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MACOLOGICAL REVIEWS

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 **The Structural Characterization of β-Endorphin and

Related Peptide Hormones and Neurotransmitters*[,] † EG97/86/3804-0291\$02.00/0**

MACOLOGICAL REVIEWS

The Structural Characterization of β -Endorphin and

Related Peptide Hormones and Neurotransmitters*^{*} †

JOHN W. TAYLOR and EMIL THOMAS KAISER Related Peptide Hormones and Neurotransmitters*' †
JOHN W. TAYLOR and EMIL THOMAS KAISER

LABOR AND INVITES AND IN THE REAL OF AND MULTER S
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 I. Introduction
A LARGE NUMBER of peptides and proteins having
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hormone have been identified and their amino acid se Hormone Haven Communism Controller and the LARGE NUMBER of peptides and proteins having
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hormone have been identifie A LARGE NUMBER of peptides and proteins having
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of neurotransmitter or neuromodulator to circulatory
hormone have been identified and their amino acid se-
quences elucid important pharmacological functions ranging from that
of neurotransmitter or neuromodulator to circulatory
hormone have been identified and their amino acid se-
quences elucidated. On considering the structures of
these p of neurotransmitter or neuromodulator to circulatory
hormone have been identified and their amino acid se-
quences elucidated. On considering the structures of
these polypeptides in their biologically active forms, it
appe hormone have been identified and their amino acid sequences elucidated. On considering the structures of weakness of these polypeptides in their biologically active forms, it appears that there are three general categorie quences elucidated. On considering the structures
these polypeptides in their biologically active forms
appears that there are three general categories. The f
group consists of short peptides, such as the five am
acid resi these polypeptides in their biologically active forms,
appears that there are three general categories. The fi
group consists of short peptides, such as the five ami
acid residue opioids [Met⁵]-enkephalin and [Leu⁵]-e
 appears that there are three general categories. The first group consists of short peptides, such as the five amino acid residue opioids [Met⁵]-enkephalin and [Leu⁵]-enkephalin, where essentially the whole structure co group consists of short peptides, such as the five amino
acid residue opioids [Met⁵]-enkephalin and [Leu⁵]-en-
kephalin, where essentially the whole structure consti-
tutes the specific recognition site that determine acid residue opioids [Met⁵]-enkephalin and [Leu⁵]-enkephalin, where essentially the whole structure constitutes the specific recognition site that determines their interactions with cell surface receptors and possibly kephalin, where essentially the whole structure contutes the specific recognition site that determines thinteractions with cell surface receptors and possisted by their environ-
other molecules. The active conformations of tutes the specific recognition site that determines their
interactions with cell surface receptors and possibly
other molecules. The active conformations of these pep-
tides will be determined almost entirely by their envi interactions with cell surface receptors and possibly
other molecules. The active conformations of these pep-
tides will be determined almost entirely by their environ-
ment, and these interactions may readily be probed
th other molecules. The active conformations of these j
tides will be determined almost entirely by their envir
ment, and these interactions may readily be pro
through the systematic investigation of a large num
of synthetic tides will be determined almost entirely by their environ-
ment, and these interactions may readily be probed
through the systematic investigation of a large number
of synthetic analogues, often incorporating conforma-
tio posterial studies of synthetic analogues, often incorporating conformational restrictions (70a, 135a), in the manner of classical potential potential studies of small organic molecules. An-
other category of polypeptide ho

complex structures that are large enough to be stabilized 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, formation of a hydrohobic core. Compounds of this type, such as insulin or growth hormone, may have interactional as insulin or growth hormone, may have interactional complex structures that are large enough to be stabili
in aqueous solution by multiple disulfide bonds or
formation of a hydrohobic core. Compounds of this ty
such as insulin or growth hormone, may have inter
tions with bi 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 interac-
 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 interac-
tions with binding sites that involve amino acid residues formation of a hydrohobic core. Compounds of this type,
such as insulin or growth hormone, may have interac-
tions with binding sites that involve amino acid residues
well separated in the peptide chain, but held together 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 tions 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 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 t 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 cr 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, poss 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
d 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 characterization of important residues and the other category of polypeptide hormones consists of more

* This article is the fifth in a series of reviews on various aspects of

* This article is the fifth in a series The third category of peptide hormones consists of polypeptides that have structural properties of an intermediate nature. These peptides often consist of a single polypeptides that have structural properties of an interdues or site-directed mutagenesis of the corresponding
gene.
The third category of peptide hormones consists of
polypeptides that have structural properties of an inter-
mediate nature. These peptides often consist of a si gene.
The third category of peptide hormones consists of
polypeptides that have structural properties of an inter-
mediate nature. These peptides often consist of a single
peptide chain of about 10 to 50 amino acid residue The third category of peptide hormones consists of
polypeptides that have structural properties of an inter-
mediate nature. These peptides often consist of a single
peptide chain of about 10 to 50 amino acid residues and
 polypeptides that have structural properties of an inter-
mediate 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 mo mediate 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 an 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 peptid 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 pre-
sents special problems. Although they are usuall 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 so 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 sy sents special problems. Although they are usually syn-
thetically accessible through the standard methods of
solid-phase peptide synthesis, their length precludes a
systematic investigation of the importance of each amino
 thetically 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. Furthermor solid-phase peptide synthesis, their length precludes a
systematic investigation of the importance of each amino
acid residue through the study of analogues. Further-
more, the effects of such structural modifications on
a systematic investigation of the importance of each amino
acid residue through the study of analogues. Further-
more, the effects of such structural modifications on
activities may be difficult to interpret. Elements of sec

other category of polypeptide hormones consists of more

* 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, * 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. opioid pharmacology which has been arranged
W. Kosterlitz, Aberdeen, and Eric J. Simon,
Consulting Editors. Earlier articles of this series
35: 33-53, 69-83, 219-281, and 283-323, 1983.
† Partial support of our research wa Existential support of our research was been supported with the field of Simon, New York, acting as is 33-53, 69-83, 219-281, and 283-323, 1983.

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Foundation (E. T. K.), by a grant from the Dow Chemical Company
Foundation (E. T. K.), and by Foundation Foundation Foundation Foundation Foundation Foundation (E. T. K.), by a grant from Foundation (E. T. K.), and by a fellow Rosita Winston Foundation (J. W. T.).

TAYLOR AND KAISER
aqueous solution are likely to be stabilized by the inter-
actions of peptides in this category with their functional monolaye TAYLOR 4
aqueous solution are likely to be stabilized by the inter-
actions of peptides in this category with their functional
environment. Single amino acid substitutions will then rayLOR AND I
aqueous solution are likely to be stabilized by the inter-
actions of peptides in this category with their functional
environment. Single amino acid substitutions will then
have both direct effects on these in have both direct effects on the stabilized by the inter-
actions of peptides in this category with their functional
environment. Single amino acid substitutions will then
have both direct effects on these interactions and aqueous solution are likely to be stabilized by the inter-
actions of peptides in this category with their functional
environment. Single amino acid substitutions will then
have both direct effects on these interactions an actions of peptides in this category with their function
environment. Single amino acid substitutions will the
have both direct effects on these interactions and indirect
effects arising from their role in the formation of 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 infor-
mation regarding, for example, the receptor-bound 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 infor-
mation regarding, for example, the receptor-bound effects arising from their role in the formation of such
structures, and they are unlikely to provide any infor-
mation regarding, for example, the receptor-bound con-
formation of the natural peptide. This review describe 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 func mation 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 de-
sig **II. Amphiphilic Secondary Structure**
II. Amphiphilic Secondary Structure
II. Amphiphilic Secondary Structure
II. Amphiphilic Secondary Structure
I. been suggested that the functional environ

It has been suggested that the functional environment and study of appropriate synthetic peptide models.

II. Amphiphilic Secondary Structure

It has been suggested that the functional environment

any peptide acting at bi sign and study of appropriate synthetic peptide models. not

II. Amphiphilic Secondary Structure

It has been suggested that the functional environment len

of any peptide acting at biological interfaces such as a ligc

pr 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, II. Amphiphilic Secondary Structure
It has been suggested that the functional environment
of any peptide acting at biological interfaces such as a
liprotein or cell surface will often be amphiphilic $(76, 77)$.
In other w 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 usu of any peptide acting at biological interfaces such as a liprotein or cell surface will often be amphiphilic (76, 77). In other words, the expression of activity will usually virvolve binding at the interface between the h protein or cell surface will often be amphiphilic (76, 77). and the words, the expression of activity will usually virtually virtually involve binding at the interface between the hydrophobic corre of a structure and its In other words, the expression of activity will usually viro
involve binding at the interface between the hydrophobic coil
core of a structure and its aqueous surroundings. This resi-
type of anisotropic environment is li 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 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 hydr 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 hyd formation of discrete segments of secondary structure in
peptide hormones of the third category discussed above,
if these structures result in the segregation of hydropho-
bic and hydrophilic amino acid residues in the pep amphiphilicity. these structures result in the segregation of hydropho-
c and hydrophilic amino acid residues in the peptide
ain into separate domains creating a complementary
phiphilicity.
The formation of amphiphilic secondary structure

bic and hydrophilic amino acid residues in the peptide
chain into separate domains creating a complementary
amphiphilicity.
The formation of amphiphilic secondary structures stru
and the properties they exhibit have been 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 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 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 hy-
drophil 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). number of model peptide systems. An amphiphilic β amphistrand will result from alternating hydrophobic and hyperiodrophilic amino acid residues in the linear sequence (17). acid Model peptides with this type of sequenc strand will result from alternating hydrophobic and hy-
drophilic amino acid residues in the linear sequence (17).
Model peptides with this type of sequence that consist to
of more than about six residues have circular di drophilic 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 conte 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 pept of more than about six residues have circular dichroism resylcom (CD) spectra indicative of a high β strand content which and results from the pronounced tendency of these peptides free to self-associate forming amphip (CD) spectra indicative of a high β strand content which and results from the pronounced tendency of these peptides free to self-associate forming amphiphilic β sheets (17, 34, fer 118, 123, 124). In aqueous solutio results from the pronounced tendency of these peptides from the self-associate forming amphiphilic β sheets (17, 34, fell 118, 123, 124). In aqueous solution, these β sheets can glaself-associate to bury their hydro to self-associate forming amphiphilic β sheets (17, 118, 123, 124). In aqueous solution, these β sheets cself-associate to bury their hydropholic faces, or the will bind very tightly at amphiphilic interfaces such t self-associate to bury their hydrophobic faces, or they an initial search for potential regions of amphiphilic
will bind very tightly at amphiphilic interfaces such as secondary structure in peptide hormones can be made
t 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 lipopro-
teins or the air-water interface, where extremely sta will bind very tightly at amphiphilic interfaces such a
the surfaces of phospholipid vesicles or serum lipopro
teins or the air-water interface, where extremely stable
monolayers are formed. Longer sequences of alternating the surfaces of phospholipid vesicles or serum lip
teins or the air-water interface, where extremely s
monolayers are formed. Longer sequences of altern
hydrophobic and hydrophilic residues are usually
cult to solubilize Model peptides that can form amphiphilic a-helical

monolayers are formed. Longer sequences of alternating

hydrophobic and hydrophilic residues are usually diffi-

cult to solubilize in aqueous solutions (118, 123).

Mod

hydrophobic and hydrophilic residues are usually difficult to solubilize in aqueous solutions (118, 123). dia
Model peptides that can form amphiphilic α -helical and
structures have also been studied. In this case, the Model peptides that can form amphiphilic α -helical astructures have also been studied. In this case, the distribution of the hydrophobic and hydrophilic amino acid the residues in the linear sequence of a peptide that 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 be
rise to such a structure will depend on the size a tribution 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 residues in the linear sequence of a peptide that gives been
rise to such a structure will depend on the size and shape clu
of the hydrophobic domain formed. Peptides of this type rele
that are about 20 residues long and rise to such a structure will depend on the size and shape clud
of the hydrophobic domain formed. Peptides of this type rele
that are about 20 residues long and can form α helices (GF
with a hydrophobic domain lying pa of the hydrophobic domain formed. Peptides of this type relevant are about 20 residues long and can form α helices (G) with a hydrophobic domain lying parallel to the helix pol axis along one side of the helix also sel 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 with a hydrophobic domain lying parallel to the helix polaxis along one side of the helix also self-associate in ogcaprecies solution (33, 52). Discrete aggregated forms such side as tetramers are observed, and they have 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

tides will bind to phospholipid surfaces and form stable
monolayers at the air-water interface, and they behave D KAISER
tides 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 D KAISER
tides 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 t shows increase the situations. Increases the situations at the sin-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 hel tides 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 surfac 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-t 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 o 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 posi 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 o 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 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 hydropholic domain of
the helical structure prevents self-association, but 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 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 the helical structure prevents self-association, but do
not markedly affect binding to phospholipid vesicles
monolayer stability (53). There has been no systema
study of the dependence of these interactions on t
length of not markedly affect binding to phospholipid vesicle
monolayer stability (53). There has been no syster
study of the dependence of these interactions or
length of the peptide chain. However, studies of ho
ligopeptides in th 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 homoo-
ligopeptides in the helix-promoting solvent trifluoroeth-
 study of the dependence of these interactions on the length of the peptide chain. However, studies of homoo-
ligopeptides in the helix-promoting solvent trifluoroeth-
anol suggest that, with suitable stabilization by the ligopeptides in the helix-promoting solvent trifluoroeth-
anol suggest that, with suitable stabilization by the en-
vironment, the transition from predominantly random
coil to α -helix will occur in peptides that are 10 anol 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 t vironment, 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 amphip $\begin{array}{c} \text{coil to }\alpha\ \text{residues}\ \text{l} \ \text{informati}\ \alpha\ \text{helices}\ \text{83, 86).} \ \text{With}\ \text{th} \end{array}$ sidues long (118). This length is consistent with the
formation available for the stabilization of amphiphilic
helices at hydrophobic-hydrophilic interfaces (34, 52,
, 86).
With the probable exception of proline residues,

cult to solubilize in aqueous solutions (118, 123). diagrams to identify segments where the hydrophobic Model peptides that can form amphiphilic α -helical and hydrophilic residues are segregated in separate dostructure length of the peptide chain. However, studies of homoo-
ligopeptides in the helix-promoting solvent trifluoroeth-
anol suggest that, with suitable stabilization by the en-
vironment, the transition from predominantly rand information available for the stabilization of amphiphil
 α helices at hydrophobic-hydrophilic interfaces (34, 5

83, 86).

