Stereoselective Interactions of Organic Cations with the Organic Cation Transporter in OK Cells

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Recent studies have suggested that certain organic cations, such as pindolol and the diastereomers, quinine and quinidine, may be stereoselectively secreted by the kidney in humans. The goal of this study was to determine if the enantiomers of pindolol, verapamil, and disopyramide and the diastereomers, quinine and quinidine, interact stereoselectively with the organic cation transporter in the brush border membrane of the opossum kidney cell line. All organic cations tested inhibited the uptake of tetraethylammonium (TEA). The IC_{50} values (mean ± SD) were as follows: quinine (17 ± 2 μM), quinidine (51 ± 13 μM), *S*-(−)-pindolol (23 ± 4 μM), *R*-(+)-pindolol (30 ± 4 μM), *S*-(−)-verapamil (0.4 ± 0.04 μM), *R*-(+)-verapamil (7 ± 2 μM), *R*-(−)-disopyramide (27 ± 4 μM), and *S*-(+)-disopyramide (66 ± 12 μM). Each individual organic cation pair showed significant stereoselective differences in their IC_{50} values, with quinine, *S*-(−)-pindolol, *S*-(−)-verapamil, and *R*-(−)-disopyramide being the more potent species. Both enantiomers of pindolol, quinine, and quinidine appear to exhibit simple competitive inhibition of TEA uptake based upon a derived slope similar to 1.0, using a sigmoidal inhibition model. The enantiomers of verapamil and disopyramide exhibited a slope of much less than 1.0, suggesting a more complex interaction of these organic cations with the TEA transporter. Our results suggest that organic cations stereoselectively interact with the organic cation transporter in the brush border membrane of OK cells. Stereoselective interactions with the organic cation transporter may be responsible for the stereoselective renal clearance of basic drugs known to occur in humans.

KEY WORDS: tetraethylammonium; OK cells; organic cation transport; quinine; quinidine; pindolol; verapamil; disopyramide.

INTRODUCTION

There are many examples of differences in pharmacological activity between enantiomeric compounds (3–5). Stereoselective metabolism of exogenous substances has been extensively studied and documented (3–5). In addition, the stereoselective renal transport of endogenous substances such as glucose and amino acids has been widely acknowledged for many decades (6–8). Only recently has the stereoselective renal elimination of drugs been described (1,2, 9,10). In humans, the unbound renal clearance of the organic cation, *R*-(+)-pindolol, is approximately 30% less than that of the *S*-(−)-enantiomer (1), and the unbound renal clearance of quinidine is four times that of quinine, its diastereomer (2). These studies suggest that organic cations may also be stereoselectively handled in the kidney.

Many exogenous organic cations are actively secreted by the proximal tubule of the kidney (11–14). The organic cation transport system responsible for this secretion appears to be composed of two separate transporters, one at each membrane. Transport of organic cations from the blood into the cell across the basolateral membrane occurs by a passive electrogenic carrier-mediated system. Organic cations are transported from the cell into the tubule lumen through the brush border membrane by a secondary active, electroneutral mechanism, referred to as the organic cation/proton antiporter (13,15–18). A proton gradient appears to drive the uphill flux of organic cations into the lumen. Therefore the mechanisms which may be responsible for the stereoselective action secretion of organic cations in the kidney are stereoselective interactions with the transporters at either membrane. In addition, stereoselective renal metabolism and renal reabsorption mechanisms may also be involved.

In vitro studies to determine the mechanism of the stereoselective secretion of pindolol and the diastereoisomers, quinine and quinidine, have produced conflicting results. A previous study from our laboratory in isolated rabbit renal brush border membrane vesicles suggested no stereoselective interactions of these organic cations with the brush border membrane transporter (19). This same approach was undertaken by Benadyan *et al.* (20) using canine renal brush border membrane vesicles. They determined that quinidine was 10 times more potent than quinine in inhibiting the uptake of the organic cation N^1-methylnicotinamide (NMN). In contrast, Wong *et al.* observed that quinine was significantly more potent than quinidine in inhibiting the uptake of the organic cation, amantidine, in rat renal cortical slices and proximal tubule suspensions (21). Both preparation reflect events at the basolateral membrane.

