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REVIEW ARTICLE

Current Concepts in Intestinal Peptide Absorption

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Abstract: Today there is considerable interest in oral peptide delivery. However, oral administration of peptides is limited by a low bioavailability and a high variability in plasma levels. A review is given of the literature describing the major barriers in peptide absorption, the basic mechanisms of intestinal peptide transport, the experimental models and the pharmaceutical approaches currently used in the investigation of peptide and protein absorption processes.

Keywords: peptide absorption; dipeptide carrier; brush border membrane; M-cell, Caco-2 cell

INTRODUCTION

Endogenous peptides play a predominant role in the regulatory processes of almost all body functions as enzymes, hormones, neuropeptides or neurotransmitters and cytokines. General characteristics of endogenous peptides are on the one hand a high specificity and potency of their effects, but on the other hand a rapid degradation, which is necessary for flexible regulation.

In medicine, there is a rapidly growing interest in the therapeutic use of natural peptides and their analogues. For some of the possible indications of peptide drugs a long-term treatment is required and, for these indications in particular, an oral drug delivery would be advantageous. As a consequence, efforts were initiated to achieve oral peptide administration [1], and understanding the basic mechanisms of intestinal absorption of peptides and proteins has become a scientific challenge, starting with the discovery of free amino acids in the gastrointestinal (GI) lumen by van Slyke and Meyer in 1912. But it was due to discovery of mixed peptidase activity in the small intestinal mucosa by O. Cohnheim in 1901 that for quite a long time it was a commonly accepted opinion that peptides undergo rapid and complete proteolytic degradation in the lumen or in the cells of the GI tract and are absorbed only as free amino acids [2]. However, an increasing number of observations of orally active peptides and proteins suggested that the absorption of intact peptides is possible. The area of peptide absorption started to regain more and more attractiveness stimulated by observations that indicated the uptake of intact small peptides [3–5]. Originally, mainly indirect observations based on the determination of peripheral activity of orally administered peptides implicated the availability of the intact molecules [6, 7], but, based on measurement of plasma concentrations, there was more direct evidence suggesting that the gastrointestinal mucosa is able to absorb significant amounts of peptides after oral ingestion [8–13]. These findings were supported by clinical observations with patients being unable to absorb free amino acids but able to absorb peptides [14]. However, bioavailabilities of orally administered peptides are generally low, enforcing up to now non-oral routes of delivery in general. Therefore, a new challenge has risen, especially to pharmacists, to apply galenic means to increase peptide permeation through the intestinal wall. Effective modulation of

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absorption is only possible if the basic mechanisms of permeation of a given drug through an epithelial barrier are fully understood. Therefore, this review will address several aspects:

(1) physicochemical and metabolic constraints for drug absorption;
(2) the morphological barriers and basic physiological mechanisms determining intestinal peptide and protein absorption;
(3) experimental *in vitro* models to study intestinal peptide and protein absorption;
(4) recent pharmaceutical approaches modulating peptide absorption.

PHYSICOCHEMICAL AND METABOLIC CONSTRAINTS

As well as the size (hydrodynamic radius) of the molecule, an important requirement for (*passive* or *diffusional*) enteral absorption of a drug is its solubility in the aqueous phase of the gut lumen at the site of absorption in proximity to the enterocyte membrane. To enter the enterocyte membrane, the drug molecule has to break its hydrogen bonds to the aqueous phase (desolvatization) and to interact with the lipid phase of the membrane, requiring at least a moderate lipophilicity. An optimum of lipophilicity for passive absorption is obtained with octanol/water partition coefficients of 10–1000; a further increase does not increase absorption, since the rate-limiting step is the penetration of the aqueous mucus and *unstirred water layer* at the surface of enterocytes and other epithelial and endothelial cells [15–17].

Before reaching the cell surface, a given drug has to traverse the intestinal mucous layer. The intestinal mucus covers the luminal side of the small intestine and is secreted by the goblet cells in the GI-tract. It has to be considered as some kind of molecular filter of 100–150 μm thickness with an exclusion size of 600–800 Da, above which absorption is relatively low. Mucus represents an extracellular glycoprotein layer including immobilized water, with a turnover rate of 12–24 h. Recent experiments with various peptides showed a direct relation between mucous diffusion *in vitro*, the lipophilicity of the test compound and the amount of drug absorbed *in vivo* [18–21]. One aspect making the direct correlation between *in vitro* models and *in vivo* absorption difficult is the continuous turnover and the variable thickness of the intestinal mucous layer. A recent review [22] gives an excellent summary of the effects of gastrointestinal mucus on the absorption of various drugs.

Hydrophilicity or polarity is, for weak acids or bases, affected by the electrical charge of the molecule that is influenced markedly by its pK_a and the local pH in the gut lumen (*pH partition hypothesis*). It is important to realize that, more than the luminal pH, the local pH in the micromilieu at the absorption site is the determining factor influencing permeation [23, 24]. Small cations can also penetrate the epithelial barrier through the aqueous pores in the tight junctions between two enterocytes (*paracellular absorption*).

Passive absorption of peptides increases in general not strictly with lipophilicity but rather is inversely correlated with the number of possible hydrogen bonds between the drug and the aqueous phase of the gut lumen [16, 25]. Reversible or irreversible derivatization of the peptide bonds by *N*-acylation can increase drug absorption as shown for thyrotropin-releasing hormone [26, 27].

