0031-6997/86/3804-0291$02.00/0
PHARMACOLOGICAL REVIEWS

Vol. 38, No. 4
Printed in U.S.A.

The Structural Characterization of β-Endorphin and Related Peptide Hormones and Neurotransmitters*, †

JOHN W. TAYLOR and EMIL THOMAS KAISER

Laboratory of Bioorganic Chemistry and Biochemistry, The Rockefeller University, New York, New York 10021

I. Introduction

A LARGE NUMBER of peptides and proteins having important pharmacological functions ranging from that of neurotransmitter or neuromodulator to circulatory hormone have been identified and their amino acid sequences elucidated. On considering the structures of these polypeptides in their biologically active forms, it appears that there are three general categories. The first group consists of short peptides, such as the five amino acid residue opioids [Met^5]-enkephalin and [Leu^5]-enkephalin, where essentially the whole structure constitutes the specific recognition site that determines their interactions with cell surface receptors and possibly other molecules. The active conformations of these peptides will be determined almost entirely by their environment, and these interactions may readily be probed through the systematic investigation of a large number of synthetic analogues, often incorporating conformational restrictions (70a, 135a), in the manner of classical pharmacological studies of small organic molecules. Another category of polypeptide hormones consists of more complex structures that are large enough to be stabilized in aqueous solution by multiple disulfide bonds or the formation of a hydrohobic core. Compounds of this type, such as insulin or growth hormone, may have interactions with binding sites that involve amino acid residues well separated in the peptide chain, but held together in a particular conformation by the tertiary structure of the molecule. In these cases, synthetic analogues will be more difficult to prepare, but it may be possible to draw conclusions directly from studies of solution or crystal structures with the aid of only a few analogues, possibly including species variants and analogues prepared by direct chemical modification of specific amino acid residues or site-directed mutagenesis of the corresponding gene.

The third category of peptide hormones consists of polypeptides that have structural properties of an intermediate nature. These peptides often consist of a single peptide chain of about 10 to 50 amino acid residues and will usually contain no disulfide bridges, or at most only one. The characterization of important residues and the biologically active conformation(s) of these peptides presents special problems. Although they are usually synthetically accessible through the standard methods of solid-phase peptide synthesis, their length precludes a systematic investigation of the importance of each amino acid residue through the study of analogues. Furthermore, the effects of such structural modifications on activities may be difficult to interpret. Elements of secondary and tertiary structure that are not present in

* This article is the fifth in a series of reviews on various aspects of opioid pharmacology which has been arranged with the help of Hans W. Kosterlitz, Aberdeen, and Eric J. Simon, New York, acting as Consulting Editors. Earlier articles of this series appeared in volume **35:** 33–53, 69–83, 219–281, and 283–323, 1983.

† Partial support of our research was by USPHS program project grant HL-18577 (E. T. K.), by a grant from the Dow Chemical Company Foundation (E. T. K.), and by a fellowship from the Norman and Rosita Winston Foundation (J. W. T.).

aqueous solution are likely to be stabilized by the interactions of peptides in this category with their functional environment. Single amino acid substitutions will then have both direct effects on these interactions and indirect effects arising from their role in the formation of such structures, and they are unlikely to provide any information regarding, for example, the receptor-bound conformation of the natural peptide. This review describes how these structures may often be identified and their functions and importance characterized through the design and study of appropriate synthetic peptide models.

II. Amphiphilic Secondary Structure

It has been suggested that the functional environment of any peptide acting at biological interfaces such as a protein or cell surface will often be amphiphilic (76, 77). In other words, the expression of activity will usually involve binding at the interface between the hydrophobic core of a structure and its aqueous surroundings. This type of anisotropic environment is likely to induce the formation of discrete segments of secondary structure in peptide hormones of the third category discussed above, if these structures result in the segregation of hydrophobic and hydrophilic amino acid residues in the peptide chain into separate domains creating a complementary amphiphilicity.

The formation of amphiphilic secondary structures and the properties they exhibit have been studied in a number of model peptide systems. An amphiphilic β strand will result from alternating hydrophobic and hydrophilic amino acid residues in the linear sequence (17). Model peptides with this type of sequence that consist of more than about six residues have circular dichroism (CD) spectra indicative of a high β strand content which results from the pronounced tendency of these peptides to self-associate forming amphiphilic β sheets (17, 34, 118, 123, 124). In aqueous solution, these β sheets can self-associate to bury their hydrophobic faces, or they will bind very tightly at amphiphilic interfaces such as the surfaces of phospholipid vesicles or serum lipoproteins or the air-water interface, where extremely stable monolayers are formed. Longer sequences of alternating hydrophobic and hydrophilic residues are usually difficult to solubilize in aqueous solutions (118, 123).

Model peptides that can form amphiphilic α-helical structures have also been studied. In this case, the distribution of the hydrophobic and hydrophilic amino acid residues in the linear sequence of a peptide that gives rise to such a structure will depend on the size and shape of the hydrophobic domain formed. Peptides of this type that are about 20 residues long and can form α helices with a hydrophobic domain lying parallel to the helix axis along one side of the helix also self-associate in aqueous solution (33, 52). Discrete aggregated forms such as tetramers are observed, and they have a high α-helical content, although the monomeric peptides have very little ordered structure in aqueous solution. These peptides will bind to phospholipid surfaces and form stable monolayers at the air-water interface, and they behave as monomers with an α-helical structure in both of these situations. Increasing the fraction of the surface of the helical structure that is hydrophobic from one-third to two-thirds causes an increase in the strength of all of these interactions (33). The incorporation of a positively charged residue into the sequence so that it will occupy a position in the center of the hydrophobic domain of the helical structure prevents self-association, but does not markedly affect binding to phospholipid vesicles or monolayer stability (53). There has been no systematic study of the dependence of these interactions on the length of the peptide chain. However, studies of homooligopeptides in the helix-promoting solvent trifluoroethanol suggest that, with suitable stabilization by the environment, the transition from predominantly random coil to α-helix will occur in peptides that are 10 to 15 residues long (118). This length is consistent with the information available for the stabilization of amphiphilic α helices at hydrophobic-hydrophilic interfaces (34, 52, 83, 86).

With the probable exception of proline residues, where the amino groups cannot participate in hydrogen bonding, the actual identity of the hydrophobic and hydrophilic residues that constitute an amphiphilic secondary structure appears to be a much less important determinant of the type of secondary structure formed in an amphiphilic environment, i.e., helix or β sheet, than the periodicity with which they occur (34, 79). The amino acid content may, however, make a significant difference to the overall stability of the structure formed. In this respect, the conformation parameters described by Chou and Fasman and others (29, 54, 100), which describe the frequencies with which each residue type occurs in different secondary structures in the crystal structures of globular proteins, may be a useful guide. Nevertheless, an initial search for potential regions of amphiphilic secondary structure in peptide hormones can be made (*a*) by scanning the linear sequences of residues for regions of alternating hydrophobic and hydrophilic residues that might form β sheets and (*b*) by projecting the sequences on helical net (37) or helical wheel (135) diagrams to identify segments where the hydrophobic and hydrophilic residues are segregated in separate domains on the helix surface that might form α helices. In this way, potential amphiphilic helical structures have been identified in many different peptide hormones, including β-endorphin, calcitonin, glucagon, corticotropin releasing factor (CRF), growth hormone-releasing factor (GRF), parathyroid hormone (PTH), and pancreatic polypeptide; as well as a number of structurally homologous peptides (*vide infra*). These structures differ considerably from one another in their general characteristics, such as the size and shape of the hydrophobic domain if a regular α-helical conformation were adopted, the type of charged residues and their distribution on the

hydrophilic side of the helix, the number of aromatic residues on the hydrophobic side, the overall length of the helix, and the number of apparent "mistakes" in its amphiphilicity, i.e., residues that have been defined as hydrophilic which occur in the hydrophobic domain and *vice versa*. In contrast to the common occurrence of amphiphilic α helices, relatively few peptide hormones that contain regions of potential amphiphilic β strand structure have been identified. These include leuteinizing hormone releasing hormone (LHRH) and dynorphin A(1–17), and they involve sequences of 10 or fewer amino acid residues, as expected from the intractable nature of model peptides with longer structures of this type.

The great variety in the general characteristics of these amphiphilic structures suggests that they can contribute to the functional diversity of the peptide hormones. However, their common occurrence also implies that they will determine certain general aspects of peptide hormone action that may be related to the properties of the amphiphilic model peptides described above. For example, an amphiphilic α helix or β strand might bind to a complementary site on a protein receptor, either causing signal transmission directly or else positioning other parts of the hormone in the correct orientation for their interactions with the receptor to result in signal transmission. This type of protein-protein interaction resembles the self-association of the model peptides and is expected to be sensitive to the nature of the residues on the hydrophobic face of the amphiphilic structure. The complex formed by a peptide ligand bound to its protein receptor should, when considered as a whole, have similar structural properties to globular proteins consisting of a continuous peptide chain, and amphiphilic α helices and β sheets are commonly found on the surface of these structures (39, 128).

A second possibility is that amphiphilic secondary structures might interact with the phospholipid surfaces of cells. Depending on the equilibrium dissociation constant for the peptide binding, which might be 1 μM or even lower, and the rate at which the bound peptide can diffuse on the surface of the cell, this type of interaction could serve to enhance the rate at which cell surface receptors are located: the three-dimensional search that would otherwise be required would become a two-step search involving adsorption to any point on the cell surface, which represents a relatively large target to find, followed by diffusion in only two dimensions on that surface (1, 6a, 62, 75, 80). Ultimately, binding to the receptor might then involve another part of the peptide molecule, with the amphiphilic secondary structure remaining at the phospholipid-water interface. The partitioning of a peptide hormone between the aqueous phase and cell surfaces by phospholipid binding could, alternatively, serve to limit its distance or rate of diffusion from the point of release, and may also either protect the peptide from attack by proteolytic enzymes or lead to more rapid and, possibly, specific breakdown by membrane-associated proteases. All of these possibilities could have a dramatic effect on the pharmacokinetics observed.

Since the hydrophobic-hydrophilic interface of a phospholipid membrane surface is approximately planar, an amphiphilic helix having a hydrophobic domain lying parallel to the helix axis would be expected to be ideal for binding to this surface (141), and indeed α helices of this type are apparently ubiquitous to the apolipoproteins which coat the phospholipid surfaces of serum lipoproteins (127, 151), and commonly occur in peptide toxins that act primarily at the phospholipid surfaces of cells (2, 3, 33). It might be possible for peptides that cannot form this type of hydrophobic domain in an α-helical conformation to form another sterically accessible structure, such as a π helix or a 3_{10} helix (36), in order to bind to phospholipids (76, 77). (In a π helix, a regular pattern of hydrogen bonding exists linking carbonyl oxygens and amide nitrogens of the nth and n+5th amino acid residues in the peptide chain, respectively, and in a 3_{10} helix the residues in positions n and n+3 are linked. These helical structures are otherwise analogous to the regular α-helical structure in which the nth and n+4th residues are hydrogen bonded.) This contrasts with the likely possibilities for binding to a protein receptor, since the only other helical structure that is known to occur in globular proteins, besides regular or distorted forms of the α helix, is the 3_{10} helix, and extended structures of this type are rare (128). However, protein-protein interactions may involve hydrophobic domains of a different shape, possibly twisting around the helix surface (28a, 38a). Thus it may be that peptide hormones like β-endorphin and GRF, which can form amphiphilic π helices of the type suitable for binding to planar amphiphilic interfaces (77), will adopt differential helical conformations in different environments.

In addition to the amphiphilic β-sheet forming peptides mentioned above, evidence for a third type of amphiphilic structure being involved in phospholipid binding has been obtained for adrenocorticotrophic hormone (ACTH) residues 1–24 (62). In this case, the phospholipid-bound peptide is proposed to consist of the hydrophobic amino-terminal segment of the peptide in an α-helical conformation buried perpendicularly to the phospholipid surface and connected to the hydrophilic carboxy-terminal segment lying in the plane of the phospholipid surface in an extended form.

Segments of amphiphilic secondary structure might also be stabilized by interactions with other soluble components of the biological milieu, including proteins, lipids, and ions. This in turn might induce the formation of more structure in the peptide through interactions of other parts of the molecule with the hydrophobic face of that secondary structure. However extensive the interactions are, any such increase in conformational rigidity is likely to result in a corresponding decrease in the susceptibility of the peptide towards the actions of solu-

ble proteolytic enzymes (78). Alternatively, the interactions with other components in solution might include the formation of tight complexes that could fulfill a function in themselves, as is suspected of the binding of amphiphilic α-helical peptides to a hydrophobic domain in calmodulin (31).

III. The Study of Peptide Models

The properties and potential functions of amphiphilic secondary structures suggest that it might be possible to replace these segments of peptide hormones with nonhomologous amino acid sequences chosen to preserve their most important features, perhaps in an idealized form, and still retain the activities of the natural peptide (76, 77). By comparing the physicochemical and pharmacological properties of peptide models of this type to those of the natural hormone, structure-function relationships may be developed that determine the importance of each feature. To the extent that homology with the natural sequence of a particular structural element can be minimized and the activities which depend on it can still be reproduced, evidence of the active conformation of the hormone will also be obtained by this modelling approach (78). Eventually, and without recourse to the synthesis of a vast array of synthetic analogues, it should be possible to design peptide models that have enhanced specificities and potencies or more desirable pharmacokinetic properties, possibly incorporating nonnatural amino acid residues or even nonpeptidic chains.

Synthetic peptide models have previously been used to investigate the functional structures of peptides and proteins which interact primarily with phospholipid surfaces, including the serum apolipoproteins (52, 53, 118a, 124, 166) and the bee venom toxin melittin (33, 35). In these cases, regions of the natural polypeptides which had the potential to form amphiphilic α-helical structure were identified. The functions of these structural segments were then investigated by studying synthetic analogues that incorporated peptide sequences having, as a first priority, minimal homology to the natural sequences, but which retained the ability to form an amphiphilic structure in the α-helical conformation. Particular attention was given to the reproduction in the model peptides of such features of the natural structures as the length of the potential helix, the size and shape of its hydrophobic domain, and the distribution of basic and acidic residues that would carry positive and negative charges in the physiological pH range on the hydrophilic face of the helix. Amino acid residues such as leucine, lysine, glutamic acid, and glutamine were chosen to construct the model helical structures because of their propensity for helix formation in globular proteins (29). It was expected that the physicochemical properties of the natural polypeptides that were dependent on the amphiphilic structure would be enhanced by this form of structural idealization, and that this might also lead to an enhancement of their biological functions. This approach has proven extremely successful, in that a 22-residue model peptide and its dimeric analogue were able to reproduce all of the salient physicochemical and biological properties of the 243-residue serum apolipoprotein A-I, including its function as an essential cofactor in the action of lecithin:cholesterol acyltransferase (52, 118a, 166). Also, a melittin analogue with very little homology to the bee venom peptide was prepared which lysed unilamellar phospholipid vesicles and erythrocytes at low concentrations and activated phospholipase A_2 (33, 35). In each of these studies, it was possible to identify the active amphiphilic conformations of the natural polypeptides in their functional environments and also to gain some understanding of the importance of particular features of these structures, only because the modelling approach was applied and both the physicochemical and the biological properties of the natural and model peptides were studied in parallel.

In the remainder of this article, the application of this approach to studies of β-endorphin, calcitonin, and glucagon is reviewed, and its potential application to other related hormones is also discussed. An enormous body of literature describing the more conventional studies aimed at the structural characterization of these peptide hormones already exists. Some of this work is reviewed here in support of the conclusions drawn from the properties of the peptide models, particularly in the case of β-endorphin. However, it is worth emphasizing the efficiency with which the peptide modelling approach described here can lead to a comprehensive understanding of the structures of these intermediate-sized peptides in their functional environments. Often, when extensive data concerning the properties of the conventional structural analogues are considered, it only serves to illustrate the inadequate and time-consuming nature of other, more established methods.

IV. β-Endorphin

The biosynthesis of endogenous opioid peptides involves the proteolytic processing of three distinct precursor peptides (69). The 31-residue peptide β-endorphin is derived from proopiomelanocortin; several copies of [Met^5]-enkephalin and small [Met^5]-enkephalin-containing peptides as well as [Leu^5]-enkephalin are derived from proenkephalin A; and several [Leu^5]-enkephalin-containing peptides, including the neoendorphins, dynorphin A (dynorphin 1–17) and dynorphin B (rimorphin), are derived from proenkephalin B. These peptides all contain the [Met^5]-enkephalin or the [Leu^5]-enkephalin sequence at their amino termini. The enkephalins alone contain sufficient specificity for potent binding to opioid receptors with concomitant agonist activity. However, the carboxy-terminal extensions found in the other opioid peptides result in important differences in their properties. For example, the selectivity of the enkephalins for δ-opioid receptors is not ob-

served for β-endorphin which has similar affinities for δ- and μ-opioid receptors (103, 124a), or for the proenkephalin B products which are selective for κ-opioid receptors and bind to the δ and μ receptors less tightly (28, 30, 157). The carboxy-terminal segments of β-endorphin and dynorphin A have also been shown to confer a resistance to degradation on the amino-terminal enkephalin structure (4, 30, 107, 147), which is otherwise rapidly hydrolysed *in vivo* (139). In the case of β-endorphin and also, possibly, dynorphin A(1–17) this property in particular is consistent with a function as a circulating hormone upon release into the bloodstream from the pituitary.

When the structures of mammalian and avian β-endorphins (66) are compared (figure 1), the [Met^5]-enkephalin sequence at the amino terminus is conserved, as expected. Overall length is also conserved, but the remainder of the sequences show more variations, with differences observed at residue positions 6, 9–12, 15, 23, 25–27, and 31. Nevertheless, the existence of a conserved "hydrophobic core" in residues 13–25 has been noted (81). Two β-endorphin-like peptides from salmon have also been characterized, which are slightly shorter and less homologous but otherwise share these conserved features (81).

A thorough structural characterization of β-endorphin by the peptide modelling approach has been performed using six peptide models (figure 2) that were designed and studied sequentially (rather than in parallel) (11, 12, 78, 126, 145–147). The design of these peptides was based on the division of the hormone into three separate structural units: an opioid receptor recognition site at the amino terminus (the [Met^5]-enkephalin sequence in residues 1–5) that is connected via a hydrophilic link (residues 6–12) to a potential amphiphilic helix in residues 13–29 (147). The amino-terminal segment was retained in all of the model peptide structures, since it was expected to have highly specific interactions with opioid receptors, and single residue deletion and substitution analogues have confirmed this expectation (*vide infra*). The hydrophilic linking region appeared to have little propensity for formation of secondary structure on the basis of predictive parameters (29). Nor is there any particular distribution of charged residues that might have a strong influence on interactions with opioid receptors, as do the multiple basic residues in dynorphin A(1–13), for example (27). Peptide segments consisting of alternating serine and glycine residues (peptides 2 and 4) or residues of the nonnatural amino acid γ-amino-γ-hydroxymethylbutyric acid (S-isomer, in peptide 6) that

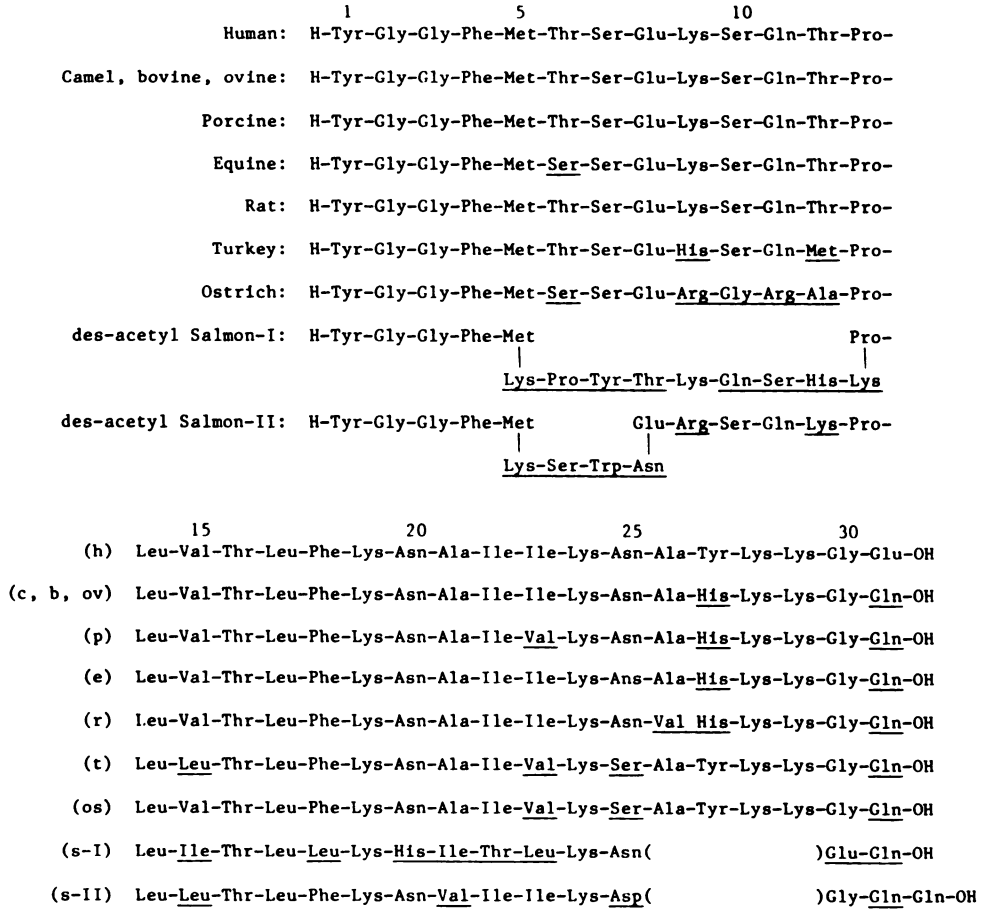

FIG. 1. Amino acid sequences of naturally occurring β-endorphin homologues. The structures are taken from refs. 66 and 81 and are aligned for maximum homology with the human sequence. Differences from the human sequence are *underlined*.

