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Biologically Active Peptides: Prospects for Drug Development

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Biologically active peptides: prospects for drug development

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Biologically active peptides are typified by their ubiquity of distribution, their high receptor affinity and an almost infinite diversity of structure. For these reasons, considerable effort is now being expended to elucidate the possible role of peptides in brain function. This effort has been stimulated by the discovery of a number of new endogenous peptides, such as the enkephalins, endorphins, vasoactive intestinal peptide and neurotensin. At present, there is no clearly defined role for these peptides, although they may form an important basis for the chemical coding of various brain functions, including pain, mood and memory.

At present, the potential for drug development of peptide agonists remains in fairly circumscribed areas such as analgesia, pituitary hormone control, and gastro-intestinal motor and secretory control. Peptide antagonists may provide a vast field for future development, although only one area, that of antifertility drugs based on LHRH antagonists, shows any promise of immediate success.

Industrial research approaches to new peptide agonists and antagonists mainly rely at present on rational drug design through structural analogies. Other fruitful approaches to be considered are the screening of natural microbial and plant products and the possible application of genetic engineering techniques.

Introduction

A large number of peptides have now been isolated and characterized from various mammalian sources. The rapid progress in this field has led to the realization that peptides may have a considerable involvement in the control of numerous physiological processes. This has raised the question as to whether peptides, peptide analogues or drugs interfering with peptide systems may have therapeutic potential. It is true to say that until now only limited applications have been found for 'peptide therapy', excluding those peptides of microbial origin with antibiotic activity. Naturally occurring peptides, such as angiotensin, vasopressin and oxytocin, have found limited applications because of their effects on smooth muscle, but these are exceptional. I wish to limit this short review to a consideration of the possible ways in which we may be able to exploit the knowledge that is accruing about the physiological role of small peptides and particularly of those peptides of neuronal origin. This is not intended to be an exhaustive review and, for this reason, only a few original references are given and the attention of the interested reader is directed towards the review bibliography at the end of this chapter for further reading in this field.

Classification of peptides

A list of the known 'small' biologically active peptides is given in table 1. These can be roughly grouped according to their main site of location. Thus, the first group, comprising oxytocin to β-endorphin, is largely concentrated in the pituitary gland, while the group containing cholecystokinin to gastric inhibitory peptide (GIP) is largely concentrated in the gastro-intestinal tract. However it is now clear that many peptides have an ubiquitous distribution as can be seen from table 1. The evolutionary and biological significance of the

presence of the same peptides in the gastro-intestinal tract and brain has been discussed by Pearse (1978) in relation to his a.p.u.d. (amine precursor and uptake decarboxylation) concept. The practical significance is that we can no longer think of peptide hormones as having one specific function. Indeed, the distinctions between hormones, neurosecretory hormones and neurotransmitters, have become so blurred as to be meaningless except in an operational sense when one is dealing with one specific action. Thus, vasopressin, which is classically referred to as a neurosecretory hormone when released from the posterior pituitary to act on the kidney, may also be a neurotransmitter at sites in the brain stem and spinal cord (Swanson 1977; Hökfelt *et al.* 1978). Similarly, the classical action of cholecystokinin (CCK) is to contract the gall bladder and cause release of pancreatic digestive enzymes, but the presence of CCK and of CCK carboxy-terminal fragments in the brain hints at a neurotransmitter function for these peptides (Rehfield 1978).

TABLE 1. IDENTIFIED BIOLOGICALLY ACTIVE PEPTIDES IN MAMMALS

	distribution			
peptide	pituitary	brain	g.i. tract	other
oxytocin	×	×	.	.
vasopressin	×	×	.	.
vasotocin	.	.	.	pineal
ACTH	×	×	.	.
α-MSH	×	×	.	.
β-MSH	×	×	.	.
β-endorphin	×	×	.	.
somatostatin	.	×	×	ganglia
TRH	.	×	×	.
LHRH	.	×	.	ganglia
substance P	.	×	×	ganglia
enkephalins	×	×	×	ganglia
neurotensin	.	×	×	.
carnosine	.	×	.	.
cholecystokinins	.	×	×	pancreas
gastrins	×	.	×	nerves
secretin	.	.	×	.
VIP	.	×	×	.
GIP	.	.	×	.
glucagon	.	×	×	pancreas
angiotensin-II	.	×	.	precursors in
bradykinin	.	×	.	blood and tissues

