Dipeptide Uptake and Transport Characteristics in Rabbit Tracheal Epithelial Cell Layers Cultured at an Air Interface

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Purpose. To determine the functional presence of a H^+/peptide cotransport process in rabbit tracheal epithelial cell layers cultured at an air-interface and its contribution to transepithelial dipeptide transport.

Methods. Rabbit tracheocytes were isolated, plated on Transwells, and cultured at an air-interface. After 5 or 6 days in culture, uptake and transepithelial transport of carnosine were examined.

Results. Carnosine uptake by tracheocytes was pH-dependent and was saturable with a Michaelis-Menten constant of 170 μM. Moreover, carnosine uptake was inhibited 94% by Gly-L-Phe, 28% by β-Ala-Gly, but not at all by Gly-D-Phe or by the amino acids β-Ala and L-His. Unexpectedly, transepithelial carnosine transport at pH 7.4 (i.e., in the absence of a transepithelial pH gradient) was similar in both the apical-to-basolateral (*ab*) and basolateral-to-apical (*ba*) directions. Lowering the apical fluid pH to 6.5 reduced *ab* transport 1.6 times without affecting *ba* transport, consistent with predominantly paracellular diffusion of carnosine under an electrochemical potential gradient.

Conclusions. The kinetic behavior of carnosine uptake into cultured tracheal epithelial cell layers is characteristic of a H^+-coupled dipeptide transport process known to exist in the small intestine and the kidney. Such a process does not appear to be rate-limiting in the transport of carnosine across the tracheal epithelial barrier.

KEY WORDS: tracheal epithelial cell layer; transport; peptide transporter; carnosine.

INTRODUCTION

Proton-coupled transport processes are known to exist in the small intestine and kidney to mediate the absorption and reabsorption, respectively, of di- and tripeptides as well as peptidomimetics such as β-lactam antibiotics (1–3), angiotensin-converting enzyme inhibitors (4), and renin inhibitors (5). By contrast, there have been few reports on oligopeptide transport in the lung. Helliwell et al. (6) demonstrated that D-Phe-L-Ala was transported from the air spaces to the vascular spaces in the perfused rat lungs 2.2 times faster than D-Phe-D-Ala. The same group (7) demonstrated that D-Phe-L-Ala was taken up by H^+-coupled dipeptide transport systems in brush border membrane vesicles of freshly isolated rat lung type II pneumocytes. Moreover, Morimoto et al. (8) observed asymmetric transepithelial transport of Gly-L-Phe at 1 mM in primary cultured rat alveolar type II cell monolayers. There is, therefore, evidence for the possible existence of dipeptide transporters in the distal respiratory tract. However, whether they also exist in the tracheal epithelium, which is morphologically and functionally different from the alveolar epithelium (9,10), is not known to date.

Thus, the purpose of the present study was to determine the functional existence of H^+-coupled dipeptide transport process in the rabbit tracheal epithelial cell layers cultured at an air-interface, that resembles the native tissue in their morphological characteristics and bioelectric properties (15), and the contribution of such a process to transepithelial dipeptide transport. Carnosine (β-Ala-L-His) served as a model substrate. This hydrolysis-resistant dipeptide has been shown to be transported via oligopeptide transporters in the small intestine and the kidney (11–14).

MATERIALS AND METHODS

Materials

Carnosine and ^{3}H-labeled carnosine (specific activity, 185 MBq/mmol) were purchased from Sigma (St. Louis, MO) and Moravek Biochemicals (Brea, CA), respectively. β-Ala-Gly, Gly-L-Phe, Gly-D-Phe, β-alanine, L-histidine, sodium azide, 2,4-dinitrophenol (DNP), and carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP) were obtained from Sigma (St. Louis, MO). PC-1 medium, the culture medium, was obtained from Hycor Biomedical (Portland, ME). Tissue culture-treated Transwells (0.45 μm, 12 mm O.D.) were obtained from Costar (Cambridge, MA). Other culture reagents and supplies were obtained from GIBCO (Grand Island, NY). Prior to cell plating, Transwells were treated for 4 h with 6.6 μg/cm^2 rat tail collagen type I, 2.2 μg/cm^2 human fibronectin (Collaborative Biomedical Products, Bedford, MA), and 10 μg/cm^2 bovine serum albumin (Sigma Chemical, St. Louis, MO) in 0.25 ml of PC-1 medium. All other chemicals used were of the highest purity available.

