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The Stimulative Effect of Diffusion Potential on Enoxacin Uptake across Rat Intestinal Brush-border Membranes

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Abstract—Evidence of a membrane potential dependence for enoxacin uptake by rat intestinal brush-border membrane vesicles has been found. The transient overshooting uptake of enoxacin disappeared in the voltage-clamped brush-border membrane vesicles in the presence of an outward H^+-gradient. Momentary dissipation of the H^+-gradient itself by carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone (FCCP) did not affect the uptake of enoxacin. In contrast, enoxacin uptake was depressed by an interior positive K^+-diffusion potential induced by valinomycin. Furthermore, not only the outward H^+-gradient but also an inward Cl^--gradient caused a stimulating effect on enoxacin uptake, and the stimulation by the Cl^--gradient was dissipated by using voltage-clamped membrane vesicles. These results indicate that enoxacin transportation across the brush-border membrane is dependent on the ionic diffusion potential. On the other hand, neither Gly-Gly nor guanidine had any effect on enoxacin uptake by the membrane vesicles in the presence of an inward (for Gly-Gly) or outward (for guanidine) H^+-gradient as a driving force for each transport system. Therefore, it seems that enoxacin transport through the intestinal epithelia does not participate in the carrier-mediated transport systems for Gly-Gly and guanidine.

Enoxacin, an orally active quinolone antibiotic, was found to be absorbed rapidly (Somogyi & Bochner 1988; Toothaker 1989) and extensively (Chang et al 1988) from the intestinal lumen. For the intestinal absorption mechanisms of quinolone antibiotics, a saturable Michaelis–Menten process for ofloxacin following rat intestinal recirculation (Prieto et al 1988) and the inhibition behaviour of dipeptides (Gly-Gly and Gly-Phe) on sparfloxacin in rat jejunal loops (Yamaguchi et al 1991) have been reported. However, the details of the transport mechanisms for these new quinolone antibiotics across the brush-border membrane of epithelial cells are unclear.

In our previous study (Iseki et al 1992), we demonstrated that the degree of uptake of the cationic form (at pH 5·5) of enoxacin was higher than that of the zwitterionic form (at pH 7·5) for the rat intestinal brush-border membrane vesicles. It is well known that the physiological pH of the intestinal lumen surface is 5·5 (Lucas et al 1976; Lucas 1983; Shimada & Hoshi 1987; Takuwa et al 1985b), and that enoxacin exists as the cationic form in a medium with this pH value. We also observed that enoxacin uptake by intestinal brush-border membrane vesicles was stimulated by a valinomycin-induced K^+-diffusion potential (interior negative) and an outward H^+-gradient (Iseki et al 1992). Moreover, we have shown that several organic cations were taken up into the intestinal brush-border membrane vesicles dependent upon the ionic diffusion potential (Sugawara et al 1992; Iseki et al 1993). We also showed that the overshoot uptake of disopyramide, a cationic compound, by an outward H^+ gradient in rat intestinal brush-border membrane vesicles was due to an interior negative H^+-diffusion potential, whereas in the renal brush-border membrane, the H^+-gradient itself (H^+ antiport system) was considered to be the driving force for the stimulation of uptake (Takahashi et al 1993). However, for enoxacin, it still remains unclear whether the H^+-coupled transport systems such as oligopeptides-H^+ symport (Ganapathy & Leibach 1983, 1985; Ferraris et al 1988) and guanidine-H^+ antiport (Miyamoto et al 1988) will affect the transport of this compound across the intestinal brush-border membrane. Therefore, this report presents the results of an investigation undertaken to clarify further details about the membrane-potential-dependence of the uptake of enoxacin by rat intestinal brush-border membrane vesicles. In addition, we have also studied the effect of glycylglycine and guanidine on the enoxacin uptake in the presence of an inward or an outward H^+-gradient as the driving force of each transporter.

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Materials and Methods

Materials

Enoxacin was kindly donated by Dainippon Pharmaceutical Co. Ltd (Osaka, Japan). Cimetidine, valinomycin, and carbonyl cyanide *p*-(trifluoromethoxy)phenylhydrazone (FCCP) were purchased from Sigma Chemicals (St Louis, MO, USA). All other chemicals were of the highest grade available and were used without further purification.

Preparation of the brush-border membrane vesicles

Brush-border membrane vesicles were prepared from small intestine of male Wistar rats, 170–220 g, by a $CaCl_2$ precipitation method (Kessler et al 1978) as described previously (Iseki et al 1992). The membrane vesicles were

usually preloaded in the buffer used for the uptake studies. The composition of each buffer is given in the legends of the figures.

