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# The Transport Mechanism of an Organic Cation, Disopyramide, by Brush-border Membranes. Comparison Between Renal Cortex and Small Intestine of the Rat

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**Abstract**—The characteristics of disopyramide uptake in brush-border membrane vesicles isolated from rat renal cortex and small intestine were investigated. Transport of disopyramide into an osmotically reactive intravesicular space was observed with notable binding to the membrane surface. An outwardly directed $H^+$ gradient stimulated disopyramide uptake, resulting in a transient uphill transport in both brush-border membranes. As for the renal brush-border membrane, the $H^+$ gradient itself appeared to be the driving force for this stimulation of uptake. These findings suggest that disopyramide-$H^+$ antiport is the mechanism of disopyramide action in renal cell membrane. The initial uptake was saturable ($K_m$ and $V_{max}$ of 68·0 μM and 1·25 nmol (mg protein)$^{-1}$/30 s, respectively). The stimulation of disopyramide uptake by an outward $H^+$ gradient in rat intestinal brush-border membrane was due to an interior negative $H^+$-diffusion potential. A $K^+$-diffusion potential (interior negative) enhanced disopyramide uptake. These results suggest that there are different mechanisms of disopyramide uptake for renal and intestinal brush-border membrane vesicles.

The brush-border membrane of the proximal tubular cells of the kidney possesses a specific transport system that accepts tetraethylammonium (Takano et al 1984; Wright & Wunz 1987), $N^1$-methylnicotinamide (Wright 1985; Sokol et al 1985), cimetidine (Takano et al 1985; Gisclon et al 1987; McKinney & Kunnemann 1987) and procainamide (McKinney & Kunnemann 1985) as substrates. The transport process occurs via an organic cation-$H^+$ antiport mechanism (Inui et al 1985; Rafizadeh et al 1986, 1987; Sokol et al 1988; Hori et al 1989), and has been implicated in the active secretion of organic cations by the kidney in-vivo due to the lower pH of the proximal tubular fluid compared with the intracellular pH of the tubular cells (Aronson 1983; Ives 1985).

In contrast, there are few reports concerning the carrier-mediated transport sytems of organic cations in the small intestinal brush-border membrane. Turnheim & Lauterbach (1977, 1980) and Turnheim et al (1977) have reported that the secretion system of a quarternary ammonium compound was present in guinea-pig intestine. The most recent study concerning rabbit intestinal brush-border membrane vesicles has shown that the uptake of guanidine into these vesicles may very well participate in proton coupled antiport systems (Miyamoto et al 1988). Despite these implications, there is little information available as to the similarity or the dissimilarity of organic cation in these organs.

In our previous study (Itoh et al 1992), we mentioned that an organic cation, disopyramide, an anti-arrhythmic agent, accumulated in the rat renal cortex slice in a saturable manner. To obtain further evidence concerning the molecular mechanisms underlying the secretion of disopyramide by the proximal tubules, we studied the uptake of this drug by rat renal brush-border membrane vesicles as presented in this paper.

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## Materials and Methods

### *Materials*

Disopyramide, probenecid, $N^1$-methylnicotinamide, famotidine, valinomycin, and carbonyl cyanide *p*-trifluoromethoxy phenylhydrazone (FCCP) were purchased from Sigma Chemicals (St Louis, MO, USA). Cimetidine was purchased from Aldrich Chemicals (Milwaukee, WI, USA). Tetraethylammonium chloride, and quinine sulphate were obtained from Wako Pure Chemical Industries, Ltd (Japan). *p*-Chlorodisopyramide, and mono-*N*-dealkyldisopyramide were obtained from Searle & Co. (Tokyo, Japan). Guanidine was obtained from Nacalai Tesque, Inc. (Kyoto, Japan). [$^{14}$C]Tetraethylammonium bromide (sp. act. 137 MBq mmol$^{-1}$) was procured from NEN Research Products (Boston, MA, USA). All other chemicals were of the highest grade available and were used without further purification.

