Biomembrane Permeability of Peptides: Strategies to Improve Their Mucosal Uptake

Andreas Bernkop-Schnürch[1]* and Andreas E. Clausen[1]

[1]*Institute of Pharmaceutical Technology and Biopharmaceutics, Center of Pharmacy, University of Vienna, Althanstr. 14, A-1090 Vienna, Austria*

Abstract: In order to gain a therapeutic response after mucosal administration peptide drugs have to permeate the absorption membrane based on the mucus layer (**I**) and the epithelial tissue (**II**) in significant quantities. The peptide drug transport across the membrane can be improved by the use of mucolytic agents and the permeation enhancers. The generation of novel, more potent permeation enhancers, based on an improved knowledge of the absorption membrane in combination with the appropriate delivery systems will strongly improve the bioavailability of mucosally applied peptide drugs.

Key words: mucosal membrane; peptide drug delivery; absorption barrier; permeation enhancer; mucolytic agents;

1. INTRODUCTION

Within the last decade numerous therapeutic peptides and proteins as well as the vaccines based on (poly)peptides have appeared in the pharmaceutical arena. The majority of such drugs and vaccines are administered invasively which is cost intensive, often complex, difficult and occasionally dangerous. According to this, there is both a great scientific interest and a medical need for the development of non-invasive peptide and protein delivery systems. Among them, mucosal delivery systems focusing on the nasal [1], buccal [2], pulmonal [3], and even peroral route [4] of the application have already been proved successful. Products such as nasal delivery systems for oxitocin or calcitonin and pulmonal delivery systems for insulin have already reached the market; others are in phase I and phase II clinical trials.

Although there are numerous further peptide and protein drugs for which a non-invasive administration would be highly beneficial, the development of appropriate delivery systems is strongly limited by the poor bioavailability encountered with the transmucosal way of absorption. Although the bioavailability strongly depends on the structure and size of the peptide drug, in most cases not more than 0.1 – 5% of the peptide drug reaches the systemic circulation in the biologically active form. In order to gain sufficient blood concentrations after mucosal application various barriers have to be overcomed. These barriers include the enzymatic barrier (**I**) based on secreted and membrane bound peptidases and the absorption barrier (**II**). Strategies to overcome the enzymatic barrier include the use of enzyme inhibitors [5], formulations such as nanoparticles and liposomes protecting the incorporated peptide drug from an enzymatic attack [6] and delivery systems targeting on the colon, where the enzymatic activity is comparatively low [7]. Once the active drug has reached the absorption barrier its uptake may be hindered by the mucus layer barrier (**IIa**) caused by the three-dimensional network of mucus glycoproteins covering mucosal membranes and the tissue barrier (**IIb**). A detailed analysis of the absorption barrier representing an important part of the whole 'enemy's strength' will therefore be given within this review. It should provide the basis for the improvement of already existing strategies and for the development of new systems in order to lower the barrier of the membrane for therapeutic peptides.

Moreover, it is the aim of this review to give a critical overview of the well-established strategies and devices focusing on the improvement of membrane permeability. They include the co-administration of mucolytic compounds, low molecular mass permeation enhancers and multifunctional polymers displaying both mucoadhesive and permeation enhancing properties. In addition, as the design and features of the delivery system itself have a great impact on the permeation of peptide drugs through the membrane, these aspects will be discussed within this review as well.

2. CHARACTERISATION OF THE ABSORPTION BARRIER

2.1. Mucus Layer Barrier

The mucus layer barrier being often underestimated in mucosal peptide administration is based on the mucus gel layer covering mucosal epithelia. The water content of the mucus layer has been determined to be around 83% [8]. The most important component of the mucus layer are glycoproteins with a relative molecular mass range of 1-40 x 10^6 Da [9]. These so-called mucins possess a linear protein core of high threonine and serine content being glycosilated by oligosaccharide side chains. The protein core of many

*Address correspondence to this author at the Center of Pharmacy, Institute of Pharmaceutical Technology and Biopharmaceutics, University of Vienna, Althanstr. 14, A-1090 Vienna, Austria Tel.: ++43-1-4277-55413, Fax.: ++43-1-4277-9554,
e-mail: andreas.bernkop-schnuerch@univie.ac.at

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mucins displays N- and/or C-terminally located cysteine-rich subdomains, which are connected with each other via intra- and/or intermolecular disulfide bonds. Generally, mucins may be classified into membrane-bound (**I**) and secretory (**II**) forms. Membrane-bound mucins being attached to the epithelial cell layer possess a hydrophilic membrane-spanning domain, while secreted mucins are continuously released from cells as well as glands undergoing immediately thereafter a polymerisation process. The polymerisation is over all based on an oxidative intermolecular disulfide bond formation. This so formed three-dimensional network gives the mucus layer its high viscosity and stability. Nevertheless, the mucus layer is continuously eroded by enzymatic and mechanical challenges on the surface. Both the mucus secretion and mucus erosion are influenced by various factors, such as mucus secretagogues, mechanical stimuli or stress, thus leading to a highly variable turnover. The average thickness of, for example the occular, buccal and intestinal mucus gel layer in humans is 40 µm [10], 70 µm [11] and between 80 µm – 200 µm [12], respectively.

