International Journal of Pharmaceutics, 75 (1991) 97–115

ADONIS 037851739100307L

IJP 02512

Invited Reviews

Peptide and protein drugs: I. Therapeutic applications, absorption and parenteral administration

X.H. Zhou and A. Li Wan Po

Drug Delivery Research Group, The School of Pharmacy, The Queen's University of Belfast, 97 Lisburn Road, Belfast BT9 7BL (U.K.)

(Received 20 February 1991)
(Modified version received 4 May 1991)
(Accepted 10 May 1991)

Key words: Peptide delivery; Protein delivery; Stability; Bioavailability; Absorption barrier; Proteolytic activity; Absorption enhancer; Proteinase inhibitor; Liposome

Summary

In this first part of a two-part review of peptide and protein drugs, the pertinent terminology is introduced and the therapeutic applications of those drugs summarised. Their absorption and the methodology commonly used for study on it are discussed. Approaches to optimising delivery of the peptide and protein drugs are highlighted.

Introduction

With the recent advances in recombinant DNA technology, the commercial production of proteins and peptides for pharmaceutical purpose is now routine. The list of available therapeutic agents produced by this technology is expanding rapidly to include interferon, macrophage activation factors, tissue plasminogen activator, neuropeptides and experimental agents that may have potential in cardiovascular disease, inflammation, contraception and so on. Unfortunately, protein and peptide drugs possess some chemical and physical properties, including molecular size, susceptibility to proteolytic breakdown, rapid plasma clearance, immunogenicity and denaturation, which make them unsuitable for delivery using the normal absorption routes and in particular, the oral route. In part one of this review protein and peptide drugs are considered with particular emphasis on their pharmacological profiles, potential routes of delivery and their associated problems.

Recent major reviews on the subject include the general article by Gardner (1984) on the intestinal absorption of intact peptides and proteins and that by Humphrey and Ringrose (1986) on the absorption, metabolism and excretion of peptide and related drugs. In a further review, Lee (1988) discussed enzymic barriers to peptide and protein absorption. Banga and Chien (1988)

Correspondence: A. Li Wan Po, Drug Delivery Research Group, The School of Pharmacy, The Queen's University of Belfast, 97 Lisburn Road, Belfast BT9 7BL, U.K.

broadened the scope and considered systemic delivery of those agents in general.

Terminology

Peptide or protein drugs are derived from amino acids by peptide bond linkages. Proteins are large peptides. Peptides containing less than eight amino acid residues are called small peptides. Peptide drugs in this group include enalapril, lisinopril and thyroid releasing hormone analogues. The term polypeptide drugs refers to peptide drugs with eight or more amino acid residues and includes cyclosporin, leuproline and luliberin. Polypeptide drugs containing from about 50 to as many as 2500 amino acid residues are named protein drugs. These include insulin, growth hormone and interferons. Some protein drugs, such as insulin or IgG containing two or more polypeptide chains, are called oligomeric proteins and their component chains are termed subunits or protomers.

Therapeutic Uses of Peptide and Protein Drugs

Peptide and protein drugs can be conveniently classified according to their activity profiles as follows:

Enzymes

Some exogenous enzymes have been used as enzyme replacement therapy in the treatment of enzyme deficiency diseases such as lysosomal storage and mannosidosis (Table 1). Because enzyme deficiency in humans is usually genetic in origin, enzyme replacement is often the only available therapy. Some exogenous enzymes have also been utilized in the treatment of diseases other than inborn enzyme deficiency. Good examples include t-PA (tissue plasminogen activators), urokinase and streptokinase. These enzymes activate circulating plasminogen and fibrin clot-associated plasminogen equally well and, because of this, they have been marketed in the U.K. and U.S.A. (Robinson and Sobel, 1986; *British National Formulary*, 1989). Thrombin-like enzymes of snake venoms have also been developed for dissolving blood clots through enhanced release of fibrinopeptides from fibrinogen (Kornalik, 1985).

Hormones

Hormones represent the largest class of protein or peptide drugs used in medical therapy. All hormones have 'target cells' on which they act and these may be located in a specific organ or be more widely distributed in the body. Some hor-

TABLE 1

Therapeutic application of some enzymes

Enzymes	Therapeutic application	Reference
Adenosine deaminase	Enzyme deficiency	Hershfield et al. (1987)
Dextranase	Lysosomal storage	Colley and Ryman (1974)
β-Fructofuranosidase	Storage disease	Gregoriadis and Ryman (1972b)
α-Mannosidase	Mannosidosis	Patel and Ryman (1974) Fishman and Citri (1975)
L-Asparaginase	Cancer	Abuchowski et al. (1984)
β-Glucosidase	Adult Gaucher's disease	Braidman and Gregoriadis (1976)
Tissue plasminogen activators	Thrombosis	Robinson and Sobel (1986)
Urokinase	Thrombosis	Robinson and Sobel (1986)
Streptokinase	Thrombosis	Robinson and Sobel (1986)
Thrombin-like enzymes of snake venoms	Thrombosis	Kornalik (1985)

mones like luliberin (luteinizing hormone releasing hormone, LHRH) function solely to bring about the release of other hormones from different endocrine glands. It is also well known that many hormones act by means of a second messenger and quite often this is cyclic AMP (cAMP) which is formed from ATP. On reaching its receptor in the cell membrane, the hormone causes the release of cAMP, which is the actual regulator of the metabolic process. In this way, the physiological effect of one molecule of the hormone is amplified many times (Wills, 1985). Because hormones are very specific and a tiny amount can produce large pharmacological effects, they are ideal for biotechnological development which is more suitable for relatively small outputs. Perhaps the best known hormone drug is insulin which has been used as an endocrinotherapeutic agent since the 1920's (Banting and Best, 1922).

Enzyme inhibitors

Enzyme inhibitors have been used as drugs for a long time. These include proteins such as aprotinin, and peptide drugs such as enalapril and lisinopril. Captopril is an inhibitor of angiotensin converting enzyme (ACE), which catalyses in vivo generation of angiotensin II from the decapeptide, angiotensin I, to constrict arterioles and increase cardiac output, leading to hypertension in man. Captopril is now a widely used antihypertensive agent (Romankiewicz et al., 1983). Enalapril and lisinopril are subsequent developments which are also becoming widely adopted for the treatment of hypertension and congestive heart failure (Todd and Heel, 1986; Lancaster and Todd, 1988).

Antimicrobial agents

A number of antimicrobial agents are peptide drugs, for example, the penicillins, cephalosporins, polymyxin B sulphate, actinomycin and bleomycin. Structurally, these drugs are small peptides, mostly containing a non-peptide moiety. All of these antimicrobial drugs are microbial metabolites.

Immunomodulating peptides and proteins

Endogenous immunomodulating agents

These agents are now produced by molecular genetic approaches. Well-known examples are the interferons (IFNs) which are families of inducible secretory proteins produced by eukaryotic cells in response to viral and other stimuli. Interferons are not directly antiviral but they act prophylactically by inducing antiviral proteins. These protect cells from viral infection by inhibiting virus-directed translation and transcription (Moore and Dawson, 1989). Another example is interleukin-2 (IL-2) which exerts its biological effect through cell surface receptors on activated T and B cells and on NK cells (natural killer cell). Interleukin-2 has been administered clinically in attempts to restore immunocompetence in patients suffering from the acquired immunodeficiency syndrome (AIDS), and to improve the immunocompetence of cancer patients (Dawson and Moore, 1989).

Exogenous immunomodulating agents

Some exogenous immunomodulating agents are also used to promote immunocompetence in man. For example, cyclosporin (CS-4), a cyclic undecapeptide which is isolated from *Tolypocladium inflatum* Gams, is widely used as an immunosuppressive (Calne et al., 1978; Cantarovich et al., 1987; Mehta et al., 1988; Borel, 1989), whereas muramyl dipeptide has been used as an immunological adjuvant (Kreuger et al., 1984; Bomford, 1989).

Vaccines

Vaccines derived from the infective microorganisms are introduced into the mammalian body to induce antibody formation against the pathogens. Well-known examples include measles vaccine and polio vaccine. It is anticipated that an increasing number of such vaccines will be biotechnologically produced, to give more specific and pronounced antigenic responses.