The distribution of CCK and its fragments neatly illustrates what may be a common biological mechanism of molecular adaptation (figure 1). Thus, the parent 33 residue CCK exists mainly in the gut, where it acts as an exocrine hormone, whereas CCK 12, CCK 8 and CCK 4 carboxy-terminal sequences are the main types found in the brain. It is the carboxy-terminal octapeptide and pentapeptide sequences in CCK and gastrin that confer biological activity on these molecules, and it appears that organisms have taken advantage of this to evolve other functions for the shorter, but still active, sequences of the parent compound. Similar molecular adaptations are apparent with ACTH/MSH† and β-endorphin/enkephalin sequences, and other possible adaptations might involve substance P and neurotensin, although smaller active sequences of these peptides have yet to be identified as normal tissue constituents.

† ACTH, adenocorticotropic hormone; MSH, melanocyte stimulating hormone.

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Physiological roles of peptides

Some biological actions can be readily identified and studied. Examples of these are the vasoconstrictor effects of angiotensin and vasopressin and the vasodilator effects of substance P, bradykinin, VIP and neurotensin. However, the greatest potential for exploiting peptides lies in their ability to affect a wide range of peripheral and central nervous mechanisms, and these actions are not so readily defined. Table 2 illustrates some of the central brain mechanisms with which various peptides have been identified. It should be emphasized that, in most cases, the suggested involvement is highly speculative and the evidence tenuous.

Table 2. The possible involvement of peptides in a number of central processes

TRH, thyrotropin releasing hormone; LHRH, luteinizing hormone releasing hormone; SOM, somatostatin; ENK, methionine^5 and leucine^5 enkephalin; β-END, β-endorphin; SP, substance P; VAS, vasopressin; NT, neurotensin; OXY, oxytocin; CCK, cholecystokinin peptides; ACTH, adrenocorticotrophin peptides; β-MSH, melanophore stimulating hormone.

c.n.s. mechanism	peptides
hypothalamic–pituitary control	TRH, LHRH, SOM, ENK, β-END, SP, VAS
motor control	SP, ENK
cardiovascular control	SP, ENK, NT
respiratory control	SP, ENK, NT
temperature control	SP, ENK, NT
pain and sensory mechanism	SP, ENK, β-END, SOM, NT, VAS, OXY
arousal, motivation and mood	ACTH, β-MSH, TRH, β-END, ENK
reward and learning	ACTH, VAS, OXY, ENK, β-END
eating behaviour	CCK
water drinking behaviour	ANG-II
sexual behaviour	LHRH, β-END
memory	VAS, ACTH, β-END, OXY

The chemical characterization of these peptides has allowed studies that show that specific peptides are localized in certain areas that can be linked with specific central activities, e.g. the association of somatostatin and substance P with some primary afferent sensory neurons, and the concentrations of the enkephalins, TRH and neurotensin-containing neurons at the central terminations of these afferent sensory neurons. Thus, the immunohistochemical studies provide direct evidence for the association of peptides with pain pathways (Hökfelt *et al.* 1977), and similar correlations can be made with such processes as motor, respiratory, cardiovascular and temperature control. Injection of peptides into various brain areas has been used to demonstrate effects on these and other processes. However, the latter type of experiment only tells us that a peptide can or cannot produce such an effect; a positive result does not prove that this is what normally occurs or that the particular peptide is involved.

Thus, the induction of bizarre behaviour and body immobility by β-endorphin does not necessarily prove that this opiate peptide is involved in disturbed mental behaviour (Bloom *et al.* 1976; Jacquet & Marks 1976). Such effects do give clues for further research, however, and, for the opiate peptides, further basic and clinical studies do support the concept of some involvement of these peptides in psychiatric states (Berger 1978).

