Peptide Drug Delivery

Peptide and Protein Group Colloquium in conjunction with the Royal Society of Chemistry
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Introductory remarks

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Peptide and protein drugs have an endogenous origin, are very potent and effective even at a low concentration and produce minimum side-effects. Hence there is a great surge in the rate of discovery of new drugs, isolation (synthesis) and characterization. Since oral administration of these drugs is not practical because of degradation and non-absorption in the gastrointestinal tract, the peptides are administered by parenteral routes, which are not very satisfactory since the drugs are degraded at the site of injection and during the hepatic first-pass their therapeutic response is short lived. Thus the current challenge that we face concerns the development of alternative methods to the painful injection for administration of these drugs. The routes under investigation involve oral, nasal, colonic, rectal and vaginal mucosa.

Oral delivery can be successful if the two major obstacles, enzymic degradation and absorption in the gastrointestinal tract, are overcome. To avoid proteolysis of the peptides in the gut, various approaches are being tried out. These include: co-administration of the peptide with inhibitors of proteolytic enzymes; delivery of the drug to a site in the gastrointestinal tract where the enzyme activity is minimal; enhancement of absorption of the peptide before proteolysis occurs; encapsulation of the drug within liposomes; and structural modification of the peptide to prevent its proteolysis.

The strategies for enhancing absorption of the peptide drugs across the epithelial barrier include co-administration of surfactant, mucolytic agents, etc., modification of peptide structure to increase its lipophilicity and encapsulation of the drug in lipid vesicles. The orally administered peptide is likely to enter the portal circulation and be reduced by hepatic first-pass metabolism. The rectal route, on the other hand, offers a number of advantages over the oral route. These include: reduced proteolytic activity, reduced hepatic first-pass metabolism, low buffer capacity, lymphatic delivery and amenable to adjuvant-enhanced absorption. However, this route may not prove popular with patients.

The transdermal route is an attractive alternative, since it offers a number of advantages over oral and nasal routes. It has no serious problems of local proteolytic degradation and hepatic first-pass metabolism and may provide better control of delivery and maintenance of the therapeutic level of drug over a prolonged period of time. This route may prove the most popular with the recipient, since transdermal formulation of peptide drugs can be easily applied to and removed from different parts of the body’s skin.

Thus there are choices available in the development of alternative routes of administration of peptide drugs, but the formulation of proteins and peptides into effective dosage forms for these routes has become a formidable task. The papers presented in this Colloquium discuss various approaches that have been researched for the development of safe and effective peptide delivery. It must be admitted that many problems still exist; hopefully, these are not beyond the ingenuity of man to solve.

Received 16 March 1989

Molecular sieving, receptor processing and peptidolysis as major determinants of peptide pharmacokinetics in vivo

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Regulatory peptides and proteins carry specific messages between cells. The transmission channel is the extracellular space. The specificity of these molecules in vitro is primarily a property of the fit between the peptide and its receptor. However, the biological action of a peptide in a physiological milieu is equally strongly affected by transport and elimination processes.

Transport processes determine which cells are potentially accessible; elimination processes determine the duration of action of the molecule and can also limit access to and egress from certain environments. Transport and elimination processes depend qualitatively and quantitatively on (a) tissue

Abbreviation used: ANP, atrial natriuretic peptide.

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type and precise cellular environment, and (b) peptide structure. Thus, the peptide carries information which determines not only the receptor type, which can be activated, but also a specific pattern of distribution and elimination.

Understanding the peptide action, therefore, requires a theoretical model based on a rather detailed knowledge at the level of tissue ultrastructure of mechanisms, rates and specificities of transport and elimination. This information is also pertinent to the design of peptides and peptide-delivery systems, especially if mucosal and other external membranes are included in the model.

This article discusses some of the parameters which should be included in such a model. A summary of much of the information presented is contained in Fig. 1 and Table 1.

Physical properties of peptides

In this article, the term peptide will be used to refer to regulatory peptides and proteins of $M_r$ from 300 up to 30000. Molecules of $M_r$ up to 3000 usually have a flexible structure in the aqueous phase and are water soluble because of polar peptide bonds and polar side-chains. Larger molecules have a folded structure which internalizes most of the non-polar residues, resulting once again in a water-soluble molecule. Non-polar groups can occur on the surface of folded peptides, but are usually dispersed so that large patches which could lead to aggregation are not present. The net charge and total hydrophobic contents of peptides can vary appreciably.

