Distribution Characteristics of Levofloxacin and Grepafloxacin in Rat Kidney

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Purpose. To elucidate the renal distribution of quinolones, we examined the uptake of levofloxacin and grepafloxacin *in vivo* and in rat renal cortical slices.

Methods. The plasma and various tissue concentrations of levofloxacin and grepafloxacin were measured after a bolus injection in rats, and tissue uptake clearance was calculated. Transport characteristics of quinolones in rat renal cortical slices were evaluated.

Results. The tissue distribution of levofloxacin and grepafloxacin in the kidney was greater than in any other tissue, and the tissue uptake clearances of levofloxacin and grepafloxacin in the kidney cortex were 1.2 and 4.6 ml/min/g tissue, respectively. The uptake of levofloxacin and grepafloxacin in rat renal cortical slices was concentrative, as indicated by slice/medium ratios of 2.3 and 9.6 at 60 min, respectively. The uptake of levofloxacin and grepafloxacin in rat renal cortical slices showed saturation, and was significantly inhibited in the presence of quinidine ($p < .05$), but not of tetraethylammonium or *p*-aminohippurate.

Conclusions. Renal distribution of levofloxacin and grepafloxacin may be mediated by a specific transport system for quinolones, distinct from the organic cation and organic anion transport systems in the kidney.

KEY WORDS: levofloxacin; grepafloxacin; quinolone antibacterial drugs; renal transport; tissue distribution; cortical slices.

INTRODUCTION

Many quinolone antibacterial drugs are well absorbed in the intestine and eliminated by metabolism and urinary excretion. Quinolone antibacterial drugs possess both a carboxylic group and a cationic amine group, and these drugs are zwitterions at physiological pH. Levofloxacin, one such drug, is mainly excreted from blood into urine as the unchanged form in humans (1). We showed previously that renal handling of levofloxacin in rats involved glomerular filtration, tubular secretion and reabsorption, and that tubular secretion of levofloxacin was potently inhibited by cimetidine (2). We also reported that transcellular transport of levofloxacin from the basolateral to apical side was much greater than that in the opposite direction in a pig kidney epithelial cell line, LLC-PK_1, and that the basolateral-to-apical transport and cellular accumulation of levofloxacin were not inhibited in the presence of either tetraethylammonium or *p*-aminohippurate (3,4).

On the other hand, grepafloxacin, a recently developed quinolone drug, is mainly excreted into bile as metabolites (5). Sasabe *et al.* (6) reported that the uptake of grepafloxacin in isolated hepatocytes occured via carrier-mediated active transport, a process distinct from the transport of bile acids, organic anions, organic cations or neutral steroids. It is likely that there exist some specific transport systems in both liver and kidney. Recently, we reported concentrative accumulation of grepafloxacin in LLC-PK_1 monolayers (4). However, there have been no reports about the distribution of levofloxacin and grepafloxacin in the kidney.

In this study, in order to characterize the uptake mechanism of quinolones into the kidney from blood, we examined and compared the processes for the uptake of levofloxacin and grepafloxacin *in vivo* and in rat renal cortical slices.

MATERIALS AND METHODS

Materials

D-[^{3}H]Mannitol (828.8 GBq/mmol) was purchased from Du Pont-New England Nuclear Research Products (Boston, MA). [^{14}C]Levofloxacin (1.07 GBq/mmol) and unlabeled levofloxacin were kindly supplied by Daiichi Pharmaceutical Co., Ltd. (Tokyo, Japan) (Fig. 1). [^{14}C]Grepafloxacin (1.17 GBq/mmol) and unlabeled grepafloxacin were gifts from Otsuka Pharmaceutical Co., Ltd. (Tokyo, Japan) (Fig. 1). Cimetidine, tetraethylammonium, quinidine, and *p*-aminohippurate were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). All other chemicals used were of the highest grade available.