With the probable exception of proline residues, whe

the amino groups cannot participate in h α helices at hydrophobic-hydrophilic interfaces (34, $\{83, 86\}$).
With the probable exception of proline residues, where animo groups cannot participate in hydrogen boning, the actual identity of the hydrophobic and 83, 86).
With the probable exception of proline residues, where
the amino groups cannot participate in hydrogen bond-
ing, the actual identity of the hydrophobic and hydro-
philic residues that constitute an amphiphilic se With the probable exception of proline residues, wh
the amino groups cannot participate in hydrogen bo
ing, the actual identity of the hydrophobic and hyd
philic residues that constitute an amphiphilic second
structure app 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 o ing, the actual identity of the hydrophobic and hydrophilic residues that constitute an amphiphilic secondary
structure appears to be a much less important determi-
nant of the type of secondary structure formed in an
amp philic residues that constitute an amphiphilic secondary
structure appears to be a much less important determi-
nant of the type of secondary structure formed in an
amphiphilic environment, i.e., helix or β sheet, than 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 nant 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 differe 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. 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 Cho 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 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 di respect, the conformation parameters described by Cho
and Fasman and others (29, 54, 100), which describe th
frequencies with which each residue type occurs in di
ferent secondary structures in the crystal structures of
gl 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, 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 ferent 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 an initial search for potential regions of amphiphilic 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 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 se (*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 (13 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 an dues 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 domai sequences on helical net (37) or helical wheel (135)
diagrams to identify segments where the hydrophobic
and hydrophilic residues are segregated in separate do-
mains on the helix surface that might form α helices. diagrams to identify segments where the hydrophobic
and hydrophilic residues are segregated in separate do-
mains on the helix surface that might form α helices. In
this way, potential amphiphilic helical structures ha 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, mains on the helix surface that might form α helices. In
this way, potential amphiphilic helical structures have
been identified in many different peptide hormones, in-
cluding β -endorphin, calcitonin, glucagon, cor this way, potential amphiphilic helical structures have
been identified in many different peptide hormones, in-
cluding β -endorphin, calcitonin, glucagon, corticotropin
releasing factor (CRF), growth hormone-releasing 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
po cluding 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 structures differ co releasing factor (CRF), growth hormone-releasing fa
(GRF), parathyroid hormone (PTH), and pancr-
polypeptide; as well as a number of structurally ho
ogous peptides (*vide infra*). These structures differ
siderably from one (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, 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 do ogous 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 adopt

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STRUCTURAL CHARACTERIZATION
hydrophilic side of the helix, the number of aromatic
residues on the hydrophobic side, the overall length of STRUCTURAL CHARACTERIZATIO
hydrophilic side of the helix, the number of aromatic b
residues on the hydrophobic side, the overall length of co
the helix, and the number of apparent "mistakes" in its STRUCTURAL CHARACTERIZATION
the helix, the number of aromatic bresidues on the hydrophobic side, the overall length of co
the helix, and the number of apparent "mistakes" in its of
amphiphilicity, i.e., residues that have hydrophilic side of the helix, the number of aromatic bresidues on the hydrophobic side, the overall length of cothe helix, and the number of apparent "mistakes" in its of amphiphilicity, i.e., residues that have been defi hydrophilic side of the helix, the number of aromatic bracesidues on the hydrophobic side, the overall length of courthe helix, and the number of apparent "mistakes" in its obsemphiphilicity, i.e., residues that have been the helix, and the number of apparent "mistakes" in its observed.

amphiphilicity, i.e., residues that have been defined as Since the hydrophobic-hydrophilic interface of a phos-

hydrophilic which occur in the hydrophobi 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 hormone amphiphilicity, i.e., residues that have been defined as
hydrophilic which occur in the hydrophobic domain and
pice versa. In contrast to the common occurrence of
amphiphilic α helices, relatively few peptide hormones
 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 stra *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 leute amphiphilic α helices, relatively few peptide hormones path that contain regions of potential amphiphilic β strand for structure have been identified. These include leuteinizing the hormone releasing hormone (LHRH) that contain regions of potential amphiphilic β strand for structure have been identified. These include leuteinizing the hormone releasing hormone (LHRH) and dynorphin to $A(1-17)$, and they involve sequences of 10 or structure have been identified. These include leuteini
hormone releasing hormone (LHRH) and dynor
A(1–17), and they involve sequences of 10 or fewer an
acid residues, as expected from the intractable nature
model peptides Framone releasing hormone (LHRH) and dynorphin te
 $1-17$), and they involve sequences of 10 or fewer amino

id residues, as expected from the intractable nature of

todel peptides with longer structures of this type.

Th

 $A(1-17)$, 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 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 contribu moder peptides with longer structures of this type.

The great variety in the general characteristics of these

to the functional diversity of the peptide hormones.

However, their common occurrence also implies that

they amphiphilic structures suggests that they can contribute hot the functional diversity of the peptide hormones.
However, their common occurrence also implies that they will determine certain general aspects of peptide phorm 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 am However, their common occurrence also implies that to
they will determine certain general aspects of peptide pair
hormone action that may be related to the properties of general
the amphiphilic model peptides described ab 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 bi 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, eithe the amphiphilic model peptides described above. For a example, an amphiphilic α helix or β strand might bind 3to a complementary site on a protein receptor, either Trausing signal transmission directly or else posit 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 orienta 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 sig causing signal transmission directly or else positioning reg
other parts of the hormone in the correct orientation for
their interactions with the receptor to result in signal like
transmission. This type of protein-protei 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 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 resi transmission. This type of protein-protein interaction the resembles the self-association of the model peptides and in is expected to be sensitive to the nature of the residues of on the hydrophobic face of the amphiphilic 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
p 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 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
consis The complex formed by a peptide ligand bound to its
protein receptor should, when considered as a whole, fer-
have similar structural properties to globular proteins (2)
consisting of a continuous peptide chain, and amphi protein receptor should, when consider
have similar structural properties to
consisting of a continuous peptide c
philic α helices and β sheets are comm
surface of these structures (39, 128).
A second possibility is we similar structural properties to globular proteins (

msisting of a continuous peptide chain, and amphi-

iilic α helices and β sheets are commonly found on the

rface of these structures (39, 128).

A second pos

consisting of a continuous peptide chain, and amphi-
philic 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 philic 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. De surface of these structures (39, 128). phinding and the secondary of structures might interact with the phospholipid surfaces I of cells. Depending on the equilibrium dissociation contribution stant for the peptide bindin structures might interact with the phospholipid surfaces
of cells. Depending on the equilibrium dissociation con-
stant for the peptide binding, which might be 1 μ M or
even lower, and the rate at which the bound peptid of cells. Depending on the equilibrium dissociation con-
stant for the peptide binding, which might be $1 \mu M$ or
even lower, and the rate at which the bound peptide can
diffuse on the surface of the cell, this type of int stant for the peptide binding, which might be 1μ M 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 su 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
inceptors are located: the three-dimensional searc 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-ste 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
surfac 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, 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, ca
followed by diffusion in only two dimensions on that search involving adsorption to any point on the cell partace, which represents a relatively large target to find, collowed by diffusion in only two dimensions on that partace (1, 6a, 62, 75, 80). Ultimately, binding to the 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 pept followed by diffusion in only two dimensions on the surface (1, 6a, 62, 75, 80). Ultimately, binding to the receptor might then involve another part of the peptic molecule, with the amphiphilic secondary structure remainin receptor might then involve another part of the peptide also be stabilized by interactions with other soluble com-
molecule, with the amphiphilic secondary structure re-
maining at the phospholipid-water interface. The par molecule, with the amphiphilic secondary structure re-
maining at the phospholipid-water interface. The parti-
tioning of a peptide hormone between the aqueous phase
and cell surfaces by phospholipid binding could, alter-
 molecule, with the amphiphilic secondary structure re-
maining at the phospholipid-water interface. The parti-
tioning of a peptide hormone between the aqueous phase
and cell surfaces by phospholipid binding could, alter-
 maining at the phospholipid-water interface. I he parti-
tioning of a peptide hormone between the aqueous phase
and cell surfaces by phospholipid binding could, alter-
natively, serve to limit its distance or rate of diffu peptide from and cell surfaces by phospholipid binding could, alteratively, serve to limit its distance or rate of diffusion that
from the point of release, and may also either protect the act
peptide from attack by proteo and cell surfaces by phospholipid binding could, alternatively, serve to limit its distance or rate of diffusio
from the point of release, and may also either protect the
peptide from attack by proteolytic enzymes or lead

STRUCTURAL CHARACTERIZATION OF PEPTIDE HORMONES 293
hydrophilic side of the helix, the number of aromatic brane-associated proteases. All of these possibilities on or PEPTIDE HORMONES
brane-associated proteases. All of these possibilities
could have a dramatic effect on the pharmacokinetics ON OF PEPTIDE HORMONES
brane-associated proteases. All of these possibilitie
could have a dramatic effect on the pharmacokinetic
observed. observed.

Since the hydrophobic-hydrophilic interface of a phosbrane-associated proteases. All of these possibilities
could have a dramatic effect on the pharmacokinetics
observed.
Since the hydrophobic-hydrophilic interface of a phos-
pholipid membrane surface is approximately planar could have a dramatic effect on the pharmacokinetics
observed.
Since the hydrophobic-hydrophilic interface of a phos-
pholipid membrane surface is approximately planar, an
amphiphilic helix having a hydrophobic domain lyin for binding to this surface (141), and indeed α helices of Since the hydrophobic-hydrophilic interface of a phos-
pholipid 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 pholipid membrane surface is approximately planai
amphiphilic helix having a hydrophobic domain l
parallel to the helix axis would be expected to be i
for binding to this surface (141), and indeed α helice
this type ar 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 apolipo 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 apolipopro-
teins which coat the phospholipid surfaces of 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 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 cel teins 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 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 acc 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 helical conformation to form another sterically accessible 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 exist 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 oxy-
gens and amide nitrogens of the nth 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, a pattern of hydrogen bonding exists linking carbonyl oxy-
gens 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 gens 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 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 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 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 recep 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 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 distort 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 e 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, prote 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 the α helix, is the 3₁₀ helix, and extended structures
of this type are rare (128). However, protein-protein
interactions may involve hydrophobic domains of a dif-
ferent shape, possibly twisting around the helix 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 h (28a, 38a). Thus it may be that peptide hormones like β -(28a, 38a). Thus it may be that pept
endorphin and GRF, which can if
helices of the type suitable for bindi
philic interfaces (77), will adopt diff
formations in different environment
In addition to the amphiphilic β endorphin and GRF, which can form amphiphilic π
helices of the type suitable for binding to planar amphi-
philic interfaces (77), will adopt differential helical con-
formations in different environments.
In addition t 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 oxy-
gens and amide nitrogens of the nth

philic interfaces (77), will adopt differential helical cor
formations in different environments.
In addition to the amphiphilic β -sheet forming per
tides mentioned above, evidence for a third type of am
phiphilic stru 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 (ACT In addition to the amphiphilic β -sheet forming t
tides mentioned above, evidence for a third type of a
phiphilic structure being involved in phospholipid bi
ing has been obtained for adrenocorticotrophic horm
(ACTH) re tides mentioned above, evidence for a third type of a
phiphilic structure being involved in phospholipid bin
ing has been obtained for adrenocorticotrophic hormo
(ACTH) residues 1-24 (62). In this case, the phosph
lipid-bo ing 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 -h (ACTH) residues 1–24 (62). In this case, the phospho-
lipid-bound peptide is proposed to consist of the hydro-
phobic amino-terminal segment of the peptide in an
 α -helical conformation buried perpendicularly to the
pho mpid-bound peptide is proposed to consist of the nydropholic amino-terminal segment of the peptide in a α -helical conformation buried perpendicularly to the phospholipid surface and connected to the hydrophil carboxy-t helical conformation buried perpendicularly to the
nospholipid surface and connected to the hydrophilic
rboxy-terminal segment lying in the plane of the phos-
olipid surface in an extended form.
Segments of amphiphilic sec carboxy-terminal segment lying in the plane of the phos-
pholipid surface in an extended form.
Segments of amphiphilic secondary structure might

phospholipid surface and connected to the hydrophili
carboxy-terminal segment lying in the plane of the phos
pholipid surface in an extended form.
Segments of amphiphilic secondary structure migh
also be stabilized by inte pholipid surface in an extended form.
Segments of amphiphilic secondary structure might
also be stabilized by interactions with other soluble com-
ponents of the biological milieu, including proteins, lip-
ids, and ions. T Segments of amphiphilic secondary structure might
also be stabilized by interactions with other soluble com-
ponents of the biological milieu, including proteins, lip-
ids, and ions. This in turn might induce the formation 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 ponents of the biological milieu, including proteins, lijids, 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 ids, 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 inter 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 inter-
actions are, any such increase in conformational rigi other parts of the molecule with the hydrophobic face of that secondary structure. However extensive the inter-
actions are, any such increase in conformational rigidity
is likely to result in a corresponding decrease in t

TAYLO
ble proteolytic enzymes (78). Alternatively, the inter
tions with other components in solution might inclu TAYLOR AND
ble proteolytic enzymes (78). Alternatively, the interac-
tions with other components in solution might include
the formation of tight complexes that could fulfill a TAYLOR AND K
ble proteolytic enzymes (78). Alternatively, the interac-
tions with other components in solution might include
the formation of tight complexes that could fulfill a
function in themselves, as is suspected of function in the protections with other components in solution might include
tions with other components in solution might include
the formation of tight complexes that could fulfill a
function in themselves, as is suspect ble proteolytic enzymes (78). Alternatively, the interac-
tions 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 tions 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 doma mphiphilic α -helical peptides to a hydrophobic domain
calmodulin (31).
III. The Study of Peptide Models
The properties and potential functions of amphiphilic
condary structures suggest that it might be possible to