(a) Peptide Models of β_h-Endorphin

```
                                5                  10                  15
Peptide  1:  H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-
Peptide  2:  H-Tyr-Gly-Gly-Phe-Met-Ser-Gly-Ser-Gly-Ser-Gly-Ser-Pro-Leu-Leu-
Peptide  3:  H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Leu-
Peptide  4:  H-Tyr-Gly-Gly-Phe-Met-Ser-Gly-Ser-Gly-Ser-Gly-Ser-Pro-Leu-Leu-
Peptide  5:  H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Gln-Leu-
Peptide  6:  H-Tyr-Gly-Gly-Phe-Met-[NH.CH(CH2OH).CH2.CH2.CO]4--Pro-Leu-Leu-

                      20                  25                  30
(1) Thr-Leu-Phe-Lys-Gln-Leu-Leu-Lys-Gln-Leu-Gln-Lys-Leu-Leu-Gln-Lys-OH
(2) Gln-Leu-Trp-Gln-Lys-Leu-Leu-Lys-Gln-Leu-Gln-Lys-Leu-Leu-Gln-Lys-OH
(3) Lys-Leu-Leu-Gln-Lys-Leu-Leu-Leu-Gln-Lys-Leu-Phe-Lys-Gln-Lys-Gln-OH
(4) Leu-Lys-Trp-Leu-Gln-Gln-Lys-Gln-Leu-Leu-Gln-Leu-Lys-Lys-Leu-Leu-OH
(5) Leu-Lys-Leu-Leu-Gln-Leu-Leu-Lys-Leu-Leu-Gln-Lys-Phe-Gln-Lys-Gln-OH
(6) Lys-Leu-Leu-Gln-Lys-Leu-Leu-Leu-Gln-Lys-Leu-Phe-Lys-Gln-Lys-Gln-OH
```

(b) Peptide Models of Calcitonin

```
                                5                  10                  15
Peptide  7:  H-Cys-Gly-Asn-Leu-Ser-Thr-Cys-Leu-Leu-Gln-Gln-Trp-Gln-Lys-Leu-
Peptide  8:  H-Cys-Ser-Asn-Leu-Ser-Thr-Cys-Leu-Leu-Gln-Gln-Leu-Gln-Lys-Leu-

                      20                  25                  30
(7) Leu-Gln-Lys-Leu-Lys-Gln-Leu-Pro-Arg-Thr-Asn-Thr-Gly-Ser-Gly-Thr-Pro-NH2
(8) Leu-Gln-Lys-Leu-Lys-Gln-Tyr-Pro-Arg-Thr-Asn-Thr-Gly-Ser-Gly-Thr-Pro-NH2
```

(c) Peptide Models of Glucagon

```
                                5                  10                  15
Peptide  9:  H-His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-
Peptide 10:  H-His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-

                      20                  25
(9) Ser-Arg-Arg-Leu-Gln-Glu-Leu-Leu-Gln-Leu-Ala-Leu-Gln-Thr-NH2
(10) Ser-Arg-Arg-Leu-Gln-Glu-Phe-Leu-Gln-Trp-Ala-Leu-Gln-Thr-NH2
```

FIG. 2. Amino acid sequences of the peptide models. Part of the structure of peptide 5 is *underlined* to indicate where D-amino acid residues were used in its construction rather than amino acid residues of the natural L configuration.

should mimic the length, hydrophilicity, and proposed lack of structure of this domain were incorporated into some of the model peptides. In other models (peptides 1, 3, and 5), the natural sequence of β_h-endorphin‡ in this region was retained.

The potential amphiphilic helix in β_h-endorphin was shown to have a hydrophobic domain that covers approximately half of the helix surface and lies along the length of the helix either parallel to the axis in a π-helical conformation, or with a clockwise twist around the helix in an α-helical conformation (figure 3). Structure-breaking (29, 54, 100) proline and glycine residues (positions 13 and 29) were proposed to define the ends of this helix when it forms, with the proline residue participating in the formation of the hydrophobic domain. In all of the model peptides, a proline residue was retained in case this residue is important in restricting the relative orientations of the different structural domains of β-endorphin on opioid receptors. All of the model peptides contained structural models of the amphiphilic helical domain which are illustrated by the α-helical net diagrams in figure 4. In these model structures, the natural residues were replaced by sequences consisting mostly of leucine, lysine, and glutamine residues. These residues were chosen for their propensity to adopt an α-helical conformation (29, 54, 100) and to provide hydrophobic, basic hydrophilic, and neutral hydrophilic elements of the model structures, respectively. In the design of peptide 1, these residues were used to replace the natural sequence in residues 20–31 so that an amphiphilic α helix with a hydrophobic domain lying parallel to the helix axis could be formed throughout residues 13–31. Peptide 2 was designed to form a similar structure to peptide 1, but with even less homology to the natural sequence. In peptides 3 and 6, identical model amphiphilic helices were used which exactly reproduced

‡ The β-endorphin structures corresponding to particular species variants are referred to, where appropriate, by the use of subscripts as follows: h = human; c = camel; b = bovine; ov = ovine; p = porcine; e = equine; r = rat; t = turkey; os = ostrich; and s = salmon.

the shape of the hydrophobic domain in the natural structure in either the α or the π conformation. In peptide 4, the same amino acid residues were used as in peptide 2, but in a rearranged sequence chosen so that a helix in residues 13–31 would *not* be amphiphilic. In peptide 5, only D-amino acid residues were used in positions 13–31 in a linear sequence chosen to allow formation of a left-handed α helix with similar characteristics to the natural right-handed α-helical structure, but not the π-helical structure. Aromatic residues were also incorporated into positions in the model helical structures that correspond to the phenylalanine residue in position 18 of β_h-endorphin (peptides 2 and 4) and the tyrosine residue in position 27 (peptides 3, 5, and 6). When Corey-Pauling-Koltun (CPK) models were examined, these residues were found to be prominent features of the hydrophobic face of the proposed amphiphilic structure, and they were considered likely to have important interactions with receptors that might not be reproduced by leucine residues substituted into the identical positions.

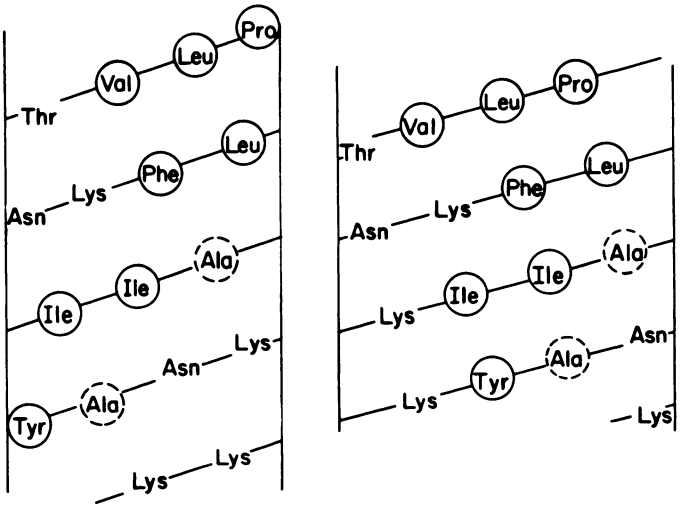

FIG. 3. Helical net diagrams of β_h-endorphin residues 13–29. This type of diagram is the equivalent of wrapping a piece of paper once around the cylindrical surface of the helical structure and marking on it the position of the α carbon atoms of the amino acid residues. The vertical edges of such a diagram would be connected on the helix surface and parallel to the helix axis. In this case, the diagrams are used to compare the distributions of the amino acid side chains of β-endorphin residues 13–29 on the surfaces of a regular α helix (*left*) and a π helix (*right*). The hydrophobic residues are *circled*.

A. Physicochemical Properties

In water and buffered saline solutions at neutral pH, the CD spectra of β_h-endorphin, β_p-endorphin, and β_c-endorphin indicate the formation of very little recognizable secondary structure, although hydrodynamic studies suggest that β_c-endorphin is at least partially folded (68, 126, 147, 164). Equilibrium centrifugation assays indicate that β-endorphin is monomeric at a concentration of 40 μM in aqueous saline solution at neutral pH, indicating that the potential amphiphilic helical structure does not readily promote self-association of the peptide (147). In attempts to identify preferred types of secondary structure that might be induced in β-endorphin by interactions with its receptors, the effects of added lipids and different solvents have also been tested. In trifluoroethanol and methanol solutions, and in the presence of negatively charged (but not neutral) lipids that have been implicated in opioid receptor binding, such as phosphatidyl serine or cerebroside sulfate (101), α-helical structure is induced in the different mammalian β-endorphins tested (68, 155, 164). Furthermore, the effects of methanol or cerebroside sulfate on amino-terminal and carboxy-terminal deletion analogues of β_h-endorphin showed that the induced helical structure lies in the carboxy-terminal half of the molecule corresponding to the potential amphiphilic structure (154). The ability of β_h-endorphin to form insoluble monolayers of modest stability on the surface of saline solutions is also indicative of its potential to form an amphiphilic structure. A detailed analysis of the properties of these monolayers showed that the relatively small area occupied by the molecules (14 Å^2/residue) was consistent with a helical

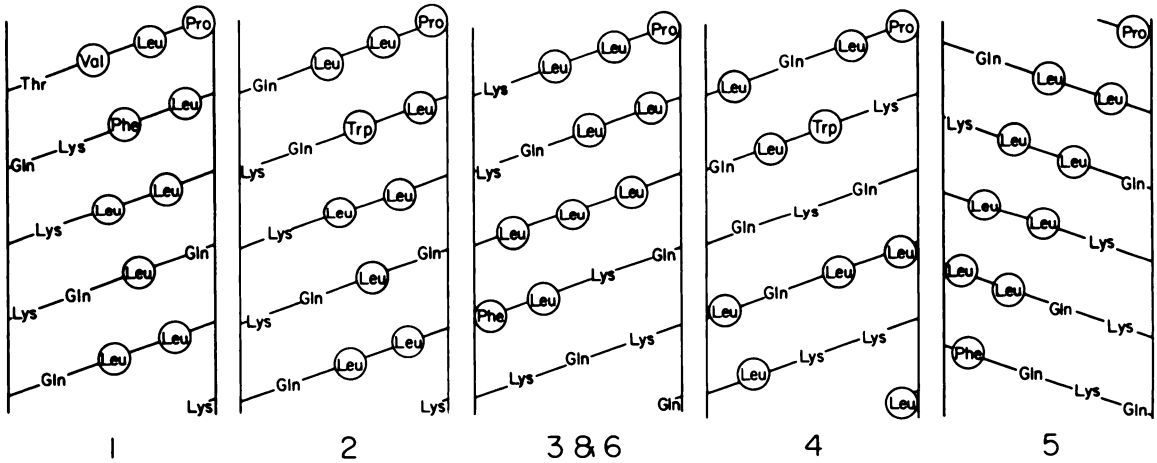

FIG. 4. Helical net diagrams of residues 13–31 of peptides 1–6. The carboxy-terminal portions of the β-endorphin peptide models are compared in the right-handed (peptides 1–4 and 6) and left-handed (peptide 5) α-helical conformations. The hydrophobic residues are *circled*.

structure in about half of the molecule occupying the surface with the rest of the peptide structure probably extending into the aqueous subphase and fully hydrated (126). In general, therefore, the physicochemical properties of β-endorphins are consistent with the potential importance of induced amphiphilic structure in peptide hormones of this type, and with the structural hypothesis used in the design of the β-endorphin model peptides.

In contrast to β_h-endorphin, the CD spectra of peptides 1, 2, 3, 5, and 6 in buffered saline solutions at neutral pH all showed concentration-dependent mixtures of helix and random-coil structure, indicative of self-association and concomitant stabilization of their amphiphilic helical structures (table 1). Furthermore, the spectra obtained for peptide 5 were consistent with the stabilization of a left-handed α-helical structure upon self-association, corresponding to the presence of only D-amino acids in its carboxy-terminal sequence. The self-association of peptides 1 and 2 at lower concentrations than peptides 3, 5, and 6 was attributed to the design of the amphiphilic α helices in the earlier peptide models to create a hydrophobic domain lying parallel to the α helix axis instead of having a clockwise twist. Thus, the lack of self-association observed for β_h-endorphin results partly from its inability to form an α helix of this type. However, additional properties of residues 13–31 of the natural peptide must further inhibit helix formation compared to peptides 3, 5, and 6, and the presence of β-branched residues (Val^{15}, Thr^{16}, Ile^{22}, and Ile^{23}), which may be conformationally restricted on a helix surface, as well as residues with little propensity for helix formation (Asn^{20}, Asn^{25}, Tyr^{27} and Gly^{30}) may be important in this respect. The properties of the peptide models of β-endorphin at the air-water interface were also consistent with the formation of a more stable helical structure than β-endorphin has in this environment (table 1). Even the most conservative peptide models (peptides 3 and 6), where all of the general features of the potential amphiphilic α-helical structure in β_h-endorphin were retained, formed very stable monolayers with high collapse pressures. The surface area occupied by these two model peptides (16 $Å^2$/residue) was similar to that of β_h-endorphin, however, indicating that similar conformations were adopted. Substitution of γ-amino-γ-hydroxymethylbutyric acid residues for the natural sequence of the hydrophilic linking region had essentially no effect on self-association or monolayer formation. The nonamphiphilic peptide 4 was monomeric with little helical structure at a concentration of 10 μM, and insoluble at higher concentrations, and monolayers formed by peptide 4 were much less stable than those formed by its parent compound, peptide 2. These results demonstrated the importance of the amphiphilic carboxy-terminal structure in determining conformational properties of these peptides in solution and at amphiphilic interfaces.

B. Resistance to Enzymatic Inactivation

As we have discussed, the presence of amphiphilic structure in peptide hormones may increase their resistance to proteolytic degradation and cause nonspecific interactions with cell surfaces, and such properties will markedly affect pharmacokinetic behavior. The resistance of the amino-terminal residues of β-endorphin to purified amino-peptidases and to the enkephalinases in brain homogenates relative to the rapidly degraded enkephalins has been demonstrated in a variety of experiments 4, 30, 59, 107, 120, 147). In aqueous solution, this property appears to be related to the formation of some type of tertiary structure involving interactions of the amino- and carboxy-terminal ends of the molecule (7, 91, 120). However, direct interactions of the amphiphilic carboxy terminus with proteolytic enzymes may also be important, as the proteolysis of [Met^5]-enkephalin by a solubilized rat brain aminopeptidase is inhibited by addition of β_h-endorphin, and β_h-endorphin(1–17) is a much less effective inhibitor of this degradation (71).

The relative resistance towards proteolysis of the peptide models in the presence of diluted rat brain homogenates at 37°C has been compared to that of β_h-endorphin (table 2). This property correlated well with the

TABLE 1

Physicochemical properties of β_h-endorphin and peptides 1–6[a]

Peptide	Self-association in aqueous solution[b]	θ_{222} (deg cm^2/dmol)[b,c]		Monolayer collapse pressure (dyn/cm)[b]
		Monomer	Oligomer	
β_h-Endorphin	None at 40 μM	−1,800		7
Peptide 1	Above 200 μM	−9,000	−15,750	24
Peptide 2	Above 3 μM	−11,400	−13,900	24
Peptide 3	Above 10 μM	−6,450	−10,650	21
Peptide 4	None at 10 μM[d]	−2,800		11
Peptide 5	Above 10 μM	4,300[e]	8,500[e]	15
Peptide 6	Above 10 μM	−3,950	−8,750	22

[a] Data are compiled from refs. 11, 12, 126, and 145–147.

[b] The aqueous phase contained 160 mM KCl, buffered at pH 7.4.

[c] Increasing negative values are indicative of increasing right-handed helical structure in peptides consisting of helical and random coil structures only.

[d] Insoluble above this concentration.

[e] Contained left-handed helical structure.

TABLE 2
Resistance to enzymatic inactivation of β_h-endorphin and peptides 1–6[a]

Peptide	Assay of degradation in rat brain homogenates		Enzymatic inactivation of peptides in smooth muscle assays	
	Initial yield (%)[b]	Subsequent degradation[c]	GPI assay	RVD assay
β_h-Endorphin	70	Rapidly degraded[d]	Resistant[d]	Rapidly inactivated[d]
Peptide 1	10	Resistant	Resistant	Resistant
Peptide 2	20	Resistant	Resistant	Resistant
Peptide 3	20	Slowly degraded	Resistant	Resistant
Peptide 4	30	Rapidly degraded	Rapidly inactivated	Rapidly inactivated
Peptide 5	10	Resistant	Resistant	Resistant
Peptide 6	10	Slowly degraded	Resistant	Resistant

[a] Data are compiled from refs. 11, 12, 126, and 145–147.
[b] After centrifugation of suspensions containing the peptides (10 μM) and diluted homogenates to remove particulate matter.
[c] Upon incubation of the peptide-brain homogenate suspensions at 37°C.
[d] Compared to β_h-endorphin, [Met^5]-enkephalin degradation/inactivation is considerably more rapid.

tendencies of these peptides to adopt amphiphilic conformations through self-association or binding to suitable interfaces. At concentrations of 10 μM, the nonamphiphilic peptide 4 was degraded at about the same rate as β_h-endorphin, whereas its parent compound, peptide 2, as well as peptides 1 and 5 were apparently completely resistant to proteolysis. Again, peptides 3 and 6 displayed identical behavior intermediate between that of the natural peptide and the highly helical peptides 1 and 2. This assay, which involved centrifugation of the incubating suspensions followed by high-pressure liquid chromatography (HPLC) quantification of the amount of each peptide recovered in the supernatants, also demonstrated that the model peptides were mostly bound to the rat brain membranes, since recoveries were as low as 10–30% at zero time. In contrast, β_h-endorphin could be recovered in high yields initially, although it was subsequently degraded. Other strong interactions with cell membranes have been demonstrated for peptides 1 and 2, which are able to lyse erythrocytes at concentrations similar to those observed for the bee venom peptide, melittin, and also disrupt unilamellar phospholipid vesicles under certain conditions (144). No similar effects were observed for β_h-endorphin.

C. Receptor Binding Properties

The synthesis of tritiated β_h-endorphin has allowed the direct characterization of its binding sites (87). Many subsequent binding studies of β-endorphin analogues have employed this radiolabel, with the probable advantage that their relevance to β-endorphin activities in vivo is more likely, but with the disadvantage that the relationship between β-endorphin structure and opioid receptor selectivity is not determined. One exception was a comparison of the inhibitory potencies of carboxy-terminal deletion analogues of β_h-endorphin on the binding of ^{3}H-dihydromorphine (a ligand selective for μ-opioid receptors), ^{3}H-[Leu^5]-enekphalin (δ receptors), and ^{3}H-β_h-endorphin to rat brain membranes (45). The binding potency relative to that of β_h-endorphin increased 1.75-fold for β_h-endorphin(1–30) and decreased steadily with decreasing chain length to 0.008 times that of β_h-endorphin for β_h-endorphin (1–15), when ^{3}H-β_h-endorphin was used as the receptor label. A similar trend in the binding potencies was observed when the other radioligands were used, but β_h-endorphin analogues shorter than β_h-endorphin (1–27) displayed a greater preference for binding to the ^{3}H-[Leu^5]-enkephalin-labelled receptors than the ^{3}H-dihydromorphine-labelled ones. These results indicate a role for the carboxy-terminal region in determining both the potency and the δ/μ selectivity of β_h-endorphin to opioid receptors.

