Dipeptide Transport Across Rat Alveolar Epithelial Cell Monolayers

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The transepithelial transport and metabolism of two model peptides, glycyl-D-phenylalanine (Gly-D-Phe) and glycyl-L-phenylalanine (Gly-L-Phe), across primary cultured monolayers of rat alveolar epithelial cells were studied. These tight monolayers (>2000 Ω-cm^2) exhibited type I pneumocyte morphological and phenotypic characteristics. A reverse-phase HPLC was used to monitor the appearance of parent dipeptides and their metabolites (D- or L-Phe) in the receiver fluid. The apparent permeability coefficient (P_{app}) for Gly-D-Phe was about 1.6×10^{-7} cm/sec at both 1 and 10 m*M* and in both the apical-to-basolateral (AB) and the basolateral-to-apical (BA) directions. In contrast, the P_{app} of Gly-L-Phe at 1 m*M* was about two times higher than that at 10 m*M* in the AB direction. The P_{app} of Gly-L-Phe in the BA direction at either concentration was about the same (about 1.4×10^{-7} cm/sec). Whereas no metabolite was detected during Gly-D-Phe transport, the proportions of a metabolite, L-Phe, observed at 4 hr in the basolateral receiver fluid for 1 and 10 m*M* apical donor Gly-L-Phe accounted for 83 and 77% of the estimated total Gly-L-Phe (i.e., L-Phe + Gly-L-Phe), respectively. The corresponding values in the BA direction were 40 and 19% of the estimated total Gly-L-Phe in the apical receiver reservoir. Metabolism of Gly-L-Phe was significantly reduced in the presence of 3 μ*M* actinonin (an inhibitor relatively specific for aminopeptidase M) in the apical but not the basolateral fluid. Under all experimental conditions, the monolayers remained intact, as indicated by no appreciable changes in the bioelectric parameters of transepithelial potential difference and electrical resistance. The above data provide evidence for cellular metabolism of Gly-L-Phe as well as paracellular restricted diffusional transport of intact Gly-D-Phe and Gly-L-Phe and comparatively lower transcellular transport of Gly-L-Phe across the rat alveolar epithelial cell monolayer.

KEY WORDS: pulmonary absorption; alveolar epithelial monolayer; dipeptide transport; aminopeptidase.

INTRODUCTION

The pulmonary route is an attractive alternative to the oral route for peptide and protein delivery. Features of the respiratory tract favoring drug absorption include a large surface area (approximately 143 m^2 in adult human lungs), a thin epithelial barrier (1 μm), extensive vascularization (blood flow being about the same as the cardiac output) (1), and possibly low proteolytic activity (2–4). Peptides and proteins that have been investigated for systemic delivery via the respiratory tract include insulin (5,6), human growth hormone (2,7), leuprolide (8), dDAVP[9] (9), and several others (3,4,10–14). However, the majority of these studies was performed in isolated tracheal or whole-lung preparations. Neither method provides a precise appreciation of the mechanisms and pathways underlying alveolar epithelial peptide and protein transport, due simply to the complex anatomical arrangement of multiple potential transport barriers (e.g., trachea, bronchus, bronchiolar and alveolar epithelial barriers in parallel). Other difficulties inherent in whole-lung approaches are the lack of information on the precise surface area and distribution volumes for solute transport, the presence of unstirred layers, and the inaccessibility of the airspace for accurate sampling of the applied dose.

The above drawbacks can be overcome to some extent by using epithelial cell monolayers cultured from different regions of the respiratory tract. To date, peptide transport across the isolated alveolar epithelial barrier has not been studied in detail, because of the lack of a suitable *in vitro* model system. The recent development of tight (>2000 Ω-cm^2) monolayers of primary cultured rat pneumocytes, which exhibit morphological and phenotypic characteristics of type I pneumocytes, has made it possible to evaluate the mechanisms and pathways of alveolar epithelial peptide transport. Rat type II cells cultured on tissue culture-treated polycarbonate filter membranes have been demonstrated to exhibit type I cell-like morphology (i.e., bulging nuclei with thin cytoplasmic extensions) (15) and react to type I pneumocyte-specific monoclonal antibodies starting from about the third day of culture (16). These features are of particular relevance to systemic drug delivery via the lung, since over