Recently we characterized the transport of an unmetabolized organic cation, tetraethylammonium (TEA) in monolayers of the continuous renal cell lines, OK (22). This cell line appears to express an organic cation transporter within the brush border membrane with characteristics closely matching those in other mammalian species, including humans (15). The expressed transporter has a much higher affinity for the model organic cations, TEA and NMN, than observed in isolated membrane vesicles from various species (13,18–20,23–25). These characteristics suggest that the OK cell line is an excellent model to study potential stereoselective organic cation transport mechanisms. The goal of this study was to determine if various organic cations (Fig. 1) including the enantiomers of pindolol, verapamil, and disopyramide and the diastereoisomers, quinine and quinidine, interact stereoselectively with the organic cation transporter in the brush border membrane of OK cells. Our rational was that if these substances interacted stereoselectively with the organic cation transporter, the IC_{50} values should reflect this interaction. All of the organic cations tested inhibited TEA accumulation across the brush border membrane. Our results suggest a stereoselective interaction of these organic cations with the brush border membrane organic cation

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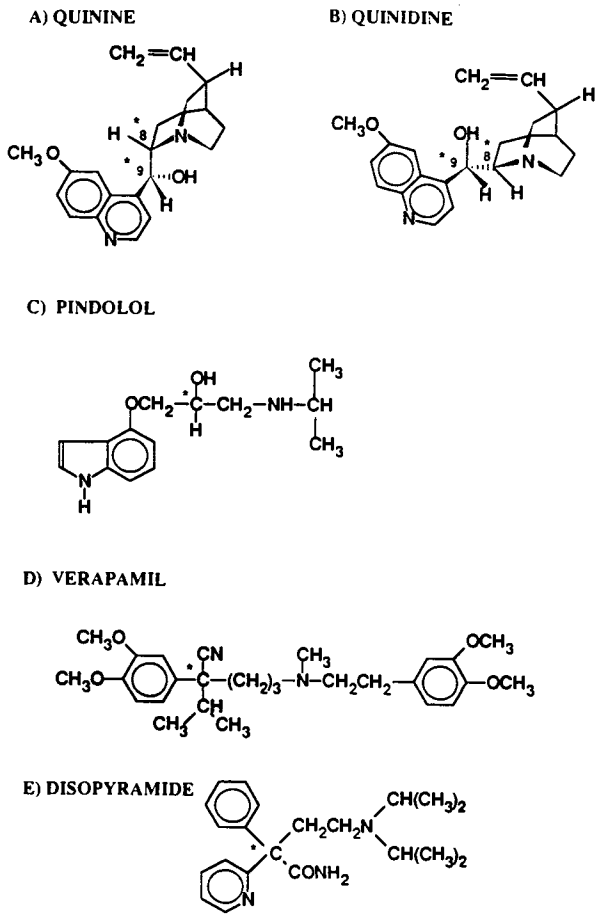

Fig. 1. Structures of (A) quinine, (B) quinidine, (C) pindolol, (D) verapamil, and (E) disopyramide. The chiral carbons are indicated by asterisks.

transporter, with *S*-(−)-pindolol, *S*-(−)-verapamil, *R*-(−)-disopyramide, and quinine being the more potent species.

MATERIALS AND METHODS

Cell Culture

Cell culture was carried out as described previously (22). OK cells were maintained on plastic tissue culture flasks (75 cm^2) at 37°C in humidified 5% CO_2/95% air atmosphere. The growth medium was Eagle's minimum essential medium with Earle's balanced salt supplemented with 2 m*M* glutamine, 100 IU/mL penicillin, 100 μg/mL streptomycin, 0.25 μg/mL fungizone, and 10% (vol/vol) fetal bovine serum. All studies were performed with cell monolayers between the 80th and the 90th passage. For transport studies, the cells were subcultured in 12-well cluster dishes (4 cm^2) following trypsinization with 0.05% trypsin containing 0.02% EDTA. The seeding density was 4×10^4 cells/cm^2 and the medium was changed every other day.

