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Interactions of quinidine and quinine and (+)- and (−)-pindolol with the organic cation/proton antiporter in renal brush border membrane vesicles

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In the past decade there have been a number of studies describing stereoselective biotransformation of drugs and the implications to the rational use of racemic compounds [1, 2]. Recently, stereoselective renal elimination of drugs has also been described [3, 4]. In a study from this laboratory, we observed that the renal clearance of (−)-pindolol was approximately 30% greater than that of the (+)-enantiomer in normal volunteers who received a single oral dose of racemic pindolol [3]. Notterman *et al.* [4] observed that the renal clearance of quinidine was approximately 4-fold greater than that of its diastereomer, quinine, in normal volunteers. Although diastereomers, unlike enantiomers, may have very different physical chemical properties, the investigators pointed out that the physical chemical properties of quinine and quinidine, including melting point, oil:water partition coefficient and pK_a, are very similar. Thus, they suggested that the large differences in renal clearances were due to the different spacial arrangements of the two molecules.

In addition to filtration, the renal handling of compounds may involve secretion, reabsorption and biotransformation. Interactions with chiral macromolecules, such as transport proteins involved in secretory or reabsorptive processes, may be stereoselective. For example, a number of endogenous molecules such as glucose and various amino acids are known to interact stereoselectively with transport systems in the proximal tubule [5, 6]. In previous clinical studies [3, 4], the renal clearance of unbound quinine and quinidine, as well as of (+)- and (−)-pindolol, was greater than the glomerular filtration rate, suggesting that these compounds are actively secreted by the kidney. Since these compounds are basic, one possible explanation for the stereoselective renal clearance is that the compounds interact stereoselectively with the organic cation transport system, a system responsible for the active secretion of a number of basic compounds [7]. Recent studies have suggested that there is an organic cation/proton antiporter in the renal brush border membrane which may be responsible for the active secretion of organic cations [8–16]; however, to date there have been no studies examining the stereoselectivity of the system. In the present work, we addressed the questions of whether (+)- and (−)-pindolol and quinine and quinidine interact with the organic cation/proton antiporter in the renal brush border membrane and whether the interaction of these compounds is stereoselective.

Methods

Brush border membrane vesicles (BBMV*) were prepared by the method of Booth and Kenny [17] as modified in our laboratory [13]. Briefly, the method involves divalent cation aggregation of basolateral membranes and intracellular organelles followed by purification of the brush border membranes by differential centrifugation. Using this method, the activity of maltase, a specific enzyme marker for brush border membranes, is enhanced between 8- and 14-fold in the final membrane preparation in comparison to the initial homogenate whereas the activity of the basolateral enzyme marker, Na^+-K^+-ATPase, is either not enhanced or only slightly enhanced [13].

To determine whether quinine and quinidine and the enantiomers of pindolol interacted with the organic cation/proton antiporter and to ascertain whether the interaction was stereoselective we studied the inhibitory effect of each compound in BBMV on the transport of N^1-methylnicotinamide (NMN), a model substrate for the organic cation/proton antiporter [7, 11, 12]. The experiments were carried out in a pH gradient as follows: 10 μL of vesicles (20 mg protein/mL) that had been preincubated for 60 min with pH 6.0 HK buffer [150 mM KCl and 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES)] or pH 6.0 MK buffer [150 mM KCl and 10 mM 4-morpholineethanesulfonic acid (MES)] was incubated at 25° with 20–40 μL pH 7.5 HK buffer containing [^{3}H]NMN (2.4 μM) for 5, 8, 15 and 30 sec. For the inhibition studies, [^{3}H]NMN (2.4 μM) and various amounts of (+)-pindolol, (−)-pindolol, quinine or quinidine were added to the pH 7.5 HK buffer and incubated with vesicles for 8 sec and 2 hr. The mechanism by which quinine interacted with the transport of NMN was evaluated by carrying out Michaelis–Menten studies in which the rate of NMN transport, evaluated at 8 sec, was studied as a function of concentration in the presence and absence of 2 μM quinine. The uptake of NMN was determined by rapid vacuum filtration and liquid scintillation counting.