Primary Culture of Rabbit Tracheal Epithelial Cells

The primary culture procedure of rabbit tracheal epithelial cells has been published elsewhere (15). The studies utilizing rabbits described in this report conformed to the Guiding Principles in the Care and Use of Animals (DHEW Publication, NIH 80-23). Briefly, excised rabbit trachea was cut into small pieces and incubated in 0.2% protease XIV (Sigma, St. Louis, MO) in a minimum essential medium (S-MEM) at 37°C for 90 min. Epithelial cells were gently scraped off, dispersed in S-MEM containing 10% fetal bovine serum (FBS) and 0.5 mg/ml deoxyribonuclease I (Sigma, St. Louis, MO), and centrifuged at 200× *g* for 10 min at room temperature. The isolated cells were washed with S-MEM containing 10% FBS twice and filtered through a 40 μm cell strainer. These purified cells were pelleted at 200× *g* for 10 min and resuspended in PC-1 medium supple-

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mented with 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 50 μg/ml gentamicin, and 1 μg/ml fungizone. These tracheocytes were plated on fibronectin/collagen-pretreated Transwells at a density of 1.3×10^6 cells/cm^2 and cultured in a humidified atmosphere of 5% CO_2 and 95% air at 37°C. The volumes of apical and basolateral culture media were 0.5 and 1.5 ml, respectively. From day 2 onward, the cells were cultured with their apical surface exposed directly to air with 0.8 ml culture medium bathing the basolateral side. Uptake experiments were conducted after 5 or 6 days in primary culture.

Uptake Experiments

All uptake experiments were performed at either 37°C in a humidified atmosphere of 5% CO_2 and 95% air or 4°C in a cold room. After removing the culture medium, 0.5 ml (pH 5.5, 6.5, or 7.4) and 1.5 ml (pH 7.4) of bicarbonated Ringer's solution was added to the apical and basolateral sides, respectively. The Ringer's solution comprised 1.8 mM $CaCl_2$, 0.81 mM $MgSO_4$, 5.4 mM KCl, 116.4 mM NaCl, 0.78 mM NaH_2PO_4, 5.55 mM glucose, 25 mM $NaHCO_3$, and 15 mM N-[2-hydroxyethyl]piperazine-N'-2-ethanesulfonic acid (HEPES) (pH 7.4) or 2-[N-morpholino]ethanesulfonic acid (MES) (pH 5.5 and 6.5). The uptake experiment was initiated by adding 0.74 MBq/ml of ^{3}H-carnosine in varying concentrations of unlabeled carnosine to the apical donor side (0.2 ml total donor volume), while 1 ml of basolateral fluid was added. In some experiments, cell layers were pretreated with metabolic inhibitors, i.e., sodium azide, DNP, and FCCP, for 30 min prior to carnosine uptake studies. At 10, 20, 30, and 60 min, the dosing solution was suctioned off, and the membrane filter with the cells was washed with ice-cold pH 7.4 Ringer's solution, followed by solubilization in 0.5 ml of 0.5% Triton X-100 solution (Sigma, St. Louis, MO) at room temperature overnight. Ten μl of the resulting solution was taken for protein determination using a Bio-Rad DC protein assay kit (Bio-Rad Laboratories, Hercules, CA), while the remainder was used for liquid scintillation counting.

The initial uptake rate was estimated from the slope of a plot of amount of carnosine transported vs. time. The Michaelis-Menten constant (Km) and the maximal saturable uptake rate (Vmax) were estimated by nonlinear regression against uptake rate-concentration profile using the following equation:

$$V = Vmax \times C/(Km + C) + P \times C$$

where V, C, and P are total uptake rate, applied concentration, and nonspecific uptake rate, respectively. A nonlinear regression program MULTI (16) was used for this calculation.