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[9] Abbreviations used: *A*, surface area (cm^2); AB, apical to basolateral; ANOVA, analysis of variance; BA, basolateral to apical; C_o, initial ($t = 0$) concentration of a given molecule (mol/L); dDAVP, 1-deamino-8-D-arginine vasopressin; D-Phe, D-phenylalanine; dQ/dt, solute transfer rate (mol/sec); EMEM, Earle's minimum essential medium; ERS, epithelial resistance (measuring) system; Gly-D-Phe, glycyl-D-phenylalanine; Gly-L-Phe, glycyl-L-phenylalanine; HEPES, *N*-[2-hydroxyethyl]piperazine-*N*′-[2-hydroxypropanesulfonic acid]; HPLC, high-performance liquid chromatography; IgG, immunoglobulin G; *J*, steady-state unidirectional flux; K_i, inhibitory constant (m*M*); K_m, apparent Michaelis–Menten constant at which the half-maximal value of a given kinetic parameter (e.g., flux) is attained (m*M*); L-Phe, L-phenylalanine; MRS, modified Ringer's solution; NBS, newborn bovine serum; OD, outer diameter; P_{app}, apparent permeability coefficient (cm/sec); PD, electrical potential difference across epithelial cell monolayer (mV; apical as reference); TEER, transepithelial electrical resistance (Ω-cm^2); V_{max}, a maximal value of kinetic process (e.g., flux) in Michaelis–Menten-type kinetics.

95% of the absorptive area in its distal airspace is covered with type I cells. The present study was conducted to evaluate the extent of transport and metabolism of two model dipeptides, glycyl-D-phenylalanine (Gly-D-Phe) and glycyl-L-phenylalanine (Gly-L-Phe), across monolayer cultures of rat alveolar epithelial cells.

MATERIALS AND METHODS

Materials

Gly-D-Phe, Gly-L-Phe, and actinonin were purchased from Sigma Chemical Co. (St. Louis, MO). Cell culture media and supplies were obtained from GIBCO (Grand Island, NY). All other chemicals were of the highest purity available commercially.

Preparations of Solutions

Dipeptides (1 or 10 m*M* Gly-D-Phe or Gly-L-Phe), L-Phe (1 or 10 m*M*), and actinonin (3 μ*M*), either separately or in combination, were dissolved in a modified Ringer's solution (MRS; pH 7.4 and 300 mOsm/kg in osmolality), which contained 1.80 m*M* $CaCl_2$, 0.81 m*M* $MgSO_4$, 5.40 m*M* KCl, 116.4 m*M* NaCl, 0.782 m*M* NaH_2PO_4, 5.55 m*M* glucose, 15 m*M* *N*-[2-hydroxyethyl]piperazine-*N*′-[2-hydroxypropanesulfonic acid] (HEPES), 25 m*M* $NaHCO_3$, and 0.075 m*M* bovine serum albumin (Fraction V, fatty acid-free). Prior to each transport experiment, the MRS solution was preequilibrated by bubbling in 95% O_2/5% CO_2 for 30 min.

Cell Culture

The primary cell culture procedure for routinely generating alveolar epithelial cell monolayers has been published elsewhere (17–20). Briefly, lungs from male specific pathogen-free Sprague–Dawley rats (100–150 g, Charles River Laboratories, Wilmington, MA) were isolated, lavaged, perfused, and enzymatically digested using porcine pancreatic elastase (2.5 U/ml, Worthington, Freehold, NJ). Elastase activity was quenched with newborn bovine serum (NBS). Crude lung cell mixtures were then filtered sequentially through Nitex membranes (150 and 35 μm, Tetko, Elmsford, NY) and pelleted (100*g*, 10 min, room temperature). The cell pellet was resuspended in Earle's minimum essential medium (EMEM), plated onto rat immunoglobulin G (IgG)-coated hydrophobic surface (20) at about 10^6 cells/10 mL, and incubated at 37°C for 1 hr. Cells containing Fc receptors, mostly macrophages and leukocytes, were allowed to bind immobilized IgG, whereby nonsticking type II pneumocytes were collected and pelleted at 100*g* for 10 min at room temperature. These partially purified cells, comprised of >90% type II pneumocytes (the rest being mostly macrophages and leukocytes), were >95% viable by trypan blue exclusion. They were then cultured on tissue culture-treated polycarbonate filter membrane (Transwell, 0.4 μm; 12-mm OD; Costar, Cambridge, MA) at a plating density of 1.2×10^6/cm^2. A modified EMEM (pH 7.4) supplemented with 10% NBS, 100 U/mL penicillin, 100 ng/mL streptomycin, 0.1 μ*M* dexamethasone, 2 m*M* L-glutamine, and 10 m*M* HEPES, was used for primary culture of rat type II pneumocytes. Cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2. By the second day, the monolayer became confluent, comprised of >95% type II cells. Meanwhile, nonsticking cells (mostly leukocytes) were effectively removed by replacement of culture medium on both sides. On day 4, these monolayers were used for transport studies.

