Journal of Pharmacokinetics and Biopharmaceutics, Vol. 26, No. 3, 1998

Competitive Inhibition of *p*-Aminohippurate Transport by Quinapril in Rabbit Renal Basolateral Membrane Vesicles

Wiyada Akarawut[1] and David E. Smith[1,2]

Received October 8, 1997—Final May 26, 1998

The mechanism of quinapril's interaction with the organic anion transporter was characterized by studying its effect on the transport of p-*aminohippurate (PAH) in rabbit renal basolateral membrane vesicles (BLMV). Cis-inhibition studies demonstrated that quinapril was a specific and potent inhibitor of PAH. The* K_i *of quinapril was about 20 μM, a value similar to that of probenecid and eight-times lower than the* K_m *value of 165 μM for PAH. Even though quinapril resulted in trans-inhibition of PAH uptake during counterflow studies, kinetic studies revealed that quinapril was a competitive inhibitor of PAH transport. This latter finding suggests that quinapril and PAH share a common binding site on the transporter. Overall, the results indicate that quinapril is a high-affinity inhibitor of the organic anion transporter in renal BLMV, and that drug–drug interactions may occur with other organic anions at the basolateral membrane of proximal cells.*

KEY WORDS: competitive inhibition; *p*-aminohippurate; quinapril; kidney; basolateral membrane vesicles.

INTRODUCTION

Angiotensin converting enzyme (ACE) inhibitors have proved to be effective not only in patients with high renin hypertension but also in many hypertension patients who have normal levels of plasma renin activity (1). More recently, ACE inhibitors have played a prominent role in treating patients with congestive heart failure, myocardial infarction, and diabetic nephropathy. These drugs act by inhibiting conversion of the relatively inactive angiotensin I to the potent vasoconstrictor angiotensin II, as well as by

This work was supported in part by the Upjohn Research Award Fund, College of Pharmacy, The University of Michigan, and by National Institutes of Health Grant R01 GM35498.
[1]College of Pharmacy and Upjohn Center for Clinical Pharmacology, 1310 E. Catherine Street, The University of Michigan, Ann Arbor, Michigan 48109–0504.
[2]To whom correspondence should be addressed.

0090-466X/98/0600-0269$15.00/0

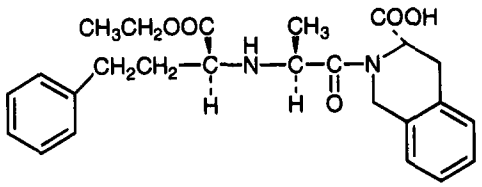

Fig. 1. Chemical structure of quinapril.

inhibiting the degradation of bradykinin, a potent vasodilator peptide. The long-standing view of the renin–angiotensin–aldosterone (RAA) system is that of a classical endocrine system in which circulating levels of hormone are important. However, recent evidence suggests that this traditional view is a gross oversimplification and should be expanded to include local (tissue) RAA systems.

Quinapril (Fig. 1) is an ACE inhibitor that is formulated as an alkyl-ester prodrug for improved bioavailability and drug performance (2). Once absorbed, it is primarily de-esterified to its pharmacologically active metabolite, quinaprilat. Being an amphoteric drug (possessing an amino group, pK_a 5.4 and a carboxyl group, pK_a 2.8), quinapril is completely ionized at its carboxyl group and completely unionized at its amino group, at physiological *p*H. Thus, quinapril is anionic in nature and would be expected to utilize the organic acid transport system of proximal tubular cells. This contention is supported by studies in the isolated perfused rat kidney (3,4) in which substantial net transport was observed for quinapril across the basolateral membrane, a process inhibited by organic anions (i.e., probenecid, *p*-aminohippurate) but not by organic cations (tetraethylammonium, quinine). Whole organ studies are limited, however, in that they describe only net processes and do not provide a precise mechanistic basis for drug transport at a single membrane.

It is important to characterize the renal handling of ACE inhibitors since this route of elimination will not only influence access to RAA sites in the kidney but may also impact on the drugs' systemic exposure and potential for drug interactions. Thus, the renal uptake of quinapril was characterized by studying its effect on the transport of *p*-aminohippurate (PAH), a prototypical organic anion, in rabbit basolateral membrane vesicles.

MATERIALS AND METHODS

Materials

[^{3}H]PAH (4.9 Ci/mmol) was purchased from DuPont-NEN (Boston, MA). Quinapril and quinaprilat were gifts from Parke-Davis (Ann Arbor,

MI). Enalapril and enalaprilat were gifts from Merck (Rahway, NJ). All other chemicals were of the highest purity available.

Membrane Preparation

Basolateral membrane vesicles (BLMV) were isolated from rabbit kidneys using a modification of the Percoll gradient method (5,6). A male New Zealand White rabbit (2–3 kg) was anesthetized with xylazine (10 mg/kg, im) and ketamine (44 mg/kg, im). Kidneys were perfused *in situ* via the renal artery with an ice-cold solution containing 140 mM NaCl and 10 mM KCl. The kidneys were excised, decapsulated, and placed in an ice-cold perfusion solution. The cortex was removed, weighed, and minced. Typically, about 10 g of tissue per rabbit were obtained. The minced cortex was homogenized in sucrose buffer (250 mM sucrose and 10 mM triethanolamine-HCl, *p*H 7.6; 10 ml/g of tissue) using a glass-Teflon homogenizer (Glas-Col, Model 099C K44) at speed setting 30 (~1400 rpm), 25 strokes. A 0.5 ml aliquot of the homogenate was saved at 4°C for protein and marker enzyme assays. Unless otherwise noted, the entire procedure was carried out on ice or at 4°C. A Sorvall RC-5C Plus refrigerated centrifuge (SS-34 fixed-angle rotor) was used for all centrifugations.