Tissue Distribution in Rats

The animal experiments were performed in accordance with the *Guidelines for Animal Experiments of Kyoto University.* Male Wistar rats weighing 200–220 g were anesthetized with sodium pentobarbital. Tracer amounts of [^{14}C]levofloxacin (0.5 μmol/kg, 533 kBq/ml) or [^{14}C]grepafloxacin (0.47 μmol/kg, 602 kBq/ml) were administered as a bolus via the catheterized right femoral vein. Blood samples were collected at 0.5, 1, 1.5, 2, 2.5, and 3 min from the left femoral artery. Three minutes after the injection, several tissue specimens were collected immediately after sacrificing of rats (7). The excised tissues were gently washed, weighed, and homogenized in 3 volumes of 0.9% NaCl. The aliquots (100 μl) of blood and tissues were solubilized in 0.5 ml of NCS II (Amersham International, Buckinghamshire, UK), and the radioactivity was determined in 5 ml of ACS II (Amersham) by liquid scintillation counting.

Estimation of Tissue Uptake Clearance

The plasma concentration at 0 min was extrapolated assuming that the concentration data could be fitted to the two-compartment model. The area under the plasma concentration-time curve until 3 min (AUC_{0-3min}) was calculated by the trapezoidal rule. The tissue uptake clearance of levofloxacin and grepafloxacin were calculated by dividing the tissue accumulation at 3 min by the AUC_{0-3min}.

Uptake in Renal Cortical Slices

Renal cortical slices from male Wistar rats (200–230 g) were stored in ice-cold oxygenated incubation buffer composed

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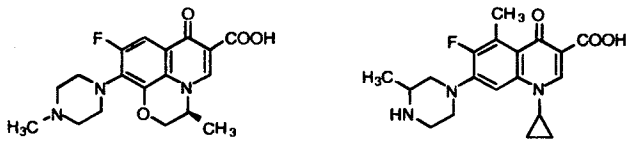

LEVOFLOXACIN GREPAFLOXACIN

Fig. 1. Chemical structure of levofloxacin and grepafloxacin.

of 120 mM NaCl, 16.2 mM KCl, 1 mM $CaCl_2$, 1.2 mM $MgSO_4$, and 10 mM NaH_2PO_4/Na_2HPO_4, pH 7.5 (8). Slices weighing 50–80 mg were randomly selected and placed for incubation in a flask containing 3 ml of the incubation buffer with [^{14}C]levofloxacin (0.5 μM, 0.54 kBq/ml or 0.1 μM, 0.11 kBq/ml) or [^{14}C]grepafloxacin (0.5 μM, 0.58 kBq/ml or 0.1 μM, 0.12 kBq/ml). The uptake of [^{14}C]levofloxacin or [^{14}C]grepafloxacin was carried out at 25°C under an atmosphere of 100% oxygen. D-[^{3}H]Mannitol (0.5 μM, 3.7 kBq/ml, or 0.1 μM, 0.74kBq/ml) was used to calculate the extracellular trapping and nonspecific uptake of [^{14}C]levofloxacin or [^{14}C]grepafloxacin, as well as to evaluate the viability of slices. In the ATP-depleted condition, slices were preincubated for 15 min in the incubation buffer containing 20 mM 2-deoxy-D-glucose and 10 mM sodium azide, and then incubated in the same buffer containing [^{14}C]levofloxacin or [^{14}C]grepafloxacin. In the anaerobic condition, slices were preincubated for 15 min under an atmosphere of 100% nitrogen, and then incubated in the incubation buffer containing [^{14}C]levofloxacin under a nitrogen atmosphere. After incubation for a specified period, each slice was rapidly removed from the flask, washed twice with 3 ml of ice-cold incubation medium, blotted on filter paper, weighed, and solubilized in 0.5 ml of NCS II (Amersham). Then the radioactivity was determined in 5 ml of ACS II (Amersham) by liquid scintillation counting.

Estimation of Kinetic Parameters

In rat renal cortical slices, the kinetic parameters for levofloxacin and grepafloxacin uptake were calculated using the following equation: $V_o = V_{max} \cdot S/(K_m + S) + K_d \cdot S$, where V_o is the uptake rate of the drug (μmol/g tissue/60 min), S is the drug concentration in the medium (mM), K_m is the Michaelis constant (mM), V_{max} is the maximum uptake rate by the saturable process (μmol/g tissue/60 min) and K_d is the coefficient of simple diffusion (μmol/g tissue/60 min/mM). The uptake data were fitted to the above equation by nonlinear least squares regression analysis. To estimate the inhibitory constant (K_i), the uptake data in the presence of an inhibitor were fitted to the following equation: $V_o = V_{max} \cdot S/\{K_m(1 + I/K_i) + S\} + K_d \cdot S$, where I is the inhibitor concentration (mM). The K_m, V_{max}, and K_d values were those obtained in the absence of inhibitors.