Each data point in each experiment was determined as the mean of three replicates. For each study at least three experiments were performed in three separate membrane preparations. Unless otherwise specified, the data are presented as mean ± SEM. Statistical differences were determined by a Student's *t*-test. The IC_{50} of each enantiomer was calculated from a Dixon plot (1/rate versus inhibitor concentration) as the absolute value of the abscissa intercept. Because the Dixon plots were curvilinear at very high concentrations of quinine and quinidine, the data were analyzed without the highest concentration (100 μM). To obtain transport parameters and to determine the mechanism involved in the inhibitory effects of quinine, the data were fit to a Michaelis–Menten model as follows

$$\text{rate} = V_{max} \cdot C/(K_m + C)$$

where V_{max} is the maximal transport rate; Km represents the concentration needed to reach half of the V_{max} and C is the concentration of NMN in the extravesicular solution. The fitting procedure used was the FIT FUNCTION Procedure on the PROPHET computer system, which is an iterative nonlinear least-squares regression program that allows for weighting of the data based upon the variance (PROPHET Statistics, National Institutes of Health). The data were also fit to the following model:

$$\text{rate} = V_{max} \cdot C/(K_m + C) + F \cdot C$$

* Abbreviations: HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; NMN, N^1-methylnicotinamide; MES, 4-morpholineethanesulfonic acid; TEA, tetraethylammonium; HK buffer, buffer containing KCl (150 mM) and HEPES (10 mM); and MK buffer, buffer containing KCl (150 mM) and MES (10 mM); BBMV, brush border membrane vesicles.

where F is the coefficient for the linear, nonsaturable transport of NMN across BBMV. However, because F was not significantly different from zero in all of the fits, the first model was used.

Results and Discussion

Consistent with previous studies of organic cation transport in renal BBMV [7–9, 11, 13–16], we observed an overshoot phenomenon in which the uptake of NMN, stimulated by the pH gradient ($pH_{in}:pH_{out} = 6.0:7.5$), temporarily exceeded the equilibrium uptake. To determine an appropriate time to obtain an accurate initial rate, we examined the uptake of NMN over the first 30 sec (Fig. 1). The uptake was apparently linear from 0 to 15 sec in the presence of a pH gradient; therefore, transport at 8 sec was used as the unidirectional initial rate throughout the rest of the experiments.

Figure 2 depicts the transport rate of NMN determined at 8 sec as a function of concentration of the inhibitors. The studies were carried out in the presence of a pH gradient ($pH_{in}:pH_{out} = 6.0:7.5$). Both enantiomers of pindolol as well as both quinine and quinidine inhibited the initial rate of transport of NMN. Quinine and quinidine were considerably more potent than (+) and (−)-pindolol in inhibiting NMN uptake. The IC_{50} values of the inhibitors were: (+)-pindolol = 0.14 ± 0.02 mM, (−)-pindolol = 0.12 ± 0.02 mM, quinine = 2.5 ± 0.8 μM, and quinidine = 2.4 ± 0.5 μM. There was no statistically significant difference between the IC_{50} values of the enantiomers of pindolol or the IC_{50} values of quinine and quinidine. None of the compounds inhibited the uptake of NMN at equilibrium, suggesting that the compounds do not inhibit binding or alter vesicle volume.

We examined the nature of the interaction of quinine with the transporter for NMN in BBMV (Fig. 3). The data demonstrate that NMN was transported by a saturable mechanism in BBMV. The apparent K_m of NMN was 2.61 ± 0.33 mM and the V_{max} was 116.8 ± 8.2 pmol/mg·sec. Consistent with a competitive mechanism, quinine (2 μM) significantly increased the apparent K_m of NMN to 11.2 ± 1.56 mM ($P < 0.05$), but did not significantly affect the apparent V_{max} (139.3 ± 14.8 pmol/mg·sec).

The specific structural requirements for organic cation transport in the kidney have been established in earlier studies in whole tissue preparations such as isolated cortical slices [7, 18]. Methodological advances in the isolation and