For small peptides such as cyclosporin, hydrophobicity appears to be the most important physicochemical characteristic influencing the diffusion through the mucus gel layer [13, 14], whereas the molecular size of larger peptide drugs, i.e. a molecular mass above 2 kDa, is mainly responsible for their very poor diffusion. According to the equation for the diffusion coefficient, the radius of the molecule indirectly correlates with the diffusion coefficient.

Equation: $$D = \frac{Tk}{6\pi\eta\gamma}$$

D: diffusion coefficient; T: absolute temperature; k: Boltzmann constant; η: viscosity; r: radius of the molecule.

The mucus layer barrier therefore increases tremendously for large peptide drugs. For example, Allen *et al*. showed that molecules as small as vitamin B_{12} (MM: 1.35 kDa) easily permeate a mucus layer 1 mm thick, but myoglobin (MM: 17 kDa) cannot [15]. These findings were confirmed by our research group suggesting a molecular mass cut-off in the range of 10 kDa [16]. Though the largest tested peptide exhibiting a molecular mass of 67 kDa still permeated the mucus layer to some extent, however, an absolute molecular mass cut-off for macromolecules doesn't seem to exist. Investigations on the diffusion of proteins exhibiting a molecular mass of up to 186 kDa through native porcine mucus confirmed the theory of no absolute molecular mass cut-off. Lysozyme (14.4 kDa), rennet (~35 kDa), bovine serum albumin (68 kDa), and glucose oxidase (186 kDa) exhibited in mucus still 3-7% of the diffusion coefficient of the same proteins in buffer [17].

2.2. Tissue Barrier

In order to overcome biological tissue barriers, molecules have to pass the epithelium which can be achieved by several pathways as demonstrated in Fig. (**1**). Among these routes the paracellular route is the main way of absorption for hydrophilic compounds such as the peptide drugs. Whether the administered compounds will be transported through the transcellular or paracellular route will be judged by the physical and the chemical properties of the drug. Highly lipophilic compounds diffuse passively across the barriers by the transcellular pathway, whereas hydrophilic, membrane-impermeable protein- and peptide drugs diffuse to a higher extent through the paracellular pathway, which is controlled by the tight junctions [18]. The paracellular flux of compounds occurs strictly by passive diffusion. Only a few drugs will be transported by active transport systems. An example of the active transport is the intestinal absorption of di- and tripeptides by the oligopeptide transporter which is a carrier mediated process [18-20]. Although either the transcellular or the paracellular route can be the favoured way of mucosal uptake, in most cases both routes are involved in the absorption process. Muranishi, for instance, investigated

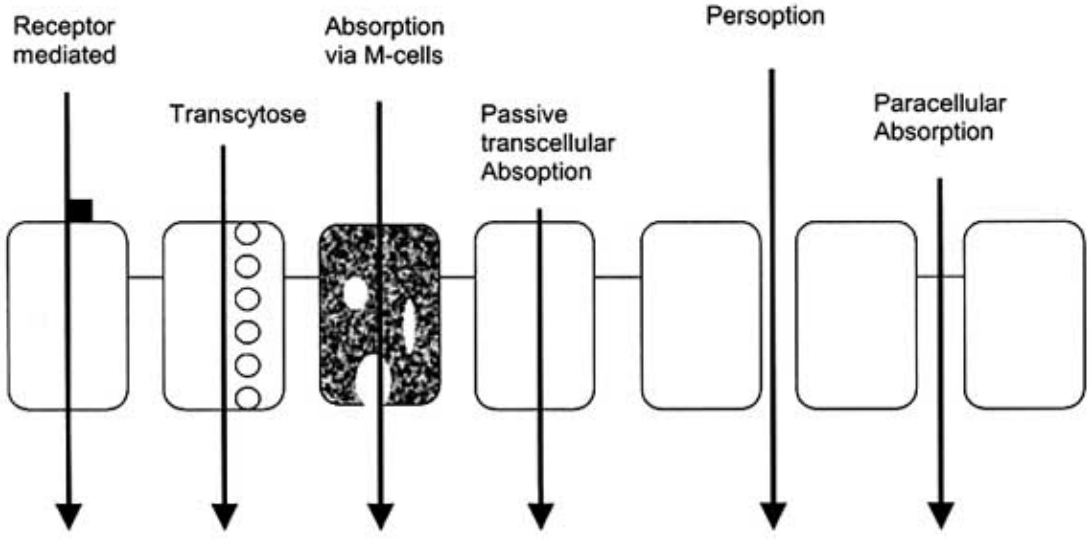

Fig. (1). Possible ways of drug permeation through biological membranes.