Statistical Analysis

The statistical significance of differences between mean values was analyzed using the non-paired t-test. Multiple comparisons were performed using the Sheffé's test following ANOVA. Differences were considered significant at $p < .05$.

RESULTS

Tissue Distribution of [^{14}C]Levofloxacin and [^{14}C]Grepafloxacin in Rats

The initial uptake of quinolones was determined in various tissues. The plasma concentrations of [^{14}C]levofloxacin and [^{14}C]grepafloxacin to 3 min after intravenous administration are shown in Fig. 2. The $AUC_{0-3\ min}$ of [^{14}C]levofloxacin and [^{14}C]grepafloxacin was 5.15 ± 0.17 and 1.36 ± 0.13 nmol·min/ml (mean ± S.E., n = 4), respectively. Table 1 shows the tissue concentration and the tissue uptake clearance of [^{14}C]levofloxacin and [^{14}C]grepafloxacin in rats. The tissue concentration of [^{14}C]levofloxacin was high in both the kidney cortex and medulla, and that of [^{14}C]grepafloxacin was also high in both kidney cortex and medulla. The tissue/plasma concentration ratio and the tissue uptake clearance indicate that both [^{14}C]levofloxacin and [^{14}C]grepafloxacin are markedly accumulated in the kidney. Therefore, we focused on the uptake mechanism(s) of these quinolones in renal proximal tubules, and also performed further uptake studies using rat renal cortical slices.

Uptake of [^{14}C]Levofloxacin and [^{14}C]Grepafloxacin in Rat Renal Cortical Slices

Figure 3 shows the time course of the uptake of [^{14}C]levofloxacin and [^{14}C]grepafloxacin in rat renal cortical slices.

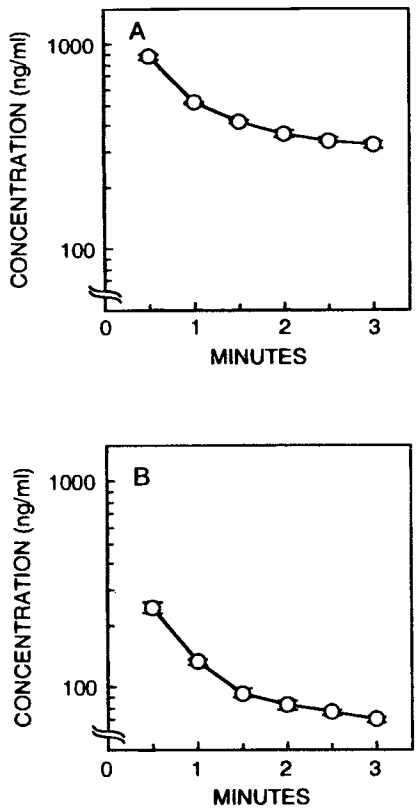

Fig. 2. The plasma concentration curve of levofloxacin (A) and grepafloxacin (B) in rats after intravenous injection. [^{14}C]Levofloxacin (0.5 μmol/kg) or [^{14}C]grepafloxacin (0.47 μmol/kg) was administered as a bolus via the catheterized right femoral vein. Blood samples were collected at 0.5, 1, 1.5, 2, 2.5, and 3 min from the left femoral artery after the injection. Each point represents the mean ± S.E. of four rats.