Transport Measurements

The determination of uptake of TEA into OK cells was carried out as previously described (22) at the seventh day of subculture. The growth medium was gently aspirated and each monolayer was rinsed three times with 1 mL of phosphate-buffered saline (PBS), pH 7.4. To initiate uptake, 500 μL of PBS containing 4.8 μ*M* [^{14}C]TEA and inhibitors at the desired concentrations was added to each well. The uptake was carried out at room temperature for 1 min and stopped by aspiration of the uptake medium. The cell monolayers were immediately washed three times with ice-cold PBS. The cells were solubilized in 1 mL of 0.5% Triton X-100 and an aliquot was used for radioactivity determination. The protein concentration was determined by the Bio-Rad Protein Assay Kit, with bovine serum albumin as the standard.

Data Analysis

For each organic cation tested, the IC_{50} or the concentration of the respective organic cation which causes a 50% reduction in the uptake of TEA was determined. The IC_{50} was estimated by a sigmoidal inhibition model (26) using FIT FUNCTION, an iterative nonlinear least-squares regression program in the National Institutes of Health PROPHET system, to the following equation:

$$V = V_o/[1 + (I/IC_{50})^n]$$

where V is the uptake of TEA in the presence of the inhibitor, V_o is the uptake of TEA in the absence of inhibitor, I is the inhibitor concentration, and n is the slope which determines the steepness of the sigmoidal inhibition curve. All experiments were conducted in four different OK cell monolayer wells. Data are presented as mean ± SD, and where no SD bars are shown, the SD is encompassed within the point. Statistical significance was determined by the unpaired Student's t test. Results were considered statistically different at a probability of $P < 0.05$.

Materials

[^{14}C]TEA (56 mCi/mmol) was purchased from Wizard Labs (Davis, CA). *R*-(−)- and *S*-(+)-disopyramide were resolved as previously described to greater than 98% purity (27). *S*-(−)- and *R*-(+)-verapamil were generously provided by Dr. Wendel L. Nelson, Department of Medicinal Chemistry, School of Pharmacy, University of Washington. Both isomers of verapamil were greater than 97% optically pure. Quinine and quinidine were purchased from Sigma. *S*-(−)- and *R*-(+)-pindolol were gifts from Sandoz Inc. (East Hanover, NJ). Quinine, quinidine, and the enantiomers of pindolol were used without further purification.

RESULTS

We determined the uptake of [^{14}C]TEA across the brush border membrane of OK cell monolayers in the presence of three pairs of enantiomers: pindolol, verapamil, and disopyramide and the diastereoisomers, quinine and quinidine (Fig. 1). The uptake of TEA (as percentage of TEA uptake in the absence of inhibitors) versus the concentration of each separate isomer is shown in Fig. 2. All individual isomers inhibited the initial uptake of TEA with progressively increasing concentrations of inhibitors. The estimated IC_{50} and slope values for each individual isomer are listed in Table I. Quinine, *S*-(−)-pindolol, *S*-(−)-verapamil, and *R*-(−)-disopyramide were all significantly more potent than their

