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Review article

Factors and strategies for improving buccal absorption of peptides

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Abstract

Peptides and polypeptides have important pharmacological properties but only a limited number (e.g. insulin, oxytocin, vasopressin) have been exploited as therapeutics because of problems related to their delivery. The buccal mucosa offers an alternative route to conventional, parenteral administration. Peptides are generally not well absorbed through mucosae because of their molecular size, hydrophilicity and the low permeability of the membrane. Peptide transport across buccal mucosa occurs via passive diffusion and is often accompanied by varying degrees of metabolism. This review describes various approaches to improve the buccal absorption of peptides including the use of penetration enhancers to increase membrane permeability and/or the addition of enzyme inhibitors to increase their stability. Other strategies including molecular modification with bioreversible chemical groups or specific formulations such as bioadhesive delivery systems are also discussed.

Keywords: Buccal mucosa; Peptide absorption; Enhancement peptide permeation; Buccal absorption mechanism; Buccal peptide delivery

1. Introduction

Peptides are currently emerging as a major class of therapeutic drugs. Many are endogenous compounds regulating endocrine and other physiological processes in the body. Oligopeptides with less than ten amino acids are not readily absorbed through the gastrointestinal tract because of their high degradation by proteolytic enzymes or their sensitivity to acidic media. Therefore, they are usually not suitable for oral administration, and are mostly delivered by parenteral administration and due to their short biological half-life, repeated injections are often required. Non-parenteral routes of administration need to be developed in order to minimize side effects produced by constant injection. Nasal, ocular, vaginal, rectal and buccal mucous membranes have all been evaluated as potential alternative routes for peptide absorption. Mucosal delivery was found to be much less efficient than parenteral administration due to a combination of poor membrane permeability and metabolization at the site of absorption [1]. However, the buccal route has the advantage of avoiding the first-pass effect and non-exposure of the drug to the gastro-intestinal tract secretions. Furthermore, there are few proteolytic enzymes as compared to oral administration [2] and in addition, the buccal mucosa is highly vascularized.

To date, a wide variety of polypeptidic drugs have been evaluated for buccal absorption [3]. Some of these include gastrointestinal peptides (secretin, substance P), pancreatic hormones (insulin, glucagon), anterior pituitary polypeptides (adrenocorticotropins, growth hormone), posterior pituitary oligopeptides (oxytocin, vasopressin and their analogues), hypothalamic-releasing-hormones (protirelin, gonadorelin, growth hormone-releasing factor hormone, somatostatin) and their derivatives (e.g. the gonadorelin agonists, buserelin, histrelin and nafarelin), as well as enkephalins, calcitonin and interferons [4–6]. Peptide absorption is limited because of their instability, low diffusivity (due to their molecular weight and hydrophilicity) and rapid metabolism. Other factors such as immunogenicity, biocompatibility, aggregation and adsorption may also affect transport [7]. Chemical modification can be essential in order to improve stability by minimizing enzymatic cleavage. In addition, small peptides may be derivatized to produce prodrugs which possess favourable physico-chemical properties in comparison to the parent compound by rendering the peptide more lipophilic thus facilitating absorption [8,9]. Bioreversible derivatization may protect small peptides against degradation by peptidases present at the mucosal absorption barrier. Another promising approach is the use

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of novel permeation enhancers in order to minimize irritation to the mucosal membranes. However, on account of the buccal mucosa's ability of rapid recovery, the toxicological issue with respect to absorption enhancers may not be as significant a factor as with other mucosal sites [10–12].

The accessibility of this absorption site over other sites and the high acceptance by the patient also favour the buccal mucosa as a site for systemic drug delivery.

2. Peptide absorption properties

2.1. Structure

2.1.1. Molecular weight and size

Molecular weight and size influence the diffusivity of the drug through the epithelial layer. As a general rule, very large molecules have lower diffusivities. Indeed, small molecules (<75–100 Da) appear to cross the mucosa barrier rapidly [13]. However, permeability falls off markedly molecular size increases. Several authors have investigated the effects of molecular weight upon mucosal absorption of various hydrophilic compounds [14–16]. It was found that the apparent permeability coefficient of fluorescein isothiocyanate dextrans (FITCD), a neutral polysaccharide, decreased as molecular weight increased [15–17]. Generally, buccal absorption decreased exponentially with molecular weights above 300 Da [18]. Peptides and proteins have a very large dispersion in their molecular weight (MW) compared to most conventional drugs, ranging from less than 600 to greater than 100 000 Da so that a direct comparison is not possible [19]. Human in vivo studies showed that peptides such as protirelin (MW: 362) and oxytocin (MW: 1007) crossed the human buccal mucosa barrier, whereas buserelin (MW: 1239) and calcitonin (MW: 3500) did not [20]. This led Merkle et al. [10] to propose that the transfer of peptides with molecular weights above 500–1000 Da through buccal mucosa would require use of an absorption enhancer.

2.1.2. Conformation, stereospecificity and immunogenicity

Unlike conventional drugs, peptidic drugs may have primary, secondary and tertiary structures and in solution, may adopt several different conformations depending upon their size [21–23]. One of the potential problems is the preservation of the pharmacologically active conformation during the process of formulation and sterilization. Furthermore, change in conformation can influence membrane permeability [24]. During formulation, the stereospecificity of the drug must be preserved since the permeation systems are thought to be stereoselective [25,26].

Peptides are also recognized as often being immunogenic and the use of inert polymers, e.g. polyethylene glycol (PEG), dextran, polyvinylpyrrolidone (PVP) and albumin for peptide delivery has been shown to increase resistance to proteolysis and simultaneously decrease peptide immunogenicity [23,27,28].

2.1.3. Electrostatic charges

The charge distribution on the chain may be even more important than the value of the partition coefficient in the prediction of buccal permeability of peptides. Terminal charges on zwitterionic peptides have a negative effect on membrane permeability even though the effective partition coefficient is relatively high [25]. The effect of charge density can be modified to promote peptide absorption by changing the pH of the medium and thus the degree of ionization of the permeant [29].

At physiological pH or at a pH above the isoelectric point (p*I*), the epithelial proteins are negatively charged and are selective to positively charged solutes [30], (below the p*I*, the opposite holds). At the isoelectric point, the membrane is non-discriminating to either ion [31]. The charge discriminating effect of the epithelia is believed to have a significant impact on the absorption of charged drugs. Absorption insulin which is negatively charged was found to be excluded from the aqueous paracellular pathway, whereas positively charged peptides, such as thyrotropin-releasing hormone, were taken up predominantly via this pathway [30].

Although not firmly established, mucosae are considered to be perm-selective towards positively charged [30–32].

