Annu. Rev. Pharmacol. Toxicol. 1994. 34:321–41

ABSORPTION OF PEPTIDE AND PEPTIDOMIMETIC DRUGS

G. L. Amidon and H. J. Lee[1]
College of Pharmacy, The University of Michigan, Ann Arbor, Michigan 48109-1065

KEY WORDS: oral peptide drug absorption, mucosal cell permeability, GI metabolism, liver metabolism, prodrugs, alternative routes

INTRODUCTION

Peptide drug delivery has been of considerable interest for the past 10 to 15 years, due in part to the availability of therapeutic proteins on a commercial scale. The advent of biotechnology and advances in peptide synthesis have made the synthesis of small peptides almost routine. These two factors augmented by the development of receptor-based screening procedures have led to a large number of drug discovery programs focused on peptide-type drugs. For the purposes of this review, we define a peptide-type drug as a drug composed of amino acids or amino acid analogs whose synthesis is based on some analogy with natural peptides or proteins. The focus of this report is principally oral delivery because this is the preferred route of drug administration, though comparative results for other routes are presented.

Oral drug absorption is usually considered to be drug absorbed into the systemic circulation. This is the most relevant definition for the majority of drugs that are active by parenteral administration. However, the most basic definition of drug absorption would be drug absorbed into the gastrointestinal tissue, because once past the intestinal mucosal cell brush border, the drug may be considered to be in the body. This view emphasizes that the processes limiting systemic availability must also

[1]Also affiliated with: TSRL, Inc., 540 Avis Drive, Ann Arbor, Michigan 48108.

0362-1642/94/0415-0321$05.00

include first-pass metabolism in the gut and the liver as well as the intestinal membrane permeation.

Metabolism is generally recognized as a particularly significant barrier for peptides and proteins given the high functional capacity of the gut and liver to hydrolyze peptide bonds. However, the oral absorption of therapeutic agents such as the β-lactam antibiotics, ACE inhibitors, and cyclosporin indicates that peptide-based drug design can be successful. In this chapter we review the various factors influencing the oral systemic availability of peptide and peptide-type compounds.

ORAL DRUG ABSORPTION

General Considerations

Oral drug absorption is controlled by the three principal factors determining mass lost from the intestinal lumen: permeability, reaction (i.e. chemical or enzymatic instability), and transit (1, 2). In addition, for drugs that are dosed above their solubility, the solubility and dissolution rate of the solid drug may be important (3). Since the permeability, reaction rate, transit, and solubility of the drug can vary with position and time in the intestine, modeling drug absorption is a complex nonlinear and time-dependent math-

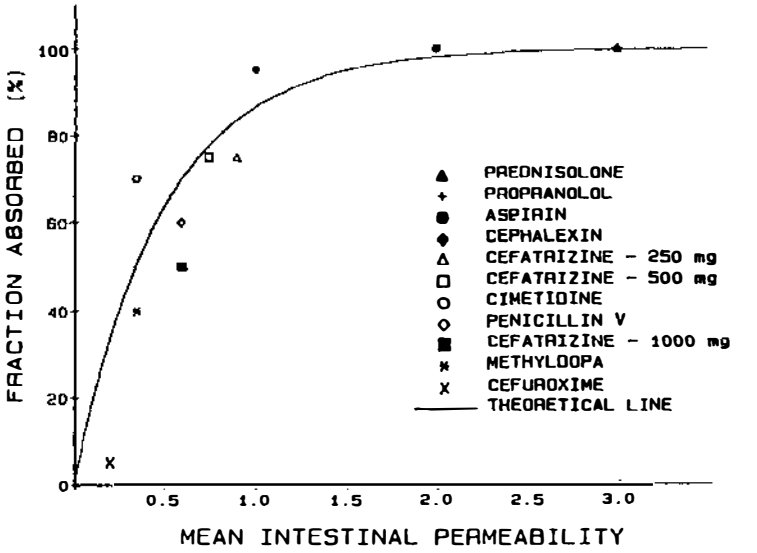

Figure 1 Plot of the fraction of the dose absorbed (%) vs the mean dimensionless intestinal wall permeability. Wall permeabilities were calculated from steady-state rat intestinal perfusion experiments.

ematical problem. However, several broad generalizations and correlations follow from the analysis of simpler, more idealized models. First, for drugs that have no intestinal instability and are dosed below their solubility, the fraction of the dose absorbed can be correlated with their intestinal membrane permeabilities (Figure 1) (1). This correlation is based on estimated extent of absorption, not systemic availability (see below). In addition, the permeabilities are for the rat intestinal jejunum, while the fraction absorbed is from human results after oral administration and includes compounds absorbed by passive as well as carrier-mediated mechanisms. Implicit in this correlation is that the absorption mechanism in the rat jejunum must be at least qualitatively similar to that in human. The fact that the correlation holds for drugs absorbed by different mechanisms is simply due to the use of a measured permeability (1, 2). The quality of this correlation as well as further absorption mechanism information can be a useful guide in drug design (see below) (4, 5).

Metabolism

Generalizations regarding metabolism are more difficult to make because of the diversity of specificities and locations of the responsible enzymes. However, a simple mass balance analysis of transport and reaction in the intestine points out that it is not simply the reaction rate that is important, but the ratio of the reaction rate to absorption rate (or permeability) that controls absorption into the intestinal tissue (6). A drug of sufficiently high permeability will get absorbed, though it may be subsequently metabolized in the liver and/or extracted into the bile. Such enterohepatic recycling further increases the time for metabolism in the gut. For a passively absorbed drug, e.g. peptides larger than three amino acids (7), the principal determinant of the membrane permeability is the membrane/lumen partition coefficient. That is, for passively absorbed compounds:

$$P_m = KD_M/\delta_m, \qquad 1.$$

where P_m is the membrane permeability, K the membrane/luminal solution partition coefficient, D_m the membrane diffusion coefficient, and δ_m the membrane thickness. However, in making drugs more lipophilic for increased membrane permeability, it is not uncommon to produce very water-insoluble compounds. This leads to a solubility/dissolution limitation.

Solubility and Dose

Recent analysis of the dissolution and absorption processes in the gut have pointed out the importance of dose, solubility, and dissolution rate in determining the maximum extent of absorption (8). While the complexity of the gut prevents accurate modeling today, it is clear that micronization

(e.g. griseofulvin, digoxin) and lipid formulation (e.g. cyclosporin, digoxin) can improve the extent of drug absorption; consequently these limits are significant and need further quantification. The basic new parameter (in addition to dissolution and permeability) is the dose number, D_0;

$$D_0 = Dose/V_0C_s, \qquad 2.$$

where *Dose* is drug oral dose, C_s the solubility, and V_0 the initial volume in which the drug dissolves in the gut. For a drug such as griseofulvin, this number is estimated to be over 100. Consequently, drug design and drug dosing must be done recognizing the finite volume and residence time in the gut, and that lipophilicity must be optimized.

MUCOSAL CELL PERMEABILITY

The mucosal cell permeability, P_m, determines the rate of entry into the mucosa across the brush-border membrane. A variety of techniques have been used to study this process (9). We focus on results using the perfusion method because this method measures the overall mass transfer coefficient controlling absorption, P_m, and an analogous experimental procedure can be used in humans (10), allowing for the future development of useful animal to human general correlations. In addition to the general passive diffusion considerations applicable to all drugs noted above, peptide absorption requires consideration of size (molecular
transport.