Transport Experiments

All transport experiments were performed at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. Prior to each experiment, the tracheal epithelial cell layers were washed with bicarbonated Ringer's solution and allowed to equilibrate with 0.5 ml of fresh bathing medium on the apical side and 1.5 ml of the medium on the basolateral side in the incubator. After 60 min of preincubation, a known concentration of carnosine was added to either the apical or the basolateral donor fluid to yield a final concentration of 0.074 MBq/ml ^{3}H-carnosine and 10 μM unlabeled carnosine. Two hundred μl of the receiver fluid was taken at 30, 60, 120, 180, and 240 min for radioactivity assay and immediately replaced with an equal volume of fresh Ringer's solution. The cumulative amount of carnosine appearing in the receiver fluid was plotted against time. From the observed steady-state flux (J, mol/cm^2/sec), the apparent permeability coefficient (Papp, cm/sec) of carnosine was estimated from the linear portion of each plot, according to the equation: $J = Papp \times C_o$, where C_o (mol/cm^3) is the initial concentration of the dipeptide concentration in the donor fluid.

RESULTS

Carnosine Uptake

Since carnosine uptake into cultured rabbit tracheal epithelial cells was linear for 60 min, 20 min was chosen as the incubation time to estimate the initial uptake rate in subsequent uptake experiments (data not shown). The tracheal cell layers appeared to be rather robust to various pH changes over the range studied. The potential difference (PD) of the cell layers was 56.2 ± 2.2 mV (n = 4) at pH 5.5, 63.6 ± 12.1 mV (n = 96) at pH 6.5, and 67.0 ± 1.4 mV (n = 3) at pH 7.4, while the corresponding transepithelial electrical resistance (TEER) values were 0.93 ± 0.06 kohm.cm^2, 1.09 ± 0.18 kohm.cm^2, and 0.90 ± 0.05 kohm.cm^2.

Concentration Dependence

Over the 0.1–10 mM concentration range, carnosine uptake into cultured rabbit tracheal epithelial cells at 37°C consisted of a saturable component and a linear component (Fig. 1). A Km of 170 μM and a Vmax of 210 pmol/20 min/mg protein were estimated for the saturable process ($r^2 = 0.97$). Nonspecific uptake rate at 37°C was 78 pmol/20 min/mg protein/mM, which was slightly higher than that at 4°C (52 pmol/20 min/

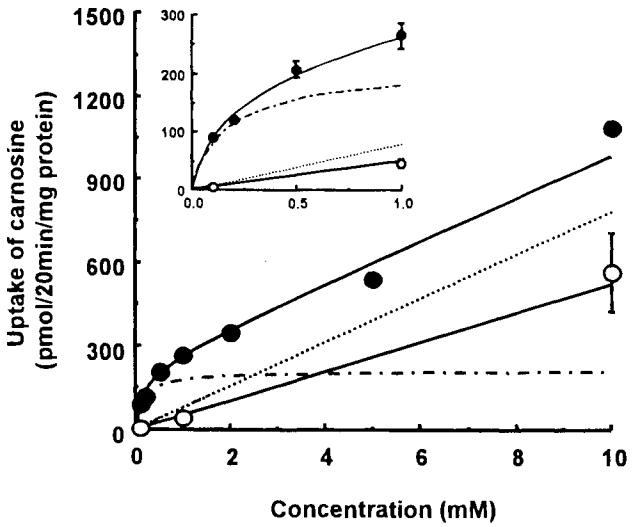

Fig. 1. Concentration dependence of carnosine uptake by rabbit tracheal epithelial cell layers (apical pH 6.5, basolateral pH 7.4) at 37°C (●) and 4°C (○). Each point represents the mean ± s.e.m. (n = 4). Solid lines were obtained by nonlinear regression using MULTI (15). Specific (dashed) and non-specific (dotted) components are also shown in this figure. The inset shows the magnification of the data observed at lower concentrations.

Table 1. Effect of 10 mM Dipeptides and Amino Acids on Carnosine Uptake by Rabbit Tracheal Epithelial Cells

Inhibitor	Carnosine uptake[a] (pmol/ 20 min/mg protein)	% Inhibition
None	11.89 ± 0.66	—
β-Ala-Gly	8.62 ± 0.72*	28
Gly-L-Phe	0.72 ± 0.13**	94
Gly-D-Phe	10.02 ± 0.26	16
β-Alanine	11.75 ± 0.23	1
Histidine	11.16 ± 1.00	6

[a] Each value represents mean ± s.e.m. (n = 4). Statistical analysis was performed by one-way analysis of variance, followed by Bonferroni multiple comparison tests (*P < 0.05 and **P < 0.01).

mg protein/mM). A linear relation between uptake and concentration was observed.

