# Peptides Are Better Absorbed from the Lung than the Gut in the Rat

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## INTRODUCTION

The clinical application of peptide drugs is being considered for the treatment of hypertension [renin inhibitors (1)], AIDS [HIV protease inhibitors (2)], and pain [enkephalin and endorphin (3)]. A limitation of these compounds is their intrinsically poor absorption from the gut, even when protected from digestion (4). The intrapulmonary route of administration represents an alternative to oral administration. Presently, however, little systematic information is available on the absorption of proteins and peptides via the lung, even though extracellular peptidase activity is much lower in the lung than the gastrointestinal tract and the surface area available for absorption is large (5).

The intrapulmonary absorption of xenobiotics appears to be a complex function of their size, concentration, charge, molecular conformation, partition coefficients (lipophilicity), and interactions with the lung epithelia (5). Studies demonstrate that the lung has both active and passive absorption pathways, similar to the intestine, and may have a greater capacity for absorption. Because peptidase activity is lower in the lung than in the gut and because intrapulmonary delivery avoids "first-pass" clearance, the lung could be an attractive route for peptide delivery. Since little is known about the chemical properties that govern peptide absorption from the lung, the present study was undertaken. Absorption of a series of model phenylalanine analogues was assessed after intrapulmonary administration and the results were compared to those obtained earlier with the same peptides after oral administration in the rat (6).

## MATERIALS AND METHODS

### Model Peptide Series

*Peptides.* The preparation of the $^{14}$C-radiolabeled peptides (Fig. 1) has been described previously (7,8). Specific activity of the individual peptides was 106–110 μCi/μmol. Each peptide was brought to >98% purity by preparative thin-layer chromatography (TLC) (7,8) immediately prior to use. This process minimized the small amount of degradation (<10%) that occurred during storage.

The first series of peptides increased successively in size, molecular weight, lipophilicity, and hydrogen bond number. The second series decreased in hydrogen bond number, while all other aforementioned parameters remained essentially the same.

The peptides were stored at −20°C in methanol–chloroform in order to maintain chemical stability and were blown down in a nitrogen stream to a clean and dry form prior to the study.

*Peptide Formulation.* Each animal given an intrapulmonary (IT) dose received 0.004 μmol of the peptide (600,000 dpm) dissolved in 50:50 vehicle of absolute ethanol and 5% dextrose in a total volume of 25 μl.

The vehicle for the intravenous (IV) study was 0.1 ml of absolute ethanol diluted with 6.9 ml of 5% dextrose. A total volume of 1 ml/rat was injected via the tail vein for a dose of 0.004 μmol.

*Dial-Urethane Anesthetic.* Preparation of the anesthetic began by mixing 40 g urethane, 40 g ethyl urea, and 10 g 5,5-diallyl barbituric acid. Next a solution of 50 mg disodium salt of EDTA dissolved in 10 ml sterile water was added to the mixture and heated with tap water until completely dissolved. The solution was then cooled at room temperature and the volume was brought up to 100 ml with sterile water.

*Animals.* Sprague–Dawley male rats (500–600 g) from the Charles River Corporation were used. Animals were fasted overnight but allowed access to water.

*Animal Procedure I: Bile and Urine Collection.* Rats were anesthetized by intraperitoneal injection of 0.2 to 0.5 mg/kg of dial-urethane. Once unconscious, a longitudinal incision was made along the ventral side of the neck to expose the trachea and polyethylene tubing (1.67-mm I.D. and 2.42-mm O.D.) was inserted through a transverse incision made between two cartilaginous rings. The cannula was then secured with a nylon ligature.

Next a midline incision was made into the abdomen to expose the bile duct, which was then cannulated with polyethylene tubing (0.28-mm I.D. and 0.61-mm O.D.). The bladder was then voided by gentle massage and a drop of cyanoacrylate adhesive was used to seal the urethral opening. After closing the abdominal incisions with autoclips, rats were then placed on a padded bench top and covered by towels to maintain the core temperature throughout the study.

Intravenous injections were made via the tail vein. The intrapulmonary (IT) injection of the peptide was made by placing the formulation directly into the trachea cannula with a Hamilton syringe. Next the solution was aerosolized and propelled into the lungs. This was accomplished with a pressurized (27-psi) mix of chlorofluorocarbons 11 and 12 packaged in 22-ml Presspart aluminum cans fitted with Valois 50-μl valves. The KN1 adapted actuator was fitted with a stainless-steel cannula of appropriate gauge size to fit into the trachea cannula.