Absorption of Peptide and Protein Drugs

Analytical problems

Several methods have been employed for studying the absorption of peptide and protein

drugs. However, high molecular weight proteins and polypeptides present some unique difficulties. Techniques such as gel filtration and ion-exchange HPLC usually have to be used. Even so, it is still very difficult to assay them in the presence of body fluids such as blood and urine. In such cases, radioassays or radioimmunoassays are often the most appropriate and hence, these techniques have been widely used in the measurement of the bioavailability of peptide or protein drugs. However, radioassays may be non-specific, and many chemical assay procedures may by themselves influence the conformation of protein drugs, thereby causing the loss of their biological activities. The entity being chemically assayed may not be the biologically active moiety and in such cases, in vitro or in vivo bioassays are often used during absorption studies. For protein/peptide hormones, the measurement of pharmacological responses may be the assay method of choice. For enzymes or enzyme inhibitors, specific enzyme reactions may be the best analytical method. The bioavailability of immunomodulating and antimicrobial agents may be evaluated using some specific animal models and indicator microorganisms. For example, the prophylactic

TABLE 2

Instability of protein and peptide drugs

Effect factor	Protein or peptide drugs	Reference
Physical instability		
Aggregation	Interferon-γ	Hsu and Arakawa (1985)
		Arakawa et al. (1987)
	Bovine growth hormone	Brems et al. (1986)
		Brems et al. (1988)
Precipitation	Insulin	Brennan et al. (1985)
		Lougheed et al. (1980)
Chemical instability		
β Elimination	Lysozyme	Nashef et al. (1977)
	Phosvitin	Sen et al. (1977)
Deamidation	Bovine growth hormone	Lewis and Cheever (1965)
	Human growth hormone	Lewis et al. (1970)
		Becker et al. (1988)
	Insulin	Berson and Yalow (1966)
		Fisher and Porter (1981)
	r-Immunoglobulin	Minta and Painter (1972)
	Epidermal growth factor	Diaugustine et al. (1987)
	Prolactin	Graf et al. (1970)
	Gastrin releasing peptide	McDonald et al. (1983)
	ACTH	Graf et al. (1971)
		Bhatt et al. (1990)
Disulphide exchange	Lysozyme	Volkin and Klibanov (1987)
	Ribonuclease A	Zale and Klibanov (1986)
Racemization	ACTH	Geiger and Clarke (1987)
		Meinwald et al. (1986)
Oxidation	Corticotropin	Dedman et al. (1961)
	α-, β-Melanotropins	Dixon (1956)
	Parathyroid hormone	Tashjian et al. (1964)
	Gastrin	Morley et al. (1965)
	Calcitonin	Riniker et al. (1968)
	Corticotropin releasing factor	Vale et al. (1981)

TABLE 3

Liposomes as peptide and protein carrier

Liposome composition	Peptide or protein	Route	Animal model	Reference
Phosphatidyl-choline : cholesterol 7 : 2	semipurified glucocerebroside β-glucosidase	i.v.	man	Belchetz et al. (1977)
Phosphatidyl-choline : cholesterol 7 : 7	highly purified glucocerebroside β-glucosidase	i.v.	man	Gregoriadis et al. (1982)
Phosphatidyl-choline : cholesterol : phosphatidic acid 7 : 2 : 1	bacterial amyloglucosidase	i.v.	man	Tyrell et al. (1976)
Dimyristoyl phosphatidyl-choline : choles-terol : dicetyl phosphate 1 : 0.75 : 0.11	cholera toxin human malaria sporozoite antigen	i.v.	rabbit	Alving et al. (1986)
Phosphatidyli-nositol	insulin	i.v.	mouse rat	Dapergolas and Gregoriadis (1976)
Phosphatidyl-choline : choles-terol : dicetyl phosphate 10 : 2 : 1	insulin	oral	rat	Patel and Ryman (1976)
Phosphatidyl-choline : choles-terol : dicetyl phosphate 3 : 9 : 1	insulin	oral	rat	Tanaka et al. (1975)
Phosphatidyl-choline : phos-phatidylserine 7 : 3	muramyl peptide	i.v.	mouse guinea-pig	Fidler et al. (1985)
Phosphatidyl-choline : choles-terol : dicetyl phosphate 7 : 1 : 2	lysozyme			Sessa and Weissmann (1970)
Phosphatidyl-choline : choles-terol : phospha-tidic acid 20 : 1.5 : 0.2	adenovirus type 5 hexon protein	i.v.	mouse	Six et al. (1988)
Phosphatidyl-choline : choles-terol : phospha-tidic acid 7 : 1 : 2	lysozyme			Sessa and Weissmann (1970)

(continued)

TABLE 3 *(continued)*

Liposome composition	Peptide or protein	Route	Animal model	Reference
Microcapsules	catalase	i.s.	mouse	Chang and Poznansky (1968)
Phosphatidylcholine : cholesterol : dicetyl phosphate 7:2:1	amyloglucosidase	i.v.	rat	Gregoriadis and Ryman (1972a)
Phosphatidylcholine : cholesterol : phosphatidic acid 7:2:1	yeast invertase	i.v.	rat	Gregoriadis and Ryman (1972b)
Phosphatidylcholine : cholesterol : phosphatidic acid 7:2:1	neuraminidase	i.v.	rat	Gregoriadis et al. (1974a)
Phosphatidylcholine : cholesterol : phosphatidic acid 7:2:1	dextranase	i.v.	rat	Colley and Ryman (1974)
Phosphatidylcholine : cholesterol : phosphatidic acid 7:2:1	α-mannosidase	i.v.	rat	Patel and Ryman (1974)
Dipalmitoylphosphatidylcholine	α-amylase		amoeba	Batzri and Korn (1975)
Phosphatidylcholine : cholesterol : phosphatidic acid 7:2:1	horseradish peroxidase		rat	Wisse and Gregoriadis (1975)
Phosphatidylcholine : cholesterol : Phosphatidic acid 7:2:1	asparaginase	i.v.	mouse	Neerunjun and Gregoriadis (1976)
Phosphatidylinositol	glucose oxidase	i.v.	mouse	Dapergolas et al. (1976)
Phosphatidylcholine : cholesterol 7:2	albumin	i.v.	rat	Gregoriadis and Neerunjun (1974)
Phosphatidylcholine : cholesterol : phosphatidic acid 7:2:1	albumin	i.v.	man	Gregoriadis et al. (1974b)

(continued)

TABLE 3 *(continued)*

Liposome composition	Peptide or protein	Route	Animal model	Reference
Phosphatidyl-choline : cholesterol : dicetyl phosphate 6 : 6 : 2	albumin	i.v.	mouse	Heath et al. (1976)
Phosphatidyl-choline : cholesterol : dicetyl phosphate 7 : 2 : 1	diphtheria toxoid	i.v.	mouse	Gregoriadis and Alison (1974)
Phosphatidyl-choline : cholesterol : phosphatidic acid 7 : 2 : 1	fetuin	i.v.	rat	Gregoriadis and Neerunjun (1975)
Phosphatidyl-choline : cholesterol : phosphatidic acid 4 : 2 : 1	anti-α-glucosidase	i.v.	rat	De Barsy et al. (1975)
Phosphatidyl-choline : cholesterol : phosphatidylethanolamine 10 : 10 : 1	monoclonal anti-Thy1 IgG1	i.v.	mouse	Debs et al. (1987)
Distearoylphosphatidylcholine: (2-puridyldithio)-propionol-dipalmitoylphosphatidylcholine: cholesterol 0.99 : 0.01 : 1	monoclonal anti-Thy1 IgG1	i.v.	mouse	Wolff and Gregoriadis (1984)
[a] Liposomes	TRH	i.v.	cat	Kumashiro et al. (1986)
Phosphatidyl-choline : phosphatidylserine 7 : 3	superoxide dismutase	intratracheal injection	rat	Padmanabhan et al. (1985)
Phosphatidylcholine: phosphatidic acid 15.3 : 0.1	factor VIII	oral	man	Sakuragawa et al. (1985)
Dipalmitoyl-phosphatidyl-choline : cholesterol : *m*-maleimidobenzoyl-(dipalmitoyl-phosphatidyl)-ethanolamine 25 : 17.5 : 2.5	subunits of monoclonal IgM	i.p.	mouse	Hashimoto et al. (1986)

[a] The composition of liposomes was not mentioned in the paper.

usefulness of intranasal IFN-β against rhinovirus infection was determined using healthy volunteers or animals (Higgins et al., 1986).

Assay methods available for monitoring the absorption of small peptide drugs are freely available and routine methods include reverse-phase HPLC, TLC, and fluorescence techniques.

Stability

Irrespective of which dosage form is used, peptide or protein decomposition may be a problem. Drug breakdown can take place both in the formulation and when present in tissue fluids. First pass metabolism and enzymic breakdown are discussed in greater detail further on. Non-enzymic breakdown may be of two types: chemical and physical changes. Physical changes include aggregation and precipitation and are usually induced by high concentrations of co-solvents which may be used in some formulations or by injudicious choice of ionic strengths. Loss of conformation not only leads to poor absorption but also to loss of activity. Chemical changes include β-elimination, deamidation, disulphide exchange, racemization and oxidation. Examples of peptides and proteins which have been reported to be unstable are shown in Table 2 along with some of the reported mechanisms of breakdown.