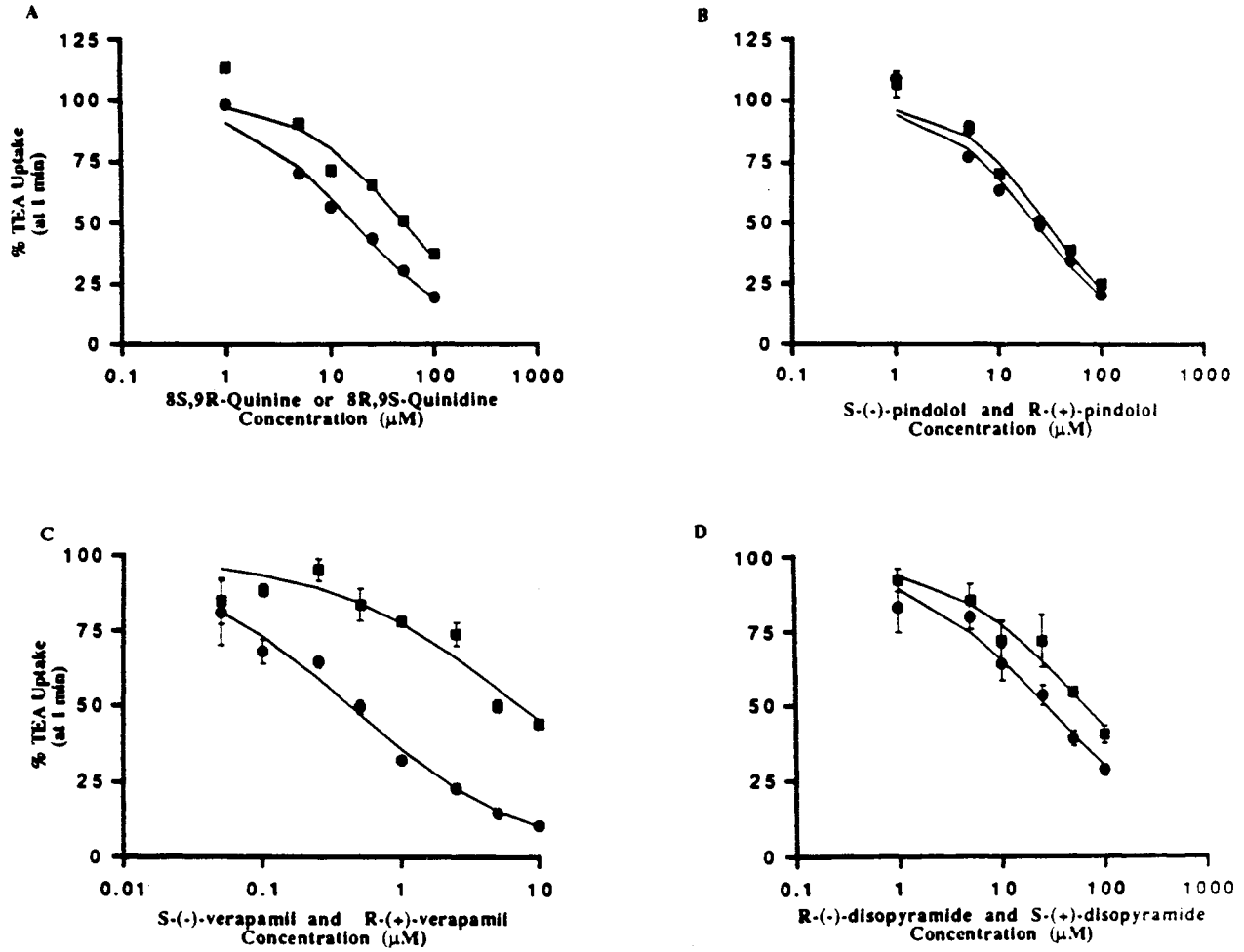

Fig. 2. The inhibition of TEA uptake at 1 min in OK cells by (A) quinine (circles) and quinidine (squares); (B) *S*-(−)-pindolol (circles) and *R*-(+)-pindolol (squares); (C) *S*-(−)-verapamil (circles) and *R*-(+)-verapamil (squares); and (D) *R*-(−)-disopyramide (circles) and *S*-(+)-disopyramide (squares). Data are expressed as the percentage (mean ± SD) of maximum TEA uptake in the absence of inhibitors versus the log inhibitor concentration. The curves were generated by computer fit of the data as described in the text. The uptake of TEA in the absence of inhibitors ranged from 140 to 240 pmol/mg protein (mean ± SD of 187 ± 49).

corresponding isomer. Notably, *S*-(−)-verapamil was over 18-fold more potent than *R*-(+)-verapamil as an inhibitor of TEA uptake. Between enantiomeric pairs, verapamil appears to be the most potent species. Both enantiomers of pindolol and the diastereoisomers, quinine and quinidine, appear to be competitive inhibitors (slope ~1) for the TEA transporter. However, the interactions of verapamil and disopyramide with the organic cation transporter appears to be more complex (slope <1).