2.2. Physico–chemical properties

2.2.1. Solubility, hydrophilicity and partition coefficient

Peptides, being amphoteric, usually have complex solubility versus pH profiles. Usually, the aqueous solubility is minimal at the isoelectric point where the drug is neutral or has no net charge. It is also strongly dependent upon pH, metallic ions, ionic strength and temperature. Unless the N- and C-termini are blocked through cyclization, amide formation or esterification, peptides are very hydrophilic with a very low octanol-water partition coefficient (Table 1) [18,33–37]. Therefore, one way to improve peptide absorption by a passive diffusion mechanism is to increase their lipophilicity (see Section 3). Although the octanol–water partition coefficient is a simple parameter that may

Table 1
Lipophilicity of selected peptides; from Lee [1]

Peptide	Partition coefficient (*n*-octanol/buffer, pH 7.4)
Insulin	0.0215
Thyrotropin-releasing hormone	0.0376
Luteinizing hormone-releasing hormone	0.0451
Glucagon	0.0633
Substance P	0.275
Met-enkephalin	0.0305
Leu-enkephalin	1.12

predict mucosal permeability, its correlation with absorption of peptides is not always observed as buccal bioavailability of peptides models varies parabolically with their lipophilicity [18,38]. It is worth noting that the permeation properties can be modified to a significant extent by the formation of hydrogen-bonds between peptides and the tissue [39–41].

It is generally recognized from human buccal absorption data that the absorption of drugs from whole oral cavity obeys the pH-partition hypothesis which implies a passive diffusion mechanism [42,43], where the absorption rate is directly proportional to the concentration of drug molecules in the free form.

The degree of ionization of a permeant is a function of both its pK_a and the local pH at the mucosal surface. Absorption of peptides has been shown to be maximal at a pH at which they are mostly non-ionized, tailing off as the degree of ionization increases. However, it has also been shown that the permeability coefficient of protirelin through rabbit buccal mucosa in vitro was independent of the pH of the peptidic solution [44].

2.2.2. Aggregation, self association and hydrogen bonding

The intrinsic properties of peptides can be modified by self-aggregation. Toniolo et al. [45] and Touitou et al. [46] report that insulin usually exhibits aggregation, with human insulin aggregating more readily than pig or cow insulin [47]; ionic ingredients and phenolic preservatives accelerate the aggregation of insulin and zinc insulin complexes are more stable than zinc-free insulin. In an extensive study concerning the effects of additives, it has been reported that non-ionic surfactants such as Pluronic F68 appear to be promising stabilizers [48].

The capacity for some peptides to form intermolecular hydrogen bonds with water has been reintroduced as a predictor of peptide absorption [49–51] since self association may involve formation of intermolecular H-bonds and hydrophobic interactions [7,19,47]. The addition of hydroxyl groups generally promotes hydrogen bonding with solvating water leading to concomitant decrease in the partition coefficient and the ability to penetrate a lipidic membrane therefore resulting in a decrease in permeability [18,39]. However, the presence of hydroxyls can sometimes lead to an increased permeability. This is most likely due to the formation of cyclic intramolecular hydrogen bonds [52–54].

Cyclization of peptides appeared to reduce hydrogen bonding, increasing lipophilicity and reducing hydrodynamic radius.

2.3. Buccal mucosa permeability

2.3.1. Structural characteristics of buccal mucosa

The oral mucosa can be subdivided according to the major regions in the oral cavity, a so-called non-keratinized area consisting of the floor of the mouth (sublingual), the buccal mucosa (cheeks), and a keratinized area comprising the gum (gingiva), the palatal mucosa, and the inner side of the lips. The mucous membranes have a total area of 100 cm^2 and show differences in structure, thickness and blood flow depending on their location within the oral cavity. The buccal mucosa consists principally of two components: the epithelium and the underlying connective tissue (Fig. 1).

The interface between these two layers is formed by the basal complex. The rapid turn-over of the epithelial cells relative to the skin is an important feature of the oral cavity that affects drug absorption by continually changing permeability characteristics.

The buccal epithelium is a non-keratinized squamous layer of cells, 500–600 μm in thickness, composed of strata of different cell types with varying degrees of maturity. The upper most superficial region, is comprised of flattened compact layers of differentiated cells, about 150 μm thick. Epithelial cohesion in the superficial layers is ensured by the lipid and glycolipid contents extruded from the cellular MCG in the intercellular space. Deeper into the epithelium, lies the malpighian layer, which contains cells at various stages of differentiation. Here, cells are less flattened and are loosely held together by desmosomes and there is less tortuosity as compared to upper layers. The epithelium terminates with the basal lamina, a proteinaceous fibrous matrix of 1–2 μm thickness. The buccal epithelium is highly vascularized and the papillary contour of the basal region permits efficient vascularization of the cells. There is still debate about the role of the upper 50 μm of the epithelium [44,55–57]. In addition, the basal lamina, with a thickness of only 1 μm, may also represent a permeability barrier [26,58–62]. In contrast, the lamina propria is not generally thought to function as a barrier. Its structure is insufficiently dense to exclude even relatively large molecules, and its hydrated matrix should facilitate the passage of hydrophilic penetrants [60]. It is conceivable that the mucus above the epithelium might also act as an absorption barrier because of its many anionic and cationic functional groups attached to the mucins, thus reducing the approach of charged molecules [63]. On the one hand, hydration of the mucous membranes, due to the contact with saliva, may strongly facilitate drug permeation. However, the mucus layer is small relative to other barriers that peptides encounter during their passage through the buccal mucosa. Squier et al. [63,64] examined the route taken by hydrophilic (fluorescent) compounds (FITC dextrans and horseradish peroxidase) applied to the surface and in different oral regions and found that the *permeability barrier* was situated in the intercellular region of the superficial layers of the epithelium. Peptide absorption through buccal mucosa is highly dependent on the application site. It is therefore important in the design of transmucosal drug delivery systems to clarify regional differences of permeability properties in the oral cavity. Buccal mucosae are readily accessible and localization of a dosage form on a defined surface area over extended periods will not only maximize absorption, but