Uptake studies

The uptake study was performed by a rapid filtration technique using a Millipore Filter (HAWP, 0·45 μm, 25 mm diam.) which was pretreated with 0·3% polyethylenimine to avoid nonspecific adsorption to the filter, as described previously (Iseki et al 1992). As a blank, a membrane vesicle-free incubation medium was handled in an identical manner.

Analytical methods

Enoxacin was analysed by HPLC (Hitachi L-6000, Hitachi, Tokyo, Japan) with a reversed-phase column (Inertsil, ODS, 5 μm, 25 cm × 4 mm i.d.) as described previously (Iseki et al 1992). Protein levels were determined by the method of Lowry et al (1951) with bovine serum albumin as a standard.

Results

The effects of ionophores on the uptake of enoxacin

The role of an inside-negative membrane potential on enoxacin uptake by brush-border membrane vesicles in the presence of an outward H^+-gradient was investigated. Fig. 1 shows the time course of enoxacin uptake in the presence of an outward H^+-gradient by normal and voltage-clamped brush-border membrane vesicles. Under these conditions (Miyamoto et al 1988; Iseki et al 1993; Takahashi et al 1993),

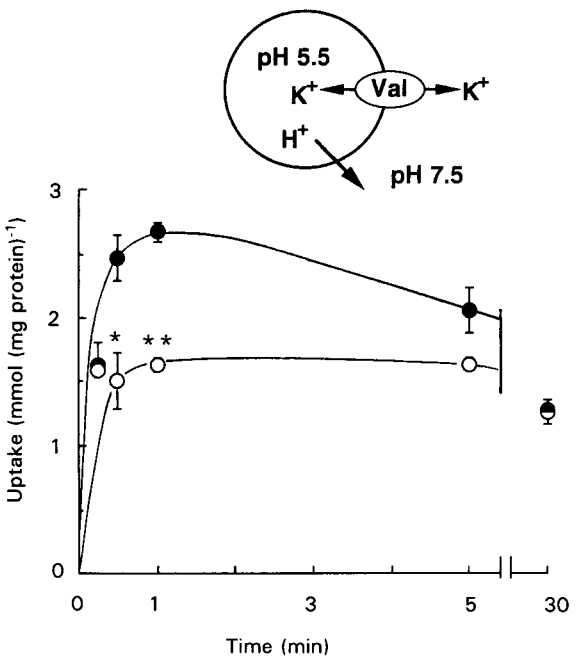

FIG. 1. Time course of enoxacin uptake by voltage-clamped brush-border membrane vesicles. Membrane vesicles (20 μL) were preincubated in 100 mM potassium gluconate, 100 mM D-mannitol and 20 mM Mes-Tris buffer (pH 5·5) with (○) or without (●) valinomycin. Uptake studies were performed by adding 100 μL transport buffer containing 100 mM potassium gluconate, 100 mM D-mannitol, 0·6 mM enoxacin and 20 mM HEPES-Tris buffer (pH 7·5). Each point represents the mean ± s.e.m. of three measurements. $*P < 0.05$, $**P < 0.001$ compared with control.

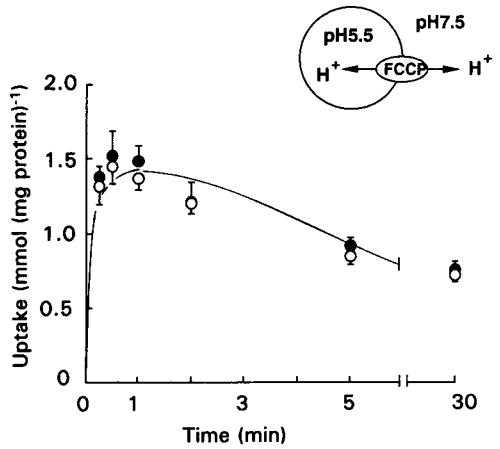

FIG. 2. Effect of an outward H^+-gradient on enoxacin uptake in the presence or absence of FCCP by intestinal brush-border membrane vesicles. Membrane vesicles were preloaded with 100 mM potassium gluconate, 100 mM D-mannitol and 20 mM Mes-Tris buffer (pH 5·5) in the presence (○) or absence (●) of FCCP. Transport solution was 0·6 mM enoxacin containing 100 mM potassium gluconate, 100 mM D-mannitol and 20 mM HEPES-Tris buffer (pH 7·5). Each point represents the mean ± s.e.m. of three or five determinations.