Molecular Weight

It is generally believed that the absorption of high mol wt compounds is low. However, there have been few efforts to quantitate the decline in absorption and the mechanism responsible, probably due to the limited availability of test compounds. One area of importance, that of oral vaccines, works effectively through the gut-associated lymphoid tissue (GALT) but (probably) delivers very small amounts of "drug" to the systemic circulation; this area lies outside the scope of this review. One set of compounds for which there is a data base in both humans and animals is the polyethylene glycols (PEGs). The PEGs have the almost unique property of maintaining a relatively constant hydrophilic-lipophilic balance as the mol wt is increased. PEG 4000 has been used as a nonabsorbable marker of integrity and fluid secretions in human and animal perfusions for many years. Recently we compared the oral and nasal absorption of PEG's 600, 1000, and 2000 using an HPLC assay that separated the various oligomers following urinary excretion in the rat (11). The results shown in Figure 2 exhibit a sharp fall off in absorption above mol wt 700. These results are in agreement with the results of human studies performed via intestinal perfusion and urinary

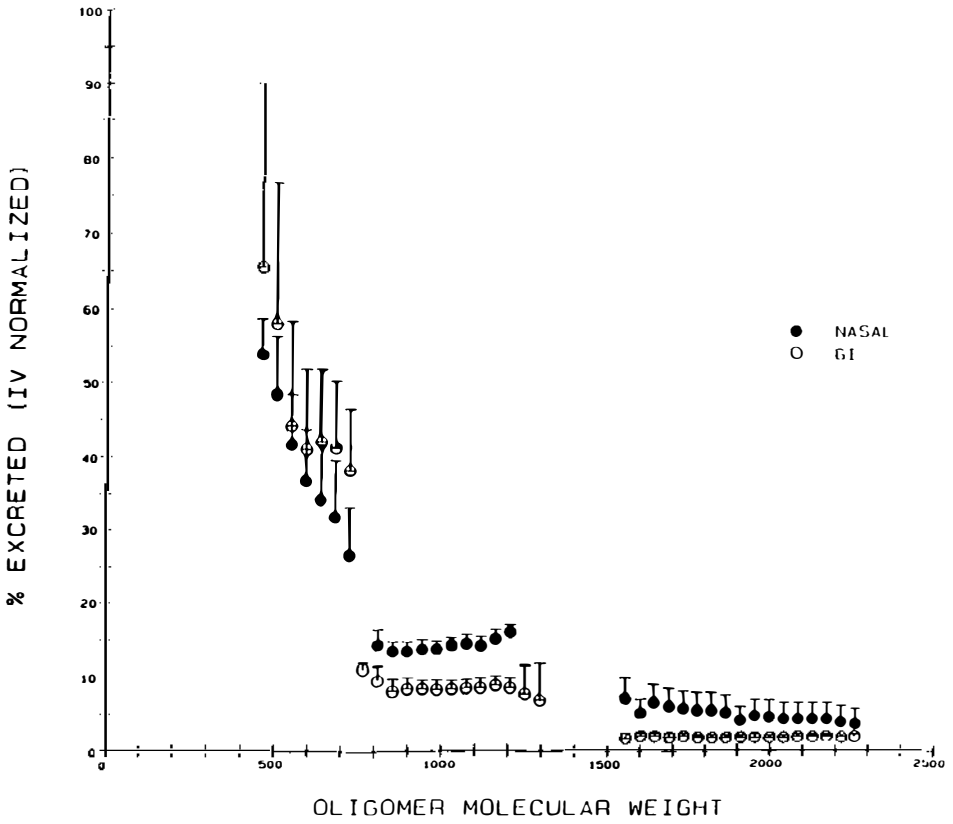

Figure 2 Excretion of oligomers of PEG 600–2000 following nasal and gastrointestinal administration in the rat (normalized to mean intravenous excretion values). Error bars represent the standard error of the mean. Nasal: n = 4 (PEG 600), 3 (PEG 1000), and 3 (PEG 2000). GI: n = 8 (PEG 600), 4 (PEG 1000), and 3 (PEG 2000).

excretion (12, 13). The mechanisms responsible for this sharp decrease have not been firmly established. For passive absorption, Equation 1 suggests that a decrease in either diffusivity or partitioning is responsible. Since the mucosal cell brush border membrane and its associated glycocalyx and mucin coating are relatively intact in the perfusion model and are presumably the functional barrier during normal drug absorption, diffusion and partitioning into each of the components of this composite membrane must be considered.

A systematic study of the absorption of peptides of increasing mol wt has yet to be reported. However, the drug cyclosporin (Figure 3) provides for some optimism regarding oral absorption of larger peptides. This cyclic natural product has mol wt 1200 and a log partition coefficient of 3

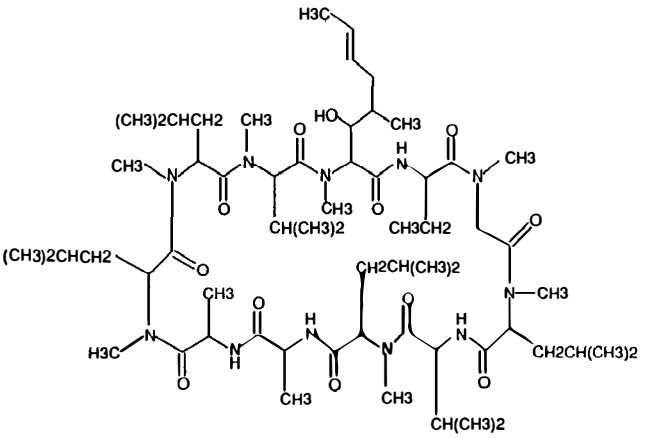

Figure 3 Cyclosporin structure.

(octanol/water). The extent of its oral absorption in humans is reported to be 23% including dosing in an oil delivery system (14). In addition, intestinal metabolism may be contributing to the low systemic availability (15). While the results of the human studies on cyclosporin cannot be directly compared to the PEG studies, the extent of absorption argues that the partition coefficient effect on membrane transport is still operative in this molecular weight range (Equation 1). Consequently, the extrapolation of the mol wt dependence on the extent of absorption to an absorption of 10–20% for mol wt 2000–3000 may be possible. Several drugs of importance have systemic availabilities of less than 20–30%, e.g. labetalol, morphine, nicardipine, and propranolol (16). The mol wt dependence of drug absorption (and hepatic extraction) should receive increasing attention in the future in order to define the limits via this route. Nevertheless, one can conclude that oral absorption is possible for peptides of at least mol wt 1200, and this provides over 10^{15} possible therapeutic candidates, using only natural amino acids.