The relative importance of the [Met^5]-enkephalin segment of β_h-endorphin that is suggested by its sequence conservation in the natural peptides is supported by a number of binding studies showing that single amino acid residue deletions or modifications in this region can drastically alter the affinity of β_h-endorphin for its binding sites in rat brain membranes (32, 65, 98, 168). The contribution of the carboxy-terminal region to binding potency is less, but it has been shown to correlate well with α helix formation in 75% trifluoroethanol (64). However, considerable evidence suggests that high binding *potency* does not *require* α-helical structure in the carboxy-terminal region. For example, the potency of [Des-Gln^{11},Leu^{19},Asn^{20},Ile^{22}]-β_h-endorphin for binding ^{3}H-β_h-endorphin-labelled sites is similar to that of β_h-endorphin itself (98). More strikingly, cyclic β_h-endorphin analogues having disulfide bonds connecting a cysteine residue in position 11, 14, 17, or 21 to a cysteine residue in position 26 have 1 to 4 times the binding potency of β_h-endorphin in these assays (8).

The role of charged residues in determining the receptor-binding potencies of β_h-endorphin is well established. Positive charges in the β_h-endorphin structure lie at the amino terminus and in five lysine residues, of which four are in the carboxy-terminal half of the molecule. These positive charges are essential for high potency in radio receptor binding assays. N$^\alpha$-acetyl-β_h-endorphin has drastically reduced potencies relative to β_h-endorphin in

several such assays (94, 96), and amino-terminal acetylation may be an important mechanism for inactivation of β-endorphin in vivo (167). Citraconylation of the lysine residues in β_p-endorphin, which replaces their positive charges with negative charges at neutral pH, also drastically reduced the ability of that peptide to displace ^{3}H-naloxone or ^{3}H-dihydromorphine from their rat brain membrane binding sites (57). The negative charges in β_h-endorphin lie in glutamic acid residues in positions 8 and 31, the latter having two negative charges as it is at the carboxy terminus. A large number of analogues have been studied in which these negative charges have been removed, and this always results in an increased potency in radioreceptor binding assays that use ^{3}H-β_h-endorphin to label rat brain membranes. For example, substitution of residue 8 by a glutamine residue increases the binding potency of β_h-endorphin deletion analogues comprising residues 1–9 (159), residues 1–17 (55), and residues 1–28 (56), as well as β_h-endorphin itself (95). Similarly, removal of one or both of the carboxyl groups in position 31 by extension of the peptide chain and/or replacement with amide groups or other nonacidic residues results in enhanced binding potency (93, 97, 158, 160).

The potencies of the β-endorphin model peptides 1 through 6 for displacing the specific binding of ^{3}H-[D-Ala^2,D-Leu^5]-enkephalin and ^{3}H-dihydromorphine to guinea pig brain membranes have been compared to those of β_h-endorphin under conditions in which the radioligands should selectively label δ- and μ-opioid receptors, respectively (58a). The results of these experiments are summarized in table 3. Peptides 1, 3, and 5, which have the natural sequence in residues 1–12 connected to different model amphiphilic helical structures, were all able to reproduce the δ/μ receptor selectivity of β_h-endorphin rather closely. Their potencies in each assay ranged from almost equal to that of β_h-endorphin (peptide 5) to nearly an order of magnitude greater than β_h-endorphin (peptide 3), and they correlate well with the number of positive charges in their helical segments. These results indicate that the general features of the potential amphiphilic helix in residues 13–29 of β-endorphin are sufficient to determine the effect on δ/μ receptor selectivity of this structure, and that the shape of its hydrophobic domain is not critical (peptide 1). Most striking is the ability of a left-handed α-helical segment of the appropriate design (peptide 5) to reproduce the effects in binding assays on both potency and receptor selectivity of the natural structure.

TABLE 3

Binding potencies relative to β_h-endorphin of peptides 1–6 to radiolabeled opiate receptors in guinea pig brain membranes[a]

Peptide	Overall charge at neutral pH	Binding potency[b]		Receptor selectivity (μ/δ)
		δ receptors[c]	μ receptors[d]	
β_h-Endorphin	+3	1	1	1
Peptide 1	+4	2.2	2.9	1.3
Peptide 2	+4	0.6	60	100
Peptide 3	+5	7.1	6.9	0.97
Peptide 4	+4	0.6	2.0	0.33
Peptide 5	+4	1.5	1.3	0.87
Peptide 6	+5	2.3	4.3	1.87

[a] Data are compiled from refs. 11, 12, 126, and 145–147.
[b] Potency = IC_{50} (β-endorphin)/IC_{50} (peptide).
[c] Selectively labelled using ^{3}H-[D-Ala^2,D-Leu^5]-enkephalin.
[d] Selectively labelled using ^{3}H-dihydromorphine.

A comparison of the binding assay results for peptides 3 and 6 shows quite clearly that four residues of γ-amino-γ-hydroxymethylbutyric acid can be substituted for the natural structure of the hydrophilic linking region in β-endorphin with only minor effects on δ/μ receptor selectivity and potency. This suggests that residues 6–12 of β-endorphin do not have strong interactions with these opioid receptors and mainly serve to connect the two binding segments in residues 1–5 and 13–29 on the receptor surfaces. The striking μ receptor selectivity of peptide 2, which also has a model linking segment (alternating serines and glycines), was earlier thought to indicate a role in μ receptor binding for this region of β-endorphin. However, the binding properties of peptide 6 imply that some other aspect of the design of peptide 2 is responsible for its high μ-opioid receptor affinity. One possibility is that the tryptophan placed in the middle of the hydrophobic domain of its carboxy-terminal α helix has important interactions with these opioid receptors (but not the δ receptors). Evidence that the size and somewhat polar character of the tryptophan side chain can affect likely protein-protein interactions involving an amphiphilic helix was provided by the self-associating properties of peptide 2 compared to peptide 1, as the tryptophan-containing peptide self-associates at more than 10-fold higher concentrations. A tryptophan residue in a similar position in a calcitonin model peptide had the same inhibitory effect on self-association (*vide infra*).

The sensitivity of μ receptor binding to changes in the helical region of these opioid peptides was further demonstrated by the much lower μ receptor affinity of peptide 4 relative to peptide 2. In comparison, the δ receptor binding is seen to be quite insensitive to major changes in the design of the model peptides. The abilities of peptide 4 to displace both δ and μ receptor ligands are in agreement with the studies of cyclic analogues cited earlier, indicating that the amphiphilic α-helical structure in the carboxy terminus is not required for high potency in either binding assay.

Recently, ligands that are more specific for μ-, δ-, and κ-opioid receptors have been described (58b), allowing more discriminating assays of binding to each receptor type to be developed. We have recently used the improved assays described by Corbett et al. (30) to compare the μ and κ receptor binding properties of peptides 3 and 6 with those of β-endorphin (144). In these assays, peptides 3 and 6 again displayed very similar characteristics, being about 10 times more potent than β_h-endorphin in the μ receptor assays and about 3 times more potent in the κ

receptor assays. These peptide models at least are, therefore, also able to reproduce the high selectivity of β_h-endorphin for μ receptors *versus* κ receptors (124a).

D. Opioid Activities in Smooth Muscle Assays

The opiates and opioid peptides inhibit electrically stimulated contractions in a variety of smooth muscle preparations. These actions appear to result from the activation of presynaptic opioid receptors and consequent inhibition of neurotransmitter release (109). Structural analogues of β-endorphin have mostly been investigated for their activities in preparations of the guinea pig ileum (GPI), mouse vas deferens (MVD), and rat vas deferens (RVD). Multiple opioid receptor types are present in the GPI and MVD, which appear to correspond to the δ, μ-, and κ-opioid receptors of membrane preparations from the central nervous system. The MVD is particularly rich in δ receptors, and the GPI has mostly μ receptors, although dynorphin A and related peptides act with high potency on κ receptors in these tissues (169).

The opioid activities of β-endorphin on the MVD and GPI are critically dependent on the [Met^5]-enkephalin region of the molecule. [Des-Tyr^1]-β_h-endorphin and N$^\alpha$-acetyl-β_h-endorphin have no activity on either tissue, and [D-Ala^2]-β_h-endorphin and [Leu^5]-β_h-endorphin have much lower GPI/MVD potency ratios than β_h-endorphin, indicating more δ receptor selectivity (138). On the GPI, β_c-endorphin with Tyr^1, Phe^4, or Met^5 substituted by the corresponding D-amino acid residues showed much reduced potency (163), whereas a similar substitution for Lys^9 or Phe^{18} in [Phe^{27},Gly^{31}]-β_h-endorphin had very little effect (165). In fact, the GPI is strikingly insensitive to changes in the β-endorphin structure outside of the [Met^5]-enkephalin segment, and the small changes in potency that are observed do not have any obvious structural correlate. Thus, the potencies of β_h-endorphin-(1–5)-(16–31) and β_c-endorphin-(1–5)-(28–31) are 1.35 and 0.35 times that of β_c-endorphin, respectively (92), and the corresponding relative potency of [Des-Gln^{11},Leu^{14},Asn^{20},Ile^{22}]-β_h-endorphin is 1.38 (98). Single residue deletions or substitutions in this region have even less effect (9, 97, 98).

[Met^5]-enkephalin is very potent in MVD assays, but only moderately active on the GPI, whereas β-endorphin has similar activities on both tissues (103). This again suggests that the carboxy-terminal extension of [Met^5]-enkephalin in β-endorphin may moderate its opioid receptor selectivity. Graf et al. have shown that, in a series of carboxy-terminal deletion analogues of β_p-endorphin, the GPI/MVD potency ratio decreases as the peptide chain length decreases, although the potencies on either one tissue fluctuate in a random fashion (60). These authors point out that the changes in this selectivity parallel the changes in α helicity they observed for these analogues in trifluoroethanol, and they suggest that the receptor-bound conformation of β-endorphin has α-helical structure in residues 13–29. These arguments are based on relatively small changes in potencies on the GPI and MVD, but are in good agreement with other studies (74, 138). They do not, however, correlate well with the results of the binding studies performed on carboxy-terminal deletion analogues using radioactive δ and μ agonists as receptor labels that were mentioned earlier (45).

In contrast to the GPI, MVD, and all other muscle preparations that have been characterized to date, the inhibition of electrically stimulated contractions of the RVD is mediated by receptors that exhibit a puzzling selectivity for β_h-endorphin among the known opioid peptides (90, 137). These receptors have been termed ϵ-opioid receptors. No opioid agonist activity on the RVD was originally reported for morphine, [Met^5]-enkephalin, or dynorphin A(1–13), even at high concentrations, and [D-Ala^2,D-Leu^5]-enkephalin was only weakly active (72, 137). Morphine antagonizes the effects of β-endorphin although with less potency than naloxone. More recently, however, agonist activities have been reported for [Met^5]-enkephalin, [Leu^5]-enkephalin, and other peptide products of proenkephalin A (but not the κ-selective proenkephalin B products) in the presence of mixtures of protease inhibitors (30, 133). In fact, the 50% inhibitory concentration (IC_{50}) values for the enkephalins in this assay (approximately 500 nM) were similar to those previously observed for β_c-endorphin in the absence of protease inhibitors, but still an order of magnitude higher than that of β_h-endorphin (IC_{50} = 40 nM). Furthermore, the necessity to invoke a new type of opioid receptor in order to explain the activities of opioids in the RVD assay is disputed by many researchers, and evidence suggesting that μ-opioid receptors mediate the activities of opioids on this tissue has also been presented (108a, 142a). Differences in the activities of various μ agonists in this assay compared to others such as the GPI and MVD assays are attributed to differences in the numbers of spare receptors present in each tissue; fewer spare receptors in the RVD make this tissue more sensitive to the intrinsic activities of opioids that bind to these receptors, so that opioids which stimulate signal transduction poorly behave as antagonists in this tissue. Interestingly, this intrinsic activity can be altered by varying the calcium ion concentrations used in the tissue bath for the RVD assay, and at low calcium ion concentrations, morphine behaves as a partial agonist (66b), in agreement with the suggested importance of the level of receptor activation. However, whether a novel type of receptor or differences in intrinsic activity provide the explanation, the high potency of β_h-endorphin among the known opioid peptides makes the RVD assay an important one for examining potentially important structural features of that hormone and the abilities of analogues to reproduce those features.

The inhibitory action of β_h-endorphin in the RVD assay is reversed by proteolytic degradation (137). This

also makes the reported activities of many β-endorphin analogues difficult to interpret in terms of their activities on the opioid receptor of the RVD, since the effects of adding protease inhibitors have not usually been tested and, as in the case of the enkephalins, inactive analogues may have higher potencies when they are protected from proteolytic degradation. Nevertheless, the importance of the amino-terminal segment of β_h-endorphin has been clearly demonstrated: N^{α}-acetyl-β_h-endorphin, [Des-Tyr^1]-β_h-endorphin, and β_h-endorphin(6–31) are all inactive, and the N^{α}-acetyl derivative in particular is not likely to be more susceptible to proteolysis than the unmodified peptide (138).

The role of the carboxy terminus has been investigated by Schulz et al. using carboxy-terminal deletion analogues of β_p-endorphin (138). Relatively potent effects (50 nM ≤ IC_{50} ≤ 200 nM) were observed for β_p-endorphin and deletion analogues consisting of residues 1–29, residues 1–27, residues 1–25, or residues 1–23. Shorter peptides, however, had potencies on the RVD which diminished rapidly with decreasing length: the IC_{50} of β_p-endorphin(1–21) was greater than 2 μM, that for β_p-endorphin(1–19) was greater than 50 μM, and the IC_{50} values of shorter analogues were apparently too high to be measured. Essentially the same results were obtained by Huidobro-Toro et al., who also showed that β_h-endorphin-(1–5)-(16–31) was inactive at least up to a concentration of 15 μM (72). These authors suggested, on this basis, that the opioid receptor in the RVD recognizes two distinct sites in the β-endorphin molecule, one at the amino terminus and one in residues 19–23. They also suggested that the region of the β-endorphin molecule between these two recognition sites is important in restricting the β-endorphin molecule to the correct conformation for expressing activity through this opioid receptor, and they proposed that this conformation involves the helical structure described by Wu et al. for β-endorphin residues 13–24 (154).

The six peptide models of β-endorphin have been examined for their activities in GPI and RVD assays (table 4). Their potencies on the GPI showed no apparent relation to the basis of their design. In particular, the nonamphiphilic peptide 4 was the most potent of the model peptides tested in this assay, and peptides 1 and 2 had potencies that differed by an order of magnitude despite their similar design. This behavior is similar to the results obtained for other β-endorphin analogues, as described earlier. Since these results do not show a direct correlation with the potencies in μ-opioid receptor binding assays, it is possible that some of the analogues are interacting with other types of opioid receptors in the GPI. This is a question that has not generally been addressed, and it is critical to the interpretation of the observed potencies in terms of potential receptor-bound conformations of β-endorphin.

In contrast, the potencies of the β-endorphin model peptides on the RVD have provided considerable information concerning the probable conformation of β-endorphin that is required for full agonist activity upon binding to the opioid receptor in this tissue. In view of the effects of protease inhibitors on the activities of the enkephalins in this assay, the loss of activity observed for carboxy-terminal deletion analogues shorter than β_p-endorphin(1–23) might have been the result of increased proteolytic degradation. Whether this is the case or not, the activities of the peptide models in relation to the general principles of their design provide a strong argument for the importance of a carboxy-terminal binding site for the opioid receptors of the RVD. In particular, the nonamphiphilic peptide 4 inhibited RVD contractions only at high concentrations and in a nonopioid manner, possibly by a postsynaptic mechanism similar to that of other peptide hormones (121, 122). The inhibitory effects of peptide 4 could not be reversed by naloxone, but were slowly reversed with time by some other mechanism such as proteolytic degradation. These results suggested the necessity for an amphiphilic helical structure in the carboxy-terminal segment of β-endorphin for potent agonist activity on the opioid receptors of the RVD. The relatively high activities observed for the other peptide models, all of which were naloxone reversible and resistant to proteolytic degradation or other mechanisms of inactivation, strongly supported this argument. Even with a left-handed amphiphilic α helix included in its design, peptide 5 was approximately equipotent to β_p-endorphin, although it exhibited a mixed agonist-antagonist behavior. The identical activities of peptides 3 and 6 further defined the carboxy-terminal receptor binding site by eliminating the hydrophilic linking region in residues 6–12 from consideration. In combination with the studies of carboxy-terminal deletion analogues, these results therefore limit the essential part of this binding site to β-endorphin residues 13–23. A comparison of the linear sequences of the model peptides in this region with that of β-endorphin shows that they almost certainly must adopt an α-helical conformation on the receptor surface; if these residues were

TABLE 4

Opiate agonist activities of β_h-endorphin and peptides 1–6 in GPI and RVD assays in vitro[a]

Peptide	IC_{50} (nM)[b]	
	GPI	RVD
β_h-Endorphin	61 ± 13[c]	41 ± 2
Peptide 1	16 ± 2	61 ± 18
Peptide 2	151 ± 21	450 ± 15
Peptide 3	30 ± 10	267 ± 48
Peptide 4	10 ± 1	No activity[d]
Peptide 5	30 ± 12	225 ± 51
Peptide 6	Not tested	206 ± 51

[a] Data are compiled from refs. 11, 12, 126, and 145–147.

[b] Concentration causing 50% of maximal inhibition of electrically stimulated contractions.

[c] Mean ± SE.

[d] Effects at high concentrations were not naloxone reversible.

in an extended conformation, there would be no obvious relationship between structure and opioid activity in the RVD assay. However, peptide 1 has a potency similar to that of β_h-endorphin and significantly higher than that of the other active peptide models, suggesting that the RVD opioid receptor may have additional specificity for certain side chains on the surface of this helix. In this context, the phenylalanine side chain in residue position 18, which forms a prominent feature of the surface of the helix on its hydrophobic face, has been proposed as a likely candidate (146). This residue is conserved in peptide 1, but replaced by a leucine residue in the less active peptides 3 and 6, and by a tryptophan residue in peptide 2, which has an even lower potency similar to that of the enkephalins in the presence of proteolytic inhibitors. Alternatively, these potency differences may be related to structure on the carboxy-terminal side of this essential binding site, and the high potency of peptide 1 may be fortuitous. For example, the lower potency of β_c-endorphin compared to β_h-endorphin results from changes in residues 27 and 31 only (72).

The actions of the model peptides on the GPI and RVD also exhibited a clear relationship between their amphiphilic design and physicochemical properties on the one hand and their rates of action and susceptibility to proteolysis on the other hand. The time courses of the RVD responses to some of these peptides are shown in figure 5. The effects of β_h-endorphin on both tissues reached a maximum within about 2–4 min after addition of doses close to its IC_{50} value. Similar doses of peptides 1 and 2, which interacted very strongly with phospholipids and the air-water interface and self-associated at low concentrations, took as long as 20 min to reach their maximal effects. Peptides 3 and 6, which have weaker amphiphilic properties because of the shape of the hydrophobic domain formed by their residues 13–29 in an α-helical conformation, exhibited similar behavior to β-endorphin in that their maximal effects were achieved only 2–4 min after the addition of each dose. However, all four of these model peptides were apparently resistant to the proteolytic enzymes present in these tissues. In the RVD, these enzymes degrade β-endorphin quite rap-

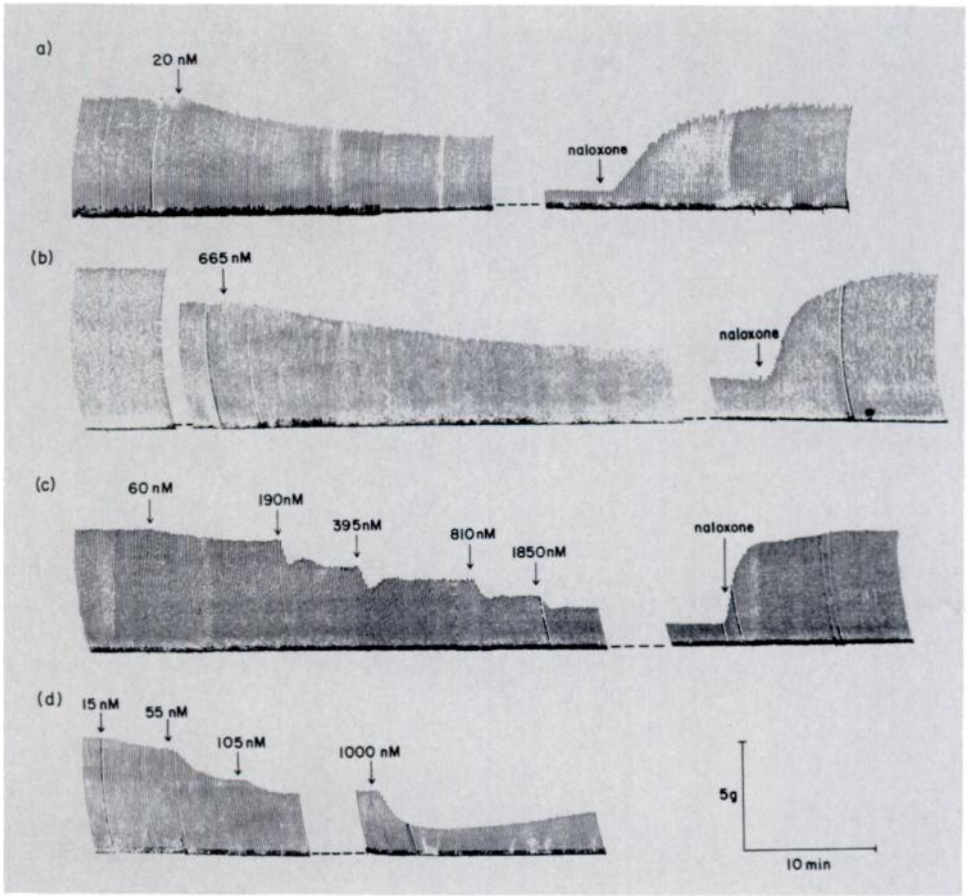

FIG. 5. Time courses of peptide actions on the RVD (144, 145). *a*, effect of adding 20 nM peptide 1 to the tissue bath, followed by the subsequent naloxone reversal of the maximum inhibitory effect of peptide 1 on the electrically stimulated contractions. *b*, the initially equilibrated RVD contractions in the absence of peptide, followed by the effect of increasing the concentration of peptide 2 in the tissue bath from 165 to 665 nM, and then the subsequent naloxone reversal of the maximum effect of this peptide. *c*, effects of stepwise increases in the concentration of peptide 3 in the tissue bath and their subsequent reversal by naloxone addition. *d*, effects of stepwise increases in the concentration of β_h-endorphin in the tissue bath, followed by the addition of 1 μM β_h-endorphin demonstrating the subsequent reversal of the actions of this peptide through enzymatic inactivation (137).

idly causing a reversal of its inhibitory effects (figure 5; and ref. 137). The same is true to a lesser extent for the GPI, which can inactivate the enkephalins quite rapidly in the absence of proteolytic enzyme inhibitors, but does not degrade β-endorphin so readily. The resistance to inactivation displayed by these model peptides in both assays (table 2) corresponded to their resistance to proteolysis in the presence of whole rat brain homogenates. In agreement with its lack of amphiphilicity and more rapid degradation by the rat brain enzymes, the actions of peptide 4 on *both* the GPI and, at high concentrations, the RVD were quite rapidly reversed with time, indicating that the structure of this model peptide provides it with no protection from proteolytic enzymes.