The homogenate was centrifuged at 3000 rpm for 10 min. The supernatant was saved, and the loose pellet was resuspended in half the original volume of sucrose buffer, homogenized 10 strokes at speed setting 30, and centrifuged at 3000 rpm for 10 min. The supernatants were combined and centrifuged at 11,000 rpm for 15 min. The supernatant was saved for subsequent use. The upper fluffy layer of the pellet was aspirated by a small volume of supernatant (≈1–2 ml) using a glass Pasteur pipette. The suspension of the fluffy pellet was further homogenized 10 strokes at speed setting 30, combined with the previously saved supernatant, and centrifuged at 18,000 rpm for 30 min. The upper fluffy layer of the pellet was resuspended in 25 ml of fresh sucrose buffer and homogenized 10 strokes at speed setting 30. A 25 ml aliquot of fresh sucrose buffer was added to the homogenized suspension and mixed. Each 25 ml of the resulting suspension was poured into two centrifuge tubes containing 3.5 ml of Percoll. A 1.5 ml aliquot of sucrose buffer was added to each tube to make a total volume of 30 ml. The suspension was gently mixed and centrifuged at 20,000 rpm for exactly 1 hr without a brake. The first 6 ml were discarded from the top of the resulting Percoll gradient and the following 8 ml (7–14 ml fractions) were pooled as crude basolateral membranes. The pooled fraction was centrifuged at 50,000 rpm for 1 hr (Beckman L8-70M ultracentrifuge, 70.1 Ti rotor). The Percoll formed a glassy pellet and the membranes formed a fluffy pellet on top of the Percoll. The fluffy pellet was resuspended in 8 ml of sucrose buffer

using a syringe with a 25-gauge needle, and centrifuged at 50,000 rpm for 45 min. The pellet was resuspended in 25 ml of sucrose buffer. A 250 μl aliquot of 1 M $MgCl_2$ was added (final concentration 10 mM). The suspension was stirred occasionally for 20 min and centrifuged at 5000 rpm for 15 min. The pellet was resuspended in 25 ml of sucrose buffer. Magnesium was removed by adding 1.25 ml of 100 mM EDTA to make a final concentration of 5 mM. The suspension was centrifuged at 20,000 rpm for 20 min. The pellet was suspended in 25 ml of an appropriate loading buffer and centrifuged at 20,000 rpm for 20 min. The resulting pellet, which contained the purified basolateral membranes, was resuspended in a small volume of loading buffer for transport studies using a syringe with a 25-gauge needle. The BLMV were prepared the day before use, stored at 4°C overnight and used within 3 days.

Marker Enzyme and Protein Assays

Na^+/K^+-ATPase, a marker for basolateral membranes, was determined according to Jørgensen and Skou (7) with phosphate determined by the method of Fiske and Subbarow (8). Orientation of BLMV was determined by comparing total ATPase and Na^+/K^+-ATPase (ouabain sensitive) activity in intact vesicles and those disrupted by the addition of deoxycholate, as described by Goldinger *et al.* (6). Cross-contamination of BLMV with brush border membranes was assessed by measuring the activity of alkaline phosphatase (ALP) using a commercially available kit (Sigma Diagnostics, Kit no. 245, St. Louis, MO). Protein was measured according to the method of Bradford (9) using γ-globulin as standard.

Uptake Measurements

Uptake studies were conducted by a rapid filtration technique (10) using a 10-place filtering manifold (Hoefer Scientific Instruments, San Francisco, CA). All transport measurements were made at 22°C. Typically, BLMV were suspended in about 1–2 ml loading buffer to give a final protein concentration of 10 mg/ml and incubated for 1 hr at room temperature before uptake measurements. The reaction was initiated by adding 90 μl uptake medium containing [^{3}H]PAH (0.5 μCi) and varying concentrations of unlabeled PAH ($\pm$ inhibitor) into a 10 μl vesicle suspension. At appropriate times, the incubation was terminated by addition of 2 ml ice-cold stop solution (200 mM mannitol, 50 mM K gluconate, and 10 mM Hepes, *p*H 7.5). The contents were immediately filtered under vacuum through a prewetted Millipore filter (PHWP, 0.3 μm) and washed three times with 2 ml ice-cold stop solution. The radioactivity remaining on the filters was counted by standard liquid scintillation technique after dissolution in 8 ml

scintillation cocktail (Ready Protein, Beckman Instruments, Fullerton, CA). Correction for nonspecific binding was performed by running a zero time in the presence of vesicles where radiolabeled substrate and stop solution were added simultaneously. This value was subtracted from the uptake data. Investigators have shown that PAH does not bind significantly (< 10%) to the membrane vesicles (6,11), therefore [^{3}H]PAH retained on the filters after blank correction represents intravesicular PAH.

For studies in the presence of glutarate and an inwardly-directed Na^+ gradient (i.e., Na^+/glutarate-coupled PAH transport), vesicles were suspended in buffer *p*H 7.5 containing 200 mM mannitol, 50 mM K gluconate, and 10 mM Hepes. Uptake was initiated by mixing 10 μl vesicle suspension with 90 μl uptake medium, *p*H 7.5, containing [^{3}H]PAH in 100 mM NaCl, 50 mM K gluconate, 10 mM Hepes, and 10 μM glutarate.

Uptake measurements conducted under voltage clamped conditions were performed using the K^+ ionophore, valinomycin, and equal concentrations of K^+ on both sides of the membrane. Valinomycin stock solution (1 mg/ml in ethanol) was added to the vesicle suspension 30 min before uptake measurements for a final concentration of 3 μg/mg protein. Equal volumes of ethanol were added in control experiments. The ethanol volume was always less than 3% of the total.

For trans experiments, various test compounds were loaded into vesicles at the final step of membrane preparation. Uptake was then performed in the absence of Na^+ and glutarate gradients and under voltage clamped conditions. Uptake medium was of the same composition as loading buffer but without test compounds. For trans-experiments using a 10-fold dilution, 10 μl vesicle suspension was incubated with 90 μl uptake medium containing [^{3}H]PAH. For subsequent studies using a 20-fold dilution, 10 μl vesicle suspension was incubated with 190 μl uptake medium containing [^{3}H]PAH. Control uptakes were performed in unloaded vesicles.

Data Analysis

Unless otherwise specified, data are reported as mean ± standard deviation from at least three different membrane preparations, with each preparation conducted in triplicate. Statistical analyses were performed using a Student's paired *t* test or ANOVA (SYSTAT v5.03, Systat, Inc., Evanston, IL), as appropriate. Pairwise comparisons were made using Tukey's test. A probability of $p \leq 0.05$ was considered statistically significant. Nonlinear regression analysis was performed using SCIENTIST (v2.01, MicroMath Scientific Software, Salt Lake City, UT) and a weighting factor of unity. Goodness of fit was determined by evaluating the coefficient of determination (r^2), the standard error of parameter estimates, and by visual inspection of the residuals.

For dose–response studies, the inhibitory effect can be described by the model

$$E = E_0 - \frac{I_{max} \cdot I}{IC_{50} + I} \tag{1}$$

where E is the observed uptake, E_0 is the uptake in the absence of inhibitor, I_{max} is the maximum inhibition, I is the inhibitor concentration and IC_{50} is the concentration that causes 50% inhibition of the maximal drug effect. If the drug is capable of abolishing the uptake, then $I_{max} = E_0$ and Eq. (1) reduces to

$$E = E_0 \cdot \left(\frac{IC_{50}}{IC_{50} + I} \right) \tag{2}$$

The IC_{50} was estimated for each membrane preparation by fitting the data to Eq. (2) using nonlinear regression.