Carrier-Mediated Transport

The permeability and absorption of di- and tripeptides have been studied extensively. The initial pioneering work of Matthews (for review see 7) focused on natural peptides. However, interest in the past ten years has focused on drugs that are analogs of di- and tripeptides. The β-lactam antibiotics have been studied most extensively, while the ACE inhibitors have received more recent attention, and a few renin inhibitors have been reported to utilize this carrier (17). Studies have focused mainly on mechanism, i.e. establishing carrier-mediated transport for particular compounds

Table 1 Summary of permeability data for some dipeptides and peptide drugs β-lactams and ACE inhibitors[a]

Compounds	K_m(mM)	J^*_{max}(mM)	P^*_c	P^*_m	Inhibitor
Peptides					
Carnosine	12.9	6.62	0.51	—	—
Phe-Gly	1.29	6.87	5.33	—	-
β-Lactams					
Ampiciliin	15.80	11.78	0.75	0.00	Amoxicillin
Amoxicillin	0.058	0.044	0.558	0.76	Cephradine
Cyclacillin	14.00	16.30	1.14	0.00	-
Cefaclor	16.1	21.3	1.32	0.00	-
Cefadroxil	5.9	8.4	1.43	0.00	Cyclacillin, Gly-Gly
Cefatrizine	0.58	0.73	1.25	0.20	-
Cephalexin	7.2	9.1	1.30	0.00	Cyclacillin, Gly-Gly
Cephradine	1.48	1.57	1.06	0.30	Cephalexin, Gly-Leu
Cefixime	0.031	0.016	0.184	0.52	Carnosine, Gly-Pro
ACE inhibitors					
Captopril	5.9	12.3	2.08	1.00	Gly-Gly, Cephradine
Enalapril	0.07	0.13	1.9	0.35	Tyr-Gly, Cephradine
Lisinopril	0.082	0.032	0.39	0.00	Tyr-Gly, Cephradine
SQ29,852	0.080	0.160	2.00	0.25	Tyr-Gly, Cephradine

[a] *—dimensionless parameter; K_m—Michaelis-Menten constant in millimolar; J^*_{max}—maximal transport rate; $P^*_c = J^*_{max}/K_m$—the carrier transport parameter; P^*_m—dimensionless passive parameter. Taken from Refs. 94 and 95.

and on the kinetics of this cotransport system. We briefly summarize the extensive literature. While the initial reports suggesting that β-lactam absorption was carrier mediated (18, 19) were published over 20 years ago, thorough investigation of the carrier-mediated transport began only about 10 years ago (20, 21). Table 1 presents a summary of the carrier transport parameters for a variety of peptide-type drugs. The parameters in the table were determined by nonlinear regression of the measured intestinal wall permeability, P_w, to either of the following alternative expressions:

$$P_w = J^*_{max}(K_m + C_w) + P_m$$

or

$$P_w = P^*_c/[1 + (C_w/K_m)] + P_m, \text{ where}$$
$$P^*_c = J^*_{max}/K_m.$$

Here, J^*_{max} and K_m are the carrier maximum flux and transport affinity, respectively, P^*_m is the passive membrane permeability, and P^*_c is the carrier permeability at $C << K_m$ (21). These parameters are overall apparent

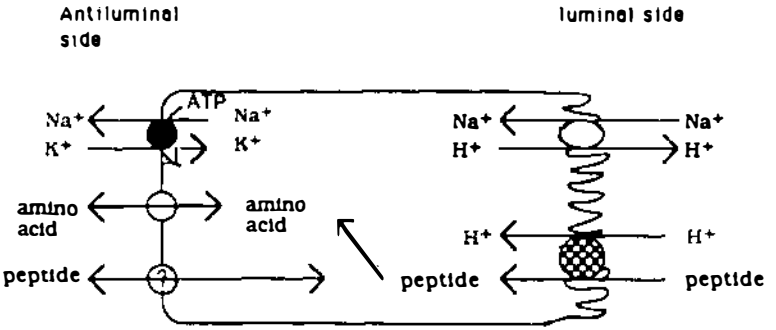

Figure 4 The transport of di- and tripeptides are energized by a proton (H^+) gradient across the brush border membrane. Referred to the model proposed by Ganapathy & Leibach (24).

transport parameters for the transport process. Detailed kinetic analysis of the carrier transport process analogous to those done for some sugar and amino acid transports has not been reported (22, 23). However, the transport process has been studied in intestinal and kidney brush-border membrane vesicles and has been shown to be a system in which the peptide is cotransported with a proton (24). The pH gradient in the intestine is maintained by the sodium/proton exchanger, while the lumen of the intestine is acidic relative to the cell cytosol. The sodium/potassium ATPase in the basal lateral membrane maintains the low cytosolic sodium concentration needed to provide the driving forces for this and the other sugar and amino acid transporters. Figure 4 summarizes this transport scheme adapted from Ganapathy & Leibach (24).

In all cases for the compounds reported in Table 1, the test compound exhibited a simple competitive inhibition pattern with other peptide compounds from the table and with various natural peptides. Another point to note from the results in Table 1 is that there is much less variation in P_c than in the determined J_{max} and K_m values. This is due in part to the perfusion methodology that determines the P_c value most precisely, i.e. the wall permeability when $C << K_m$. For correlations with overall drug absorption such as shown in Figure 1, this is the parameter of primary interest. However, dose-dependent drug absorption may be observed at high oral doses as has been observed (25). More detailed kinetic molecular insight must await further studies of the transporter kinetics in vesicle systems and the expression and cloning of the transporter(s). There have been several reports of successful expression of the transporter in the oocyte model (26–28).

METABOLISM

In addition to transport, another key factor playing an important role in the absorption of peptide and peptide-type drugs is metabolism. The metabolism of peptide and peptide-type drugs usually results in loss of efficacy; thus, identification of the true site of loss needs careful assessment. The sites for the presystemic hydrolysis of peptide and peptide-type drugs following oral administration are the gastrointestinal lumen, mucosal cells (including brush border membrane and cytosol), and the liver. Extensive studies on identification of participating enzymes in the metabolism of peptides and peptide-type drugs have been in progress for several years. This is because a major design goal in the synthesis of biologically active peptides and related drugs, whether derived from naturally occurring compounds or not, is to achieve metabolic stability, enhanced oral bioavailability, and prolonged duration of action. However, achievement of metabolic stability with a peptide structure does not always guarantee a long biological half-life. Peptide drugs that are stable to peptidase activity may still undergo metabolism by other detoxification systems in the body. For example, the cyclic undecapeptide cyclosporin is metabolized to at least nine metabolites (29). Biotransformation includes mono- and dihydroxylation and N-demethylation at various sites on the cyclosporin molecule and is thought to involve the cytochrome P-450 monoxygenase system because compounds known to inhibit these enzymes increase cyclosporin blood levels. Nevertheless, enzymatic hydrolysis is the dominant cause of metabolism of peptide drugs, and we focus on these metabolic sites in the gastrointestinal tract and liver.

Gastrointestinal Tract

LUMINAL ENZYMES The gastrointestinal luminal enzymes involved in protein digestion include pepsin, trypsin, α-chymotrypsin, elastase, and carboxypeptidases A and B. The specificities of these enzymes are respectively: hydrophobic, basic, large hydrophobic, small aliphatic and aromatic, and basic C-terminal amino acids (30). Table 2 gives typical substrates for these enzymes. Because these enzymes autodigest as they traverse the intestine, oral peptide delivery strategies that focus on ileal or colonic delivery are intended, in part, to deliver the drug past the region of highest luminal enzyme activity. This strategy can reduce the luminal metabolism component limiting oral peptide delivery. In order for this strategy to be successful, the drug must exhibit good membrane permeability in the ileum and colon. This probably requires good passive permeability and solubility properties. If the peptide absorption mechanism is carrier-mediated and the transporter

Table 2 Luminal proteolytic enzymes and example substrates

Enzyme	Substrate
Pepsin	Z-His-Phe-Phe-OMe
Trypsin	Benzoyl arginine methyl ester (BAEE)
α-Chymotrypsin	Benzoyl tryosine ethyl ester (BTEE)
Elastase	Ala-Ala-Ala methyl ester
Carboxypeptidase A	Hippuryl phenylalanine
Carboxypeptidase B	Hippuryl arginine

is expressed only in the small intestine, this strategy likely will not be successful. Some success with this strategy has been reported (31).