### *Preparation of the brush-border membrane vesicles*

Renal brush-border membrane vesicles were isolated from the renal cortex of male Wistar rats, 200–250 g, by the calcium precipitation method (Evers et al 1978). Rat intestinal brush-border membrane vesicles were prepared according to the method of Kessler et al (1978) as described in our previous paper (Iseki et al 1989).

### *Uptake experiments*

The uptake of substrates by the freshly isolated membrane

vesicles was measured at 25°C by a rapid filtration technique (Iseki et al 1992). The membrane filter (HAWP 0·45 μm pore size, 2·5 cm diam, Millipore, Japan) was pretreated with 0·3% polyethylenimine to avoid nonspecific adsorption to the filter.

In the assay, the uptake was initiated by mixing 40 μL of membrane vesicle suspension with 200 μL of the transport buffer containing substrates. The specific conditions for each experiment are given in the figure legends. As blanks, the membrane-free incubation media were handled in an identical manner.

*Analytical procedures*

The filter, which trapped the vesicles containing disopyramide, was put into 2 mL 0·02 M NaOH and extracted with 5 mL chloroform. After shaking and centrifugation, 3 mL of the organic layer was taken and evaporated under vacuum. The residue was reconstituted in 200 μL of mobile phase containing the internal standard (*p*-chlorodisopyramide) and the concentration of disopyramide was determined by HPLC according to Meffin et al (1977), using a liquid chromatograph (L-6000, Hitachi Ltd, Japan) equipped with an L-4000 UV detector (Hitachi Ltd, Japan) with detection set at 214 nm. Separation was achieved on a reversed phase column (Inertsil ODS, 5 μm. 4·6 mm i.d. × 250 mm) using a mobile phase consisting of methanol:10 mM $KH_2PO_4$ (40:60) at a flow rate of 0·9 mL $min^{-1}$. [$^{14}$C]Tetraethylammonium on the filter was determined by standard liquid scintillation counting methods. Protein was determined by the method of Lowry et al (1951) using bovine serum albumin as a standard. Statistical analysis was evaluated by Student's *t*-test. Kinetic constants were calculated by use of nonlinear regression analysis and by fitting the values for uptake rate and substrate concentration into a Michaelis–Menten equation.

## Results

*Effects of an outwardly directed $H^+$ gradient and a membrane potential on disopyramide uptake*

To investigate the effect of an outwardly directed $H^+$ gradient on the disopyramide uptake in rat renal and intestinal brush-border membrane vesicles, the disopyramide uptakes in the presence and absence of a $H^+$ gradient were compared. The initial uptake rates of disopyramide into renal brush-border membrane vesicles, with an indication of active transport, were greater when measured in the presence of an outwardly directed $H^+$ gradient (pH $5{\cdot}5_{in}$ > pH $7{\cdot}5_{out}$) than in the absence of an $H^+$ gradient (Fig. 1A). In the presence of an $H^+$ gradient, there was a transient accumulation of disopyramide inside the vesicles above the equilibrium value, demonstrating an uphill transport. The intravesicular concentration of disopyramide at the peak of overshoot was approximately three times that of the equilibrium value. This $H^+$ gradient-stimulated disopyramide uptake was not due to the influence of the $H^+$ gradient on the intravesicular space, since the equilibrium value, which depends on the intravesicular volume, remained the same in the presence or in the absence of the $H^+$ gradient concerning disopyramide uptake.

In the case of intestinal brush-border membrane vesicles, disopyramide uptake was also rapid in the presence of an outward $H^+$ gradient ($pH_{in} = 5{\cdot}5$; $pH_{out} = 7{\cdot}5$) and exhibited a transient 'overshoot' phenomenon, although the maximum uptake of disopyramide was only 1·5-fold the equilibrium value (Fig. 1B).