Consideration of the effects listed in table 2 might lead one to the conclusion that it might be extremely difficult, if not impossible, to produce selective effects on 'target' peptide systems since there appears to be a plurality of overlapping effects and actions. However, if we assume that all these peptides act through specific receptor interactions and that there are differences

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in both the 'quality' and the 'quantity' of the effects, then the prospect is somewhat brighter. Table 2 does allow us to identify some areas of potential drug development, such as pain control and treatment of behavioural disorders and of cardiovascular and motor disease states such as hypertension and Parkinson's disease.

A knowledge of the physiological role of a particular peptide, although an advantage, is not an absolute necessity when considering drug design. Indeed, in the non-peptide field, such planned developments have been more than outnumbered by unplanned or serendipitous discoveries. Thus, the discovery of antipsychotic drugs such as the phenothiazines and butyrophenones actually contributed towards our knowledge of the involvement of the catecholamines in manic states. Thus, drug development aided physiological understanding, rather than vice versa. A similar example has occurred with the narcotic antagonists such as naloxone and naltrexone. These drugs were originally developed as opiate receptor antagonists for the treatment of narcotic overdosage and respiratory depression. However, these antagonists proved invaluable in the identification of the enkephalins (Hughes 1975) and in revealing the existence of endogenous opioid peptide systems. These antagonists are now being evaluated as possible therapeutic agents in such conditions as obesity, hypotensive shock and manic-depressive disorders.

In the following sections I will discuss the possible avenues of drug development, giving selected examples.

TABLE 3. POSSIBLE METHODS OF MODULATING PEPTIDE ACTION

(For meanings of symbols see table 2.)

drug mechanism	example
receptor agonists	TRH antidepressants LHRH fertility agents ENK analgesics
receptor antagonists	LHRH antifertility agents ENK/β-END antipsychotics SP analgesics ANG-II antihypertensives BK analgesics
inhibitors of breakdown or synthesis	ANG-II, BK } antihypertensives

DRUG DESIGN

(*a*) *Agonists and antagonists*

There are several possible approaches to drug design (table 3) that can be borrowed from other known biological systems. The most straightforward approach is to produce analogues that either mimic the parent peptide and act as agonists or occupy the peptide receptor to act as an antagonist. Even with this approach, a decision has to be made whether to modify the parent peptide or to develop non-peptide analogues. There is evidence that either approach can yield compounds with suitable activity. The best examples of non-peptide analogues acting at peptide receptors come from studies on the opiates. In this case, the number of non-peptide narcotic analgesic agonist compounds greatly exceeds the number of opioid peptide

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analogues. Similarly, the only potent antagonists available for blocking opiate receptors are derivatives of oxymorphone, benzomorphans and other narcotic analgesic structures.

To date, it appears that the search for active peptide agonists analogues has been much more successful than that for peptide antagonists. Thus, at the present time, specific receptor antagonists are available only for the opioid peptides, angiotensin, luteinizing hormone releasing factor and vasopressin. This partly explains why there have been such rapid advances in our understanding of the opioid peptides compared with the longer known peptides, such as substance P, somatostatin and TRH (thyrotropin releasing hormone). In most cases, it is impossible

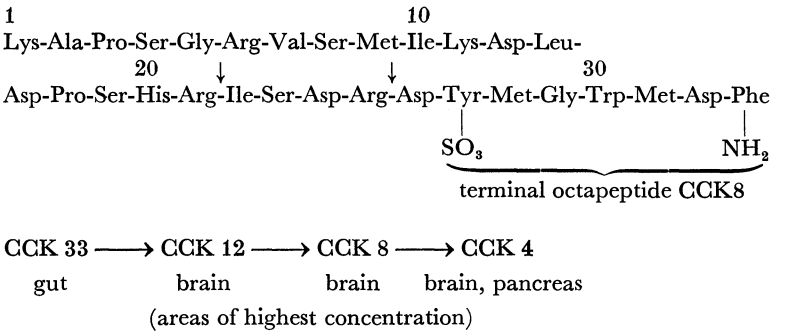

FIGURE 1. Possible molecular adaptation of cholecystokinin from exocrine hormone to putative neurotransmitter.