E. Analgesic Activities

The analgesic activities of the opiates and opioid peptides are mediated by opioid receptors in the central nervous system and are usually accompanied by a variety of naloxone-reversible effects including catalepsy, hyperactivity, "wet-dog" shakes, and Straub tail (13, 73, 149). Intracerebrally administered β_h-endorphin causes potent and relatively long-lasting analgesia in a variety of mammals, including humans (18, 48, 102). In comparison, morphine is 18–33 times less potent in different analgesic assays (102), and even high doses of the enkephalins elicit only a weak and transient effect when administered intracerebroventricularly (6, 23, 25). The low potency of the enkephalins in these assays is certainly due in part to their rapid degradations in vivo, but many stable analogues have lower potencies than β-endorphin, indicating that selectivity for opioid receptor types and/or intrinsic activity is also important.

A great many β-endorphin analogues have been tested for their analgesic potencies, usually in mice assayed by the hot-plate method or in rats assayed by the tail-flick method, after intracerebroventricular administration. The results indicate that the whole peptide sequence is involved in determining this activity, but again the effect of proteolytic inactivation is difficult to assess, and the time courses of the effects are not generally presented, so that a detailed interpretation of the results is not possible. N^{α}-acetyl-β_h-endorphin (32), [D-Tyr^1]-β_c-endorphin (163), [$Des\text{-}Gly^2$]-β_c-endorphin (98), and β_c-endorphin(6–31) (99) all have little or no activity, and a variety of other modifications of single residues in the enkephalin segment of β-endorphin drastically reduce its potency, thus establishing the vital importance of this region (161, 163). Residues 6–31 of β-endorphin show less sensitivity to single residue changes or other modifications including deletions (9, 10, 94–96, 98, 160, 165), and some such analogues have greater potency than the corresponding natural structure (*vide infra*). However, β_h-endorphin(1–27) and shorter carboxy-terminal deletion analogues have considerably reduced potencies (32, 45), and β_h-endorphin-(1–5)-(16–31) and β_c-endorphin-(1–5)-(28–31) have only 0.003 and 0.001 times the potency of β_c-endorphin, respectively (99).

Lee and Smith have proposed a model for β-endorphin interactions with the analgesic receptor, which explains the apparent involvement of the whole β-endorphin molecule in determining its analgesic potency and involves α-helical structure in the carboxy-terminal region (89). They suggested that the analgesic receptor consists of both protein and lipid, and that the amino terminus of β-endorphin interacts with an enkephalin-binding site in the protein part of the receptor, and the carboxy terminus of β-endorphin interacts with the lipid part. The antagonism of morphine- and β-endorphin-induced analgesia by β_c-endorphin(6–31) and β_c-endorphin(20–31), but not by β_h-endorphin(1–15) (88), is cited as evidence for the carboxy-terminal site, and α-helical structure in this region is suggested in view of the helix-stabilizing effects of phosphatidyl serine and cerebroside sulfate on β-endorphin solutions (155). More recently, β_h-endorphin(1–27) has also been determined to be a potent antagonist (4 times more potent than naloxone) for β_h-endorphin-induced analgesia (66a). This action was predicted on the basis of the relatively tight binding of this peptide to ^3H-β_h-endorphin-labelled receptors in rat brain (0.3 times as potent as β_h-endorphin in competitive displacement assays), compared to its weak analgesic action (0.02 times the potency of β_h-endorphin). Thus, the removal of four residues from the carboxy-terminal end of β-endorphin may have diminished the intrinsic activity of the hormone at analgesic receptors to a greater extent than the binding affinity has been affected, whereas in β_h-endorphin(1–15), too much of the carboxy-terminal binding site has apparently been lost to allow even nonproductive binding to these receptors, also in agreement with the brain receptor binding assays (45).

In recent years, Li and coworkers have investigated the analgesic potencies of more than 50 different β-endorphin analogues with one or more single-residue modifications in an effort to develop more potent and longer lasting effects. Very few modifications have produced more potent peptides. These peptides and their potencies relative to β_h-endorphin are as follows: [Gln^8]-β_h-endorphin, 2.45 (95); [Trp^{27}]-β_h-endorphin, 3.72 (96); [Tyr^{31}]-β-endorphin, 1.16 (160); [Phe^{27},Gly^{31}]-β-endorphin, 1.19 (165); [Gly^{31}]-β_h-endorphin amide, 2.25 (97); [Gly^{31}]-β-endorphin-Gly, 2.17 (97); [Gly^{31}]-β-endorphin-Gly-Gly amide, 1.08 (93); [Gln^8,Gly^{31}]-β_h-en- (162); [Ala^8,Gln^{31}]-β_h-endorphin, 1.16 (162); and [Val^8,Gln^{31}]-β_h-endorphin, 1.21 (162). All of these peptides differ from β_h-endorphin in one or more of just three different residue positions: 8, 27, and 31; modifications of other residues always resulted in lower analgesic potencies. Furthermore, the effects of different modifications were not always additive. For example, substitution of either a D-threonine residue in position 2

of [Phe^{27},Gly^{31}]-β_h-endorphin or a D-lysine residue in position 9 gave analgesic potencies 0.21 times that of β_h-endorphin in each case, but the potency of [D-Thr^2,D-Lys^9,Phe^{27},Gly^{31}]-β_h-endorphin was 0.42 times that of β_h-endorphin (9, 165). The incorporation of individual D-amino acid residues into the β-endorphin structure has not led to more prolonged analgesic activities either. Equal doses of [D-Ala^2]-β_c-endorphin and β_c-endorphin have the same potencies and similar lengths of action, and other inversions of configuration caused a considerable loss of analgesic potency with no prolonged activity reported (9, 56, 65, 161, 163, 165).

A study of the analgesic potencies of several of the naturally occurring β-endorphin structures proved to be much more informative than such assays of analogues chosen essentially at random (66). The potencies were ordered as follows: camel = equine > ostrich = human > salmon I = turkey. However, despite many differences in the amino acid sequences of these peptides, their potencies are all very high and lie within a narrow range, the camel and equine endorphins being approximately 3 times as potent as the salmon I and turkey endorphins. The relatively high potency of the salmon peptide is particularly surprising in view of its structure at the carboxy terminus relative to β_h-endorphin residues 26–31. The critical role of these residues in the analgesic activities of the human peptide is apparently compensated for by other aspects of the structure of β_s-endorphin I. The high potencies of the ostrich and salmon I peptides also indicate that the hydrophilic segments of their structures corresponding to residues 6–12 of the human peptide do not interact strongly with the receptor surfaces involved, since their sequences in this region are mostly nonhomologous to the mammalian structures. This suggests a probable role for residues 6–12 as a linker connecting essential binding sites in the β-endorphin structure at the amino-terminal and carboxy-terminal ends, as was proposed for binding to δ and μ receptors in guinea pig brain membranes as well as for activity on the opioid receptors of the RVD.

The multiple variations in the sequences of the natural β-endorphins and their high analgesic potencies suggested that the approach of studying peptide models would be a particularly appropriate method for developing structure-activity relationships with this assay. When peptides 1–6 were tested by the hot-plate method for analgesic activity in mice after intracerebroventricular administration, peptides 3, 5, and 6 all produced potent and long-lasting antinociception, as shown in figure 6. These activities were dose dependent and were naloxone reversible and accompanied by other opioid behavioral effects, including catalepsy and Straub tail. In view of the minimal sequence homology compared to the natural endorphins of the modelled structures in these active peptides (and especially the nonnatural structures of the hydrophilic linking region in peptide 6 and the left-handed amphiphilic α-helical region in peptide 5), these results provide a very convincing demonstration that the general features of their design are sufficient to allow diffusion within the central nervous system to the appropriate sites without degradation, followed by binding with agonist activity to the analgesic receptors. Compared to β_h-endorphin, these peptides were about 0.1–0.3 times as potent when the maximal effects were compared. This represents a relative potency comparable to several β-endorphin analogues having sin-

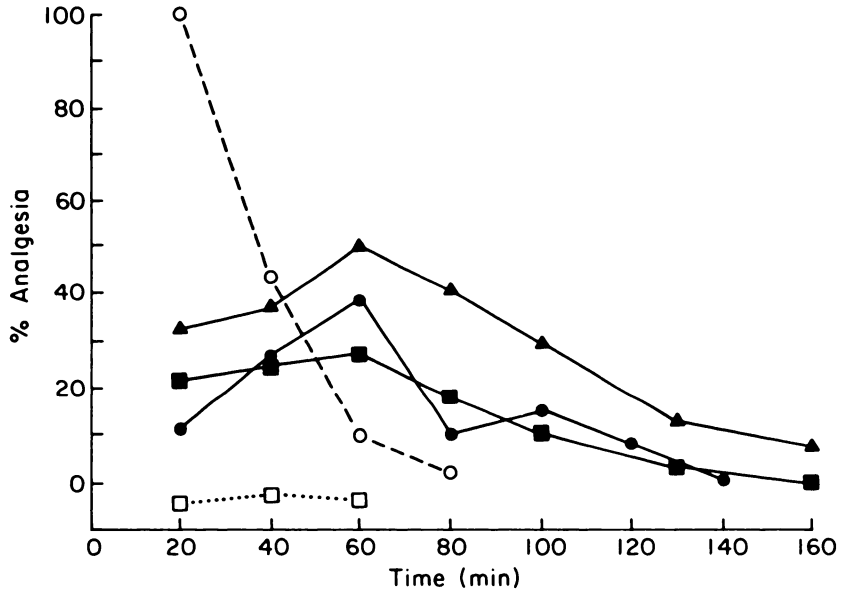

FIG. 6. Time courses of the analgesic effects on mice of β_h-endorphin and peptides 3, 5, and 6. The hot-plate assay was used to determine the analgesic effects of equal doses (3 μg) of β_h-endorphin (○), peptide 3 (▲), peptide 5 (■), or peptide 6 (●) compared to saline controls (□), as a function of the time after their intracerebroventricular injection into different groups of mice (11, 126, 146).

gle residue changes of a conservative nature compared to the natural sequences (9, 10, 93, 94, 98, 165) and indicates that, as would be expected, the model structures are unable to duplicate more specific features of the natural hormones that might result in higher potencies if they could be identified and incorporated. Diffusion to the receptors apparently required longer for the active model peptides, as they all produced their maximal effects at about 40–80 min after administration, compared to about 10–20 min for β_h-endorphin. This behavior may be a result of greater nonspecific binding to cell surfaces, as was observed in the assays of proteolytic degradation in the presence of rat brain homogenates discussed earlier, and may be an additional factor reducing their apparent potency. However, the loss of activity of the model peptides as a result of proteolytic degradation, N^{α}-acetylation, or other mechanisms was also considerably slower than that of β_h-endorphin, even when equal doses were compared. This again corresponds to the relative behaviors of these peptides in the in vitro assays (table 2).

Peptides 1, 2, and 4 produced no antinociceptive effects when tested in the same assay. This was expected for peptide 4, which may not have been able to bind to the necessary opioid receptors and was certainly more susceptible to inactivation by the endogenous enzymes as a result of its nonamphiphilic structure. However, the lack of activities in peptides 1 and 2 was surprising, since these peptides were resistant to degradation and reproduced the affinities for opioid receptors of β_h-endorphin in binding assays, as well as its potent opioid activities in the GPI and RVD assays. It is possible that the shape of the hydrophobic domain in the potential amphiphilic α helix formed by residues 13–31 of these model peptides, which lies parallel to the helix axis, is inappropriate for interactions with the receptors mediating analgesic activity. All of the active peptides in this assay were designed to form an amphiphilic α helix with a hydrophobic domain that twists around the helix in a clockwise direction, corresponding more precisely to the corresponding feature in β_h-endorphin. Another possibility related to this difference, though, is that the much stronger amphiphilic properties that arise from this feature of the design of peptides 1 and 2 resulted in a strong nonspecific adsorption to cell surfaces that prevented their diffusion to receptors from the site of injection. The marked differences in the time courses of the actions of peptides 1 and 2 compared to peptides 3 and 6 in the GPI and RVD are consistent with this explanation.

F. Future Peptide Models of β-Endorphin

Now that the structural characterization of β-endorphin in terms of the [Met^5]-enkephalin segment, the hydrophilic link in residues 6–12, and the carboxy-terminal amphiphilic α helix has been thoroughly tested by the peptide modelling approach, it will be interesting to see if future peptide models will be able to retain the resistance to proteolytic degradation exhibited by peptides 3 and 6, for example, while reproducing the abilities of β-endorphin to diffuse rapidly to receptors with minimal cell surface adsorption. Other features of the β-endorphin structure that still need to be investigated include the length requirements of the hydrophilic linking region and the possible importance of certain specific residues such as the proline in position 13 and the aromatic side chains on the helix surface in positions 18 and 27. In conjunction with the studies already performed, enough information would then be available to allow the rational design of peptides having greater potencies than β-endorphin and more desirable receptor specificities and pharmacokinetic properties.

V. Calcitonin and CGRP

The calcitonin and calcitonin gene-related peptide (CGRP) amino acid sequences are encoded in the same gene (1a, 132). Production of either one peptide or the other through the enzymatic processing of precursor molecules occurs in a tissue-specific manner as a result of alternative processing of the mRNA. Mammalian calcitonin is produced in the thyroid and is released in response to elevations in the serum calcium concentration. By inhibiting the activity of bone-resorbing cells (osteoclasts) and reabsorption of calcium and phosphate in the renal tubule, the hormone reduces calcium levels to normal (148). Calcitonin receptors have been identified in cell membrane preparations of bone, kidney, the central nervous system, and human cancer cell lines by radioreceptor binding assays in vitro (46, 47, 108, 119, 130). The pharmacological potencies of this hormone and its analogues have been assessed in competitive binding assays to these receptors, as well as by measuring activation of the adenylate cyclase second messenger system in the same membrane preparations (except the brain) in vitro (106), and by determining the hypocalcemic response to s.c. or i.v. injections of the hormone in vivo (84). CGRP is produced in specific areas of the central and peripheral nervous systems and has been postulated to be a neurotransmitter (19). As such, its actions are likely to be more related to its point of release than the circulatory calcitonin. These include potent vasodilatory effects and presynaptic actions on the RVD similar to those of β-endorphin, as well as potential roles in nociception and other behaviors (19, 132).

Calcitonin structures from a variety of sources have been characterized (figure 7; ref. 104). All are 32 amino acid residues long, contain an amino-terminal loop resulting from a disulfide bridge between cysteine residues in positions 1 and 7, and have an amidated proline residue at their carboxy termini. The amino-terminal loop is highly conserved, with species variations observed in position 2 (glycine or serine) only. However, deletion of the serine residue in position 2 of salmon calcitonin (140) or replacement of the cystine structure of eel calcitonin with an aminosuberic acid residue in position 7

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Human:	H-Cys	Gly	Asn	Leu	Ser	Thr	Cys	Met	Leu	Gly	Thr	Tyr	Thr	Gln	Asp-
Rat:															
Salmon II:		Ser									Lys	Leu	Ser		
Salmon III:		Ser						Val			Lys	Leu	Ser		
Salmon I:		Ser						Val			Lys	Leu	Ser		Glu
Eel:		Ser						Val			Lys	Leu	Ser		Glu
Porcine:		Ser						Val		Ser	Ala		Trp	Arg	Asn
Bovine:		Ser						Val		Ser	Ala		Trp	Lys	
Ovine:		Ser						Val		Ser	Ala		Trp	Lys	

	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
(h)	Phe	Asn	Lys	Phe	His	Thr	Phe	Pro	Gln	Thr	Ala	Ile	Gly	Val	Gly	Ala	Pro-NH_2
(r)	Leu										Ser						
(s-II)	Leu	His		Leu	Gln				Arg		Asn	Thr		Ala		Val	
(s-III)	Leu	His		Leu	Gln				Arg		Asn	Thr		Ala		Val	
(s-I)	Leu	His		Leu	Gln		Tyr		Arg		Asn	Thr		Ser		Thr	
(e)	Leu	His		Leu	Gln		Tyr		Arg		Asp	Val		Ala		Thr	
(p)	Leu		Asn			Arg		Ser	Gly	Met	Gly	Phe		Pro	Glu	Thr	
(b)	Leu		Asn	Tyr		Arg		Ser	Gly	Met	Gly	Phe		Pro	Glu	Thr	
(o)	Leu		Asn	Tyr		Arg	Tyr	Ser	Gly	Met	Gly	Phe		Pro	Glu	Thr	

FIG. 7. Amino acid sequences of naturally occurring calcitonin homologues. The entire human calcitonin sequence is shown, as well as the differences from this sequence in the structures of calcitonins from other species (104).

cyclized to the amine of residue 2 (114) results in no loss of activity. A cyclic structure is nevertheless required, as reduction of the disulfide bridge in human calcitonin with or without methylation of the cysteine sulfhydryls decreases activity dramatically (129). The carboxy terminus is also essential, and deletion of the carboxy-terminal proline amide, leaving either an amide or the free acid in position 31, diminishes activity (142).

Studies of deletion analogues of porcine calcitonin have demonstrated that partial structures are generally devoid of activity (142). One exception to this is [Des-Tyr[22]]-salmon calcitonin I which has the same activity as the unmodified peptide (47). Nevertheless, the naturally occurring calcitonins display a considerable degree of sequence variation between the disulfide loop at the amino terminus and the carboxy-terminal proline amide residue. In this region, only the leucine residue in position 9 and the glycine in position 28 are invariant, yet all of these peptides are highly active according to the in vivo assay of hypocalcemic response. Several years ago, it was recognized that the central portions of the natural calcitonins could form amphiphilic α-helical structures that were similar to those formed by the serum apolipoproteins in that their hydrophobic domains lay along one side of the helix parallel to its axis (51). These structures appeared likely to extend from residue 8 immediately after the amino-terminal loop, to residue 22 which is commonly followed by a helix-breaking proline residue in position 23 as well as several residues of low helix-forming propensity, such as glycine, serine, and asparagine, in positions 24–31. Furthermore, Fukushima in our laboratory (51) was able to correlate the potencies of the natural calcitonins, as well as several synthetic analogues (63), with a parameter describing the relative amphiphilic α helicity of these structures that took into account the helix-forming propensity (29) of their constituent residues as well as the net difference in hydrophobicities (38) of their hydrophobic and hydrophilic faces. More recently, a similar correlation with amphiphilicity alone has also been made (44), using the method of Eisenberg's laboratory to calculate relative hydrophobic moments (39).