*Effects of an outwardly directed $K^+$ diffusion potential*

The role of an experimentally-induced negative membrane potential on disopyramide uptake in renal and intestinal brush-border membrane vesicles was investigated. In these experiments, the interior negative membrane potential was generated by valinomycin, a $K^+$ ionophore, in the presence of an outward $K^+$ gradient (Table 1). The valinomycin-induced $K^+$-diffusion potential had no effect on the disopyramide uptake into renal brush-border membrane vesicles.

*Effect of FCCP on disopyramide uptake*

We also studied disopyramide uptake in the presence of an outward-directed $H^+$ gradient as well as under the experimental conditions in which the $H^+$ gradient was effectively dissipated (Table 1). Membrane vesicles were preloaded with

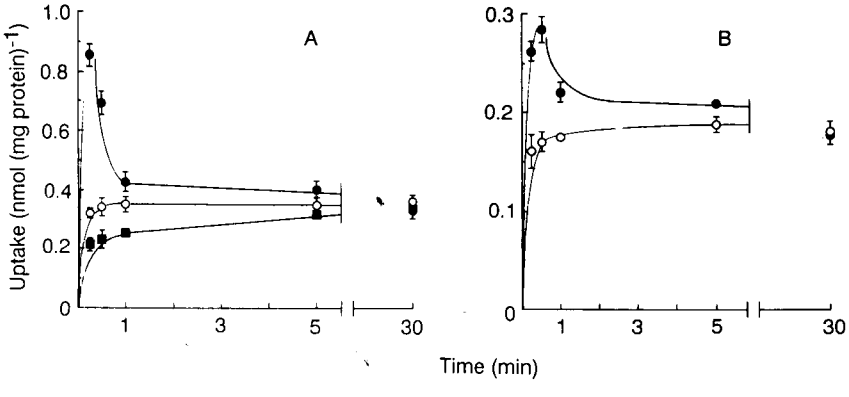


FIG. 1. Time-course of disopyramide uptake in the presence or the absence of an outwardly directed $H^+$ gradient. Brush-border membrane vesicles isolated from renal cortex (A) and small intestine (B) of rat were preloaded with 100 mM D-mannitol, 100 mM KCl and either 20 mM HEPES/Tris, pH 7·5 (○, ■) or 20 mM Mes/Tris, pH 5·5 (●). Uptake buffer was 100 mM D-mannitol, 100 mM KCl and either 20 mM HEPES/Tris, pH 7·5 (○, ●) or 20 mM Mes/Tris, pH 5·5 (■). Concentration of disopyramide was 50 μM. Each point represents the mean ± s.e. of four determinations.

Table 1. Effects of inside-negative membrane potential on disopyramide uptake.

| Incubation condition | | | | Uptake | | | |
|---|---|---|---|---|---|---|---|
| Intravesicular | pH | Extravesicular | pH | Kidney nmol (mg protein)$^{-1}$ at 15 s | % | Intestine nmol (mg protein)$^{-1}$ at 15 s | % |
| | | $K^+$-diffusion potential | | | | | |
| $K^+ = 100$ mM | 7·5 | $K^+ = 0$ mM | 7·5 | 0·272 ± 0·017 | 100 | 0·190 ± 0·010 | 100 |
| $K^+ = 100$ mM | 7·5 | $K^+ = 0$ mM (Valinomycin) | 7·5 | 0·257 ± 0·010 (NS) | 94 | 0·241 ± 0·018 (NS) | 127 |
| | | $H^+$-diffusion potential | | | | | |
| | 5·5 | | 7·5 | 0·830 ± 0·033 | 100 | 0·290 ± 0·023 | 100 |
| | 5·5 | | 7·5 (FCCP) | 0·416 ± 0·038 ($P < 0·01$) | 50 | 0·315 ± 0·029 (NS) | 109 |