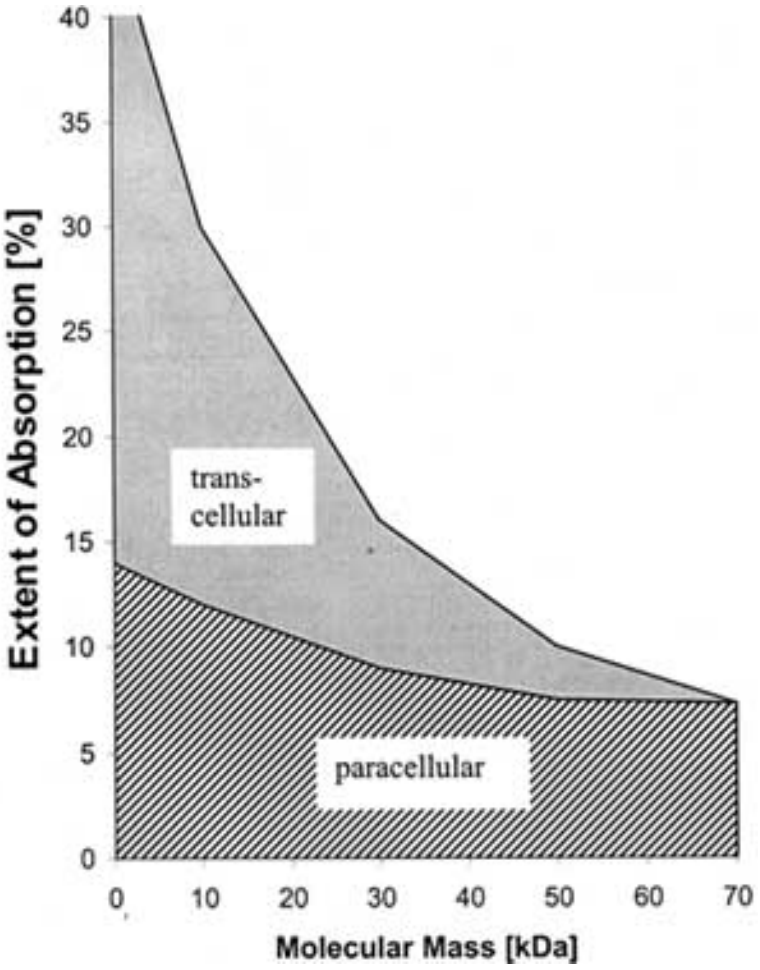

Fig. (2). Ratio of transcellular and paracellular mucosal uptake of dextrans in dependence on their molecular mass (Adapted from Muranishi (21)).

the mucosal absorption of dextrans with different molecular mass influencing the uptake [21]. The results of this study are shown in Fig. (**2**). For the paracellular route of absorption, it was demonstrated that molecules with a radius above 15 Å are poorly transported through the tight junctions, which represent the limiting gate fence area for the paracellular pathway [22, 23]. In order to understand the influence of tight junctions on the paracellular absorption, it is useful to summarise the so far known function of the proteins regulating and/or influencing the gate fence function of the tight junctions.

As shown in Tab. 1 [24-35] the proteins can generally be divided into transmembrane- and intracellular proteins. Among the transmembrane proteins occludin was the first are which could be identified [24]. It is widely expressed, essentially by all the epithelial and endothelial tissues and has also been reported to be expressed by neurons and astrocytes [24, 36, 37]. Occludin is a 60-65 kDa protein that was shown to express two extracellular loops from amino acid 81-124 and 184-227. These loops express several tyrosine and glycine residues which can be influenced enzymatically [38]. These two extracellular loops of occludin are believed to provide the adhesiveness of the junctional barrier. The C- and N-termini of the protein are located in the cytoplasm.

Another family of proteins also expressing two extracellular loops are the claudins. The originally identified proteins are claudin-1 and -2 expressing a molecular mass of 22-24 kDa [25]. Actually there is no evidence that claudin-1 or -2 and occludin directly interact with each other. At least some of the claudins are able to mediate cell adhesion in a Ca^{2+} -independent manner [39]. The function of the claudins seems to be the selection of ions passing through the paracellular barrier [40].

The third known transmembrane protein at the tight junction, JAM (junctional adhesion molecule), is a member of the Ig superfamily and thus is structurally very distinct from occludin or the claudins. The influence of JAM on tight junctional integrity is so far not completely understood although strong evidence is given for its role in cell-cell adhesion [41].

Focusing on the intracellular area of the tight junctions many proteins as listed in Tab. 1 have been identified. Specially three proteins namely ZO-1, ZO-2 and ZO-3 are

Table 1. Proteins of the Tight Junctions

Transmembrane Proteins			Intracellular Proteins		
Protein	**Function**	**Ref.**	**Protein**	**Function**	**Ref.**
Occludin	cell-cell adhesion	(24)	ZO-1	direct interaction with occludin	[27]
Claudin-1	selection of ions passing the tight junctions	(25)	ZO-2	direct interaction with occludin	[28]
Claudin-2	selection of ions passing the tight junctions	(25)	ZO-3	direct interaction with occludin	[29]
JAM	cell-cell adhesion	(26)	AF-6	involved in cell signalling pathways	[30]
			Cingulin	unknown	[31]
			7H6 Antigen	unknown	[32]
			Symplecin	unknown	[33]
			Fodrin	unknown	[34]
			P130	unknown	[35]

expressed at this region specified as scraffolding proteins. These three proteins belong to a large family of proteins known as the MAGUKs (Membrane Associated Guanine Kinases) [29]. ZO-1 seems to play a central role as it directly interacts, on the one hand with the C-terminal trail of occludin [42] and on the other hand with ZO-2, ZO-3, AF-6 and actin [43]. According to the knowledge about the wide area of tight junctional proteins and of some of their functions, the gate fence area can be characterised as shown in Fig. (**3**).