METABOLIC BARRIERS

The first barrier following oral uptake of peptides and proteins is a mixture with the acidic secretions of the stomach. In addition to the very poor permeability of the gastric mucosa, the complex gastric juice containing proteolytic pepsins and hydrochloric acid results in an acidic protein hydrolysis at pH values between 2 and 5, preferably of aspartate-containing proteins. Whereas most larger proteins are highly susceptible to gastric proteolysis, smaller peptides are surprisingly stable in this environment. Therefore, the predominant function of the stomach has to be seen in large protein abridgement.

The second barrier to be overcome consists of luminal enzymes in the upper small intestine: Pancreatic proteases are secreted into the duodenum, such as trypsin and chymotrypsin, elastase and carboxypeptidase A and B. These enzymes exhibit their highest activity at pH values around 8 and cooperate by distinct substrate specificities making almost each class of peptide susceptible to proteolysis, thereby degrading 30–40% of proteins in the duodenal content to small peptides with two to six amino acid residues within 10 min [28, 29]. Recent investigations demonstrated an up to 10-fold increased bioavailability of oxytocin analogue octa- and nonapeptides in the absence of pancreatic juice in pigs [30]. Whereas most of the larger proteins are completely degraded to small fragments, a variety of

smaller peptides exists with an extraordinary stability against these luminal proteases. Well-known examples are the cyclic hepta-, octa- and decapeptides of the poisonous *Amanita* mushrooms leading to intoxication by absorption of intact peptides [31, 32], synthetic hexa- and octapeptide analogues of somatostatin [33, 34], of vasopressin [12] or cyclosporin A [35, 36]. There are also small linear peptides being stable against luminal proteases, such as di- and triglycines [37, 38].

The other luminal secretion, the biliary fluid, seems to have less influence on peptide and protein stability, although it may also strongly affect absorption. There are examples in the literature, both for inhibitory [39] as well as for absorption promoting activity of bile [40], but these effects seem to depend rather on the formation of mixed micellar complexes than on proteolytic activity of biliary constituents.

Probably the major absorption-limiting enzymatic barrier in the GI tract is formed by peptidases located in the brush border membrane and the cytosol of the enterocytes. Although it must be assumed that these enzymes are able to split peptides consisting of more than four or five amino acids, they predominantly degrade tetra, tri- and dipeptides [11, 41, 42]. There seems also to be a gradual increase of brush border peptidase activity from the upper duodenum to the lower ileum [43–45], paralleling the increasing amount of small peptide fragments resulting from luminal enzymatic degradation of large peptides and proteins. The majority of peptidases being selective for tripeptides are located within the brush border membrane, whereas dipeptides are better absorbed through the membrane and are then degraded predominantly by cytosolic proteases [46–48]. There is evidence that small peptides containing proline, hydroxyproline or D-amino acid residues are preferentially absorbed into the portal vein [49, 50]. In addition, certain amino acids seem to have a regulatory effect upon brush border peptidase activity [51, 52]. Only a little information is available about the extent to which proteolytic enzymes are present in the basolateral membrane of enterocytes and how they affect peptide absorption [53].

TRANSPORT MECHANISMS INVOLVED IN PEPTIDE AND PROTEIN ABSORPTION

Several mechanisms have to be considered for intestinal peptide uptake: besides passive diffusion through the enterocytes and via paracellular spaces, cytotic mechanisms as well as carrier-mediated uptake have been observed (Figure 1). In the late 1960s and early 1970s the first successful experiments were performed indicating the presence of distinct active transport systems for small peptides, that were different from the recently identified amino acid transport systems [54, 55]. These fascinating findings stimulated the interest in the absorption of small peptides and today it is generally accepted that peptides of two or three amino acids in length can be taken up through the brush border membrane of enterocytes by carrier-mediated, pH-dependent processes. These carriers are characterized by saturability and the capability to transport small peptides against a concentration gradient into the cell interior, thereby requiring energy supply. There is some disagreement on whether these carrier(s) are also dependent on Na^+, as it has been described for amino acids and sugars. *In vivo* and *in situ* experiments suggested an enhancing contribution of extracellular Na^+ to peptide uptake [56–58]. Membrane vesicles studies, however, point to a Na^+-independent transport [59, 60]. The inwardly directed H^+ gradient ($[H^+]_{out} > [H^+]_{in}$), required for dipeptide transport, is maintained by the Na^+/H^+ exchanger in the brush border membrane. As an explanation for the observed discrepancy between *in vivo* and *in vitro* experiments, the coupling of the Na^+/H^+-antiporter to the basolateral Na^+/K^+-ATPase in the intact tissue has been proposed [61, 62]. The role of Ca^{2+} in peptide absorption is not completely understood. There is evidence that calcium channel blockers enhance the rate of transport, which might be explained by a regulatory function of Ca^{2+} at the Na^+/H^+-antiporter [63, 64]. Numerous studies have now confirmed the presence of the pH-dependent peptide transporter and characterized its substrate requirements [65–67]. A large number of experiments demonstrated the feasibility of oral administration of peptides or peptide mimetics, and some of them, such as dopamin-prodrugs, some β-lactam antibiotics, renin inhibitors, TRH analogues and inhibitors of angiotensin-converting enzyme, have been demonstrated to use the dipeptide carrier system [68–76].