III. The Study of Peptide Models
The properties and potential functions of amphiphilic in calmodulin (31). A-I
actional methods and potential functions of amphiphilic
The properties and potential functions of amphiphilic
secondary structures suggest that it might be possible to
replace these segments of pept III. The Study of Peptide Models
The properties and potential functions of amphiphilis
secondary structures suggest that it might be possible to
replace these segments of peptide hormones with non-
homologous amino acid se The properties and potential functions of amphiphilic
secondary structures suggest that it might be possible to
replace these segments of peptide hormones with non-
homologous amino acid sequences chosen to preserve
their The properties and potential functions of amphiphilic to secondary structures suggest that it might be possible to underplace these segments of peptide hormones with non-
homologous amino acid sequences chosen to preserve secondary structures suggest that it might be possible to
replace these segments of peptide hormones with non-
homologous amino acid sequences chosen to preserve I
their most important features, perhaps in an idealized
for replace these segments of peptide hormones with non
homologous amino acid sequences chosen to preserve their most important features, perhaps in an idealize
form, and still retain the activities of the natural peptic
(76, their most important features, perhaps in an idealize
form, and still retain the activities of the natural peptic
(76, 77). By comparing the physicochemical and pha
macological properties of peptide models of this type
tho form, and still retain the activities of the natural pepti(76, 77). By comparing the physicochemical and phimacological properties of peptide models of this type those of the natural hormone, structure-function retionships (76, 77). By comparing the physicochemical and phar-
macological properties of peptide models of this type to
those of the natural hormone, structure-function rela-
applionships may be developed that determine the impor-
 macological properties of peptide models of this type to
those of the natural hormone, structure-function rela-
tionships may be developed that determine the impor-
tance of each feature. To the extent that homology with
t those of the natural hormone, structure-function rela-
tionships may be developed that determine the impor-
tance of each feature. To the extent that homology with
the natural sequence of a particular structural element
ca tionships may be developed that determine the imp
tance of each feature. To the extent that homology w
the natural sequence of a particular structural eleme
can be minimized and the activities which depend or
can still be tance 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 conforthe 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 confor-
mation of the hormone will also be obtained by this
mod can be minimized and the activities which depend on it
can still be reproduced, evidence of the active confor-
mation of the hormone will also be obtained by this
modelling approach (78). Eventually, and without re-
course 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, i mation of the hormone will also be obtained by this remodelling approach (78). Eventually, and without recourse to the synthesis of a vast array of synthetic aid analogues, it should be possible to design peptide models ha modelling approach (78). Eventually, and without
course to the synthesis of a vast array of synthe
analogues, it should be possible to design peptide moc
that have enhanced specificities and potencies or m
desirable pharma course to the synthesis of a vast array of synthe
analogues, it should be possible to design peptide mot
that have enhanced specificities and potencies or m
desirable pharmacokinetic properties, possibly incor
rating nonna analogues, it should be possible to design peptide models
that have enhanced specificities and potencies or more
desirable pharmacokinetic properties, possibly incorpo-
rating nonnatural amino acid residues or even nonpepat have enhanced specificities and potencies or more
sirable pharmacokinetic properties, possibly incorpo-
tring nonnatural amino acid residues or even nonpep-
 β -ei
ic chains.
Synthetic peptide models have previously b

desirable pharmacokinetic properties, possibly incorpo-
rating nonnatural amino acid residues or even nonpep-
tidic chains.
Synthetic peptide models have previously been used to
scriptive structures of peptides and of the rating 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 phospholi tidic chains.
Synthetic peptide models have previously been used to
investigate the functional structures of peptides and
proteins which interact primarily with phospholipid sur-
faces, including the serum apolipoproteins Synthetic peptide models have previously been used to scri-
investigate the functional structures of peptides and of the
proteins which interact primarily with phospholipid sur-
faces, including the serum apolipoproteins (investigate the functional structures of peptides and of proteins which interact primarily with phospholipid surfaces, including the serum apolipoproteins (52, 53, 118a, da 124, 166) and the bee venom toxin melittin (33, 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 whi faces, 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 s 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 structura these cases, regions of the natural polypeptides which the had the potential to form amphiphilic α -helical structure mowere identified. The functions of these structural segments were then investigated by studying synt had the potential to form amphiphilic α -helical structure
were identified. The functions of these structural seg-
ments were then investigated by studying synthetic an-
alogues that incorporated peptide sequences havin 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 ments were then investigated by studying synthetic alogues that incorporated peptide sequences having, a first priority, minimal homology to the natural quences, but which retained the ability to form an a phiphilic struc alogues 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. Particu first priority, minimal homology to the natural se-
quences, but which retained the ability to form an am-
phiphilic structure in the α -helical conformation. Partic-
ular attention was given to the reproduction in the phiphilic structure in the α -helical conformation. Partic-
ular attention was given to the reproduction in the model [M
peptides of such features of the natural structures as the tail
length of the potential helix, the ular 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 basi 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 negati 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 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
l 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 con face of the helix. Amino acid residues such as leucine, pelysine, glutamic acid, and glutamine were chosen to construct the model helical structures because of their properties of the physicochemical properties of the achi lysine, glutamic acid, and glutamine were chosen to costruct the model helical structures because of their p
pensity for helix formation in globular proteins (29).
was expected that the physicochemical properties of the
na struct the model helical structures because of their pr
pensity for helix formation in globular proteins (29).
was expected that the physicochemical properties of th
natural polypeptides that were dependent on the amph
phi pensity for helix formation in globular proteins (29). It bind was expected that the physicochemical properties of the action atural polypeptides that were dependent on the amphi-
philic structure would be enhanced by this

their most important features, perhaps in an idealized active amphiphilic conformations of the natural polypep-
form, and still retain the activities of the natural peptide tides in their functional environments and also t D KAISER
enhancement of their biological functions. This approach
has proven extremely successful, in that a 22-residue) KAISER
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 D KAISER
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 b represent of their biological functions. This approach as proven extremely successful, in that a 22-resid model peptide and its dimeric analogue were able reproduce all of the salient physicochemical and biological propert 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, has proven extremely successiul, in that a 22-residue
model peptide and its dimeric analogue were able to
reproduce all of the salient physicochemical and biolog-
ical properties of the 243-residue serum apolipoprotein
A-I reproduce all of the salient physicochemical and biolog-
ical properties of the 243-residue serum apolipoprotein
A-I, including its function as an essential cofactor in the
action of lecithin:cholesterol acyltransferase (5 ical 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 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 l 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 l 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, 3 to the bee venom peptide was prepared which ly
unilamellar phospholipid vesicles and erythrocytes at
concentrations and activated phospholipase A_2 (33, ;
In each of these studies, it was possible to identify
active amp 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 natura 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 polypep-
tides in their functional environments and also In each of these studies, it was possible to identify the
active amphiphilic conformations of the natural polypep-
tides in their functional environments and also to gain
some understanding of the importance of particular 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
app tides in their functional environments and also to gair
some understanding of the importance of particular fea
tures of these structures, only because the modelling
approach was applied and both the physicochemical and
the some understanding of the im
tures of these structures, on
approach was applied and bot
the biological properties of th
tides were studied in parallel.
In the remainder of this art res of these structures, only because the modelling
proach was applied and both the physicochemical and
e biological properties of the natural and model pep-
les were studied in parallel.
In the remainder of this article,

approach was applied and both the physicochemical and
the biological properties of the natural and model pep-
tides were studied in parallel.
In the remainder of this article, the application of this
approach to studies o the biological properties of the natural and model pep-
tides were studied in parallel.
In the remainder of this article, the application of this
approach to studies of β -endorphin, calcitonin, and glu-
cagon is review tides were studied in parallel.

In the remainder of this article, the application of this

approach to studies of β -endorphin, calcitonin, and glu-

cagon is reviewed, and its potential application to other

related h In the remainder of this article, the application of this
approach to studies of β -endorphin, calcitonin, and glu-
cagon is reviewed, and its potential application to other
related hormones is also discussed. An enormo approach to studies of β -endorphin, calcitonin, and glu-
cagon is reviewed, and its potential application to other
related hormones is also discussed. An enormous body
of literature describing the more conventional stu cagon 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 peptid related hormones is also discussed. An enormous boot of literature describing the more conventional studiaimed at the structural characterization of these peptic hormones already exists. Some of this work is reviewed here 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 pro 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 prop-
erties of the peptide models, particularly in the ca hormones already exists. Some of this work is reviewed
here in support of the conclusions drawn from the prop-
erties of the peptide models, particularly in the case of
 β -endorphin. However, it is worth emphasizing the 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 des erties 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 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 ciency with which the peptide modelling approach d
scribed here can lead to a comprehensive understandin
of the structures of these intermediate-sized peptides
their functional environments. Often, when extensive
data conc scribed 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 struc of the structures of these intermediate-sized peptides in
their functional environments. Often, when extensive
data concerning the properties of the conventional struc-
tural analogues are considered, it only serves to ill their functional environm
data concerning the proper
tural analogues are conside
the inadequate and time-
more established methods.

Iv. **\$-Endorphin**

quences, but which retained the ability to form an am-
phiphilic structure in the α -helical conformation. Partic-
phiphilic structure in the α -helical conformation. Partic-
is derived from proopiomelanocortin; sever charges in the physiological pH range on the hydrophilic phin), are derived from proenkephalin B. These
face of the helix. Amino acid residues such as leucine, peptides all contain the [Met⁵]-enkephalin or the [Leu⁵]-
 e inadequate and time-consuming nature of other,

ore established methods.
 I **V**. β-**Endorphin**

The biosynthesis of endogenous opioid peptides in-

lves the proteolytic processing of three distinct pre-The biosynthesis of endogenous opioid peptides involves the proteolytic processing of three distinct pre-**TV.** β **-Endorphin**
The biosynthesis of endogenous opioid peptides in-
volves the proteolytic processing of three distinct pre-
cursor peptides (69). The 31-residue peptide β -endorphin
is derived from proopiomelanocor **is derived from Proof in the protection of the protector of the protector of the protector periods** (69). The 31-residue peptide β -endorphin is derived from proopiomelanocortin; several copies of $[Met⁵]$ -enkephali The biosynthesis of endogenous opioid peptic
volves the proteolytic processing of three distinc
cursor peptides (69). The 31-residue peptide β -end
is derived from proopiomelanocortin; several co
[Met⁵]-enkephalin and volves the proteolytic processing of three distinct pre-
cursor peptides (69). The 31-residue peptide β -endorphin
is derived from proopiomelanocortin; several copies of
[Met⁵]-enkephalin and small [Met⁵]-enkephalin cursor peptides (69). The 31-residue peptide β -end
is derived from proopiomelanocortin; several co
[Met⁵]-enkephalin-and small [Met⁵]-enkephali
taining peptides as well as [Leu⁵]-enkephalin are of
from proenkepha taining peptides as well as $[Leu⁵]$ -enkephalin are derived
from proenkephalin A; and several $[Leu⁵]$ -enkephalin-
containing peptides, including the neoendorphins, dy-
norphin A (dynorphin 1–17) and dynorphin B ([Met⁵]-enkephalin and small $[Met⁵]$ -enkephalin-cotaining peptides as well as $[Leu⁵]$ -enkephalin are derivation proenkephalin A; and several $[Leu⁵]$ -enkephal containing peptides, including the neoendorphins taining peptides as well as [Leu⁵]-enkephalin are derived
from proenkephalin A; and several [Leu⁵]-enkephalin-
containing peptides, including the neoendorphins, dy-
norphin A (dynorphin 1-17) and dynorphin B (rimor-
p from proenkephalin A; and several [Leu⁵]-enkephalin-
containing peptides, including the neoendorphins, dy-
norphin A (dynorphin 1–17) and dynorphin B (rimor-
phin), are derived from proenkephalin B. These
peptides all c containing peptides, including the neoendorphins, dy-
norphin A (dynorphin 1–17) and dynorphin B (rimor-
phin), are derived from proenkephalin B. These
peptides all contain the $[Met^5]$ -enkephalin or the $[Leu^5]$ -
enkephali norphin A (dynorphin 1–17) and dynorphin B (rimor-
phin), are derived from proenkephalin B. These
peptides all contain the $[Met⁵]$ -enkephalin or the $[Leu⁵]$ -
enkephalin sequence at their amino termini. The en-
ke phin), are derived from proenkephalin B. These
peptides all contain the [Met⁵]-enkephalin or the [Leu⁵]-
enkephalin sequence at their amino termini. The en-
kephalins alone contain sufficient specificity for potent
bin peptides all contain the [Met⁵]-enkephalin or the [Leu⁵]-enkephalin sequence at their amino termini. The enkephalins alone contain sufficient specificity for potent binding to opioid receptors with concomitant agonist 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 t kephalins alone contain sufficient specificity for pot
binding to opioid receptors with concomitant agon
activity. However, the carboxy-terminal extensis
found in the other opioid peptides result in import
differences in 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

aspet

STRUCTURAL CHARACTERIZATION O
served for β -endorphin which has similar affinities for δ -
and μ -opioid receptors (103, 124a), or for the proenke- by th STRUCTURAL CHARACTE
served for β -endorphin which has similar affinities fo
and μ -opioid receptors (103, 124a), or for the proen
phalin B products which are selective for κ -opioid rec STRUCTURAL CHARACTERI
served for β -endorphin which has similar affinities for
and μ -opioid receptors (103, 124a), or for the proenk
phalin B products which are selective for κ -opioid rece
tors and bind to the $\$ served for β -endorphin which has similar affinities for δ -
and μ -opioid receptors (103, 124a), or for the proenke-
phalin B products which are selective for *k*-opioid recep-
tors and bind to the δ and μ re served for β -endorphin which has similar affinities for δ -
and μ -opioid receptors (103, 124a), or for the proenke-
phalin B products which are selective for κ -opioid recep-
tors and bind to the δ and μ and μ -opioid receptors (103, 124a), or for the proenke-
phalin B products which are selective for κ -opioid recep-
tors and bind to the δ and μ receptors less tightly (28,
30, 157). The carboxy-terminal segmen phalin B products which are selective for κ -opioid rectors and bind to the δ and μ receptors less tightly (30, 157). The carboxy-terminal segments of β -endorp and dynorphin A have also been shown to conferesi tors and bind to the δ and μ receptors less tightly (28, an 30, 157). The carboxy-terminal segments of β -endorphin 78 and dynorphin A have also been shown to confer a oriesistance to degradation on the amino-term 30, 157). The carboxy-terminal segments of β -endorphin 78, and dynorphin A have also been shown to confer a on resistance to degradation on the amino-terminal enkeph-
alin structure (4, 30, 107, 147), which is otherwis and dynorphin A have also been shown to conferentiation of the amino-terminal enker-
resistance to degradation on the amino-terminal enker-
alin structure (4, 30, 107, 147), which is otherwise rapic-
hydrolysed *in vivo* 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 propert alin structure (4, 30, 107, 147), which is otherwise rapid
hydrolysed in vivo (139). In the case of β -endorphin an
also, possibly, dynorphin A(1-17) this property in partic
ular is consistent with a function as a circu tary. so, possibly, dynorphin A(1-17) this property in particular is consistent with a function as a circulating home upon release into the bloodstream from the pitury.
When the structures of mammalian and avian β -en-
rphins

dorphins (66) are compared (figure 1), the [Met^{5]}
dorphins (66) are compared (figure 1), the [Met⁵]
kephalin sequence at the amino terminus is consermone upon release into the bloodstream from the pituitary.