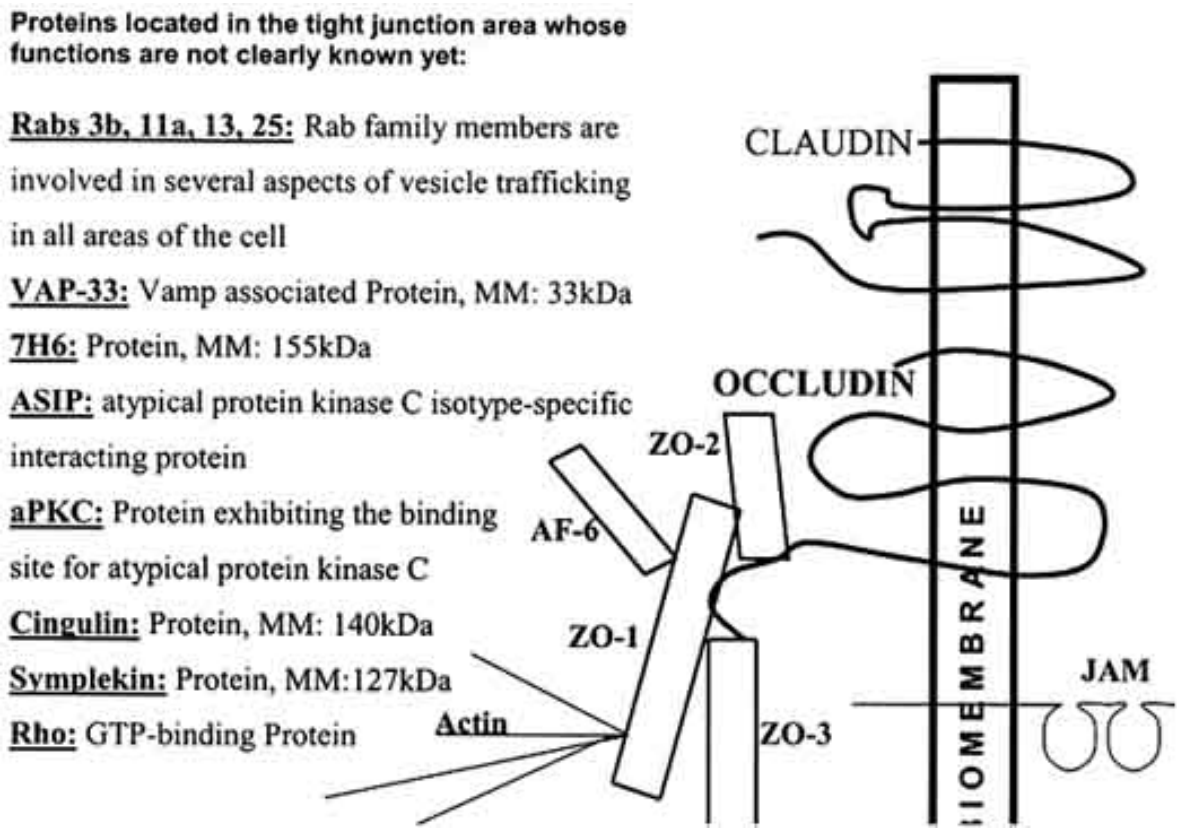

Fig. (3). Molecular components of the tight junctions.

AF-6 = protein containing one domain being able to interact with ZO-proteins and two domains that can disrupt this interaction

ZO-1, ZO-2, ZO-3 = zona occludens proteins 1, 2, 3

JAM = junctional adhesion molecule

3. AUXILIARY AGENTS

3.1. Mucolytic Compounds

In order to lower the mucus layer barrier the co-administration of mucolytic compounds might be helpful. Detergents, sulfhydryl compounds and mucolytic enzymes are reported to display a mucolytic activity.

Detergents such as Triton-X100 (t-octylphenoxypolyethoxyethanol), ambroxol (2-amino-3,5-dibromo-N-[trans-4-hydroxycyclohexyl]benzylamine) or Tweens (polyoxyethylenesorbitan) are believed do exhibit a mucolytic activity. Their effect, however, seems to be in many cases quite weak and insufficient. Investigations on the mucolytic activity of Triton-X100 and Tween, for example, showed in our laboratories no significant mucolytic effect of these compounds [44].