Table 1. Tissue Distribution of Levofloxacin and Grepafloxacin After the Intravenous Bolus Administration in Rats

Tissues	Levofloxacin			Grepafloxacin		
	Concentration (nmol/g tissue)	Ratio[a]	CL_{tissue}[b] (ml/min/g tissue)	Concentration (nmol/g tissue)	Ratio[a]	CL_{tissue}[b] (ml/min/g tissue)
Plasma	0.9 ± 0.0	1.0	—	0.2 ± 0.0	1.0	—
Brain	0.03 ± 0.00	0.03 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.1 ± 0.00	0.02 ± 0.00
Heart	1.2 ± 0.0	1.5 ± 0.0	0.2 ± 0.0	1.6 ± 0.1	9.3 ± 0.5	1.2 ± 0.1
Lung	0.8 ± 0.0	1.0 ± 0.1	0.2 ± 0.0	2.3 ± 0.1	13.0 ± 0.6	1.7 ± 0.2
Liver	2.5 ± 0.1	3.0 ± 0.1	0.5 ± 0.0	1.7 ± 0.2	10.0 ± 1.2	1.3 ± 0.3
Small Intestine	1.0 ± 0.1	1.3 ± 0.2	0.2 ± 0.0	1.0 ± 0.1	5.9 ± 0.7	0.8 ± 0.2
Spleen	1.3 ± 0.0	1.6 ± 0.0	0.3 ± 0.0	1.6 ± 0.1	9.0 ± 0.5	1.2 ± 0.2
Kidney Cortex	6.2 ± 0.8	7.5 ± 0.7	1.2 ± 0.2	6.1 ± 0.4	35.1 ± 2.6	4.6 ± 0.6
Kidney Medulla	10.4 ± 1.1	12.6 ± 1.3	2.0 ± 0.3	5.8 ± 0.4	33.2 ± 1.6	4.4 ± 0.5

Note: Each value represents the mean ± S.E. of four rats.
[a] Levofloxacin and grepafloxacin ratios were calculated as tissue/plasma concentration.
[b] The tissue uptake clearance (CL_{tissue}) of levofloxacin and grepafloxacin were calculated by dividing the tissue concentration of the quinolones at 3 min by the AUC_{0-3min}.

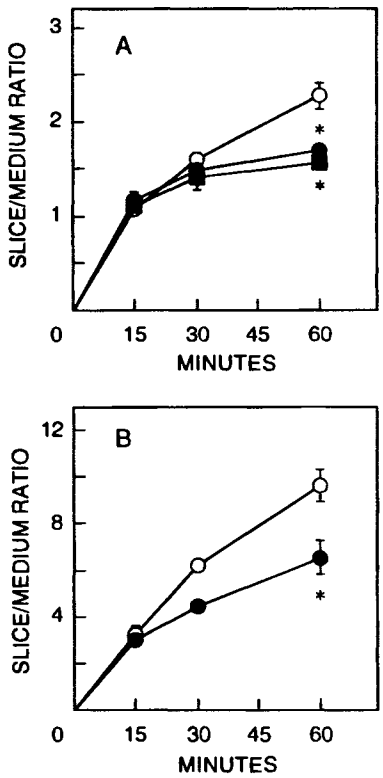

Fig. 3. The slice/medium ratio of levofloxacin and grepafloxacin in rat renal cortical slices. Cortical slices were incubated at 25°C in buffer containing 0.5 μM [^{14}C]levofloxacin (A) or 0.5 μM [^{14}C]grepafloxacin (B) in the absence (control, ○) or presence of 20 mM 2-deoxy-D-glucose and 10 mM sodium azide (●) and a nitrogen atmosphere (■). D-[^{3}H]Mannitol was used to estimate the extracellular trapping and nonspecific uptake of [^{14}C]levofloxacin or [^{14}C]grepafloxacin. Each point represents the mean ± S.E. of three slices. * $P < .05$, significantly different from control.

The slice/medium ratio of [^{3}H]mannitol was approximately 0.4 under all conditions tested up to 60 min. The uptake of both quinolones gradually increased with time under the control conditions. The slice/medium ratios of [^{14}C]levofloxacin and [^{14}C]grepafloxacin were 2.3 ± 0.1 and 9.6 ± 0.7 (mean ± S.E., n = 3) at 60 min, respectively. The concentrative accumulations of [^{14}C]levofloxacin and [^{14}C]grepafloxacin corresponded to the tissue/plasma concentration ratios of these drugs *in vivo*. The slice/medium ratio of the quinolones at 60 min was significantly decreased by treatment with 2-deoxy-D-glucose and sodium azide or a nitrogen atmosphere ($p < .05$).