Parenteral routes of delivery

For systemic delivery of peptide and protein drugs, parenteral administration is currently almost universally required in order to achieve consistent therapeutic activities. This is because of the drugs' susceptibility to breakdown by gastric acid and the proteolytic enzymes in the gastrointestinal tract. In addition, peptides and proteins are high-molecular-weight substances and thus do not easily cross the intestinal mucosa. Therefore, the oral bioavailabilities of most intact peptides and proteins are very low.

Of the parenteral routes, only intravenous (i.v.) administration is usually efficient in delivering protein and peptide drugs to the systemic circulation. For example, optimal blood levels of protein or peptide drugs, such as γ-globulin (Buckley, 1982), can be achieved by the intravenous route. Generally, intramuscular or subcutaneous injections are less efficient due to the absorption and diffusion barriers presented by the muscle mass and connective tissues under the skin. However, insulin can be efficiently administered by subcutaneous injections (Nora et al., 1964; Koivisto and Felig, 1978) although hydrolysis is still significant (Berger et al., 1979).

While most peptide/protein drugs can be efficiently delivered to the systemic circulation by parenteral injections, poor disposition profiles lead to sub-optimal therapeutic benefits without high dosing frequencies. Such frequent injections, besides being unpleasant to the patients, also lead to usual complications such as thrombophlebitis and tissue necrosis.

In attempts to improve the disposition profile and the efficiency of delivery of parenterally administered peptides and protein drugs, many investigators have reported on liposomal systems. Examples of enzymes and monoclonal antibodies which have been formulated as liposomal systems for intravenous administration are shown in Table 3. Also included are some liposomal systems intended for oral administration. Despite the extensive evaluation of such systems and experimental results (Gregoriadis, 1976; Goosen, 1987) indicating that insulin absorption is greatly enhanced in animals by liposomal encapsulation of the hormone, no insulin liposomal system is currently in commercial use.

One biodegradable implant in current use in humans in goserelin acetate formulated in a biodegradable matrix of lactide-glycolide co-polymer. Systems which are designed with an enzymically controlled feed-back mechanism have also been described. Fischel-Ghodsian et al. (1988), for example, reported on an insulin system consisting of insulin and glucose oxidase dispersed in an ethylene/vinyl acetate polymer matrix. In the presence of glucose oxidase, glucose is converted into gluconic acid. This acid lowers the pH and increases the solubility of entrapped insulin which is then released faster. Consequently, some feedback control between glucose and insulin is thereby established.

General Approaches to Optimizing Absorption and Disposition

To optimize the absorption of high-molecular-weight protein and peptide drugs across absorption barriers, several approaches are available: (i) inhibition of their enzymic degradation; (ii) increasing their permeability across the relevant membrane; and (iii) improving their resistance to breakdown by structural modification.

Inhibitors of proteolytic enzymes

Protease inhibitors have been known for several years to increase the absorption of protein drugs (Laskowski et al., 1958). Table 4 lists the different protease inhibitors which have been used in investigations of the delivery of peptide and protein drugs.

Aprotinin, a bovine pancreatic kallikrein inhibitor, consists of a single-chain polypeptide containing 58 amino acid residues with a molecular weight of 6500 (Kassell et al., 1965). It has been used to inhibit plasmin, trypsin, chymotrypsin and various intracellular proteases (Trautschold et al., 1967). It was demonstrated, in an early study, that when insulin and aprotinin were injected together into a loop of the jejunum, a significant drop in blood glucose was observed. In contrast, no significant drop in blood glucose was found when the insulin was injected alone (Laskowski et al., 1958). Similar results have also been reported by several other workers (Berger et al., 1980; Fredenberg et al., 1981; William et al., 1983; Dandona et al., 1985; Linde and Gunnarson, 1985; Owens et al., 1988). However, some recent studies provided conflicting results, at least with respect to insulin and calcitonin absorption by nasal administration (Hanson et al., 1986; Aungst and Rogers, 1988). When the effects of laureth-9, sodium salicylate, Na_2EDTA and aprotinin on insulin absorption via the rectal, nasal and buccal tissues were examined by Aungst and Rogers (1988), aprotinin was found to be ineffective, either alone or in combination with laureth-9. Hanson and his co-workers (1986) examined the effects of several protease inhibitors, including bile salt, fatty acid derivative, aprotinin, kallikrein inhibitor, RG-1, bestatin, fusidic acid, chemostatin, benzamidine, chymotrypsin inhibitor, trypsin inhibitor III-0 and leupeptin on intranasal delivery of calcitonin, and found that aprotinin in vitro did not inhibit proteolytic activity of nasal extracts. In vivo the inhibitor did not

TABLE 4

Inhibitors of proteolytic enzymes used in investigation of the delivery of peptide and protein drugs

Compound	Route	Peptide studied	Animal model	Reference
Aprotinin	intestinal	insulin	rat	Ziv and Kidron (1987), Laskowski et al. (1958)
		RNase	rat	Ziv and Kidron (1987)
	s.c. [a]	insulin	man	Owens et al. (1988), Linde and Gunnarsson (1985), Berger et al. (1980)
Soybean trypsin inhibitor	intestinal	insulin	rat	Ziv and Kidron (1987)
		RNase	rat	Ziv and Kidron (1987)
FK-448 [b] (chymotrypsin inhibitor)	intestinal	insulin	rat	Yokoo et al. (1988)
Boroleucine [c]	nasal	Leu-enkephalin	rat	Hussain et al. (1989)
Borovaline [c]	nasal	Leu-enkephalin	rat	Hussain et al. (1989)

[a] Subcutaneous delivery.

[b] 4-(4-Isopropylpiperazinocarbonyl)phenyl-1,2,3,4-tetrahydro-1-naphthoate methanesulphonate.

[c] α-Aminoboronic acid derivatives.

enhance the serum calcium drop observed. These results are supported by the study of Deurloo et al. (1989). The co-administration of sodium taurodihydrofusidate with aprotinin also failed to increase significantly insulin bioavailability in rabbits via the nasal route. Clearly, further studies are required to define better the effects of aprotinin on the absorption of peptide and protein drugs.

More recently, α-aminoboronic acid derivatives, such as boroleucine, which are potent and reversible inhibitors of aminopeptidase, have been used to stabilize peptide drugs during their intranasal absorption (Hussain et al., 1989). When these inhibitors were compared with other known peptidase inhibitors, bestatin [an inhibitor of leucine aminopeptidase, aminopeptidase B, and aminopeptidase N (Suda et al., 1976)] and puromycin [an inhibitor of aminopeptidase B and N but not leucine aminopeptidase (McDonald et al., 1964)], using leucine enkephalin as substrate in rat nasal perfusate, it was found that bestatin and puromycin were less effective than boroleucine, even at concentrations 100- and 1000-times higher, respectively.

Absorption enhancers

The use of absorption enhancers has been studied extensively, particularly with respect to insulin absorption. These enhancers can be divided into several groups as listed in Table 5.

Despite extensive use, it is very difficult to make a judgement about the relative efficacy of these bioenhancers in promoting peptide or protein absorption because the results were obtained in different laboratories using different assay methods and different experimental conditions. However, it is clear that the bioavailability of most peptide and protein drugs administered by any non-parenteral route may be significantly enhanced by some of these compounds (see Tables in part II of this review).

The value of a particular enhancer depends on the route of administration used. For example, the bioavailability of ocular insulin was found to be significantly enhanced by saponin, whereas enhancement by glycocholate, which was a potentially good enhancer for nasal and rectal peptide and protein drug absorption, was found to be only slight (Chiou and Chuang, 1989).

The mechanisms of action of the peptide absorption enhancers are not clearly known, but several possibilities have been postulated. The first is increased solubility of the drugs brought about by the enhancers because proteins and peptides usually form aggregates in aqueous solutions. In the presence of enhancers, dissociation takes place to form monomers which are better absorbed. A second mechanism is the protection of the peptide and protein drugs from potential proteolytic hydrolysis. Both bile salts (Hirai et al., 1981b; Hanson et al., 1986; Zhou and Li Wan Po, 1991) and derivatives of fusidic acid (Deurloo et al., 1989) are known to inhibit proteolytic degradation of the drugs by nasal homogenates. Thirdly, binding between peptide or protein and enhancer to produce a better-absorbed entity may be a possibility. Although the effects of absorption enhancers such as glycocholate (Hirai et al., 1981b) and sodium cholate (Zhou and Li Wan Po, 1991) on the absorption of insulin by nasal delivery are thought to be partly due to inhibition of protease, recent work suggests that compared to aminopeptidase inhibitors such as bestatin and amastatin, cholate and its analogues are not very efficient (Hanson et al., 1986). Cholate and its analogues may also enhance the absorption of peptide and protein drugs by binding to insulin (Zhou and Li Wan Po, 1991). This would prevent the formation of enzyme-substrate complex to undergo the necessary conformational change which aligns the catalytic site on the protease with the susceptible bond of the substrate. Cholate and its analogues may possibly also promote the absorption of proteins by selectively denaturing the enzymes, although this is unlikely as it is difficult to identify the basis for the necessary selectivity.