Two peptide models of calcitonin (peptides 7 and 8; figure 2) have been designed and synthesized based on the above analysis of calcitonin, namely its division into three structurally distinct regions: a loop formed by linking the cysteine residues in positions 1 and 7; a potential amphiphilic α-helical segment in residues 8–22; and a nonhelical segment connecting the helix to the carboxy-terminal proline amide in residues 23–31 (111,

112). In both peptide models, the amphiphilic α-helical structure only was modelled, using leucine, lysine, and glutamine residues to maximize the α-helix-forming potential of the structure, and in such a way as to minimize homology with any of corresponding natural sequences. In each case, all of the general characteristics of the natural structures were retained, including the length and amphiphilicity of the helix; the size, shape, and orientation relative to the rest of the peptide of its hydrophobic domain; and the occurrence of mostly neutral or basic residues on its hydrophilic side. The amphiphilic helical segments of peptides 7 and 8 differed from each other and the natural structures in certain more specific aspects: a single acidic residue lying on the edge of the hydrophobic domain commonly occurs in position 15 of the calcitonin structures and was replaced by leucine residues in the model peptides to idealize the amphiphilicity; peptide 7 incorporated a tryptophan residue in the middle of the hydrophobic domain, whereas peptide 8 did not have this aromatic residue but did have a tyrosine residue in position 22, where an aromatic residue is common to all of the natural structures. The model structures in peptides 7 and 8 are compared to the α-helical structure postulated for salmon calcitonin I residues 8–22 by means of helical wheel diagrams in figure 8.

In aqueous, salt solutions at neutral pH, salmon calcitonin I has a CD spectrum typical of mixtures of α-helical and random structures. No concentration dependency has been observed, and the peptide appears to be monomeric according to analysis by equilibrium centrifugation at concentrations near 1 mM (111, 112). A low amount of helical structure is normally present in aqueous solutions of calcitonins, but this increases upon interactions with phospholipids and helix-promoting solvents (21, 43, 44). Despite its interactions with phospholipid micelles, salmon calcitonin I does not bind to unilamellar egg lecithin vesicles (111). It does, however, form stable monolayers of moderately high collapse pressure (12 dyn/cm) at the air-water interface, indicating the ability of the peptide to adopt an amphiphilic structure in the correct environment (112). The lack of self-association and inability to interact with phospholipid vesicles, when compared to those properties of the serum apolipoproteins and related model peptides, may be a result of the shorter length of the potential amphiphilic helix in residues 8–22 of the calcitonins (four turns of helix structure compared to at least six) or to the hydrophilic elements on its hydrophobic face, such as the acidic residue in position 15 or tyrosine hydroxyl groups. Alternatively, the hydrophobic domain of the helix may be masked in aqueous solution by the other structural domains in the peptide through formation of some tertiary structure, as is suggested by immunological studies indicating that the amino and carboxy termini of human calcitonin are in close proximity (24).

Compared to salmon calcitonin I, the physicochemical properties of peptide models 7 and 8 were indicative of more stable helical structures with stronger amphiphilic properties, as expected from their design. Thus, both peptides self-associated in aqueous solution at 1 mM concentrations to form trimers according to equilibrium centrifugation studies, and even in the monomeric form were more helical than the natural peptide; monolayers formed by peptides 7 and 8 were stable up to surface pressures greater than 20 dyn/cm, and these peptides were more compact and less compressible than the natural structure in this environment; peptides 7 and 8 both interacted strongly with unilamellar egg lecithin vesicles, disrupting their structure and forming mixed peptide and lipid aggregates (111, 112). The most pronounced differences in the behavior of these model peptides compared to each other were seen in their CD spectra. The spectrum given by peptide 8 showed increasing α helix content above 1 μM concentrations, indicative of self-association and concomitant stabilization of the amphiphilic helical structure in that peptide, and consistent with the observed trimerization at a concentration of 1 mM. In contrast, peptide 7 gave concentration-independent spectra in the concentration range between 100 nM and 100 μM, indicating that the observed self-association occurred at higher concentrations or without significant stabilization of helical structure. In either case, the difference was similar to that of the β-endorphin model

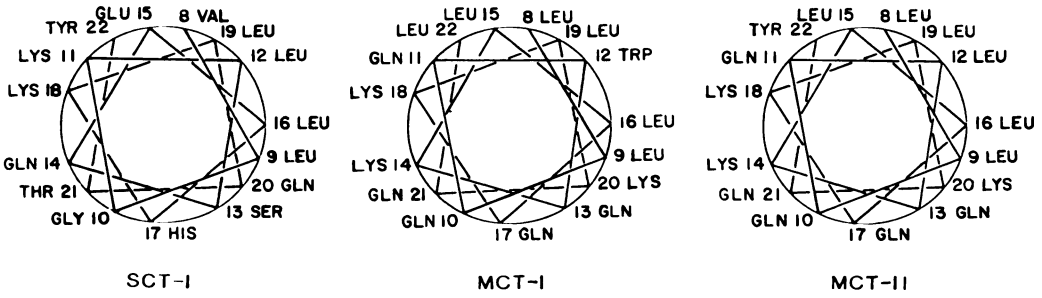

FIG. 8. Helical wheel diagrams comparing the distributions of the amino acid residue side chains in positions 8–22 of salmon calcitonin I (*SCT-I*), peptide 7 (*MCT-I*), and peptide 8 (*MCT-II*) on the surface of a regular α helix. In these diagrams the α carbon atoms of the residues in the helical structures are labelled and connected by *straight lines*. The resultant *spiral* is then viewed along the helix axis from the amino-terminal end.

peptide 2 compared to peptide 1, and it is attributed likewise to the presence of a tryptophan residue on the hydrophobic face of the helical structure having a disruptive effect on potential helix-helix interactions (111).

The peptide models of calcitonin were both compared to salmon calcitonin I in assays of binding to rat brain membranes using ^{125}I-labelled salmon calcitonin I to radiolabel calcitonin receptors, and in assays of their hypocalcemic effect in rats 1 h after s.c. injection (111, 112). Similar results were obtained in each assay. Salmon calcitonin I was slightly more potent than peptide 8 in the binding assay, and these two peptides were approximately equipotent in the in vivo assay. Peptide 7 was about 10–20 times less potent in each assay than the salmon peptide, which corresponds to a similar potency to porcine calcitonin, the most active of the mammalian structures tested (119). The close similarity of the pharmacological behavior of peptide 8 to that of salmon calcitonin I was further demonstrated in binding assays to rat kidney cortical membranes and assays of adenylate cyclase activation in the same tissue (111). In both of these assays, the model peptide and the salmon peptide were equipotent. Overall, therefore, the pharmacological assays of these model peptides provide a convincing demonstration that peptides designed to have idealized amphiphilic α-helical structures in residues 8–22 of the calcitonin structure can reproduce the most potent and specific actions of the natural hormones. As already discussed, the minimal homology of these model structures to the corresponding natural sequences is also very strong evidence that residues 8–22 are in the α-helical conformation on the calcitonin receptor surface.

A further test to establish that amphiphilicity in the helical structure is essential for high activity may now be attempted through the study of appropriate nonamphiphilic calcitonin model peptides corresponding to the β-endorphin model peptide 4. However, the lower activity of peptide 7 compared to salmon calcitonin I and peptide 8 has already indicated that receptor interactions are sensitive to changes in the hydrophobic face of the amphiphilic helix involving aromatic residues. Again, the tryptophan residue in position 12 of peptide 7 is most strongly implicated, in view of its disruptive effect on self-association as well as the nonessential nature (47) of the tyrosine residue in position 22 of peptide 8 and salmon calcitonin I. This effect is similar to previous observations of Maier et al., who suggested that aromatic residues in positions 12, 16, and 19 were responsible for the lower activities of the mammalian calcitonins in the hypocalcemic assay, and showed that sequential substitution of leucine residues in all three positions increased the potency of human calcitonin in this assay significantly (105). Sensitivities of this sort to the nature of the residues on the hydrophobic face are clearly in support of the importance of amphiphilicity in the helical structure.

In view of the increased helical structure and amphiphilic properties of peptide 8 in particular compared to salmon calcitonin I, it would be interesting to determine the time course of its hypocalcemic effects in vivo, to see if the onset and subsequent diminution of this activity are slower relative to the salmon peptide, just as was observed for the antinociceptive effects of peptides 3, 5, and 6 in vivo relative to the less structured and less amphiphilic β_h-endorphin. Since the assays of peptides 7 and 8 were performed under conditions of optimal activity of salmon calcitonin I (84), it may well be that one or both of the model peptides are more active and/or longer lasting than the salmon peptide. The functional role of the carboxy-terminal residues 23–31 also remains to be studied. If these residues simply serve a linking role connecting the helix in residues 8–22 to the proline amide carboxy-terminal residue, it should be possible to design a simple model structure, perhaps consisting of nonnatural amino acid residues such as were used in peptide 6, which would adequately perform the same role. The multiple amino acid substitutions seen in this region of the natural sequences suggest that this should be possible, and that no specific interactions with receptor surfaces are required of these residues. One alternative possibility, that these residues lay along the surface of the helix in salmon calcitonin I, has already been suggested (78), and a study of peptide models with constrained conformations could determine the importance of this type of tertiary structure.

The primary structures of mammalian CGRPs bear little resemblance to those of the calcitonins (113, 132). However, there is a striking homology in terms of their general structures (78). The residues immediately following the amino-terminal loop formed by the disulfide bridge between cysteines in position 2 and 7 of CGRP are capable of forming an amphiphilic α helix with similar characteristics to the one formed by the calcitonins (figure 9). As with the calcitonins, the hydrophobic domain covers a little less than half of the helix surface and lies along its length parallel to the helix axis. The charge distribution on the hydrophilic side is also similar, consisting mostly of neutral or basic residues, one acidic residue being present in the rat structure. Some aspects of this amphiphilic structure in CGRP differ from the corresponding calcitonin structure, however. If it is assumed that the proline residue in position 29 of CGRP fulfills the helix-breaking function of proline 23 in most of the calcitonins, then the CGRP helix is six residues longer than the calcitonin structure. Interestingly, the carboxy-terminal segment of CGRP that connects this putative helix to a phenylalanine amide residue in the terminal position would then be one residue shorter than the corresponding calcitonin structure, so that the 36-residue CGRP and the 32-residue calcitonin, stretched out but containing these helical structures, would have similar overall lengths. On the other hand, extension of the helical structure to the proline in position 23 would place a hydrophobic valine residue (position 22) on the

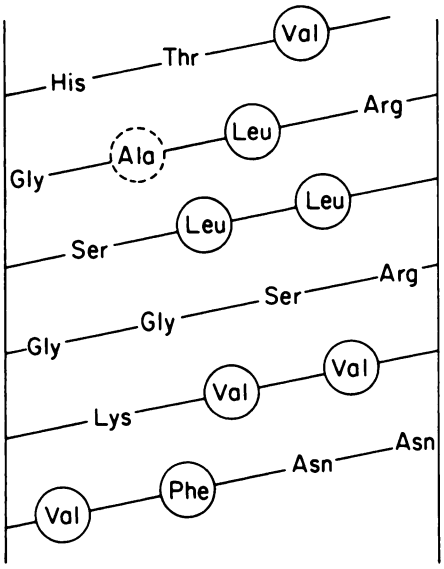

FIG. 9. Helical net diagram of human CGRP residues 8–28. The distribution of the amino acid side chains on the surface of a regular α helix is shown. The hydrophobic residues are *circled*.

hydrophilic side of the structure, suggesting that a shorter helix might be favored (78). Another difference in this putative CGRP structure is the presence of a hydrophilic serine residue in the middle of the hydrophobic domain. This residue is also part of a 5-residue sequence in positions 17–21 that includes two glycines and is expected to have very little propensity for formation of helical structure (29). Given the capacity of cell surfaces to adsorb peptides containing this type of amphiphilic structure in large quantities at relatively low concentrations, it is reasonable to speculate that some type of helix destabilization may be more important in the longer amphiphilic structure potentially formed by CGRP compared to the calcitonins, in order that it may function in vivo. The presence of a certain number of hydrophilic residues on the hydrophobic face of an amphiphilic helix does not prevent such structures from forming at the air-water interface or phospholipid surfaces, as has been demonstrated previously for CRF, sauvagine, and GRF (*vide infra*), as well as a peptide model of the serum apolipoproteins (53). In keeping with the above analysis, the formation of stable monolayers at the air-water interface has been demonstrated for rat CGRP, and these monolayers collapse at a surface pressure similar to the monolayer collapse pressure of salmon calcitonin I (78). Analysis of the role of this potential amphiphilic α-helical structure in determining the physicochemical and pharmacological properties of CGRP by the peptide modelling method is under way in our laboratory and should prove informative.

VI. Glucagon and Related Peptides

Glucagon is produced by the A cells of the pancreatic islets of Langerhans (125), and its release from the pancreas is regulated by a variety of factors including glucose, insulin, and somatostatin, which inhibit release, and fatty acids, fasting, stress and exercise, which increase plasma levels (58). Glucagon acts primarily at cell surface receptors in the liver where binding results in stimulation of the adenylate cyclase second messenger system and increased rates of glycogenolysis and lipolysis (131). Analysis of glucagon binding to isolated hepatocytes and hepatic plasma membranes using ^{125}I-labelled glucagon as a radioligand indicates that there are two distinct populations of receptors having different affinities for the hormone (15).

Mammalian glucagon has a highly conserved amino acid sequence consisting of 29 residues. Significant sequence homology with several other peptide hormones, including secretin, vasoactive intestinal peptide (VIP), peptide histidine isoleucine (PHI), gastrin, and GRF, has been noted (5, 143, 143b), suggesting that these peptides might have similar active structures (figure 10). Compared to the invariant mammalian structure, avian and fish glucagon homologues have a small number of sequence differences involving conservative substitutions of hydrophilic residues only (70). Receptor binding, adenylate cyclase activation, glycogenolysis, and lipolysis assays of a variety of deletion analogues indicate that residues throughout the mammalian peptide are essential for high activity (20, 41, 50, 70, 152, 153). Even [Des-His^{1}]-glucagon (70) and [Des-Asn^{28},Thr^{29}]-glucagon (41) only have a small fraction of the activity of the intact peptide, and removing residues 5–9, residues 10–15, residues 16–21, or residues 22–26 from the structure results in even lower potencies (50). Perhaps more interestingly, glucagon(5–29) was found to retain considerable receptor binding potency, although it did not elicit a biological response, indicating that it might be possible to design glucagon antagonists (50). Several partial agonists and antagonists have indeed been prepared, and their potential for clinical use in the treatment of diabetes, as well as their uses as probes for glucagon signal transduction, has been extensively discussed elsewhere (20a, 70, 99a).

Analysis of the glucagon structure using helical net diagrams indicates the potential formation of two separate hydrophobic domains by residues 5–16 and residues 17–29, if these segments adopt an α-helical conformation (figure 11). However, the two hydrophobic domains would be oriented on opposite sides of a single continuous α helix in residues 5–29. Interactions with an amphiphilic interface might, therefore, be expected to favor a discontinuity in the hydrophilic segment of the peptide that links the two amphiphilic structures, so that the hydrophobic domains would be aligned on the same side of the molecule. However, each helix would then consist of only three complete turns, suggesting that they might only be marginally stable even in a suitable environment. The crystal structure of glucagon reveals a trimeric structure which is in partial agreement with this analysis (134). Residues 1–4 have no well-defined conformation, and residues 5–29 form an α helix which is distorted at either

```
                    1              5                  10                  15
Glucagon:           H-His-Ser-Gln-Gly-Thr-Phe-Thr-Ser-Asp-Tyr-Ser-Lys-Tyr-Leu-Asp-

Secretin:           H-His-Ser-Asp-Gly-Thr-Phe-Thr-Ser-Glu-Leu-Ser-Arg-Leu-Arg-Asp-

VIP:                H-His-Ser-Asp-Ala-Val-Phe-Thr-Asp-Asn-Tyr-Thr-Arg-Leu-Arg-Lys-

PHI:                H-His-Ala-Asp-Gly-Val-Phe-Thr-Ser-Asp-Phe-Ser-Arg-Leu-Leu-Gly-

Gastrin (1-30):     H-Tyr-Ala-Gln-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met-Asp-

GRF (1-30):         H-Tyr-Ala-Asp-Ala-Ile-Phe-Thr-Asn-Ser-Tyr-Arg-Lys-Val-Leu-Gly-


                    16             20                  25
(Glucagon)          Ser-Arg-Arg-Ala-Gln-Asp-Phe-Val-Gln-Trp-Leu-Met-Asn-Thr-NH2

(Secretin)          Ser-Ala-Arg-Leu-Gln-Arg-Leu-Leu-Gln-Gly-Leu-Val-NH2

(VIP)               Gln-Met-Ala-Val-Lys-Lys-Tyr-Leu-Asn-Ser-Ile-Leu-Asn-NH2

(PHI)               Gln-Leu-Ser-Ala-Lys-Lys-Tyr-Leu-Glu-Ser-Leu-Ile-NH2

(Gastrin)           Lys-Ile-Arg-Gln-Gln-Asp-Phe-Val-Asn-Trp-Leu-Leu-Ala-Gln-Lys-

(GRF)               Gln-Leu-Ser-Ala-Arg-Lys-Leu-Leu-Gln-Asp-Ile-Met-Ser-Arg-Gln-
```

FIG. 10. Amino acid sequences of the glucagon peptide hormone family. The sequences of the porcine hormones are shown (143b), except for GRF(1-30), which is the human structure (143). *VIP*, vasoactive intestinal peptide; *PHI*, peptide histidine isoleucine; GRF, growth hormone releasing factor.

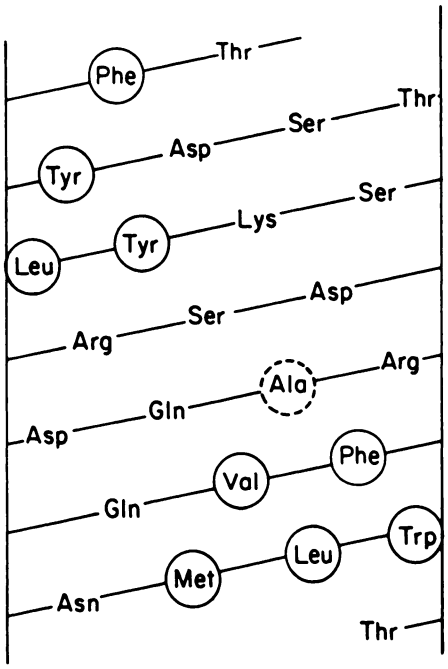

FIG. 11. Helical net diagram of mammalian glucagon residues 5–29. The distribution of the amino acid side chains on the surface of a continuous regular α helix is shown, illustrating that the hydrophobic residues (*circled*) would be segregated in two separate domains on opposite sides of the helix, if this conformation were adopted.

end in residues 5–9 and residues 26–29. In aqueous solution, glucagon has little structure in the monomeric form, but at high concentrations a concentration-dependent increase in helical structure is observed in the CD spectra which is consistent with formation of trimers (49). Helical structure is also promoted by interactions of glucagon with a variety of lipids and other surfactants, in whose presence glucagon can often form mixed micelles (16, 43, 136, 156). An analysis of partial glucagon sequences suggests that the helical structure stabilized in this way lies in residues 19–29 (156), and nuclear magnetic resonance (NMR) experiments are in agreement with this (22).

Two glucagon peptide models (peptides 9 and 10 in figure 2) with multiple changes in the carboxy-terminal region designed to enhance the helix-forming propensity and amphiphilicity of the potential α-helical structure in residues 19–29 were synthesized and studied (115, 117). These model peptides differed from one another only in that peptide 10 retained the aromatic residues in positions 22 (phenylalanine) and 25 (tryptophan) that are present in the natural sequence in this region. Compared to the peptide models of β-endorphin and calcitonin, both of these model structures have a conservative design in that no hydrophobic residues in the natural peptide have been substituted by hydrophilic residues and vice versa, and specific features in addition to the aromatic residues in peptide 10 have been conserved in both peptides, including an acidic residue in position 21 and the carboxy-terminal threonine residue.

As expected from their design, CD spectra indicated that these peptide models had more helical structure than glucagon and self-associated at lower concentrations. At the air-water interface, glucagon and peptides 9 and 10 all formed compact, incompressible monolayers of similar moderate stabilities (collapse pressures were around 10 dyn/cm) that indicated formation of amphiphilic helical structures (116). However, when the potencies of these peptides were determined in receptor bind-

ing assays using isolated rat hepatocytes and purified ^{125}I-glucagon as a radiolabel, considerable differences were observed (115, 117). Glucagon itself displaced the ^{125}I-glucagon binding over a wide range of concentrations and in a manner consistent with binding of the hormone to two noninteracting types of receptors. The calculated dissociation constants for binding to these sites were 57 pM and 41 nM, and the receptor populations (B_{max} values) were similar. Peptide 9 did not displace any of the ^{125}I-glucagon binding even at a concentration of 10 μM, but peptide 10 showed more interesting behavior, displacing about half of the ^{125}I-glucagon binding over a relatively narrow concentration range. Further analysis revealed that peptide 10 was able to bind to the high affinity binding sites of glucagon very selectively with a dissociation constant of 1.2 μM. Thus, in the presence of 10 μM peptide 10, the concentration-dependent displacement of ^{125}I-glucagon by glucagon itself was consistent with binding of the natural peptide to a single population of sites with a dissociation constant of 46 nM, corresponding to the value observed for its binding to the low affinity sites only (115). In assays of the abilities of these peptides to stimulate glycogenolysis, their inhibitory effects on the incorporation of radiolabelled carbohydrate into hepatocyte glycogen were measured and found to correspond to the observed binding to the high affinity glucagon sites (115, 117). Both glucagon and peptide 10 were able to stimulate glycogenolysis with half-maximal effects at about 18 pM and 10 nM, respectively, and peptide 9 had essentially no effect, even at a concentration of 1 μM. Peptide 10 was, however, considerably more potent than expected on the basis of the binding assay. This is the opposite behavior to that expected for a partial agonist or antagonist and suggests that this peptide has greater intrinsic activity than glucagon, although its binding affinity is considerably lower. These results are consistent with the notion expressed elsewhere (20a, 50, 99a) that the carboxy-terminal region of glucagon serves primarily to enhance receptor binding affinity, whereas the amino-terminal region is important for receptor activation.