K^+ was presented in equimolar concentrations both inside and outside the vesicles and valinomycin, a K^+ ionophore, was added to the vesicle suspension beforehand. Therefore, all the ionic diffusion potentials are instantly compensated by K^+ movement. As shown in Fig. 1, the voltage-clamped membrane vesicles exhibited a marked decrease in the overshoot uptake of enoxacin despite the presence of an outward H^+-gradient. We also examined enoxacin uptake in

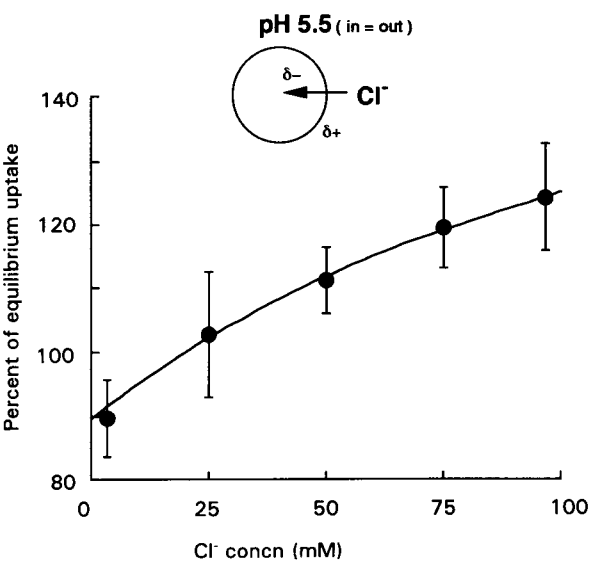

FIG. 3. Effect of an inward Cl^--gradient on the initial uptake (1 min) of enoxacin by rat intestinal brush-border membrane vesicles. Membrane vesicles were suspended in 100 mM potassium gluconate, 100 mM D-mannitol and 20 mM Mes-Tris (pH 5·5). Transport studies were performed with 20 mM Mes-Tris (pH 5·5) buffer, containing 100 mM D-mannitol and the various concentrations of potassium chloride (0–100 mM) and potassium gluconate (100–0 mM). All the osmolarities of the medium were identical to the regular assay. Each point represents the mean ± s.e.m. of six or nine measurements.

the presence of an outward H^+-gradient under experimental conditions in which the H^+-gradient was effectively dissipated. Momentary dissipation of the H^+-gradient by FCCP, a protonophore, did not affect the uptake of enoxacin (Fig. 2). Furthermore, the initial uptake of enoxacin was markedly supressed by an interior positive valinomycin-induced K^+-diffusion potential across the membrane at pH 5·5, but not at pH 7·5 (data not shown).

Effect of an inward Cl^--gradient on enoxacin uptake

Further evidence on stimulation by an intravesicular negative ionic diffusion potential was gained by determining the effect of an inward Cl^--gradient on the uptake of enoxacin by intestinal brush-border membrane vesicles. As shown in Fig. 3, at a pH of 5·5, the initial uptake of enoxacin was increased by an inward Cl^--gradient and was dependent on the Cl^- concentration of the outer medium. The increasing effect of the Cl^--gradient on the enoxacin uptake was eliminated by using voltage-clamped brush-border membrane vesicles (Fig. 4). These results are in agreement with the effects of both the valinomycin-induced K^+-diffusion potential and the FCCP-induced H^+-diffusion potential on the uptake of enoxacin (Figs 1, 2) (Iseki et al 1992).

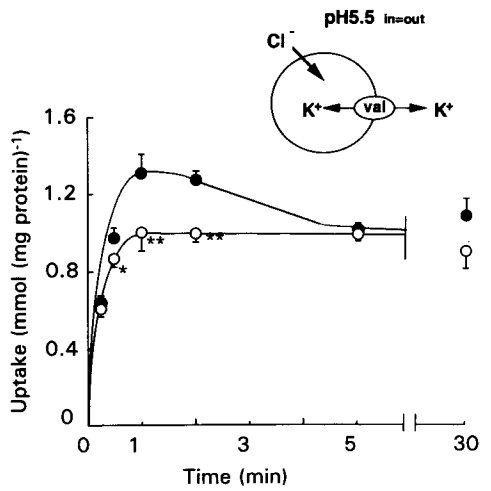

FIG. 4. Disappearance of stimulative effect of Cl^--gradient on enoxacin uptake by voltage-clamped brush-border membrane vesicles. Membrane vesicles were preincubated in 100 mM potassium gluconate, 100 mM D-mannitol and 20 mM Mes-Tris buffer (pH 5·5) with (○) or without (●) valinomycin (7 μg (mg protein)$^{-1}$). Transport buffer was composed of 100 mM KCl, 100 mM D-mannitol and 20 mM Mes-Tris buffer (pH 5·5) containing 0·6 mM enoxacin. Each point represents the mean with s.e.m. of 4–5 determinations. $*P<0·05$, $**P<0·01$ compared with corresponding experiments with valinomycin.