MUCOSAL CELL ENZYMES: BRUSH-BORDER MEMBRANE BOUND Brush-border membranes form the luminal surface of the epithelial cells of the intestine, liver, and kidney. These membranes are rich in enzymes and transporters, which are involved in the cell surface metabolism and absorption of various peptides and peptide-type drugs. Typical gastrointestinal brush-border membrane enzymes associated with GI tract are listed in Table 3 (7, 32). Brush-border enzymes can be divided into four types based on their mode of action (Table 3): e.g. aminopeptidase, carboxypeptidase, dipeptidase, and endopeptidase. Aminopeptidase, carboxypeptidase, and dipeptidase belong to exopeptidase because these enzymes hydrolyze peptides sequentially from either the aminoterminal or carboxylterminal end of the molecule. The most abundant enzymes in the intestinal brush-border membrane are aminopeptidases. Many dipeptides are hydrolyzed in the brush border, except for

Table 3 Typical intestinal brush-border membrane enzymes

Type	Specificity	Enzyme
Exopeptidase, NH_2 terminus	Many amino acids	Aminopeptidase N
	Asp or Glu	Aminopeptidase A
	Amino acid-Pro	Aminopeptidase P
	Amino acid-Pro,-Ala	Dipeptidylpeptidase IV
	γ-Glu	γ-Glutamyltransferase
Exopeptidase, COOH terminus	Many amino acids	Angiotensin-converting enzyme
	Pro, Ala, Gly	Carboxypeptidase P
Exopeptidase dipeptidase	Many amino acids	Microsomal dipeptidase
Endopeptidase	Hydrophobic	Endopeptidase-24.11
	Aromatic	Endopeptidase-2

glycyl-dipeptides especially in human jejunum (33). Aminopeptidases capable of hydrolyzing dipeptides containing proline (Pro) are absent from the brush-border membrane, while dipeptidylpeptidase IV hydrolyzes Pro-containing oligopeptides from the carboxylterminal (34). Peptides of at least eight amino acid residues may be hydrolyzed by the indigenous peptidases of the brush-border membrane (7). The oligoaminopeptidases of the brush-border membrane do not hydrolyze very large peptides and have no action on the β-chain of insulin or on bovine serum albumin (7). However, endopeptidases in the brush-border membrane are still capable of hydrolyzing proteins and large polypeptides. Although some brush-border enzymes are species- and substrate-dependent, in general, the rate of hydrolysis for peptide-type drugs in the brush border increases in the order of dipeptide > tripeptide > larger peptide (35, 36).

CYTOSOLIC ENZYMES Cytosolic peptidases are listed in Table 4, which include aminotripeptidase, prolidase, prolinase, dipeptidase, and carnosinase. Substrate specificity of these enzymes are also tabulated in Table 4 (32). Neutral dipeptides are hydrolyzed more readily than acidic or basic dipeptides. However, the possible detection of these peptides in the portal blood stream suggests that this enzyme activity is not completely effective. It is not certain whether peptides of more than thrcc amino acid residues can be hydrolyzed in the cytosol. Overall, the cytosolic enzymes prefer to hydrolyze dipeptides over tri or large peptides.

Liver

In addition to the GI sites, the liver contributes to the presystemic loss of peptide and peptide-type drugs and also is responsible for the metabolism of peptidc drugs administered peripherally. The liver metabolism of peptide and peptide-type drugs is influcnced by the route of administration (cf. oral versus intravenous) and/or physicochemical properties of peptide molecules (37–39). There is, in general, a lack of data from which to draw basic rules regarding

Table 4 Intestinal brush-border cytosol enzymes with typical substrates

Enzymes	Typical substrate
Dipeptidase	Neutral dipeptides
Aminopeptidase	Tripeptides with N-terminal Pro
Prolidase	Imidodipeptides with C-terminal Pro/Hyp
Prolinase	Imidodipeptides with N-terminal Pro/Hyp
Carnosinase	Carnosine (β-Ala-His)

hepatic metabolism of peptides and proteins. However, one rough empirical conclusion based on data currently available is that there is high hepatic extraction for smaller peptides with chain length of less than 8 amino acid residues (40). For instance, radio labeled cholecystokinin (CCK33), cholecystokinin octapeptide (CCK8), CCK8 desulfate, and CCK4 are extracted by the liver in a structurally specific manner. The fate of the extracted radio labeled peptides was studied by quantitating biliary excretion and determining the nature of the metabolites in the bile (40); CCK8 desulfate and CCK4 appeared in the bile in completely metabolized forms. In contrast, for CCK33 and CCK8, about 3% and 20%, respectively, of the major forms of the label in bile were intact (40). Transforming growth factor beta (TGFβ with three amino acids), a recently discovered polypeptide, modulates growth of normal and neoplastic cells (41). After intravenous injection of ^{125}I-TGFβ in the rat, most of the label removed by the liver (83%) was excreted into bile.

The mechanism of hepatic uptake of peptides involves two processes (42, 43)—usually passive for hydrophobic peptides and active for less hydrophobic peptides. These active processes then can be divided into two types of mechanism: endocytosis and carrier-mediated transport (42, 43). Most water-soluble peptides not cleared by specific mechanisms are believed to enter liver cells by endocytosis. The representative liver enzymes for peptide and peptide-type drugs are cathepsin (D, B, L, H) and intracellular and lysosomal proteinases (44). Some membrane-bound aminopeptidases were also found in the biliary canalicular membranes (45). Although limited information is available regarding participating liver enzymes, the characterization of enzymes and transport mechanism will aid in the future design of orally active peptides and peptide-type drugs.

PRODRUGS

The prodrug approach to improving drug absorption has generally been used to increase the lipophilicity of a drug and hence to improve its membrane permeability (46). This strategy assumes that passive transport is the mucosal membrane transport mechanism. However, the broad specificity exhibited by the peptide transporter suggests that prodrugs may be targeted for this transporter. Figure 5 shows this scheme with a polar low permeability drug being converted into a (polar) di/tri peptide-type drug and a substrate for the peptide transporter. Following transport across the mucosal cell brush border membrane, hydrolysis back to the parent drug occurs in the mucosal cell or the liver. In fact the ACE inhibitors appear to be transported in this manner (47, 48). Enalaprilat, the active dipeptide, has a very low permeability (47) and has a very low systemic availability, while the prodrug ethyl ester is well absorbed and hydrolyzed back to the active parent compound in vivo.

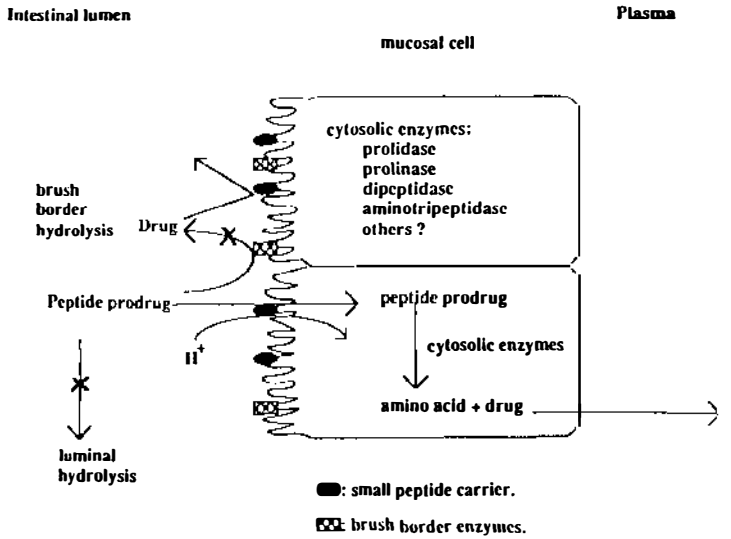

Figure 5 Schematic representation of a peptide prodrug strategy for improving oral absorption.