For kinetic studies, the concentration-dependent uptake of PAH reflected both saturable (i.e., Michaelis-Menten) and nonsaturable transport processes

$$Uptake = \frac{V_{max} \cdot C}{K_m + C} + K_d \cdot C \tag{3}$$

where V_{max} is the maximal rate of saturable uptake, K_m is the Michaelis constant, K_d is the rate constant for a nonsaturable process and C is the substrate (PAH) concentration. Kinetic parameters (i.e., V_{max}, K_m, and K_d) of PAH uptake were estimated for each membrane preparation by fitting the data to Eq. (3) using nonlinear regression.

The Michaelis-Menten equation was used to analyze the saturable transport component $[v = Uptake - K_d \cdot C = V_{max} \cdot C/(K_m + C)]$, and is conveniently transformed into one that gives a straight line. This can be done by taking the reciprocal of both sides of the equation to give

$$\frac{1}{v} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \cdot \frac{1}{C} \tag{4}$$

From a plot of $1/v$ vs. $1/C$, K_m and V_{max} could then be estimated by linear regression analysis.

Quinapril inhibited the saturable uptake of PAH in a competitive manner (see Results section). For transport in the presence of a competitive inhibitor, the kinetics become

$$v = \frac{V_{max} \cdot C}{K_m \cdot (1 + I/K_i) + C} \tag{5}$$

where K_i is the inhibition constant. Lineweaver–Burk transformation of Eq. (5) gives

$$\frac{1}{v} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \cdot \left(1 + \frac{I}{K_i}\right) \cdot \frac{1}{C} \tag{6}$$

In the presence of a competitive inhibitor, the apparent value ($K_{m.app}$) is equal to K_m $(1 + I/K_i)$.

The K_i of quinapril was determined by three different approaches: (i) by linear regression analysis of the Lineweaver–Burk plots of Eqs. (4) and (6), (ii) by simultaneous fitting of the data to Eq. (5) using nonlinear regression, and (iii) according to the method of Cheng and Prusoff (12); thus, the K_i for a competitive inhibitor can be calculated from the known IC_{50} of inhibitor, and the K_m and concentration (C) of substrate using the following relationship

$$K_i = \frac{IC_{50}}{1 + C/K_m} \tag{7}$$

RESULTS

Membrane Purity

Membrane vesicle preparations (±PAH) were evaluated with respect to enzymatic activity or enrichment ($n = 23$), contamination ($n = 24$), and orientation ($n = 18$). In particular, Na^+/K^+-ATPase activity in the isolated BLMV was enriched 13.6 ± 5.5-fold whereas ALP activity was not enriched (1.5 ± 0.4-fold). Analysis of vesicle orientation revealed $47.1 \pm 10.5\%$ right-side-out, $11.1 \pm 2.6\%$ inside-out, and $41.8 \pm 10.8\%$ open/leaky. Of the vesicles formed, over 80% were right-side-out. These values compare favorably with those reported in the literature (6,13–19) and demonstrate that the BLMV preparations were highly purified with negligible cross contamination.

Kinetics of PAH Uptake

To obtain preliminary estimates of the kinetics for PAH, its initial rate uptake (at 15 sec) was measured as a function of substrate concentration (i.e., 10–1000 μM). As shown in Fig. 2, PAH uptake appeared nonlinear and the data were best fit using a combination of saturable and nonsaturable processes. According to Eq. (3), PAH had the following transport parameters: $V_{max} = 75.7 \pm 16.6$ pmol/mg per 15 sec, $K_m = 162 \pm 13$ μM and $K_d = 0.174 \pm 0.047$ μl/mg per 15 sec. For all analyses, the coefficient of determination (r^2) was ≥ 0.994. Comparison of the V_{max}/K_m vs. K_d suggests that,

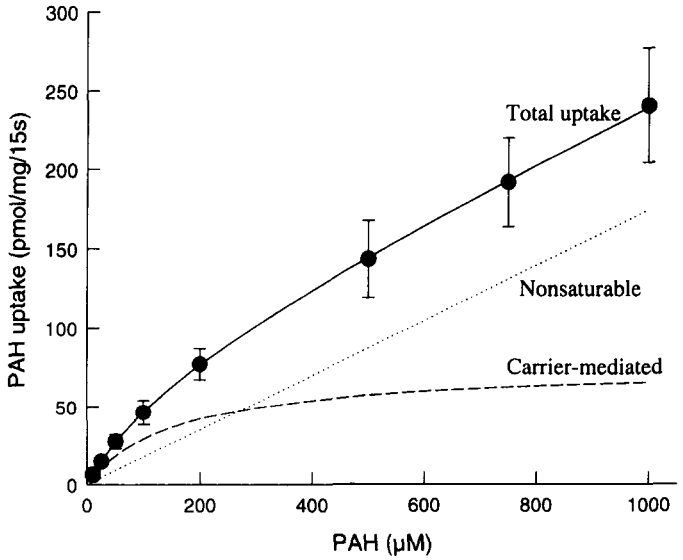

Fig. 2. Concentration-dependent uptake of PAH in BLMV. Membrane vesicles were suspended in buffer, *p*H 7.5 (200 mM mannitol, 50 mM K gluconate, and 10 mM Hepes) and uptake was initiated in buffer, *p*H 7.5 (100 mM NaCl, 50 mM K gluconate, 10 mM Hepes, and 10 μM glutarate). The 15-sec uptake of [^{3}H]PAH was measured in the presence of increasing concentrations of unlabeled drug. Data are $\bar{x} \pm$ SE from three separate membrane preparations. Lines were generated using fitted mean parameters (see text), as determined by nonlinear regression analysis.

under linear conditions, the carrier-mediated component accounts for about 73% of the total PAH uptake and the nonsaturable process accounts for about 27% of its uptake. However, at higher PAH concentrations the nonsaturable process makes a greater contribution to the overall transport process.