Transport Studies

All transport experiments were performed at 37°C in an incubator with a humidified 5% CO_2 atmosphere. Prior to adding the dipeptides, the cell monolayers were washed twice with MRS and allowed to equilibrate with the new bathing medium for 2 hr in the incubator. The apical and basolateral volumes were 0.6 and 1.5 mL, respectively, which allowed zero hydrostatic pressure gradient across the monolayer. After the equilibration period, 100 μL was sampled from both the apical and the basolateral fluids for measurement of background levels of the dipeptide and its metabolite, which turned out to be below detection limits. Next, a known concentration of either dipeptide solution was added to either the apical or the basolateral donor reservoir to yield a final concentration of 1 or 10 m*M*. In one series of experiments, 3 μ*M* actinonin, an inhibitor relatively specific for aminopeptidase M, was also present apically or basolaterally concurrently with 10 m*M* Gly-L-Phe in the donor reservoir. To estimate the transepithelial fluxes of the parent peptide and its metabolite, 100 μL of the receiver fluid was taken at 0.5, 1, 2, 3, and 4 hr for assay. In another series of experiments, 1 or 10 m*M* L-Phe alone was used in the donor fluid to determine the unidirectional fluxes of L-Phe across the monolayer. Throughout all experiments, the volumes of the apical and basolateral reservoirs were kept constant by replacing equal volume of preequilibrated, fresh MRS immediately following each sampling. The spontaneous potential difference (PD; in reference to apical side) and the transepithelial resistance (TEER) of the monolayer were monitored with a Milli Cell ERS device (Millipore, Bedford, MA) at 0 and 4 hr.

Assay

Gly-Phe and Phe were quantified using a reverse-phase high-performance liquid chromatographic system (HPLC, Shimadzu LC-6A, Shimadzu Co., Ltd., Kyoto, Japan) on a Beckman Ultrasphere C_{18} column (250 × 4.6 mm, 5 μm; Irvine, CA). Samples (100 μL) were mixed with 100 μL of acetonitrile containing 5 m*M* nadolol (an internal standard) and centrifuged at 3000*g* for 10 min at room temperature. Aliquots (up to 100 μL) of the supernatant were injected into the HPLC. The mobile phase was a mixture of acetonitrile and 0.1 *M* sodium perchlorate preadjusted to pH 2.5 with phosphoric acid. The proportion of acetonitrile in the mobile phase was increased linearly from 8 to 25% during the first 30 min, maintained at 25% for the next 5 min, and decreased linearly to 8% for another minute. The flow rate was 1.0 mL/min. Gly-Phe and Phe were monitored spectrophotometrically at 210 nm. Under these conditions, Gly-Phe, Phe, and nadolol were eluted at 10, 15, and 17 min, respectively. The detection limits for Gly-Phe and Phe were about 50 ng. The intra-($n = 5$) and interrun ($n = 7$) coefficients of variation for the assay (e.g., peak area ratio between Gly-Phe and the internal standard) were 1.10 and 3.99%, respectively.

Data Analysis

The cumulative amount of dipeptide and its metabolite in the receiver fluid was plotted as a function of time. The steady-state flux (J; mol/cm^2/sec) of Gly-Phe and Phe and the apparent permeability coefficients (P_{app}; cm/sec) of the dipeptide were estimated from the linear slope of the plot using the relations shown in Eqs. (1) and (2), respectively,

$$J = (dQ/dt)/A \tag{1}$$

$$P_{app} = J/C_o = (dQ/dt)/A/C_o \tag{2}$$

where dQ/dt is the solute transfer rate (mol/sec), A is the surface area of the membrane (1.13 cm^2; the nominal surface area of the Transwell filter), and C_o is the initial dipeptide concentration (mol/L).

The data are presented as mean ± SE (n), where n is the number of observations. Unpaired t tests were performed to contrast the group means. When appropriate, group means were compared by one-way analysis of variance (ANOVA) with post hoc Scheffe procedures. A P value <0.05 was considered to be statistically significant.

RESULTS

The time courses of Gly-D-Phe transport across rat alveolar epithelial cell monolayers are shown in Fig. 1. The cumulative appearance of Gly-D-Phe in the receiver fluid was linear at both 1 and 10 mM. No measurable lag time was observed, and no measurable metabolite was present in the receiver fluid. As shown in Table I, the P_{app} showed neither direction nor concentration dependence.