Assays of peptide 10 in particular have therefore provided good evidence that it is the high affinity glucagon receptor identified in binding assays that mediates the physiological response to glucagon. The peptide modelling approach has, so far, been less useful in identifying the receptor-bound conformation of glucagon responsible for initiating this response, since the relatively small number of conservative amino acid substitutions (six) made in the natural sequence of glucagon to give peptide 10 has resulted in potencies about three orders of magnitude lower. This does not rule out helical structure as a possibility, however, since the individual side chain functionalities on its surface could easily produce such specificity. Should this be the case, the hydrophobic face of the helix is likely to provide most of the specific interactions involved, and a modified approach to the design of peptide models in which only residues on the hydrophilic face were substituted might provide a better indication of the importance of that structure. This type of approach has recently been successfully applied to a limited extent by Krstenansky et al. in their design of the analogue [Lys^{17},Lys^{18},Glu^{21}]-glucagon (83a). This analogue was more helical in aqueous salt solutions (100 μM peptide at pH 9.2), was more potent than glucagon in receptor binding and adenylate cyclase activation assays using liver plasma membranes, and was equipotent with glucagon in an in vivo glucose release assay. These results are clearly very supportive of a functional role for the potential amphiphilic α helix in the carboxy-terminal region of glucagon, and it would seem worthwhile making more extensive substitutions of hydrophilic residues in this region of the molecule in order to test this hypothesis more rigorously.

When the peptide hormones that are homologous to glucagon (figure 10) are also analyzed for potential amphiphilic α-helical structure, helical net diagrams reveal a similar pattern to that found for glucagon in each case (as illustrated for PHI in figure 12). The character of the carboxy-terminal structure is most highly conserved and is located in residues 18–27 of each hormone except gastrin, where residues 20–29 are implicated. The same discontinuity in the two hydrophobic domains is also observed in each case. The amino-terminal amphiphilic α helix has a less consistent character, though. For example, residues 5–18 of secretin might form four turns of amphiphilic α-helical structure with a relatively small hydrophobic domain lying parallel to the helix axis, whereas residues 1–18 of PHI (figure 12) or GRF can form five complete turns of amphiphilic α helix with a somewhat larger hydrophobic domain that twists clockwise around the helix surface.

In the case of GRF, the π helix has been proposed as an alternative structure that might be adopted by that peptide at amphiphilic interfaces (77). In contrast to the α-helical structure, a π helix formed by residues 1–29 of this hormone would form a hydrophobic domain lying along one side of the helix, parallel to its axis, and covering half of its surface for almost seven complete turns. Throughout this structure, only two hydrophilic serine residues would be positioned within the hydrophobic domain, and no hydrophobic residues would occur on the hydrophilic side of the helix. In keeping with the formation of a helical structure having these characteristics, GRF was found to form very stable monolayers and to bind tightly to egg lecithin vesicles, and so it was postulated that these environments provide sufficient stabilization for this extended π-helical conformation to be adopted in preference to shorter segments of the intrinsically more stable α-helical conformation. Furthermore, a GRF analogue in which several substitutions were made to improve the suitability of the amphiphilic π-helical structure for such interactions has recently been prepared and found to have a biological potency

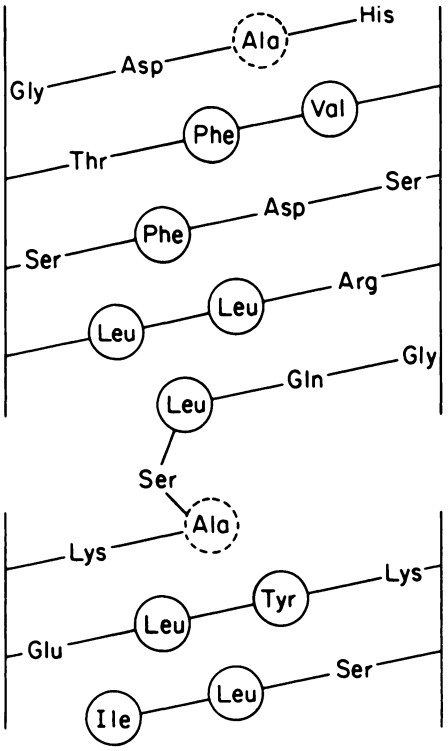

FIG. 12. Helical net diagram of porcine PHI. The distribution of the amino acid side chains of the entire peptide on the surface of a discontinuous α-helical structure is shown, illustrating that the hydrophobic residues (*circled*) are segregated in a domain lying along one side of this structure, if it is formed.

similar to that of the natural hormone (150). Therefore, although the studies performed thus far do not determine whether α-helical or π-helical structure is involved in the function of GRF, they do suggest that peptide models designed on the basis of the π-helical structure might allow useful structure-function relationships to be developed. A similar analysis of the other members of this family of homologous hormones shows that they too can form extended amphiphilic π helices, although again there are always one or two hydrophilic residues located in the hydrophobic domain formed. The approach of idealizing this type of amphiphilic structure might also be fruitful for these peptides, including glucagon, as an alternative to the possibility of limiting the nonhomologous residues in peptide models to the hydrophilic sides of the potential α-helical structures.

VII. Other Amphiphilic Peptide Hormones

Regions of potential amphiphilic secondary structure have been identified in a number of other peptide hormones and neurotransmitters or neuromodulators. However, the evidence that these conformations are actually adopted at amphiphilic interfaces and that they might be of functional importance varies from case to case, and no studies of peptide models have yet been reported. Some of these peptides are described below.

The hypothalamic hormone, CRF, which stimulates secretion of ACTH from the pituitary, as well as the structurally homologous peptide, sauvagine, isolated from frog skin, both have strong amphiphilic properties (85). Both peptides bind very tightly to unilamellar egg lecithin vesicles without disrupting their structures, a property shared by the strongly amphiphilic α-helical serum apolipoproteins, and both peptides form very stable monolayers at the air-water interface (the collapse pressures are about 19 dyn/cm) with properties typical of a compact molecular structure. CD spectra of these peptides indicate little secondary structure in aqueous solution at low concentrations, but CRF was found to aggregate at concentrations above 1 μM to form tetrameric micelles and larger aggregates all having increased helical structure. An examination of the amino acid sequences of CRF and sauvagine on helical wheel diagrams indicated that these properties probably result from formation of amphiphilic α-helical structures by residues 6–23 of CRF and residues 5–20 of sauvagine. Each amphiphilic structure has a large hydrophobic domain covering over half of the helix surface that lies parallel to the helix axis for 4–5 complete turns. Two hydrophilic residues lie within this hydrophobic domain, which is similar to the situation in the putative π-helical conformation of GRF (77), and no hydrophobic residues occur on the hydrophilic side of the helix. Another, less hydrophobic, amphiphilic α helix has been postulated for the carboxy-terminal CRF residues in positions 24–41, but no equivalent structure can be formed by sauvagine.

The crystal structure of avian pancreatic polypeptide (aPP), which consists of 36 amino acid residues, has been determined at high resolution and reveals an amphiphilic α-helical structure in residues 13–32 (14). In this case, the hydrophobic domain lies along the helix for over five complete turns, but twists around the helix slightly in an anticlockwise direction. Again, over half of the helix surface is hydrophobic. The crystal structure of aPP also provides an interesting illustration of how an amphiphilic structure in a peptide hormone might interact with other less structured parts of the sequence to protect them from enzymatic modification or hydrolysis. In this case, residues 9–12 of aPP form a β bend so that the amino-terminal residues are folded back across the hydrophobic surface of the amphiphilic α helix. Furthermore, residues 1–8 form an extended helical structure of the type found in collagen, and proline residues in positions 2, 5, and 8 are all positioned on one side of this structure and interact with the α helix in the carboxy terminus to form a hydrophobic core. Since the other residues in this segment of aPP are all essentially hydrophilic, the extended collagen-like helix in residues 1–8 with prolines in every third position represents another type of amphiphilic structure.

The aPP amino acid sequence differs from the human and other mammalian pancreatic polypeptide (PP) sequences in about half of the positions (110). Nevertheless, the general features of the amphiphilic structures appear to be conserved, and the mammalian peptides are

likely to have similar conformational properties. In particular, the hydrophobic surfaces of the amino- and carboxy-terminal helical structures might bind to separate sites on receptors or other cellular surfaces having amphiphilic interfaces as well as interacting with each other in solution. At present, the physiological functions of PP are not clear, but two peptides with about 50% homology to the PP sequences in which both of the amphiphilic helical structures are again preserved have recently been characterized and determined to have important actions, both in the central nervous system and in the periphery (110, 143a, 40). These peptides, peptide tyrosine tyrosine (PYY) and neuropeptide tyrosine (NPY), retain the prolines in positions 2, 5, and 8 of the PP sequences, and the intervening residues are hydrophilic, so that a collagen-like amphiphilic helix might again be formed. The residues in positions 9–12 have a high propensity for β-turn formation and, in particular, the glycine in position 9 of the PP sequences is preserved. Residues 13–32 of NPY or 14–32 of PYY can form an amphiphilic structure in the α-helical conformation with general properties identical to that observed in the aPP crystal structure, although there are multiple sequence differences in this region (144). The carboxy terminus is amidated in each case and contains a basic hydrophilic sequence that is highly conserved throughout this peptide family in residues 32–36. This structural organization (figure 13) is reminiscent of that in the calcitonin and CGRP peptide family, where the amphiphilic helical segment might serve to connect "active sites" at either end of the molecule and position them in the correct orientation for agonist activity on a receptor surface. The functional relationship between PYY and NPY is also similar to that between calcitonin and CGRP: PYY is located in endocrine cells and circulates in the periphery where its actions are hormonal, whereas NPY is found in very high concentrations in the brain and is a neuronal peptide present in both central and peripheral nervous systems (40, 61, 110). This analogy, therefore, suggests that the amphiphilic structures involved have properties suitable for both types of function.

Since NPY and PYY share a variety of actions that are not reproduced by the PP structure, it will be interesting to identify the specific features of the apparently similar architecture of these peptides that determine these differences. Our preliminary investigations of NPY and human PP show that both of these peptides form relatively stable monolayers at the air-water interface (collapse pressures of about 15 dyn/cm were observed) that have properties consistent with helical structure being adopted in this amphiphilic environment (144). Dimer formation has also been observed in aqueous solutions of the pancreatic polypeptides, consistent with the self-association of amphiphilic α-helical structures (26). It is very likely, therefore, that similar helical structures will be involved in cell surface binding and, possibly, will determine the receptor selectivity and in vivo stability of these peptides. Their structural homology, combined with the variety in their amino acid sequences, suggests that this family of peptides is ideally suited to characterization by the peptide modelling approach.

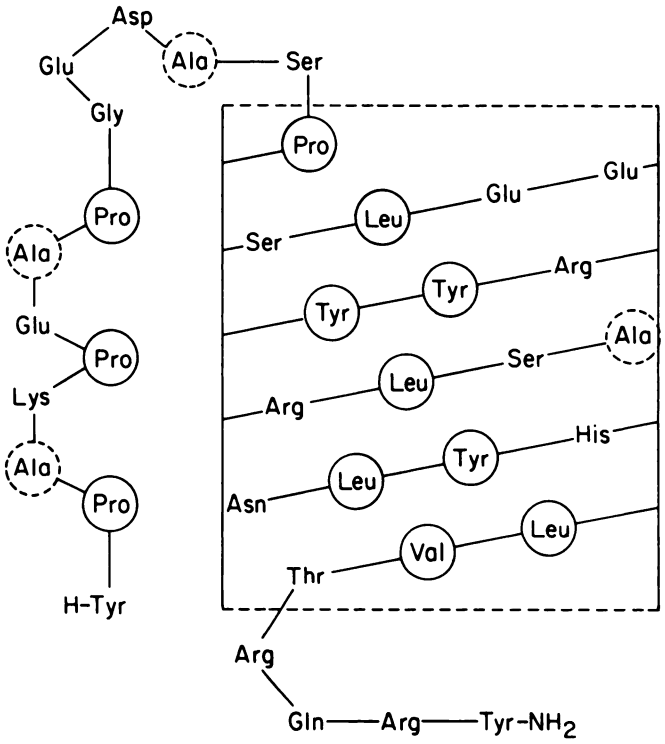

FIG. 13. Schematic representation of the potential structural domains of porcine PYY (143a). A helical net diagram illustrates the formation of a hydrophobic domain by residues 14–32 in the α-helical conformation. Another amphiphilic structure is formed by residues 1–8 in a collagen-like helical conformation (14), and a β turn formed by residues 9–12 connects these two structures. A basic, hydrophilic "tail" is formed by residues 33–36 at the carboxy terminus. Hydrophobic residues are *circled*.

Yet another peptide hormone for which a potential amphiphilic α-helical structure has been postulated is PTH (42). In an α-helical conformation, the entire active fragment of this hormone consisting of the amino-terminal 34 residues has an amphiphilic surface on which a row of mostly leucine, valine, and methionine residues forms a hydrophobic domain that is essentially one residue wide. This domain lies along the complete length of the helix (a total of nine turns) and, in the regular α-helical conformation, would twist all the way around the helix surface once in an anticlockwise direction. In order to interact with most amphiphilic interfaces, therefore, it is likely that this amphiphilic structure would contain a discontinuity or other irregularities that might better align this long hydrophobic domain along one side of the structure. Alternatively, it has been suggested that segments of the narrower 3_{10}-type helix would align this hydrophobic ridge in a suitable manner parallel to the helix axis (42). In either case, the active PTH fragment interacts with phospholipids with a concomitant increase

in helicity, indicating that amphiphilic secondary structure might contribute to cell surface binding of this hormone also (42, 43).

An extensive search of the amino acid sequences of peptide hormones reveals that segments of alternating hydrophobic and hydrophilic residues, which could form amphiphilic β strands, are very unusual. Perhaps this is not so surprising, in view of the strongly amphiphilic properties that such sequences exhibit as a result of their extensive aggregation into β-sheet structures with extremely hydrophobic surfaces (123, 124). It may be that this type of structure would be too lipophilic and might even cause the disruption of many biological structures just as the hyrophobic amphiphilic α-helical structure of melittin, the bee venom peptide, lyses erythrocytes (33, 35). An earlier proposal that LHRH might form such an amphiphilic β-strand structure (77) has been tested in our laboratory by attempting to spread the peptide on the surface of aqueous solutions containing increasing concentrations of KCl. Thus far, however, it has not been possible to induce this peptide to form monolayers at this interface, indicating that there must be a soluble folded conformation that is strongly preferred by LHRH over the extended amphiphilic structure (82). In contrast, we have found that the κ-opiate receptor selective peptide, dynorphin A(1–17), does form monolayers at the air-water interface (144). These monolayers have a similar stability to those formed by β_h-endorphin (126). Furthermore, the tendency of the dynorphin A(1–17) molecules to form extensive aggregates in this environment is typical of the type of behavior expected for an extended amphiphilic β strand structure (34, 123, 124) and, indeed, residues 7–15 of this peptide do have hydrophobic and hydrophilic residues arranged in an alternating fashion. Strong nonspecific membrane binding properties of dynorphin A(1–17) have also been described (67), and it has been demonstrated that the complete 17-residue structure has a much greater resistance to proteolytic degradation than its carboxy-terminal deletion analogues (30), suggesting that this potential amphiphilic β-strand structure might determine the properties of dynorphin A in much the same way as the corresponding helical structure does in β-endorphin. A peptide modelling approach to investigating this possibility also appears attractive.

VIII. Summary and Prospects for Future Studies

Medium-sized peptide hormones and neurotransmitters that have little or no apparent secondary or tertiary structure in aqueous solution often have the potential to form amphiphilic secondary structures which may be stabilized in the biological milieu in general, or at specific interfaces that provide a suitable complementary amphiphilic environment. We have postulated here and elsewhere that such structures may serve multiple functions in these peptides, including (*a*) limiting their receptor-bound conformations so that the structural features that are important for agonist activity are held in the correct orientation; (*b*) promoting adsorption to cell surfaces in order to enhance the efficiency of receptor location, limit diffusion, or control proteolytic processing; and (*c*) stabilizing protease-sensitive parts of these molecules to increase the duration of the messages they convey.

The approach of studying synthetic peptides incorporating models of the potential amphiphilic structures has proven extremely useful in assessing their importance and functions. In the case of β-endorphin, an amphiphilic helix at the carboxy terminus has been shown to determine the receptor specificity of the enkephalin structure to which it is connected via a hydrophilic linking region, and to protect this essential part of the message from enzymatic inactivation. In addition, evidence that the amphiphilic structure binds to receptor surfaces in the α-helical form has been obtained, certain receptors apparently having some specificity for individual side chains on its surface. The corresponding amphiphilic structure in calcitonin is also α-helical, but it lies in the middle of the hormone and connects essential structural elements at either end. As with β-endorphin, the studies of model peptides show that this α-helical structure is probably adopted on the receptor surfaces where its general characteristics, including its amphiphilicity and charge distribution, are more important than any specific side chains on its surface, although some side chain specificity may again be involved. The situation with glucagon was found to be altogether different in that most of this peptide hormone is potentially involved in amphiphilic helical structure, and the multiple amino acid substitutions made in peptide models resulted in considerable losses in potency. At present, therefore, it is not clear what conformation glucagon adopts on receptor surfaces, and it is possible that more than one conformation may be involved. Even in this situation, however, the modelling approach has proven useful in the functional characterization of glucagon binding sites, and it may still be possible to identify the receptor-bound conformation of the natural hormone through the design of more conservative peptide models, as discussed.

The modelling strategy, as it has developed so far, has placed an emphasis on optimizing the stability and amphiphilicity of the helical structures studied and on minimizing homology to the natural sequences. This approach was adopted in an attempt to simultaneously create hormone analogues that were more potent than the natural structures and identify their receptor-bound conformations. These goals may sometimes be incompatible, however, as minimizing homology in an amphiphilic structure might necessitate altering parts of a hormone that interact somewhat specifically with its receptor surface and, in these circumstances, a lower binding affinity is likely to result. Nevertheless, enhancing the amphiphilic properties of β-endorphin has produced three peptide models with longer lasting antinociceptive actions in vivo than the natural structure, dem-

onstrating that this is a powerful approach to the design of peptides with the potential for therapeutic use. Freed from the constraint of attempts to minimize homology to the natural structures, it appears quite likely that higher potencies could also be attained. On the other hand, our original hypothesis for the organization of structural domains in the β-endorphin model has, we believe, been completely validated through the design and study of the physicochemical and pharmacological properties of just six synthetic peptides, demonstrating the power of model design with minimal homology as the first priority when structural characterization is desired. We now look forward to the development of similar studies of the other amphiphilic structures discussed in this article, so that the time-consuming synthesis of multiple analogues with single residue changes can be avoided. It is also expected that, given the number of amphiphilic structures—particularly helices—that have been identified so far, some general rules relating these structures to their functions in peptide hormones and neurotransmitters will soon become evident.

REFERENCES

1. Adam, G., and Delbruck, M.: Reduction of dimensionality in biological diffusion processes. *In* Structural Chemistry and Molecular Biology, ed. by A. Rich and N. Davidson, pp. 198–215, W. H. Freeman, San Francisco, 1968.

1a. Amara, S. G., Jonas, V., Rosenfeld, M. G., Ong, E. S., and Evans, R. M.: Alternative RNA processing in calcitonin gene expression generates mRNAs encoding different polypeptide products. Nature (Lond.) **298:** 240–244, 1982.

2. Andreu, D., Merrifield, R. B., Steiner, H., and Boman, H. G.: *N*-terminal analogues of Cecropin A: synthesis, antibacterial activity, and conformational properties. Biochemistry **24:** 1683–1688, 1985.

3. Argiolas, A., and Pisano, J. J.: Bombolitins, a new class of mast cell degranulating peptides from the venom of the bumblebee *Megabombus pennsylvanicus*. J. Biol. Chem. **260:** 1437–1444, 1985.

4. Austin, B. M., and Smyth, D. G.: The NH_2-terminus of C-fragment is resistant to the action of aminopeptidases. Biochem. Biophys. Res. Commun. **76:** 477–482, 1977.

5. Bell, G. I., Santerre, R. F., and Mullenbach, G. T.: Hamster preproglucagon contains the sequence of glucagon and two related peptides. Nature (Lond.) **302,** 716–718, 1983.