Cis Effect of ACE Inhibitors on PAH Uptake

The interaction of select ACE inhibitors with the organic anion transport system was investigated by determining the extent of cis inhibition on PAH uptake. Cis refers to the inhibitor being present on the same side of the membrane as radiolabeled PAH. The 15-sec uptake of PAH (50 μM) was determined in the presence of an inwardly directed Na^+ gradient and 10 μM glutarate, and in the presence of 1 mM of test inhibitors. As shown in Fig. 3, quinapril markedly reduced the uptake of PAH to 30% of control values and appeared to be as potent as probenecid (PRO), a model inhibitor

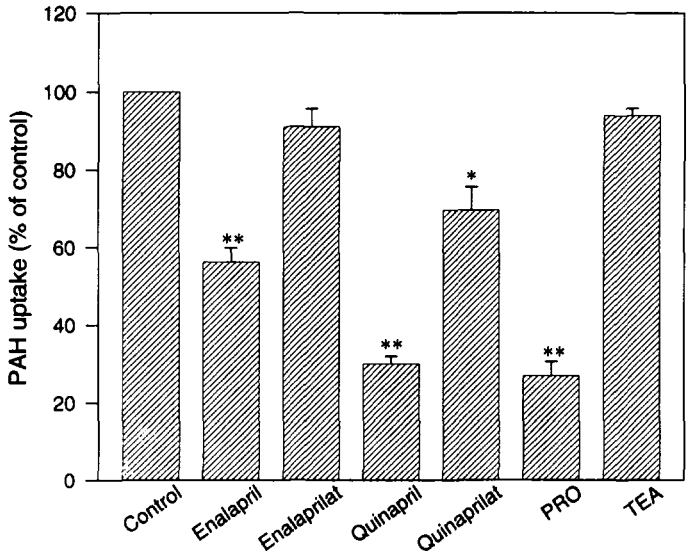

Fig. 3. Cis effect of ACE inhibitors, probenecid (PRO) and tetraethylammonium (TEA) on PAH uptake in BLMV. Membrane vesicles were suspended in buffer, *p*H 7.5 (200 mM mannitol, 50 mM K gluconate, and 10 mM Hepes) and uptake was initiated in buffer, *p*H 7.5 (100 mM NaCl, 50 mM K gluconate, 10 mM Hepes, and 10 μM glutarate). The 15-sec uptake of 50 μM [^{3}H]PAH was measured in the absence (control) and presence of 1 mM various test inhibitors. Data are $\bar{x} \pm$ SE from four separate membrane preparations. $^*p < 0.001$ and $^{**}p < 0.0002$, as compared to control.

of organic anion transport. This result suggested that quinapril and probenecid effectively blocked the carrier-mediated transport of PAH since uptake was reduced to that of the nonsaturable component. Of all ACE inhibitors tested, quinapril exhibited the strongest inhibition. The inhibitory potency of ACE inhibitors was as follows: quinapril > enalapril > quinaprilat > enalaprilat. In contrast, tetraethylammonium (TEA), a model substrate for organic cation transport, had no effect. It should be appreciated that the inhibitory effect was specific since no compound altered the equilibrium value of PAH uptake (as measured at 1 hr).

Dose–Response Effect of Quinapril on PAH Uptake

The effectiveness of quinapril as an inhibitor of PAH uptake was confirmed by constructing a dose–response curve. The 15-sec uptake of PAH (50 μM) was measured in the presence of increasing concentrations of inhibitor (i.e., 10–2000 μM). As shown in Fig. 4, quinapril inhibited PAH uptake in a concentration-dependent manner. The IC_{50}, as determined by

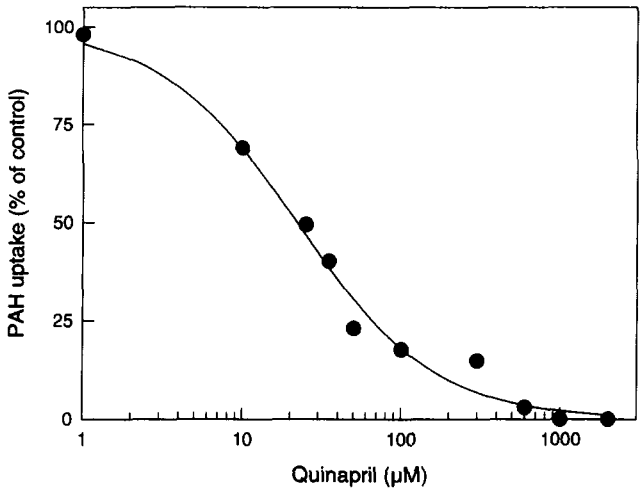

Fig. 4. Dose–response effect of quinapril on PAH transport in BLMV. Membrane vesicles were suspended in buffer, *p*H 7.5 (200 mM mannitol, 50 mM K gluconate, and 10 mM Hepes) and uptake was initiated in buffer, *p*H 7.5 (100 mM NaCl, 50 mM K gluconate, 10 mM Hepes, and 10 μM glutarate). The 15-sec uptake of 50 μM [^{3}H]PAH was measured in the presence of increasing concentrations of quinapril. Each data point is the mean of triplicate values from a typical experiment and represents only the carrier-mediated transport after correction for a nonsaturable component. The line represents a best fit of the data using nonlinear regression analysis.

Eq. (2), was 24.7 ± 2.5 μM. For all analyses, the coefficient of determination (r^2) was ≥ 0.951. The inhibition constant (K_i) of quinapril was then calculated from Eq. (7). Using known values for PAH concentration (50 μM) and K_m (162 μM), and individual IC_{50}s for inhibitor from dose–response curves, the K_i value for quinapril was estimated as 18.9 ± 1.9 μM.

Effect of Quinapril on the Kinetics of PAH Uptake

The previous studies demonstrated that quinapril interacted with the organic anion transporter by inhibiting the Na^+/glutarate-coupled uptake of PAH. This suggests that these two substrates could share a common transport pathway. To further investigate the nature of this interaction, the 15-sec uptake kinetics of increasing concentrations of PAH were examined alone and in the presence of 25 μM quinapril. As shown by the Lineweaver–Burk plot in Fig. 5, a change was observed in the slope and x intercept of the curve, but not y intercept in the presence of inhibitor. Kinetic analysis (Table I) revealed a significant increase in the apparent K_m of PAH (161 ± 35 μM for control vs. 428 ± 202 μM in the presence of quinapril;