Inhibition by Amino Acids and Dipeptides

As shown in Table 1, the constituent amino acids of carnosine, β-alanine and L-histidine, did not affect carnosine uptake by rabbit tracheocytes. By contrast, the dipeptides, Gly-L-Phe and β-Ala-Gly, inhibited carnosine uptake 94% and 28%, respectively ($p < 0.05$); whereas Gly-D-Phe was without effect ($p > 0.05$). Degradation of these dipeptides during the uptake experiments appeared to be negligible (17).

Effect of Apical Fluid pH and Metabolic Inhibitors

Carnosine uptake was maximal at pH 6.5 among the three pH's tested (Table 2). Carnosine uptake at pH 6.5 was reduced to 45% of the control in the presence of 50 μM FCCP, a proton ionophore ($p < 0.05$) (Fig. 2), and was reduced to 68% and 19% in the presence of 0.5 mM DNP and 10 mM sodium azide, respectively (Fig. 2).

Carnosine Transport

Table 2 also summarizes the apparent permeability coefficients (Papp) for transepithelial carnosine transport in both

Table 2. Effect of Apical pH on Cellular Uptake and Transepithelial Transport of Carnosine in Rabbit Tracheal Epithelial Cell Layers

	Cellular uptake		Transepithelial transport		
Apical pH	uptake rate (pmol/20min/ mg protein)	n	direction[a]	Papp[b] × 10^7 (cm/sec)	n
pH 5.5	7.72 ± 0.60	4		N.D.[c]	
pH 6.5	13.10 ± 0.84*	4	ab	0.61 ± 0.08*	6
			ba	1.11 ± 0.09	6
pH 7.4	8.74 ± 1.36	3	ab	0.95 ± 0.04	7
			ba	0.94 ± 0.04	5

Note: Each data point represents the mean ± s.e.m. Asterisk (*) means statistical difference from the others. ($P < 0.05$). Statistical analyses were performed by one-way analysis of variance followed by Bonferroni multiple comparisons test.

[a] *ab* and *ba* denote apical-to-basolateral and basolateral-to-apical directions, respectively.

[b] Papp denotes apparent permeability coefficient.

[c] Not determined.

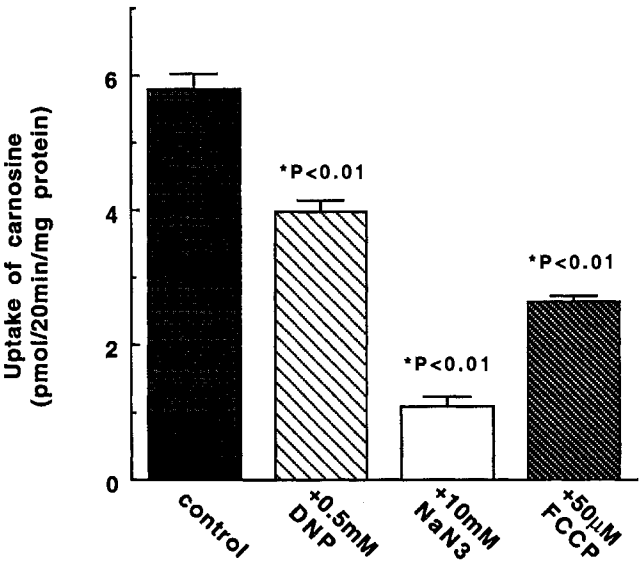

Fig. 2. Effect of metabolic inhibitors on carnosine uptake by rabbit tracheal epithelial cell layers (apical pH 6.5, basolateral pH 7.4). Metabolic inhibitors were applied apically 30 min prior to the carnosine uptake studies. Each column represents the mean ± s.e.m. (n = 4).

the presence and the absence of a transepithelial pH gradient. Carnosine transport at pH 7.4 was similar in both the apical-to-basolateral (*ab*) and the basolateral-to-apical (*ba*) directions. When the apical bathing fluid was lowered to pH 6.5, *ab* transport was reduced 1.6 times, whereas *ba* transport was unaffected. The PD of the cell monolayers was 52.1 ± 5.3 mV (n = 12) at pH 6.5 and 50.4 ± 5.8 mV (n = 12) at pH 7.4, while the corresponding TEER values were 2.15 ± 0.17 kohm.cm^2 and 1.93 ± 0.11 kohm.cm^2.