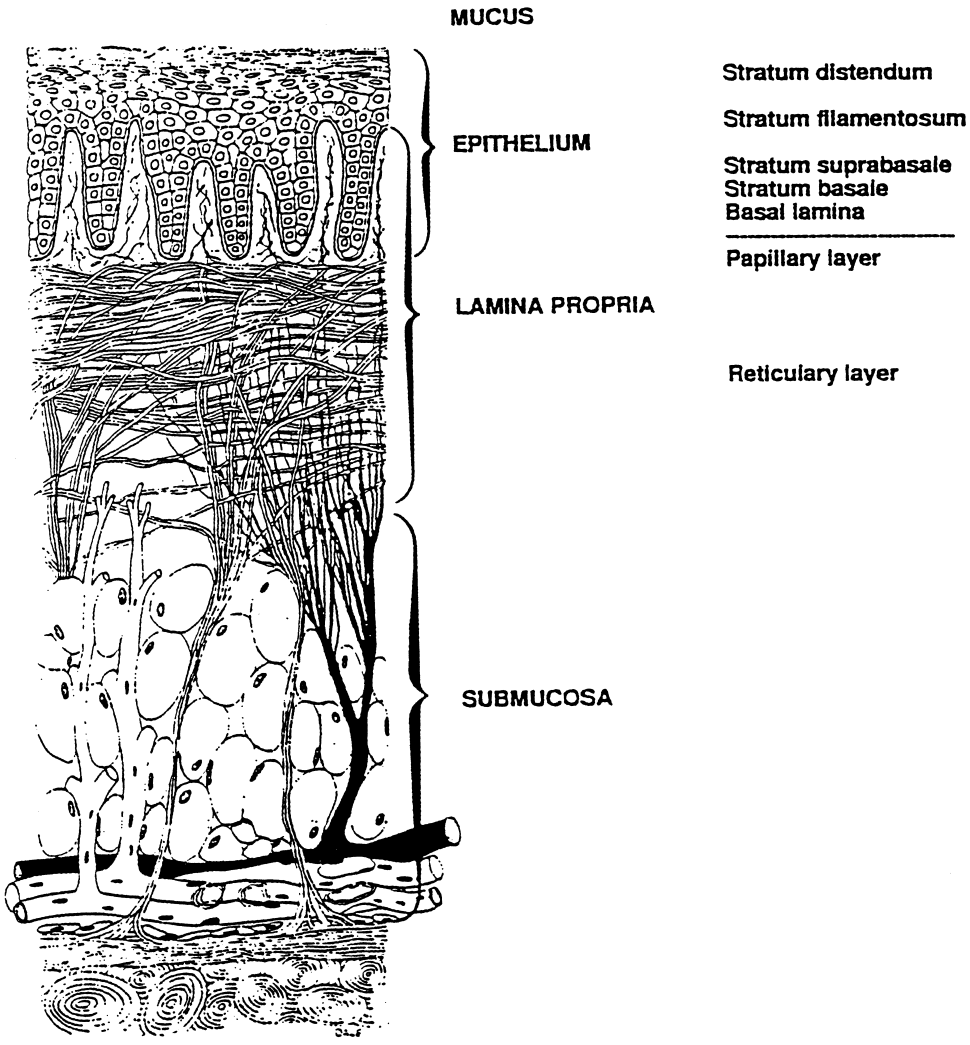

Fig. 1. Structure of buccal mucosa. From Merkle and Wolany [26].

may also provide a higher degree of control and reproducibility when compared to other mucosal delivery routes.

2.3.2. Biochemical structure

Oral mucosal tissue contains a large amount of extracellular material, which not only gives the epithelium its elasticity but is also thought to contribute to the permeability barrier. Regional differences in permeability are dependent upon epithelial thickness, the eventual presence of a keratinized epithelium and the organization of intercellular material extruded by membrane-coating granules in the upper layers of the epithelium.

Buccal mucosa contains mostly polar lipids such as phospholipids, cholesterol sulfate and glycosylceramides [66,67] (Table 2). This may result in fluidity and may create microdomains with specific properties. The non-keratinized regions have higher permeability to water and hydrophilic compounds than keratinized areas.

2.4. Biological environment

2.4.1. Sensitivity towards enzymes

The fraction of peptide reaching the systemic circulation intact will depend on its ability to cross the mucosal barrier and also on its resistance to degradation by peptidases present at both the site of administration and in the mucosal barrier. Peptides can be rapidly metabolized by proteolysis at most routes of administration. Among the mucosal routes, peptide transport through the buccal mucosa was found to be much less sensitive to degradative enzymes than nasal, vaginal and rectal administration [5,68]. Indeed, the buccal mucosa seems to be deficient in proteinases such as pepsin, trypsin and chymotrypsin present in gastric and intestinal secretion which are known to contribute to peptide hydrolysis [69]. However, since hydrolytic enzymes are ubiquitous, complete absorption of intact peptide is an exception [1,5,47,70–73]. In order to characterize the enzymatic barrier, investigators often use the enkephalins, a short

Table 2
Composition of the oral epithelia of the pig, from Ho et al. [65]

Components (values of lipids as wt.(%)	Gingiva	Palate	Floor	Buccal
Keratin	Present	Present	Absent	Absent
Phospholipids[a]	42.3	39.1	44.2	38.2
Glycosylceramides	2.1	1.8	5.8	16.5
Acylglycosylceramides	2.1	2.8	0.0	0.0
Acylceramides	0.4	0.2	0.0	0.0
Ceramides	6.6	3.3	0.7	0.8
Cholesterol	21.0	33.6	19.5	13.6
Cholesterol sulfate	2.0	1.7	3.2	7.8
Fatty acids	5.0	1.1	0.6	1.6
Triglycerides	16.9	15.9	11.1	15.7
Cholesteryl esters	1.1	0.2	15.0	5.9

[a] Major phospholipid composition includes sphingomyelin, phosphatidylcholine and phosphoethanolamine; minor ones include serine and inositol phosphatides.

peptide for which the enzymatic hydrolysis profiles are well known. Fig. 2 shows several enzymes involved in the metabolism of leucine enkephalin and the specific inhibitors which prevent their action. Comparison of the hydrolysis rates and degradation products of a given enkephalin when in contact with a tissue provide a measurement of the relative amount of these enzymes at each site.

Several proteolytic enzymes (aminopeptidases, endopeptidases, carboxypeptidases, deamidases), were found in various buccal epithelium (human, pig, monkey, rat, rabbit and cultured hamster buccal cells) [65]. Table 3 shows a list

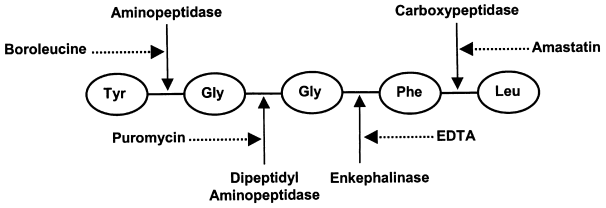

Fig. 2. Scheme for leucine–enkephalin metabolism, the enzyme involved (solid line) and inhibitors of these enzymes (dotted line). Adapted from Aungst et al. [74].

of enzymes capable of peptide degradation located in buccal mucosa.

The enzymatic barrier includes exo- and endopeptidases. Aminopeptidases (exopeptidases) present in the buccal mucosa and were shown to be primarily responsible for enkephalin degradation [69,78]. Lee and co-workers [68,72] demonstrated that proteolytic activity in mucosal tissues such as buccal mucosa against methionine enkephaline (D-Ala^2), methionine enkephalinamide, substance P, insulin and pro-insulin were comparable with that in the ileum. However, although aminopeptidase may be responsible for enkephalin degradation [69,78], it is worth noting that carboxypeptidase activity is greater in the buccal mucosa [5]. In contrast to enkephalins, aminopeptidases did not play an extensive role in the degradation of insulin. This is because despite the low proteolytic activity against insulin in the buccal mucosa, insulin absorption across this barrier was much less than that across the proteolytically more active nasal and rectal mucosae [1,80]. Unlike the mucosal barrier

Table 3
Selection of enzymes involved in the peptide degradation within buccal mucosa[a]