Chemical modification

Chemical modification is an important approach for enhancing the absorption of peptides and protein drugs, especially for peptides with fewer than ten amino acid residues. Chemical modification usually results in denaturation of

TABLE 5

Absorption enhancers for peptides and proteins

Compound	Route	Peptide	Animal model	Reference
Fatty acid				
MCFC [a]				
Caprylate	nasal	insulin	rat	Mishima et al. (1987)
Caprate	nasal	insulin	rat	
Laurate	nasal	insulin	rat	
LCFC [b]				
Oleate in PAGB [c]	rectal	insulin	rat	Morimoto et al. (1983)
Linoleate in PAGB	rectal	insulin	rat	
Linolenate in PAGB	rectal	insulin	rat	
Oleic acid	vaginal	leuprolide	rat	Okada et al. (1982)
Bile salts				
Taurocholate	nasal	insulin	rat	Hirai et al. (1981a)
Cholate	nasal	insulin	rat	Moses et al. (1983)
Deoxycholate	nasal	insulin	rat	
Glycocholate	nasal	insulin	rat	Mishima et al. (1987)
Chenodeoxycholate	nasal	insulin	rat	
Deoxycholate (aerosol)	nasal	insulin	man	Moses et al. (1984)
Glycocholate	vaginal	leuprolide	rat	Okada et al. (1982)
Cholate	rectal	insulin	rat	Ziv et al. (1981)
Deoxycholate	rectal	insulin	rat	
Cholate	intestinal	insulin	rat	Ziv et al. (1987)
	intestinal	RNase	rat	
Glycocholate	nasal	insulin	rat	Aungst et al. (1988)
	rectal	insulin	rat	
	buccal	insulin	rat	
	sublingual	insulin	rat	
Enamine derivatives of phenylglycine				
Ethylacetoacetate enamine of sodium D-glycine	rectal	insulin	rabbit	Kim et al. (1983)
Ethylacetoacetate enamine of sodium D-alanine	rectal	insulin	rabbit	
Ethylacetoacetate enamine of sodium D-leucine	rectal	insulin	rabbit	
Ethylacetoacetate enamine of sodium D-isoleucine	rectal	insulin	rabbit	
Ethylacetoacetate enamine of sodium D-phenylalanine	rectal	insulin	rabbit	
Ethylacetoacetate enamine of sodium D-phenylalanine in gelatin	rectal	insulin	dog	
Ethylacetoacetate enamine of sodium D-phenylglycinate	rectal	insulin	rabbit	Kamada et al. (1981)
Ethylacetoacetate enamine of sodium DL-phenylalanine	rectal	lysozyme	rabbit	Miyake et al. (1984)

(continued)

TABLE 5 *(continued)*

Compound	Route	Peptide	Animal model	Reference
Ethylacetoacetate enamine of sodium D-phenylglycine	rectal	lysozyme	rabbit	
Ester type				
Glycerine-1, 3-diacetoacetate	rectal	lysozyme	rabbit	Miyake et al. (1984)
1,2-Isopropylidene-glyceryl-3-acetoacetate	rectal	lysozyme	rabbit	
Ethylaceto-acetylglycolate	rectal	lysozyme	rabbit	
Polyoxyethylene 10-monolaurate	nasal	insulin	rat	Hirai et al. (1981a)
Glyceryl esters of acetoacetic acid	rectal	insulin	rabbit	Nishihata et al. (1983)
Ether type				
Polyoxyethylene 9-lauryl ether in PAGB	rectal	calcitonin	rat	Morimoto et al. (1985)
Polyoxyethylene 9-lauryl ether	rectal	insulin	dog	Shichiri et al. (1978)
Polyoxyethylene 24-cholesteryl ether	oral	ergot peptide alkaloids	rat	Urbancic-Smerkolj et al. (1987)
Polyoxyethylene 5-octyl ether	nasal	insulin	rat	Hirai et al. (1981a)
Polyoxyethylene 10-octyl ether	nasal	insulin	rat	
Polyoxyethylene 5-lauryl ether	nasal	insulin	rat	
Polyoxyethylene 9-lauryl ether	nasal	insulin	rat	
Polyoxyethylene 10-lauryl ether	nasal	insulin	rat	
Polyoxyethylene 20-lauryl ether	nasal	insulin	rat	
Polyoxyethylene 10-cetyl ether	nasal	insulin	rat	
Polyoxyethylene 20-cetyl ether	nasal	insulin	rat	
Polyoxyethylene 10-stearyl ether	nasal	insulin	rat	
Polyoxyethylene 20-stearyl ether	nasal	insulin	rat	
Polyoxyethylene 10-nonylphenyl ether	nasal	insulin	rat	
Polyoxyethylene 10-octylphenyl ether	nasal	insulin	rat	
Polyoxyethylene 24-cholesteryl ether	nasal	insulin	rat	

(continued)

TABLE 5 *(continued)*

Compound	Route	Peptide	Animal model	Reference
Polyoxyethylene 20-cetyl ether (Cetomacrogol 1000)	rectal	insulin	rat	Bar-on et al. (1981)
Polyoxyethylene 9-lauryl alcohol ether	rectal	insulin	rabbit	Ichikawa et al. (1980)
Polyoxyethylene 10-nonylphenyl ether	rectal	insulin	rabbit	
Salicylates				
3,5-Diiodosalicylate	rectal	insulin	rat	Hauss and Ando (1988)
5-Methoxysalicylate	rectal	insulin	rat	
5-Methoxysalicylate	rectal	insulin	rat	Nishihata et al. (1981)
5-Methoxysalicylate	rectal	gastrin	rat	Yoshioka et al. (1982)
		pentagastrin	rat	
5-Methoxysalicylate in PAGB (pH 5.5, M.W. 1 250 000)	rectal	calcitonin	rat	Morimoto et al. (1984)
Salicylate	rectal	human growth hormone	rat	Moore et al. (1986)
Organic acids				
Citric acid	vaginal	leuprolide	rat	Okada et al. (1982, 1983)
	vaginal	insulin	rat	
Succinic acid	vaginal	leuprolide	rat	
Tartaric acid	vaginal	leuprolide	rat	
Malonic acid	vaginal	leuprolide	rat	
Glycoside				
Saponin	nasal	insulin	rat	Hirai et al. (1981a)
Saponin	ocular	insulin	rat	Chiou and Chuang (1989)
Peptide lipid				
Surfactin	nasal	insulin	rat	Hirai et al. (1981a)
Amine				
Polyoxyethylene 5-oleylamine	rectal	insulin	rabbit	Ichikawa et al. (1980)
Anion				
Sodium lauryl sulphate	rectal	insulin	rabbit	Ichikawa et al. (1980)
Monosodium *N*-lauroyl L-glutamate	rectal	insulin	rabbit	
Derivatives of fusidic acid				
sodium tauro-dihydrofusidate	nasal	insulin	rabbit	Deurloo et al. (1989)
Sodium tauro-dihydrofusidate	nasal	insulin	sheep	Longenecker et al. (1987)
Glycyrrhetinic acid derivatives				
Glycyrrhizinic acid	nasal	insulin	rat	Mishima et al. (1989)
Glycyrrhetinic acid hydrogen succinate	nasal	insulin	rat	

[a] Medium-chain fatty acids.
[b] Long-chain fatty acids.
[c] Polyacrylic acid gel base.