DISCUSSION

In this study we determined that various organic cations interact stereoselectively with the organic cation transporter in the brush border membrane of OK cells. Stereoselective interactions of the organic cation transport system have been suggested previously to explain the stereoselective unbound renal clearance of the enantiomers of pindolol and the diastereoisomers, quinine and quinidine, in humans (1,2). These compounds are all actively secreted in the kidney and are positively charged at physiological pH. Therefore they may be transported by the organic cation transport system.

The renal handling of organic cations and bases has received considerable attention during the last decade due to the identification of many clinically relevant drug–drug interactions occurring in the kidney. Many clinically important organic cations including cimetidine, procainamide, quinine, quinidine, and pindolol are actively secreted (1,11,28,29). Drug interactions between many of these substances have been documented *in vivo* in humans. For example cimetidine has been shown to decrease the renal clearance of procainamide, triamterene, ranitidine, cephalexin, and amiloride (14,30,31). Competitive inhibition of the organic cation/proton antiporter at the brush border membrane has been suggested as the mechanism for these drug–drug interactions (14,20).

In the present study, we determined a small but statistically significant difference in the IC_{50} values of the two enantiomers of pindolol [IC_{50} for *R*-(+)-pindolol = 30 ± 40 μ*M*; *S*-(−)-pindolol = 23 ± 4 μ*M*], suggesting that there is

Table I. Estimated IC_{50} and n^a Values (Mean ± SD) for Various Organic Cations

Compound	IC_{50} (μM)	n
8*S*,9*R*-Quinine	17 ± 2	0.81 ± 0.09
8*R*,9*S*-Quinidine	51 ± 13	0.87 ± 0.23
	($P < 0.001$)	
S-(−)-Pindolol	23 ± 4	0.95 ± 0.18
R-(+)-Pindolol	30 ± 4	1.02 ± 0.16
	($P < 0.02$)	
S-(−)-Verapamil	0.4 ± 0.04	0.69 ± 0.05
R-(+)-Verapamil	7 ± 2	0.62 ± 0.12
	($P < 0.001$)	
R-(−)-Disopyramide	27 ± 4	0.64 ± 0.07
S-(+)-Disopyramide	66 ± 12	0.65 ± 0.09
	($P < 0.001$)	

[a] n is the slope. See text for equation.

a stereoselective interaction of pindolol in the brush border membrane of OK cells. These data are consistent with the previous clinical study which demonstrated that the *S*-(−)-enantiomer of pindolol had a 30% higher renal clearance than the *R*-(+) form in humans (1). Previously, we observed no significant difference in the IC_{50} values for the enantiomers of pindolol [*R*-(+)-pindolol = 140 ± 20 μM; *S*-(−)-pindolol = 120 ± 20 μM] in renal brush border membrane vesicles from rabbit, suggesting no stereoselective interaction with the organic cation/proton antiporter. The conflicting results between the two *in vitro* studies may be due to the increased affinity for both pindolol enantiomers and the organic cation TEA in OK cell monolayers.

We determined that the *S*-(−)-verapamil enantiomer was a much more potent inhibitor of TEA uptake than the *R*-(+) enantiomer [IC_{50} for *S*-(−)-verapamil = 0.4 ± 0.04 μM; *R*-(+)-verapamil = 7 ± 2 μM). Although verapamil is highly metabolized in humans, a major metabolite of verapamil (D-617), which contains the chiral center, is actively secreted by the kidney (10). Upon coadministration of the organic cation, cimetidine, the renal clearance of the S-D-617 isomer was significantly decreased in humans, whereas the clearance of R-D-617 metabolite was unaffected (10), suggesting stereoselective renal secretion. This clinical study is consistent with our data demonstrating a higher affinity of the S-antipode.