Sulfhydryl compounds, in contrast, display a high mucolytic activity by cleaving disulfide bonds which connect mucus glycoproteins with each other. A well established sulfhydryl compound of high mucolytic activity is N-acetylcysteine, which is used as an expectorant in various pharmaceutical formulations. *In vivo* studies focusing on the influence of the mucus gel layer on intestinal permeability, for instance, demonstrated a significantly higher uptake of FITC-dextran 70,000 in rats due to the co-administration of N-acetylcysteine [45]. Another potent sulfhydryl compound is dithiothreitol. In ileum and proximal colon this auxiliary agent increased the absorption and biliary recovery of a tripeptide four-fold and 70-fold over controls in rats, respectively [46]. The use of sulfhydryl compounds in combination with peptide drugs, however, remains often quite questionable. They do not exclusively cleave disulfide bonds of mucus glycoproteins but also those of the therapeutic peptide. Sulfhydryl compounds can therefore primarily be used for peptide drugs without any disulfide bonds. For example, insulin displaying three disulfide bonds within its structure is highly degraded by dithiothreitol and N-acetylcysteine [44]. Therapeutic peptides exhibiting no cysteine moieties within their primary structure such as leucine-enkephalin or gonadorelin. For however, the use of these auxiliary agents seems to be helpful. If the disulfide bonds are not accessible for the sulfhydryl compound due to the conformation of the peptide, they remain stable as well. Moreover, cysteine proteases, such as papain which is used in antiinflammatory therapy can even be activated in the presence of cysteine. Hence, the co-administration of sulfhydryl compounds lowering the mucus layer barrier for peptides has to be evaluated from case to case. If the peptide stability is guaranteed in the presence of sulfhydryl compounds, they are certainly the first choice in order to overcome this barrier.

Mucolytic enzymes bear similar problems as sulfhydryl compounds. On the one hand these enzymes exhibit a strong mucolytic activity by cleaving within the amino acid sequence of mucus glycoproteins. On the other hand, however, the proteolytic degradation of various peptide drugs cannot be avoided. Enzymes of high mucolytic activity are pronase, papain, bromelain and trypsin [44, 47].

3.2. Low Molecular Mass (LMM) Permeation Enhancers

A promising way to improve the permeation of peptide drugs through biological membranes is the use of LMM permeation enhancers. Various classes of substances have proven to be useful in improving the permeation across intact epithelial membranes. In case of the transcellular route of permeation the whole surface area of the epithelial membrane is available for the drug uptake.

Interaction of absorption enhancers with membrane lipids and/or proteins, which leads to membrane perturbation, is followed by an increase in permeability. This could be demonstrated by for example mixed micelles [48], middle chain fatty acids [49], salicylic acids [50] and acyl carnitine [51].

The paracellular route, on the other hand, bears the advantage of the "leakiness" of the cell to cell junction thereby avoiding the possibility of drug degradation within the cells. The intestinal tight junctional area can be calculated according to the following considerations. The maximum permeable molecular radius of drugs was evaluated to be 3 nm and the diameter of a epithelial cell can be estimated to be approximately 10 µm. First the area of the cell surface was calculated according to the circle equation. Thereafter, the cell diameter and the tight junctional diameter were added up to reach a total diameter of the cell plus tight junction. This new diameter was used for a second circle area equation. The total tight junctional area was reached by subtracting the area of the cell from the total area. According to this consideration the tight junctional area is limited to approximately 0.2% of the whole intestinal absorption area. In order to lower the gate fence function by the use of LMM permeation enhancers an improved absorption can be achieved by an interference in the following mechanisms:

- Chelation between enhancer and calcium/magnesium ions around tight junctions by the use of EDTA resulted in a decrease in the extracellular Ca^{2+} level leading to an opening of the tight junctions [52].
- The mechanism of sodium caprate opening the tight junctions was demonstrated to be dependent on increasing the intracellular calcium level through interaction with phospholipase C [53] leading to the activation of junctional actomyosin contraction.
- Solubilization of membrane components was demonstrated to be the underlying mechanism for permeability improvement with bile salts and nonionic, anionic and cationic surfactants [54]. *In vivo* studies on human volunteers for instance, demonstrated after oral administration of octreotide a significant increased permeation by co-administration of bile salts (Fig. **4**) [55].
- A new way of opening the tight junctions is to administer Zonula occludens toxin (Zot) elaborated by *Vibrio cholerae* [56, 57]. Zot appears to activate a complex intracellular cascade of events that regulate membrane permeability [58].

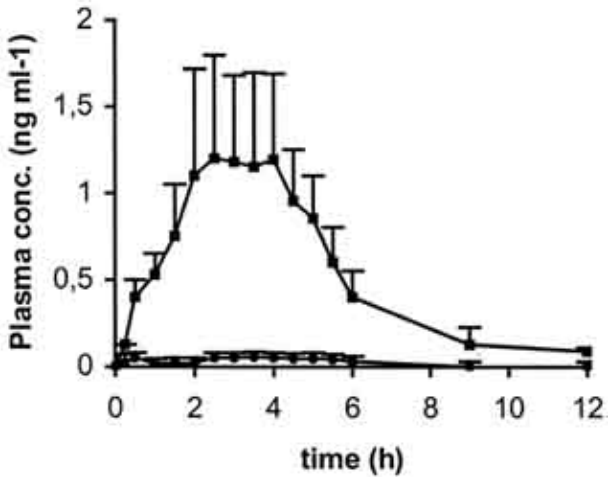

Fig. (4). Plasma profiles of octreotide in human volunteers (n=10) after oral administration of 4 mg peptide in the presence of 100 mg chenodesoxycholate (■) or 100 mg ursodeoxycholate (●). Data represent means ± S.D. (Adapted from Fricker *et al*. [55]) .