Enzyme	Substrate	Animal model	Localization	Authors	Year	Ref.
Aminopeptidase	4-Methoxy-2-naphthylamide of Leu, Ala, Arg, Glu	Human	Tissue homogenate	Tavakoli-Saberi et al.	1991	[75]
	GSH	Human	Absorption test	Hunjan and Evered	1985	[76]
	Oligoglycine	Pig	Tissue homogenate	Tucker et al.	1989	[77]
	Enkephalin, analogues	Rabbit	Tissue homogenate	Kashi and Lee	1986	[78]
	4-Methoxy-2-naphthylamide of Leu, Ala, Arg, Glu	Rabbit	Tissue homogenate	Stratford and Lee	1986	[69]
	L-Leucine-*p*-nitroanilide	Hamster, rat	Tissue homogenate	Harris and Robinson	1992	[60]
Aminopeptidase B		Porcine	Cytosol	Stratford and Lee	1986	[69]
Dipeptidyl peptidase	Enkephalin analogues	Rabbit	Tissue homogenate	Kashi and Lee	1986	[78]
Dipeptidyl carboxypeptidase	Enkephalin analogues	Rabbit	Tissue homogenate	Kashi and Lee	1986	[78]
Carboxypeptidase		Rat, hamster		Wearly et al.	1991	[5]
Deamidase	TRH	Rabbit		Dowty et al.	1992	[44]
Endopeptidase	Enkephalin analogues	Hamster, rat	Tissue homogenate	Harris and Robinson	1992	[60]
Leucine aminopeptidase	L-Leucine-*b*-naphthylamide	Rabbit, Guinea Pig	Tissue homogenate	Zhou and Po	1991	[79]
	L-Leucine-*b*-naphthylamide	Rat	Tissue homogenate	Zhou and Po	1990	[70]
Protease	Insulin	Rabbit	Tissue homogenate	Yamamoto et al.	1990	[80]
	Proinsulin	Rabbit	Tissue homogenate	Yamamoto et al.	1990	[80]
	Substance P	Rabbit	Tissue homogenate	Lee and Yamamoto	1990	[72]
		Human	Saliva	Watanabe et al.	1980	[81]
Non-specific esterases	L-Leucine-*p*-nitroanilide	Rat, hamster	Tissue homogenate	Garren and Repta	1988	[11]

[a] Leu, leucine; Ala, alanine; Arg, arginine; Glu, glutamic acid; TRH, thyrotropin-releasing hormone.

of the small intestine, the buccal mucosa lacks surface-bound peptidases [65]. Saliva, which ranges in pH from 6.8 to 7.2, contains a variety of enzymes such as esterases and carboxylesterase which could cause degradation of peptides and their prodrugs [82,83]. The low aminopeptidase activity would suggest that the buccal route offers interesting possibilities for delivering peptides and esters [70].

Another approach to characterize the proteolytic action is to incubate the peptide in mucosal tissue homogenates [1]. One obvious disadvantage using tissue homogenates is the inability to discriminate between cytosolic, membrane-bound and intercellular proteolytic activities [2,26]. Moreover, intracellular enzymes may be inaccessible for peptides which are paracellularly transported. To better understand the role of the enzymatic barrier to mucosal peptide absorption, it would be necessary to determine the type of proteases present, their location, their disposition in the mucosal cells, their substrate specificities and the probability of their contact with a peptidic drug. With respect to the possible intra- or paracellular routes of peptides absorption, the exact localization of mucosal proteolytic activity is important [84]. It should also be mentioned that degradation also occurs after passage through the mucosa from the exposure to extracellular and/or cytosolic enzymes in the blood and at the site of action.

2.4.2. Intracellular metabolism

Polypeptides and in particular smaller peptides, can be further hydrolyzed to free amino acids by intracellular peptidases. A small percentage of oligopeptides do escape the hydrolysis process and enter the blood intact if they reach the internal cellular compartment [85]. The buccal membrane is devoid of surface-bound peptidases, hence, peptide metabolism will occur intracellularly by aminopeptidases. Since most of the proteases are located within cells, observations of first-pass buccal metabolism of peptides would provide an important clue on the route by which peptides are transported intracellularly [86]. Since the proteolytic activity against leucine enkephalin and insulin is primarily cytosolic in the buccal mucosa [2], it is believed that these peptides, permeate the epithelium by way of the paracellular route, hence escaping extensive metabolism.

2.5. Transport mechanisms of peptides

Two main pathways seem to be implicated in peptide molecular transport across membranous tissues [30,32,85, 87,88]: (i) the intracellular pathway where peptides traverse the epithelium across the cells, (ii) the intercellular pathway where peptides diffuse through the intercellular lipids [56,57] (Fig. 3).

The transcellular route may involve permeation across the apical cell membrane, the intracellular space and the basolateral membrane either by passive transport (diffusion, pH partition) or by active transport (facilitated and carrier-mediated diffusion, endocytosis). The transcellular permeability of a peptide is a complex function of various physicochemical properties including size, lipophilicity, hydrogen bond potential, charge and conformation [89]. There are a few reports in the literature suggesting that small polar drugs penetrate buccal epithelium via the intracellular route [90,91]. One should also consider that transport via aqueous pores in the cell membranes of the epithelium is also possible for substances of low molar volume (80 cm^3/mol) [32].

The second route, available to substances of a wide range of molar volumes would be an intercellular route (paracellular route). Within the intercellular space, there probably exist at least two pathways, one is essentially a hydrophobic route through the lipidic bilayer, while the second is more hydrophilic and associated with the narrow aqueous regions adjacent to the polar head groups of the lipids. A consequence of these two pathways is that the substances having nearly equal solubility in water and oil, traverse using both routes [86]. Peptides are presumed to permeate through the aqueous pathways, i.e. the paracellular and aqueous pore paths. Paracellular transport occurs between the epithelial cells by passive diffusion across the intercellular junctional complex of the epithelium.

It has also been suggested that the oral mucosae contain active, carrier-mediated systems for small molecules such as

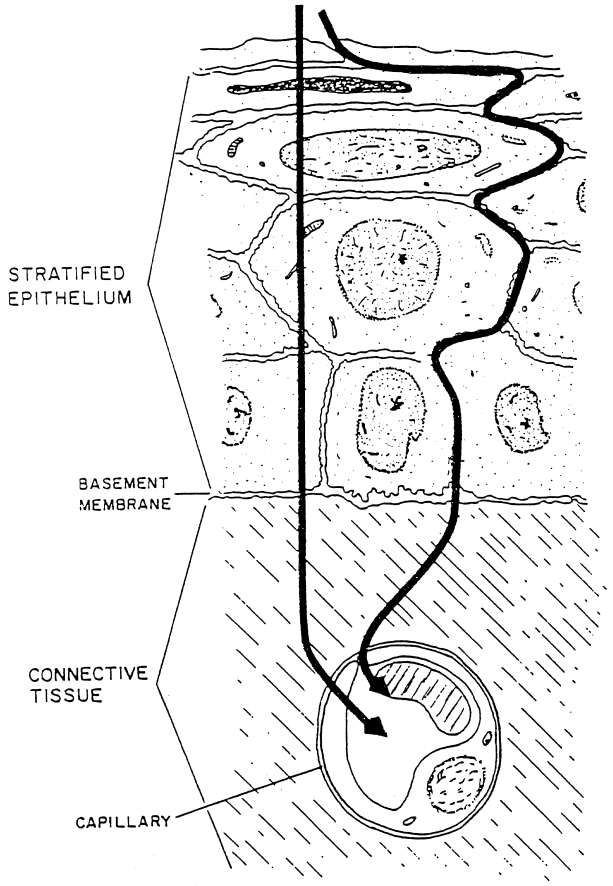

Fig. 3. Routes of transepithelial penetration; transcellular route vs. intercellular pathway. From Wertz et al. [86].

monosaccharides and amino-acids [60,92]. Small peptides such as di- and tri-peptides are thought to be transported by carrier-mediated systems [25]. But, these processes have not been fully characterized in terms of location, transport capacity or specificity in the buccal mucosa. Thyrotropin-releasing hormone (TRH) is buccally absorbed [93] but it has not been fully substantiated as to whether this tripeptide is transported by the active tripeptide system, as it is in the small intestine [94]. The similarities in buccal and intestinal stereospecific absorptions of the D- and L-forms of amino acid and glucose, have been cited as evidence that the buccal mucosa might be capable of carrier-mediated transport and may be a model membrane for the small intestine. The existence of carrier-mediated transport systems for certain compounds may explain the apparent saturation kinetics, mutual inhibition and partial sodium dependency sometimes observed [92].