Figure 2 shows the time courses of cumulative appearance of Gly-L-Phe and its metabolite (L-Phe) in the receiver fluid across rat alveolar epithelial cell monolayers. The Gly-L-Phe fluxes appeared to be linear at both 1 and 10 mM. As shown in Table II, the flux of intact Gly-L-Phe in the apical-to-basolateral (AB) direction was significantly greater ($P <$ 0.05) than that in the reverse (BA) direction with 1 mM donor Gly-L-Phe. As a result, the P_{app} of intact Gly-L-Phe at 1 mM donor Gly-L-Phe in the AB direction was significantly greater than that in the reverse direction and was also greater than those at 10 mM Gly-L-Phe in both directions. At 4 hr, the proportion of L-Phe in the basolateral receiver fluid (i.e., AB direction) was about 83 and 77% of the estimated total Gly-L-Phe transported (i.e., sum of L-Phe and intact Gly-L-Phe) at 1 and 10 mM, respectively. The corresponding percentages in the reverse (BA) direction were about 40 and 19%.

The transport of L-Phe alone across rat alveolar epithelial cell monolayers was both concentration and direction dependent (Table III). The P_{app} for L-Phe estimated in the AB direction was 12 times greater at 1 mM than that at 10 mM. The P_{app} for L-Phe was 29 to 372 times larger in the AB than the BA direction at both concentrations. There was no concentration dependence of P_{app} in the BA direction.

As to the L-Phe appearance from Gly-L-Phe administration in the donor fluid, there was a lag time of about 1 hr in the basolateral donor fluid, followed by a linear rise in

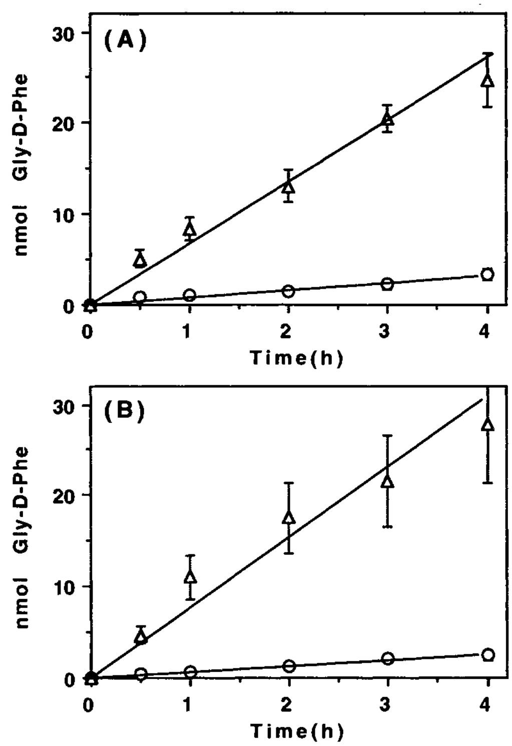

Fig. 1. Time course of accumulation of Gly-D-Phe in the receiver fluid at 1 mM (○) or 10 mM (△) dipeptide in the apical (A) or basolateral (B) donor fluid of open-circuited rat alveolar epithelial cell monolayers. Each data point with a given symbol represents the mean and the vertical bar on the symbol is the standard error of the mean. At least five measurements were made at each time point.

estimated total L-Phe (i.e., the calculated sum of L-Phe in the receiver and donor fluids), whereas that in the apical donor fluid appeared to increase linearly with no appreciable lag time (Fig. 3). The L-Phe observed in the apical donor fluid showed a plateau after about 2 hr, in contrast to the linear time course noted for estimated total apical donor L-Phe. On the other hand, the measured L-Phe in the basolateral donor fluid closely followed the time course for estimated total L-Phe in the basolateral fluid.

Although actinonin did not significantly affect the unidirectional fluxes of intact Gly-L-Phe at 10 mM (Fig. 4), the cumulative appearance of L-Phe in the basolateral fluid was significantly (P < 0.05) decreased when actinonin was

Table I. Transport Parameters for Gly-D-Phe Across Rat Alveolar Epithelial Cell Monolayers (37°C, 4 hr)[a]

Direction	Concentration (mM)	Unidirectional flux (pmol/cm^2/sec)	P_{app} ($\times 10^7$ cm/sec)
AB[b]	1	0.16 ± 0.02	1.61 ± 0.24
BA[c]	1	0.15 ± 0.03	1.54 ± 0.33
AB	10	1.48 ± 0.09	1.48 ± 0.09
BA	10	1.65 ± 0.33	1.65 ± 0.33

[a] Entries are mean ± SE (n = 5), where n is the number of observations.
[b] Parameters measured in the apical-to-basolateral direction.
[c] Parameters measured in the basolateral-to-apical direction.