Lipophilic neutral species or an ion-pair can be formed as a result of the electrostatic attraction between two oppositely charged species. Due to the formation of a complex between enhancer and the

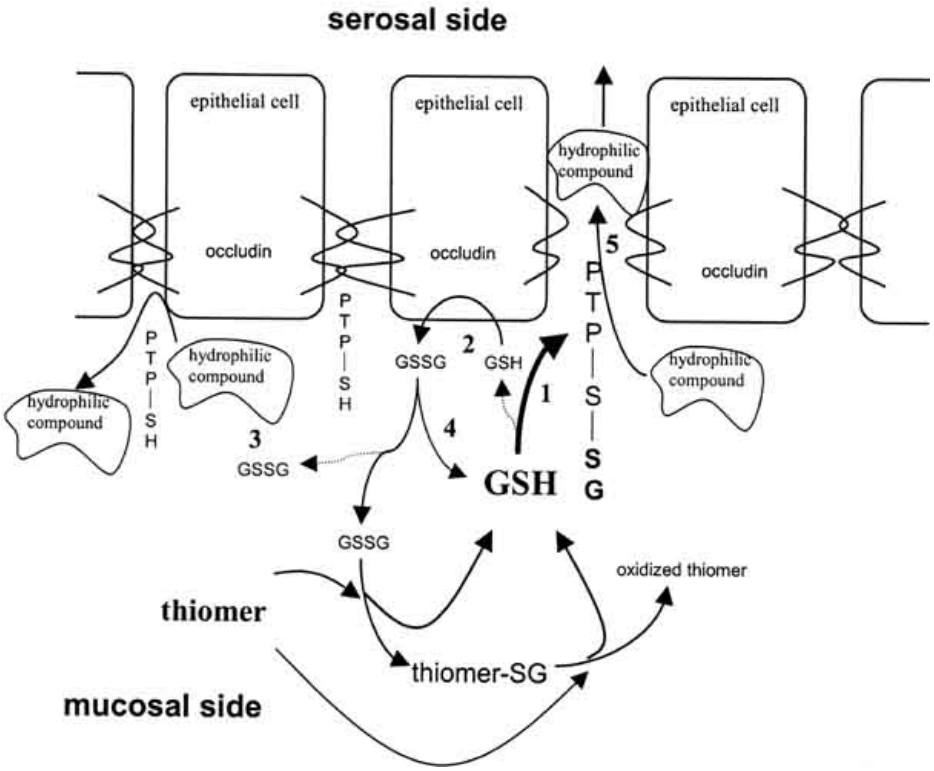

Fig. (5). Proposed mechanism of GSH induced opening of the tight junctions via inhibition of PTP. PCP-SH = polycarbophil-cysteine conjugate; PTP-SH = active form of protein tyrosine phosphatase; GSH = reduced form of glutathione; GSSG = oxidised form of glutathione. (Adapted from Clausen *et al*. [65]]

1 Inactivation of PTP via covalent attachment of GSH on the active site cysteine 215: [60]

2 Oxidation of GSH to GSSG catalyzed by the cells: [61]

3 Metabolismus of GSSG via gamma-Glutamyltransferase: [61]

4 Reduction of GSSG in the gastrointestinal mucosa by GSSG reductase: [62]

5 Increased tight junction permeability by the inhibition of PTP via a specific tyrosine phosphatase inhibitor: [63]

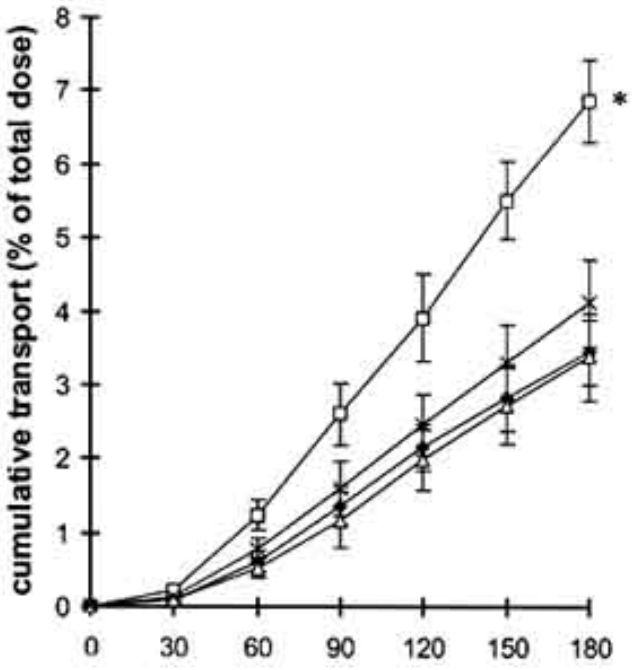

Fig. (6). Transport of fluorescence labelled bacitracin across small intestinal mucosa of guinea pigs. Transport data are expressed as percentage of the total dose of bacitracin applied to the luminal side of the mucosa. Control without enhancer (♦); 0.5% (w/v) polycarbophil (Δ); 0.5% (w/v) thiolated polycarbophil (X); 0.4% (w/v) reduced glutathione and 0.5% (w/v) thiolated polycarbophil (□); (means ± S.D.; n=3). *, differs from control, $p<0.001$. (Adapted from Clausen *et al*. [64, 65]).

peptide drug the thermodynamic activity of the drug will be increased. For example, Zhou demonstrated an improved bioavailability of insulin by the use of cholate [59].