When the structures of mammalian and avian β -en-

dorphins (66) are compared (figure 1), the [Met⁵]-en-

kephalin sequence at the amino terminus is conserve tary. per also the structures of mammalian and avian β -en-
dorphins (66) are compared (figure 1), the [Met⁵]-en-
kephalin sequence at the amino terminus is conserved, Tl
as expected. Overall length is also conserved, When the structures of mammalian and avian β -en-
dorphins (66) are compared (figure 1), the [Met⁵]-en-
kephalin sequence at the amino terminus is conserved, Th
as expected. Overall length is also conserved, but the p dorphins (66) are compared (figure 1), the [Met⁵]-en-
kephalin sequence at the amino terminus is conserved,
as expected. Overall length is also conserved, but the
remainder of the sequences show more variations, with
di kephalin 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, as expected. Overall length is also conserved, but the pr
remainder of the sequences show more variations, with ba
differences observed at residue positions 6, 9-12, 15, 23, pa
 $25-27$, and 31. Nevertheless, the existence remainder of the sequences show more variations, with biddefferences observed at residue positions 6, 9–12, 15, 23, pa
25–27, and 31. Nevertheless, the existence of a conserved hieral proposition of the salmon have conser differences observed at residue positions 6, 9-12, 15, 23, parallel 25-27, and 31. Nevertheless, the existence of a conserved have "hydrophobic core" in residues 13-25 has been noted cer (81). Two β -endorphin-like pept 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 "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 fea

STRUCTURAL CHARACTERIZATION OF PEPTIDE HORMONES 295
h has similar affinities for δ - A thorough structural characterization of β -endorphin
, 124a), or for the proenke- by the peptide modelling approach has been perfo on or PEPTIDE HORMONES 295
A thorough structural characterization of β -endorphin
by the peptide modelling approach has been performed
using six peptide models (figure 2) that were designed ON OF PEPTIDE HORMONES 295
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 (rathe 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, 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, by the peptide modelling approach has been perform
using six peptide models (figure 2) that were designe
and studied sequentially (rather than in parallel) (11, 1
78, 126, 145-147). The design of these peptides was base
on 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 separa 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 struc-
tural units: an opioid receptor recognition s 78, 126, 145–147). The design of these peptides was base
on the division of the hormone into three separate structural units: an opioid receptor recognition site at th
amino terminus (the $[Met^5]$ -enkephalin sequence in re 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 (re 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 i amino terminus (the $[Met⁵]$ -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 retain idues $1-5$) that is connected via a hydrophilic link (resi-
dues $6-12$) to a potential amphiphilic helix in residues
 $13-29$ (147). The amino-terminal segment was retained
in all of the model peptide structures, since i dues 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 r 13–29 (147). The amino-terminal segment was retained
in all of the model peptide structures, since it was ex-
pected to have highly specific interactions with opioid
receptors, and single residue deletion and substitution
 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* pected 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 litt 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 th 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 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 hav 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 re-
ce basis of predictive parameters (29). Nor is there any
particular distribution of charged residues that might
have a strong influence on interactions with opioid re-
ceptors, as do the multiple basic residues in dynorphin
 ceptors, 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 - β –25 has been noted ceptors, as do the multiple basic residues in dynorphines from salmon have A(1–13), for example (27). Peptide segments consisting slightly shorter and of alternating serine and glycine residues (pep

TAYLO
(a) Peptide Models of 6_h-Endorphin
————————————————————

		Peptide Models of B _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(CH ₂ OH).CH ₂ .CH ₂ .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				
			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-NH ₂				
				(8) Leu-Gln-Lys-Leu-Lys-Gln-Tyr-Pro-Arg-Thr-Asn-Thr-Gly-Ser-Gly-Thr-Pro-NH ₂				
		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	(9) Ser-Arg-Arg-Leu-Gln-Glu-Leu-Leu-Gln-Leu-Ala-Leu-Gln-Thr-NH ₂	25			

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

FIG. 2. Amino acid sequences of the peptide models. Part of the structu
were used in its construction rather than amino acid residues of the natura
should mimic the length, hydrophilicity, and proposed re
lack of structure were used in its construction rather than amino acid residues of the natural
should mimic the length, hydrophilicity, and proposed ret
lack of structure of this domain were incorporated into the
some of the model peptides. should mimic the length, hydrophilicity, and proposed relative of the model peptides. In other models (peptides 1, m 3, and 5), the natural sequence of β_h -endorphint in this m region was retained. should mimic the lack of structure of
some of the model p
3, and 5), the natur
region was retained
The potential am mack of structure of this aomain were incorporated into
some of the model peptides. In other models (peptides 1, n
3, and 5), the natural sequence of β_h -endorphin in this
region was retained.
The potential amphiphilic

proximately half of the helix surface and lies along the contential amphiphilic helix in β_h -endorphin was helix surface and helix surface and lies along the proximately half of the helix surface and lies along the coll The potential amphiphilic helix in β_h -endorphin was
shown to have a hydrophobic domain that covers ap-
proximately half of the helix surface and lies along the
clength of the helix either parallel to the axis in a π shown to have a hydrophobic domain that covers a
proximately half of the helix surface and lies along t
length of the helix either parallel to the axis in a
helical conformation, or with a clockwise twist arous
the helix proximately nair 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 mencar conformation, or with a clockwise twist around
the helix in an α -helical conformation (figure 3). Struc-
ture-breaking (29, 54, 100) proline and glycine residues
(positions 13 and 29) were proposed to define the the netter in an α -hencal 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 resi ture-breaking (29, 34, 100) prome and givene residues consider the model of this helix when it forms, with the proline residue repricipating in the formation of the hydrophobic domain. In all of the model peptides, a prol rticipating in the formation of the hydrophobic do-
ain. In all of the model peptides, a proline residue was
 \ddagger The β -endorphin structures corresponding to particular species
riants are referred to, where appropriat

The interaction of peptide 5 is *underlined* to indicate where D-amino acid residues
al L configuration.
The configuration in case this residue is important in restricting
the relative orientations of the different structu ture of peptide 5 is *underlined* to indicate where D-amino acid residues
al L configuration.
retained in case this residue is important in restricting
the relative orientations of the different structural do-
mains of $\$ mainstand in case this residue is important in restricting
the relative orientations of the different structural do-
mains of β -endorphin on opioid receptors. All of the
model peptides contained structural models of th retained in case this residue is important in restricting
the relative orientations of the different structural do-
mains of β -endorphin on opioid receptors. All of the
model peptides contained structural models of the mains 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 struc 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 struc-
tures, the natural residues were replaced by sequence phiphilic helical domain which are illustrated by the α -
helical net diagrams in figure 4. In these model struc-
tures, the natural residues were replaced by sequences
consisting mostly of leucine, lysine, and glutamin nencar net diagrams in rigure 4. In these model struc-
tures, the natural residues were replaced by sequences
consisting mostly of leucine, lysine, and glutamine resi-
dues. These residues were chosen for their propensity tures, the natural residues were replaced by sequences
consisting mostly of leucine, lysine, and glutamine resi-
dues. These residues were chosen for their propensity to
adopt an α -helical conformation (29, 54, 100) an dues. I nese residues were chosen for their propensity to
adopt an α -helical conformation (29, 54, 100) and to
provide hydrophobic, basic hydrophilic, and neutral hy-
drophilic elements of the model structures, respect adopt an α -nencar conformation (29, 54, 100) and to
provide hydrophobic, basic hydrophilic, and neutral hy-
drophilic elements of the model structures, respectively.
In the design of peptide 1, these residues were used are parallel to the helix axis could be formed throughout 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 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 simil 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 amphiphilic helices were used which exactly reproduced

main. In all of the model peptides, a proline residue was
 \uparrow The β -endorphin structures corresponding to particular species

variants are referred to, where appropriate, by the use of subscripts as

follows: h = hu follows: h = human; c = camel; b = bovine; ov = ovine; p = porcine; e

= equine; r = rat; t = turkey; os = ostrich; and s = salmon. \ddagger The β -endorphin structures corresponding to particulariants are referred to, where appropriate, by the use of sifollows: h = human; c = camel; b = bovine; ov = ovine; p = equine; r = rat; t = turkey; os = ostrich

 $\begin{array}{ll}\n\textbf{STRUCTURAL} \textbf{ CARACTERIZA} \\
\textbf{the shape of the hydrophobic domain in the natural} \\
\textbf{structure in either the }\alpha\ \textbf{or the π conformation.}\n\textbf{In peptide}\n\end{array}$ STRUCTURAL CHARACTERIZATIONS STRUCTURE Shape of the hydrophobic domain in the natural I structure in either the α or the π conformation. In peptide v 4, the same amino acid residues were used as in peptide f STRUCTURAL CHARACTERIZATION
the shape of the hydrophobic domain in the natural Kol-
structure in either the α or the π conformation. In peptide were
4, the same amino acid residues were used as in peptide face
2, bu the shape of the hydrophobic domain in the natural lastructure in either the α or the π conformation. In peptide 4, the same amino acid residues were used as in peptide 12, but in a rearranged sequence chosen so tha 4, the same amino acid residues were used as in peptide

2, but in a rearranged sequence chosen so that a helix in

considered likely to have important interactions with

residues 13-31 would *not* be amphiphilic. In pept 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 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 residues 13-31 would *not* be amplifying. In peptide 3, 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 th omy b-ammo acid residues were used in positions 13-31 du

in a linear sequence chosen to allow formation of a left-

handed α helical structure, but not the π -helical

structure. Aromatic residues were also incorpor manded α helix with similar characteristics to the nature
right-handed α -helical structure, but not the π -hel
structure. Aromatic residues were also incorporated is
positions in the model helical structures that right-handed α -helical structure, but not the π -helical Instructure. Aromatic residues were also incorporated into the positions in the model helical structures that correspond end to the phenylalanine residue in po positions in the model helical structures that correlations in the model helical structures that correct
to the phenylalanine residue in position 18 of β_h -
phin (peptides 2 and 4) and the tyrosine residue
position 27 (

"Thr Lys V Asn⁻... \rightarrow Lys Lys"' **Asn** $Lys \sim$ \overline{L} Lys^{\overline{A}} Execution 1998

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 markin

around the cylindrical surface of the helical structure and marking on FIG. 3. Helical net diagrams of β_h -endorphin residues 13-29. This carbon of diagram is the equivalent of wrapping a piece of paper once the around the cylindrical surface of the helical structure and marking on β_i i 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 positi type of diagram is the equivalent of wrapping a piece of paper once the around the cylindrical surface of the helical structure and marking on β it the position of the α carbon atoms of the amino acid residues. The used to compare the distributions of the amino acid residues. The state 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 comp vertical edges of such a diagram would be connecte surface and parallel to the helix axis. In this case, the used to compare the distributions of the amino acid si endorphin residues 13-29 on the surfaces of a regular α

ON OF PEPTIDE HORMONES
Koltun (CPK) models were examined, these residues
were found to be prominent features of the hydrophobic on of PEPTIDE HORMONES
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 ON OF PEPTIDE HORMONES 297
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 import 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
recep receptors that might not be remained, these residues
were found to be prominent features of the hydrophobia
face of the proposed amphiphilic structure, and they were
considered likely to have important interactions with
re face of the proposed amphiphilic structure, and they were *receptors that might not be reproduced by leucine resi-*

Free prostructured into the reproduced by leucine residues
dues substituted into the identical positions.
A. Physicochemical Properties
In water and buffered saline solutions at neutral pH,
the CD spectra of β_h -endorph A. Physicochemical Properties
In water and buffered saline solutions at neutral μ
the CD spectra of β_h -endorphin, β_p -endorphin, and
endorphin indicate the formation of very little recogn
able secondary structure, 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 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 recogniz-
able secondary structure, although hydrodynami 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 partia 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 ass able 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 conce 126, 147, 164). Equilibrium centrifugation assays indicate
that β -endorphin is monomeric at a concentration of 40
 μ M in aqueous saline solution at neutral pH, indicating
that the potential amphiphilic helical struct that β -endorphin is monomeric at a concentration of 4μ M in aqueous saline solution at neutral pH, indication
that the potential amphiphilic helical structure does no
readily promote self-association of the peptide (μ M in aqueous saline solution at neutral pH, indicat
that the potential amphiphilic helical structure does
readily promote self-association of the peptide (147).
attempts to identify preferred types of secondary str
tu that the potential amphiphilic helical structure does not
readily promote self-association of the peptide (147). In
attempts to identify preferred types of secondary struc-
ture that might be induced in β -endorphin by readily promote self-association of the peptide (14'
attempts to identify preferred types of secondary s
ture that might be induced in β -endorphin by int
tions with its receptors, the effects of added lipids
different 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 trifluor net in aqueous saline solution at neutral pH, indicating
that the potential amphiphilic helical structure does not
teadily promote self-association of the peptide (147). In
retating that might be induced in β -endorphin tions with its receptors, the effects of added lipids
different solvents have also been tested. In trifluoroa
anol and methanol solutions, and in the presence
negatively charged (but not neutral) lipids that have b
implic different solvents have also been tested. In trifluoroetlanol and methanol solutions, and in the presence negatively charged (but not neutral) lipids that have been implicated in opioid receptor binding, such as phosphiti and and methanor 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 s implicated in opioid receptor binding, such as phospha-
tidyl serine or cerebroside sulfate (101), α -helical struc-
ture is induced in the different mammalian β -endorphins
tested (68, 155, 164). Furthermore, the eff tidyl 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 ture is induced in the different mammalian β -endorphins
tested (68, 155, 164). Furthermore, the effects of metha-
nol or cerebroside sulfate on amino-terminal and car-
boxy-terminal deletion analogues of β_h -endorphi tested (68, 155, 164). Furthermore, the effects of metha-
nol or cerebroside sulfate on amino-terminal and car-
boxy-terminal deletion analogues of β_h -endorphin
showed that the induced helical structure lies in the
car boxy-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
 $\$ 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 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 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 stru β_h -endorphin to form insoluble monolayers of modest
stability on the surface of saline solutions is also indic-
ative of its potential to form an amphiphilic structure. A
detailed analysis of the properties of these mo ative 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 $(14 \text{ Å}^2/\text{residue})$ was consistent with a h

RMACOL

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structure in about half of the molecule occupying the su
surface with the rest of the peptide structure probably pe TAYLOR AND
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 p 298 TAYLOR ANI
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 physico surface with the rest of the peptide structure probably
extending into the aqueous subphase and fully hydrated
(126). In general, therefore, the physicochemical prop-
erties of β -endorphins are consistent with the pote (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 hypot hormones of this type, and with the structural hypothesis ties of β -endorphins are consistent with the potential
portance of induced amphiphilic structure in peptide
rmones of this type, and with the structural hypothesis
ed in the design of the β -endorphin model peptides.