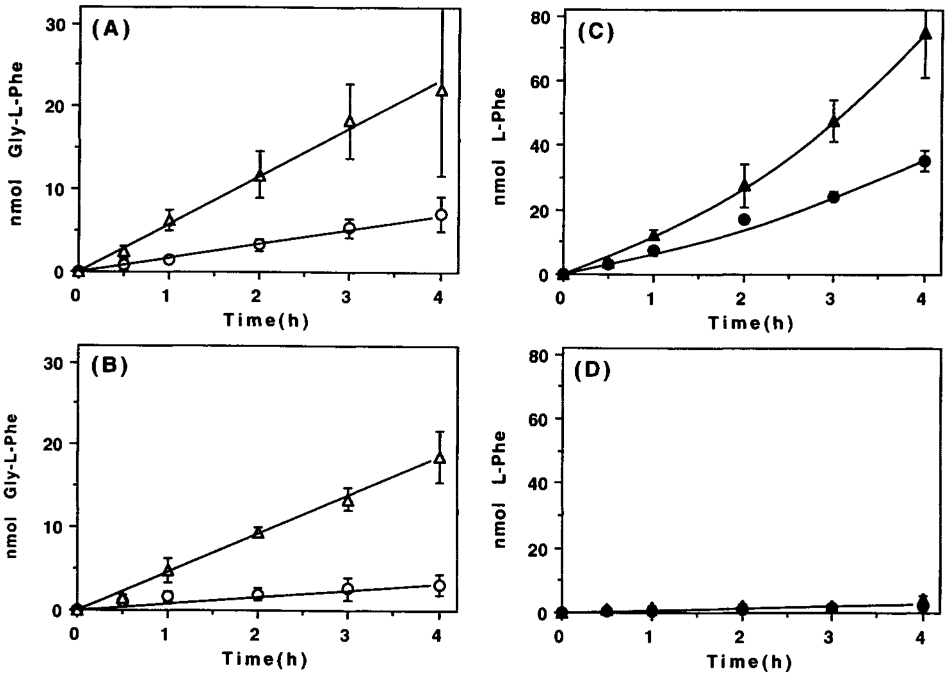

Fig. 2. Time course of accumulation of Gly-L-Phe (unfilled symbols in A and B) and L-Phe (filled symbols in C and D) in the receiver fluid at 1 m*M* (denoted ○ and ●) and 10 m*M* (denoted △ and ▲) dipeptide in the apical (A and C) or basolateral (B and D) donor fluid, respectively. Each data point with a given symbol represents the mean and the vertical bar on the symbol is the standard error of the mean. Between five and seven measurements were made at each time point.

present in the apical, but not the basolateral, fluid. In contrast, the cumulative appearance of L-Phe in the apical receiver fluid (i.e., with 10 m*M* Gly-L-Phe in the basolateral donor fluid) was not significantly affected by actinonin in either bathing fluid.

The bioelectric properties of the alveolar epithelial cell monolayers used under all experimental conditions in the present study were not statistically different ($P > 0.05$). The potential difference (PD) and transepithelial electrical resistance (TEER) observed at 4 hr, 7.3 ± 0.3 mV, and 2330 ± 56 $\Omega\text{-cm}^2$, respectively, were statistically not different from those at time 0 [PD, 9.4 ± 0.5 mV (apical side negative); TEER, 1950 ± 61 $\Omega\text{-cm}^2$; $n = 94$]. These bioelectric parameters obtained under open-circuited conditions were comparable to those estimated under short-circuited conditions in Ussing chambers on the same culture preparation (18,19).

DISCUSSION

This study demonstrates that rat alveolar epithelial cell monolayers are capable of transporting the model dipeptides, Gly-D-Phe and Gly-L-Phe. Gly-D-Phe is resistant to cellular processing and is transported as intact molecule across the monolayer via passive diffusion. On the other hand, Gly-L-Phe is susceptible to cellular metabolism by aminopeptidase activity, especially when the dipeptide is presented to the apical fluid. There appears to be a smaller, transcellular component of alveolar epithelial Gly-L-Phe transport as intact molecules in the apical to basolateral direction. The metabolically generated L-Phe appears to be translocated across the monolayer via mostly transcellular pathways in the apical to basolateral direction, while L-Phe transport in the opposite direction is much smaller in magnitude.