- The reduced form of glutathione (GSH) was also shown to improve the paracellular permeation of peptide drugs. GSH was demonstrated to inhibit protein tyrosine phosphatase (PTP) which is involved in the closing process by interacting with occludin. Due to a disulfide bond formation of GSH with the cysteine moiety in the active region opening of the tight junctions was provided as demonstrated in Fig. (**5**) [60-63] which led to an improved permeation of the model peptide drug bacitracin (Fig. **6**) [64, 65].

An overview of LMM permeation enhancers is given in table 2 [66-77]. Nevertheless some of the enhancers can cause intestinal damage or can even enter the systemic circulation due to their low molecular mass leading to the systemic toxic side-effects [78].

3.3. Polymeric Permeation Enhancers

Another class of permeation enhancers that has received lots of attention are high molecular mass polymers such as chitosan and polyacrylates [79]. They display some advantages in comparison to LMM enhancers like additional mucoadhesive properties which allow them to remain concentrated at the area of drug absorption [79]. In general the polymers can be divided into cationic and anionic polymers.

A representative member of the **cationic** polymers is the widely used chitosan. (Various studies on Caco-2 monolayers and *in vivo* rat models have shown that, the permeation enhancing effect for poorly absorbable drugs could be demonstrated) [7, 80-82]. The underlying mechanism of opening of the tight junctions by chitosan was attributed to the interaction of the positively charged amino groups with the negatively charged sialic groups of membrane-bound glycoproteins [80].

Furthermore, **anionic** polymers such as polycarbophil or carboxymethylcellulose also demonstrated permeation enhancing properties [79, 83, 84]. In contrast to the direct interaction of chitosan to the mucosal surface these two polymers were shown to express a high Ca^{2+}-binding ability [84, 85] similar to the Ca^{2+}-binding mechanism of the LMM enhancer EDTA. The depletion of Ca^{2+}-ions from the extracellular cell medium has been shown to increase the permeation of sodium-fluorescein, bacitracin, a vasopressin analogue and insulin [79, 84]. Parallel measurement of the transepithelial electrical resistance (TEER) demonstrated a decrease in TEER indicating the opening of the tight junctions.

High molecular mass polymers will not be absorbed from the mucosal barriers [86, 87], therefore systemic side-effects can be excluded. Chemical modifications of these polymers appear to improve their properties. For instance,

Table 2. Use of LMM Absorption Enhancers on Different Tissues

Absorption Enhancer	Used Peptide Drug	Site of Administration	Ref.
Polyoxyethylene-24-cholesterol ether	Octreotide	Oral	[66]
Sodium caprate	Insulin	Oral	[67]
Sodium taurodihydrofusidate	Insulin	Vaginal	[68]
Phosphato-dihydrofusidate	Leucine enkephalin	Vaginal	[69]
Sodium glycodeoxycholate	Buserelin	Buccal	[70]
Sodium salicylate	Insulin	Rectal	[71]
Na_2 EDTA	Insulin	Rectal	[71]
n-Lauryl-ß-D-maltopyranoside	Insulin	Colon	[72]
Zot	Insulin	Illeum	[73]
Phospholipids	Desmopressin	Caco-2	[74]
Taurodeoxycholate	Insulin	Nasal	[75]
Sodium tauro-24,25-dihydrofusidate	Calcitonin	Nasal	[76]
Polyoxyethylene-20-stearylether	Gonadorelin	Ocular	[77]

chitosan derivatives are not soluble at pH above 6.5, therefore their permeation enhancing effect cannot be used at values above pH 6.5. In order to overcome this problem N-trimethylation of chitosan chloride was shown to increase the solubility at higher pH [88, 89]. The use of this new trimethylated chitosan *in vivo* on rats was shown to significantly improve the absorption of octreotide after intrajejunal administration [90]. Another chemical modification is the mono-N-carboxymethylation of chitosan. This resulted in an improved permeation of low molecular mass heparin *in vitro* and *in vivo* [91].