From studies in rats and beagle dogs [10,95], it appears that peptide binding to the epithelium is an important feature for buccal absorption [58]. FITC-labelled dextran 4400, a hydrophilic compound [96], and thyrotropin [44] were used to show a slow onset of appearance of permeant in the systemic circulation and a depot-like behaviour in the oral mucosae, which were attributed to some form of binding within the mucosae [60]. However, this phenomenon is poorly understood and has not been systematically investigated.

3. Strategies to improve buccal peptide absorption

3.1. Chemical enhancers

3.1.1. Different classes of promoters used for buccal delivery

Relatively few studies have been carried out on the effect of absorption enhancers on peptide transport across buccal mucosa [88]. Most have only shown limited success (summarized in Table 4). The enhancers belong to various chemical classes including surfactants (anionic and non-ionic), bile salts, chelators, fatty acids, and alcohols.

The degree of enhancement depended on a number of factors, including the characteristics of the permeant, the composition of the delivery vehicle, and whether the tissue was pretreated with enhancer. It is also evident that the choice of an enhancer must be based on the following characteristics: effectiveness, safety, chemical inertness, lack of biological activity, and rapid reversibility (of its effects). It is fundamental for the penetration enhancer to be non-irritant, non-toxic and physiologically inactive although it seems to be less critical with the buccal epithelium than with other mucosal tissues.

It has been proposed that penetration enhancers improve mucosal peptide absorption:

1. By changing mucus rheology in reducing the viscosity and/or elasticity of mucus layer.
2. By increasing membrane fluidity and hence facilitating transcellular transport through interaction with their lipidic or proteic membrane components.
3. By facilitating paracellular transport.
4. By overcoming the enzymatic barrier for peptides: protease inhibitors for endo- and exo-peptidases are indirect potential penetration enhancers.
5. By increasing the thermodynamic activity of peptide drugs. This may be affected by the vehicle composition, which will influence solubility and micellization and also by ion-pair formation between the enhancer and the drug.

The most promising agents for buccal delivery are surfactants (Table 4). Their widespread use has created considerable interest; however, depending on the type of surfactant used, the concentration and exposure time, they can induce side effects such as protein denaturation or extraction, enzyme inactivation, swelling of tissue and extraction of lipid components.

Sodium lauryl sulfate (SLS) is an ionic surfactant, which disorganizes the entire membrane architecture affecting both protein and lipid structures. Expansion of intercellular spaces and insertion of SLS molecules into the lipid structure has also been observed [97]. SLS proved to be efficient in promoting an extensive enhancement of the buccal absorption of human calcitonin [98] and insulin [99]. Polyoxyethylene-9-lauryl ether (laureth 9), a non-ionic surfactant, was shown by Aungst and Rogers [104,105] to significantly promote insulin absorption through buccal mucosa when used at 5%, reaching approximately when 30% of the intramuscular route.

Bile salts make up another important class of natural or semi-synthetic surfactants. They have been often used to enhance the absorption of insulin across the mucous membranes from various sites depending on their lipophilicity [104,113,114]. The efficiency of insulin delivery is directly related to the lipophilicity/hydrophilicity ratio brought about hydroxy substitutions. Bile salts are thought to act by solubilizing epithelial lipids, possibly by micellization, thereby increasing the mucosal permeability. Their effect on the permeability has been reported to be reversible and dependent on their concentration. Hoogstraate et al. [115] showed that glucodeoxycholate (GDC) increased both the amount and the rate of buserelin absorption across the buccal mucosa. Moreover, a number of authors [95,105,107,108] have reported that a conjugated bile salt, sodium glycocholate, enhanced absorption of peptides. Peptide enhancement by other bile salts in other animal models has also been documented: Ebert et al. [109] in dogs, Oh and Ritschel [99] and Nakada et al. [111] in rats. Ishida et al. [108] reported a moderate bioavailability of 0.5% in dogs when insulin was administered together with sodium glycocholate in a cacao butter matrix. A study in rats [105] described a remarkable buccal bioefficacy of 25–30% as compared to an i.m. administration. In addition, Steward et al. [106] showed that sodium taurocholate was capable of

Table 4
Various classes of permeation enhancers used for buccal delivery of peptides

Class	Example	Concentration	Peptides	Authors	Year	Ref.
Synthetic surfactants	Sodium lauryl sulfate		Calcitonin	Nakada et al.	1988	[98]
	Sodium lauryl sulfate		Insulin	Oh and Ritschel	1990	[99]
	Lysophosphatidylcholin	2%	Insulin	Zhang et al.	1994	[100]
	Brij 35	1 mM	Insulin	Oh and Rischel	1990	[101]
	Various alkylglycosides	0.1–0.2 M	Insulin	Aungst	1996	[102]
	Alkylglycosides	5%	Insulin	Aungst	1994	[103]
	Quillajasaponin (quillayanin P-20)		Calcitonin	Nakada et al.	1988	[98]
Non-ionic surfactants	Laureth 9	5%	Insulin	Aungst and Rogers	1988	[104]
		0.5–5%	Insulin	Aungst and Rogers	1989	[105]
	Laureth 4	5%	Insulin			
	Polysorbate 80	5%	α-Interferon	Steward et al.	1994	[106]
	Polysorbate 20	5%	Insulin	Aungst and Rogers	1989	[105]
	PEG 8-laurate	5%	Insulin			
	PEG 4-laurate	5%	Insulin			
	Propylene glycol laurate	5%	Insulin			
	Sorbitan laurate	5%	Insulin			
	Glycerol monolaurate	5%	Insulin			
	Octoxynol-9	5%	Insulin			
	Cocomorpholine	5%	Insulin			
Steroidal surfactants	Sodium cholate	2%	Insulin	Zhang et al.	1994	[100]
Bile salts	Sodium glycocholate	5%	Insulin	Aungst et al.	1988	[107]
	Sodium glycocholate		Insulin	Ishida et al.	1981	[108]
	Sodium glycocholate	5%	Insulin	Aungst and Rogers	1989	[105]
	Sodium deoxycholate		Insulin	Oh and Ritschel	1990	[99]
	Sodium deoxycholate		Heparin	Ebert et al.	1994	[109]
	Sodium deoxycholate	5%	Insulin	Aungst and Rogers	1989	[105]
	Sodium glycodeoxycholate	0.45%	Buserelin	Hoogstraate et al.	1996	[115]
	Sodium taurocholate	1–4%	α-Interferon	Steward et al.	1994	[106]
	Sodium taurocholate	3%	Insulin	Zhang et al.	1994	[100]
	Sodium taurocholate	40 mM	Insulin	Ritschel et al.	1989	[110]
	Sodium taurocholate		Insulin	Oh and Ritschel	1990	[99]
	Various bile salts		Calcitonin	Nakada et al.	1989	[111]
	Sodium fusidate	1–5%	Insulin	Aungst and Rogers	1989	[105]
	CHAPS	1–5%	Insulin			
	BigCHAP	5%	Insulin			
Chelators	Disodium EDTA		Insulin	Aungst and Rogers	1988	[104]
	Disodium EDTA		Insulin	Oh and Ritschel	1990	[99]
	EDTA	0.067 M	Vasopressin	de Vries et al.	1990	[112]
	Sodium salicylate		Insulin	Aungst et al.	1988	[107]
Fatty acids and derivatives	Sodium myristate		Calcitonin	Nakada et al.	1988	[98]
	Sodium laurate	5%	Insulin	Aungst and Rogers	1989	[105]
	Palmitoylcarnitine	5%	Insulin			
Others	Sugar ester (sucrose palmitate) (Ryoto sugar ester P-1670)		Calcitonin	Nakada et al.	1988	[98]
	Lauroamphoglycinate	5%	Insulin	Aungst and Rogers	1989	[105]
	Lauramidopropylbetaine	5%	Insulin			
	Phosphatidylinositol	5%	Insulin			
	Phosphatidylcholine	5%	Insulin			
	Aminocaproic acid	5%	Insulin			
	Polyacrylic acid (in water)	0.25%	Insulin			
	Hyaluronidase	2900 units/ml	Insulin			
	Chondroitinase	5 units/ml	Insulin			