A recent effort to design a prodrug for this transporter utilized the poorly absorbed amino acid analog α-methyldopa as the "drug" and evaluated the transport of several peptide prodrugs (49, 50). Some of the reported results are shown in Table 5. The mucosal cell permeability was significantly increased for all the peptide prodrugs. Studies demonstrated mucosal cell hydrolysis of the peptide prodrugs and recently a significant increase in the systemic availability of α-methyldopa was observed when the prodrug α-methyldopa-Phe was administered into the ileum of rats (51). These results indicate that design of prodrugs based on transport by the peptide transporter may be a useful strategy for improving the absorption of small polar drugs.

Table 5 Kinetic parameters of L-α-methyldopa, L-phenylalanine, and L-α-methyldopa-L-phenylalanine

Compound	K_m(mM)	J^*_{max}(mM)	P^*_c
L-α-Methyldopa	18.6 (4)[a]	7.4 (1.1)	0.40 (0.04)
L-Phenylalanine	9.5 (3.1)	48 (20)	5.3 (1.4)
L-α-Methyldopa-L-phe	0.15 (0.03)	1.8 (0.27)	12.2 (0.31)

[a] Estimated standard deviation of the parameter.

ALTERNATIVE ROUTES

The development of oral delivery of peptide and peptide-type drugs has been limited, in part, by their rapid metabolism in the gastrointestinal tract and in some cases their inability to readily cross the membrane barrier. Stability of peptides to the hostile environment of the gut is an essential requirement for oral activity. Oral formulations of peptide and peptide-type drugs which have been investigated to improve presystemic stability are: (*a*) oil/water emulsion (52), (*b*) encapsulation in liposomes (53), (*c*) entrapment in polymer particles in the nanometer size range (54), and (*d*) coadministration with protease inhibitors or permeability promoters (55, 56). These approaches have increased oral activity of these peptide molecules; however, the extent of absorption is still low. The cyclic structure of cyclosporin, which is stable to the action of proteases, exhibits the greatest absorption (20–50% of dose) after administration in an olive oil–based formulation (14), especially when given with food (57). The use of GI absorption enhancers for therapeutic peptides and proteins increases bioavailability (58–60), although questions remain concerning the safety of many of the systems described in the literature. Examples of peptides that are biologically active on oral or intraluminal administration include: (*a*) lysine vasopressin, especially in the presence of trasylol protease inhibitor (61), (*b*) l-Desamino-8-D-arginine vasopressin, for which about 0.7–1% of an oral dose is absorbed by humans, compared to 11% of a nasal dose (62, 63), (*c*) SMS 201-995, a selective mini-somatostatin, a "blocked" octapeptide having three times more potency than somatostatin (64, 65), (*d*) luliberin, a decapeptide with blocked termini (66–68), (*e*) thyroliberin, a tripeptide with blocked termini (69), (*f*) insulin with hypertonic solutions, trypsin inhibitors, and/or encapsulated in liposomes (70–72).

Other mucous membranes can be used as potential sites of drug delivery and alternative routes for parenteral administration. The various membrane sites have different permeabilities to peptide molecules because of the different membrane structures and junctions between the cells. However, the non-oral routes have the advantage of avoiding the effects of stomach acid, gut proteases, and "first-pass" metabolism by the liver. Various alternate routes for systemic administration of peptide drugs have been investigated; for nonparenteral administration: buccal, vaginal, rectal, transdermal, ocular, and nasal routes, and for parenteral administration: intradermal, subcutaneous (sc), intramuscular (im), intraperitoneal (ip), intravenous (iv), and other routes. The iv, sc, im, transdermal, and nasal routes are most often used for peptide drug delivery for practical purposes. These routes are reviewed briefly below.

Parenteral Administration

For systemic delivery of large peptide drugs, parenteral administration is almost essential to achieve consistent therapeutic activities, because of the drug's susceptibility to breakdown by gastric acid and the proteolytic enzymes in the gastrointestinal tract. In addition, peptides are generally hydrophilic and relatively high mol-wt substances and thus do not easily cross the intestinal mucosa. Of the parenteral routes, only intravenous administration can be considered efficient in delivering peptide drugs to the systemic circulation. Intramuscular or subcutaneous injections are less efficient due to the absorption and diffusion barriers presented by the muscle mass and connective tissues under the skin. While most peptide/peptide-type drugs can be efficiently delivered to the systemic circulation by parenteral injections, a poor disposition profile still leads to low therapeutic concentrations which necessitates high dosing frequencies. Frequent injections, besides being unpleasant to the patients, also lead to complications such as thrombophlebitis and tissue necrosis (73). For parenteral administration of high potency hormones, consideration needs to be given to the rate of drug delivery and to the possible benefit of a sustained release of peptide (74).

Nonparenteral Administration

NASAL ROUTE The nasal mucosa has a high surface area due to numerous microvilli on the epithelial cells and is supplied with an extensive vascular network. Thus it is not surprising that the nasal cavity provides a good site for extensive absorption of both lipophilic drugs and hydrophilic drugs, which are poorly absorbed by the oral route. With coadministration of absorption enhancers to the nasal route, bioavailabilities of peptides are far greater than those in the oral route. These peptides include LH-RH, Ala^1-Lys^{17}-ACTH, cholecystokinin octapeptide, sulbenicilin, and cephalosporin. Even larger peptides or proteins seem to be absorbed. For example, insulin absorption is up to 30% in rats (75). The rapidity of absorption is also of note, and for this reason there is interest in intranasal insulin (76) as a means of achieving the desired insulin bioavailability profile. Although nasal delivery avoids the hepatic first-pass effect, the enzymatic barrier of the nasal mucosa creates a pseudo-first-pass effect. In addition to an enzymatic barrier, there also exists the permeation barrier to nasal delivery. The xenobiotic metabolic activity in the nasal epithelium has been investigated in several species including humans. The cytochrome P-450 activity in the olfactory region of the nasal epithelium is higher even than in the liver, mainly because of a three- to four-fold higher NADPH-cytochrome P-450 reductase content (77). All three major classes of hepatic inducible cyto-

chrome P-450 isozymes have been found in the nasal mucosa (78, 79). Phase II conjugation activity has also been found in the nasal epithelium (77).

The delivery of peptides and peptide-type drugs has been hindered by the peptidase and protease activity in the nasal mucosa. The predominant enzyme appears to be aminopeptidase along with other exopeptidases and endopeptidases (80). The absorption of peptide drugs can be improved by using aminoboronic acid derivatives, amastatin, and other enzyme inhibitors as absorption enhancers. It is possible that some of the surfactants, e.g. bile salts, may increase absorption by inhibiting the proteolytic enzymes, presumably by denaturation (81). Mean plasma levels of human growth hormone following intranasal administration of 1 mg/kg hGH solution alone were significantly lower than those in combination with two aminopeptidase inhibitors, 0.015% amastatin and 0.015% bestatin (80). The surfactant, sodium glycolate, provides a bioavailability of 100% for cortocotrophin-releasing hormone (CRH) but only 7.1% for growth hormone releasing hormone (GHRH).