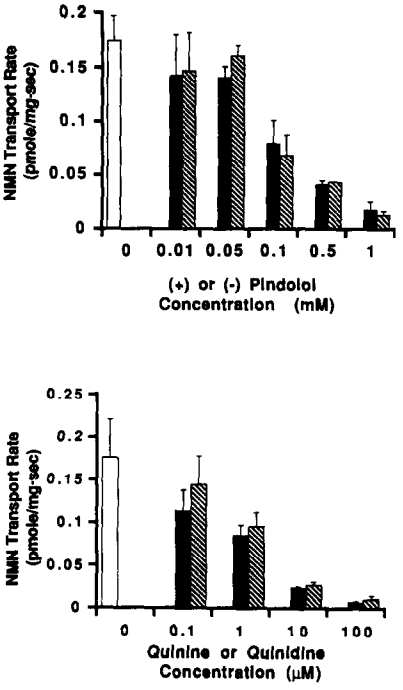

Fig. 2. Initial uptake of NMN at 8 sec in brush border membrane vesicles in the presence of various concentrations of quinine and quinidine (lower panel) and (+)- and (−)-pindolol (upper panel). The clear bar in each panel is the control, the black bars represent either quinine (lower panel) or (+)-pindolol (upper panel), and the shaded bars represent either quinidine (lower panel) or (−)-pindolol (upper panel). Vesicles were loaded with pH 6.0 buffer and incubated in pH 7.5 buffer containing 2.4 μM [^{3}H]NMN and various concentrations of inhibitors. Data are the means ± SE of three experiments carried out in three membrane preparations except for the control for the pindolol panel where six experiments are described.

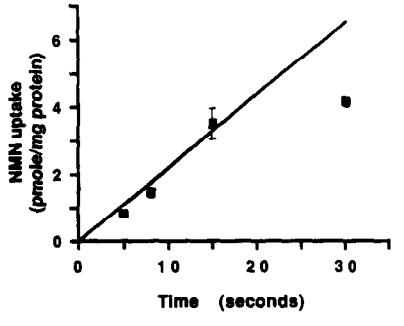

Fig. 1. pH-driven NMN uptake versus time in renal brush border vesicles prepared from rabbit renal cortex. Vesicles were loaded with pH 6.0 buffer and incubated with pH 7.5 buffer containing 2.4 μM [^{3}H]NMN. Each data point is the mean ± SE of three experiments carried out in three membrane preparations. The line represents the best fit of the first three points by linear regression analysis forced through the origin.

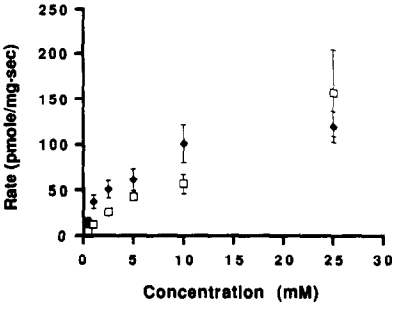

Fig. 3. Initial transport rate of NMN as a function of concentration in brush border membrane vesicles prepared from rabbit renal cortex as in Fig. 2. The diamonds represent control data and the squares represent the transport of NMN in the presence of 2 μM quinine. Data are the means ± SE of four experiments carried out in four membrane preparations.

purification of brush border and basolateral membrane vesicles have provided a powerful tool to study the individual structural requirements of the transport systems in each of the polar membranes of the renal proximal tubule. To date, the stereoselectivity of the organic cation transport system in the brush border and basolateral membrane has not been studied. In this study, we examined the effects of four chiral compounds on the transport of NMN, a model organic cation, in renal BBMV. Our goals were to establish whether organic cation transport in the brush border membrane is stereoselective and to ascertain whether the previously observed stereoselective renal clearances [3, 4] may have been due to stereoselective interactions with the organic cation/proton antiporter at the renal brush border membrane. We postulated that the organic cation/proton antiporter may be responsible for this stereoselectivity because all of the compounds were basic and all had renal clearances greater than the glomerular filtration rate, indicating that active secretion was involved. The system in the brush border membrane seemed likely to be involved because previous studies have suggested that the brush border membrane is a site of active transport of organic cations [7–16].

Because of the obvious difficulties in carrying out detailed mechanistic studies in human kidneys, the rabbit was selected as the animal model for these studies. Data in the literature suggest that there are remarkable similarities among mammalian species in the characteristics of organic cation transport in the renal brush border membrane. For example, organic cation transport in renal BBMV prepared from the cortex of rabbit [13, 15], rat [14] and dog [10] kidney is saturable. The apparent K_m of NMN transport obtained in this study in BBMV prepared from rabbit renal cortex (2.61 mM) is in agreement with the values reported previously in the literature, which range from 0.015 mM in the dog [19] to 0.63–2.01 mM in rabbit renal BBMV [10, 13, 16, 20] depending upon the pH conditions employed. Studies in BBMV prepared from the kidney of dog [11], rat [14], rabbit [13, 15, 16] and from cultured renal cells of the pig [21] have demonstrated that the uptake of the organic cations, NMN and tetraethylammonium (TEA), can be driven by an outwardly directed proton (or inwardly directed hydroxyl) gradient. Thus, the data obtained in rabbit renal BBMV should be relevant to other mammalian species including humans.