The properties of anionic polymers have also been improved due to a chemical modification, thereby generating a new type of mucoadhesive polymers. Due to the immobilization of free sulfhydryl groups onto various polymers their permeation enhancing effect on hydrophilic compounds such as sodium fluoresceine (NaFlu), bacitracin or insulin has been strongly increased [64, 84, 92]. In addition, thiolated polymers or so called **thiomers** exhibit improved mucoadhesive properties [93] which allow them to remain concentrated at the area of drug absorption. Recently, the underlying mechanism of permeation enhancement by thiomers was shown to depend on the inhibition of proteine tyrosine phosphatase (PTP). This results in a higher extent of phosphorylisated tyrosine groups on the two loops of the membrane spanning protein occludin leading to the opening of the tight junctions. Inhibition of PTP can be reached by a simple disulfide bond formation of the active site cysteine of the protein. The cysteine groups on PTP are oxidised by reduced glutathione (GSH) released by intestinal cells [60]. It is believed that reduced thiol groups on the thiomers reduce the oxidised glutathion, thereby increasing the amount of GSH at the absorption area for PTP inhibition. This results in significantly improved permeability of the tight junctions according to the mechanism shown in Fig. **5** [65].

4. DELIVERY SYSTEMS

Besides the use of auxiliary agents the whole delivery system also has a great impact on the membrane permeability of peptide drugs. Features such as the type of formulation, release kinetics and mucoadhesive properties have to be taken into consideration when designing a dosage form.

4.1. Type of Formulation

In many cases the type of formulation itself influences the peptide drug absorption. Formulations such as liposomes and nanoparticles are reported to improve mucosal peptide drug absorption. As chapter 9 of this thematic issue focuses on the development of liposomal formulations, they will not be discussed here.

Nanoparticles offer the advantage of protecting incorporated peptides from enzymatic degradation. They can cross over the mucosal membrane either through the Payer's patch and/or the paracellular route. After having reached the systemic circulation the particles are biodegraded releasing the incorporated peptide drug. The uptake of nanoparticles, however, is not overwhelming. The extent of absorption of 50 nm and 100 nm particles, for instance, was 34% and 26% from rat intestine, respectively. Particles larger than 300 nm did not reach the systemic circulation at all [94]. In addition, Norris and Sinko showed that the permeation of nanoparticles trough intestinal mucin is strongly limited for particles with a diameter greater than 300 nm [95].

4.2. Release Kinetics

The efficacy of permeation enhancers strongly depends on the ability to co-deliver them with the peptide drug in

effective concentrations at the absorbing membrane. In particular for low molecular mass permeation enhancers it would be highly advantageous, if the auxiliary agent is simultaneously released with the peptide drug. Since the peptide drug and the permeation enhancer display in most cases a different size, charge and hydrophilic/lipophilic balance, a synchronized release will be difficult to achieve. Although there are numerous delivery systems in development, which should fulfil these demands, none of them has so far reached the clinical trials.

4.3. Mucoadhesive Properties

Mucoadhesive delivery systems are able to adhere on the mucus gel layer covering mucosal membranes. These mucoadhesive properties are in many cases advantageous in order to enhance the permeation of peptide drugs through the absorption membrane, which can be explained as following:

I. Mediated by the mucoadhesive properties of the delivery system, the residence time of dosage forms on the mucosa can be prolonged, which allows a sustained drug release at the absorption membrane. Thereby, a prolonged period of drug uptake and subsequently a greater amount of total dose absorbed can be achieved.

II. An intimate contact of the delivery system with the absorption membrane can be guaranteed providing the basis for a steep concentration gradient as driving force of the drug uptake. The combination of hydroxypropyl cellulose and microcrystalline cellulose causing a locally high concentration of leuprolide or salmon calcitonin in the vicinity of the nasal mucosa surface, for instance, led to a strongly improved bioavailability of these peptide drugs [96].

III. In case of oral delivery systems, a presystemic metabolism of peptide drugs on the way between the delivery system and the membrane caused by luminally secreted proteases can be minimized.

IV. Moreover, the adhesion of polymeric delivery system to the mucosa is essential for their interaction with the membrane in order to achieve a permeation enhancing effect.

Mucoadhesive strengh of polymers is based on secondary bonds such as hydrogen bonding and ionic interactions or covalent bonds such as the formation of disulfide bonds with the mucus layer, these polymers adhere on mucosal surfaces. Polymers displaying high mucoadhesive properties are polyacrylates [93] and chitosans [97]. Their mucoadhesive properties can even be improved by the immobilization of thiol groups [93]. Based on thiol/disulfide exchange reactions with mucus-glycoproteins, new disulfide bonds are formed between the thiolated polymer and the mucus. Particles and liposomes can be coated with mucoadhesive polymers [82] or the mucoadhesive polymer can directly be used as drug carrier matrix in the delivery system [98].

5. CONCLUSION

The development of non-invasive peptide delivery systems is strongly limited by the poor biomembrane permeability of these therapeutic agents. Within the last decade our knowledge concerning different mechanisms of peptide drug absorption has enhanced tremendously. In particular, the paracellular route of uptake was studied intensively, as it is the favored way of peptide permeation. On the basis of this knowledge it should be possible to improve the efficacy of mucolytic agents, enhancers and polymers, which augment peptide transport across biological membranes and aid in attaining minimum therapeutic levels in blood. Furthermore, the design of the drug delivery system and the combination of the different types of auxiliary agents lowering the barrier function of the membrane, by acting in different ways will become more and more important. Successful developments will result in novel mucosal delivery systems for peptide drugs which will be highly appreciated by therapists and patients.

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