TRANSDERMAL ROUTE The transdermal route is an attractive alternative, because it offers a number of advantages over oral and nasal routes. It has less serious problems of local proteolytic degradation and hepatic first-pass metabolism and may provide better control of delivery and maintenance of the therapeutic level of drug over a prolonged period of time. Although the transdermal route has been thought to have low proteolytic enzyme activity, a means of increasing permeation must be found because of the general impermeability of the skin and the large molecular size of peptides and their ionic character. One approach employs iontophoresis as an active driving force for the charged molecules (82). Another approach involves the use of chemical enhancers (83–85). However, little is known about the proteolytic enzyme activity in the skin and its possible influences on mass transfer, even though other enzymes of the skin have been relatively well characterized (86). In the investigation of the effects of the nonionic surfactant, n-decylmethyl sulfoxide (NDMS), pH, and inhibitors on the metabolism and the permeation of amino acids, dipeptides, and the pentapeptide enkephalin, through hairless mouse skin (87), NDMS was found to increase the permeability of all amino acids and peptides tested. At neutral pH, the enzyme activity within the skin was such that no flux of leucine-enkephalin (YGGFL) was observed, and the donor cell concentration of YGGFL decreased rapidly. The major cleavage occurred at the Tyr-Gly bond. At pH 5.0 the metabolic activity was reduced significantly, and a substantial flux of YGGFL was observed. These results show that a com-

bination of enhancer, pH adjustment, and inhibitors can increase the transdermal delivery of peptides (87). One of the barriers to transdermal delivery of peptides is the metabolic activity of the epidermis (88). The subcellular distribution of aminopeptidases was determined in homogenates of cultured mouse keratinocytes and mouse epidermis. Soluble and membrane-bound fractions of the homogenates were analyzed separately for aminopeptidase activity (88).

The larger molecular size and charge of proteins and peptide drugs make them poor candidates for passive transdermal delivery (89). With an applied electromotive force, iontophoresis, an additional force can be applied to drive these molecules through the skin. LH-RH, a decapeptide of molecular weight 1182 and positive charge at pH 6.0, was delivered through pig skin by anodal iontophoresis. Although usually potent at very low concentrations, peptides generally have very short half-lives, necessitating frequent dosing. The achievement of the reliable transdermal delivery of peptides would offer several clinical advantages over other more conventional routes of delivery.

MISCELLANEOUS ROUTES Other routes of administration, like intravaginal and pulmonary, have shown good absorption for peptides such as LH-RH analogs (40% to 95%) (90) and insulin (up to 57%) (91). Rectal administration has also provided good delivery for tetragastrin (16% of dose) and insulin (28% of dose) in the dog (92, 93). Although rectal administration generally does not appear to compare favorably with intravaginal or intranasal routes regarding extent of absorption of peptides, these route offers a number of advantages over the oral route.

CONCLUSIONS

In this brief review, we have summarized the current state of peptide and peptide-type drug delivery. We have focused principally on oral delivery and the mechanisms of peptide permeation and metabolism. The successful delivery of peptide-type drugs via the oral route is possible. However, the mechanisms and sites of transport and metabolism must be considered and in many cases investigated further in order to design drugs to be effective via the oral route. The same is true for the other routes of administration. Both chemical modification and delivery strategies will likely be needed to develop peptide-type compounds into effective therapeutic agents.

Literature Cited

1. Amidon GL, Sinko PJ, Fleisher D. 1988. Estimating human oral fraction dose absorbed: a correlation using rat intestinal membrane permeability for passive and carrier-mediated compounds. *Pharm. Res.* 5(10):651–54
2. Sinko PJ, Leesman GD, Amidon GL. 1991. Predicting fraction dose absorbed in humans using a macroscopic mass balance approach. *Pharm. Res.* 8(8): 979–88
3. Oh D, Curl RL, Amidon GL. 1993. Estimating the fraction dose absorbed from suspensions of poorly soluble compounds in humans: a mathematical model. *Pharm. Res.* 10(2):264–70
4. Amidon GL, Sinko PJ, Hu M, Leesman GD. 1989. Absorption of difficult drug molecules; carrier-mediated transport of peptides and peptide analogs. In *Novel Drug Delivery and Its Therapeutic Application,* ed. LF Prescott, WS Nimmo, pp. 45–56. New York: Wiley
5. Amidon GL, Leesman GD, Sinko PJ, Hu M. 1988. *Proc. Int. ISSX Meet., 2nd, Kobe, Japan,* pp. 117–24. Great Britain: Taylor & Francis Ltd
6. Sinko PJ, Leesman GD, Amidon GL. 1993. Mass balance approaches for estimating the intestinal absorption and metabolism of peptides and analogues: theoretical development and applications. *Pharm. Res.* 10(2):271–75
7. Matthews DM. 1991. *Protein Absorption,* New York: Wiley
8. Oh D, 1991. *Estimating oral drug absorption in humans,* PhD thesis. Univ. Mich., Ann Arbor.
9. Fleisher D, Stewart BH, Amidon GL. 1985. Design of prodrugs for improved gastrointestinal absorption by intestinal enzyme targeting. *Meth. Enzymo.* 112: 360–81
10. Merfeld AE, Mlodozeniec AR, Cortese MA, Rhodes JB, Dressman JB, Amidon GL. 1986. The effect of pH and concentration on alpha-methyldopa absorption in man. *J. Pharm. Pharmacol.* 38:815–22
11. Donovan MD, Flynn GL, Amidon GL. 1990. Absorption of polyethylene glycols 600 through 2000: the molecular weight dependence of gastrointestinal and nasal absorption. *Pharm. Res.* 7(8):863–68
12. Shaffer CB, Critchfield FH. 1947. Absorption and excretion of solid polyethylene glycols ("carbowax" compounds). *J. Am. Pharm. Assoc.* 36: 152–57
13. Shaffer CB, Critchfield FH, Nair JH. 1950. Absorption and excretion of liquid polyethylene glycol. *J. Pharm. Sci.* 39:340–44
14. Wood AJ, Maurer G, Niederberger W, Reveridge, T. 1983. Cyclosporine-pharmacokinetics, metabolism, and drug-interactions. *Transplant. Proc.* 15:2409
15. Kolars JC, Schmiedlin-Ren P, Dobbins WO III, Schuetz J, Wrighton SA, Watkins PB. 1992. Heterogeneity of cytochrome P450 IIIA expression in rat gut epithelia. *Gastroenterology* 102: 1186–98
16. Benet LZ, Williams RL. 1990. Design and optimization of dosage regimens; pharmacokinetic data. In *The Pharmacological Basis of Therapeutics,* ed. AG Gilman, TW Rall, AS Nies, P Taylor, p. 1655. New York: Pergamon
17. Kramer W, Girbig F, Gutjahr U, Kleemann HW, et al. 1990. Interaction of renin inhibitors with the intestinal uptake system for oligopeptides and beta-lactam antibiotics. *Biochim. Biophys. Acta* 1027:25–30
18. Quay JF. 1972. Transport interaction of glycine and cephalexin in rat jejunum. *Physiologist* 15:241
19. Quay JF, Foster L. 1970. Cephalexin penetration of the surviving rat intestine. *Physiologist* 13:287
20. Nakashima E, Tsuji A, Kagatani S, Yamana T. 1984. Intestinal absorption mechanism of amino-beta-lactam antibiotics. III. Kinetics of carrier-mediated transport across the rat small intestine in situ. *J. Pharm. Dyn.* 7:452- 64
21. Sinko PJ, Amidon GL. 1988. Characterization of the oral absorption of beta-lactam antibiotics. I. Caphalsporins: determination of intrinsic mebrane absorption parameters in the rat intestine in situ. *Pharm. Res.* 5(10): 645–50
22. Stein WD. 1986. *Transport and Diffusion across Cell Membranes.* San Diego: Academic
23. Stein WD. 1990. *Channels, Carriers, and Pumps: An Introduction to Membrane Transport.* San Diego: Academic
24. Ganapathy V, Leibach F. 1985. Is intestinal peptide transport energized by a proton gradient? *Am. J. Physiol.* 249:G153–60
25. Couet WR, Reigner BG, Guedes J, Tozer TN. 1991. Theoretical model for both saturable rate and extent of absorption: simulations of cefatrizine