Investigation of the structural substrate requirements indicated that modification of the N-terminal α-amino group reduces the affinity to the peptide transporter and that γ-amino acid residues within a peptide are incompatible with the carrier [67, 77]. However, there are also examples of dipeptide analogues without an N-terminal α-amino group that are recognized by the carrier [78]. Only few studies show that modification of the C-terminal end reduces

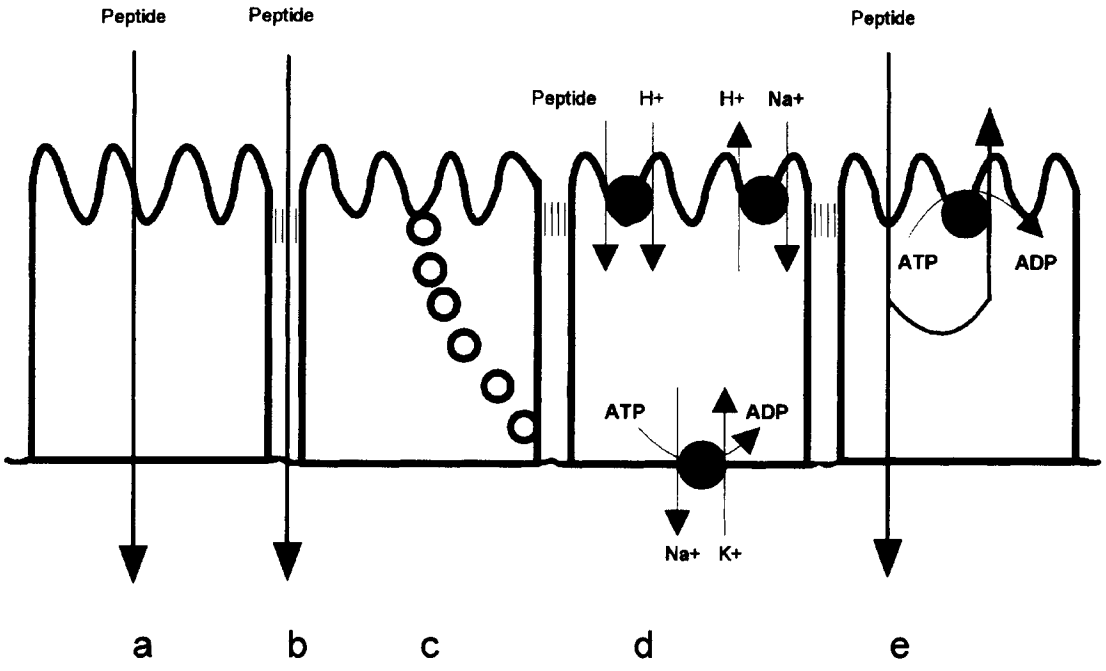

Figure 1 Basic mechanisms of intestinal peptide absorption: (a) passive diffusion through enterocytes; (b) passive diffusion via paracellular pathway; (c) cytotic mechanisms; (d) cellular uptake via the dipeptide carrier; (e) secretion back into the intestinal lumen by *p*-glycoprotein.

the affinity to the transport site [77, 79]. Dipeptides containing D-amino acids are also substrates of the carrier [80, 81]. Amino-β-lactam antibiotics that share the transport system can be regarded as peptide mimetics of D-alanyl-D-alanine. Photoaffinity labelling studies using benzylpenicillin as photolabile ligand and subsequent reconstitution experiments with brush border membrane subfractions into proteoliposomes suggest that a polypeptide with an apparent molecular weight of 127,000 Da is the carrier or part of the carrier, which is directly involved in the dipeptide transport activity in rabbit small intestine [82, 83]. Recent work demonstrated the expression cloning of that proton-coupled oligopeptide transporter [84]. By which way di- and tripeptides are further processed by enterocytes has not yet been clarified. Recent studies gave indication for an outwardly directed proton cotransport system for glycylproline in the basolateral membrane from rabbit small intestinal cells [85]. However, it remains to be clarified whether that system also plays a role in the transport of other peptides from enterocytes into the blood.

The mode of absorption of peptides with more than three amino acids is still regarded controversially and no unequivocal evidence for the participation of active carrier mechanisms has been demonstrated for larger peptides. Whereas several studies demonstrated passive diffusion for tetrapeptides [65, 86, 87], others indicate that an active component might be involved [88, 89]. The synthetic pentapeptide metkephamid has been demonstrated to penetrate rat brush border membranes by passive diffusion [90]. But there is some evidence that the orally active cyclic octapeptide analogue of somatostatin, octreotide, is at least partially absorbed via a mediating membrane transport system [39, 91]. Recent *in situ* studies in rats using fluorescence-labelled octreotide demonstrated uptake and transcellular transport of the intact peptide through enterocytes ([91], Figure 2), which was reduced in the presence of the unlabelled peptide. Other *in vitro* investigations showed that rabbit jejunum is able to absorb an intact renin-inhibiting nonapeptide [92]. Other studies reported the absorption of the intact nonapeptide vasopressin and analogues; however, these data do not really elucidate the mechanisms of uptake, i.e. by paracellular or transcellular pathways [93, 94]. *In vivo* activity of orally administered luliberin, a decapeptide luteinizing hormone releasing factor [95, 96] also indicates the intestinal absorption of intact peptide.

The most impressive example for an orally active peptide is the immunosuppressive endecapeptide cyclosporin A. Absolute bioavailabilities between < 5% and 89% have been observed in patients [97, 98]. In rats, bioavailabilities between 10% and 30% were determined after administration of the drug in olive oil [99, 100]. Uptake of cyclosporin A into enterocytes is by passive diffusion, but there is evidence that a carrier-mediated secretion into the intestinal lumen occurs in parallel [101], presumably by *p*-glycoprotein, similar to that observed in intact kidney tubular cells ([102, 103], Figure 3). Cell culture studies showed higher permeation rates for cyclosporin A across the cell monolayers from the basolateral to the apical cell side than vice versa and an increase of apical to basolateral permeation in the presence of *p*-glycoprotein substrates [101]. Initial findings of a dose-dependent absorption have been explained by the used oily formulation and effects of altered gastric emptying [100]. Several other studies found the absorbed fraction was independent of the administered dose [104, 105]. The absorption behaviour of cyclosporin A is certainly influenced by its most unusual physicochemical characteristics. Nine of its 11 peptide bonds are *N*-acylated, reducing the number of possible hydrogen bonds. In addition, the molecule exerts a partial β-sheet conformation that results in a peculiar high lipophilicity, therefore requiring special formulations with dispersing and emulsifying properties [105, 106].