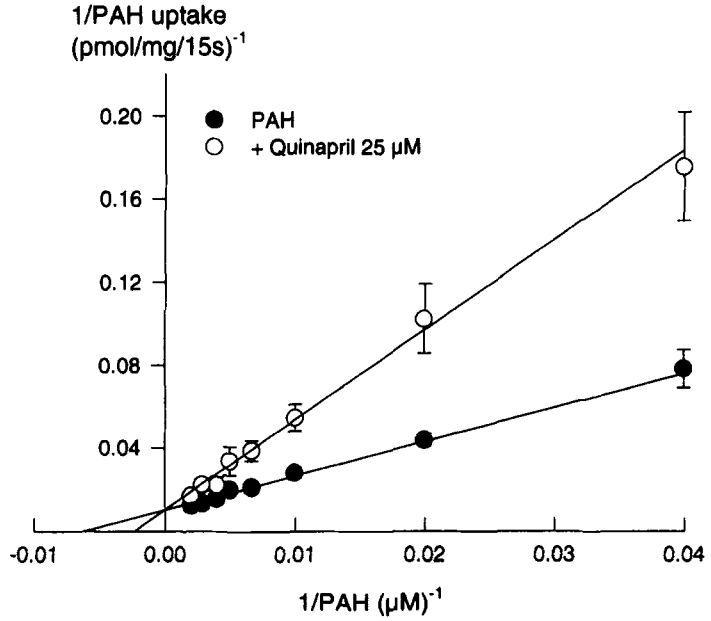

Fig. 5. Lineweaver–Burk analysis of the effect of quinapril on PAH transport in BLMV. Membrane vesicles were suspended in buffer, *p*H 7.5 (200 mM mannitol, 50 mM K gluconate, and 10 mM Hepes) and uptake was initiated in buffer, *p*H 7.5 (100 mM NaCl, 50 mM K gluconate, 10 mM Hepes, and 10 μM glutarate). The 15-sec uptake of increasing concentrations of [^{3}H]PAH was measured alone and in the presence of 25 μM quinapril. Data are $\bar{x} \pm$ SE from four separate membrane preparations and represent only the carrier-mediated transport after correction for a nonsaturable component. Lines were generated using fitted mean parameters (see Table I), as determined by linear regression analysis.

Table I. Lineweaver–Burk Analysis of the Kinetics of Quinapril Inhibition on PAH Uptake in BLMV

	Kinetic parameters[a]		
	V_{max} (pmol/mg per 15 sec)	K_m (μM)	K_i (μM)
PAH (control)	100 ± 20	161 ± 35[d]	—
+ Quinapril 25 μM	97.8 ± 13.2	428 ± 202[b,d]	17.3 ± 5.1[c]

[a] Values are $\bar{x} \pm$ SD from four separate membrane preparations. Data were fit to Eqs. (4) and (6) using linear regression.
[b] $K_{m,app} = K_m (1 + I/K_i)$.
[c] $K_i = I/[(K_{m,app}/K_m) - 1]$.
[d] $p < 0.05$, as determined using a Student's paired t test.

Table II. Nonlinear Regression Analysis of the Kinetics of Quinapril Inhibition on PAH Uptake in BLMV

	Kinetic Parameters[a]		
	V_{max} (pmol/mg per 15 sec)	K_m (μM)	K_i (μM)
PAH (control)	103 ± 18	168 ± 19	—
Simultaneous fit	105 ± 23	173 ± 48	20.2 ± 3.5

[a] Values are $\bar{x} \pm$ SD from four separate membrane preparations. Data were fit to Eq. (5) using nonlinear regression.

$p < 0.05$), whereas the value for V_{max} was not altered (100 ± 20 pmol/mg per 15 sec for control vs. 98 ± 13 pmol/mg per 15 sec in the presence of quinapril). Thus, the mechanism of inhibition was clearly of a competitive type, suggesting that quinapril and PAH share a common carrier. The K_i, as estimated from this plot using Eqs. (4) and (6), was 17.3 ± 5.1 μM.

Knowing the type of inhibition, kinetic parameters were confirmed by fitting the data to a competitive inhibition model, as described by Eq. (5). The parameter estimates (K_m and V_{max}) were comparable regardless of whether PAH uptake data were evaluated alone (i.e., fit only control data to a Michaelis-Menten equation) or by simultaneous fitting (Table II). Moreover, these values were in close agreement with those obtained from the Lineweaver–Burk plot. This analysis is graphically depicted in Fig. 6, and K_i was estimated as 20.2 ± 3.5 μM. For all analyses, the coefficient of determination (r^2) was ≥ 0.878.

K_i values determined by all three approaches (i.e., Lineweaver–Burk, nonlinear regression, dose–response) were in excellent agreement. Taken together, the K_i of 20 μM for quinapril was approximately eight times lower than the K_m of PAH. Thus, it appears that quinapril is a high affinity substrate for the basolateral organic anion transporter.

Trans Effect of Quinapril on PAH Uptake

The data indicate that quinapril is a potent competitive inhibitor of the PAH transport system due to its ability to cis inhibit PAH/glutarate exchange. Trans-stimulation is one of the criteria used to demonstrate the involvement of a carrier or whether compounds share a common carrier. Therefore, we further investigated whether quinapril was capable of trans-stimulating [^{3}H]PAH uptake, where trans refers to the presence of driving substance on the side opposite to that of radiolabeled substrate. In these experiments, [^{3}H]PAH influx was measured in unloaded vesicles (control) and in vesicles preloaded with high concentration of unlabeled PAH or quinapril under equal *p*H (in = out) and voltage clamped conditions. As shown

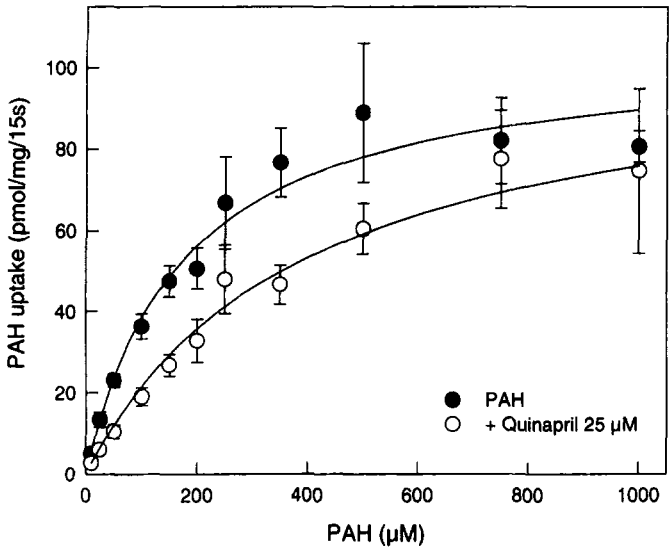

Fig. 6. Simultaneous fit of the concentration-dependent uptake of PAH in BLMV using a Michaelis-Menten equation and a competitive inhibition model. Experimental conditions were as described in Fig. 5. Data are $\bar{x} \pm$ SE from four separate membrane preparations and represent only the carrier-mediated transport after correction for a nonsaturable component. Lines were generated using fitted mean parameters (see Table II), as determined by nonlinear regression analysis.

in Fig. 7A, PAH concentrations of 1 and 4 mM were capable of trans-stimulating itself by $159 \pm 24\%$ and $182 \pm 16\%$ of control, respectively. Although not statistically significant, quinapril (at 1 and 4 mM) showed inhibitory effects with the uptake reduced to $58 \pm 12\%$ and $46 \pm 14\%$ of control, respectively. This trans effect by unlabeled PAH and quinapril was specific since the equilibrium uptake of [^{3}H]PAH (as measured at 1 hr) was not affected when vesicles were preloaded with these drugs.