Bile was collected for a total of 3 hr in 0.5-hr time in-

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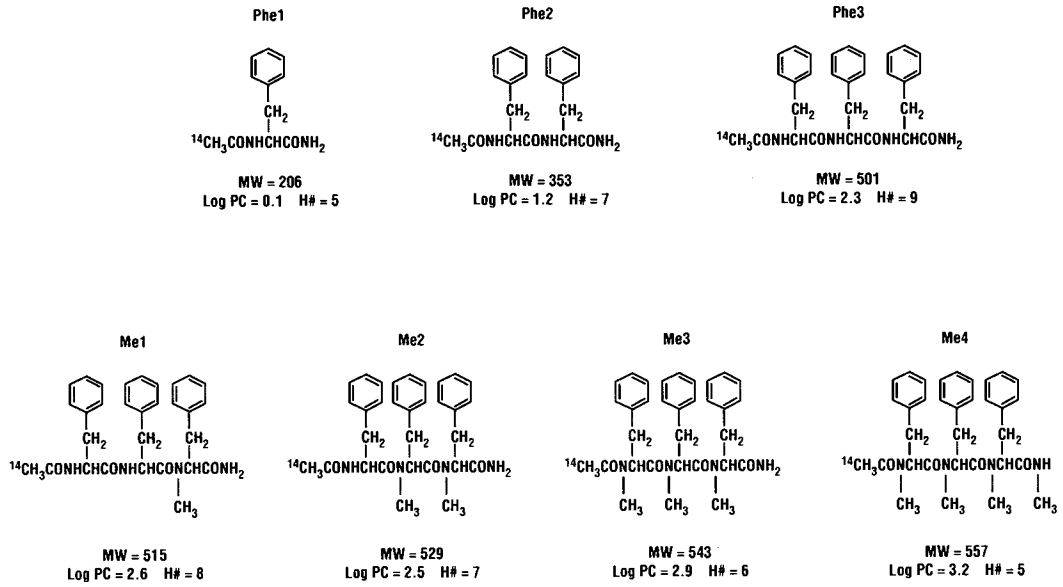


Fig. 1. Structures of the model peptides.

crements. Urine was collected at sacrifice by aspiration of the bladder contents. Aliquots (0.1 ml) of bile, urine, and stock solutions of peptide were analyzed by liquid scintillation counting.

*Animal Procedure II: Serum Collection.* All rats received both a trachea cannula (as previously described) and a carotid artery cannula. The dosages of the peptides were increased 25-fold in volumes of 30 μl for this procedure to facilitate detection of small amounts of the peptide in the sera. Five minutes after IT administration of the peptide, the carotid artery cannula was opened and the arterial blood was collected for 10 min or until death from exsanguination. The serum sample was then separated from the whole blood and treated with a Sep Pac procedure. Methanol was used to activate the column side chains, followed by 1 ml of deionized water. Serum samples (1 ml) were then added to the column, which was washed with 1 ml of methanol. Deionized water was flushed through the system, and peptides were removed with methanol. Peptides were dried under a nitrogen stream in a Pierce Reacti-Therm Heating/Stirring Module at approximately 50°C for 15–20 min. HPLC was used to analyze the serum samples for possible metabolism of the peptides as described previously (7,8).

*Statistical Methods.* Peptide absorption was calculated using the following formula:

$$\% \text{ absorption} = \frac{\text{total \% IT recovery}}{\text{total \% IV recovery}} \times 100$$

The recovery after IV administration is reflective of 100% absorption; therefore the recovery after IT administration is corrected to that of IV administration to find the overall percentage absorption.