The pharmacokinetics of the enantiomers of disopyramide are complex, involving both concentration-dependent and stereoselective binding to plasma proteins in the therapeutic plasma concentration range (9,27,32). When administered as the racemate, the unbound renal clearance of *S*-(+)-disopyramide was significantly greater than that of the *R*-(−) enantiomer in one report (9) and only slightly greater in another report (32). However, when administered as the separate enantiomers, there was no significant difference between the unbound renal clearances (32). In this report, we determined that the IC_{50} value of *R*-(−)-enantiomer of disopyramide was approximately one-half of the IC_{50} of *S*-(+)-disopyramide, suggesting a greater affinity for the *R*-(−) antipode for the TEA transporter in the brush border membrane of the OK cells.

The diastereoisomers, quinine and quinidine, have similar physical properties. In clinical studies, the renal clearance of quinidine was much greater than that of quinine (2). Because no stereoselective differences in plasma protein binding were observed and because the compounds have similar octanol–water partition coefficients and pK_a values (2), the renal filtration and passive reabsorption of these two diastereoisomers should be similar. Therefore, stereoselective active renal secretion may be the mechanism responsible for the observed renal clearances differences of quinine and quinidine.

In vitro studies performed with these two diastereoisomers are quite conflicting and are summarized in Table II. In the present study, we determine a threefold difference in the IC_{50} of the two diastereoisomers (IC_{50} quinine = 16.7 ± 1.9 μM; quinidine = 51.2 ± 13.3 μM), suggesting a stereoselective interaction, with quinine being the more potent species. In our previous study (19) we found no significant difference in the IC_{50} values of quinine and quinidine on the uptake of NMN in renal brush border membrane vesicles from the rabbit, suggesting no stereoselective interaction of these two diastereoisomers. Based upon inhibition studies, Bendayan *et al.* (20) determined that quinidine is the substance with a greater affinity in canine renal brush border membrane vesicles. The results of Wong *et al.* (21) suggest that quinine was more potent than quinidine in inhibiting the uptake of the organic cation, amantidine, in rat cortical slices and in proximal tubule suspensions. Clearly the results of the *in vivo* and the *in vitro* studies involving these diastereoisomers are confusing. These conflicting data may be a result of species differences in transport mechanisms or in differences in experimental methods. In addition, the *in vitro* studies examine the potential stereoselective events occurring at only one membrane face, whereas the stereoselective renal clearance differences observed in the *in vivo* studies are a result of the events at both membrane faces and potential renal cellular drug metabolism occurring in concert.

In summary, our results suggest stereoselective interactions of various organic cations with the organic cation transporter in the brush border membrane of OK cells. These results are consistent with some human clinical findings showing stereoselective renal handling of the organic cations pindolol and a metabolite of verapamil. Further studies are required to determine potential stereoselective interactions for this system. The studies to date have relied on inhibition potencies (IC_{50}) of enantiomers. Studies directly examining the transport of the enantiomers are needed, which may re-

Table II. Apparent IC_{50} or K_i Values of Quinine and Quinidine Determined by *in Vitro* Studies

Species	Technique	IC_{50} or K_i (μM)		Ref. No.
		Quinine	Quinidine	
Rabbit	BBMV	2.5 ± 0.8	2.4 ± 0.5	19
Dog	BBMV	7.0	0.7	20
Rat	Proximal tubule suspensions	32 ± 3	84 ± 11	21
Rat	Cortical slices	368 ± 28	780 ± 84	21
Opossum	Cells	17 ± 2	51 ± 13	This paper

veal differences in binding and translocation rates. In addition, no study has specifically addressed potential interactions occurring at the basolateral membrane or potential stereoselective renal cellular metabolism.

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