on other transport systems, the uptake of choline and D-glucose were studied in the presence of benzyl alcohol. We previously demonstrated the existence of the choline transport system driven by an interior-negative membrane potential in renal BBM, and showed that the transport system is different from the H^+/organic cation antiport system (5). The uptake of choline in the presence of an interior-negative membrane potential was inhibited by benzyl alcohol in a dose-dependent manner (Fig. 5). The Na^+ gradient-dependent uptake of D-glucose was also inhibited by benzyl alcohol and its IC_{50} was 24.3 mM. This value was similar to that reported previously (7). The apparent inhibitory potency of benzyl alcohol on these transport systems was in the following order; H^+/organic cation antiport > Na^+/glucose cotransport > membrane potential-dependent choline transport.

In summary, the present results suggest that benzyl alcohol increases BBM fluidity which leads to a decrease in TEA transport in renal BBM. Thus, membrane fluidity should be an important factor regulating the organic cation transport in the kidney. These findings represent useful information for further

study on the role of membrane lipids in the transport of ionic drugs across renal epithelium, under either physiological or pathophysiological conditions.

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