data. *J. Pharmaco. Biopharm.* 19(3): 271–86
26. Miyamoto Y, Thompson YG, Howard EF, Ganapathy V, Leibach FH. 1991. Functional expression of the intestinal peptide-proton co-transporter in *Xenopus laevis* oocytes. *J. Biol. Chem.* 265:4742–45
27. Saito H, Ishii T, Inui, K. 1993. Expression of human intestinal dipeptide transporter in *Xenopus laevis* oocytes. *Biochem. Pharmacol.* 45:776–79
28. Oh D, Amidon GL, Sadee W. 1992. Endogenous peptide transporter and exogenous proton/peptide co-transporter exressed in *Xenopus* oocytes. *Pharm. Res.* 9(10):S82 (Suppl.)
29. Maurer G. 1985. Metabolism of cycosporine. *Transplant. Proc.* 17(1): 19 (Suppl.)
30. Banerjee PK, Amidon GL. 1985. Design and prodrugs based on enzyme-substrate specificity. In *Design of Prodrugs,* ed. H Bundgaard, pp. 93–133. Amsterdam/New York/Oxford: Elsevier
31. Lee HJ, Amidon GL, 1993. Peptide oral delivery employing a selective enzyme inhibitor and a specific absorption site: I. [D-ala^2, D-leu^5] enkephalin. *Proc. Int. Symp., Delivery of Protein Drugs—The Next 10 Years, Kyoto*
32. Bai JPF, Amidon GL. 1992. Structural specificity of mucosal-cell transport and metabolism of peptide drugs: implication for oral peptide drug delivery. *Pharm. Res.* 9(8):969–78
33. Steinhardt HJ, Adibi SA. 1986. Kinetics and characteristics of absorption from an equimolar mixture of 12 glycyl-dipeptides in human jejunum. *Gastroenterology* 90:577
34. Morita A, Chung YC, Freeman HJ, Erickson RH, Sleisenger MM, Kim YS. 1983. Intestinal assimilation of a proline-containing tetrapeptide. Role of a brush border membrane postproline dipeptidyl aminopeptidase IV. *J. Clin. Invest.* 72:610
35. Wojnarowska F, Gray GM. 1975. Intestinal surface peptide hydrolases: identification and characterization of three enzymes from rat brush border. *Biochim. Biophys. Acta* 403:147
36. Kania RK, Santiago NA, Gray GM. 1977. Intestinal surface amino-oligopeptidases. II. Substrate kinetics and topography of the active site. *J. Biol. Chem.* 252:4929
37. Coffey RJ, Kost LJ, Lyons RM, Moses HL, LaRusso NF. 1987. Hepatic processing of transforming growth factor beta in the rat. Uptake, metabolism, and biliary excretion. *J. Clin. Invest.* 80:750–57
38. Hagenbuch B, Stieger B, Foguet M, Lübbert H, Meier P. 1991. Functional expression, cloning, and characterization of the hepatocyte Na^+/bile acid cotransport system. *Proc. Natl. Acad. Sci. USA* 88:10629–33
39. Premont RT. 1987. Glucagon receptor: structure and function. In *Peptide Hormone Receptors,* ed. MY Kalimi, JR Hubbard, pp. 129–77. New York: Walter de Gruyter
40. Parant M, Parant F, Chedid L, Yapo A, et al. 1979. Fate of the synaptic immunoadjuvant, muramyl dipeptide (14C-labelled) in the mouse. *Int. J. Immuno. Pharmacol.* 1:35
41. Pradayrol L, Jornvall H, Mutt, V. Ribet A. 1980. N-terminally extended somatostatin: the primary structure of somatostatin-28. *FEBS Lett.* 109:55
42. Meijer DKFJ. 1987. Current concepts on hepatic transport of drugs. *Hepatology* 4:259–68
43. Silverstein SC, Steinman RM, Cohn ZA. 1977. Endocytosis. *Annu. Rev. Biochem.* 46:669–722
44. Neurath H. 1989. The diversity of proteolytic enzymes. In *Proteolytic Enzymes: A Practical Approach,* ed. RJ Beynon, JS Bond, pp. 1–13. New York: IRL
45. Boyer JL, Meier PJ. 1990. Preparation of basolateral (sinusoidal) and canalicular plasma membrane vesicles for the study of hepatic transport processes. *Methods Enzymol.* 192:34–45
46. Hadgraft J. 1985. Structure activity relationships of percutaneous absorption of prodrugs. In *Design of Prodrugs,* ed. H. Bundgaard, pp. 271–89. New York: Elsevier
47. Friedman DI, Amidon GL. 1989. Passive and carrier-mediated intestinal absorption components of two angiotensin converting enzyme (ACE) inhibitor prodrugs in rats: enalapril and fosinopril. *Pharm. Res.* 6(12):1043–47
48. Friedman DI, Amidon GL. 1989. Intestinal-absorption mechanism of dipeptide angiotensin converting enzyme-inhibitors of the lysyl-proline type—lisinopril and SQ-29.852. *J. Pharm. Pharmacol.* 78(12):995–98
49. Tsuji A, Tamai I, Nakanishi M, Amidon GL. 1990. Mechanism of absorption of the dipeptide alpha-methyldopa-phe in intestianl brush-border membrane vesicles. *Pharm. Res.* 7(3): 308–9
50. Bai JP, Hu M, Subramanian P, Mos-

berg HI, Amidon GL. 1992. Utilization of peptide carrier system to improve intestinal absorption: targeting prolidase as a prodrug-converting enzyme. *J. Pharm. Sci.* 81(2):113–16