It is possible that the inhibition caused by quinapril was due to a significant amount of the drug carried over from the uptake medium according to our study design. For example, the final concentration would be 100 μM when vesicles preloaded with 1 mM quinapril were diluted 10-fold. This residual amount of quinapril would be present on the cis face of the membranes and could result in cis inhibition of [^{3}H]PAH influx. To rule out this possibility, we performed another experiment using vesicles loaded with 50 μM quinapril followed by a 20-fold dilution. Glutarate, an effective counter ion of PAH (20), was also tested. Similar to our previous results, [^{3}H]PAH uptake was enhanced to 120% and 153% of control by intravesicular PAH and glutarate, respectively, but was reduced to 65% of control in

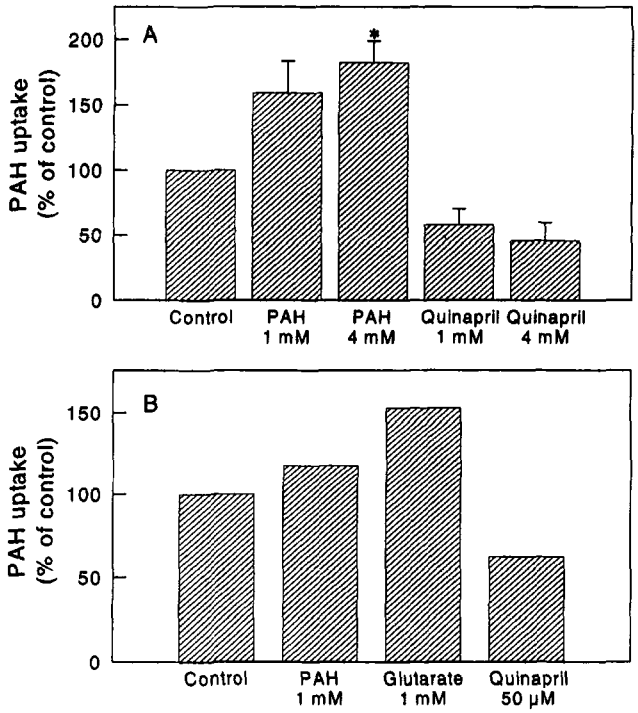

Fig. 7. Trans-effect of PAH, quinapril and glutarate on PAH uptake in BLMV. Membrane vesicles were suspended in buffer, *p*H 7.5 (200 mM mannitol, 50 mM K gluconate, and 10 mM Hepes) without loading compounds (control) or with unlabeled PAH (1 and 4 mM), quinapril (1 and 4 mM), and glutarate (1 mM). Valinomycin was added to the vesicles (3 μg/mg protein) 30 min prior to the uptake measurements. Uptake medium was of the same composition as loading buffer but without test compounds. (A) Uptake was measured at 2 min by diluting vesicles 10-fold with uptake medium containing 50 μM [^{3}H]PAH. Data are $\bar{x} \pm$ SE ($n = 3$); $^*p < 0.05$, as compared to control. (B) Experimental conditions were the same as in Panel A except that vesicles were diluted 20-fold. Data are the mean of triplicate values from one membrane preparation.

quinapril-loaded vesicles (Fig. 7B). Therefore, the observed trans effect by quinapril was not a manifestation of cis inhibition.

DISCUSSION

Renal tubular secretion is a multistep process involving the uptake of drug across the basolateral membrane, diffusion through the proximal cell, and exit across the brush border membrane. Organic anion uptake has been

characterized as a tertiary active transport (21,22). In this regard, Na^+/K^+-ATPase creates an electrochemical potential difference for Na^+ (out > in) at the basolateral membrane. Dicarboxylates (i.e., α-ketoglutarate) are then cotransported with Na^+ down its concentration gradient. Finally, α-ketoglutarate is exchanged for an organic anion resulting in its net uptake. Competitive inhibition among organic anions may occur at this tertiary step, thereby altering the proportional relationship between plasma and urine drug levels.

With notable exceptions, ACE inhibitors (including quinapril) are cleared predominantly by the kidney. Yet, few studies have rigorously examined the precise mechanisms of renal transport for these drugs. Instead, previous studies have defined the overall or net renal clearance of ACE inhibitors using whole animal or isolated organ models. Renal clearance models may, still, be further complicated by the substantial intrarenal metabolism of ACE inhibitor prodrugs once they reach the intracellular space of proximal tubular cells (3,4,23). As a result, we have characterized the interaction of quinapril with the organic anion transporter by studying its effect on the transport of PAH in BLMV. Studies in isolated basolateral membranes are advantageous in that they are free from the influence of intracellular events such as metabolism, sequestration, and binding. They are also devoid of complicating extracellular events such as blood flow and protein binding.

In the present study, we demonstrated that PAH transport is inhibited strongly by quinapril and that this effect is specific since the equilibrium value of PAH uptake is not altered. The IC_{50} and K_i values of quinapril ($\approx$25 and 20 μM, respectively) are much lower than the K_m of PAH ($\approx$165 μM), indicating that quinapril is a high-affinity inhibitor of the basolateral organic anion transport system. Further, the drug is as potent as probenecid, a typical inhibitor of PAH transport. In this regard, the K_i of quinapril falls within the range of values (K_is of 15–50 μM) reported in the literature for probenecid (24–26). With respect to mechanism, kinetic studies reveal that quinapril acts as a competitive inhibitor of PAH transport. This result further verified that quinapril competes with PAH at the same binding site for transport across the basolateral membrane into proximal cells. It should be appreciated that the K_m value of PAH ($\approx$165 μM) determined in this investigation was in good agreement with previous studies in kidney. Others have reported K_m values of 175 μM in rat BLMV (27), 144 μM in bovine BLMV (25), and 110 μM in isolated rabbit proximal tubules (26,28). The carrier-mediated component of PAH uptake estimated in this study (i.e., 73% of total uptake) is similar to the 72% value reported in dog BLMV (29) and the 70% value reported in isolated rabbit renal proximal tubules (26).