The data obtained in this study suggest that both (+)- and (−)-pindolol and quinine and quinidine interact with the organic cation/proton antiporter in the renal brush border membrane. The enantiomers of pindolol were considerably less potent than either quinine or quinidine. The IC_{50} of both pindolol enantiomers was approximately 0.1 mM which is considerably lower than the K_m of NMN obtained in this study (2.61 mM) and is similar to the K_m of TEA (0.12–0.549 mM) obtained in recent studies in rabbit renal BBMV [15, 22–24] (Table 1). The IC_{50} of the interaction of the quinine diastereomers was about 2 μM, indicating that both compounds are considerably more potent than pindolol, TEA and NMN but are similar in potency to cimetidine [8] and amiloride [25]. Table 1 lists some of the physical chemical properties of pindolol, TEA, NMN, cimetidine, procainamide, amiloride, quinine and quinidine along with their apparent K_m or IC_{50} values for organic cation transport in the brush border membrane. As indicated by their high partition coefficients, quinine and quinidine are clearly more lipophilic than the other organic cations. This high degree of lipophilicity may explain in part their potency.

Quinine is considered a potent, specific inhibitor of organic cation transport and is used frequently to determine whether a compound is transported by the organic cation/proton antiporter [7, 27]. Accordingly, we examined the mechanism by which quinine interacted with the transport system for NMN. The data obtained in this study demonstrated that quinine inhibited the uptake of NMN in BBMV by an apparently competitive mechanism, i.e. the V_{max} of NMN was unchanged by quinine whereas the apparent K_m of NMN was increased (Fig. 3).

In summary, neither quinine or quinidine nor (+)- or (−)-pindolol stereoselectively inhibited the transport of NMN in the brush border membrane of rabbit kidney. If the results obtained in rabbit kidney are applicable to human kidney, it appears that the previously observed stereoselective renal clearance of these compounds was not due to stereoselective interactions with the organic cation/proton antiporter in the renal brush border membrane. Recently, we observed that pindolol does not interact stereoselectively with the system involved in the reabsorption of basic amino acids in the brush border membrane of the rabbit renal tubule [30]. Other mechanisms of stereoselective transport for these compounds cannot be ruled out by these data. Because radiolabeled ligands are not available, the actual transport of each enantiomer could not be studied. Therefore, it is possible that different translocation rates across the brush border membrane or interactions with transport pathways within the brush border membrane not studied here could cause the

Table 1. Physical properties and affinities of organic cations to the organic cation transport system in rabbit renal BBMV

Compound	pK_a	Partition coefficient*	K_m or IC_{50} (mM)
NMN	Quaternary	Quaternary	0.015–2.61 [10, 13, 16, 19, 20]†
TEA	Quaternary	Quaternary	0.12–0.549 [15, 22–24]
Quinine	4.1, 8.4 [4]	39 [4]	0.0025
Quinidine	4.3, 8.5 [4]	36 [4]	0.0024
(+)-Pindolol‡	8.8 [26]	0.41 [26]	0.14
(−)-Pindolol‡	8.8 [26]	0.41 [26]	0.12
Cimetidine	7.1 [27]	2.5 [27]	0.0048 [8]
Amiloride§	8.4 [25]	0.054 [28]	0.0075 [25]
Procainamide‖	9.3 [9]	0.79 [29]	0.54 [9]

* The partition coefficients were determined in octanol and buffer, pH 7.0.
† Values in brackets indicate reference numbers.
‡ The partition coefficient of pindolol was determined at pH 9.0.
§ The partition coefficient of amiloride was determined in octanol:water, not buffered.
‖ The partition coefficient of procainamide was determined at pH 7.4.

observed stereoselective renal clearance differences. Other mechanisms such as stereoselective biotransformation or stereoselective interactions with organic cation transport pathways in the basolateral membrane also need to be explored.

Note added in proof: Bendayan *et al.* recently reported (Bendayan R, Sellers EM and Silverman M, Inhibition kinetics of cationic drugs on N^1-methylnicotinamide uptake in brush border membrane vesicles from the dog kidney cortex. *Can J Physiol Pharmacol* **68**: 467–475, 1990) a slight stereoselective inhibitory effect of quinine and quinidine upon the uptake of NMN in BBMV in the dog. They determined a K_I for quinine of 7.0 μM and a K_I for quinidine of 0.7 μM. The statistical significance between these K_I values was not reported. These values, however, are within the range of values determined in this study.

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