Larger peptides and proteins may also permeate the gastrointestinal lumen once they are sufficiently stabilized against proteolytic degradation. The most likely pathway via transcytotic vesicles has been shown by ultrastructural imaging for horseradish peroxidase having a molecular weight of about 40 kDa [107]. However, the rate of membrane permeation decreases markedly with increasing molecular weight and the total measure of peroxidase crossing the intestinal wall is extremely low as demonstrated in Ussing chamber experiments [108]. It amounts to about 3 pmol/h/cm^2. Some large proteins such as ferritin, consisting of 24 subunits with a total molecular weight of about 445 kDa, are taken up by receptor-mediated endocytosis, but they target cellular lysosomes rather than permeate the basolateral membrane [109]. Interestingly, in the neonatal mammalian intestine, the low levels of proteolytic enzymes and an increased cytotic capacity allow more intact macromolecules like milk protein antigens, bovine serum albumin or β-lactoglobulin to bind to the epithelium and to be absorbed [110–112]. A cell type which

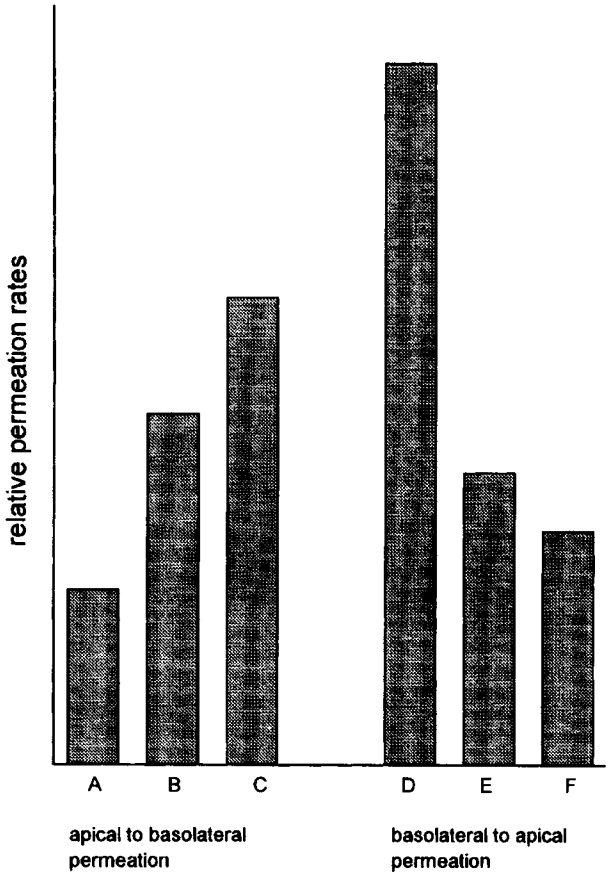

Figure 3 Influence of *p*-glycoprotein upon the permeation of cyclosporin A across Caco-2 cell monolayers. Relative permeation rates in the absence and in the presence of other *p*-glycoprotein substrates: A, control; B, in the presence of 50 μM vinblastine; C, in the presence of 50 μM daunomycin; D, control; E, in the presence of 50 μM vinblastine; F, in the presence of 50 μM daunomycin.

seems to be peculiarly equipped for such endocytotic processes is the M-cell overlying the lymphoid follicles of Peyer's patches. These cells possess only a limited number of microvilli, a rather meagre glycocalix and only few lysosomes. They are enabled for transcellular transport of macromolecules, like lectins or IgA [109, 110, 113]. However, the extent of proteins reaching the systemic circulation is under debate [114] and can generally be regarded to be very low.

Another distinct endocytotic mechanism is involved in the absorption of vitamin B_{12}. Vitamin B_{12} is bound to intrinsic factor, a 44 kDa glycoprotein secreted from gastric parietal cells. This complex binds to specific receptors at the luminal membranes of ileocytes and is internalized [115]. However, the

overall capacity of this specific system seems also to be extremely low, making it of lower interest for a broad therapeutic application.

Besides the involvement of membrane carrier proteins and receptors in peptide and protein absorption, other mechanisms have also to be taken into consideration. The intercellular tight junctional complexes are the subject of intensive research as modulators of paracellular transport. They certainly also play a role in the absorption of peptides. According to Pappenheimer [116], the paracellular pathway is available to neutral or positively charged peptides of sizes up to 2000 Da, including releasing hormones [95, 117] or 1-deamino-8-D-arginine vasopressin [118]. It is a well-established fact that the opening of epithelial tight junctions is dependent on the presence of Ca^{2+} [119, 120]. Presumably, Ca^{2+}-dependent cell adhesion molecules in the tight junctional region, which interact with actin filaments of the cytoskeleton, regulate the permeability of that pathway [121, 122]. Regulation of the junctional tightness seems also to be linked to the luminal D-glucose concentration and a stimulatory effect of D-glucose, but also of D-xylose, has been observed for the paracellular permeation of a synthetic nonapeptide and octreotide ([123, 124], Figure 4) in animals. No effect was seen when the permeation of octreotide was determined in the presence of L-glucose or other carbohydrates, such as D-fructose. Although in a series of investigations similar observations were made for other, non-peptide drugs [125–127], it is still under debate whether these observations can be transposed to the situation in humans *in vivo*. A recent review excellently describes in detail the various theoretical concepts of paracellular solute absorption [128]. It compares animal and human studies and comes to the conclusion that that physiological approach to increasing the paracellular diffusion of hydrophilic compounds in quantitative amounts might not be feasible for optimizing oral drug delivery of low permeability compounds in humans.