In membrane vesicles, the definitive criteria for showing a compound as a substrate for a specific transport system is the ability to exhibit both cis inhibition and trans stimulation (30). In general, carrier-mediated transport involves four steps (31): first, binding of the substrate at one face of the membrane; second, translocation of the loaded carrier from one face of the membrane to the other; third, dissociation of the substrate; and fourth, reorientation of the empty carrier back to the opposite face of the membrane. To secure trans stimulation, it is assumed that translocation is slow relative to the binding and dissociation steps, and that migration of the empty carrier is slow relative to migration of the loaded carrier. Thus, a large concentration of substrate on one side of the membrane can accelerate the transport of a smaller concentration of substrate present on the opposite side of the membrane by increasing the availability of free carrier (i.e., trans stimulation). Our results showing that high concentrations of PAH and glutarate inside the vesicles are capable of trans-stimulating [^{3}H]PAH uptake are consistent with this concept. In contrast, the lack of a trans stimulatory effect by quinapril (at high and low concentrations) might suggest that the drug binds to the transporter but is not translocated. In conflict with this possibility, however, are the *in vivo* and organ perfusion studies (2–4,32) clearly indicating that quinapril undergoes tubular secretion. In addition, kinetic studies verifying quinapril as a competitive inhibitor of PAH confirmed that the two substrates share a common binding site. Finally, it should be noted that the absence of trans stimulation for a substrate does not necessarily mean that translocation does not occur. For example, counterflow studies have revealed a lack of trans stimulation for N^1-methylnicotinamide (NMN) by NMN and procainamide (PCA) by PCA in rabbit renal BLMV (33). Trans inhibitory effects were observed for NMN by 1-methyl-4-phenylpyridinium (MPP^+) in canine renal BBMV (31). Nevertheless, it was subsequently demonstrated that MPP^+ was indeed transported in rabbit renal BBMV by a pathway shared by TEA and NMN (34).

Quinapril's high affinity for the organic anion transporter suggests that potential drug–drug interactions may readily occur with other organic anions. This belief is supported by an *in vivo* rat study (35) in which quinapril substantially increased cefdinir's area under the curve and half-life, but decreased its clearance as a result of competition at the tubular anion transporter. Captopril had similar effects on cefdinir pharmacokinetics, but of lesser magnitude. Human studies have also suggested that many ACE inhibitors are renally cleared by tubular secretion along with glomerular filtration (2,32,36). They appear to utilize the nonspecific organic acid secretory pathway and, as a result, probenecid pretreatment has been shown to significantly reduce the renal clearance of captopril (37), enalapril, and

enalaprilat (38). Therefore, knowing the precise mechanism of renal disposition for quinapril is of clinical importance, and should prove valuable in optimizing drug therapy while avoiding unwanted drug interactions.

In conclusion, quinapril is found to be a potent inhibitor of the Na^+/glutarate-coupled transport of PAH in rabbit renal BLMV. Quinapril's affinity ($K_i \approx 20\ \mu M$) is on order of that for probenecid and was about eight times higher than that for PAH ($K_m \approx 165\ \mu M$). Kinetic studies reveal that quinapril acts as a competitive inhibitor of PAH, suggesting the two substrates share a common binding site on the transporter. Overall, the results indicate that quinapril is a high-affinity inhibitor of the organic anion transporter in renal BLMV, and that drug–drug interactions may occur with other organic anions at the basolateral membrane of proximal cells.

ACKNOWLEDGMENTS

We thank Dr. Marilyn Morris for help in the preparation of renal membrane vesicles.

REFERENCES

1. E. K. Jackson and J. C. Garrison. Renin and angiotensin. In P. B. Molinoff, R. W. Ruddon, and A. G. Gilman (eds.), *The Pharmacologic Basis of Therapeutics*, 9th ed., McGraw-Hill, New York, 1996, pp. 733–758.
2. A. N. Wadworth and R. N. Brogden. Quinapril: A review of its pharmacological properties, and therapeutic efficacy in cardiovascular disorders. *Drugs* **41**:378–399 (1991).
3. A. R. Kugler, S. C. Olson, and D. E. Smith. Disposition of quinapril and quinaprilat in the isolated perfused rat kidney. *J. Pharmacokin. Biopharm.* **23**:287–305 (1995).
4. A. R. Kugler, S. C. Olson, and D. E. Smith. Tubular transport mechanisms of quinapril and quinaprilat in the isolated perfused rat kidney: Effect of organic anions and cations. *J. Pharmacokin. Biopharm.* **24**:349–368 (1996).
5. V. Scalera, Y. Huang, B. Hildmann, and H. Murer. A simple isolation method for basal-lateral plasma membranes from rat kidney cortex. *Membrane Biochem.* **4**:49–61 (1981).
6. J. M. Goldinger, D. S. Khalsa, and S. K. Hong. Photoaffinity labeling of organic anion transport system in proximal tubule. *Am. J. Physiol.* **247**:C219-C227 (1984).
7. P. L. Jørgensen and J. C. Skou. Purification and characterization of (Na^+–K^+)-ATPase in preparations from the outer medulla of rabbit kidney. *Biochim. Biophys. Acta* **233**:366–380 (1971).
8. C. H. Fiske and Y. Subbarow. The colorimetric determination of phosphorous. *J. Biol. Chem.* **66**:375–400 (1925).
9. M. M. Bradford. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **72**:248–254 (1976).
10. U. Hopfer, K. Nelson, J. Perrotto, and K. J. Isselbacher. Glucose transport in isolated brush-border membrane from rat small intestine. *J. Biol. Chem.* **248**:25–32 (1973).
11. J. Eveloff. *p*-Aminohippurate transport in basal-lateral membrane vesicles from rabbit renal cortex: Stimulation by *p*H and sodium gradient. *Biochim. Biophys. Acta* **897**:474-480 (1987).
12. Y.-C. Cheng and W. H. Prusoff. Relationship between the inhibition constant (Ki) and the concentration which causes 50% inhibition (IC_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* **22**:3099–3108 (1973).