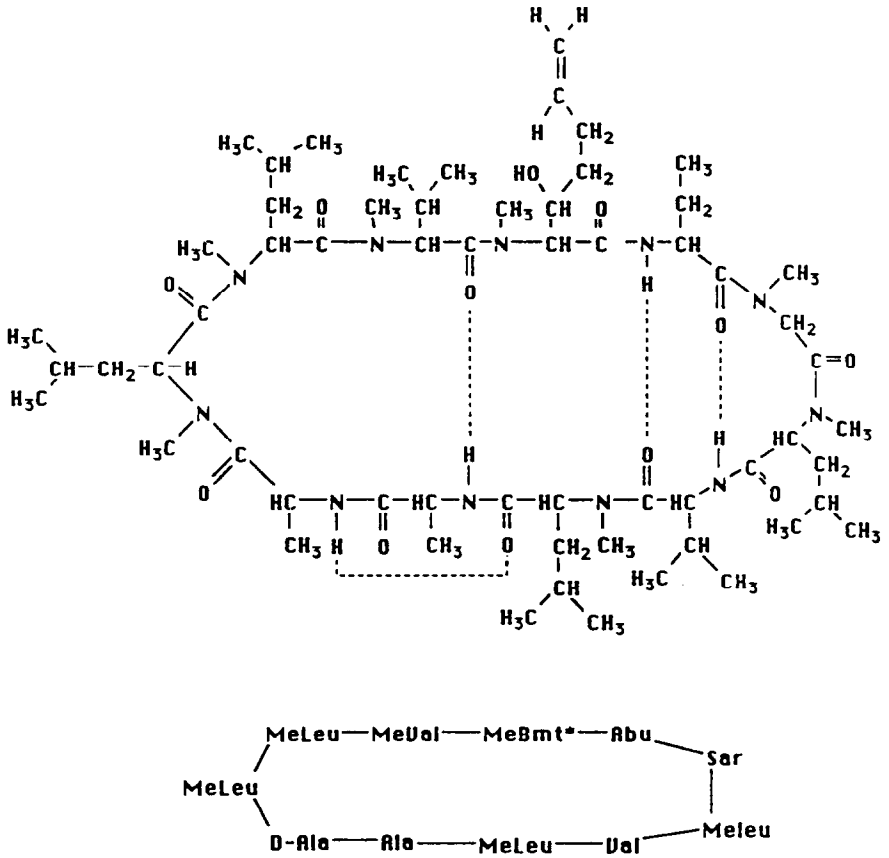

Fig. 1. Structure of cyclosporin (CS-A). MeBmt*, (4*R*)-4-[(*E*)-2-butenyl]-4-methyl-L-threonine.

the bioactive protein or polypeptide. However, a modified luteinizing hormone releasing hormone (LHRH), a decapeptide, and a modified tripeptide, thyrotrophin releasing hormone (TRH), were found to be active when given orally to rat and man. Both of these hypothalamic regulatory hormones contain a pyroglutamyl residue at the amino end, which renders them relatively resistant to enzymic hydrolysis (Masson et al., 1979). The possibility of formation of pyroglutamyl residues by cyclization of the N-terminal glutamine warrants more attention, because the pyroglutamyl residue is hydrolysis-resistant and may promote the gastrointestinal entry of intact peptide hormone into the systemic circulation. Peptides such as 1-deamino-8-D-arginine vasopressin and cyclosporin (Fig. 1) containing D-amino acids may lead to sequences which are relatively stable to enzymatic hydrolysis. Peptides with a high proline content or with a phosphate residue are also relatively resistant to enzymatic hydrolysis (Gardner, 1984).

Some peptide drugs can be converted into prodrugs. A good example is enalapril, which was found to be orally well absorbed and is metabolized to the active form, enalaprilat, in the liver. In contrast, the parent drug, enalaprilat is very poorly absorbed via the oral route (Ip and Brenner, 1987).

Conclusion

Peptide and protein drugs will form an increasingly important part of the range of therapeutic agents available for the control of human dis-

eases. The problems associated with the need to use the parenteral route for most of those drugs present formidable challenges for the formulation scientist. Some promising avenues have been discussed in this review. Alternatives to the parenteral route are discussed in greater detail in part II of this review.

References

Abuchowski, A., Kazo, G.M., Verhoest, C.R. Jr, Van Es, T., Kafkewitz, D., Nucci, M.L., Vian, A.J. and Davis, F.F., Cancer therapy with chemically modified enzymes. I. Antitumor properties of polyethylene glycoasparaginase conjugates. *Cancer Biochem. Biophys.*, 7 (1984) 175–183.

Alving, C.R., Richards, R.L., Moss, J., Alving, L.I., Clements, J.D., Shiba, T., Kotani, S., Wirtz, R.A. and Hockmeyer, W.T., Effectiveness of liposomes as potential carriers of vaccines: applications to cholera toxin and human malaria sporozoite antigen. *Vaccine*, 4 (1986) 166–173.

Arakawa, T., Hsu, Y.R. and Yphantis, D.A., Acid unfolding and self-association of recombinant *Escherichia coli* derived human interferon gamma. *Biochemistry*, 26 (1987) 5428–5432.

Aungst, B.J., Rogers, N.J. and Shefter, E., Comparison of nasal, rectal, buccal, sublingual and intramuscular insulin efficacy and the effects of the bile salt absorption promoter. *J. Pharm. Exp. Ther.*, 244 (1988) 23–27.

Aungst, B.J. and Rogers, N.J., Site dependence of absorption-promoting actions of laureth-9, Na salicylate, NaEDTA, and aprotinin on rectal, nasal and buccal insulin delivery. *Pharm. Res.*, 5 (1988) 305–309.

Banga, A.K. and Chien, Y.W., Systemic delivery of therapeutic peptides and proteins. *Int. J. Pharm.*, 48 (1988) 15–50.

Banting, F.G. and Best, C.H., Insulin: Its chemistry and physiology. *J. Lab. Clin. Med.*, 7 (1922) 464–484.

Bar-on, H., Berry, E.M., Eldor, A., Kidron, M., Lichtenberg, D. and Ziv, E., Enteral administration of insulin in the rat. *Br. J. Pharmacol.*, 73 (1981) 21–24.

Batzri, S. and Korn, E.D., Interaction of phospholipid vesicles with cells. *J. Cell. Biol.*, 66 (1975) 621–634.

Becker, G.M., Tackitt, P.M., Bromer, W.W., Lefeber., D.S. and Riggin, R.M., Isolation and characterization of a sulfoxide and a desamide derivative. *Biotech. Appl. Biochem.*, 10 (1988) 326–337.

Belchetz, P.E., Crawley, J.W.C., Braidman, I.P. and Gregoriadis, G., Treatment of Gaucher's disease with liposome entrapped glucocerebroside: β-glucosidase. *Lancet*, ii (1977) 116–123.

Berger, M., Cuppers, H.J., Halban, P.A. and Offord, R., The effect of aprotinin on the absorption of subcutaneously injected regular insulin in normal subjects. *Diabetes*, 29 (1980) 81–83.

Berger, M., Hallban, P.A., Girardier, L., Seydoux, J., Offord, R.E. and Renold, A.E., Absorption kinetics of subcutaneously injected insulin. Evidence for degradation at the injection site. *Diabetologia*, 17 (1979) 97–99.

Berson, S.A. and Yalow, R.S., Deamidation of insulin during storage in frozen state. *Diabetes*, 15 (1966) 875–879.

Bhatt, N.P., Patel, K. and Borchardt, R.T., Chemical pathways of peptide degradation. I. Deamidation of adrenocorticotropic hormone. *Pharm. Res.*, 7 (1990) 593–599.

Bomford, R., Immunological adjuvants. In Dale, M.M. and Foreman, J.C. (Eds.), *Textbook of Immunopharmacology*, Blackwell, Oxford, 1989, pp. 320–323.

Borel, J.F., Cyclosporin. In Dale, M.M. and Foreman, J.C. (Eds), *Textbook of Immunopharmacology*, Blackwell, Oxford, 1989, pp. 300–311.

Braidman, I. and Gregoriadis, G., Preparation of glucocerebroside: β-glucosidase for entrapment in liposomes and treatment of patients with adult Gaucher's disease. *Biochem. Soc. Trans.*, 4 (1976) 259–261.

Brems, D.N., Plaisted. S.M., Kauffman, E.W. and Havel, H.A., Characterization of an associated equilibrium folding intermediate of bovine growth hormone. *Biochemistry* 25 (1986) 6539–6543.

Brems, D.N., Plaisted. S.M., Havel, H.A. and Tomich, C.S., Stabilization of an associated folding intermediate of bovine growth hormone by site-directed mutagenesis. *Proc. Natl. Acad. Sci. USA*, 85 (1988) 3367–3371.

Brennan, J.R., Gebhart, S.S. and Blackard, W.G., Pump-induced insulin aggregation. A problem with the biostator. *Diabetes*, 34 (1985) 353–359.

British National Formulary, British Medical Association & Royal Pharmaceutical Society of Great Britain, The Pharmaceutical Press, London, 1990, Vol. 20, pp. 90–94.

Buckley, R.H., Long term use of intravenous immune globulin in patients with primary immunodeficiency diseases in adequacy of current dosage practices and approaches to the problem. *J. Clin. Immunol.*, 2 (1982) 155–162.