A large number of studies have been performed to understand how the structure influences the absorption of such peptides that permeate the GI wall via the paracellular route or by passive diffusion through the cell membranes. It was shown that the permeability of water-soluble peptides may be determined by their potential to form hydrogen bonds with their aqueous environment rather than by their lipophilicity [129, 130]. For a short review explaining the relationship between overall intestinal permeability and pharmacokinetic absorption rate constants see [131].

Additional factors that are relevant for peptide absorption are gastric emptying being dependent on fasted or fed states and intestinal transit time. Both parameters are species-dependent and comparison of various animal modes have shown that the dog represents a relatively good model, coming close to the situation in humans [132]. It has been shown that gastric release of particles (size >0.5 mm) with

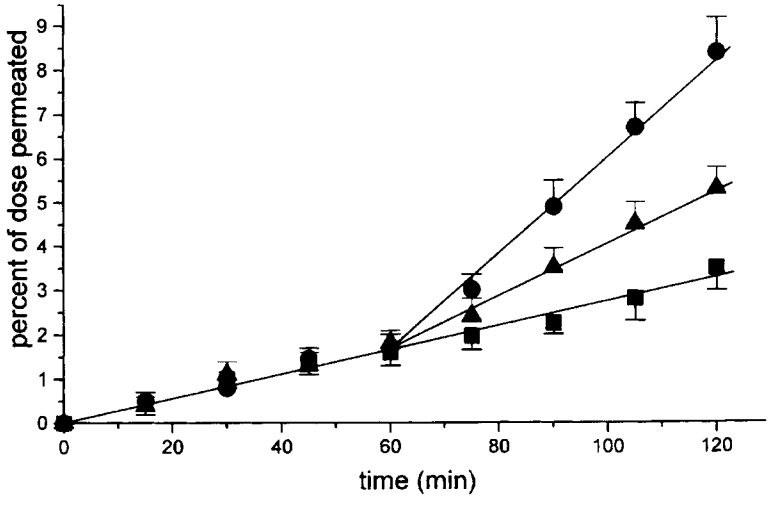

Figure 4 Carbohydrate-induced paracellular permeation of octreotide across Caco-2 cell monolayers. 0–60 min: permeation of octreotide in the absence of carbohydrates in the apical incubation medium; 60–120 min: permeation in the absence of carbohydrate (■) or in the presence of 20 mM D-glucose (●) or 20 mM D-xylose (▲) in the apical incubation medium. Data from [128].

the liquid phase depends on a randomly occurring migrating motor complex in the fasted state [133]. As a consequence, the time of intestinal dissolution will become highly variable, especially for highly lipophilic compounds. In the fed state, the gastric residence time is normally prolonged, thereby ensuring a better dissolution of the drug. If an administered peptide is not subject to extensive gastric degradation, a better absorption should occur compared with the fasted state. For a review on the influence of gastric emptying and intestinal transit time see also [131].

EXPERIMENTAL *IN VITRO* MODELS TO STUDY INTESTINAL PEPTIDE AND PROTEIN ABSORPTION

Intestinal absorption may be dependent on a variety of distinct factors such as dissolution in the GI lumen, interaction with intestinal secretions, GI motility, degradation by luminal or cell associated enzymes and transport processes through the intestinal tissue. A discrimination of these processes in *in vivo* studies is not possible. Although observations in humans and animals gave manifold evidence for peptide absorption, most of the underlying basic mechanisms were detected and proven in *in vitro* systems. Initially, much information was gained from balance studies in isolated tissue preparations like the everted intestinal sac model introduced by Wilson and Wiseman in 1954 [134]. Intestinal segments are inverted so that the apical side of the enterocytes is oriented to the exterior medium, collecting the absorbed material into the closed sac. One major experimental disadvantage of that system is the difficulty of maintaining cellular viability of the isolated tissue over a longer experimental time interval. In addition, blood flow is not maintained, leading to an unphysiological accumulation of absorbed compound within the tissue. Developing the technique of isolated tissue further, Ussing chamber systems became popular [135], especially in studying electrical changes associated with transepithelial transport processes.

Isolated enterocytes can be obtained from intestinal villi by mechanic disruption and enzymatic treatment of the tissue [136]. However, their use as model systems is limited due to membrane damage, loss of cellular polarity and cell viability. Therefore, efforts focused on the preparation of isolated brush border membranes and basolateral membranes, which can be obtained from cell homogenates by the divalent cation precipitation and differential centrifugation method [137, 138]. During their isolation the membranes form vesicles which allow the separate manipulation of intra- and extravesicular media and the investigation of transmembrane movement without interference of intracellular processes. Ion-dependencies, pH effects and a possible influence of membrane potentials on transport rates can easily be investigated. By use of that system a real breakthrough in the understanding of membrane transport could be achieved by identifying the driving forces for cellular uptake from the intestinal lumen of many compounds, including the transport of carbohydrates, amino acids and small peptides [39, 67–69, 74, 90].