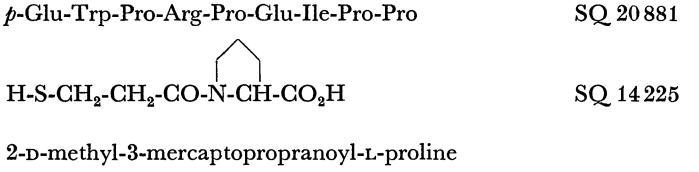

FIGURE 2. Angiotensin-converting enzyme inhibitors.

to predict the possible application of an antagonist beyond its obvious value in basic studies on the physiology and pharmacology of a peptide. With antagonists for angiotensin and LHRH (luteinizing hormone releasing hormone) there is the hope of producing effective blood pressure lowering agents and antifertility agents respectively. Studies with opiate antagonists suggest that they may have some value in certain psychiatric states, but even here the currently available drugs may not have the required specificity, since there appear to be different types of opiate receptors (Lord *et al.* 1977). Indeed, it may be expected that peptide receptors will, in general, prove to be heterogeneous in their conformational or structural requirements for ligand activation.

The existence of heterogeneous populations of receptors will certainly aid the development of specific agonists or antagonists. Such receptor specificity has been demonstrated already for analogues of TRH and somatostatin. Thus, des-Asn^{5}(D-Trp^{8}, D-Ser^{13})somatostatin is much more potent (*ca.* 20 times) than the parent compound in inhibiting insulin secretion, and much less potent than somatostatin in inhibiting glucagon and growth hormone secretion. Similarly, L-*N*-(2-oxopiperidon-6-ylcarbonyl)histidyl-L-thiazolidine-4-carboxamine, known as MK-771, an analogue of TRH, is equipotent in its pituitary releasing actions, but much more potent than the parent compound in its behavioural actions (Yarborough 1979). Similar differences

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have previously been observed for different TRH analogues in their effects in increasing prolactin release and synthesis. Thus, 3-*N*-Me(His)-TRH has equiactive effects on both these parameters, while the analogue *p*-Glu-His-Pro-NH$(CH_2)_6NH_2$ is 15 times more active in stimulating synthesis than in stimulating release. These observations do not necessarily indicate the existence of multiple receptors, but they do show that analogues with selective activity can be prepared.

The development of highly active agonist analogues of the enkephalins as analgesics is a good example of peptide drug development. The parent sequence Tyr^{1}Gly^{2}Gly^{3}Phe^{4}Met^5(Leu^5) has relatively weak *in vivo* actions even when injected directly into the brain. In contrast, the larger, 31-residue, opioid peptide β-endorphin is from 20–50 times more potent as an analgesic than morphine. These differences in potencies between the small and large opioid peptides is, at least partly, due to the rapid metabolism of enkephalin by aminopeptidases, carboxypeptidases, and probably also by a dipeptidyl carboxypeptidase. Thus, the peptide bonds 1–2, 3–4 and 4–5 are vulnerable to hydrolysis. It has been found that modification of the enkephalin molecule at positions 2, 4 and 5 considerably reduces metabolism, with the production of analogues with considerably enhanced potency (Morley 1980). Thus, the analogue (D-Ala^2, Me-Phe^4, Met(O)-ol^5)-enkephalin is 10 times more potent than morphine as an analgesic when injected intracerebrally and it is almost equipotent when given orally. However, reduction of metabolism is probably not the entire story, since there is evidence that receptor specificity may be altered in these analogues (Beaumont & Hughes 1979). In general, three considerations are uppermost in designing such analogues: (1) reduction of metabolism; (2) increase in membrane transport for oral absorption and brain penetration; and (3) maintenance of receptor specificity. This is a complicated equation and, at the present time, there is no indication that stable enkephalin analogues will have any advantage as analgesics over the stable morphine-type analgesics and their cogeners.