Inhibitory effect of dipeptide and guanidine on enoxacin uptake

To clarify whether any endogenous transport system participates in enoxacin uptake by brush-border membrane, the effects of glycylglycine and guanidine on enoxacin uptake by intestinal brush-border membrane vesicles were investigated. The former is a substrate of the dipeptide-H^+ symport system in the intestinal brush-border membrane (Takuwa et al 1985a); the latter is reported to be transported via an H^+-antiport system which recognizes the endogenous organic cations such as 5-hydroxytryptamine, dopamine and polyamines in the intestinal brush-border membrane (Miyamoto et al 1988). As shown in Table 1, however, the uptake of enoxacin by brush-border membrane vesicles was not affected by glycylglycine or guanidine even in the presence of an H^+-gradient as a driving force for each substrate (an inward H^+-gradient for glycylglycine, an outward H^+-gradient for guanidine), whereas other newer quinolones, such as ofloxacin and sparfloxacin, have been reported to be transported via a carrier-mediated process (Prieto et al 1988; Yamaguchi et al 1991).

Discussion

The results obtained from the present study give further evidence for an interior negative ionic diffusion potential-dependent uptake mechanism of enoxacin in intestinal brush-border membranes. The stimulation effect of an outward H^+-gradient on the enoxacin uptake disappeared in the voltage-clamped brush-border membrane vesicles, the effect of the H^+-gradient was not affected by the addition of FCCP, and not only an outward H^+-gradient but also an inward Cl^--gradient caused the overshoot uptake of enoxacin, and the Cl^--gradient-dependent overshooting was dissipated by the voltage-clamping treatment.

In previous reports (Iseki et al 1992, 1993; Sugawara et al 1992; Takahashi et al 1993), the permeation of several organic cations across brush-border membranes was dependent upon an interior negative H^+- or K^+-diffusion potential, although their zwitterionic derivatives were not entirely

Table 1. The initial uptake of enoxacin by intestinal brush-border membrane vesicles in the presence of glycylglycine and guanidine.

	% Control uptake		
	$pH_{in}=pH_{out}=5·5$	$pH_{in}=7·5$, $pH_{out}=5·5$	$pH_{in}=5·5$, $pH_{out}=7·5$
Glycylglycine			
5 mM	114±13	—	
10 mM	131±15	112±15	
Guanidine			
5 mM			107±6
10 mM			106±7

Membrane vesicles were preincubated in 100 mM D-mannitol, 100 mM potassium gluconate and either 20 mM HEPES-Tris (pH 7·5) or 20 mM MES-Tris (pH 5·5) buffer. Transport studies were performed with the buffer containing 100 mM potassium gluconate, 100 mM D-mannitol, 0·5 mM enoxacin in the presence or absence or Gly-Gly and guanidine.

affected by the ionic diffusion potentials. Therefore, it is suggested that a cationic form of enoxacin with the undissociated carboxyl group ($pK_{a_1}=6{\cdot}2$) and the protonated piperazinyl group ($pK_{a_2}=8{\cdot}8$) is more permeable than the zwitterionic form, as suggested in a previous paper (Iseki et al 1992).

In the present study, it was found that the uptake mechanism of enoxacin did not participate in a common transport system between dipeptides and other newer quinolones (sparfloxacin and ofloxacin). Wise et al (1986) have reported that the most rapid absorption occurred with ciprofloxacin and ofloxacin, and the slowest absorption with enoxacin, although the rates of absorption of these drugs from the intestinal tract are relatively rapid. The lesser contribution of carrier-mediated transport systems to enoxacin absorption, as described above, and the binding characteristics of enoxacin might cause it to have the slowest absorption. It has been clarified that there is a close relationship between the membrane binding (static interaction) and the transport properties (dynamic interaction) in the transport processes of organic cations into the membrane (Iseki et al 1992; Sugawara et al 1992). A recent report (Miyauchi et al 1993) showed that the criteria for the presence of carrier-mediated transport, such as mutually competitive inhibition and saturability, occurred in a phospholipid bilayer containing no proteins. Considering these results, we conclude that enoxacin is transported via a membrane potential-dependent permeation route for organic cations in the intestinal brush-border membrane. This permeation mechanism seems to work in combination with the membrane surface-binding, which may have a relative specificity among organic cations.

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