The standard error of the mean (SE) was calculated for the percentage recoveries of each model peptide after both IV and IT administration for each study day using the following formula:

$$\text{SE of absorption} = \sqrt{\left(\frac{\text{\% IT Rec}}{\text{\% IV Rec}}\right)^2 \left[\left(\frac{\text{SE of \% IT Rec}}{\text{\% IP Rec}}\right)^2 + \left(\frac{\text{SE of \% of IV Rec}}{\text{\% IV Rec}}\right)^2\right]} \times 100$$

where Rec is recovery. When comparing absorption of two different compounds, differences were considered significant if

$$\frac{\text{(absorption of peptide 1) } - \text{ (absorption of peptide 2)}}{\sqrt{\text{(SE of absorption of peptide 1) } + \text{ (SE of absorption of peptide 2)}}} > 1.96$$

## RESULTS

Following IV administration, the Phe1–3 series of peptides exhibited a biliary recovery that ranged from 2.5 to 73.0%, while recovery in the urine was inversely related, with a range of 26.9 to 8.7% (Table I). The methylated series had a biliary recovery that ranged from 75.1 to 90.7% and an inverse urinary recovery ranging from 0.5 to 13.4%. The total percentage recovery (bile + urine) had a narrow range of 81.7 to 88.6% for six of the seven peptides. Phe1 had a lower recovery of 29.5%, suggesting that it is handled differently from the other six peptides. Earlier studies (6) had demonstrated that the peptides were not transformed in the sera after IV administration.

Table I. Recovery of Model Peptides After Intravenous or Intrapulmonary Administration (Mean ± SE)

| Peptide | % Recovery | | Total % recovery | % absorption |
|---|---|---|---|---|
| | In bile | In urine | | |
| Phe1 | | | | |
| IV | 2.5 ± 28 | 26.9 ± 3.1 | 29.5 ± 3.3 ($n$ = 4) | — |
| IT | 2.0 ± 15 | 13.6 ± 1.8 | 15.5 ± 1.9 ($n$ = 10) | No estimate possible |
| Phe2 | | | | |
| IV | 65.2 ± 1.5 | 17.2 ± 2.1 | 82.4 ± 1.1 ($n$ = 4) | — |
| IT | 54.8 ± 6.4 | 13.4 ± 1.7 | 66.9 ± 7.4 ($n$ = 8) | No estimate possible |
| Phe3 | | | | |
| IV | 73.0 ± 4.4 | 8.7 ± 1.4 | 81.7 ± 4.1 ($n$ = 4) | — |
| IT | 47.9 ± 3.6 | 6.1 ± 1.7 | 54.0 ± 4.5 ($n$ = 9) | No estimate possible |
| Me1 | | | | |
| IV | 75.1 ± 3.2 | 13.4 ± 1.1 | 88.5 ± 2.2 ($n$ = 4) | — |
| IT | 54.1 ± 3.6 | 5.0 ± 1.1 | 58.3 ± 3.6 ($n$ = 9) | 65.9 ± 4.4 |
| Me2 | | | | |
| IV | 84.4 ± 1.6 | 4.2 ± .7 | 88.6 ± 1.6 ($n$ = 4) | — |
| IT | 50.6 ± 6.6 | 1.4 ± .3 | 51.9 ± 6.5 ($n$ = 8) | 58.6 ± 7.5 |
| Me3 | | | | |
| IV | 90.7 ± 1.4 | 0.6 ± .3 | 91.6 ± 1.4 ($n$ = 4) | — |
| IT | 50.4 ± 4.2 | 0.2 ± .1 | 50.5 ± 4.2 ($n$ = 8) | 55.1 ± 4.6 |
| Me4 | | | | |
| IV | 86.8 ± 1.5 | 0.7 ± .3 | 87.4 ± 1.5 ($n$ = 4) | — |
| IT | 59.6 ± 5.5 | 0.5 ± .1 | 59.9 ± 5.6 ($n$ = 9) | 68.5 ± 6.5 |

Serum collections from rats given IT doses of the model peptides (Animal Procedure II) were examined by HPLC analysis to determine whether metabolism had occurred during absorption across the pulmonary epithelia. Two radioactive species, one corresponding to the retention time of the authentic peptide, were observed in sera from rats given IT Phe2. A single radioactive peak with a retention time different from that of authentic peptide was found in sera from rats given IT Phe3 (Fig. 2). For the other five peptides, only peaks with retention times corresponding to authentic peptide were found. Since transformation of Phe2 and Phe3 might have occurred prior to transport across the lung, absorption estimates could not be reliably made.

Absorption estimates of Phe1 were likewise unreliable because recovery by the IT route was incomplete by the termination of the study (Fig. 3b), in contrast to the other peptides (Fig. 3a).