Efforts to cultivate 'normal' absorptive small intestinal cells have had only limited success [139, 140]. However, a novel approach was introduced by the use colon carcinoma-derived cell lines, which are known to undergo enterocytic differentiation in culture once they are grown on permeable filter supports. The highest attention amongst cell lines such as HT29, HT81, T84 and SW116 has been paid to the Caco-2 cell culture model, because of its intriguing similarity to small intestinal epithelial cells [141–143]. These cells exhibit a polar organization with distinct apical and basolateral plasma membrane areas, they possess most of the active transport systems observed in normal tissue and their metabolic capacity is comparable to that of small intestinal enterocytes. Most proteolytic enzymes found in normal enterocytes have been detected in Caco-2 cells, like dipeptidylpeptidase IV, leucine aminopeptidase and others. Finally, they represent a human tissue. Consequently, Caco-2 cells grown on permeable filters have been used to study all transport mechanisms described in normal enterocytes including peptide permeation. In particular, the contribution of the di/tri-peptide carrier to the absorption of small peptide drugs and peptidomimetics has been demonstrated in a series of excellent studies, as well as the contribution of paracellular permeation to overall transport rates, e.g. with peptidomimetic thrombin and renin inhibitors, TRH analogues, cephalosporins, L-α-methyldopa derivatives or HIV protease inhibitors [73, 144–149]. Comparability of the Caco-2 cell model, other *in vitro* models (membrane vesicles) and *in vivo* observations has been found [130, 150, 151]; however, extrapolating correlations from one class of compounds to another has to be done very carefully and cross-validation of experimental systems is recommended [152, 153]. One major difference between the Caco-2 cell system and the *in vivo* situation is the lack of a mucous layer covering the luminal side of the *in vitro*

system. Therefore, attempts have been initiated to study drug permeation in cell culture systems using cells with a mucous production [154], such as subclones of HT29 cells.

In addition, there is evidence from several laboratories that the Caco-2 cell line exists in a variety of subpopulations with diverging attributes [155, 156]. Both morphological features and transport characteristics were different in cells of different origin but otherwise identical culture conditions. The observed variation in permeability characteristics of different Caco-2 cell clones may be the result of selection processes depending on the respective culture conditions. The data allow the conclusion that the Caco-2 cell line consists of subpopulations with a varying degree of differentiation. They suggest that direct comparison between cells of different origin should be made.

The cell model is also used to elucidate the basic requirements for passive and paracellular permeation of peptides as well as in the investigation of enhancer principles to improve peptide absorption, such as carbohydrates, surfactants or bile salts [124, 145, 157, 158].

RECENT PHARMACEUTICAL APPROACHES MODULATING INTESTINAL PEPTIDE ABSORPTION

The first line of attempts to improve peptide absorption is to increase drug solubility, especially for very lipophilic compounds, such as cyclosporin A, by use of new drug formulations on the basis of bile [40, 159], olive oil [160], water-in-oil [161] or oil-in-water microemulsions [98]. These approaches resulted in an increased absorption and for the latter in a decreased variability of absorption. Other approaches appropriate for passively absorbed peptides are the use of absorption enhancers: to improve intestinal and nasal transmucosal transport of peptides chelators like EDTA, bile salt derivatives (glychocholate, chenodeoxycholate, deoxycholate, taurocholate and sodium-taurodihydrofusidate) [158, 162–166], ionic and non-ionic tensides (polyoxyethylene ether, cetomacrogol, Myrj 45, crown ethers, laureth-9) [98, 167–171] as well as various fatty acids (oleic acid, capric acid) or salicylates [172, 173] have been used. These enhancers improve absorption by different partly unknown mechanisms, such as increased paracellular transport by an opening of intercellular tight junctions, inhibition of metabolism, decrease in viscosity of mucus or increased solubility. However, they act non-specifically and increase absorption for various compounds, also including potentially toxic constituents of the gut lumen. In addition, some of these enhancers are known to irritate the mucosa [166, 174–178], and recovery of mucosa integrity certainly depends on the type and dose of enhancer used. In addition, only a little information is available on the consequences of chronic application of unspecific absorption enhancers on epithelial tissues [179, 180].

Another approach to increasing the absorbed fraction of peptides is to prevent intraluminal degradation [181–185]: after *in situ* administration of insulin in isolated duodenal loops or *in vitro* in everted sac experiments, an increased absorption could be observed after co-administration of trypsin inhibitors. However, these approaches do not offer attractive solutions for a practical peptide delivery, but rather underline the importance of intraluminal metabolism. For a short review about the use of protease inhibitors see [186].

One more way to circumvent metabolic degradation is to target the peptide to the distal part of the gut (colon) that exhibits less proteolytic enzyme activity. The feasibility of this approach was shown for insulin and desmopressin, which were embedded into biodegradable polymers. Such polymers may consist of azo-crosslinked copolymers of styrene and hydroxyethylmethacrylate that are degraded due to colonic microbial azoreductase activity at the azo bonds in the polymer backbone, or of pH-sensitive polyacrylic polymers [187, 188].

An additional possibility of polymer application in oral peptide delivery is the use of bioadhesive polymers in order to prolong the transit time of a given peptide in the absorption region of the GI tract. Plant lectins, such as from tomato or beans, have also been shown to be approximate for that purpose; however, in each case potential pharmacological effects and effects on cell viability have to be clarified [189–192]. This approach may be of value especially for peptides with a narrow absorption window; however, it has to be taken into consideration that a prolonged residence time in the GI tract also means an increased exposure to proteolytic enzymes.