Calne, R.Y., White, D.J.G., Thiru, S., Evens, D.B., McMaster, P., Dunn, D.C., Craddock, G.N. and Penthow, B.D., Cyclosporin A in patients receiving renal allografts from cadaver donors. *Lancet*, ii (1978) 1323–1327.

Cantarovich, M., Hiesse, C., Lockiec, F., Charpentiec, B. and Fries, D., Confirmation of the interaction between cyclosporine and the calcium channel blocker nicardipine in renal transplant patients. Clin. Nephrol., 28 (1987) 190–193.

Chang, T.M.S. and Poznansky, M.J., Semi-permeable microcapsules containing catalase for enzyme replacement in catalaseaemic mice. *Nature*, 218 (1968) 243–244.

Chiou, G.C.Y. and Chuang, C.Y., Improvement of systemic absorption of insulin through eyes with absorption enhancers. *J. Pharm. Sci.*, 78 (1989) 815–818.

Colley, C.M. and Ryman, B.E., A model for a lysosomal storage disease and a possible method of therapy. *Biochem. Soc. Trans.*, 2 (1974) 871–872.

Dandona, P., Mier, A., Boag, F., Chappell, M. and Beckett, A.G., Aprotinin induced lipohypertrophy and glomerulonephritis in an insulin dependent diabetic. *Diabetes Res.*, 2 (1985) 213–226.

Dapergolas, G. and Gregoriadis, G., Hypoglycaemic effect of

liposome-entrapped insulin administered intragastrically into rats. *Lancet*, ii (1976) 824–827.

Dapergolas, G., Neerunjun, E.D. and Gregoriadis, G., Penetration of target areas in the rat by liposome-associated bleomycin, glucose-oxidase and insulin. *FEBS Lett.*, 63 (1976) 235–239.

Dawson, M.M. and Moore, M., Interleukin-2. In Dale, M.M. and Foreman, J.C. (Eds), *Textbook of Immunopharmacology*, Blackwell, Oxford, 1989, pp. 340–346.

De Barsy, T., Devos, P. and Van Hoof, F., The cellular distribution of liposomes in the liver of newborn rats. *Biochem. Soc. Trans.*, 3 (1975) 159–160.

Debs, R.J., Heath, T.D. and Papahadjopoulos, D., Targeting of anti-Thy 1.1 monoclonal antibody conjugated liposome in Thy 1.1 mice after intravenous administration. *Biochim. Biophys. Acta*, 901 (1987) 183–190.

Dedman, M.L., Farmer, T.H. and Morris, C.J.O., Pituitary adrenocorticotropin (II) oxidation-reduction properties of the hormone. *Biochem. J.*, 78 (1961) 348–352.

Deurloo, M.J.M., Hermens, W.A.J.J., Romeyn, S.G., Verhoef, J.C. and Merkus, F.W.H.M., Absorption enhancement of intranasally administered insulin by sodium taurodihydrofusidate (STDHF) in rabbits. *Pharm. Res.*, 6 (1989) 853–856.

Diaugustine, P.R., Gibson, B.W., Aberth, W., Kelly, M., Ferrua, C.M., Tomooka, Y., Brown, C.F. and Walker, M., Evidence for isoaspartyl' (deamidated) forms of mouse epidermal growth factor. *Anal. Biochem.*, 165 (1987) 420–429.

Dixon, H.B.F., The melanophore-stimulating activity of corticotropin. *Biochim. Biophys. Acta*, 19 (1956) 392–394.

Fidler, I., Brown, N.O. and Hart, I.R., Species variability for toxicity of free and liposome-encapsulated muramyl peptides administered intravenously. *J. Biol. Resp. Modif.*, 4 (1985) 298–309.

Fischel-Ghodsian, F., Brown, L., Mathiowitz, E., Brandenburg, D. and Langer, E., Enzymically controlled drug delivery. *Proc. Natl. Acad. Sci. USA*, 85 (1988) 2403–2406.

Fisher, B.V. and Porter, P.B., Stability of bovine insulin. *J. Pharmacol.*, 33 (1981) 203–206.

Fishman, Y., and Citri, N., L-Asparaginase entrapped in liposomes: preparation and properties. *FEBS Lett.*, 60 (1975) 17–20.

Fredenberg, G.R., White, N., Cataland, S., O'Dorisio, T. M., Sotos, J.F. and Santiago, J.V., Diabetes responsive to intravenous but not subcutaneous insulin: Effectiveness of aprotinin. *N. Engl. J. Med.*, 305 (1981) 363–368.

Gardner, M.L.G., Intestinal assimilation of intact peptides and proteins from the diet — a neglected field? *Biol. Rev.*, 59 (1984) 289–331.

Geiger, T. and Clarke, S., Deamidation, isomerization, and racemization at asparaginyl and aspartyl residues in protein degradation. *J. Biol. Chem.*, 262 (1987) 785–794.

Goosen, M.F.A., Insulin delivery systems and the encapsulation of cells for medical and industrial use. *CRC. Crit. Rev. Biocompat.*, 3 (1987) 1–24.

Graf, L. Cseh, G., Nagy, I. and Kurcz, M., Evidence for deamidation of prolactin monomer. *Acta Biochim. Biophys. Acad. Sci. Hung.*, 5 (1970) 299–303.

Graf, L., Bajusz, S., Patthy, A., Barat, E. and Cseh, G., Revised amide location for porcine and human adrenocorticotropic hormone. *Acta Biochim. Biophys. Acad. Sci. Hung.*, 6 (1971) 415–418.

Gregoriadis, G., The carrier potential of liposomes in biology and medicine. *New Engl. J. Med.*, 295 (1976) 704–710.

Gregoriadis, G. and Alison, A.C., Entrapment of proteins in liposomes prevents allergic reactions in pre-immunised mice. *FEBS. Lett.*, 45 (1974) 71–74.

Gregoriadis, G. and Neerunjun, E.D., Control of the rate of hepatic uptake and catabolism of liposome-entrapped proteins injected into rats: possibility therapeutic applications. *Eur. J. Biochem.*, 47 (1974) 179–185.

Gregoriadis, G. and Neerunjun, E.D., Homing of liposomes to target cells. *Biochem. Biophys. Res. Commun.*, 65 (1975) 537–544.

Gregoriadis, G. and Ryman, B.E., Fat of protein-containing liposomes injected into rats: an approach to the treatment of storage diseases. *Eur. J. Biochem.*, 24 (1972a) 485–491.

Gregoriadis, G. and Ryman, B.E., Liposomal localization of β-fructofuranosidase-containing liposomes injected into rats: some implications in the treatment of genetic disorder., *Biochem. J.*, 129 (1972b) 123–133.

Gregoriadis, G., Putman, D. and Louis, L., Comparative fate and effect of non-entrapped and liposome-entrapped neuraminidase injected into rats. *Biochem. J.*, 140 (1974a) 323–330.

Gregoriadis, G., Swain, C.P. and Wills, E.J., Drug-carrier potential of liposomes in cancer chemotherapy. *Lancet*, i (1974b) 1313–1316.

Gregoriadis, G., Weereratne, H., Blair, H. and Bull, G.M., Liposomes in Gaucher type I disease: use in enzyme therapy and the creation of an animal model. *Prog. Clin. Biol. Res.*, 95 (1982) 681–701.

Hanson, M., Gazdick, G., Cahill, J. and Augustine, M., Intranasal delivery of the peptide, salmon calcitonin. In Davis, S.S., Illum, L. and Tomlinson, E. (Eds), *Delivery Systems for Peptides*, Plenum, New York, 1986, pp. 233–242.

Hashimoto, Y., Sugawara, M., Kamiya, T. and Suzuki, S., Coating of liposomes with subunits of monoclonal IgM antibody and targeting at the liposomes. *Methods Enzymol.*, 121 (1986) 817–828.

Hauss, D.J. and Ando, H.Y., The influence of concentration of two salicylate derivatives on rectal insulin absorption enhancement. *J. Pharm. Pharmacol.*, 40 (1988) 659–661.

Heath, T.D., Edwards, D.C., Ryman, B.E., The adjuvant properties of liposomes in cancer chemotherapy. *Lancet*, i (1976) 1313–1316.

Hershfield, M.S., Buckley, R.H., Greenberg, M.L., Melton, A.L., Schiff, R., Hatem, C., Kurtzberg, J., Markert, M.L., Kobagashi, R.H., Kobagashi, A.L. and Abuchowski, A., Treatment of adenosine deaminase deficiency with polyethylene glycol-modified adenosine deaminase. *N. Engl. J. Med.*, 316 (1987) 589–597.

Higgins, P.G., Al-Nakib, W., Willman, J. and Tyrrell, D.A.J., Interferon-B_{ser} as prophylaxis aganist experimental rhinovirus infection in volunteers. *J. Interferon Res.*, 6 (1986) 153–158.