Distribution and transport

Because of their water solubility and $M_r$, most peptides remain in the extracellular space unless active mechanisms of transport or elimination come into play. The extracellular space can be divided into: plasma, the interstitial space of tissue, lymph and cerebrospinal fluid, fluid bathing mucosal surfaces, and other minor components. These compartments are separated from each other by multicellular membranes. Transport across these endothelial and epithelial membranes can occur by a number of mechanisms.

Passive mechanisms apply to all peptides and are often sufficient to account for major distribution characteristics. This account, therefore, concentrates on these, but it is important to note that individual peptides may be actively transported across certain membranes.

Mucosal membranes. The nasal membranes have been shown to be highly permeable to peptides of $M_r$ below 1000 (for a quantitative literature survey, see [1]). Charge and hydrophobicity are not important determinants. The small intestine is much less permeable to peptides in this $M_r$ range [2]. The rectum is permeable to peptides and may be similar to the nasal membranes. For higher-$M_r$ peptides, adjuvants can dramatically improve nasal bioavailability [3].

Capillary endothelial membranes. Investigations with inert polar substances show that the capillary membranes of different tissues vary appreciably in permeability [4]. Substances have rapid access to the interstitial space of most of the internal organs, a notable exception being the brain. Permeability of the capillaries of muscle and skin is intermediate.

Distribution volumes and plasma pharmacokinetics. A circulating peptide will rapidly equilibrate with the interstitial space of major internal organs. The peripheral tissues, muscle and skin, however, constitute a major component of the total volume of distribution. As a result of slower exchange with this volume, the decay of blood concentration would be expected to show two phases. Experimental investigations often show biphasic decay curves and when these data are compiled and analysed it is found that the volumes of distribution are close to those predicted and that the exchange rates are dependent on $M_r$.

This shows that the results obtained for inert model compounds also apply to a first approximation to peptides. Properties other than $M_r$ (e.g. charge and polarity) do not play a major role in determining distribution volumes or rates (C. McMartin, unpublished work).

Summary of distribution kinetics. The distribution of polar molecules is largely determined by permeability through multicellular membranes. These membranes act as molecular sieves with a permeability dependent mainly on the size of the permeant. Peptide kinetics, therefore, depend on the $M_r$ characteristic of the membranes involved, the volumes of vascular and interstitial fluid and the flow rate through the appropriate tissue beds.

Uses and limitations of the simple distribution model. Specific peptides can exhibit specialized active transport mechanisms. In addition, sequestration by receptors may modify volume of distribution. Therefore, the general simple model presented above should not be taken as more than a starting point for the study of an individual peptide. However, it may serve as a useful basis for the development of a model in which specific transport characteristics are superimposed on non-specific ones.

Clearance

Clearance is a term which signifies irreversible elimination of a drug from the body. For a peptide which cannot passively diffuse across cell membranes, the definition of clearance can usually be more precisely defined as irreversible elimination from the extracellular space. Thus, rapid distribution from plasma into the interstitial space of tissues is not a clearance mechanism because it is reversible (note, however, that distribution may become a rate-limiting step for clearance if rapid elimination takes place in the interstitial space).
for the rapid inactivation of many small peptides

space). Three major clearance mechanisms must be considered. These mechanisms have a totally different physical basis and have remarkably different properties.

**Glomerular filtration.** Clearance by glomerular filtration probably occurs with most peptides of $M_{r}$ less than 10,000. Measurements with charged and uncharged dextrans show that, in addition to $M_{r}$, charge has an important influence on filtration efficiency, fractional clearance being largest for cations and smallest for anions [5]. This relationship is likely to apply to peptides and proteins and is of physiological significance enabling albumin, which is a polyanion, to be retained 100 times more efficiently than a neutral molecule of equivalent effective radius. The capacity of this mechanism to eliminate molecules has an upper limit so that a molecule distributed throughout the extracellular space which is cleared only by filtration will have a half-life of at least 40 min. For most peptides much shorter half-lives are observed, implying that other more efficient elimination processes are in operation.

**Cleavage of the peptide bond.** Peptidases are responsible for the rapid inactivation of many small peptides in vivo [6]. Half-lives can be in the order of seconds [7], but are typically minutes. Although inactivation can take place in plasma, rapid inactivation usually results from the action of tissue-bound enzymes. These are ectoenzymes, i.e., they reside on the outer face of the cell membrane. A number of these peptidases have been studied in detail, and it is becoming clear that they are cell and tissue specific and that each has a characteristic and very different tissue distribution [8].