The use of particulate delivery systems for peptide delivery has also been investigated, but there are only a few examples of an effective peptide or protein permeation resulting in therapeutically significant blood or plasma levels. Nanoparticles may have the advantage of preventing peptides from proteolytic degradation in the GI lumen and preserving their pharmacological activity. Insulin-loaded nanocap-

sules of < 300 nm size, that were composed by interfacial polymerization of isobutyl 2-cyanoacrylate around a lipidic phase were orally administered [193, 194]. In fasted diabetic rats they reduced glycaemia; in normal rats and dogs glucose-induced peaks of hyperglycaemia decreased after administration of insulin-loaded nanocapsules. Scanning electron microscopy observations indicated that the nanocapsules were preferentially absorbed via Peyer's patches in the ileum, giving evidence that particulate peptide delivery is feasible. But proof of an economic application of that concept in man has still to be shown.

The oral administration of liposomal formulations, e.g. of insulin or calcitonin, is still the subject of discussion, although pharmacological effects have been demonstrated [195–199]. One major drawback of that approach is the low stability of liposomes in the GI tract, resulting in a rather undefined mixture of peptide and phospholipid reaching the cell surface.

The most promising approach, however, is to improve peptide absorption by synthesis of new analogues that are smaller in size, and more lipophilic and metabolically stable. An increase in lipophilicity was obtained by esterification of enalaprilate (octanol/water partition coefficient of 0.01) to enalapril (octanol/water partition coefficient 0.22), resulting in an improvement in bioavailability up to about 60% [200]. However, it has to be emphasized that an increase in lipophilicity does not generally improve the intestinal absorption rate. Metabolic stabilization could be achieved by introduction of D-amino acids such as for desmopressin [201], octreotide [33] or cyclosporin A [202], modification of peptide bonds by introduction of pseudo peptide (ψ) bonds as for bombesin antagonists [203] or derivatization of the carboxyterminal end [33].

CONCLUSION AND FUTURE TRENDS

The increasing knowledge about the cellular mechanisms of solute transport has improved our understanding of the absorption of peptide drugs and is used to rationally design peptide or peptidomimetic drugs that can be transported by the cellular carrier system(s). Many approaches to achieve intestinal peptide absorption are currently under investigation, but a variety of questions still remain to be solved. The presently available data demonstrate that the specificity of the active intestinal peptide carrier(s) is limited to small-sized peptides. Therefore, there is a clear trend for the rational design of peptides up to four amino acids in length or peptidomimetics having an increased affinity to the carrier binding site without loss or even an increase in pharmacological potency. Attempts in that direction are novel TRH analogues or thrombin inhibitors [73, 144] or HIV proteinase inhibitors [204–206], although for the latter class of compound large species differences and variability in bioavailability have been found. It has not yet been clarified whether these peptidomimetics are substrates of the intestinal peptide carrier system.

The absorption mechanisms for larger peptides are still under debate, and two major challenges are how to use the identified pathways for drug administration that results in reproducible plasma levels, and how to transpose the knowledge about the basic mechanisms of absorption into drug formulations that provide efficient delivery, in ways that can easily be handled from a technical point of view and are cost effective. Understanding the relative contribution of passive transport through the GI wall and the modes of efficiently enhancing absorption via those pathways without tissue damage will certainly influence the development of new delivery systems, such as of novel microspheric particles, that are biodegradable and induce a pulsatile release of peptides across the epithelial barrier by a reversible separation of the tight junctions [207]. There is research going on to develop novel absorption enhancers based on naturally occurring compounds, which are well tolerated and are further processed by the body without damaging the cellular environment, e.g. glycerides or acylcarnitins [208, 209]. Although today efficient oral peptide delivery is only feasible for highly potent peptide hormones, the present concepts give a reason for the development of new strategies offering efficient oral administration of peptide drugs.

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(a) (b)