Hirai, S., Yashiki, T. and Mina, H., Mechanisms for the enhancement of the nasal absorption of insulin by surfactants. *Int. J. Pharm.*, 9 (1981a) 173–184.

Hirai, S., Yashiki, T. and Mina, H., Effect of surfactants on the nasal absorption of insulin in rats. *Int. J. Pharm.*, 9 (1981b) 165–172.

Hsu, Y.R. and Arakawa, T., Structural studies on acid unfolding and refolding of recombinant human interferon gamma. *Biochemistry*, 24 (1985) 7959–7963.

Humphrey, M.J. and Ringrose, P.S., Peptides and related drugs: A review of their absorption, metabolism, and excretion. *Drug Metab. Rev.*, 17 (1986) 283–310.

Hussain, M.A., Shenvi, A.B., Rowe, S.M. and Shefter, E., The use of α-aminoboronic acid derivatives to stabilize peptide drugs during their intranasal absorption. *Pharm. Res.*, 6 (1989) 186–189.

Ichikawa, K., Ohata, I., Mitomi, M., Kawamura, S., Maeno, H. and Kawata, H., Rectal absorption of insulin suppositories in rabbits. *J. Pharm. Pharmacol.*, 32 (1980) 314–318.

Ip, D.P. and Brenner, G.S., Enalapril maleate. *Anal. Profiles Drug Substances*, 16 (1987) 207–243.

Kamada, A., Nishihata, T., Kim, S., Yamamoto, M. and Yata, N., Study of enamine derivatives of phenylglycine as adjuvants for the rectal absorption of insulin. *Chem. Pharm. Bull.*, 29 (1981) 2012–2019.

Kassell, B., Radicevic, M., Ansfield, M.J. and Laskowski, M., The basic trypsin inhibitor of bovine pancreas. IV. The linear sequence of the 58 amino acids. *Biochem. Biophys. Res. Commun.*, 18 (1965) 255–258.

Kim, S., Kamada, A., Higuchi, T. and Nishihata, T. Effect of enamine derivatives on the rectal absorption of insulin in dogs and rabbits. *J. Pharm. Pharmacol.*, 35 (1983) 100–103.

Koivisto, V.A. and Felig, P., Effects of leg exercise on insulin absorption in diabetic patients. *N. Engl. J. Med.*, 298 (1978) 77–83.

Kornalik, F., The influence of snake venom enzymes on blood coagulation, *Pharmacol. Ther.*, 29 (1985) 353–405.

Kreuger, J.M., Walter, J., Karnovsky, M.L., Chedid, L., Choay, J.D., Lefrancier, J.P. and Lederer, E., Muramyl peptides-variation of somnogenic activity and structure. *J. Exp. Med.*, 159 (1984) 68–75.

Kumashiro, H., Mori, N., Fukatsu, T. and Sato, T., Application of liposome-entrapped drugs. *Jap. J. Psychiatry*, 40 (1986) 367–368.

Lancaster, S.G. and Todd, P.A., Lisinopril, A preliminary review of its pharmacodynamic and pharmcokinetic properties, and therapeutic use in hypertension and congestive heart failure. *Drugs*, 35 (1988) 646–669.

Laskowski, M.J., Haessler, H.A., Miech, R.P. and Peanasky, R.J., Effect of trypsin inhibitor on passage of insulin across the intestinal barrier. *Science*, 127 (1958) 1115–1116.

Lee, V.H.L., Enzymatic barriers to peptide and protein absorption. *CRC. Crit. Rev. Ther. Drug Carr. Syst.*, 5 (1988) 69–97.

Lewis, U.J. and Cheever, E.V., Evidence for two types of conversion reactions for prolactin and growth hormone. *J. Biol. Chem.*, 240 (1965) 247–252.

Lewis, U.J., Cheever, E.V. and Hopkins, W.C., Kinetic study of the deamidation of growth hormone and prolactin. *Biochim. Biophys. Acta*, 214 (1970) 498–508.

Linde, B. and Gunnarsson, R., Influence of aprotinin on insulin absorption and subcutaneous blood flow in type 1 (insulin-dependent) diabetes. *Diabetologia*, 28 (1985) 645–648.

Longenecker, J.P., Moses, A.C., Flier, J.S., Silve, R.D., Carey, M.C. and Dubovi, E.J., Effects of sodium taurodihydrofusidate on nasal absorption of insulin in sheep. *J. Pharm. Sci.*, 76 (1987) 351–355.

Lougheed, W.D., Woulfe-Flanagan, H., Clement, J.R. and Albisser, A.M., Insulin aggregation in artificial delivery systems. *Diabetologia*, 19 (1980) 1–9.

Masson, M.A., Moreau, O., Debuire, B., Han, K.-K., Morier, E. and Rips, R., Evidence for the resistance of thyrotropin-releasing hormone (TRH) and pseudo-hormone, pyroglutamyl histidyl amphetamine, to degradation by enzymes of the digestive tract in vitro. *Biochemie*, 61 (1979) 847–852.

McDonald, J.K., Reilly, T.J. and Ellis, E., The hydrolysis of amino acyl-β-naphthylamides by plasma aminopeptidase. *Biochem. Biophys. Res. Commun.*, 16 (1964) 135–140.

McDonald, T.J., Jornvall, H., Tatemoto, K. and Mutt, V., Identification and characterization of variant forms of the gastrin-releasing peptide (GRP). *FEBS Lett.*, 156 (1983) 349–356.

Mehta, M.U., Venkataramanan, R., Rurckart, G.J., Ptachcinski, R.J., Delamos, B., Stachak, S., Van Thiel, D.H., Iwatsuki, S. and Starzl, T.E., Effect of bile on cyclosporin absorption in liver transplant patients. *Br. J. Clin. Pharmacol.*, 25 (1988) 579–584.

Meinwald, Y.C., Stimson, E.R. and Scheraga, H.A., Deamidation of the asparaginyl-glycyl sequence. *Int. J. Peptide Protein. Res.*, 28 (1986) 79–84.

Minta, J.O. and Painter, R.H., Chemical and immunological characterization of the electrophoretic components of the Fc fragment of human immunoglobulin G. *Immunochemistry*, 9 (1972) 821–832.

Mishima, M., Wakita, Y. and Nakano, M., Studies on the promoting effects of medium chain fatty acid on the nasal absorption of insulin. *J. Pharmacobio-Dyn.*, 10 (1987) 624–631.

Mishima, M. Okada, S., Wakita, Y. and Nakano, M., Promotion of nasal absorption of insulin by glycyrrhetinic acid derivatives. I. *J. Pharmacobio-Dyn.*, 12 (1989) 31–36.

Miyake, M., Nishihata, T., Wada, N., Takeshima, E. and Kamada, A., Rectal absorption of lysozyme and heparin in rabbits in the presence of non-surfactant adjuvants. *Chem. Pharm. Bull.*, 32 (1984) 2020–2025.

Moore, J.A., Pletcher, S.A. and Ross, M.J., Absorption en-

hancement of growth hormone from the gastrointestinal tract of rats. *Int. J. Pharm.*, 34 (1986) 35–43.

Moore, M. and Dawson, M.M., Interferons. In Dale, M.M. and Foreman, J.C. (Eds.), *Textbook of Immunopharmacology*, Blackwell, Oxford, 1989, pp. 324–339.

Morimoto, K., Akatsuchi, H., Aikawa, R., Morishita, M. and Morisaka, K., Enhanced rectal absorption of [Asu^{1,7}]-eel calcitonin in rats using polyacrylic acid aqueous gel base. *J. Pharm. Sci.*, 73 (1984) 1366–1368.

Morimoto, K., Akatsuchi, H., Morisaka, K. and Kamada, A., Effect of non-ionic surfactants in a polyacrylic acid gel base on the rectal absorption of [Asu^{1,7}]-eel calcitonin in rats. *J. Pharm. Pharmacol.*, 37 (1985) 759–760.

Morimoto, K., Kamiya, E., Takeeda, T., Nakamoto, Y. and Morisaka, K., Enhancement of rectal absorption of insulin in polyacrylic acid aqueous gel bases containing long chain fatty acid in rats. *Int. J. Pharm.*, 14 (1983) 149–157.

Morley, J.S., Tracey, H.J. and Gregory, R.A., Structure-function relation in the active c-terminal tetrapeptide sequence of gastrin. *Nature*, 207 (1965) 1356–1359.

Moses, A.C., Gordon, G.S., Carey, M.C. and Flier, J.S., Insulin administered intranasally as an insulin-bile salt aerosol. *Diabetes*, 32 (1983) 1040–1047.