DISCUSSION

The present study demonstrated that the uptake characteristics of carnosine by cultured rabbit tracheal epithelial cells were similar to those reported for dipeptide uptake in the intestinal and the renal epithelia (12–14). Thus, carnosine uptake was inhibited by other dipeptides such as Gly-L-Phe and β-Ala-L-His (Table 1). In spite of its closer structural similarity to carnosine, β-Ala-Gly was less inhibitory than Gly-L-Phe. This pattern is consistent with the observation of Daniel et al. (18) in rat renal brush-border membrane vesicles (BBMV) that dipeptides with a β-amino acid in the NH_2-terminus seemed to show a much lower affinity towards the dipeptide transporter. Moreover, carnosine uptake was significantly decreased by either lowering the temperature (Fig. 1) or treating the cell layers with metabolic inhibitors (Fig. 2), indicating involvement of active transport process(es).

As has been shown in the intestinal and the renal epithelia, carnosine uptake by tracheal epithelial cells was driven by an inwardly directed pH gradient (Table 2). Such a gradient may be established by the apically located Na^+/H^+ exchanger in tracheocytes (19,20), that are suggested to render a pH of 6.9 for the airway surface liquid (21). Carnosine uptake by the tracheal epithelial cells was maximal at pH 6.5 among the pH tested. The lower uptake of carnosine at pH 5.5 relative to pH 6.5 may be attributed to its increased protonation at the lower

pH. At pH 5.5, carnosine would be 96% protonated, as compared with 68% at pH 6.5 and 21% at pH 7.4. Brandsch et al. (22) demonstrated that lowering the apical pH increased the Vmax for intestinal and renal dipeptide transport without affecting the Km.

Carnosine uptake in tracheal epithelial cells was saturable with a Km of 170 μM, being closer in value to the Km of 1.1 mM in the rabbit kidney (14) than the Km of 9.3 mM in the rabbit small intestine (12) and being comparable to the Ki of 250 μM for the competitive inhibition by carnosine of the high affinity system for ceftibuten uptake in the rat renal BBMV (23). PEPT2 has been identified in human (24), rabbit (25), and rat (26) kidneys as a high-affinity dipeptide transporter. Interestingly, the mRNA of PEPT2 was also detected in the lung (25,26), making it attractive to postulate that the dipeptide transporter activity found in this study may conceivably be PEPT2-related.

In spite of the possible existence of dipeptide transporters in the tracheal epithelial cells, the transport of carnosine across cultured tracheal cell monolayers did not show the expected asymmetry at pH 7.4 (Table 2). The Papp was, in fact, comparable to that of mannitol (1.2×10^{-7} cm/sec), implying that carnosine, like Gly-L-Phe (17), may be translocated primarily by paracellular diffusion. Even more surprisingly, in the presence of an inwardly directed transepithelial H^+ gradient at pH 6.5, the *ab* transport of carnosine was reduced 1.6 times, despite a 1.5-fold increase in dipeptide uptake into tracheocytes (Table 2). Nevertheless, since carnosine became more protonated by lowering apical pH (68% and 21% protonation at pH 6.5 and 7.4, respectively), the transepithelial electric potential gradient (i.e., lumen negative) across the cell layers will hinder the electrodiffusion of (protonated) caronsine via paracellular pathways. Based on the Goldman's approximated solution to the Nernst-Planck equation (27), the overall aparent paracellular permeability coefficient ($Papp^{para}$) is expressed as,

$$Papp^{para} = f \times P^{+,para} + (1 - f) \times P^{\pm,para}$$
$$= P^{\pm,para} \times \{f \times \kappa/(1 - exp(-\kappa)) + (1 - f)\}$$