The absorption of the methylated series ranged from 55.1 to 68.5% (Table I). Statistical evaluation indicated that the percentage absorption of Me2 and Me3 were significantly different from that of Me4 and Me1. Since the methylated

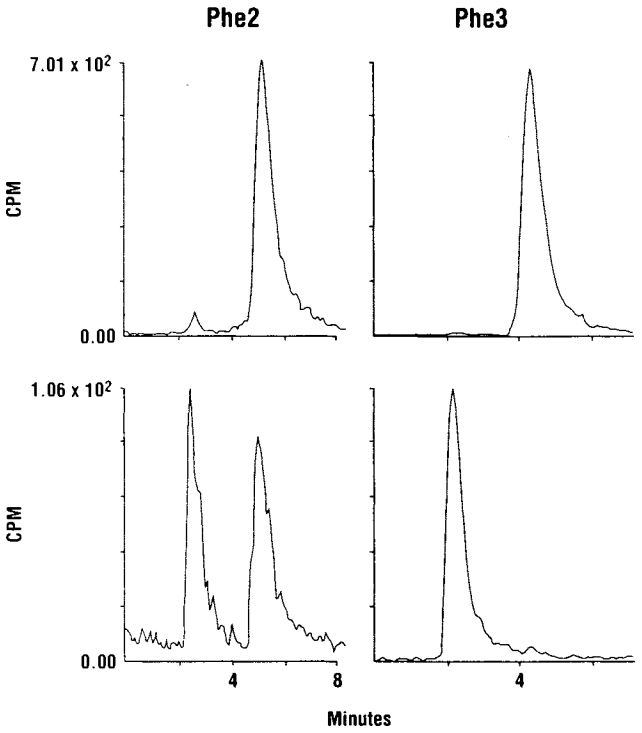


Fig. 2. HPLC profile of Phe2 and Phe3 in buffer (top) or in sera taken from rats (bottom).

peptides differed little in molecular weight and lipophilicity, no correlation could be explored between the absorption estimates and the molecular weight or log partition coefficient. Although the peptides in the methylated series ranged in hydrogen bond number from five to eight, no correlation was observed between this parameter and absorption (Fig. 4).

## DISCUSSION

The peptides used in this study were prepared from the unnatural D-amino acid isomer of phenylalanine specifically to stabilize them toward enzymatic hydrolysis (7). Consis-

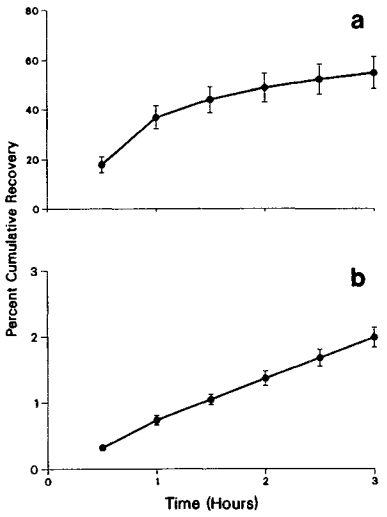


Fig. 3. Biliary recovery of Me1 (a) and Phe1 (b) over time. Each point represents the mean ± SE of four rats.

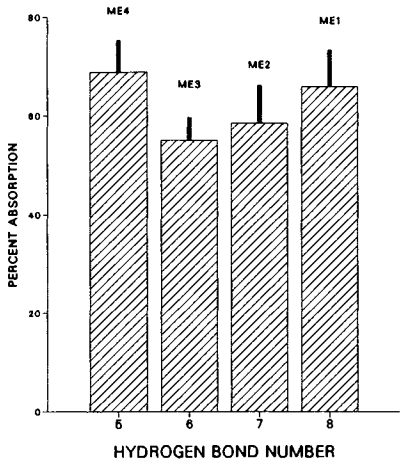


Fig. 4. Absorption of the methylated peptides as a function of hydrogen bond number. Each bar represents the mean ± SE of four rats.

tent with this goal, in earlier *in vitro* (7,8) and *in vivo* (6) intestinal absorption studies, no evidence of metabolism during transport was observed. However, metabolism of the *N*-methylated peptides was seen during passage through the liver and kidney (6). In the present study, the presence of multiple radioactive peaks in the sera after Phe2 and Phe3 administration suggest that they are metabolized during absorption across the pulmonary epithelium. Alkylation of the amide bonds seems to stabilize Phe3 to this activity. While the mechanism of this metabolism is presently unknown, these results suggest that the lung and the gut of the rat possess different abilities to metabolize these peptides.