51. Hu M, Subramanian P, Mosberg HI, Amidon GL. 1989. Use of the peptide carrier system to improve the intestinal absorption of L-alpha-methyldopa: carrier kinetics, intestinal permeabilities, and in vitro hydrolysis of dipeptidyl derivatives of L-alpha-methyldopa. *Pharm. Res.* 6(1):66–70
52. Shichiri M, Kamon R, Yoshida M, Etani N, et al. 1975. Short-term treatment of alloxan-diabetic rats with intrajejunal administration of water-in-oil-in-water insulin emulsions. *Diabetes* 24:971
53. Gregoriadis G. 1976. The carrier potential of liposomes in biology and medicine. *N. Engl. J. Med.* 295:704
54. Couvreur P, Kante B, Lenaerts V, Scailteur V, Roland M, Speiser P. 1980. Tissue distribution of antitumor drugs associated with polyalkylcanoacrylate nanoparticles. *J. Pharm. Sci.* 69:199–202
55. Ziv E, Kidron M, Berry E, Baron H. 1981. Bile salts promote the absorption of insulin from the rat colon. *Life Sci.* 29:803
56. Fujii S, Yokoyama T, Ikegaya K, Sato F, Yokoo N. 1985. Promoting effect of the new chynotrypsin inhibitor FK-448 on the intestinal absorption of insulin in rats and dogs. *J. Pharm. Pharmacol.* 37:545
57. Ptachcinski R, Venkataramanan R, Rosenthal J, Burckart G, Taylor R, Hakala T. 1985. The effect of food on cyclosporine absorption. *Transplantation* 40:174
58. Lee VHL, Yamamoto A, Kompella UB. 1991. Mucosal penetration enhancers for facilitation of peptide and protein drug absorption. *CRC Crit. Rev Ther. Drug Carrier Syst.* 8:91–192
59. Muranashi S. 1990. Absorption enhancers. *CRC Crit. Rev. Ther. Drug Carrier Syst.* 7:1–33
60. Ritschel WA. 1991. Microemulsions for improved peptide absorption from the gastrointestinal tract. *Methods Find. Exp. Clin. Pharmacol.* 13:205–20
61. Saffran M, Franco-Saenz R, Kongm A, Papahadjopoulos D, Szoka F. 1979. A model for the study of the oral administration of peptide hormones. *Can. J. Biochem.* 57:548–53
62. Lundin S, Vilhardt H. 1986. Absorption of intragastrically administered DDAVP in conscious dogs. *Life Sci.* 38:703–9
63. Vilhardt H, Lundin S. 1986. Biological effect and plasma concentrations of DDAVP after intranasal and peroral administration to humans. *Gen. Pharmacol.* 17:481–83
64. Bauer W, Briner U, Doepfner W, Haller R, et al. 1982. SMS 201–995: a very potent and selective octapeptide analogue of somatostatin with prolonged action. *Life Sci.* 31:1133–40
65. Fuessl HS, Domin J, Bloom SR. 1987. Oral absorption of the somatostatin analogue SMS 201–995: theoretical and practical implications. *Clin. Sci.* 72: 255–57
66. Amoss M, Rivier J, Guillemin R. 1972. Release of gonadotropins by oral administration of synthetic LRF or a tripeptide fragmnet of LRF. *J. Clin. Endocrinol. Metab.* 35:175–77
67. Humphrey RR, Dermody WC, Brink HO, Bousley FG, et al. 1973. Induction of luteinizing hormone (LH) release and ovulation in rats, hamsters, and rabbits by synthetic luteinizing hormone-releasing factor (LRF). *Endocrinology.* 92:1515–26
68. Nishi N, Arimura A, Coy DH, Vilchez-Martinez JA, Schally AV. 1975. The effect of oral and vaginal administration of synthetic LH-RH and [D-ALA-6, DES GLY-10-NH2]-LH-RH ethylamide on serum LH levels in ovariectomized, stroid blocked rats. *Proc. Soc. Exp. Biol. Med.* 148:1009–12
69. Orniston BJ. 1972. Clinical effects of TSH release after i.v. and oral administration in normal volunteers and patients with thyroid discase. In *Thyrotropin Releasing Hormone,* ed. R Hall, I Werner, H Holgate, pp. 45–52. Basel: Karger
70. Laskowski M, Haessler HA, Miech RP, Peanasky RJ, Laskowski M. 1958. Effect of trypsin inhibitor on passage of insulin across the intestinal barrier. *Science* 127:1115-16
71. Fitzgerald JF, Kottmeier PK, Adamsons RJ, Butt KM, et al. 1968. Effect of hypertonic solutions on intestinal mucaosal integrity. *Surg. Forum.* 19: 297–99
72. Patel HM, Ryman BE. 1976. Oral administration of insulin by encapsulation within liposomes. *FEBS Lett.* 62:60–63
73. Zhou XH, Po ALW. 1991. Peptide and protein drugs. Therapeutic applications, absorption and parenteral administration. *Int. J. Pharmaceutics* 75: 97–115

74. Hutchinson FG, Furr BJ. 1985. Biodegradable polymers for the sustained release of peptides. *Biochem. Soc. Trans.* 13:520
75. Hirai S, Yashiki T, Matsuzawa T, Mima H. 1981. Absorption of drugs from the nasal-mucosa of rat. *Int. J. Pharm.* 7:317
76. Schade DS, Eaton RP. 1985. Insulin delivery: how, when, and where. *N. Engl. J. Med.* 312:1120
77. Sarkar MA. 1992. Drug metabolism in the nasal mucosa. *Pharm Res.* 9(1): 1–9
78. Baron J, Burke JP, Guengerich FP, Jakoby WB, Voigt JM. 1988. Sites for xenobiotic activation and detoxication within the respiratory tract: implications for chemically induced toxicity. *Toxicol. Appl. Pharmacol.* 93: 493–505
79. Ding X, Coon MJ. 1988. Purification and characterization of two unique forms of cytochrome P-450 from rabbit nasal microsomes. *Biochemistry* 27: 8330–37
80. O'Hagan DT, Critchley H, Faraj NF, Fisher AN, et al. 1990. Nasal absorption enhancers for biosynthetic human growth hormone in rats. *Pharm. Res.* 7:772–6
81. Eppstein DA, Longenecker JP. 1988. Alternative delivery sytems for peptides and proteins as drugs. *Crit. Rev. Ther. Drug Carrier Syst.* 5:99–139
82. Chien YW, Liu J, Sun Y, Siddiqui O. 1987. Facilitated transdermal transport of insulin. *J. Pharm. Sci.* 76:341–45
83. Banerjee PS, Ritschel WA. 1989. Transdermal permeation of vasopressin. 1. Influence of pH, concentration, shaving and surfactant on in vitro permeation. *Int. J. Pharm.* 49:189–97
84. Kazim M, Weber C, Strausberg L, LaForet G, et al. 1984. Treatment of diabetes in mice with topical application of insulin to the skin. *Diabetes* 33:181A (Suppl)
85. Banerjee PS, Ritschel WA. 1989. Transdcrmal permcation of vasopressin. 2. Influence of azone on in vitro and in vivo permeation. *Int. J. Pharm.* 49:199–204
86. Potts RO, McNeil SC, Desbonnet CR, Wakshull E. 1989. Transdermal drug transport and metabolism. II. The role of competing kinetic events. *Pharm. Res.* 6:119–24
87. Choi H, Flynn GL, Amidon GL. 1990. Transdermal delivery of bioactive peptides: the effect of n^{-} decylmethyl sulfoxide, pH, and inhibitors on enkephalin metabolism and transport. *Pharm. Res.* 7(11):1099–106
88. Shah PK, Borchardt RT. 1991. A comparison of petidase activities and peptide metabolism in cultured mouse keratinocytes and neonatal mouse epidermis. *Pharm. Res.* 8(1):70–75
89. Heit MC, Williams PL, Jayes FL, Chang SK, Riviere JE. 1993. Transdermal iontophoretic peptide delivery: in vitro and in vivo studies with luteinizing hormone releasing hormone. *J. Pharm. Sci.* 82(3):240–44
90. Okada H, Yamazaki I, Ogawa Y, Hirai S, et al. 1982. Vaginal absorption of a potent luteinizing hormone-releasing hormone analog (leuprolide) in rats I: absorption by various routes and absorption enhancement. *J. Pharm. Sci.* 71:1367
91. Yoshida H, Kumura KO, Hori R, Anmo T, Yamaguchi H. 1979. Absortpion of insulin delivered to rabbit trachea using aerosol dosage form. *J. Pharm. Sci.* 68:670
92. Jennewein HM, Waldock F, Konz W. 1974. The absorption of tetragastrin from different sites in rates and dogs. *Arzneim. Forsch.* 24:1225
93. Yagi T, Hakui N, Yamasaki Y, Kawamori R, et al. 1983. Insulin suppository: enhanced rectal absorption of insulin using an enamine derivative as a new promoter. *J. Pharm. Pharmacol.* 35:177
94. Friedman DI, Amidon GL. 1990. Characterization of the intestinal transport parameters for small peptide drugs. *J. Control. Rel.* 13:141–46
95. Oh D, Sinko PJ, Amidon GL. 1992. Characterization of the oral absorption of several aminopencicillins: determination of intrinsic membrane absorption parameters in the rat intestine in situ. *Int. J. Pharm.* 85:181–87