Table II. Transport Parameters for Gly-L-Phe Across Rat Alveolar Epithelial Cell Monolayers (37°C, 4 hr)[a]

Direction	Concentration (m*M*)	Unidirectional flux (pmol/cm²/sec)	P_{app} (10^7 cm/sec)	Appearance of L-Phe (pmol/cm²/sec)
AB[b]	1	0.33 ± 0.08	3.29 ± 0.81	2.02 ± 0.23
BA[c]	1	0.15 ± 0.07	1.58 ± 0.72	0.13 ± 0.03
AB	10	1.38 ± 0.36	1.38 ± 0.36	4.61 ± 0.81
BA	10	1.29 ± 0.17	1.29 ± 0.17	0.24 ± 0.06

[a] Entries are mean ± SE ($n = 5$), where n is the number of observations.
[b] Parameters measured in the apical-to-basolateral direction.
[c] Parameters measured in the basolateral-to-apical direction.

Table III. Transport Parameters for L-Phe Across Rat Alveolar Epithelial Cell Monolayers (37°C, 4 hr)[a]

Direction	Concentration (mM)	Unidirectional flux (pmol/cm^2/sec)	P_{app} ($\times 10^7$ cm/sec)
AB[b]	1	8.520 ± 0.850	85.2 ± 8.50
BA[c]	1	0.023 ± 0.006	0.23 ± 0.06
AB	10	7.43 ± 0.13	7.43 ± 0.13
BA	10	0.25 ± 0.03	0.25 ± 0.03

[a] Entries are mean ± SE (n = 3), where n is the number of observations.
[b] Parameters measured in the apical-to-basolateral direction.
[c] Parameters measured in the basolateral-to-apical direction.

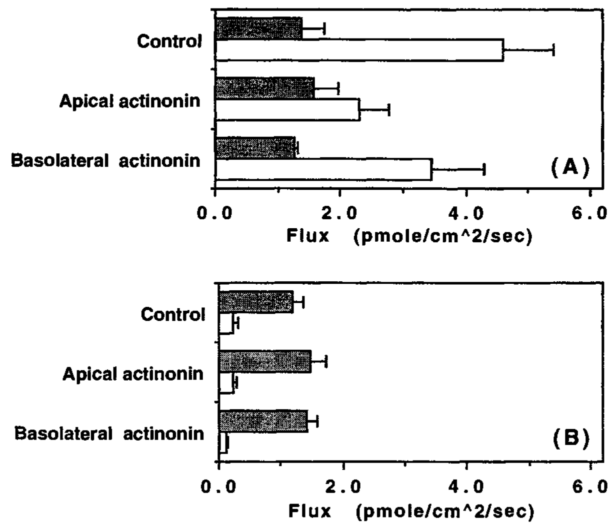

Fig. 4. Effects of actinonin (3 μM) on unidirectional fluxes of Gly-L-Phe measured across the alveolar epithelial cell monolayer. The donor concentration of Gly-L-Phe for these experiments was 10 mM. Also included for comparison are the appearance rates of L-Phe in the receiver fluid. Filled bars represent the intact Gly-L-Phe flux, whereas open bars show the appearance rates of L-Phe in the receiver fluid under the same experimental conditions. A refers to transport in the AB direction and B refers to transport in the BA direction.

Mammalian alveolar epithelium which lines the distal airspaces of the lung consists of type I (covering >95% of the surface) and type II pneumocytes. It is widely accepted that the alveolar epithelial barrier is very tight and limits the passive leak of solutes and water for efficient gas exchange. The alveolar epithelial model utilized in this study exhibits type I cell-like phenotype and tight (>2000 Ω-cm^2) monolayer characteristics with a PD of about 7 mV (apical-side negative). Overall, these bioelectric properties did not change appreciably in the presence of the dipeptides or L-Phe in either bathing fluid of the monolayer for 4-hr flux experiments.