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 p normones 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 differentiative of 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 he-

lix and random-coil structure, indicative 1, 2, 3, 5, and 6 in buffered saline solutions at neutral $\frac{1}{2}$ pH all showed concentration-dependent mixtures of helix and random-coil structure, indicative of self-association and concomitant stabilization of their pri all showed concentration-dependent mixtures of he-
lix and random-coil structure, indicative of self-associa-
tion and concomitant stabilization of their amphiphilic
helical structures (table 1). Furthermore, the spec tion and concomitant stabilization of their amphiphilic
helical structures (table 1). Furthermore, the spectra
obtained for peptide 5 were consistent with the stabili-
zation of a left-handed α -helical structure upon s mencal structures (table 1). Furthermore, the spectra
obtained for peptide 5 were consistent with the stabili-
zation of a left-handed α -helical structure upon self-
association, corresponding to the presence of only D zation 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 concentratio 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 amino acids in its carboxy-terminal sequence. The self-
association of peptides 1 and 2 at lower concentrations strip
than peptides 3, 5, and 6 was attributed to the design of and
the amphiphilic α helices in the earli association of peptides 1 and 2 at lower concentrations structure in peptide hormones may increase their resist-
than peptides 3, 5, and 6 was attributed to the design of ance to proteclytic degradation and cause nonspeci 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 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 -en to create a nyuropholoc domain lying paramet to the α m
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 However, additional properties of residues 13–31 of the kephalins has been demonstrated in a variety of experi-
natural peptide must further inhibit helix formation ments 4, 30, 59, 107, 120, 147). In aqueous solution, th However, additional properties of residues 13-31 of the kendtural peptide must further inhibit helix formation mompared to peptides 3, 5, and 6, and the presence of β -
branched residues (Val¹⁵, Thr¹⁶, Ile²², and matural peptide must further infinited helix formation in
compared to peptides 3, 5, and 6, and the presence of β -
branched residues (Val¹⁵, Thr¹⁶, Ile²², and Ile²³), which
may be conformationally restricted on branched residues (Val¹⁵, Thr¹⁶, Ile²², and Ile²³), whimay be conformationally restricted on a helix surface, well as residues with little propensity for helix formation (Asn²⁰, Asn²⁵, Tyr²⁷ and Gly³⁰) may may be conformationally restricted on a helix surface, as
well as residues with little propensity for helix formation 91,
(Asn²⁰, Asn²⁵, Tyr²⁷ and Gly³⁰) may be important in this car
respect. The properties of the (Asn²⁰, Asn²⁵, Tyr²⁷ and Gly³⁰) may be important in this carboxy terminus with proteolytic enzymes may also be respect. The properties of the peptide models of β -endor-
phin at the air-water interface were also respect. The properties of the peptide models of β -endor-
phin 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 phin at the air-water interface were also consistent with formation of a more stable helical structure than endorphin has in this environment (table 1). Even the most conservative peptide models (peptides 3 and where all 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 amph endorphin has in this environment (table 1). Even th
most conservative peptide models (peptides 3 and 6)
where all of the general features of the potential amphi
philic α -helical structure in β_h -endorphin were retai

surface with the rest of the peptide structure probably peptides (16 \AA^2 /residue) was similar to that of β_h -endor-
extending into the aqueous subphase and fully hydrated phin, however, indicating that similar confor TAYLOR AND KAISER
pying the sures. The surface area occupied by these two model peptides (16 A²/residue) was similar to that of β_h -endor-
peptides (16 A²/residue) was similar to that of β_h -endor-
phin, however, indicating that similar conformation b KAISER
sures. The surface area occupied by these two model
peptides (16 \AA^2 /residue) was similar to that of β_h -endor-
phin, however, indicating that similar conformations
were adopted. Substitution of γ -aminosures. The surface area occupied by these two
peptides (16 Å²/residue) was similar to that of β_1
phin, however, indicating that similar confor:
were adopted. Substitution of γ -amino- γ -h
methylbutyric acid resi sures. The surface area occupied by these two model
peptides (16 \AA^2 /residue) was similar to that of β_h -endor-
phin, however, indicating that similar conformations
were adopted. Substitution of γ -amino- γ -hydro 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 non-
amphiphilic peptide 4 was monomeric with little helical methylbutyric acid residues for the natural sequence of
the hydrophilic linking region had essentially no effect
on self-association or monolayer formation. The non-
amphiphilic peptide 4 was monomeric with little helical on self-association or monolayer formation. The non-
amphiphilic peptide 4 was monomeric with little helical
structure at a concentration of 10 μ M, and insoluble at
higher concentrations, and monolayers formed by pep-
 on sen-association or monolayer formation. The non-
amphiphilic peptide 4 was monomeric with little helical
structure at a concentration of 10 μ M, and insoluble at
higher concentrations, and monolayers formed by pep-
t structure at a concentration of 10 μ M, 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 th structure at a concentration of 10 μ M, and insoluble
higher concentrations, and monolayers formed by pe
tide 4 were much less stable than those formed by
parent compound, peptide 2. These results demonstrat
the importa higher concentrations, and monolayers formed by pep-
tide 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 tide 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 pe *B. Resistance of the amphiphilic castructure in determining conformations*
 B. Resistance to Enzymatic Inactivation
 B. Resistance to Enzymatic Inactivation

As we have discussed the presence these peptides in solution and at amphiphilic interfaces.

B. Resistance to Enzymatic Inactivation

As we have discussed, the presence of amphiphilic

Fructure in determining conformational properties of
ese peptides in solution and at amphiphilic interfaces.
Resistance to Enzymatic Inactivation
As we have discussed, the presence of amphiphilic
ructure in peptide hormone B. Resistance to Enzymatic Inactivation
As we have discussed, the presence of amphiphilic
structure in peptide hormones may increase their resist-
ance to proteolytic degradation and cause nonspecific
interactions with cel 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 mark 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 resist-
 ance to proteolytic degradation and cause nonspecific
interactions with cell surfaces, and such properties will
markedly affect pharmacokinetic behavior. The resist-
ance of the amino-terminal residues of β -endorphin t meractions with cen surfaces, and such properties win
markedly affect pharmacokinetic behavior. The resist-
ance of the amino-terminal residues of β -endorphin to
purified amino-peptidases and to the enkephalinases in
b markeury 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 enkeph purified amino-peptidases and to the enkephalinases in
brain homogenates relative to the rapidly degraded en-
kephalins has been demonstrated in a variety of experi-
ments 4, 30, 59, 107, 120, 147). In aqueous solution, th brain homogenates relative to the rapidly degraded en-
kephalins has been demonstrated in a variety of experi-
ments 4, 30, 59, 107, 120, 147). In aqueous solution, this
property appears to be related to the formation of s kephalins 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 ments 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 (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 amphiphili 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 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 carboxy terminus with proteolytic enzymes may
important, as the proteolysis of $[Met^5]$ -enkephal
solubilized rat brain aminopeptidase is inhibited
dition of β_h -endorphin, and β_h -endorphin(1-17) is
less effective inhi portant, as the proteolysis of $[Met^5]$ -enkephalin by a
lubilized rat brain aminopeptidase is inhibited by ad-
tion of β_h -endorphin, and β_h -endorphin(1–17) is a much
se effective inhibitor of this degradation (71).
T solubilized rat brain aminopeptidase is inhibited by a dition of β_h -endorphin, and β_h -endorphin(1-17) is a mu
less effective inhibitor of this degradation (71).
The relative resistance towards proteolysis of the pe

dition of β_h -endorphin, and β_h -endorphin(1-17) is a multes effective inhibitor of this degradation (71).
The relative resistance towards proteolysis of the p
tide models in the presence of diluted rat brain hom
enat The relative resistance towards proteolysis of the pep-
tide models in the presence of diluted rat brain homog-
enates at 37°C has been compared to that of β_h -endor-
phin (table 2). This property correlated well with t

		TABLE 1	Physicochemical properties of β_h -endorphin and peptides 1–6 ^{α}	
	Self-association		θ_{222} (deg cm ² /dmol) ^{b, c}	Monolayer collapse pressure
Peptide	in aqueous solution ^b	Monomer	Oligomer	$(dvn/cm)^b$
β_{h} -Endorphin	None at $40 \mu M$	-1.800		η
Peptide 1	Above $200 \mu M$	$-9,000$	$-15,750$	24
Peptide 2	Above $3 \mu M$	-11.400	-13.900	24
Peptide 3	Above $10 \mu M$	$-6,450$	$-10,650$	21
Peptide 4	None at $10 \mu M^d$	$-2,800$		11
Peptide 5	Above $10 \mu M$	4.300°	8.500°	15
Peptide 6	Above $10 \mu M$	-3.950	$-8,750$	22

TABLE ¹

⁶ The aqueous phase contained 160 mM KCl, buffered at pH 7.4.
⁶ Increasing negative values are indicative of increasing right-handed helical structure in peptides consisting of helical and random coil
structures only. **Structures only.** The aqueous phase contained 160 mM K

Increasing negative values are indicative

structures only.

Insoluble above this concentration.

Contained left-handed helical structure.

ARMACOLOGICAL REVIEW

aspet

After centritugation of suspensions containing the peptides (10 μ M

Clypon incubation of the peptide-brain homogenate suspensions at

d'Compared to β_h -endorphin, [Met⁵]-enkephalin degradation/inactive

tendencies Formations of these peptides to adopt amphiphilic contendencies of these peptides to adopt amphiphilic conformations through self-association or binding to suitable interfaces. At concentrations of 10μ M, the nonant tendencies of these peptides to adopt amphiphilic coformations through self-association or binding to suit ble interfaces. At concentrations of 10μ M, the nonaphiphilic peptide 4 was degraded at about the same r tendencies of these peptides to adopt amphiphilic con-
formations through self-association or binding to suita-
ble interfaces. At concentrations of 10 μ M, the nonam-
phiphilic peptide 4 was degraded at about the same tendencies of these peptides to adopt amphiphilic conformations through self-association or binding to suitable interfaces. At concentrations of 10 μ M, the nonamphiphilic peptide 4 was degraded at about the same rate a formations through self-association or binding to suita-
ble interfaces. At concentrations of 10 μ M, the nonam-
phiphilic peptide 4 was degraded at about the same rate
as β_h -endorphin, whereas its parent compound, p ble interfaces. At concentrations of 10 μ M, the nonam-
phiphilic 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 com phiphilic 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 as p_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 resistant to proteolysis. Again, peptides 3 and 6 displied intical behavior intermediate between that of the ural peptide and the highly helical peptides 1 and 2.
assay, which involved centrifugation of the incubasy
suspen identical behavior intermediate between that of the nat-
ural peptide and the highly helical peptides 1 and 2. This
assay, which involved centrifugation of the incubating
on
suspensions followed by high-pressure liquid ch draft peptide and the fightly neitcal peptides 1 and 2. This
assay, which involved centrifugation of the incubating
suspensions followed by high-pressure liquid chromatog-
raphy (HPLC) quantification of the amount of each
 suspensions followed by high-pressure liquid chromatog-
raphy (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 raphy (HPLC) quantification of the amount of each δ/ρ
peptide recovered in the supernatants, also demonstrated
that the model peptides were mostly bound to the rat
methranic membranes, since recoveries were as low as 1 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
re brain membranes, since recoveries were as low as $10-$ constant 30% at zero time. In contrast, β_h -endorphin could be nu recovered in high yields initially, although it was subsequently degraded. Other strong interac 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, 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
si quently 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,
melitt 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 vesicle z, which are able to lyse ery is
similar to those observed for
melittin, and also disrupt uni
icles under certain conditions
were observed for β_h -endorphin **c.** *Receptor Binding Properties* C. *Receptor Binding Properties*

were observed for β_h -endorphin.

C. Receptor Binding Properties [I]

The synthesis of tritiated β_h -endorphin has allowed ³H

the direct characterization of its binding sites (87). Many en

subsequent binding stud 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 r The synthesis of tritiated β_h -endorphin has allow
the direct characterization of its binding sites (87). Ma
subsequent binding studies of β -endorphin analog
have employed this radiolabel, with the probable adva
tage The synthesis of tritiated β_h -endorphin has allowed ^of-
the direct characterization of its binding sites (87). Many
subsequent binding studies of β -endorphin analogues
have employed this radiolabel, with the proba subsequent binding studies of β -endorphin analogues
have employed this radiolabel, with the probable advan-
tage that their relevance to β -endorphin activities in vivo
is more likely, but with the disadvantage that 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 rela-
tionship between β -endorphin structure and o Let us 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 in the selectivity is not determined. One exception was
a comparison of the inhibitory potencies of carboxy-
terminal deletion analogues of β_h -endorphin on the bind-
ing of ³H-dihydromorphine (a ligand selective for a comparison of the inhibitory potencies of carboxy-
terminal deletion analogues of β_h -endorphin on the bind-
ing of ³H-dihydromorphine (a ligand selective for μ -
opioid receptors), ³H-[Leu⁵]-enekphalin (δ

and diluted homogenates to remove particulate matter.
 \sum .

creased 1.75-fold for β_h -endorphin(1-30) and decreased

steadily with decreasing chain length to 0.008 times that on is considerably more rapid.

creased 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 3H - β_h creased 1.75-fold for β_h -endorphin (1-30) and decrease
steadily with decreasing chain length to 0.008 times that
of β_h -endorphin for β_h -endorphin (1-15), when ³H- β
endorphin was used as the receptor label. A creased 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 ³H- β_h -
endorphin was used as the receptor label creased 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 ³H- β_h -
endorphin was used as the receptor label. steadily with decreasing chain length to 0.008 times that
of β_h -endorphin for β_h -endorphin (1–15), when ³H- β_h -
endorphin was used as the receptor label. A similar trend
in the binding potencies was observed whe of β_h -endorphin for β_h -endorphin (1-15), when ³H- 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 ana endorphin was used as the receptor label. A similar tr
in the binding potencies was observed when the ot
radioligands were used, but β_h -endorphin analog
shorter than β_h -endorphin (1–27) displayed a gree
preference f radioligands were used, but β_h -endorphin analoshorter than β_h -endorphin (1-27) displayed a grepreference for binding to the ³H-[Leu⁵]-enkephali belled receptors than the ³H-dihydromorphine-lab ones. These resu shorter than β_h -endorphin (1–27) displayed a greater
preference for binding to the ³H-[Leu⁵]-enkephalin-la-
belled receptors than the ³H-dihydromorphine-labelled
ones. These results indicate a role for the carbox preference for binding to the ³H-[Leu⁵]-enkepha
belled receptors than the ³H-dihydromorphine-la
ones. These results indicate a role for the carbox
minal region in determining both the potency ar
 δ/μ selectivity o illed receptors than the ³H-dihydromorphine-labelled
nes. These results indicate a role for the carboxy-ter-
inal region in determining both the potency and the
 μ selectivity of β_h -endorphin to opioid receptors.
T

Les under certain conditions (144). No similar effects However, considerable evidence suggests that high bind-

ing potency does not require α -helical structure in the

carboxy-terminal region. For example, the potency ones. These results indicate a role for the carboxy-ter-
minal region in determining both the potency and the
 δ/μ selectivity of β_h -endorphin to opioid receptors.
The relative importance of the [Met⁵]-enkephalin s minal region in determining both the potency and the
 δ/μ selectivity of β_h -endorphin to opioid receptors.