Values are means ± s.e. from three determinations. In the first set of experiments in which the effects of a $K^+$-diffusion potential were studied, membrane vesicles were preloaded with 100 mM D-mannitol, 100 mM KCl, buffered with 20 mM HEPES–Tris, pH 7·5 and the uptake buffer was 100 mM D-mannitol, 100 mM NaCl, buffered with 20 mM Tris, pH 7.5. The initial uptake rate of disopyramide measured for a 15 s incubation period in the presence and absence of valinomycin, was 7 μg (mg protein)$^{-1}$. In the second set of experiments in which the effects of a $H^+$-diffusion potential were studied, membrane vesicles were preloaded with 100 mM D-mannitol, 100 mM KCl, buffered with 20 mM Mes-Tris, pH 5·5 and the uptake buffer was 100 mM D-mannitol, 100 mM KCl, buffered with 20 mM HEPES-Tris, pH 7·5. The initial uptake rates were measured for a 15 s incubation period in the presence and absence of 50 μM FCCP. Final concentration of disopyramide in both sets of experiments was 50 μM. NS; not significant.

100 mM KCl plus 100 mM mannitol buffered with 20 mM Mes-Tris, pH 5·5. The uptake buffer was 100 mM KCl plus 100 mM mannitol, buffered with 20 mM HEPES-Tris, pH 7·5. Disopyramide uptake was measured in the presence and in the absence of FCCP. In the absence of FCCP, a transient accumulation of disopyramide inside the vesicles against a concentration gradient was observed in response to the outward-directed $H^+$ gradient in both renal and intestinal brush-border membrane vesicles. In the presence of FCCP, however, disopyramide uptake was markedly decreased, and there was no evidence of active accumulation of disopyramide to renal brush-border membrane vesicles, as the $H^+$ gradient was effectively dissipated by the FCCP (Table 1). In the intestinal brush-border membrane vesicles, the uphill transport of disopyramide occurred even in the presence of FCCP.

*Disopyramide uptake in voltage-clamped brush-border membrane vesicles*

The non-involvement of an $H^+$-diffusion potential in disopyramide uptake in renal brush-border membrane vesicles was further substantiated by the examination of the $H^+$ gradient-dependent disopyramide uptake in voltage-clamped membrane vesicles. Table 2 shows that concentrative uptake of disopyramide in response to an outwardly directed $H^+$ gradient can be demonstrated, even in these voltage-clamped renal membrane vesicles, thus indicating that an outwardly directed $H^+$ gradient itself acts as the driving force for disopyramide uptake. On the other hand, the voltage-clamped brush-border membrane vesicles of rat intestine have exhibited a significant decrease of overshoot phenomenon in the presence of an outward $H^+$ gradient. These results agreed well with the effect of FCCP from a viewpoint of comparison between renal and intestinal brush-border membranes.

*Kinetic analysis of the disopyramide-$H^+$ antiport system in the renal brush-border membrane*

The concentration dependence of the initial uptake (15 s) rate was studied over the disopyramide concentration range of 0·025–4·0 mM. In these experiments, disopyramide uptake (15 s) at the equilibrium pH (pH 7·5) was used to determine the nonsaturable diffusion component at each substrate concentration. The rate of carrier-mediated uptake, which was determined by subtracting the diffusional component from the total uptake, was adequately described by simple Michaelis–Menten kinetics (Fig. 2). Lineweaver–Burk transformation of the corrected data resulted in values for $K_m$

Table 2. Disopyramide uptake into voltage-clamped membrane vesicles of kidney and intestine in the presence of an outwardly directed $H^+$ gradient.

| | Uptake (nmol (mg protein)$^{-1}$) Time (min) | | |
|---|---|---|---|
| Vesicle conditions | 0·25 | 0·5 | 30 |
| Kidney | | | |
| Control BBMV (without valinomycin) | 0·824 ± 0·032 | 0·643 ± 0·045 | 0·296 ± 0·038 |
| Voltage-clamped BBMV (with valinomycin) | 0·785 ± 0·054 | 0·610 ± 0·066 | 0·228 ± 0·029 |
| Intestine | | | |
| Control BBMV (without valinomycin) | 0·315 ± 0·019 | | 0·199 ± 0·028 |
| Voltage-clamped BBMV (with valinomycin) | 0·273 ± 0·011* | | 0·212 ± 0·024 |