where $P^{+,para}$ is that for the protonated species of carnosine, $P^{\pm,para}$ is that for its deprotonated species, f is the fraction of the protonated species, and $\kappa = zF\Delta\Psi/RT$. In the last relationship, z, F, $\Delta\Psi$, R, and T are the net charge of protonated carnosine (+1), Faraday constant (9.65×10^4 Coul/mol), potential difference (50.4 mV at pH 6.5 and 52.1 mV at 7.4), universal gas constant (8.31 J/mol/K), and absolute temperature (310°K), respectively. Since carnosine is 68% and 21% protonated at pH 6.5 and 7.4, respectively, $Papp^{para}$ in the *ab* direction at apical pH 6.5 should theoretically be 1.6-times smaller than that at apical pH 7.4. This prediction was borne out experimentally, indicating that carnosine is predominantly translocated across tracheal epithelial barrier by paracellular diffusion. In other words, transcellular transport is negligible. Based on a Km of 170 μM, a Vmax of 210 pmol/20min/mg protein, and a protein content of ~0.18 mg/1.13 cm^2, the permeability coefficient of 10 μM carnosine across apical cell membranes would be on the order of 1.5×10^{-7} cm/sec, which were about 2 times larger than the Papp that was observed. This theoretical consideration raises the interesting alternate possibility that basolateral efflux of the accumulated carnosine may be rate-limiting for its overall transport. Indeed, Inui et al. (28) demonstrated that efflux of cephalosporins across the basolateral membranes in Caco-2 cells, that was mediated by a yet-to-be-characterized transport system (29), was rate-limiting in the overall transepithelial transport of the drug.

In conclusion, carnosine appears to be taken up into cultured rabbit tracheal epithelial cells via a dipeptide transporter, whereas its transport across the cell monolayer seems to occur mainly via the paracellular pathway. The evidence is that transepithelial carnosine transport at pH 7.4 is symmetrical in both directions and that, in accordance with the prediction based on the Nernst-Planck equation, its transport at pH 6.5 is lower than that at pH 7.4. This suggests that basolateral efflux of carnosine may be the rate-limiting step in the overall transepithelial transport of carnosine in the tracheal epithelial barrier.

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REFERENCES

1. T. Okano, K. Inui, H. Maegawa, M. Takano, and R. Hori. H^+ coupled uphill transport of aminocephalosporins via the dipeptide transport system in rabbit intestinal brush-border membranes. *J. Biol. Chem.* **261**:14130–14134 (1986).
2. A. Tsuji, I. Tamai, H. Hirooka, and T. Terasaki. Beta-lactam antibiotics and transport via the dipeptide carrier system across the intestinal brush-border membrane. *Biochem. Pharmacol.* **36**:565–567 (1987).
3. H. Daniel and S. A. Adibi. Transport of β-lactam antibiotics in kidney brush border membrane: determinants of their affinity for the oligopeptide/H^+ symporter. *J. Clin. Invest.* **92**:2215–2223 (1993).
4. M. Hu and G. L. Amidon. Passive and carrier mediated intestinal absorption components of captopril. *J. Pharm. Sci.* **77**:1007–1011 (1988).
5. W. Kramer, F. Girbig, U. Gutjahr, H. W. Kleeman, I. Leipe, H. Urbach, and A. Wagner. Interaction of renin inhibitors with the intestinal uptake system for oligopeptides and β-lactam antibiotics. *Biochim. Biophys. Acta* **1027**:25–30 (1990).
6. P. A. Heilliwell, D. Merdith, C. A. R. Boyd, J. R. Bronk, N. Lister, and P. D. Bailey. Tripeptide transport in rat lung. *Biochim. Biophys. Acta* **1190**:430–434 (1994).
7. D. Meredith and C. A. R. Boyd. Dipeptide transport characteristics of the apical membrane of rat lung type II pneumocytes. *Am. J. Physiol.* **269**:L137–L143 (1995).
8. K. Morimoto, H. Yamahara, V. H. L. Lee, and K. J. Kim. Dipeptide transport across rat alveolar epithelial cell monolayers. *Pharm. Res.* **10**:1668–1674 (1993).
9. R. G. Breeze and E. B. Wheeldon. The cells of the pulmonary airways. *Am. Rev. Respir. Dis.* **116**:705–777 (1997).
10. S. I. Rennard, J. D. Beckmann, and R. A. Robbins. Biology of airway epithelial cells. In R. G. Crystal, J. B. West, P. J. Barnes, N. S. Cherniack, and E. R. Weibel (eds.), *THE LUNG: Scientific Foundations*, Raven Press, New York, 1991, pp. 157–167.
11. D. M. Matthews, J. M. Addison, and D. Burston. Evidence for active transport of the dipeptide carnosine (β-alanyl-L-histidine) by hamster jejunum in vitro. *Clin. Sci. Mol. Med.* **46**:693–705 (1974).
12. V. Ganapathy and F. H. Leibach. Peptide transport in rabbit kidney: study with L-carnosine. *Biochim. Biophys. Acta* **691**:362–366 (1982).
13. V. Ganapathy and F. H. Leibach. Role of pH gradient and membrane potential in dipeptide transport in intestinal and renal brush-