Moses, A.C., Flier, J.S., Gordon, G.S., Silver, R.D. and Carey, M., Transnasal insulin delivery: structure-function studies of absorption enhancing adjuvants. *Clin. Res.*, 32 (1984) 245A.

Nashef, A.S., Osuga, D.T., Lee, H.S., Ahmed, A.I., Whitaker, J.R. and Feeney, R.E., Effects of alkali on proteins. Disulfides and their products. *J. Agric. Food Chem.*, 25 (1977) 245–251.

Neerunjun, E.D. and Gregoriadis, G., Tumour regression with liposome-entrapped asparaginase: some immunological advantages. *Biochem. Soc. Trans.*, 4 (1976) 133–134.

Nishihata, T., Rytting, J.H., Higuchi, T. and Caldwell, L., Enhanced rectal absorption of insulin and heparin in rats in the presence of non-surfactant adjuvants. *J. Pharm. Pharmacol.*, 33 (1981) 334–335.

Nishihata, T., Kim., S., Morishita, S., Kamada, A., Yata, N. and Higuchi, T., Adjuvant effects of glyceryl esters of acetoacetic acid on rectal absorption of insulin and inulin in rabbits. *J. Pharm. Pharmacol.*, 35 (1983) 100–103.

Nora, J.J., Smith, D.M. and Cameron, J.R., The route of insulin administration in the management of Diabetes Mellitus. *J Pediatr.*, 64 (1964) 547–551.

Okada, H., Yamazaki, I., Yashiki, T. and Mima, H., Vaginal absorption of a potent luteinizing hormone-releasing hormone analog (leuprolide) in rats. I: absorption by various routes and absorption enhancement. *J. Pharm. Sci.*, 71 (1982) 1367–1371.

Okada, H., Yamazaki, I., Ogawa, Y., Hirai, S., Yashiki, T. and Mima, H., Vaginal absorption of a potent luteinizing hormone-releasing hormone analog (leuprolide) in rats. II: Mechanism of absorption enhancement with organic acids. *J. Pharm. Sci.*, 72 (1983) 75–78.

Owens, D.R., Vora, J.P., Birtwell, Luzio, S. and Hayes, T.M., The influence of aprotinin on regional absorption of soluble human insulin. *Br. J. Clin. Pharmacol.*, 25 (1988) 453–456.

Padmanabhan, R.V., Gudapaty, R., Liener, I.E., Schwartz, B.A. and Hoidal, J.R., Protection against pulmonary oxygen toxicity in rats by the intratracheal administration of liposome-encapsulated superoxide dismutase or catalase. *Am. Rev. Respir. Dis.*, 132 (1985) 164–167.

Patel, H.M. and Ryman, B.E., α-Mannosidase in zinc-deficient rats: possibility of liposomal therapy in mannosidosis. *Biochem. Soc. Trans.*, 2 (1974) 1014–1017.

Patel, H.M. and Ryman, B.E., Oral administration of insulin within liposome. *FEBS Lett.*, 62 (1976) 60–63.

Riniker, V.B., Neher, R., Maier, R., Kahnt, F.W., Byfield, P.G.H., Gudmundsson, T.V., Galante, L. and MacIntyre, I., Human calcitonin. I. Isolation and characterization. *Helv. Chim. Acta*, 51 (1968) 1738–1742.

Robinson, A.K. and Sobel, B.E., Activator of plasminogen. In Davis S.S., Illum, L. and Tomlinson, E. (Eds), *Delivery Systems for Peptides*, Plenum, New York. 1986, pp. 295–304.

Romankiewicz, J.A., Brogden, R.N., Heel, R.C., Speight, T.M. and Avery, G.S., Captopril: an update review of its pharmacological properties and therapeutic efficacy in congestive heart failure. *Drugs*, 25 (1983) 6–40.

Sakuragawa, N., Niiya, K. and Kondo, S.-I., Oral administration of factor VIII concentrate preparation in von Willebrand's disease. *Thromb. Res.*, 38 (1985) 681–685.

Sen, A., Sherr, C.J. and Todaro, G.J., Phosphorylation of murine type C viral p12 proteins regulates their extent of binding to the homologous viral RNA. *Cell*, 10 (1977) 489–496.

Sessa, G. and Weissmann, G. Incorporation of lysozyme into liposomes. *J. Biol. Chem.*, 245 (1970) 3295–3301.

Shichiri, M., Yamasaki, Y., Kawamori, R., Kikuchi, M., Hakui, N. and Abe, H., Increased intestinal absorption of insulin: an insulin suppository. *J. Pharm. Pharmacol.*, 30 (1978) 806–808.

Six, H.R., Anderson, C. and Kasel, J.A., Further studies of liposomes as carriers of purified protein vaccines. In (Gregoriadis, G. Ed.), *Liposomes as Drug Carriers*, Wiley New York, 1988, pp. 197–206.

Suda, H., Aoyagi, T., Takeuch, T. and Umezawa, H., Inhibition of aminopeptidase B and leucine aminopeptidase by Bestatin and its stereoisomer. *Arch. Biochem. Biophys.*, 177 (1976) 196–200.

Tanaka, T., Taneda, K., Kobayashi, H., Okumura, K., Muranishi, S. and Sezaki, H., Application of liposomes to the pharmaceutical modification of the distribution characteristics of drugs in the rat. *Chem. Pharm. Bull.*, 23 (1975) 3069–3074.

Tashjian, A.H., Ontjes, D.A. and Munson, P.L., Alkylation and oxidation of methionine in bovine parathyroid hormone-effects on hormonal activity and antigenicity. *Biochemistry*, 3 (1964) 1175–1182.

Todd, P.A. and Heel, R.C., Enalapril, a review of its pharma-

codynamic and pharmacokinetic properties, and therapeutic use in hypertension and congestive heart failure. *Drugs*, 31 (1986) 198–248.

Trautschold, I., Werle, E. and Zickgraf-Rudel, G., Trasylol. *Biochem. Pharmacol.*, 16 (1967) 59–72.

Tyrell, P.A., Ryman, B.E., Keeton, B.R. and Dubowitz, V., Use of liposomes in treating type II glycogenosis. *Br. Med. J.*, 27 (1976) 88–96.

Urbancic-Smerkolj, J., Lenardic, A. and Kopitar, Z., Enhancement of bioavailability of some ergot peptide alkaloid in the rat. *Yugoslav. Physiol. Pharmacol. Acta*, 23 (1987) 127–134.

Vale, W., Spiess, J., Rivier, C. and Rivier, J., Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and endorphin. *Science*, 213 (1981) 1394–1397.

Volkin, D.B. and Klibanov, A.M., Thermal destruction processes in proteins involving cystine residues. *J. Biol. Chem.*, 262 (1987) 2945–2950.

Willian, G., Pickup, J.C., Bowcock, S., Cooke, E. and Keen, N., Subcutaneous aprotinin causes local hyperaemia. *Diabetologia*, 24 (1983) 91–94.

Wills, E.D., Hormones: a summary of their structures and functions, In Wills, E.D. (Ed.), *Biochemical Basis of Medicine*, Pitman, Bristol, 1985, pp. 196–220.

Wisse, E. and Gregoriadis, G., The uptake of liposomes by the rat liver RES. *J. Reticuloendothel. Soc.*, 18 (1975) 10a.

Wolff, B. and Gregoriadis, G., The use of monoclonal anti-Thy1 IgG1 for the targeting of liposomes to akr-a cells in vitro and in vivo. *Biochim. Biophys. Acta*, 802 (1984) 259–273.

Yokoo, N., Fujii, S. and Suzuki, T., Promoting effect of the chymotrypsin inhibitor FK-448 on the intestinal absorption of insulin in rats. *Yakugaku Zasshi*, 108 (1988) 164–169.

Yoshioka, S., Caldwell, L. and Higuchi, T., Enhanced rectal bioavailability of poly peptides using sodium-5-methoxysalicylate as an absorption promoter. *J. Pharm. Sci.*, 71 (1982) 593–594.

Zale, S.E. and Klibanov, A.M., Why does ribonuclease irreversibly inactivate at high temperature? *Biochemistry*, 25 (1986) 5432–5444.

Zhou, Z.H. and Li Wan Po, A., Effects of cholic acid and other enhancers on the bioavailability of insulin from a subcutaneous site. *Int. J. Pharm.*, 69 (1991) 29–41.

Ziv, E., Kidron, M., Berry, E.M. and Bar-on, H, Bile salts promote the absorption of insulin from the rat colon. *Life Sci.*, 29 (1981) 803–809.

Ziv, E., Lior, O. and Kidron, M., Absorption of protein via the intestinal wall. A quantitative model. *Biochem. Pharmacol.*, 36 (1987) 1035–1039.