The relative importance of the [Met⁵]-enkephalin seg-

ment of β_h -endorphin that is suggested by its s δ/μ selectivity of β_h -endorphin to opioid receptors.
The relative importance of the [Met⁵]-enkephalin seg-
ment of β_h -endorphin that is suggested by its sequence
conservation in the natural peptides is supporte The relative importance of the [Met⁵]-enkephalin seg-
ment 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
 ment of β_h -endorphin that is suggested by its sequence conservation in the natural peptides is supported by number of binding studies showing that single amin acid residue deletions or modifications in this region ca d 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 i 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 bind-
ing sites in rat brain membranes (32, 65, 98, acid residue deletions or modifications in this region can
drastically alter the affinity of β_h -endorphin for its bind-
ing sites in rat brain membranes (32, 65, 98, 168). The
contribution of the carboxy-terminal regio 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 correl Ing sites in rat brain membranes (32, 63, 36, 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 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 bind-
ing potency does not require α -helical structure with α helix formation in 75% trifluoroethanol (64).
However, considerable evidence suggests that high bind-
ing *potency* does not *require* α -helical structure in the
carboxy-terminal region. For example, the pote However, considerable evidence suggests that high bind-
ing potency does not require α -helical structure in the
carboxy-terminal region. For example, the potency of
[Des-Gln¹¹,Leu¹⁹,Asn²⁰,lle²²]- β_h -endorphin carboxy-terminal region. For example, the potency of [Des-Gln¹¹,Leu¹⁹,Asn²⁰,Ile²²]- β_h -endorphin for binding ³H- β_h -endorphin-labelled sites is similar to that of β_h -endorphin itself (98). More strikingly [Des-Gln¹¹, Leu¹⁹, Asn²⁰, Ile²²]- β_h -endorphin for binding ³H- β_h -endorphin-labelled sites is similar to that of β_h -endorphin itself (98). More strikingly, cyclic β_h -endorphin analogues having disulfid ³H- 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 t phin analogues having disulfide bonds connecting a cysphin analogues having distinct bonds connecting a cystein
tesidue in position 11, 14, 17, or 21 to a cystein
residue in position 26 have 1 to 4 times the bindin
potency of β_h -endorphin in these assays (8).
The role of

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 recep-
tor-binding potencies of β_h -endorphin is well established.
Pos potency of β_h -endorphin in these assays (8).
The role of charged residues in determining the recep-
tor-binding potencies of β_h -endorphin is well established.
Positive charges in the β_h -endorphin structure lie at 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, o tor-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 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 potenc receptor binding assays. N^a-acetyl- β_h -endorphin has

SOO TAYLOR AND KAISER
several such assays (94, 96), and amino-terminal acety- potential
lation may be an important mechanism for inactivation phin are s 300 TAYLOR ANI
several such assays (94, 96), and amino-terminal acety-
lation may be an important mechanism for inactivation
of β -endorphin in vivo (167). Citraconylation of the TAYLOR AND I
several such assays (94, 96), and amino-terminal acety-
lation may be an important mechanism for inactivation
of β -endorphin in vivo (167). Citraconylation of the sel
lysine residues in β -endorphin, whi several such assays (94, 96), and amino-terminal acety-
lation may be an important mechanism for inactivation
of β -endorphin in vivo (167). Citraconylation of the
lysine residues in β_p -endorphin, which replaces thei several such assays (94, 96), and amino-terminal acety-
lation may be an important mechanism for inactivation
of β -endorphin in vivo (167). Citraconylation of the
lysine residues in β_p -endorphin, which replaces thei lation may be an important mechanism for inactivation philof β -endorphin in vivo (167). Citraconylation of the sele lysine residues in β_p -endorphin, which replaces their hydpositive charges with negative charges at 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 t rat brain membrane binding sites (57). The negative selection of that peptide to of the displace 3 H-naloxone or 3 H-dihydromorphine from their effect brain membrane binding sites (57). The negative selections is 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 r rat brain membrane binding sites (57). The negative selectivity of the natural structure.
charges in β_h -endorphin lie in glutamic acid residues in A comparison of the binding assay results for peptides
positions 8 and positions 8 and 31, the latter having two negative charges charges in β_h -endorphin lie in glutamic acid residues in Apositions 8 and 31, the latter having two negative charges 3 and as it is at the carboxy terminus. A large number of γ -h₁ analogues have been studied in wh 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 i as it is at the carboxy terminus. A large number of γ analogues have been studied in which these negative relatives that γ charges have been removed, and this always results in γ an increased potency in radiorece analogues have been studied in which these negative
charges have been removed, and this always results in en
an increased potency in radioreceptor binding assays that ti
use ³H- β _h-endorphin to label rat brain membr an increased potency in radioreceptor binding assays that tivuse ³H- β _h-endorphin to label rat brain membranes. For β -example, substitution of residue 8 by a glutamine residue opincreases the binding potency of use ³H- 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), resid example, substitution of residue 8 by a glutamine residue op
increases the binding potency of β_h -endorphin deletion bin
analogues comprising residues 1-9 (159), residues 1-17 rec
(55), and residues 1-28 (56), as well a increases the binding potency of p_h -endorphin detection
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 t 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 th (55), and residues 1–28 (56), as well as β_h -endorphin pitself (95). Similarly, removal of one or both of the tecarboxyl groups in position 31 by extension of the peptide in chain and/or replacement with amide groups or itself (95). Similar
carboxyl groups in p
chain and/or replae
monacidic residues 1
(93, 97, 158, 160).
The potencies of The position of the peptide

in and/or replacement with amide groups or other endo

inacidic residues results in enhanced binding potency imp

3, 97, 158, 160).

The potencies of the β -endorphin model peptides 1 poss

the specific binding potency

monacidic residues results in enhanced binding potency imp

(93, 97, 158, 160). is re

(93, 97, 158, 160). is re

through 6 for displacing the specific binding of ³H-[D-

the Ala²,D-Leu⁵ (93, 97, 158, 160). is re

The potencies of the β -endorphin model peptides 1 poss

through 6 for displacing the specific binding of ³H-[D-

Ala²,D-Leu⁵]-enkephalin and ³H-dihydromorphine to has

guinea pig brai The potencies of the β -endorphin model peptides 1 potency through 6 for displacing the specific binding of ³H-[D-
Ala²,D-Leu⁵]-enkephalin and ³H-dihydromorphine to has guinea pig brain membranes have been compa through 6 for displacing the specific binding of ³H-[D-Ala²,D-Leu⁵]-enkephalin and ³H-dihydromorphine to guinea pig brain membranes have been compared to those of β_h -endorphin under conditions in which the radi Ala²,D-Leu⁵]-enkephalin and ³H-dihydromorphine guinea pig brain membranes have been compared those of β_h -endorphin under conditions in which tradioligands should selectively label δ - and μ -opioid ceptors, r 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 re-
ceptors, respectively (58a). The results of these exp those of ρ_h -endorphiful under conditions in which the radioligands should selectively label δ - and μ -opioid re ceptors, respectively (58a). The results of these experiments are summarized in table 3. Peptides 1, 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 ments 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 selectivi which have the natural sequence in residues 1-12 con-
nected to different model amphiphilic helical structures,
were all able to reproduce the δ/μ receptor selectivity of
 β_h -endorphin rather closely. Their potencies mected to different model amplifying fields structures, there all able to reproduce the δ/μ receptor selectivity of in β_h -endorphin rather closely. Their potencies in each as-
say ranged from almost equal to that of were all able to reproduce the δ/μ receptor selectivity of in β_h -endorphin rather closely. Their potencies in each as-
say ranged from almost equal to that of β_h -endorphin (peptide 5) to nearly an order of magnit β_h -endorphin rather closely. Their potencies in each as-
say 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

Se results indicate that the general features of the
TABLE 3
Binding potencies relative to β_h *-endorphin of peptides 1–6 to*
radiolabeled opiate receptors in guinea pig brain membranes^e *rading potencies relative to* β_h *-endorphin of peptides 1–6 to***
***radiolabeled opiate receptors in guinea pig brain membranes⁴* radiolabeled opiate receptors in guinea pig brain membranes^a

Peptide	Overall charge		Binding potency ^b	Receptor selectivity
	at neutral pH		δ receptors ^c μ receptors ^d	(μ/δ)
β_h -Endorphin	$+3$	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). ' Selectively labelled using ³ H-[D-Ala ² ,D-Leu ⁵]-enkephalin. ^d Selectively labelled using ³ H-dihydromorphine.			

potential amphiphilic helix in residues 13–29 of β -endor-
phin are sufficient to determine the effect on δ/μ receptor b KAISER
potential amphiphilic helix in residues 13–29 of β -endor-
phin are sufficient to determine the effect on δ/μ receptor
selectivity of this structure, and that the shape of its by KAISER
potential amphiphilic helix in residues 13–29 of β -endor-
phin are sufficient to determine the effect on δ/μ receptor
selectivity of this structure, and that the shape of its
hydrophobic domain is not crit potential amphiphilic helix in residues 13–29 of β -endor-
phin 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 (pept potential amphiphilic helix in residues 13–29 of β -endor-
phin 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 (pept phin 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 -he 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 reproduc hydrophobic domain is not critic
striking is the ability of a left-hane
of the appropriate design (peptide
effects in binding assays on both
selectivity of the natural structure.
A comparison of the binding assa SUTIKING IS the aboutly of a left-handed α -nencal segment
of the appropriate design (peptide 5) to reproduce the
effects in binding assays on both potency and receptor
selectivity of the natural structure.
A comparison effects in binding assays on both potency and receptor
selectivity of the natural structure.
A comparison of the binding assay results for peptides

selectivity of the natural structure.
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 A comparison of the binding assay results for peptide 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 regi 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 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 residu matural structure of the hydrophinc intempregnon in p-
endorphin with only minor effects on δ/μ receptor selec-
tivity and potency. This suggests that residues 6–12 of
 β -endorphin do not have strong interactions wit endorphin with only inflor effects on ∂/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 tivity 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 th p-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 o 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 (al-

ternating serines and glycines), was earlier thought poince the some of the striking μ receptor burston with a receptor perceptor sand mainly serve to connect the two
binding segments in residues 1–5 and 13–29 on the
receptor surfaces. The striking μ receptor selectiv ternating 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 o 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 rece 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 t 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-termi 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 opi 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 cha the hydropholic 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 cha (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-associa 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 can attect 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 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 t represents in reading a small in the secure of the two binding segres and mainly serve to connect the two binding segres and a 13-20 on the receptor surfaces. The striking μ receptor selectivity of represide 2, which a

(peptide 5) to nearly an order of magnitude greater than helical region of these opioid peptides was further dem-
 β_h -endorphin (peptide 3), and they correlate well with onstrated by the much lower μ receptor affini The sensitivity of μ receptor binding to changes in the than 10-fold higher concentrations. A tryptophan residum in a similar position in a calcitonin model peptide has the same inhibitory effect on self-association (*vide infra*). The sensitivity of μ receptor binding to c 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 The same inhibitory effect on seff-association (*bide infra)*.
The sensitivity of μ receptor binding to changes in the
helical region of these opioid peptides was further dem-
onstrated by the much lower μ receptor In 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, th onstrated 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 abi 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 li 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 -hel peptide 4 to displace both δ and μ
agreement with the studies of
earlier, indicating that the amph
ture in the carboxy terminus is
potency in either binding assay.
Recently, ligands that are more 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

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 ture 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 discrim potency in either ointing 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 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 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 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 a 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 char

ARMACOLOGICAL REVIEW!

STRUCTURAL CHARACTERIZATION OF PEPTIDE HORMONES 301

 $\begin{array}{lll} \textbf{STRUCTURAL} & \textbf{CHARACTER} \\\textbf{receptor assays. These peptide models at least are, the}\\ \textbf{fore, also able to reproduce the high selectivity of }\beta\end{array}$ STRUCTURAL CHARACTERIZA
receptor assays. These peptide models at least are, there-
fore, also able to reproduce the high selectivity of β_{h} -
endorphin for μ receptors versus κ receptors (124a). endorphin for *endorphin* for *endorphin* for *μ* receptor *ssays*. These peptide models at least are, the fore, also able to reproduce the high selectivity of endorphin for *μ* receptors *versus κ* receptors (124a). *Preceptor assays.* These peptide models at least as fore, also able to reproduce the high selectivierdorphin for μ receptors versus κ receptors (12
D. Opioid Activities in Smooth Muscle Assays
The opiates and opi

D. Opioid Activities in Smooth Muscle Assays
The opiates and opioid peptides inhibit electrically
stimulated contractions in a variety of smooth muscle 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

prepa D. Opioid Activities in Smooth Muscle Assays
The opiates and opioid peptides inhibit electrically can
stimulated contractions in a variety of smooth muscle are
preparations. These actions appear to result from the eartivat 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 conse-
quent stimulated contractions in a variety of smooth muscle
preparations. These actions appear to result from the
activation of presynaptic opioid receptors and conse-
quent inhibition of neurotransmitter release (109). pre
Str preparations. These actions appear to result from the earlier (45).
activation of presynaptic opioid receptors and conse-
quent inhibition of neurotransmitter release (109). preparations that have been characterized to da rat vas deferens (RVD). Multiple opioid receptor types Structural analogues of p-endorphin have mostly been inhorsely
investigated for their activities in preparations of the RV
guinea pig ileum (GPI), mouse vas deferens (MVD), and select
rat vas deferens (RVD). Multiple opio mvestigated for their activities in preparations of the
guinea pig ileum (GPI), mouse vas deferens (MVD), and vas deferens (RVD). Multiple opioid receptor typ
are present in the GPI and MVD, which appear
correspond to the brane preparations from the central nervous system. The correspond to the δ , μ -, and κ -opioid receptors of mem-
brane preparations from the central nervous system. The or MVD is particularly rich in δ recepto are present in the GTT and MVD, which appear to decorrespond to the δ , μ -, and κ -opioid receptors of mem-
brane preparations from the central nervous system. The of
MVD is particularly rich in δ receptors, an correspond to the δ , μ -, and κ -opioid receptors of mem-
brane preparations from the central nervous system. The
MVD is particularly rich in δ receptors, and the GPI has
[mostly μ receptors, although dynorp brane preparat

MVD is particus

mostly μ recep

peptides act w

tissues (169).