Membrane vesicles were preloaded in the medium containing 100 mM KCl plus 100 mM D-mannitol buffered with 20 mM Mes-Tris (pH 5·5). The vesicles (40 μL) were incubated with 200 μL of 100 mM KCl, 100 mM D-mannitol, 60 μM disopyramide and 20 mM HEPES–Tris (pH 7·5) in the presence (voltage-clamped BBMV) or the absence (control BBMV) of valinomycin (7 μg (mg protein)$^{-1}$). Each value represents the mean ± s.e. from four determinations. *$P < 0·01$ compared with control.

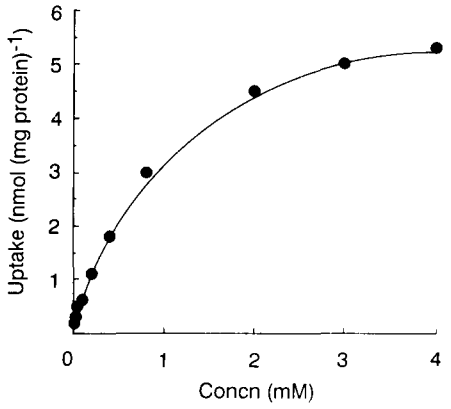


FIG. 2. Concentration dependence of disopyramide uptake into rat renal brush-border membrane vesicles. Uptake studies were performed in the same medium as described in Fig. 1. The $H^+$ gradient-dependent uptake rate at each time point was determined by subtracting the uptake value at equilibrium (pH $7{\cdot}5_{in=out}$) from the total uptake value.

Table 3. Trans-stimulation of disopyramide uptake by various organic cations in renal brush-border membrane of rats.

| Organic cations | Uptake of disopyramide nmol (mg protein)$^{-1}$ at 15 s | % |
|---|---|---|
| Control | 0·835 ± 0·53 | 100·0 |
| Tetraethylammonium | 0·940 ± 0·88 | 112·6 |
| $N^1$-Methylnicotinamide | 0·991 ± 0·03* | 118·7 |
| Cimetidine | 0·926 ± 0·028 | 110·9 |
| Famotidine | 0·934 ± 0·042* | 111·9 |
| Guanidine | 1·055 ± 0·050** | 126·3 |
| Quinine | 0·910 ± 0·019 | 109·0 |
| Mono-*N*-dealkyldisopyramide | 0·848 ± 0·019 | 101·6 |
| *p*-Chlorodisopyramide | 0·845 ± 0·046 | 101·2 |

Membrane vesicles were preincubated with 100 mM KCl, 100 mM D-mannitol and 20 mM HEPES–Tris (pH 7·5) in the presence or the absence (control) of 1 mM organic cations. Uptake of 50 $\mu$M disopyramide was assayed in 100 mM KCl, 100 mM D-mannitol and 20 mM HEPES–Tris (pH 7·5). Each value represents the mean ± s.e. of four determinations. *$P < 0{\cdot}05$, **$P < 0{\cdot}01$ compared with control.

(affinity constant) and $V_{max}$ (maximum velocity) of 68·0 $\mu$M and 1·25 nmol (mg protein)$^{-1}$/30 s, respectively.

*Trans-stimulation effect of disopyramide uptake*

To substantiate the presence of a carrier system for disopyramide in renal brush-border membrane, we investigated whether the presence of various organic cations inside the vesicles are capable of stimulating the uptake of disopyramide in the presence of an $H^+$ gradient (pH $5{\cdot}5_{in}$ > pH $7{\cdot}5_{out}$) (Table 3). The uptake of disopyramide measured with 15 s incubation was significantly greater in the vesicles preloaded with $N^1$-methylnicotinamide, guanidine, famotidine than in the control vesicles.