Concentration Dependence of Levofloxacin and Grepafloxacin Uptake

The uptake of levofloxacin and grepafloxacin in renal cortical slices was examined as a function of substrate concentration. The uptake of [^{14}C]levofloxacin (Fig. 4A) and [^{14}C]grepafloxacin (Fig. 4B) approached saturation at high substrate concentrations. The apparent kinetic parameters (K_m, V_{max} and K_d) for levofloxacin and grepafloxacin uptake in rat renal cortical slices are summarized in Table 2.

Mutual Inhibition of [^{14}C]Levofloxacin and [^{14}C]Grepafloxacin Uptake in Rat Renal Cortical Slices

Next, mutual inhibition studies of levofloxacin and grepafloxacin were performed. The concentrations of grepafloxacin tested ranged from 0.1 to 20 mM; high concentration were not tested because of the solubility limitation. [^{14}C]Levofloxacin uptake was inhibited in a concentration-dependent fashion by unlabeled grepafloxacin (Fig. 5A). The K_i value of unlabeled grepafloxacin against [^{14}C]levofloxacin uptake was comparable to the K_m value of grepafloxacin (Table 2). Similarly, [^{14}C]grepafloxacin uptake was also inhibited in a concentration dependent fashion by unlabeled levofloxacin (Fig. 5B). The K_i value of unlabeled levofloxacin was nearly equal to the K_m value for levofloxacin (Table 2).

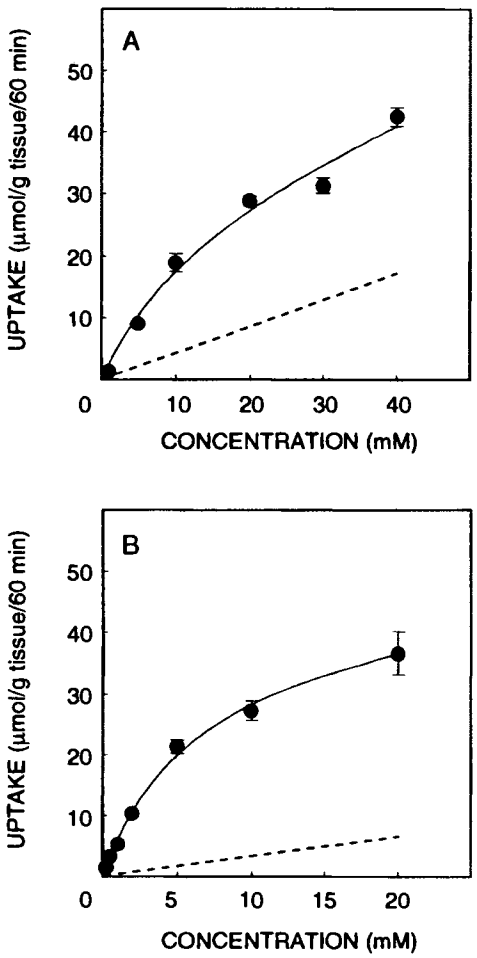

Fig. 4. Concentration-dependence of levofloxacin and grepafloxacin uptake in rat renal cortical slices. Cortical slices were incubated for 60 min at 25°C in buffer containing various concentrations of [^{14}C]levofloxacin (A) or [^{14}C]grepafloxacin (B). D-[^{3}H]Mannitol was used to estimate the extracellular trapping and nonspecific uptake of [^{14}C]levofloxacin or [^{14}C]grepafloxacin. The solid and dotted lines represent the estimated overall and nonsaturable uptake, respectively. Each point represents the mean ± S.E. of three slices.