(*b*) *Indirectly acting peptides*

The indirect modulation of peptidergic systems may have several advantages compared to the use of directly acting analogues. Thus, if the concentration of a particular peptide, or its release, can be raised or lowered, then a desired effect may be obtained without altering receptor specificity. With this approach there is also the option of designing either non-peptide- or peptide-containing compounds for the desired effect. The development of inhibitors for angiotensin converting enzyme illustrates the potential of this approach; both SQ 20881 and SQ 14255 inhibit the conversion of the precursor angiotensin-I to the active octapeptide angiotensin-II. SQ 14225 is orally active, and preliminary clinical trials indicate that it is an effective antihypertensive agent. The rationale for this approach was that inhibition of the formation of angiotensin-II, which is a potent vasoconstrictor agent, might act to reduce blood pressure. However, these peptidase inhibitors also block the degradation of bradykinin, a potent vasodilator. Thus, the clinical effectiveness of SQ 14255 is possibly due to a dual effect, reduction of angiotensin vasoconstrictor activity and potentiation of bradykinin vasodilator activity.

There is reason to believe that there is considerable scope for further applications of this type. For example, although it was originally believed that the enkephalins were mainly degraded by a variety of rather non-specific aminopeptidases, there is now evidence (Malfroy *et al.* 1978) that a specific dipeptidyl carboxylase may limit the activity of neuronally released

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enkephalin. Specific inhibitors of this membrane-bound enzyme may prove a better alternative to the development of stable enkephalin analogues. There is little known about the normal physiological inactivation processes for other brain peptides; unfortunately, most work in this field has been carried out on tissue homogenates, or even supernatants, and these results can have little relevance to the normal physiological processes in intact tissues. There is a pressing need to identify the mechanisms responsible for the synaptic inactivation of a number of putative peptide neurotransmitters, including substance P, neurotensin, TRH, somatostatin and cholecystokinin. There is also a complete lack of knowledge regarding the nature of the processing enzymes involved in the formation of these active peptides, of which there may be several for each precursor protein. Until these are known, there is little hope of rational drug design along the lines carried out for inhibitors of angiotensin converting enzyme.

Conclusions

Research and development in the peptide field is not new, but it is likely that previous disappointments in this field contributed to a certain lack of enthusiasm for this area. Several factors may now be responsible for changing this attitude; these are: the major improvements made in peptide chemistry and synthesis; the discovery of new peptides with potent peripheral and central activities; and the first glimmers of success in designing potentially useful drugs. Among the latter, one must cite the work carried out on LHRH agonists and antagonists and the development of angiotensin receptor blocking agents and angiotensin converting enzyme inhibitors. There must now be considerable cause to be optimistic about further advances in these fields.

No doubt classical chemical methods allied to the new peptide technologies will be the methods of choice for preparing small (less than 30 residue) peptide analogues. DNA cloning techniques may eventually provide a means for producing commercial quantities of the larger proteins, but this still appears to be some way into the future. However, DNA cloning has already had a major impact on basic aspects of peptide biology, most notably in the study of peptide precursor molecules. The use of cloning techniques to produce peptide precursors may prove to be invaluable for the study of the biosynthetic mechanisms and of the cleavage enzymes. I would predict that this will be an extremely vital area of research, since there can be little hope of therapeutic manipulation until we understand these processes and the enzymes involved. The angiotensin converting enzyme work is a beautiful example of the potential value of this approach.

Little more need be said regarding the strategy and techniques for agonist analogue development, since, here, the major problems have been delineated and, in some cases, overcome. However, I would single out the development of peptide antagonists as being one of the most pressing problems and one that may well pay handsome dividends in terms of basic knowledge and possible therapeutic benefits. There is now a possibility of developing non-peptide drugs as antagonists, and every effort should be made to combine the talents of classical organic and peptide chemists in this area.

In conclusion, it is worth remembering that the peptides, in terms of numbers, represent the major mechanism of internal chemical communication. We are still largely ignorant of their function in the brain, but the diversity of the peptide structures and their information content must surely make them very attractive candidates for drug development.

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