In any event, estimates of absorption of Phe2 and Phe3 cannot be made with confidence since isotope recovered in the bile and urine may reflect transport of the metabolite rather than the parent peptide. Further, the incomplete recovery of Phe1 over the time course of the study makes its absorption estimate unreliable as well. Thus, the results for the methylated series were the only ones from which reasonable absorption estimates could be made.

Pulmonary absorption was similar with all the methylated peptides. This is in striking contrast to our earlier studies in cultured cells and rat intestine, where transport was shown to be dependent upon the number of hydrogen-bonding amide groups in the molecule which needed to be desolvated in order for the peptide to enter and diffuse across the intestinal mucosa (6–8). *In vivo,* a linear increase in intestinal absorption from 12.9 to 44% was seen as the number of *N*-methyl groups was increased, concomitant with a decrease in the hydrogen bond number of the peptide (6).

All peptides were better absorbed from the lung than the gut, consistent with earlier observations that lipid-insoluble molecules are more rapidly transported across the lung than gut epithelia (9). In the rat intestinal transport studies, a second contribution, possibly due to molecular size, was also noted (6). Since the only peptides that could be reliably evaluated in the study described herein were close in molecular weight, no discrimination on the basis of that parameter could be observed. Others, however, have reported that small peptides were more readily transported across pulmonary epithelia than large ones (10).

In summary, these results suggest that the IT route could be a viable alternate route of absorption for peptide delivery. Relative to absorption from the intestine of the rat, the methylated peptides were absorbed to a significantly greater extent. However, the potential advantage to be gained from avoidance of "first-pass" liver clearance by this route must be balanced by the ability of the lung to metabolize peptides by pathways not observed in the intestine. The desirability of the pulmonary administration of a peptide will have to be examined on a case-by-case basis.

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## REFERENCES

1. W. J. Greenlee. Renin inhibitors. *Pharm. Res.* 4:364–372 (1987).
2. M. I. Johnston, H. S. Allaudeen, and N. Sarver. HIV proteinase as a target for drug action. *TITS* 10:305–307 (1989).
3. J. S. Morley. Structure-relationship activity of enkephalin-like peptides. *Annu. Rev. Pharmacol. Toxicol.* 20:81–106 (1980).
4. M. J. Humphrey. The oral bioavailability of peptides and related drugs. In S. S. Davis, L. Ilum, and E. Tomilinson (eds.), *Delivery Systems for Peptide Drugs,* Plenum, New York, 1986.
5. R. W. Niven, F. Rypacek, and P. R. Byron. Solute absorption from the airways of the isolated rat lung. III. Absorption of several peptidase-resistant, synthetic polypeptides: poly-(2-hydroxyethyl)-aspartamides. *Pharm. Res.* 7:990–994.
6. M. S. Karls, B. D. Rush, K. F. Wilkinson, P. S. Burton, and M. J. Ruwart. Desolvation energy: A major determinant of absorption but not clearance of peptides in rats. *Pharm. Res.* 8:1477–1481 (1991).
7. R. A. Conradi, A. R. Hilgers, P. S. Burton, and N. F. Ho. The influence of peptide structure on transport across Caco-2 cells. *Pharm. Res.* 8:1453–1459 (1991).
8. R. A. Conradi, A. R. Hilgers, P. S. Burton, and N. F. Ho. The influence of peptide structure on transport across Caco-2 cells. II. Peptide bond modification which results in improved permeability. *Pharm. Res.* 9:431–435 (1992).
9. S. J. Enna and L. S. Shanker. Absorption of saccarides and urea from the rat lung. *Am. J. Physiol.* 12:409–413 (1972).
10. R. W. Niven, F. Rypacek, and P. R. Byron. Solute absorption from the airways of the isolated rat lung. III. Absorption of several peptidase-resistant, synthetic polypeptides: Poly-(2-hydroxyethyl)-aspartamides. *Pharm. Res.* 7:990–994 (1990).