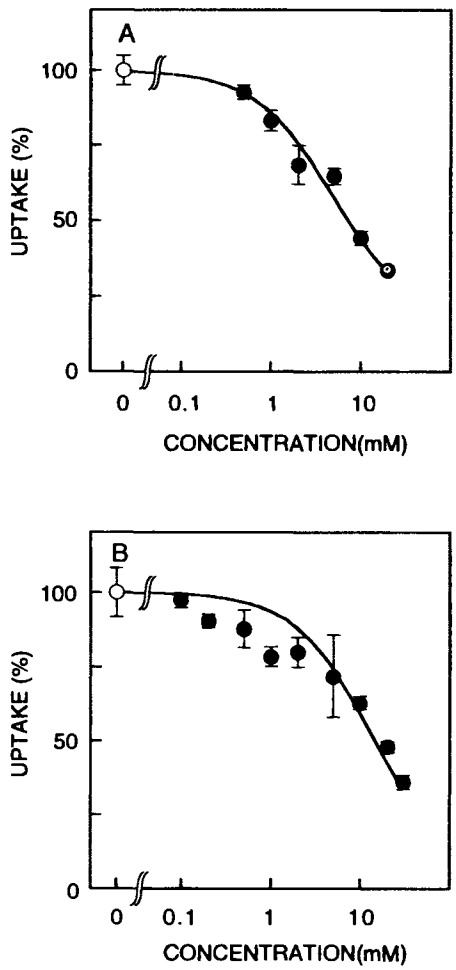

Fig. 5. Mutual inhibition of the uptake for levofloxacin and grepafloxacin in rat renal cortical slices. Cortical slices were incubated for 60 min at 25°C in buffer containing 0.5 μM [^{14}C]levofloxacin (A) or 0.5 μM [^{14}C]grepafloxacin (B) in the absence or presence of unlabeled grepafloxacin and levofloxacin, respectively. D-[^{3}H]Mannitol was used to calculate the extracellular trapping and nonspecific uptake of [^{14}C]levofloxacin or [^{14}C]grepafloxacin. [^{14}C]Levofloxacin uptake in the absence of grepafloxacin was 1.1 ± 0.1 nmol/g tissue/60 min. [^{14}C]Grepafloxacin uptake in the absence of levofloxacin was 4.8 ± 0.4 nmol/g tissue/60 min. Each symbol represents the mean ± S.E. of three to six slices.

Effect of Ionic Drugs on [^{14}C]Levofloxacin and [^{14}C]Grepafloxacin Uptake

We examined the effects of several ionic drugs on [^{14}C]levofloxacin and [^{14}C]grepafloxacin uptake (Fig. 6). Quinidine tended to inhibit the uptake of both quinolones in a concentration-dependent manner and significantly inhibited the uptake at 5 mM ($p < .05$). However, other cationic drugs, cimetidine and tetraethylammonium, and an anionic drug, *p*-aminohippurate, did not significantly influence the uptake of levofloxacin and grepafloxacin.

DISCUSSION

To date, several quinolone antibacterial drugs have been developed, some of which are eliminated into the urine or the bile as the metabolized form. In the present study, we focused on the distribution of levofloxacin and grepafloxacin in rat kidney *in vivo* and in renal cortical slices *in vitro*, and on the interaction of these quinolones with renal organic cation and/or organic anion transporters.

Levofloxacin and grepafloxacin were well distributed into a number of tissues except brain, as shown by tissue/plasma concentration ratios greater than unity (Table 1). The octanol to water partition coefficients (log *P*) of levofloxacin and grepafloxacin were −0.42 and 0.66, respectively. Therefore, it was considered that higher tissue uptake clearance of grepafloxacin than of levofloxacin was due to higher lipophilicity of grepafloxacin. Furthermore, the uptake clearance of both quinolones in the kidney was much higher than in any other tissues, suggesting an active transport mechanism of quinolones in the kidney (Table 1).

Uptake studies with rat renal cortical slices also indicated active transport of levofloxacin and grepafloxacin (Fig. 3). The tissue binding of quinolones was not a major component in

Table 2. Apparent Kinetic Parameters for Levofloxacin and Grepafloxacin Uptake in Rat Renal Cortical Slices

	K_m (mM)	V_{max} (μmol/g tissue /60 min)	K_d (μmol/g tissue /60 min/mM)	K_i (mM) against [^{14}C]levofloxacin	K_i (mM) against [^{14}C]grepafloxacin
Levofloxacin	13.9	31.0	0.45	—	13.5
Grepafloxacin	6.2	42.4	0.21	4.7	—

the concentrative accumulation, because the kinetic analysis showed that the saturable uptake of levofloxacin and grepafloxacin was much greater than that attributable to simple diffusion of each drug. Both quinolones in rat renal cortical slices are suggested to be retained as the unchanged form, because we could not detect metabolites of the quinolones for up to 60 min by TLC analysis (data not shown). In addition, the renal slice technique primarily reflects events associated with the basolateral membranes (9). The kinetic analysis showed that levofloxacin and grepafloxacin were mutually inhibitory, with K_i values comparable to the respective K_m values (Table 2). Therefore, we considered that the uptake of levofloxacin and grepafloxacin in rat renal cortical slices was mediated by the same transport system as that mediating basolateral uptake of levofloxacin and grepafloxacin in the kidney.