13. J. L. Kinsella, P. D. Holohan, N. I. Pessah, and C. R. Ross. Isolation of luminal and antiluminal membranes from dog kidney cortex. *Biochim. Biophys. Acta* **552**:468–477 (1979).
14. R. A. Reynolds, J. Wald, P. D. McNamara, and S. Segal. An improved method for isolation of basolateral membranes from rat kidney. *Biochim. Biophys. Acta* **601**:92–100 (1980).
15. M. I. Sheikh, U. Kragh-Hansen, K. E. Jørgensen, and H. Røigaard-Petersen. An efficient method for the isolation and separation of basolateral-membrane and luminal-membrane vesicles from rabbit kidney cortex. *Biochem. J.* **208**:377–382 (1982).
16. E. F. Boumendil-Podevin and R. A. Podevin. Isolation of basolateral and brush border membranes from the rabbit kidney cortex: Vesicle integrity and membrane sidedness of the basolateral fraction. *Biochim. Biophys. Acta* **735**:86–94 (1983).
17. Z. Talor, R. M. Gold, W. C. Yang, and J. A. L. Arruda. Anion exchanger is present in both luminal and basolateral renal membranes. *Eur. J. Biochem.* **164**:695–702 (1987).
18. S. H. Wright and T. M. Wunz. Succinate and citrate transport in renal basolateral and brush border membranes. *Am. J. Physiol.* **253**:F432–F439 (1987).
19. S. A. Hilden, C. A. Johns, W. B. Guggino, and N. E. Madias. Techniques for isolation of brush-border and basolateral membrane vesicles from dog kidney cortex. *Biochim. Biophys. Acta* **983**:77–81 (1989).
20. J. B. Pritchard. Coupled transport of *p*-aminohippurate by rat kidney basolateral membrane vesicles. *Am. J. Physiol.* **255**:F597–F604 (1988).
21. D. C. Brater, P. P. Sokol, S. D. Hall, and T. D. McKinney. Disposition and dose requirements of drugs in renal insufficiency. In D. W. Seldin and G. Giebisch (eds.), *The Kidney: Physiology and Pathophysiology*, 2nd ed., Raven, New York, 1992, pp. 3671–3695.
22. J. B. Pritchard and D. S. Miller. Proximal tubular transport of organic anions and cations. In D. W. Seldin and G. Giebisch (eds.), *The Kidney: Physiology and Pathophysiology*, 2nd ed., Raven, New York, 1992, pp. 2921–2945.
23. I. A. M. de Lannoy, R. Nespeca, and K. S. Pang. Renal handling of enalapril and enalaprilat: Studies in the isolated red blood cell-perfused rat kidney. *J. Pharmacol. Exp. Ther.* **251**:1211–1222 (1989).
24. K. J. Ullrich, G. Rumrich, and S. Klöss. Contraluminal organic anion and cation transport in the proximal renal tubule: V. Interaction with sulfamoyl- and phenoxy diuretics, and with β-lactam antibiotics. *Kidney Int.* **36**:78–88 (1989).
25. C. Schmitt and G. Burckhardt. *p*-Aminohippurate/2-oxoglutarate exchange in bovine renal brush-border and basolateral membrane vesicles. *Pflügers Arch.* **423**:280–290 (1993).
26. S. Shpun, K. K. Evans, and W. H. Dantzler. Interaction of α-KG with basolateral organic anion transporter in isolated rabbit renal S3 proximal tubules. *Am. J. Physiol.* **268**:F1109–F1116 (1995).
27. D. A. Griffiths, S. D. Hall, and P. P. Sokol. Interaction of 3′-azido–3′-deoxythymidine with organic ion transport in rat renal basolateral membrane vesicles. *J. Pharmacol. Exp. Ther.* **257**:149–155 (1991).
28. W. H. Dantzler, K. K. Evans, and S. H. Wright. Kinetics of interactions of para-aminohippurate, probenecid, cysteine conjugates and *N*-acetyl cysteine conjugates with basolateral organic anion transporter in isolated rabbit proximal renal tubules. *J. Pharmacol. Exp. Ther.* **272**:663–672 (1995).
29. F. G. M. Russel and W. G. Vermeulen. Effect of substituted benzoylglycines (hippurates) and phenylacetylglycines on *p*-aminohippurate transport in dog renal membrane vesicles. *Pharm. Res.* **11**:1829–1833 (1994).
30. P. D. Holohan and C. R. Ross. Mechanisms of organic cation transport in kidney plasma membrane vesicles: 1. Countertransport studies. *J. Pharmacol. Exp. Ther.* **215**:191–197 (1980).
31. P. P. Sokol, P. D. Holohan, and C. R. Ross. The neurotoxins 1-methyl-4-phenylpyridinium and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine are substrates for the organic cation transporter in renal brush border membrane vesicles. *J. Pharmacol. Exp. Ther.* **242**:152–157 (1987).

32. E. J. Begg, R. A. Robson, R. R. Bailey, K. L. Lynn, G. J. Frank, and S. C. Olson. The pharmacokinetics and pharmacodynamics of quinapril and quinaprilat in renal impairment. *Br. J. Clin. Pharmacol.* **30**:213–220 (1990).
33. P. P. Sokol and T. D. McKinney. Mechanism of organic cation transport in rabbit renal basolateral membrane vesicles. *Am. J. Physiol.* **258**:F1599-F1607 (1990).
34. K. D. A. Lazaruk and S. H. Wright. MPP^+ is transported by the TEA^+–H^+ exchanger of renal brush-border membrane vesicles. *Am. J. Physiol.* **258**:F597–F605 (1990).
35. A. Jacolot, M. Tod, and O. Petitjean. Pharmacokinetic interaction between cefdinir and two angiotensin-converting enzyme inhibitors in rats. *Antimicrob. Agents Chemother.* **40**:979–982 (1996).
36. J. B. Kostis, J. J. Raia, Jr., E. A. DeFelice, J. A. Barone, and R. G. Deeter. Comparative clinical pharmacology of ACE inhibitors. In J. B. Kostis and E. A. DeFelice (eds.), *Angiotensin Converting Enzyme Inhibitors*, Alan R Liss, New York, 1987, pp. 19–54.
37. S. M. Singhvi, K. L. Duchin, D. A. Willard, D. N. KcKinstry, and B. H. Migdalof. Renal handling of captopril: Effect of probenecid. *Clin. Pharmacol. Ther.* **32**:182–189 (1982).
38. F. H. Noormohamed, W. R. McNabb, and A. F. Lant. Pharmacokinetic and pharmacodynamic actions of enalapril in humans: Effect of probenecid pretreatment. *J. Pharmacol. Exp. Ther.* **253**:362–368 (1990).