delivering a dose-dependent increase in α-interferon absorption and produced a greater than 10-fold improvement in the bioavailability. Bile salts may also reduce proteolytic enzyme activity.

There is little information on the effect of fatty acids on the buccal delivery of peptides. It has been shown that the effect of fatty acids depends on the presence and the position of double bonds, isomer type (*cis*- or *trans*-), chain length and the degree of branching. Unsaturated acids are usually more disruptive than their saturated counterparts having the same carbon number [105,116,117]. Sodium laurate (C_{12}) and myristate (C_{14}) have been used to promote the absorption of insulin [105] and calcitonin [98] through rat buccal mucosa in vivo.

1-Dodecylazacycloheptan-2-one (Azone) has been used to promote the oral mucosal absorption of octreotide (Sandostatin) [95]. Azone is a hydrophobic substance specifically developed as a skin penetration enhancer showing no protein interaction. It was demonstrated that Azone only enhanced intercellular drug diffusion [118]. If you consider the structure of buccal mucosa, the mechanism of Azone is probably the same in skin and in buccal mucosa. Hadgraft et al. [119] proposed that Azone was able to form ion pairs with anionic drugs thereby promoting their permeation.

A different approach adopted by Squier [120] and subsequently by Aungst and Rogers [105] was to modify enzymatically the surface by phospholipase, hyaluronidase, neuraminidase and chondroitinase in order to increase the buccal permeability to horseradish peroxidase in vitro. Chondroitinase was the most efficient agent with negligible tissue damage.

3.1.2. Vehicles and adjuvants (co-solvent)

In the most simple forms of application, a drug can be dissolved or dispersed in a solvent to improve transport. Basically, the effects can be categorized as follows: (a) change in the thermodynamic activity (by increasing the degree of saturation in the vehicle); (b) increasing the drug solubility in the epithelial barrier of the buccal mucosa (i.e. facilitate partitioning of drug from the vehicle in the mucosa). It is often difficult to distinguish between possible modes of action since several causes affect the global process.

Because of the similarities between buccal mucosa and the skin, chemical enhancers and vehicles that increase transdermal delivery have also been used on the buccal mucosa (Table 5). Ten percent lauric acid in propylene glycol was the most effective for buccal insulin absorption [105]. Ethanol at different concentrations (5 and 30%) was also effective in improving peptide absorption [106,121]. Furthermore, Mollgard et al. [122] showed that two or more permeation enhancers when applied together could act synergistically.

3.2. Enzyme inhibitors

To date, there has only been a limited evaluation of enzyme inhibitors in buccal mucosal delivery in contrast to the extensive work done with respect to nasal delivery [7,123]. Table 6 lists some protease inhibitors, such as aprotinin [80,104], bestatin [11,69], puromycin [69] and bile salts [74,101], which have been tested and shown to stabilize peptides against buccal mucosal enzymes.

Yamamoto et al. [80] demonstrated that 0.01% aprotinin, a serine protease inhibitor, reduced the metabolism of insulin and proinsulin by approximately 70–80% within 2.5 h in homogenates of the albino rabbit buccal mucosa, which would otherwise have occurred at a rapid rate. Moreover, the in vivo application of bestatin and puromycin with 4-methoxy-2-naphthylamides of leucine, alanine, arginine and glutamic acid reduced the overall enzymatic activity. However, despite their action on peptide stability in buccal tissue homogenates, these compounds did not display any significant effects upon buccal administration in vivo [1]. For example, Aungst and Rogers [102,103] observed no improvement of buccal insulin bioefficacy in rats, upon co-administration with either aprotinin or a peptidase-inhibiting pentapeptide (Z-Gly-Pro-Leu-Gly-Pro). Peptidase inhibitors can be used alone or in combination with permeation enhancers to overcome both enzymatic and physicochemical barriers to permeation [74]. Moreover, some permeation enhancers may also increase the stability of peptides by altering the conformation of the peptide, rendering it less accessible to enzymatic degradation. Garren and Repta [11] studied the simultaneous diffusion and metabolism of leucine-*p*-nitroanilide, a substrate for aminopeptidase, across excised hamster cheek pouch. It was fully hydrolysed as it diffused through the buccal mucosal barrier. However, in the presence of an aminopeptidase inhibitor (bestatin), its metabolism was substantially reduced. Combinations of enzymatic inhibitors have been used to better stabilize peptides because more than one enzyme is implicated in their degradation. Such an approach has been successful in promoting oral absorption of the peptides pentagastrin and an analogue of the nonapeptide renin inhibitor and insulin [72].

Peptide stability can also be improved and enzymatic metabolism reduced, to some extent, by modification of the peptide's structure. For example, substitution of unnatural amino acids, i.e. D-amino acids for L-amino acids, in

Table 5
Buccally administered using non aqueous vehicles with or without adjuvant

Vehicle	Peptide	Concentration	Authors	Year	Ref.
Dimethylsulfoxide (DMSO)	Insulin	50 units/kg	Aungst and Rogers	1988	[105]
N-Methylpyrrolidone (NMP)	Insulin	50 units/kg			
5% lauric acid in NMP	Insulin	10 units/kg			
5% Decylmethylsulfoxide in NMP	Insulin	10 units/kg			
5% lauric acid in NMP in propylene glycol (PG)	Insulin	10 units/kg			
10% lauric acid in PG	Insulin	10 units/kg			
5% ethanol	α-Interferon		Steward et al.	1994	[106]
30% ethanol	Trp-Leu	6 mg/ml	Veuillez et al.	1998	[121]