As quinolone molecules in the physiological conditions (pH 7.4) are zwitterions, they might be transported via the organic cation and/or anion transport systems present in the proximal tubules. In the present study, typical substrates for the organic cation transporter, tetraethylammonium and cimetidine, and a typical anionic substrate, *p*-aminohippurate, had no inhibitory effect on the uptake of either quinolone in rat renal cortical slices even at concentrations of 10 mM (Fig. 6). We have reported that levofloxacin inhibits the transport of tetraethylammonium via the apical and basolateral organic cation transporters expressed in the kidney epithelial cell line, LLC-PK$_1$ (3). We also reported that the basolateral-to-apical transport and cellular accumulation of levofloxacin were not inhibited by either tetraethylammonium or *p*-aminohippurate (4). These findings led us to consider that quinolones might be transported by neither the basolateral organic cation nor anion transport systems. Stop-flow peritubular capillary perfusion studies indicated that the basolateral uptake of ofloxacin was not inhibited by tetraethylammonium or probenecid, consistent with the present results (10). Interestingly, quinidine (5 mM) significantly inhibited the uptake of levofloxacin and grepafloxacin (Fig. 6). We also found that quinidine significantly inhibited the basolateral-to-apical transport of levofloxacin in LLC-PK$_1$ monolayers (4). The interaction of quinidine with the transport of levofloxacin and grepafloxacin in the basolateral membranes should be characterized in the future.

There have been some reports that quinolones can be recognized by active transport systems for uptake from blood in liver and small intestine. Sasabe *et al.* (6) reported that the uptake of quinolones in isolated hepatocytes occurs via carrier-mediated active transport, which is distinct from the mechanism involved in the transport of bile acids, organic anions, organic cations or neutral steroids. They also reported that the K_m value of grepafloxacin uptake by isolated rat hepatocytes was 173 μM, which is much smaller than the K_m found in the present study. The transporters which mediate the uptake of quinolones from the blood to the tissue might be different in kidney and liver. Griffiths *et al.* (11) suggested that an active transporter mediates the uptake of ciprofloxacin from the basolateral side of the intestinal epithelial cell line, Caco-2. The transporters which contribute to the removal of quinolones from the blood into various tissues remain to be clarified.

In conclusion, the present findings suggest that the basolateral transport of levofloxacin and grepafloxacin is mediated by a specific active transport system, distinct from the organic cation and anion transport systems in the kidney.

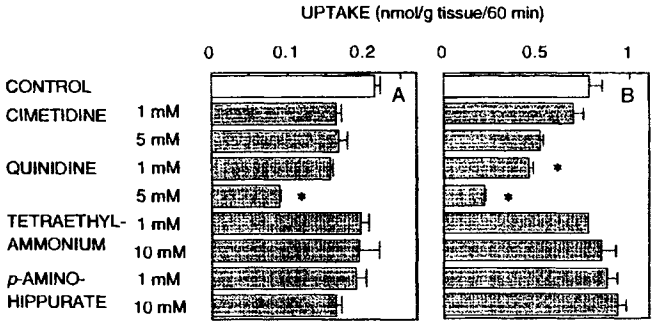

Fig. 6. Effect of various ionic drugs on the levofloxacin and grepafloxacin uptake in rat renal cortical slices. Cortical slices were incubated for 60 min at 25°C in buffer containing 0.1 μM [^{14}C]levofloxacin (A) or 0.1 μM [^{14}C]grepafloxacin (B) in the absence (open columns) or presence (dotted columns) of various ionic drugs. D-[^{3}H]Mannitol was used to calculate the extracellular trapping and nonspecific uptake of [^{14}C]levofloxacin or [^{14}C]grepafloxacin. Each column represents the mean ± S.E. of three slices, or two in the presence of 1 mM tetraethylammonium for grepafloxacin uptake. * $P < .05$, significantly different from control.

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