Direct comparison of our alveolar epithelial monolayer resistance measurement with that which exists *in vivo* is not possible, due largely to the complex branching arrangement

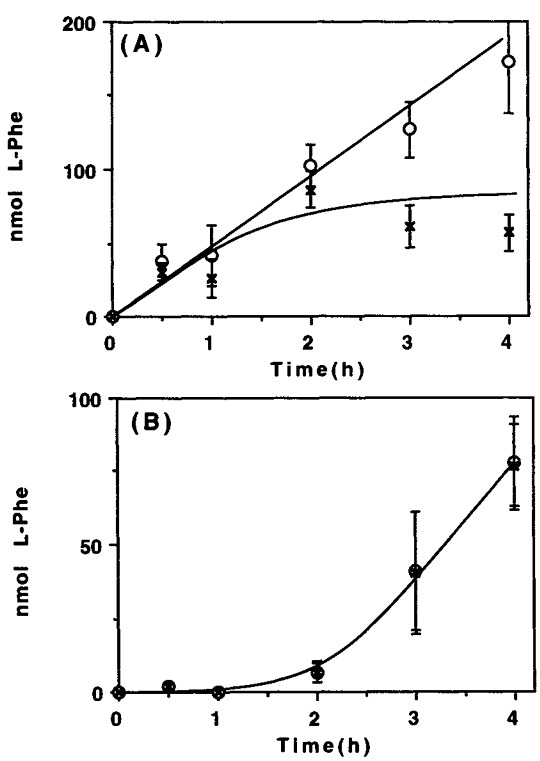

Fig. 3. Time course of accumulation of L-Phe formed from 10 mM Gly-L-Phe in the AB (A) or BA direction (B) across rat alveolar epithelial cell monolayers. (○) Sum of L-Phe in the receiver and donor reservoirs; (x) L-Phe in the donor reservoir only.

in the lung. However, information on the transtissue resistance of simpler amphibian lungs, which are lined with a continuous alveolar epithelium, is available. For example, excised *Rana catesbeiana* alveolar epithelium (21) exhibits a TEER of about 2000 Ω-cm^2, whereas the isolated *Xenopus* alveolar epithelium (22) exhibits a TEER of 1000 Ω-cm^2, which are comparable to that obtained in the present study, 2330 Ω-cm^2. The barrier resistance of the alveolar epithelial cell monolayer is about an order of magnitude greater than that of other mucosal barriers, such as Caco-2 cells, nasal, tracheal, and jejunal epithelia, whose resistance ranges from 20 to 600 Ω-cm^2 (23,24).

The lack of concentration and direction dependence in Gly-D-Phe (MW 222) transport strongly suggests a simple diffusion mechanism for its transport. The absence of detectable D-Phe in the receiver fluid indicates that Gly-D-Phe is translocated intact across the monolayer without undergoing cellular processing. Given that its P_{app} of 1.6×10^{-7} cm/sec is comparable to that of ^{14}C-mannitol [a paracellular marker, 1.8×10^{-7} cm/sec (18)], restricted diffusion via a paracellular pathway is likely to be the predominant transport mechanism for alveolar epithelial Gly-D-Phe transport. Interestingly, Caco-2 cell monolayers exhibited a P_{app} for phenylalanylglycine of about 10^{-6} cm/sec (24) and a P_{app} for mannitol of about 1.9×10^{-6} cm/sec (25). These higher permeabilities probably reflect the lower barrier resistance of Caco-2 cell monolayers than the tighter alveolar epithelial cell monolayers.

The P_{app} for the transport of intact Gly-L-Phe across the monolayer is of a similar magnitude to that for Gly-D-Phe transport. However, Gly-L-Phe at a lower concentration (<1 mM) may also be translocated transcellularly as indicated by the asymmetry in P_{app} (Table II), where the flux of intact

Gly-L-Phe in the apical-to-basolateral (AB) direction was about twice the flux in the opposite direction. At a higher concentration (i.e., 10 mM Gly-L-Phe), the apparent asymmetry in intact dipeptide flux collapsed, indicating predominantly paracellular transport of the molecule across the monolayer. The translocation of intact Gly-L-Phe transport in the basolateral-to-apical (BA) direction was the same at 1 and 10 mM and its P_{app} was comparable to that for ^{14}C-mannitol (see above), suggesting that intact Gly-L-Phe in the BA direction occurs predominantly by passive diffusion via paracellular pathways across the monolayer.