Table 6
Enzyme inhibitors used to control buccal peptide degradation

Enzyme inhibitor	Substrate	Authors	Year	Ref.
Aprotinin	Insulin	Aungst and Rogers	1988	[104]
	Proinsulin	Yamamoto et al.	1990	[80]
Z-Gly-Pro-Leu-Gly-Pro	Insulin	Aungst and Rogers	1989	[105]
Bestatin	4-Methoxy-2-naphthylamides of leucine, alanine, arginine and glutamic acid	Tavakoli-Saberi and Audus	1993	[124]
		Stratford and Lee	1986	[69]
	Leucine-*p*-nitroanilide	Garren and Repta	1988	[11]
p-Chloromercuriphenylsulfonic acid	Proinsulin	Yamamoto et al.	1990	[80]
Puromycin	4-Methoxy-2-naphthylamides of leucine, alanine, arginine and glutamic acid	Tavakoli-Saberi and Audus	1993	[124]
		Stratford and Lee	1986	[69]
Sodium glycocholate	Insulin	Aungst	1994	[74]
Brij-35	Insulin	Oh and Ritschel	1990	[101]

the primary structure. Other methods include the introduction of conformational constraints by reversing the direction of the peptide backbone [7] or inverting the chirality of each amino acid. Several examples may be cited. The substitution of D-Ala for L-Gly at the N-terminal position in methionine enkephalin and the amidation of its C-terminal methionine produce a more stable analogue Tyr-D-Ala-Phe-Gly-Met-NH_2 [125].

3.3. Lipophilicity modification

As shown above, an important factor for the passage of peptide through biological membranes is the hydrophobicity. A potential useful approach is the chemical modification of peptides to produce prodrugs and analogues. However, this approach is more useful for small peptides with fewer than ten amino acid residues [71]. The idea behind such conversion, is to protect the peptides against degradation by enzymes present at the mucosal barrier and to render them more lipophilic, thereby improving peptide transport [126].

3.3.1. Acylation

One method to increase lipophilicity is through conjugation of the N-terminal with lipophilic molecules by acylation or alkylation [1,72,126] (Fig. 4). The conjugation of fatty acids to peptides has been used to improve lipophilicity.

Many studies on peptide acylation have been carried out concerning intestinal route. It was shown previously that new acyl derivatives of peptide drugs such as tetragastrin [129–132], TRH [133–136], calcitonin [137], insulin [138,139], lysozyme [140] and DADLE (enkephalin analogue) [141] improved their enteral absorption while retaining their pharmacological activities. Bungaard et al. [142] synthesized various peptide derivatives of TRH, LHRH, neurotensin, pepsin, gastrin, fibrinopeptides and collagen containing the pyroglutamyl group. It was found that lipophilic modification of these peptides resulted in a significant increase in their enteral absorption by overcoming the poor permeability and enzymatic instability of the native agents. Asada et al. [139] acylated bovine-insulin with one or two of the short fatty acids (6 carbons), caproic acid, medium length (12 carbons), lauric acid and/or long chain (16 carbons) palmitic acid. The observed enhancement was attributed to the increased lipophilicity and also to inhibition of insulin self association [143]. It is possible that the increased absorption of insulin by acylation may be related to the aggregation of its molecules. Although the usefulness of chemical derivatisation has been clearly established for the gastro-intestinal tract, to date, there are only a few examples for the buccal route (Fig. 4).

Veillard et al. [127,128] evaluated a lauryl-TRH tripeptide derivative in order to increase buccal absorption in vitro and in vivo. In that study, it was shown that a lauryl derivative of a tripeptide (RP-56142) was absorbed from a buccal patch. In contrast, a myristoylated model dipeptide synthesized by Veuillez et al. [121], was found to be so lipophilic that it accumulated in the buccal epithelium and was unable to cross the entire mucosal barrier.

3.3.2. Prodrugs

Prodrugs are a particular case of chemical modification where the peptide has no pharmacological activity itself and only elicits activity after conversion to the parent drug. For example, lauryl-TRH was gradually converted to TRH [134,135] in the plasma. Such a modification is only interesting if the modified and inactive peptide can be quantita-

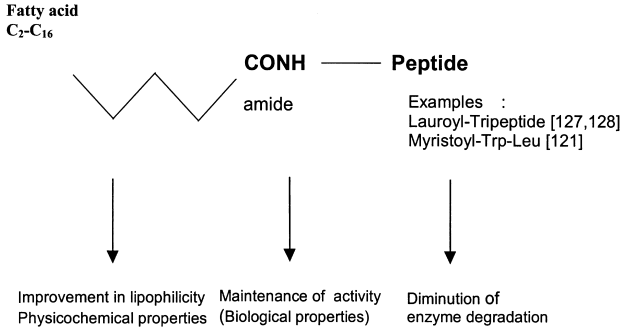

Fig. 4. Conjugation of acyl chain to amino terminus of peptide.

tively converted to its active form. Thus, bioreversible transformation may protect peptides against degradation by peptidases present at the mucosal absorption barrier, render the peptides more lipophilic and hence facilitate the absorption [144].

3.3.3. Conjugation with polymers

Structural modifications can be obtained by the covalent conjugation of peptides to polymers. Conjugation may also increase peptide solubility, stability, and plasma half-life and decrease the immunogenicity because the polymer would partially or totally cover the immunogenic sites of the peptides [145]. Increased lipophilicity was achieved by conjugation with lipophilic polymers such as polystyrene-co-maleic acid/anhydride (SMA) [146].

Chemical modification of peptides has also been reported with several macromolecules such as polyethyleneglycol (PEG), poly(styrene maleic acid), copolymer (SM), albumin and dextrans [147]. Generally, these polymers must of course be water-soluble, biocompatible, and non-immunogenic. Such approaches, although promising on paper, have not yet been extensively tested on buccal mucosa.

3.3.4. Methylation

Hydrogen bond potential and the lipophilicity of peptides can be modified by methylation. This often leads to conformational change of the peptides and thereby may increase the permeability of the drug across the cell membrane. For example, when four methyl groups were added to the peptide, $AcPhe_3NH_2$, it was found that the penetration rate through Caco-2 cell membrane was significantly increased [50]. The derivatives must be cleaved spontaneously or enzymatically in the blood following their absorption to release the parent bioactive peptide [142,148]. Masking of the carboxylic acid function in the acylated amino acids, by forming 5-oxazolidinones derivatives which are considerably more lipophilic than the parent compounds at physiological pH, increases the peptide's ability to penetrate biomembranes [148].

3.4. Formulation design

Essentially, research has revolved around the development of strategies extending of the lifetime of the drug and hence improving its efficacy. The drug delivery system must ensure the release of the drug in a controlled manner and at a sufficiently high concentration to the mucosal surface. Several approaches have been considered using buccal delivery systems.

3.4.1. Non-'attached' drug delivery systems

Three types of non-attached drug delivery systems can be defined: (i) fast-dissolving tablet dosage forms, (ii) chewing-gum formulations and (iii) microporous hollow fibres. Few studies have been reported concerning buccal peptide absorption. Burnside et al. [149] described a microporous hollow fiber of polysulfone (molecular weight cut-off: 500 000 Da) intended to be placed in the buccal cavity for the delivery of histrelin, an LHRH agonist. Preliminary experiments showed that the delivery rate could be adjusted and prolonged for up to 6 h. However, the lack of intimate contact with the mucosa may not be favourable for peptide absorption because of eventual enzymatic degradation in saliva. Despite some interesting results, non-attached buccal mucosal drug delivery has many drawbacks due to the local physiological environment, e.g. the presence of saliva and the intake of foods or liquids.