The opioid a MVD is particularly rich in δ receptors, and the GPT has
mostly μ receptors, although dynorphin A and related 13
peptides act with high potency on κ receptors in these
tissues (169).
The opioid activities of β

tissues (169).
The opioid activities of β -endorphin on the MVD and
GPI are critically dependent on the [Met⁵]-enkephalin
region of the molecule. [Des-Tyr¹]- β_h -endorphin and N^o-
acetyl- β_h -endorphin have no a The option activities of p-endorphin on the MVD and GPI are critically dependent on the $[Met⁵]$ -enkephalin region of the molecule. [Des-Tyr¹]- β_h -endorphin and N⁻accetyl- β_h -endorphin have more activity on eit acetyl- β_h -endorphin have no activity on either tissue, of p
and [D-Ala²]- β_h -endorphin and [Leu⁵]- β_h -endorphin tor;
have much lower GPI/MVD potency ratios than β_h -
this
endorphin, indicating more δ recep and $[D-Ala^2]$ - β_h -endorphin and $[Leu^6]$ - 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 Tyr¹, Phe⁴, or M have much lower GPI/MVD potency ratios than β_h -
endorphin, indicating more δ receptor selectivity (138).
On the GPI, β_c -endorphin with Tyr¹, Phe⁴, or Met⁵
substituted by the corresponding D-amino acid resid endorphin, indicating more δ receptor selectivity
On the GPI, β_c -endorphin with Tyr¹, Phe⁴, o
substituted by the corresponding D-amino acid r
showed much reduced potency (163), whereas a
substitution for Lys⁹ On the GPI, β_c -endorphin with Tyr¹, Phe⁴, or Met⁵ prosubstituted by the corresponding D-amino acid residues the showed much reduced potency (163), whereas a similar the substitution for Lys⁹ or Phe¹⁸ in [Phe substituted by the corresponding D-amino acid residues
showed much reduced potency (163), whereas a similar
substitution for Lys⁹ or Phe¹⁸ in [Phe²⁷,Gly³¹]- β_h -endor-
phin had very little effect (165). In fact, substitution for Lys⁹ or Phe¹⁸ in [Phe²⁷,Gly³¹]- β_h -endor-
phin had very little effect (165). In fact, the GPI is
strikingly insensitive to changes in the β -endorphin
structure outside of the [Met⁵]-enkepha phin had very little effect (165). In fact, the GPI
strikingly insensitive to changes in the β -endorph
structure outside of the [Met⁵]-enkephalin segment, a
the small changes in potency that are observed do r
have an strikingly insensitive to changes in the β -endorphin-
structure outside of the [Met⁵]-enkephalin segment, an
the small changes in potency that are observed do no
have any obvious structural correlate. Thus, the poter structure outside of the [Met⁵]-enkephalin segment, an
the small changes in potency that are observed do no
have any obvious structural correlate. Thus, the potencies of β_h -endorphin-(1-5)-(16-31) and β_c -endorphin the small changes in potency that are observed do not 1⁴ 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 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 relat cies 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, of
respectively (92), and the corresponding relative potency rec
of [Des-Gln¹¹,Leu¹⁴,Asn²⁰,Il b)-(26-31) are 1.33 and 0.35 times that of p_c -endorphin,
respectively (92), and the corresponding relative potency
of [Des-Gln¹¹,Leu¹⁴,Asn²⁰,Ile²²]- β_h -endorphin is 1.38 (98).
Single residue deletions or subst of [Des-Gln¹¹,Leu¹⁴,Asn²⁰,Ile²²]- β_h -endorphin is 1.38 (98).

Single residue deletions or substitutions in this region

have even less effect (9, 97, 98).

[Met⁵]-enkephalin is very potent in MVD assays, but

only moderately active on the GPI, whereas β -endorphin [Met^v]-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 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 [Met⁵]-
enkephalin in β -endorphin may moderate its op suggests that the carboxy-terminal extension of [Met⁵]-
enkephalin in β -endorphin may moderate its opioid re-
ceptor selectivity. Graf et al. have shown that, in a series
of carboxy-terminal deletion analogues of β enkephain in p-endorphin may moderate its option 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 ceptor 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 of carboxy-terminal detector analogues of p_p -endorprint,
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
a the GPI/MVD potency ratio decreases as the peptide exchain length decreases, although the potencies on either the one tissue fluctuate in a random fashion (60). These in authors point out that the changes in this selectiv chain length decreases, although the potencies on either thone tissue fluctuate in a random fashion (60). These im authors point out that the changes in this selectivity strifluoroethanol, and they suggest that the recept 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 h

ica! structure in residues 13-29. These arguments are based on relatively small changes in potencies on the GN OF PEPTIDE HORMONES 301
ical 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, howev ical 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 ical 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 th 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 studies (74, 138). They do not, however, correlate well with the results of the binding studies performed on th the results of the binding studies performed on
rboxy-terminal deletion analogues using radioactive δ
d μ agonists as receptor labels that were mentioned
rlier (45).
In contrast to the GPI, MVD, and all other mus and μ agonists as receptor labels that were mentioned

are present in the GPI and MVD, which appear to opioid receptors. No opioid agonist activity on the RVD tissues (169). however, agonist activities have been reported for
The opioid activities of β -endorphin on the MVD and [Met⁵]-enkephalin, [Leu⁵]-enkephalin, and other peptide
GPI are critically dependent on the [Met substitution for Lys⁹ or Phe¹⁸ in [Phe²⁷, Gly³¹]- β_h -endor-
phin had very little effect (165). In fact, the GPI is assay is disputed by many researchers, and evidence
strikingly insensitive to changes in the β Single residue deletions or substitutions in this region receptors, so that opioids which stimulate signal trans-
have even less effect $(9, 97, 98)$.
[Met⁵]-enkephalin is very potent in MVD assays, but terestingly, thi 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 contrac 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 peptides (90, 137). These receptors have been termed ϵ 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 RVD is mediated by receptors that exhibit a puzzl
selectivity for β_h -endorphin among the known opi
peptides (90, 137). These receptors have been termed
opioid receptors. No opioid agonist activity on the R
was original 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, [Met⁵]-enke peptides (90, 137). These receptors have been termed ϵ -
opioid receptors. No opioid agonist activity on the RVD
was originally reported for morphine, [Met⁵]-enkephalin,
or dynorphin A(1–13), even at high concentratio opioid receptors. No opioid agonist activity on the RVD
was originally reported for morphine, [Met⁵]-enkephalin,
or dynorphin A(1-13), even at high concentrations, and
[D-Ala²,D-Leu⁵]-enkephalin was only weakly acti was originally reported for morphine, [Met⁵]-enkephalin,
or dynorphin A(1-13), even at high concentrations, and
[D-Ala²,D-Leu⁵]-enkephalin was only weakly active (72,
137). Morphine antagonizes the effects of β -e or dynorphin A(1–13), even at high concentrations, and
[D-Ala²,D-Leu⁵]-enkephalin was only weakly active (72,
137). Morphine antagonizes the effects of β -endorphin
although with less potency than naloxone. More rec [D-Ala²,D-Leu⁵]-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 f 137). Morphine antagonizes the effects of β -endorphin
although with less potency than naloxone. More recently,
however, agonist activities have been reported for
[Met⁵]-enkephalin, [Leu⁵]-enkephalin, and other pept although with less potency than haloxone. More recently,
however, agonist activities have been reported for
[Met⁵]-enkephalin, [Leu⁵]-enkephalin, and other peptide
products of proenkephalin A (but not the *x*-selectiv [Met⁵]-enkephalin, [Leu⁵]-enkephalin, and other peptide
products of proenkephalin A (but not the *k*-selective
proenkephalin B products) in the presence of mixtures
of protease inhibitors (30, 133). In fact, the 50% i 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% inhibi-
tory concentration (IC₅₀) values for the enkephal proenkephalin B products) in the presence of mixtures
of protease inhibitors (30, 133). In fact, the 50% inhibi-
tory concentration (IC₅₀) values for the enkephalins in
this assay (approximately 500 nM) were similar to of protease inhibitors (30, 133). In fact, the 50% inhibitory concentration (IC₅₀) values for the enkephalins in this assay (approximately 500 nM) were similar to those previously observed for β_c -endorphin in the abs tory concentration (IC₅₀) values for the enkephalins is
this assay (approximately 500 nM) were similar to thos
previously observed for β_c -endorphin in the absence oprotease inhibitors, but still an order of magnitude 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 (IC₅₀ = 40 nM). 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 (IC₅₀ = 40 nM). Furthermore,
the necessity to invoke a new type of opioi protease inhibitors, but still an order of magnitude higher
than that of β_h -endorphin (IC₅₀ = 40 nM). Furthermore,
the necessity to invoke a new type of opioid receptor in
order to explain the activities of opioids i than that of β_h -endorphin (IC₅₀ = 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 evidenc 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 activiti 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 (108 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 suggesting that μ -opiola 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 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 s 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 th MVD assays are attributed to differences in the numbe
of spare receptors present in each tissue; fewer spa
receptors in the RVD make this tissue more sensitive
the intrinsic activities of opioids that bind to the
receptors 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 transd receptors in the RVD make this tissue more sensitive the intrinsic activities of opioids that bind to thes receptors, so that opioids which stimulate signal transduction poorly behave as antagonists in this tissue. In tere the intrinsic activities of opioids that bind to these receptors, so that opioids which stimulate signal trans-
duction poorly behave as antagonists in this tissue. In-
terestingly, this intrinsic activity can be altered b receptors, so that opioids which stimulate signal traduction poorly behave as antagonists in this tissue.
terestingly, this intrinsic activity can be altered by va
ing the calcium ion concentrations used in the tiss
bath f was originally reported for morphine, [Met²]-enkephalin, or dynorphin A(1-13), even at high concentrations, and [D-Ala²₁-D-kephalin was only weakly active (72, [37). Morphine antagonizes the effects of β -endorphi terestingly, this intrinsic activity can be altered by vary-
ing the calcium ion concentrations used in the tissue
bath for the RVD assay, and at low calcium ion concen-
trations, morphine behaves as a partial agonist (66b ing 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
 trations, 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 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 amon 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
impor 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 structura 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 the known opiold peptides makes the KVD 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

302 TAYLOR AND KAISER
also makes the reported activities of many β -endorphin
analogues difficult to interpret in terms of their activities $\frac{Opiate}{a}$ 302 TAYLOR AN
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 TAYLOR AND
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 no 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 b 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 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 importan 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 importan and, as in the case of the enkephalins, inactive analogumay have higher potencies when they are protected fro
proteolytic degradation. Nevertheless, the importance
the amino-terminal segment of β_h -endorphin has bee
cle 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, 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-
Tyr¹]- β_h -endorphin, and β_h -endorphin(6–31 the amino-terminal segment of β_h -endorphin has been
clearly demonstrated: N^{α} -acetyl- β_h -endorphin, [Des-
Tyr¹]- β_h -endorphin, and β_h -endorphin(6–31) are all in-
active, and the N^o-acetyl derivative in p clearly demonstrated: N^{α}
Tyr¹]- β_h -endorphin, and β_h
active, and the N"-acetyl d
likely to be more suscepti
unmodified peptide (138).
The role of the carboxy te The role of the Carboxy terminus has been investigated
tive, and the N^a-acetyl derivative in particular is not
ely to be more susceptible to proteolysis than the
modified peptide (138).
The role of the carboxy terminus

active, and the N["]-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 car 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 ana-
logues of β_p -endorphin (138). The role of the carboxy terminus has been investigated
by Schulz et al. using carboxy-terminal deletion ana-
logues of β_p -endorphin (138). Relatively potent effects
(50 nM \leq IC₅₀ \leq 200 nM) were observed for and deletion analogues consisting of residues 1-29, residues 1-27, residues 1-25, or residues 1-23. Shorter pep by Schulz et al. using carboxy-terminal deletion ana-
logues of β_p -endorphin (138). Relatively potent effects
(50 nM \leq IC₅₀ \leq 200 nM) were observed for β_p -endorphin
and deletion analogues consisting of res logues of β_p -endorphin (138). Relatively potent effection and ϵ IC₅₀ \leq 200 nM) were observed for β_p -endorph and deletion analogues consisting of residues 1–29, redues 1–27, residues 1–25, or residues 1–23. $(50 \text{ nM} \leq IC_{50} \leq 200 \text{ nM})$ were observed for β_{p} -endorphin
and deletion analogues consisting of residues 1–29, resi-
dues 1–27, residues 1–25, or residues 1–23. Shorter pep-
ides, however, had potencies on the R and deletion analogues consisting of residues 1-29, resi-
dues 1-27, residues 1-25, or residues 1-23. Shorter pep-
tides, however, had potencies on the RVD which dimin-
ished rapidly with decreasing length: the IC₅₀ of dues 1-27, residues 1-25, or residues 1-23. Shorter pep-
tides, however, had potencies on the RVD which dimin-
ished rapidly with decreasing length: the IC₅₀ of β_{p} -
endorphin(1-21) was greater than 2 μ M, that fo ides, nowever, nad potencies on the KVD which dimin-
ished rapidly with decreasing length: the IC_{50} of β_p -
endorphin(1–21) was greater than 2 μ M, that for β_p -
endorphin(1–19) was greater than 50 μ M, and the be measured. Taplet measured. The measured rapidly with decreasing length. the $1C_{50}$ of p_p -
endorphin(1-21) was greater than 50μ M, and the IC₅₀
values of shorter analogues were apparently too high to
be measured endorphin(1–21) was greater than 2 μ M, that for
endorphin(1–19) was greater than 50 μ M, and the I
values of shorter analogues were apparently too high
be measured. Essentially the same results were obtain
by Huidobr endorphin(1-19) was greater than 50 μ M, and the I
values of shorter analogues were apparently too high
be measured. Essentially the same results were obtain
by Huidobro-Toro et al., who also showed that β_h -end
phinvalues 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 -endor-
phin-(1-5)-(16-31) was inactive at least up to a c be measured. Essentially the same results were obtained
by Huidobro-Toro et al., who also showed that β_h -endor-
phin-(1-5)-(16-31) was inactive at least up to a concen-
tration of 15 μ M (72). These authors suggested phin-(1-5)-(16-31) was inactive at least up to a concentration of 15 μ M (72). These authors suggested, on this me basis, that the opioid receptor in the RVD recognizes site two distinct sites in the β -endorphin mole tration of 15 μ M (72). These authors suggested, on this basis, that the opioid receptor in the RVD recognizes stwo distinct sites in the β -endorphin molecule, one at the amino terminus and one in residues 19–23. The between these two receptor in the KVD 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
b 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 confo suggested that the region of the β -endorphin molecule
between these two recognition sites is important in re-
stricting the β -endorphin molecule to the correct confor-
mation for expressing activity through this opi between these two recognition sites is important in stricting the β -endorphin molecule to the correct confination for expressing activity through this opioid rector, and they proposed that this conformation involue the stricting the β -endorphin momation for expressing activities, and they proposed that the helical structure describing residues $13-24$ (154).
The six peptide models ation for expressing activity through this opioid recep-
one, and they proposed that this conformation involves me
e helical structure described by Wu et al. for β -endor-
sulin residues 13-24 (154).
The six peptide mod