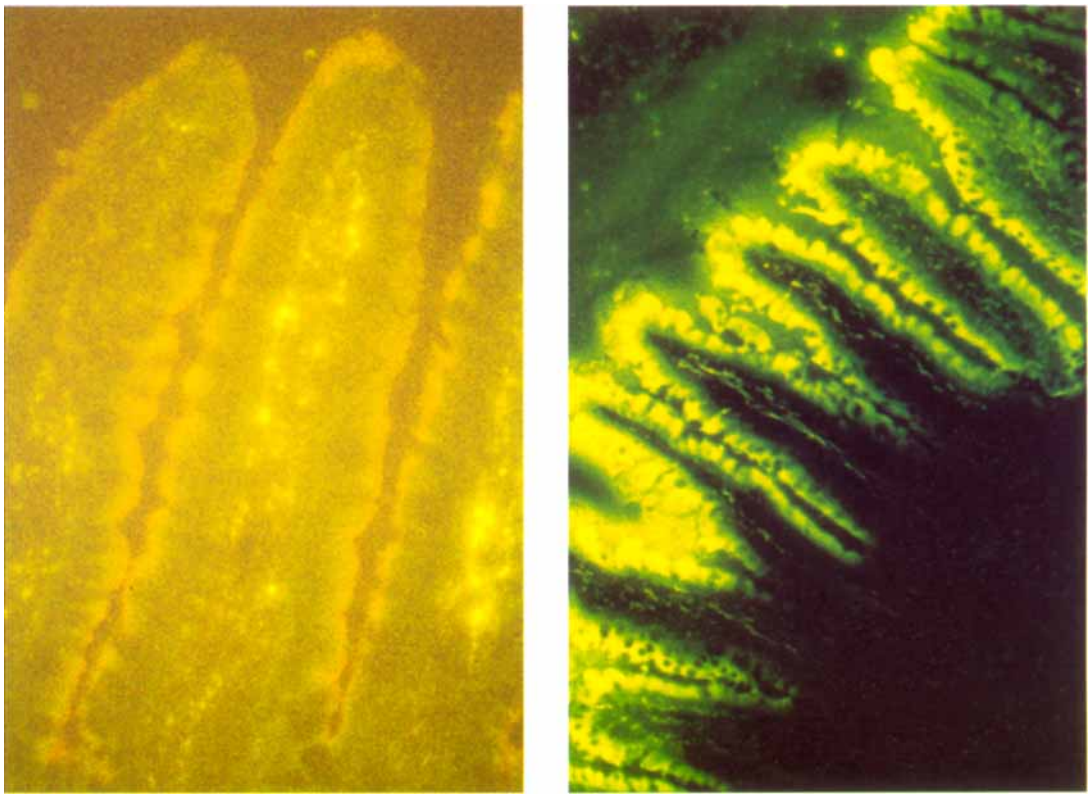

Figure 2. (a) Self fluorescence of a 5 µm cryosection of rat jejunum. Original magnification (×253). (b) Fluorescence distribution in a 5 µm cryosection of rat jejunum after 10 min incubation with 100 µM fluorescence-labelled octreotide. The slice was embedded to prevent artificial refractive luminescence. No fluorescence is detected in goblet cells. Original magnification (×105), see also [91].

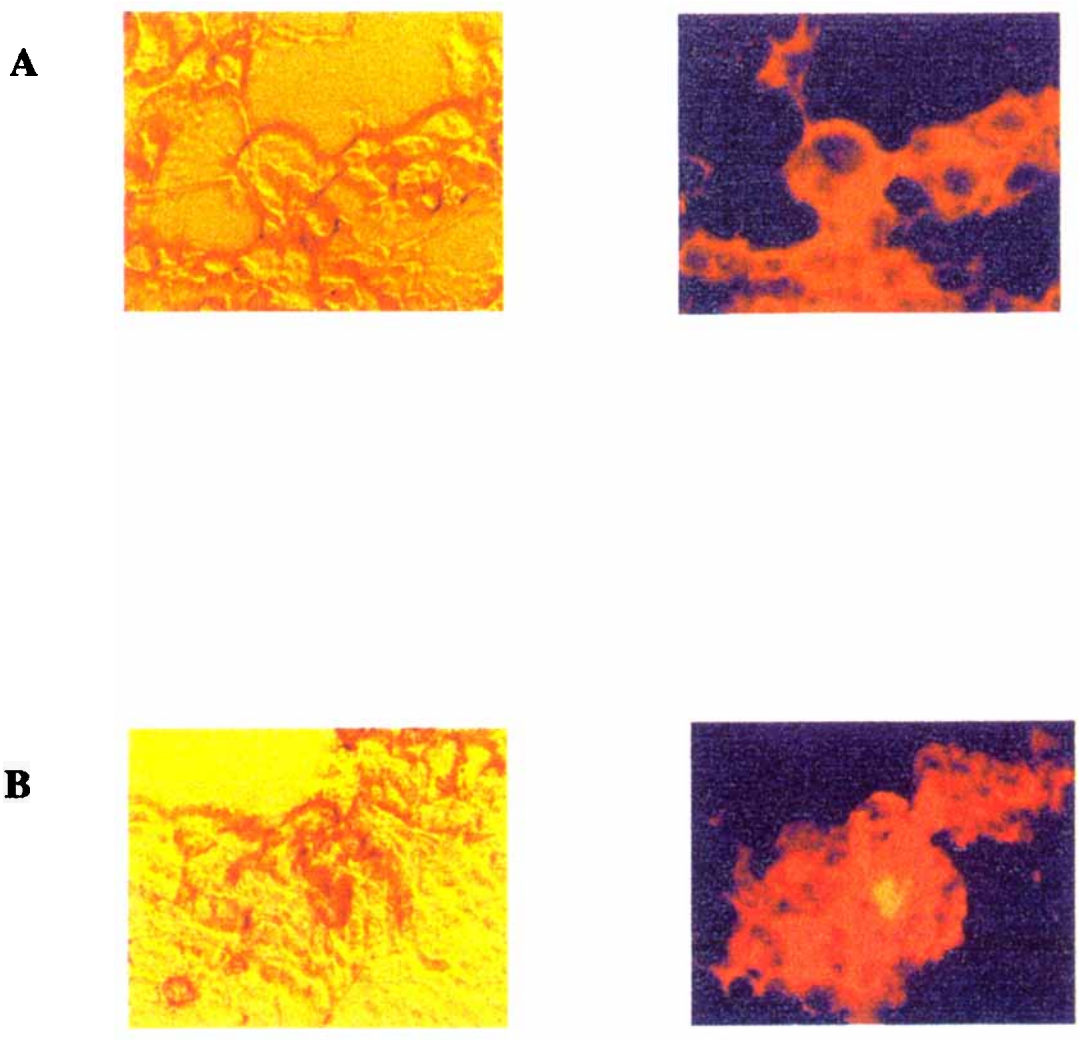

Figure 4. Interaction with NG108-15 cell of (A) Enk6D immobilized on DMPC liposomes after 1.5 h incubation and (B) Enk3D coimmobilized with D6NT on DMPC liposomes after 1 h of incubation. Optical micrograph (left) and fluorescence micrograph (right).