6. Belluzzi, J. D., Grant, N., Garsky, V., Sarantakis, D., Wise, C. D., and Stein, L.: Analgesia induced *in vivo* by central administration of enkephalin in rat. Nature (Lond.) **260:** 625–626, 1976.

6a. Berg, H. C., and Purcell, E. M.: Physics of chemoreception. Biophys. J. **20:** 193–219, 1977.

7. Bewley, T. A., and Li, C. H.: Tertiary structure in deletion analogues of human β-endorphin: resistance to leucine aminopeptidase action. Biochemistry **24:** 6568–6571, 1985.

8. Blake, J., Ferrara, P., and Li, C. H.: β-Endorphin: synthesis and radioligand binding activity of analogs containing cystine bridges. Int. J. Pept. Protein Res. **17:** 239–242, 1981.

9. Blake, J., Tseng, L.-F., Chang, W.-C., and Li, C. H.: The synthesis and opiate activity of human β-endorphin analogs. Int. J. Pept. Protein Res. **11:** 323–328, 1978.

10. Blake, J., Tseng, L.-F., and Li, C. H.: Synthesis and analgesic activity of human β-endorphin analogs substituted at positions 17, 18, or 19. Int. J. Pept. Protein Res. **15:** 167–170, 1980.

11. Blanc, J. P., and Kaiser, E. T.: Biological and physical properties of a β-endorphin analog containing only D-amino acids in the amphiphilic helical segment 13–31. J. Biol. Chem. **259:** 9549–9556, 1984.

12. Blanc, J. P., Taylor, J. W., Miller, R. J., and Kaiser, E. T.: Examination of the requirement for an amphiphilic helical structure in β-endorphin through the design, synthesis, and study of model peptides. J. Biol. Chem. **258:** 8277–8284, 1983.

13. Bloom, F., Segal, D., Ling, N., and Guillemin, R.: Endorphins: profound behavioral effects in rats suggest new etiological factors in mental illness. Science (Wash. DC) **194:** 630–632, 1976.

14. Blundell, T. L., Pitts, J. E., Tickle, I. J., Wood, S. P., and Wu, C.-W.: X-ray analysis (1.4 Å resolution) of avian pancreatic polypeptide: small globular protein hormone. Proc. Natl. Acad., Sci. USA **78:** 4175–4179, 1981.

15. Bonnevie-Nielsen, V., and Tager, H. S.: Glucagon receptors on isolated hepatocytes and hepatocyte membrane vesicles. J. Biol. Chem. **258:** 11313–11320, 1983.

16. Bornet, H., and Edelhoch, H.: Polypeptide hormone interaction. J. Biol. Chem. **246:** 1785–1792, 1971.

17. Brack, A.: β-Structures of alternating polypeptides and their possible role in chemical evolution. BioSystems **9:** 99–103, 1977.

18. Bradbury, A. F., Smyth, D. G., Snell, C. R., Birdsall, N. J. M., and Hulme, E. C.: C fragment of lipotropin has a high affinity for brain opiate receptors. Nature (Lond.) **260:** 793–795, 1976.

19. Brain, S. D., Williams, T. J., Tippins, J. R., Morris, H. R., and MacIntyre, I.: Calcitonin gene-related peptide is a potent vasodilator. Nature (Lond.) **313:** 54–56, 1985.

20. Brandenberg, D., and Saunders, D.: Chemical structure and biological activity of hormones and related compounds. *In* Amino Acids, Peptides, and Proteins, ed. by R. C. Sheppard, vol. 13, pp. 363–366, The Royal Chemical Society, Burlington House, London, 1982.

20a. Bregman, D. A., Trivedi, D., and Hruby, V. J.: Glucagon amino groups: evaluation of modifications leading to antagonism and agonism. J. Biol. Chem. **255:** 11725–11731, 1980.

21. Brewer, H. B., Jr., and Edelhoch, H.: A conformation study of porcine thyrocalcitonin. J. Biol. Chem. **245:** 2402–2408, 1970.

22. Brown, L. R., Bosch, C., and Wutrich, K.: Location and orientation relative to the micelle surface for glucagon in mixed micelles with dodecylphosphocholine. Biochem. Biophys. Acta **642:** 296–312, 1981.

23. Buscher, H. H., Hill R.-C., Romer, D., Cardinaux, F., Closse, A., Hauser, D., and Press, J.: Evidence for analgesic activity of enkephalin in the mouse. Nature (Lond.) **261:** 423–425, 1976.

24. Byfield, P. G. H., Clark, M. B., Turner, K., Foster, G. V., and MacIntyre, I.: Immunochemical studies on human calcitonin M leading to information on the shape of the molecule. Biochem. J. **127:** 199–206, 1972.

25. Chang, J. K., Fong, B. T. W., Pert, A., and Pert, C. B.: Opiate receptor affinities and behavioral effects of enkephalin: structure-activity relationship of ten synthetic peptide analogs. Life Sci. **18:** 1473–1478, 1976.

26. Chang, P. J., Noelken, M. E., and Kimmel, J. R.: Reversible dimerization of avian pancreatic polypeptide. Biochemistry **19:** 1844–1849, 1980.

27. Chavkin, C., and Goldstein, A.: Specific receptor for the opioid peptide dynorphin: structure-activity relationships. Proc. Natl. Acad. Sci. USA **78:** 6543–6547, 1981.

28. Chavkin, C., James, I. F., and Goldstein, A.: Dynorphin is a specific endogenous ligand of the κ opioid receptor. Science (Wash. DC) **215:** 413–415, 1982.

28a. Chothia, C.: Principles that determine the structure of proteins. Annu. Rev. Biochem. **53:** 537–572, 1984.

29. Chou, P. Y., and Fasman, G. D.: Empirical predictions of protein conformation. Annu. Rev. Biochem. **47:** 251–276, 1978.

30. Corbett, A., Paterson, S. J., McKnight, A. T., Magnan, J., and Kosterlitz, H. W.: Dynorphin (1–8) and dynorphin (1–9) are ligands for the κ-subtype of opiate receptor. Nature (Lond.) **299:** 79–81, 1982.

31. Cox, J. A., Comte, M., Fitton, J. E., and DeGrado, W. F.: The interaction of calmodulin with amphiphilic peptides. J. Biol. Chem. **260:** 2527–2534, 1985.

32. Deakin, J. F. W., Dostrovsky, J. O., and Smyth, D. G.: Influence of N-terminal acetylation and C-terminal proteolysis on the analgesic activity of β-endorphin. Biochem. J. **189:** 501–506, 1980.

33. DeGrado, W. F., Kezdy, F. J., and Kaiser, E. T.: Design, synthesis, and characterization of a cytotoxic peptide with melittin-like activity. J. Am. Chem. Soc. **103:** 679–681, 1981.

34. DeGrado, W. F., and Lear, J. D.: Induction of peptide conformation at apolar/water interfaces. 1. A study with model peptides of defined hydrophobic periodicity. J. Am. Chem. Soc. **107:** 7684–7689, 1985.

35. DeGrado, W. F., Musso, G. F., Kezdy, F. J., and Kaiser, E. T.: Kinetics and mechanism of hemolysis induced by melittin and by a synthetic melittin analogue. Biophys. J. **37:** 329–338, 1982.

36. Dickerson, R. E., and Geis, I.: The Structure and Action of Proteins, pp. 1–120, W. A. Benjamin, Inc., London, 1969.

37. Dunnil, P.: The use of helical net diagrams to represent protein structures. Biophys. J. **8:** 865–875, 1968.

38. Edelstein, C., Kezdy, F. J., Scanu, A. M., and Shen, B. W.: Apolipoproteins and the structural organization of plasma lipoproteins: human plasma high density lipoprotein-3. J. Lipid Res. **20:** 143–153, 1979.

38a. Efimov, A. V.: Packing of α-helices in globular proteins. Layer-structure of globin hydrophobic cores. J. Mol. Biol. **134:** 23–40, 1979.

39. Eisenberg, D., Weiss, R. M., and Terwilliger, T. C.: The helical hydrophobic moment: a measure of the amphiphilicity of a helix. Nature (Lond.) **299:** 371–374, 1982.

40. Emson, P. C., and DeQuidt, M. E.: NPY—a new member of the pancreatic polypeptide family. Trends Neurosci. **7:** 31–35, 1984.

41. England, R. D., Jenkins, W. T., Flanders, K. C., and Gurd, R. S.: Noncooperative receptor interactions of glucagon and eleven analogues: inhibition of adenylate cyclase. Biochemistry **22:** 1722–1728, 1983.

42. Epand, R. M., Epand, R. F., Hui, S. W., He, N. B., and Rosenblatt, M.: Formation of water-soluble complex between the 1–34 fragment of parathyroid hormone and dimyristoylphosphotidylcholine. Int. J. Pept. Protein Res. **25:** 594–600, 1985.

43. EPAND, R. M., EPAND, R. F., ORLOWSKI, R. C., PLANIGAN, E., AND STAHL, G. L.: A comparison of the interactions of glucagon, human parathyroid hormone-(1-34) peptide, and calcitonin with dimyristoylphosphatidylglycerol and with dimyristoylphosphatidylcholine. Biophys. Chem. **23:** 39–48, 1985.
44. EPAND, R. M., EPAND, R. F., ORLOWSKI, R. C., SCHLUETER, R. J., BONI, L. T., AND HUI, S. W.: Amphipathic helix and its relationship to the interaction of calcitonin with phospholipids. Biochemistry **22:** 5074–5084, 1983.
45. FERRARA, P., AND LI, C. H.: β-endorphin. Interaction of synthetic analogs having different chain lengths with morphine and enkephaline receptors in rat brain membranes. Int. J. Pept. Protein Res. **19:** 259–262, 1982.
46. FINDLAY, D. M., DELUISE, M., MICHELANGELI, V. P., ELLISON, M., AND MARTIN, T. J.: Properties of a calcitonin receptor and adenylate cyclase in BEN cells, a human cancer cell line. Cancer Res. **40:** 1311–1317, 1980.
47. FINDLAY, D. M., MICHELANGELI, V. P., EISMAN, J. A., FRAMPTON, R. J., MOSELEY, J. M., MACINTYRE, I., WHITEHEAD, R., AND MARTIN, T. J.: Calcitonin and 1,25-dihydroxyvitamin D_3 receptors in human breast cancer cell lines. Cancer Res. **40:** 4764–4767, 1980.
48. FOLEY, K. M., KOURIDES, I. A., INTURRISI, C. E., KAIKO, R. F., ZAROULIS, C. G., POSNER, J. B., HOUDE, R. W., AND LI, C. H.: β-endorphin: analgesic and hormonal effects in humans. Proc. Natl. Acad. Sci. USA **76:** 5377–5381, 1979.
49. FORMISANO, S., JOHNSON, M. L., AND EDELHOCH, H.: Thermodynamics of the self-association of glucagon. Proc. Natl. Acad. Sci. USA **74:** 3340–3344, 1977.
50. FRANDSEN, E. K., GRONWALD, F. C., HEDING, L. G., JOHANSEN, N. L., LUNDT, B. F., MOODY, A. J., MARKUSSEN, J., AND VOLUND, A.: Glucagon: structure-function relationships investigated by sequence deletions. Hoppe-Seyler's Z. Physiol. Chem. **362:** 665–677, 1981.
51. FUKUSHIMA, D., AND KAISER, E. T.: Unpublished observations.
52. FUKUSHIMA, D., KUPFERBERG, J. P., YOKOYAMA, S., KROON, D. J., KAISER, E. T., AND KEZDY, F. J.: A synthetic amphiphilic helical docosapeptide with the surface properties of plasma apolipoprotein A-I. J. Am. Chem. Soc. **101:** 3703–3704, 1979.
53. FUKUSHIMA, D., YOKOYAMA, S., KEZDY, F. J., AND KAISER, E. T.: Binding of amphiphilic peptides to phospholipid/cholesterol unilamellar vesicles: a model for protein-cholesterol interaction. Proc. Natl. Acad. Sci. USA **78:** 2732–2736, 1981.
54. GARNIER, J., OSGUTHORPE, D. J., AND ROBSON, B.: Analysis of the accuracy and implications of simple methods for predicting the secondary structure of globular proteins. J. Mol. Biol. **120:** 97–120, 1978.
55. GARZIA, R., YAMASHIRO, D., HAMMONDS, R. G., JR., AND LI, C. H.: Synthesis and properties of human β-endorphin-(1-17) and its analogs. Int. J. Pept. Protein Res. **19:** 432–435, 1982.
56. GARZIA, R., YAMASHIRO, D., LI, C. H., AND NICOLAS, P.: β-endorphin: synthesis and properties of human β-endorphin-(1-28) and its analogs. Int. J. Pept. Protein Res. **20:** 94–198, 1982.
57. GEISOW, M. J., AND SMYTH, D. G.: Preparation and properties of lipotropin C-fragment with anionic substituents attached to the lysine residues. FEBS Lett. **81:** 431–434, 1977.
58. GERICH, J., CHARLES, M., AND GRODSKY, G.: Regulation of pancreatic insulin and glucagon secretion. Annu. Rev. Physiol. **38:** 353–388, 1976.
58a. GILLAN, M. G. C., KOSTERLITZ, H. W., AND PATERSON, S. J.: Comparison of the binding characteristics of tritiated opiates and opioid peptides. Br. J. Pharmacol. **70:** 481–490, 1980.
58b. GOLDSTEIN, A., AND JAMES, I. F.: Multiple opioid receptors: criteria for identification and classification. Trends Pharmacol. Sci. **5:** 503–505, 1984.
59. GRAF, L., COEH, G., BARAT, E., RONAI, A. Z., SZEKELY, J. I., KENNESEY, A., AND BAJUSZ, S.: Structure-function relationships in lipoproteins. Ann. NY Acad. Sci. **297:** 63–83, 1977.
60. GRAF, L., HOLLOSI, M., BARNA, I., HERMANN, I., BORVENDEG, J., AND LING, N.: Probing the biologically and immunologically active conformation of β-endorphin: studies on C-terminal deletion analogs. Biochem. Biophys. Res. Commun. **95:** 1623–1627, 1980.
61. GRAY, T. S., AND MORLEY, J. E.: Neuropeptide Y: anatomical distribution and possible function in mammalian nervous system. Life Sci. **38:** 389–401, 1986.
62. GREMLICH,, H.-U., FRINGELI, U.-P., AND SCHWYZER, R.: Conformational changes of adrenocorticotropin peptides upon interaction with lipid membranes revealed by infrared attenuated total reflection spectroscopy. Biochemistry **22:** 4257–4264, 1983.
63. GUTTMAN, S., PLESS, J., HUGUENIN, R. L., SANDRIN, E., BOSSERT, H., AND ZEHEDER, K.: Synthese von salm-calcitonin, einem hochaktiven hypocalcamischen hormon. Helv. Chim. Acta **52:** 1789–1795, 1969.
64. HAMMONDS, R. G., JR., HAMMONDS, A. S., LING, N., AND PUETT, D.: β-endorphin and deletion peptides. A correlation of opiate receptor affinity with helix potential. J. Biol. Chem. **257:** 2990–2995, 1982.
65. HAMMONDS, R. G., JR., AND LI, C. H.: β-endorphin. Opiate receptor-binding activity of synthetic analogs modified in the enkephalin segment in rat brain membrane and neuroblastoma × glioma hybrid cell. Int. J. Pept. Protein Res. **19:** 254–258, 1982.
66. HAMMONDS, R. G., JR., NICOLAS, P., AND LI, C. H.: β-endorphin: analgesic and receptor binding activity of non-mammalian homologs. Int. J. Pept. Protein Res. **19:** 556–561, 1982.
66a. HAMMONDS, R. G., JR., NICOLAS, P., AND LI, C. H.: β-Endorphin-(1-27) is an antagonist of β-endorphin analgesia. Proc. Natl. Acad. Sci. USA **81:** 1389–1390, 1984.
66b. HAYES, A. J., AND SHEEHAN, K. M. J.: Are there multiple opioid receptor types in the rat vas deferens? Br. J. Pharmacol. **88:** 276, 1986.
67. HO, W. K. K., COX, B. M., CHAVKIN, C., AND GOLDSTEIN, A.: Opioid peptide dynorphin-(1-13): adsorptive losses and potency estimates. Neuropeptides **1:** 143–152, 1980.
68. HOLLOSI, M., KAJTAR, M., AND GRAF, L.: Studies on the conformation of β-endorphin and its constituent fragments in water and trifluorethanol by CD spectroscopy. FEBS Lett. **74:** 185–189, 1977.
69. HOLLT, V.: Multiple endogenous opioid peptides. Trends Neurosci. **6:** 24–26, 1983.
70. HRUBY, V. J.: Structure-conformation-activity studies of glucagon and semisynthetic glucagon analogs. Mol. Cell. Biochem. **44:** 49–64, 1982.
70a. HRUBY, V. J.: Conformational restrictions of biologically active peptides via amino acid side chain groups. Life Sci. **31:** 189–199, 1982.
71. HUI, K.-S., GRAF, L., AND LAJTHA, A.: β-endorphin inhibits met-enkephalin breakdown by a brain aminopeptidase: structure-activity relationships. Biochem. Biophys. Res. Commun. **105:** 1482–1487, 1982.
72. HUIDOBRO-TORO, J. P., CATURAY, E. M., LING, N., LEE, N. M., LOH, H. H., AND WAY, E. L.: Studies on the structural prerequisites for the activation of the β-endorphin receptor on the rat vas deferens. J. Pharmacol. Exp. Ther. **222:** 262–269, 1982.
73. JACQUET, Y. F., AND MARKS, N.: The C-fragment of β-lipotropin: an endogenous neuroleptic or antipsychotogen? Science (Wash. DC) **194:**632–635, 1976.
74. JIBSON, M. D., AND LI, C. H.: β-ENDORPHIN. Circular dichroism of synthetic human analogs with various chain lengths in methanol solutions. Int. J. Pept. Protein Res. **18:** 297–301, 1981.
75. KAISER, E. T.: The design of peptides and proteins. Proc. NY Acad. Sci. in press, 1986.
76. KAISER, E. T., AND KEZDY, F. J.: Secondary structures of proteins and peptides in amphiphilic environments (a review). Proc. Natl. Acad. Sci. USA **80:** 1137–1143, 1983.
77. KAISER, E. T., AND KEZDY, F. J.: Amphiphilic secondary structure: design of peptide hormones. Science (Wash. DC) **223:** 249–255, 1984.
78. KAISER, E. T., LYNCH, B., AND RAJASHEKHAR, B.: The design and construction of peptides and proteins with amphiphilic secondary structures. *In* Proceedings of the Ninth American Peptide Symposium, ed. by C. M. Deber, V. J. Hruby, and K. D. Kopple, pp. 855–864, Pierce Chemical Company, USA, 1985.
79. KANELLIS, P., ROMANS, A. Y., JOHNSON, B. J., KERCRET, H., CHIOVETTI, R., JR., ALLEN, T. M., AND SEGREST, J. P.: Studies of synthetic peptide analogs of the amphipathic helix. J. Biol. Chem. **225:** 11464–11472, 1980.
80. KATCHALSKI-KATZIR, E., RISHPON, J., SAHAR, E., LAMED, R., AND HEINS, Y. I.: Enzyme diffusion and action on soluble and insoluble substrate biopolymers. Biopolymers **24:** 257–277, 1985.
81. KAWAUCHI, H., TSUBOKAWA, M., KANEZAWA, A., AND KITAGAWA, E.: Occurrence of two different endorphins in the salmon pituitary. Biochem. Biophys. Res. Commun. **92:** 1278–1288, 1980.
82. KENDALL, D. A., AND KAISER, E. T.: Unpublished observations.
83. KROON, D. J., KUPFERBERG, J. P., KAISER, E. T., AND KEZDY, F. J.: Mechanism of lipid-protein interactions in lipoproteins. A synthetic peptide-lecithin vesicle model. J. Am. Chem. Soc. **100:** 5975–5977, 1978.
83a. KRSTENANSKY, J. L., TRIVEDI, D., JOHNSON, D., AND HRUBY, V. J.: Conformational considerations in the design of a glucagon analogue with increased receptor binding and adenylate cyclase potencies. J. Am. Chem. Soc. **108:** 1696–1698, 1986.
84. KUMAR, M. A., SLACK, E., EDWARDS, A., SOLIMAN, H. A., BAGHDIANTZ, A., FOSTER, G. V., AND MACINTYRE, I.: A biological assay for calcitonin. J. Endocrinol. **33:** 469–475, 1965.
85. LAU, S. H., RIVIER, J., VALE, W., KAISER, E. T., AND KEZDY, F. J.: Surface properties of an amphiphilic peptide hormone and of its analog: corticotropin-releasing factor and sauvagine. Proc. Natl. Acad. Sci. USA **80:** 7070–7074, 1983.
86. LAU, S. Y. M., TANEJA, A. K., AND HODGES, R. S.: Synthesis of a model protein of defined secondary and quaternary structure. J. Biol. Chem. **259:** 13253–13261, 1984.
87. LAW, P.-Y., LOH, H. H., AND LI, C. H.: Properties and localization of β-endorphin receptor in rat brain. Proc. Natl. Acad. Sci. USA **76:** 5455–5459, 1979.
88. LEE, N. M., FRIEDMAN, H. J., LEYBIN, L., CHO, T. M., LOH, H. H., AND LI, C. H.: Peptide inhibitor of morphine- and β-endorphin-induced analgesia. Proc. Natl. Acad. Sci. USA **77:** 5525–5526, 1980.
89. LEE, N. M., AND SMITH, A. P.: A protein-lipid model of the opiate receptor. Life Sci. **26:** 1459–1464, 1980.
90. LEMAIRE, S., MAGNAN, J., AND REGOLI, D.: Rat vas deferens: a specific bioassay for endogenous opioid peptides. Br. J. Pharmacol. **64:** 327–329, 1978.
91. LI, C. H., AND CHUNG, D.: Distinct behavior of β-endorphin toward leucine aminopeptidase action. Int. J. Pept. Protein Res. **26:** 113–117, 1985.
92. LI, C. H., RAO, A. J., DONEEN, B. A., AND YAMASHIRO, D.: β-endorphin: lack of correlation between opiate activity and immunoreactivity by radioimmunoassay. Biochem. Biophys. Res. Commun. **75:** 576–580, 1977.
93. LI, C. H., TSENG, L.-F., FERRARA, P., AND YAMASHIRO, D.: β-endorphin:

dissociation of receptor binding activity from analgesic potency. Proc. Natl. Acad. Sci. USA **77:** 2303–2304, 1980.
94. Li, C. H., Tseng, L.-F., and Yamashiro, D.: β-endorphin: complete primary structure is required for full analgesic activity. Biochem. Biophys. Res. Commun. **85:** 795–800, 1978.
95. Li, C. H., Yamashiro, D., Hammonds, R. G., Jr., Nicholas, P., and Tseng, L.-F.: β-endorphin: replacement of glutamic acid in position 8 by glutamine increases analgesic potency and opiate receptor-binding activity. Biochem. Biophys. Res. Commun. **101:** 118–123, 1981.
96. Li, C. H., Yamashiro, D., and Nicolas, P.: β-endorphin: replacement of tyrosine in position 27 by tryptophan increases analgesic potency: preparation and properties of the 2-nitrophenylsulfenyl derivative. Proc. Natl. Acad. Sci. USA **79:** 1042–1044, 1982.
97. Li, C. H., Yamashiro, D., Tseng, L.-F., Chang, W.-C., and Ferrara, P.: β-endorphin: synthesis of analogs modified at the carboxyl terminus with increased activities. Proc. Natl. Acad. Sci. USA **76:** 3276–3278, 1979.
98. Li, C. H., Yamashiro, D., Tseng, L.-F., Chang, W.-C., and Ferrara, P.: β-Endorphin omission analogs: dissociation of immunoreactivity from other biological activities. Proc. Natl. Acad. Sci. USA **77:** 3211–3214, 1980.
99. Li, C. H., Yamashiro, D., Tseng, L.-F., and Loh, H. H.: β-endorphin: synthesis and biological activity of shortened peptide chains. Int. J. Pept. Protein Res. **11:** 154–158, 1978.
99a. Liepnieks, J., and Epand, R. M.: Preparation of N^{α}-citraconyl glucagon and evaluation of its biological properties. Biochim. Biophys. Acta **707:** 171–177, 1982.
100. Lim, V. I.: Algorithms for prediction of α-helical and β-structural regions in globular proteins. J. Mol. Biol. **88:** 873–894, 1974.
101. Loh, H. H., Cho, T. M., Wu, Y. C., Harris, R. A., and Way, E. L.: Opiate binding to cerebroside sulfate: a model system for opiate-receptor interaction. Life Sci. **16:** 1811–1818, 1975.
102. Loh, H. H., Tseng, L.-F., Wei, E., and Li, C. H.: β-Endorphin is a potent analgesic agent. Proc. Natl. Acad. Sci. USA **73:** 2895–2898, 1976.
103. Lord, J. A. H., Waterfield, A. A., Hughes, J., and Kosterlitz, H. W.: Endogenous opioid peptides: multiple agonists and receptors. Nature (Lond.) **267:** 495–499, 1977.
104. MacIntyre, I., Evans, I. M. A., Hobitz, H. H. G., Joplin, G. F., and Stevenson, J. C.: Chemistry, physiology, and therapeutic applications of calcitonin. Arthritis Rheum. **231:** 1139–1146, 1980.
105. Maier, R., Kamber, B., Riniker, B., and Rittel, W.: Analogs of human calcitonin. IV. Influence of leucine substitutions in positions 12, 16, and 19 on hypocalcemic activity in the rat. Clin. Endocrinol. **5:** 327s–332s, 1976.
106. Marcus, R., and Aurbach, G. D.: Adenyl cyclase from renal cortex. Biochim. Biophys. Acta **242:** 410–421, 1971.
107. Marks, N., Grynbaum, A., and Neidle, A.: On the degradation of enkephalins and endorphins by rat and mouse brain extracts. Biochem. Biophys. Res. Commun. **74:** 1552–1559, 1977.
108. Marx, S. J., Woodward, C. J., and Aurbach, G. D.: Calcitonin receptors of kidney and bone. Science (Wash. DC) **178:** 999–1001, 1972.
108a. Miller, L., Shaw, J. S., and Whiting, E. M.: The contribution of intrinsic activity to the action of opioids in vitro. Br. J. Pharmacol. **87:** 595–601, 1986.
109. Miller, R. J.: Peptides as neurotransmitters: focus on the enkephalins and endorphins. Pharmacol. & Ther. **12:** 73–108, 1981.
110. Miller, R. J.: New perspectives on gut peptides. J. Med. Chem. **27:** 1239–1245, 1984.
111. Moe, G. R., and Kaiser, E. T.: Design, synthesis, and characterization of a model peptide having potent calcitonin-like biological activity: implications for calcitonin structure/activity. Biochemistry **24:** 1971–1976, 1985.
112. Moe, G. R., Miller, R. J., and Kaiser, E. T.: Design of a peptide hormone: synthesis and characterization of a model peptide with calcitonin-like activity. J. Am. Chem. Soc. **105:** 4100–4102, 1983.
113. Morris, H. R., Panico, M., Etienne, T., Tippins, J., Girgis, S. I., and MacIntyre, I.: Isolation and characterization of human calcitonin gene-related peptide. Nature (Lond.) **308:** 746–748, 1984.
114. Murikawa, T., Munzkata, E., Sakakibara, S., Noda, T., and Otani, M.: Synthesis of eel-calcitonin and [$Asu^{1,7}$]-eel-calcitonin: contribution of the disfulfide bond to the hormonal activity. Experientia (Basel) **32:** 1104–1106, 1976.
115. Musso, G. F., Assoian, R. K., Kaiser, E. T., Kezdy, F. J., and Tager, H. S.: Heterogeneity of glucagon receptors of rat hepatocytes: a synthetic peptide probe for the high affinity site. Biochem. Biophys. Res. Commun. **119:** 713–719, 1984.
116. Musso, G. F., and Kaiser, E. T.: Unpublished observations.
117. Musso, G. F., Kaiser, E. T., Kezdy, F. J., and Tager, H. S.: Glucagon receptor recognition: studies with model α-helical peptides. *In* Proceedings of the Eighth American Peptide Symposium, ed. by V. J. Hruby and D. H. Rich, pp. 365–368, Pierce Chemical Co., Rockford, Illinois, 1983.
118. Mutter, M.: The construction of new proteins and enzymes—a prospect for the future? Angew. Chem. Int. Ed. Engl. **24:** 639–653, 1985.
118a. Nakagawa, S. H., Lau, H. S. H., Kezdy, F. J., and Kaiser, E. T.: The use of polymer bound oximes for the synthesis of large peptides usable in segment condensation: synthesis of a 44 amino acid amphiphilic peptide model of apolipoprotein A-I. J. Am. Chem. Soc. **107:** 7087–7092, 1985.
119. Nakamuta, H., Furukawa, S., Koida, M., Yajima, H., Orlowski, R. C., and Schlueter, R.: Specific binding of ^{125}I-salmon calcitonin to rat brain: regional variation and calcitonin specificity. Jpn. J. Pharmacol. **31:** 53–60, 1981.
120. Nicolas, P., Bewley, T. A., Graf, L., and Li, C. H.: β-Endorphin: demonstration of a tertiary structure in aqueous solution. Proc. Natl. Acad. Sci. USA **78:** 7290–7293, 1981.
121. Ohhashi, T., and Jacobowitz, D. M.: The effects of pancreatic polypeptides and neuropeptide Y on the rat vas deferens. Peptides (Fayetteville) **4:** 381–386, 1983.
122. Ohhashi, T., and Jacobowitz, D. M.: Effect of calcitonin gene-related peptide on the neuroeffector mechanisms of sympathetic nerve terminals in rat vas deferens. Peptides (Fayetteville) **6:** 987–991, 1985.
123. Osterman, D. G., and Kaiser, E. T.: Design and characterization of peptides with amphiphilic β-strand structures. J. Cell. Biochem. **29:** 57–72, 1985.
124. Osterman, D., Mora, R., Kezdy, F. J., Kaiser, E. T., and Meredith, S. C.: A synthetic amphiphilic β-strand tridecapeptide: a model for apolipoprotein B. J. Am. Chem. Soc. **106:** 6845–6847, 1984.
124a. Paterson, S. J., Robson, L. E., and Kosterlitz, H. W.: Classification of opioid receptors. Br. Med. Bull. **39:** 31–36, 1983.
125. Patzelt, C., Tager, H. S., Caroll, R. J., and Steiner, D. F.: Identification and processing of proglucagon in pancreatic islets. Nature (Lond.) **282:** 260–266, 1979.
126. Rajashekhar, B., and Kaiser, E. T.: Design of biologically active peptides with non-peptidic structural elements: biological and physical properties of a synthetic analogue of β-endorphin with unnatural amino acids in the region 6–12. Submitted for publication.
127. Rall, S. C., Jr., Weisgraber, K. H., and Mahley, R. W.: Human apolipoprotein E. The complete amino acid sequence. J. Biol. Chem. **257:** 4171–4178, 1982.
128. Richardson, J.: The anatomy and taxonomy of protein structure. Adv. Protein Chem. **35:** 167–339, 1982.
129. Riniker, B., Neher, R., Maier, R., Kahat, F. W., Byfield, P. G. H., Gudmundsson, T. V., Galante, L., and MacIntyre, L.: Menschliches calcitonin I. Isolierung und charakterisierung. Helv. Chim. Acta **51:** 1738–1742, 1968.
130. Rizzo, A. J., and Gottzman, D.: Calcitonin receptors in the central nervous system of the rat. Endocrinology **108:** 1672–1677, 1981.
131. Rodbell, M.: The role of hormone receptors and GTP-regulatory proteins in membrane transduction. Nature (Lond.) **284:** 17–22, 1980.
132. Rosenfeld, M. G., Mermond, J., Amara, S. G., Swanson, L. W., Sawchenko, P. E., Rivier, J., Vale, W. W., and Evans, R. M.: Production of a novel neuropeptide encoded by the calcitonin gene via tissue-specific RNA processing. Nature (Lond.) **304:** 129–135, 1983.
133. Sanchez-Blazquez, P., Garzon, J., Lee, N. M., and Hollt, V.: Opiate activity of peptides derived from the three opioid peptide families on the rat vas deferens. Neuropeptides **5:** 181–184, 1984.
134. Sasaki, K., Dockerill, S., Adamiak, D. A., Tickle, I. J., and Blundell, T.: X-ray analysis of glucagon and its relationship to receptor binding. Nature (Lond.) **257:** 751–757, 1975.
135. Schiffer, M., and Edmundson, A. B.: Use of helical wheels to represent the structures of proteins and to identify segments with helical potential. Biophys. J. **7:** 121–135, 1967.
135a. Schiller, P. W., and DiMaio, J.: Aspects of conformational restriction in biologically active peptides. *In* Peptides: Structure and Function, Proceedings of the Eighth American Peptide Symposium, ed. by V. J. Hruby and D. H. Rich, pp. 269–278, Pierce Chemical Co., Rockford, IL, 1983.
136. Schnieder, A. B., and Edelhoch, H.: Polypeptide hormone interaction. II. Glucagon binding to lysolecithin. J. Biol. Chem. **247:** 4986–4995, 1972.
137. Schulz, R., Fasse, E., Wuster, M., and Herz, A.: Selective receptors for β-endorphin on the rat vas deferens. Life Sci. **24:** 843–850, 1979.
138. Schulz, R., Wuster, M., and Herz, A.: Pharmacological characterization of the ε-opiate receptor. J. Pharmacol. Exp. Ther. **216:** 604–606, 1981.
139. Schwartz, J.-C.: Metabolism of enkephalins and the inactivating neuropeptidase concept. Trends Neurosci. **6:** 45–48, 1983.
140. Schwartz, K. E., Orlowski, R. C., and Marcus R.: des-Ser salmon calcitonin: a biologically potent synthetic analog. Endocrinology **108:** 831–835, 1981.
141. Segrest, J. P., Jackson, R. L., Morrisett, J. D., and Gotto, A. M., Jr.: A molecular theory of lipid-protein interactions in the plasma lipoproteins. FEBS Lett. **38:** 247–253, 1974.
142. Sieber, P., Brugger, M., Kamber, B., Riniker, B., Rittel, W., Maier, R., and Staehelin, M.: Synthesis and biological activity of peptide sequences related to porcine a-thyrocalcitonin. *In* Calcitonin 1969: Proceedings of the Second International Symposium, ed. by S. F. Taylor and G. Foster, pp. 28–38, Excerpta Medica, Princeton, NJ, 1970.
142a. Smith, C. F. C., and Rance, M. J.: Opiate receptors in the rat vas deferens. Life Sci. **33** (Suppl. 1): 327–330, 1983.
143. Spiess, J., Rivier, J., Thorner, M., and Vale, W.: Sequence analysis of a growth hormone releasing factor from a human pancreatic islet tumor. Biochemistry **21:** 6037–6040, 1982.
143a. Tatemoto, K.: Isolation and characterization of peptide YY (PYY), a candidate gut hormone that inhibits pancreatic exocrine secretion. Proc. Natl. Acad. Sci. USA **79:** 2514–2518, 1982.

143b. TATEMOTO, K., AND MUTT, V.: Isolation and characterization of the intestinal peptide porcine PHI (PHI-27), a new member of the glucagon-secretin family. Proc. Natl. Acad. Sci. USA **78:** 6603–6607, 1981.
144. TAYLOR, J. W., AND KAISER, E. T.: Unpublished observations.
145. TAYLOR, J. W, MILLER, R. J., AND KAISER, E. T.: Structural characterization of β-endorphin through the design, synthesis, and study of model peptides. Mol. Pharmacol. **22:** 657–666, 1982.
146. TAYLOR, J. W., MILLER, R. J., AND KAISER, E. T.: Characterization of an amphiphilic helical structure in β-endorphin through the design, synthesis, and study of model peptides. J. Biol. Chem. **258:** 4464–4471, 1983.
147. TAYLOR, J. W., OSTERMAN, D. G., MILLER, R. J., AND KAISER, E. T.: Design and synthesis of a model peptide with β-endorphin-like properties. J. Am. Chem. Soc. **103:** 6965–6966, 1981.
148. TEPPERMAN, J.: Calcium metabolism. *In* Metabolic and Endocrine Physiology, ed. 4, chap. 8, pp. 297–315, Year Book Medical Publishers, Inc., Chicago, 1980.
149. TSENG, L.-F., OSTWALD, T. J., LOH, H. H., AND LI, C. H.: Behavioral activities of opioid peptides and morphine sulfate in golden hamsters and rats. Psychopharmacology **64:** 215–218, 1979.
150. VELICELEBI, G., PATTHI, S., AND KAISER, E. T.: Design and biological activity of GHRF analogs with potential amphiphilic helical C-termini. Proc. Natl. Acad. Sci. USA **83:** 5397–5399, 1986.
151. WEI, C.-F., CHEN, S.-H., YANG, C.-Y., MARCEL, Y. L., MILNE, R. W., LI, W.-H., SPARROW, J. T., GOTTO, A. M., JR., AND CHAN, L.: Molecular cloning and expression of partial cDNAs and deduced amino acid sequence of a carboxyl-terminal fragment of human apolipoprotein B-100. Proc. Natl. Acad. Sci. USA **82:** 7265–7269, 1985.
152. WRIGHT, D. E., HRUBY, V. J., AND RODBELL, M.: A reassessment of structure function relationships in glucagon. J. Biol. Chem. **253:** 6338–6340, 1978.
153. WRIGHT, D. E., AND RODBELL, M.: $Glucagon_{1-6}$ binds to the glucagon receptor and activates hepatic adenylate cyclase. J. Biol. Chem. **254:** 268–269, 1979.
154. WU, C. S., LEE, N. M., LING, N., CHANG, J. K., LOH, H. H., AND YANG, J. T.: Conformation of β-endorphin analogues in cerebroside sulfate solution. Mol. Pharmacol. **189:** 302–306, 1981.
155. WU, C.-S. C., LEE, N. M., LOH, H. H., YOUNG, J. T., AND LI, C. H.: β-endorphin: formation of α-helix in lipid solutions. Proc. Natl. Acad. Sci. USA **76:** 3656–3659, 1979.
156. WU, C. S. C., AND YANG, J. T.: Helical conformation of glucagon in surfactant solutions. Biochemistry **19:** 2117–2122, 1980.
157. WUSTER, M., SCHULZ, R., AND HERZ, A.: Highly specific opiate receptors for dynorphin-(1–13) in the mouse vas deferens. Eur. J. Pharmacol. **62:** 235–236, 1980.
158. YAMASHIRO, D., FERRARA, P., AND LI, C. H.: β-endorphin: synthesis of analogs with extension at the carboxyl terminus with high radioreceptor binding activity. Int. J. Pept. Protein Res. **16:** 70–74, 1980.
159. YAMASHIRO, D., GARZIA, R., HAMMONDS, R. G., JR., AND LI, C. H.: Synthesis and properties of human β-endorphin-(1–9) and its analogs. Int. J. Pept. Protein Res. **19:** 284–289, 1982.
160. YAMASHIRO, D., LI, C. H., NICOLAS, P., AND HAMMONDS, R. G., JR.: β-endorphin. Synthesis and properties of double-headed and related analogs. Int. J. Pept. Protein Res. **19:** 348–353, 1982.
161. YAMASHIRO, D., LI, C. H., TSENG, L.-F., AND LOH, H. H.: β-endorphin: synthesis and analgesic activity of several analogs modified in positions 2 and 5. Int. J. Pept. Protein Res. **11:** 251–257, 1978.
162. YAMASHIRO, D., NICOLAS, P., AND LI, C. H.: β-endorphin: synthesis and properties of analogs modified in positions 8 and 31. Int. J. Pept. Protein Res. **20:** 43–46, 1982.
163. YAMASHIRO, D., TSENG, L.-F., DONEEN, B. A., LOH, H. H., AND LI, C. H.: β-endorphin: synthesis and morphine-like activity of analogs with D-amino acid residues in positions 1, 2, 4, and 5. Int. J. Pept. Protein Res. **10:** 159–166, 1977.
164. YANG, J. T., BEWLEY, T. A., CHEN, G. C., AND LI, C. H.: Conformation of β-endorphin and β-lipotropin: formation of helical structure in methanol and sodium dodecyl sulfate solutions. Proc. Natl. Acad. Sci. USA **74:** 3235–3238, 1977.
165. YEUNG, H.-W., YAMASHIRO, D., TSENG, L.-F., CHANG, W., AND LI, C. H.: Human β-endorphin: synthesis and biological activity of analogs with substitutions in positions 2, 9, 18, 27, and 31. Int. J. Pept. Protein Res. **17:** 235–238, 1981.
166. YOKOYAMA, S., FUKUSHIMA, D., KUPFERBEG, J. P., KEZDY, F. J., AND KAISER, E. T.: The mechanism of activation of lecithin: cholesterol acyltransferase by apolipoprotein A-I and an amphiphilic peptide. J. Biol. Chem. **255:** 7333–7337, 1980.
167. ZAKARIAN, S., AND SMYTH, D. G.: Distribution of active and inactive forms of endorphins in rat pituitary and brain. Proc. Natl. Acad. Sci. USA **76:** 5972–5976, 1979.
168. ZEORAL, M., YAMASHIRO, D., HAMMONDS, R. G., JR., AND LI, C. H.: β-endorphin: synthesis and radioreceptor binding activity of β_h-endorphin-(1–27) and its analogs. Int. J. Pept. Protein Res. **17:** 292–296, 1981.
169. ZUKIN, R. S., AND ZUKIN, S. R.: The case for multiple opiate receptors. Trends Neurosci. **7:** 160–164, 1984.