Unlike Gly-D-Phe, which remained essentially intact during transit across the monolayer, Gly-L-Phe was extensively metabolized, especially when the dipeptide was presented to the apical fluid (Table II, Figs. 2 and 3). When actinonin, an inhibitor relatively specific for aminopeptidase M with a K_i of ~0.2 μM (26,27), was added to the apical donor fluid, the concentration of a metabolite (i.e., L-Phe formed from Gly-L-Phe) in the basolateral receiver fluid was decreased by about 50% (Fig. 4A). The persistent appearance of metabolically generated L-Phe in the basolateral receiver fluid in spite of the presence of actinonin suggests that other peptidases may also be involved in the alveolar epithelial processing of the dipeptide. Since the basolateral application of actinonin reduced the appearance of L-Phe to a lesser extent than did apical application (Fig. 3), it is attractive to postulate that the "putative" aminopeptidase activity responsible for degrading Gly-L-Phe to L-Phe (and glycine) resides predominantly in the apical domain of the monolayer (e.g., apical cell plasmalemma). The apparent lack of significantly increased AB flux of intact Gly-L-Phe when actinonin was present in the apical donor fluid supports the hypothesis that Gly-L-Phe transport in either direction occurs predominantly via paracellular pathways by passive restricted diffusion. The L-Phe generated by the apically located peptidases is then translocated across the monolayer by a specialized transcellular L-Phe transport process (see below). This speculation is consistent with the 16- and 19-fold higher appearance of the metabolite (i.e., L-Phe) in the basolateral receiver fluid than in the apical receiver fluid, at 1 and 10 mM Gly-L-Phe in the donor fluid, respectively.

Another possible explanation, although less likely, for the greater transport of L-Phe formed from Gly-L-Phe metabolism in the AB direction than that in the BA direction would be intracellular processing of the dipeptides which was accumulated by cellular uptake via a small saturable process (i.e., $K_m \ll 1$ mM and $V_{max} \sim 0.3$ pmol/cm^2/sec) from the apical fluid (Tables I and II). Peculiarly, when compared to its counterpart in the small intestine (28), such a "putative," alveolar epithelial peptide–carrier system does not appear to participate in promoting net amino acid flux across the alveolar epithelium. As shown in Tables II and III, the intrinsic L-Phe flux is 42 and 1.6 times higher than that generated metabolically from Gly-L-Phe at 1 and 10 mM, respectively.

The rat alveolar epithelial cell monolayer appears to have a more than adequate capacity to translocate the metabolite (L-Phe) preferentially in the AB direction. As shown in Table III, the transport of L-Phe per se across the alveolar epithelial cell monolayer appears to be carrier-mediated (as evidenced by the concentration- and direction-dependent P_{app}). The reason for the 10 times smaller P_{app} for L-Phe (MW 165) in the BA direction than that for comparably sized mannitol (MW 180) is not clear. L-Phe transport in the BA direction probably takes place via paracellular pathways, where the higher lipophilicity of the amino acid relative to mannitol is likely the main reason for its low P_{app}. The very low L-Phe transport in the BA direction could also result from the "recycling" of L-Phe (which was transported into the apical receiver fluid) via the "saturable" process for L-Phe transport in the AB direction. Finally, the lower P_{app} for L-Phe in the BA direction also suggests that the metabolite, L-Phe, found in the apical receiver fluid probably resulted from the direct apical processing of Gly-L-Phe which had been translocated intact across the monolayer.

The high resistance (>2000 Ω-cm^2) of the alveolar epithelium restricts the leak of hydrophilic solutes and water from the vascular bed and interstitial space in the mammalian lung, thereby keeping its distal airspaces relatively fluid-free for efficient gas exchange. This high resistance barrier, however, may in turn pose a formidable but surmountable permeability barrier for paracellular peptide delivery across the alveolar epithelium into the systemic circulation. Nevertheless, the magnitude of overall systemic delivery *in vivo* via the distal pulmonary epithelial tract may still be quite large, due mainly to its large surface area available for absorption, even though the permeability of these dipeptides to the alveolar epithelial barrier is very limited.

In conclusion, the alveolar epithelial cell monolayer allows the passage of dipeptides such as Gly-D-Phe and Gly-L-Phe. We have provided evidence for the transport of Gly-L-Phe across the monolayer via paracellular pathways in parallel with a smaller transcellular component. The extent of degradation during transepithelial transport is peptide dependent, in that Gly-L-Phe is vulnerable to peptidase activity primarily present at the apical cell membrane. In contrast, Gly-D-Phe is resistant to cellular degradation and is transported intact across the monolayer by simple (restricted) diffusion via paracellular pathways. Further detailed work is required to elucidate the mechanisms for peptide/amino acid transport, such as Na^+ dependency of the amino acid/peptide transport systems and/or facilitated diffusion (29,30). The tight, rat alveolar epithelial cell monolayers may be a useful *in vitro* model for the evaluation of peptide and protein drug delivery from the distal respiratory tract.

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