It is also clear that each peptide will be inactivated by a small number of these enzymes. A number of examples show that small modifications to a peptide stabilize it and prolong its half-life in vivo or increase its potency. The selectivity of an enzyme for inactivation can also be demonstrated by the use of specific inhibitors. Investigations of the effects of specific inhibitors of neutral endopeptidase 24.11 on degradation of atrial natriuretic peptide (ANP) have shown that this is the major peptidase involved in inactivation [9]. It seems, therefore, that the structure of a peptide can determine which enzyme inactivates it. The distribution of this enzyme can be expected to impose a specific pattern on the activity of the peptide.

**Receptor-mediated processing.** Larger peptides are often cleared by receptor-mediated processes or by formation of inactive complexes. ANP is an example where both proteolytic [9] and receptor-mediated [10] processes are involved.

Other examples show that receptor-mediated clearance may be a major elimination mechanism. For example, a minor (in molecular terms) mutation of insulin reduces its receptor binding affinity and greatly increases its plasma half-life [11]. The implication that receptor-mediated clearance is playing a major role is confirmed by observations that an antibody to the insulin receptor also prolongs the half-life of insulin [12].
Receptor-mediated clearance is cell specific (e.g. TPA, which is rapidly cleared by hepatocytes [13, 14]). The clearance receptor is not necessarily the same as the receptor which is used to express biological action (an example of this is provided by ANP [10]). In addition, there are low-specificity receptor clearance mechanisms (e.g. the galactose clearance receptors which non-specifically, but very rapidly, eliminate asialoglycopeptides [15]).

Receptor-mediated clearance is more complex than enzymic degradation because once internalized the peptide and the receptor can be processed in a number of different ways. This depends on a sorting process which may recycle the peptide to the cell surface, transport it to the lysosome or transport to a part of the cell surface in contact with a different extracellular compartment (e.g. from plasma to bile in the liver [16]). Elegant methods for investigating the kinetics of peptide processing in intact tissues have been developed [17].

In addition to receptor-mediated clearance, active peptides can be rendered inactive by complex formation. Numerous examples of this are found in the regulatory cascades of complement and the clotting systems.

Conclusion

This survey is intended to give a broad view of the very different processes which co-operate to determine the behaviour of peptides and proteins in vivo. Investigation of molecules in the whole animal is inevitably complex. The growing knowledge of mechanisms of distribution and processing emphasizes how complex the system is. Nevertheless, this knowledge is beginning to provide a framework within which the observed properties of a peptide can be more effectively studied and understood. This knowledge and the new experimental possibilities arising from the development of specific enzyme inhibitors should make it easier to establish the relevant mechanisms for each peptide and to develop simple investigative animal models.

It is encouraging to note that for many peptides only a few of the potential mechanisms of clearance and distribution play a dominant role in determining the fate of the molecule.

Abbreviation used: PEG-400, poly(ethylene)glycol 400.

Transport of peptides across the gastrointestinal tract

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Introduction

The oral route for drug administration is particularly attractive because it is aesthetically natural, cheap, non-invasive, relatively free from complications arising from the need for sterile media and devices for parenteral administration and free from need of special applicators; also it may offer pharmacokinetic advantages over injection routes. However, these advantages become relevant only if sufficiently high bioavailability can be achieved; unfortunately, there is a long history of disheartening results from oral administration of peptide drugs, and many potentially interesting developments have never reached clinical usage or received their desired full fundamental investigation (e.g. oral administration of liposome-entrapped insulin), and hence these approaches have been abandoned because of early conclusions of inadequate efficacy. As will be clear from the literature cited below, there is abundant evidence that peptides, or at least some peptides, can cross the small intestine in intact and active form. But, unfortunately, the amounts are generally too small to be of pharmaceutical benefit. We now need to identify why they are so small. Is peptide hydrolysis (in the gastrointestinal lumen, within the epithelial cells, or systemically after absorption) limiting for peptide delivery, or are intestinal transport mechanisms lacking or inadequate, or is ‘passive’ intestinal permeability too small to permit adequate transepithelial passage of intact peptides? And can the small amounts absorbed be increased by acceptable therapeutic manipulations? In view of these difficulties with the small-intestinal route, much current work focuses on absorption across the colon (delivered with azo-aromatic coating which can be bacterially degraded, e.g. [1]) or rectal mucosa, often involving ‘absorption promoters’, the latter route is dealt with separately in this Colloquium by Liversidge [2], and so our account deals mainly with the small-intestinal route. We argue that small-intestinal absorption, though problematic, probably still offers more potential scope for peptide delivery than is generally presumed.

Possible routes/mechanisms for passage of peptides across intestinal mucosa

Results obtained by a variety of approaches suggest that at least four routes may be available for passage of peptides, especially small ones, across intestinal epithelia. These are (i) transcellular route via mediated transport mechanisms