border membrane vesicles from the rabbit: study with L-carnosine and glycyl-L-proline. *J. Biol. Chem.* **10**:14189–14192 (1983).
14. V. M. Rajendran, A. Berteloot, Y. Ishikawa, A. H. Khan, and K. Ramaswamy. Transport of carnosine by mouse intestinal brush-border membrane vesicles. *Biochim. Biophys. Acta* **778**: 443–448 (1984).
15. N. R. Mathias, T. W. Robison, K. J. Kim, and V. H. L. Lee. Development and characterization of rabbit tracheal epithelial cell culture models for drug transport studies. *Pharm. Res.* **12**: 1499–1505 (1995).
16. K. Yamaoka, Y. Tanigawara, T. Nakagawa, and T. Uno. A pharmacokinetic analysis program (MULTI) for microcomputer. *J. Pharmacobio-Dyn.* **4**:879–885 (1981).
17. F. Yamashita, K. J. Kim, and V. H. L. Lee. Gly-L-Phe transport and metabolism across primary cultured rabbit tracheal epithelial cell monolayers. *Pharm. Res.* **14**:238–240 (1997).
18. H. Daniel, E. L. Morse, and S. A. Adibi. Determinants of substrate affinity for the oligopeptide/H^+ symporter in the renal brush border membrane. *J. Biol. Chem.* **267**:9565–9573 (1992).
19. M. Acevedo and L. W. Steele. Na^+-H^+ exchanger in isolated epithelial tracheal cells from sheep. Involvement in tracheal proton secretion. *Exp. Physiol.* **78**:383–394 (1993).
20. A. M. Paradiso. Identification of Na^+-H^+ exchange in human normal and cystic fibrotic ciliated airway epithelium. *Am. J. Physiol.* **262**:L757–L764 (1992).
21. J. G. Widdicombe. Airway mucus. *Eur. Respir. J.* **2**:107–115 (1989).
22. M. Brandsch, C. Brandsch, M. E. Ganapathy, C. S. Chew, V. Ganapathy, and F. H. Leibach. Influence of proton and essential histidyl residues on the transport kinetics of the H^+/peptide cotransport systems in intestine (PEPT 1) and kidney (PEPT 2). *Biochim. Biophys. Acta* **1324**:251–262 (1997).
23. I. Naasani, K. Sato, K. Iseki, M. Sugawara, M. Kobayashi, and K. Miyazaki. Comparison of the transport characteristics of ceftibuten in rat renal and intestinal brush-border membranes. *Biochim. Biophys. Acta* **1231**:163–168 (1995).
24. W. Liu, R. Liang, S. Ramamoorthy, Y. J. Fei, M. E. Ganapathy, M. A. Hediger, V. Ganapathy, and F. H. Leibach. Molecular cloning of PEPT 2, a new member of the H^+/peptide cotransporter family, from human kidney. *Biochim. Biophys. Acta* **1235**:461–466 (1995).
25. M. Boll, M. Herget, M. Wagener, W. M. Weber, D. Markovich, J. Biber, W. Clauss, H. Murer, and H. Daniel. Expression cloning and functional characterization of the kidney cortex high-affinity proton-coupled peptide transporter. *Proc. Natl. Acad. Sci. USA* **93**:284–289 (1996).
26. H. Saito, T. Terada, M. Okuda, S. Sasaki, and K. Inui. Molecular cloning and tissue distribution of rat peptide transporter PEPT2. *Biochim. Biophys. Acta* **1280**:173–177 (1996).
27. D. E. Goldman. Potential, impedance, and rectification in membranes. *J. Gen. Physiol.* **27**:37–60 (1943).
28. K. Inui, M. Yamamoto, and H. Saito. Transepithelial transport of oral cephalosporins by monolayers of intestinal epithelial cell line Caco-2: Specific transport systems in apical and basolateral membranes. *J. Pharmacol. Exp. Ther.* **261**:195–201 (1992).
29. S. Matsumoto, H. Saito, and K. Inui. Transcellular transport of oral cephalosporins in human intestinal epithelial cells, Caco-2: Interaction with dipeptide transport systems in apical and basolateral membranes. *J. Pharmacol. Exp. Ther.* **270**:498–504 (1994).