*Inhibitory effect of organic cations on $H^+$ gradient-dependent disopyramide uptake*

To clarify whether the uptake of these organic cations, including disopyramide, occurs via a common transport mechanism, we investigated the inhibitory effects of several organic cations on the $H^+$ gradient-dependent uptake of disopyramide in renal brush-border membrane vesicles (Table 4). The uptake rates for disopyramide were measured with 15 s incubation in the presence of an outward $H^+$ gradient. The typical substrates of the renal organic cation-$H^+$ antiporter, such as tetraethylammonium, $N^1$-methylnicotinamide, famotidine, and guanidine, failed to inhibit

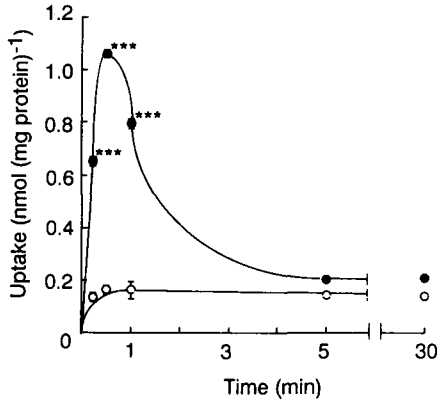


FIG. 3. Inhibitory effect of disopyramide on the uptake of [$^{14}$C]-tetraethylammonium into renal brush-border membrane vesicles. The transport study was performed in medium containing [$^{14}$C]tetraethylammonium chloride, 100 mM D-mannitol, 100 mM KCl and 20 mM HEPES-Tris, pH 7·5 in the presence (○) or the absence (●) of disopyramide. Concentrations of disopyramide and [$^{14}$C]*S*-tetraethylammonium in the incubation media were 1 mM and 50 $\mu$M, respectively. Each column represents the mean ± s.e. of three determinations. ***$P < 0{\cdot}001$ compared with control.

Table 4. Effects of various compounds on disopyramide uptake.

| Compounds (mM) | Initial uptake nmol (mg protein)$^{-1}$ at 15 s | % | Equilibrated uptake nmol (mg protein)$^{-1}$ at 30 min | % |
|---|---|---|---|---|
| Control | 0·875 ± 0·040 | | 0·392 ± 0·012 | |
| Tetraethylammonium (5) | 0·712 ± 0·054 | 81 | 0·390 ± 0·014 | 99 |
| $N^1$-Methylnicotinamide (10) | 0·719 ± 0·052 | 81 | 0·376 ± 0·024 | 96 |
| Cimetidine (5) | 0·539 ± 0·028 | 62** | 0·274 ± 0·019 | 70* |
| Famotidine (5) | 0·700 ± 0·052 | 80 | 0·291 ± 0·020 | 74* |
| Guanidine (20) | 0·726 ± 0·030 | 83 | 0·376 ± 0·006 | 96 |
| Quinine (0·4) | 0·435 ± 0·015 | 50** | 0·292 ± 0·018 | 74* |
| Mono-*N*-dealkyldisopyramide (1) | 0·656 ± 0·008 | 78* | 0·246 ± 0·019 | 63** |
| *p*-Chlorodisopyramide (1) | 0·381 ± 0·026 | 44** | 0·250 ± 0·018 | 64** |

Values are means ± s.e. from three determinations. Membrane vesicles were preloaded with 100 mM D-mannitol, 100 mM KCl, buffered with 20 mM Mes-Tris, pH 5·5. Effects of compounds on the 30 s uptake of disopyramide were studied in the presence of an outwardly directed $H^+$ gradient, using 100 mM D-mannitol, 100 mM KCl, buffered with 20 mM HEPES-Tris, pH 7·5 as the uptake buffer. The final concentration of disopyramide was 50 $\mu$M. *$P < 0{\cdot}05$, **$P < 0{\cdot}01$ compared with control.