3.4.2. 'Immobilized' drug delivery systems (bioadhesive delivery systems)

Buccal mucosal drug delivery systems designed to remain in contact with the buccal mucosa for prolonged periods have been the subject of growing interest. Such systems offer advantages over non-attached systems. These include: (i) immobilization allowing an intimate contact between the drug dosage form and the buccal mucosa; (ii) a high drug concentration, maintained at the absorptive surface for an extended period of time; (iii) the device can be immobilized specifically at the buccal mucosa, protecting the peptide from environmental degradation. A combination of different properties within a unique system can be obtained by the use of polymers and immobilization by bioadhesion or mucoadhesion. This concept has been applied to different systems including sustained release tablets, semi-solid dosage forms, films and patches [150].

Buccal mucosal bioadhesive patches are a new type of external preparation permitting an effective, local or systemic safe treatment. The most successful approach for buccal mucosal delivery of peptides has been a bioadhesive formulation. Bioadhesive systems enhance buccal peptide absorption by increasing the contact time with the mucosa. Many mucoadhesive biocompatible polymers including cellulose derivatives, poly(acrylates), gelatin, agarose as well as other naturally occurring polymers, such as hyaluronic acid and chitosan, have been described [150,151]. Merkle et al. [152–156] investigated a number of polymers and different geometries for the design of patches for the delivery of different peptides. Polyacrylic-based hydrogels have also been extensively studied. Kellaway et al. [157–159] have prepared hydrogels by reacting polyacrylic acid (MW: 450 000) with sucrose, glycerol or a non-ionic surfactant for the delivery of oxytocin. Recently, oxytocin was incorporated into a polymeric patch matrix [87] or into a custom coformulation of carbopol 974P and silicone [160]. These systems exhibited increased peptide delivery when applied directly to a freshly excised rabbit mucosa.

Bioadhesive polymers, such as polycarbophil, have also been shown to stabilize peptides by inhibiting proteolytic activity [160,161]. Carbopol 934P, 971P and 974P strongly inhibit proteolysis of insulin, calcitonin and insulin-like growth factor-I [145]. The mechanisms of bioadhesion

and methods of evaluation have been reviewed by Peppas and Buri [162]. Mucoadhesive polymers mainly improve absorption by direct binding to the mucus layer which covers the surface of the buccal epithelium. However, the duration of bioadhesion is largely limited due to the fast turnover rate of the mucus layer. Furthermore, the non-specificity of most bioadhesion processes can be strongly affected by various factors such as fluid pH, food intake and composition leading to the concomitant use of penetration enhancers as in the case of LHRH [115,163]. Various examples of bioadhesive formulations of peptides (with or without enhancers) are listed in Table 7. Permeation enhancers and enzyme inhibitors can also be added to transbuccal tablet formulations of insulin and calcitonin [101,164].

3.4.3. Liposomes

Liposomal formulations with encapsulated drugs have been investigated for buccal administration [172–176]. Applications of liposomal formulation in buccal delivery resulted in an increase of local, and a decrease of systemic, drug concentration [172]. Peptide entrapment within liposome is also possible [177]. Buccal administration of human insulin in streptozocin-diabetic rats was investigated and no significant difference in the blood glucose level profile was observed after administration of liposome-vesicles containing insulin (LEV-INS) [178]. However, Petelin et al. [179] showed that liposomes limit the transport of hydrophilic substances to the superficial layer of the epithelium. Among the hydrophilic polymers, polymethylmethacrylate was found to be the most appropriate mucoadhesive ointment for local application in the oral cavity since the liposomes were shown to be more stable in this polymer. Incorporation of protease inhibitors is one approach to improving the performance of less effective liposome peptide delivery systems; for example, aprotinin can be incorporated into Factor VIII loaded liposomes, made up of lecithin and phosphatidic acid [145].

4. Conclusions

Buccal delivery has many advantages, including the avoidance of hepatic first-pass metabolism, the membrane is robust and readily accessible and has high patient compliance and it certainly offer to the pharmacologist and clinicians the opportunity to deliver peptides. However, as the buccal mucosa is an effective permeability barrier, several strategies have been proposed to improve absorption. A number of factors define and limit the absorption of drugs from the buccal mucosa, including the histology of the mucosal site, environmental factors such as the presence of saliva, its pH and the movements of the buccal tissues, and by enzymatic activity on and in the mucosa. In addition, peptides must be stable and must be retained on the buccal mucosa for extended periods of time. Several factors have

Table 7
Different bioadhesive peptide formulations used for buccal delivery[a]

Peptide	Enhancer	Method formulation	Animal model	Ref.
Thyrotropin-releasing hormone (TRH, protirelin)		Mucoadhesive patch	Rat; in vitro, in vivo	[165,166]
		Bioadhesive patch	Rat; in vitro, in vivo	[152,156]
		Bioadhesive patch	Rat, human; in vivo	[167]
RP-56142 (lauroyl derivative of a tripeptide)		Buccal patch	Dog; in vivo	[128]
Octreotide acetate (somatostatin analogue)		Buccal patch	Beagle dog; in vivo	[10]
		Buccal patch	Cat; in vivo	[153]
Oxytocin		Buccal tablet	Human; in vivo	[72]
		Mucoadhesive patch	Rabbit; in vitro	[160]
		Polymeric patch matrix	Rabbit; in vitro	[91]
		Patch bilaminated (hydrogel)	Cat; in vitro	[157]
		Hydrogel disc	Cat; in vitro, in vivo	[158,159]
Calcitonin		Buccal tablet	Dog; in vivo	[164]
Insulin	SGC; cacao butter	Bioadhesive tablet	Beagle dog	[168]
Luteinizing hormone-releasing hormone (LHRH)	Bile salt 5%	Field-shape bilayer Mucoadhesive device	Beagle dog; in vivo	[163]
Deslorelin (LHRH agonist)		Bioadhesive polymeric matrix	Ovines; in vivo	[169]
Buserelin (LHRH agonist)	GDC 10 mM	Bioadhesive patch	Pig; in vivo	[115]
		Bioadhesive patch	Rat; in vitro	[154,167]
		Bioadhesive patch	Human, in vivo	[154]
Glucagon-like peptide		Buccal tablet	Human; in vivo	[170]
Leuenkephalin	SGC + enzyme inhibitor	Bioadhesive patch	Cat, in vitro	[171]
Octreotide (Sandostatin)	Azone® 3%	Bioadhesive patch	Beagle dog; in vivo	[95]
	SGC 4%	Bioadhesive patch	Beagle dog; in vivo	[95]
		Buccal patch	Beagle dog; in vivo	[10]
		Buccal patch	Cat; in vivo	[153]

[a] GDC, glycodeoxycholate; SGC, sodium glycocholate.

been proposed that affect buccal peptide absorption: (i) the existence of non-passive mechanisms of buccal absorption, such as carrier-mediated transport and endocytosis; (ii) the physicochemical properties of peptides that may or may not favour their transport via the paracellular pathway; (iii) the mechanism of action of penetration enhancers, and the criteria of defining local and systemic toxicities. New approaches have been suggested for improving buccal peptide absorption: (i) molecular modifications to the peptide such as synthesis of prodrugs; (ii) development of novel delivery systems.

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