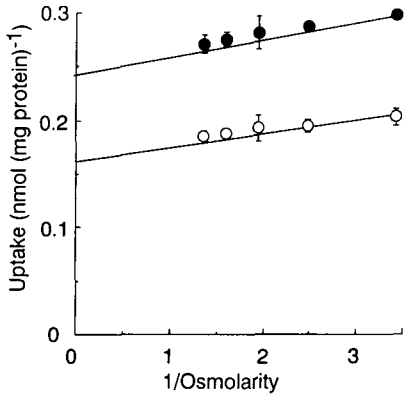


FIG. 4. Uptake of disopyramide as a function of osmolarity of the extravesicular medium in the presence (○) or the absence (●) of cimetidine. Uptakes were assayed in 20 mM HEPES-Tris, pH 7·5, 100 mM KCl and various concentrations of D-cellobiose to give the medium osmolarity. Concentrations of disopyramide and cimetidine were 50 μM and 5 mM, respectively. Each point represents the mean ± s.e. of three determinations for 30 min incubations.

disopyramide uptake even at relatively high concentrations. However, other organic cations, including disopyramide derivatives such as *p*-chlorodisopyramide and mono-*N*-dealkyldisopyramide, significantly inhibited disopyramide uptake into the membrane vesicles. Furthermore, disopyramide remarkably inhibited the $H^+$-dependent uptake of tetraethylammonium by renal brush-border membrane vesicles even at 1 mM (Fig. 3).

*Binding of disopyramide to the brush-border membrane*

To elucidate the binding inhibition between disopyramide and cimetidine, uptake of disopyramide by the vesicles after 30 min incubation was measured as a function of medium osmolarity in the presence or absence of cimetidine (Fig. 4). The y-intercept, determined by linear regression analysis, indicates a value of the binding of disopyramide at infinite osmolarity either in the presence or in the absence of cimetidine. This result showed that disopyramide binding to the membrane vesicles was decreased by the presence of cimetidine, suggesting the mutual inhibitory effect on binding between the two organic cations.

## Discussion

The results obtained from the uptake study of the renal membrane vesicles provide strong evidence for the presence of a disopyramide-$H^+$ antiport system in rat renal brush-border membranes. Lin et al (1988) have reported that there may be more than one organic cation-$H^+$ antiporter in renal brush-border membrane for the handling of organic cations. A recent study (Miyamoto et al 1989) has shown that the renal brush-border membrane possesses at least two organic cation-$H^+$ antiporters, one that accepts guanidine and cimetidine and the other with a broader substrate specificity that includes tetraethylammonium and $N^1$-methylnicotinamide. In the present study, however, it was revealed that guanidine, $N^1$-methylnicotinamide, and famotidine inside the vesicles were capable of stimulating the uptake of disopyramide (trans-stimulation), although there were less cis-inhibitory effects of these organic cations on the uptake of disopyramide by renal membrane vesicles. Furthermore, as shown in Fig. 3, the outward $H^+$-gradient-dependent uptake of tetraethylammonium into the membrane vesicles was markedly inhibited by the presence of disopyramide at even lower concentration (1 mM), in spite of the lack of effect of either the trans-stimulation or the cis-inhibition upon the disopyramide uptake. We conclude that disopyramide can be recognized as a substrate by both organic cation-$H^+$ antiporters.

The present results also demonstrate that disopyramide can be transported via organic cation-$H^+$ antiport systems in renal brush-border membranes, but not, however, in the intestinal brush-border membranes of rats. The effects of $H^+$, $K^+$-diffusion potential on the disopyramide uptake across the intestinal brush-border membrane was in agreement with our previous data, in that the cationic form of enoxacin entered into the brush-border membrane vesicles driven by the membrane potential differences (Iseki et al 1992).

In conclusion, it has been shown that the stimulation of disopyramide uptake by an outward $H^+$ gradient was associated with an organic cation-$H^+$ antiport system in rat renal brush-border membrane, whereas disopyramide uptake stimulation was caused by the effect of an interior negative $H^+$-diffusion potential in the intestinal brush-border membranes.

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