tor, and they proposed that this comformation involves
the helical structure described by Wu et al. for β-endor-
phin residues 13–24 (154).
The six peptide models of β-endorphin have been
examined for their activities in phin residues 13–24 (154). Strategy in the basis of β -endorphin have been phexamined for their activities in GPI and RVD assays of (table 4). Their potencies on the GPI showed no apparent the relation to the basis of t The six peptide models of β -endorphin have been phexamined for their activities in GPI and RVD assays of (table 4). Their potencies on the GPI showed no apparent the relation to the basis of their design. In particular examined for their activities in GPI and RVD assays of t
(table 4). Their potencies on the GPI showed no apparent the
relation to the basis of their design. In particular, the rev
nonamphiphilic peptide 4 was the most pote (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 relation to the basis of their design. In particular, the reversion
amphiphilic peptide 4 was the most potent of the other model peptides tested in this assay, and peptides 1 and this
2 had potencies that differed by an o nonamphiphilic peptide 4 was the most potent of the otlended peptides tested in this assay, and peptides 1 and the 2 had potencies that differed by an order of magnitude he despite their similar design. This behavior is s 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 analog 2 had potencies that differed by an order of magnitud despite their similar design. This behavior is similar the results obtained for other β -endorphin analogues, a described earlier. Since these results do not show a despite their similar design. I his behavior is similar to equivalent the results obtained for other β -endorphin analogues, as midescribed earlier. Since these results do not show a direct tie correlation with the pote described earlier. Since these results do not show a direct ties
correlation with the potencies in μ -opioid receptor bind-
ing assays, it is possible that some of the analogues are phi
interacting with other types of o correlation with the potencies in μ -opioid receptor bind-
ing assays, it is possible that some of the analogues are
phinteracting with other types of opioid receptors in the
In GPI. This is a question that has not gene ing 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 PI. This is a question that has not generally been
dressed, and it is critical to the interpretation of the
served potencies in terms of potential receptor-bound
nformations of β -endorphin.
In contrast, the potencies o addressed, and it is critical to the interpretation of the observed potencies in terms of potential receptor-boun conformations of β -endorphin.
In contrast, the potencies of the β -endorphin moderphides on the RVD ha

TABLE 4
endorphin and pe **KAISER**
COPIACE 4
Opiate agonist activities of β_h *-endorphin and peptides 1–6 in GPI and*
RVD assays in vitro **TABLE 4**
of B_h-endorphin and peptide
*RVD assays in vitro*⁴

	IC_{50} (nM) ^b							
Peptide	GPI	RVD						
β_h -Endorphin	$61 \pm 13^{\circ}$	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.

phin-(1-5)-(16-31) was inactive at least up to a concen-
tration of 15 μ M (72). These authors suggested, on this ment for the importance of a carboxy-terminal binding
basis, that the opioid receptor in the RVD recogniz Feptide 6 Not tested 206 ± 51
 C Data are compiled from refs. 11, 12, 126, and 145-147.

^b Concentration causing 50% of maximal inhibition of electricall

stimulated contractions.

Concerning the probable conformatio Mean \pm SE.

dorphin that is required for full agonist activity upon

dorphin that is required for full agonist activity upon

binding to the opioid receptor in this tissue. In view of Effects at high concentrations were not haloxone reversible.

mation concerning the probable conformation of β -en-

dorphin that is required for full agonist activity upon

binding to the opioid receptor in this tissue mation concerning the probable conformation of β -en-
dorphin 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 activitie mation concerning the probable conformation of β -en-
dorphin 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 activitie dorphin 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 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 shorte 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 ⁴ Effects at high concentrations were not naloxone reversible.

mation concerning the probable conformation of β -en-

dorphin that is required for full agonist activity upon

binding to the opioid receptor in this ti for carooxy-terminal detector analogues shorter than p_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 the activities of the peptide models in relation to the the activities of the peptide models in relation to
general principles of their design provide a strong are
ment for the importance of a carboxy-terminal bind
site for the opioid receptors of the RVD. In particu
the nonamp 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 contrac-
tio ment for the importance of a carboxy-terminal binding
site for the opioid receptors of the RVD. In particular,
the nonamphiphilic peptide 4 inhibited RVD contrac-
tions only at high concentrations and in a nonopioid
manner site for the opioid receptors of the RVD. In particul
the nonamphiphilic peptide 4 inhibited RVD contra
tions only at high concentrations and in a nonopio
manner, possibly by a postsynaptic mechanism simi
to that of other the nonamphiphilic peptide 4 inhibited RVD contrations only at high concentrations and in a nonopion
manner, possibly by a postsynaptic mechanism simi
to that of other peptide hormones (121, 122). The inhi
tory effects of tions only at high concentrations and in a nonopioid
manner, possibly by a postsynaptic mechanism similar
to that of other peptide hormones (121, 122). The inhibi-
tory effects of peptide 4 could not be reversed by nalox-
 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 result mechanism such as proteolytic degradation. These results suggested the necessity for an amphiphilic helical structure in the carboxy-terminal segment of β -endor-
phin for potent agonist activity on the opioid receptors one, 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 mechanism such as proteolytic degradation. These results suggested the necessity for an amphiphilic helical structure in the carboxy-terminal segment of β -endor-
phin for potent agonist activity on the opioid receptors sults suggested the necessity for an amphiphilic helical
structure in the carboxy-terminal segment of β -endor-
phin for potent agonist activity on the opioid receptors
of the RVD. The relatively high activities observe structure in the carboxy-terminal segment of β -endor-
phin 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 naloxon phin 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 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 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 other mechanisms of inactivation, strongly support
this argument. Even with a left-handed amphiphilic
helix included in its design, peptide 5 was approximate
equipotent to β_p -endorphin, although it exhibited
mixed agon this argument. Even with a left-handed amphiphili
helix included in its design, peptide 5 was approximated
equipotent to β_p -endorphin, although it exhibited
mixed agonist-antagonist behavior. The identical act
ties of helix included in its design, peptide 5 was approximate equipotent to β_p -endorphin, although it exhibited mixed agonist-antagonist behavior. The identical actities of peptides 3 and 6 further defined the carbox termina equipotent to β_p -endorphin, although it exhibited
mixed agonist-antagonist behavior. The identical activ
ties of peptides 3 and 6 further defined the carbox
terminal receptor binding site by eliminating the hydr
philic mixed agonist-antagonist behavior. The identical activities of peptides 3 and 6 further defined the carboxy-
terminal receptor binding site by eliminating the hydro-
philic linking region in residues $6-12$ from considera deletion analogues of the sential particle in the estimating the hydro-
philic linking region in residues 6–12 from consideration.
In combination with the studies of carboxy-terminal
deletion analogues, these results ther philic linking region in residues 6–12 from consideration.
In combination with the studies of carboxy-terminal
deletion analogues, these results therefore limit the es-
sential part of this binding site to β -endorphin In combination with the studies of carboxy-terminal
deletion analogues, these results therefore limit the es-
sential part of this binding site to β -endorphin residues
13-23. A comparison of the linear sequences of the 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 -endor sential 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

PHARMACOLOGICAL REVIEW

PHARMACOLOGICAL REVIEWS

STRUCTURAL CHARACTERIZATION
in an extended conformation, there would be no obvious
relationship between structure and opioid activity in the STRUCTURAL CHARACTERIZATION
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 an **RUDE STRUCTURAL CHARACTERIZATION**
 RVD assay. However, peptide 1 has a potency similar to

that of β_h -endorphin and significantly higher than that the 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 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 context, the phenylalanine side chain in residue position and the RVD opioid receptor may have additional specificity for RVD opioid receptor may have additional specificity for RVD opioid receptor may have additional spe helix of the surface of this helix. In this
certain side chains on the surface of this helix. In this fig
context, the phenylalanine side chain in residue position
18, which forms a prominent feature of the surface of the 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 to the twist of the surface 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 pep-
tide 1, but re 18, which forms a prominent reature of the surface of the original
helix on its hydrophobic face, has been proposed as a 1
likely candidate (146). This residue is conserved in pep-
tide 1, but replaced by a leucine residue likely candidate (146). This residue is conserved in pep-
tide 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 the interpretation of the state 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.
 pepticies 3 and 6, and by a tryptophan residue in peptice
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 rel 2, which has an even lower potency similar to that of the
enkephalins in the presence of proteolytic inhibitors. dro
Alternatively, these potency differences may be related α -
to structure on the carboxy-terminal side enkephains in the presence of proteolytic inhibit
Alternatively, these potency differences may be relate
to structure on the carboxy-terminal side of this esser
binding site, and the high potency of peptide 1 may
fortuito Alternatively, these potency differences may be related α -
to structure on the carboxy-terminal side of this essential enco
binding site, and the high potency of peptide 1 may be
fortuitous. For example, the lower pote to structure on the carooxy-ter-
binding site, and the high pot
fortuitous. For example, the lephin compared to β_h -endorphi
residues 27 and 31 only (72).

relationship between structure and opioid activity in the RVD also exhibited a clear relationship between their
RVD assay. However, peptide 1 has a potency similar to amphiphilic design and physicochemical properties on
t that of β_h -endorphin and significantly higher than that the one hand and their rates of action and susceptibility
of the other active peptide models, suggesting that the to proteolysis on the other hand. The time cours The actions of the mode! peptides on the GPI and ON OF PEPTIDE HORMONES 303

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 act 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 prote 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 prote RVD also eximulted 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 th the one hand and their rates of action and susceptibility 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 tis 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 RVD responses to some of these peptides are shown
figure 5. The effects of β_h -endorphin on both tiss
reached a maximum within about 2–4 min after addit
of doses close to its IC_{50} value. Similar doses of pepti
1 and 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 IC₅₀ value. Similar doses of peptides
1 and 2, which interacted very strongly with pho reached a maximum within about $2-4$ mm after addition
of doses close to its IC_{50} value. Similar doses of peptides
1 and 2, which interacted very strongly with phospho-
lipids and the air-water interface and self-assoc 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 re 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 a lipids 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 h 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 amphiphilic properties because of the shape of the hy-
drophobic domain formed by their residues 13–29 in an
 α -helical conformation, exhibited similar behavior to β -
endorphin in that their maximal effects were achi amphiphilic properties because of the shape of the hy-
drophobic domain formed by their residues 13–29 in an
 α -helical conformation, exhibited similar behavior to β -
endorphin in that their maximal effects were achi drophobic 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. Howev 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 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.

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 ele 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 elect 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 elec 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 elec 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 thi

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idly causing a reversal of its inhibitory effects (figure 5; (
and ref. 137). The same is true to a lesser extent for the t TAYLOR AND 1
idly causing a reversal of its inhibitory effects (figure 5; (1-
and ref. 137). The same is true to a lesser extent for the the
GPI, which can inactivate the enkephalins quite rapidly TAYLOR AND

idly causing a reversal of its inhibitory effects (figure 5; (1)

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 proteoly idly causing a reversal of its inhibitory effects (figure 5; (1-5 and ref. 137). The same is true to a lesser extent for the tend GPI, which can inactivate the enkephalins quite rapidly L in the absence of proteolytic enz and ref. 137). The same is true to a lesser extent for the ter GPI, which can inactivate the enkephalins quite rapidly lin the absence of proteolytic enzyme inhibitors, but does int not degrade β -endorphin so readily. 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 in the absence of proteolytic enzyme inhibitors, but does inactivation displayed by these model peptides in both
assays (table 2) corresponded to their resistance to pro-
teolysis in the presence of whole rat brain homogenates.
In agreement with its lack of amphiphilicity and mor assays (table 2) corresponded to their resistance to p
teolysis in the presence of whole rat brain homogenat
In agreement with its lack of amphiphilicity and mo
rapid degradation by the rat brain enzymes, the actio
of pept teolysis in the presence of whole rat brain homogenat
In agreement with its lack of amphiphilicity and m
rapid degradation by the rat brain enzymes, the action
of peptide 4 on both the GPI and, at high concentration
the RV In agreement with its lack of amphiphilicity and more
rapid degradation by the rat brain enzymes, the actions β
of peptide 4 on *both* the GPI and, at high concentrations, is
the RVD were quite rapidly reversed with ti rapid degradation by the rat brain enzymes, to feptide 4 on *both* the GPI and, at high conce the RVD were quite rapidly reversed with tim ing that the structure of this model peptide with no protection from proteolytic en **EXAMPLE 1 STATE OF THE STATE OF STATE 1**

ing that the structure of

With no protection from

E. Analgesic Activities

The analgesic activities

In the structure of this model peptide provides it
th no protection from proteolytic enzymes.
Analgesic Activities
The analgesic activities of the opiates and opioid pep-
les are mediated by opioid receptors in the central with no protection from proteolytic enzymes.

E. Analgesic Activities

The analgesic activities of the opiates and opioid pep-

tides are mediated by opioid receptors in the central

nervous system and are usually accompan E. Analgesic Activities

The analgesic activities of the opiates and opioid pep-

tides are mediated by opioid receptors in the central

nervous system and are usually accompanied by a variety

of naloxone-reversible effe E. Analgesic Activities of the opiates and opioid perides are mediated by opioid receptors in the central nervous system and are usually accompanied by a varie of naloxone-reversible effects including catalepsy, hyperactiv The analgesic activities of the opiates and opioid pep-
tides are mediated by opioid receptors in the central
nervous system and are usually accompanied by a variety
of naloxone-reversible effects including catalepsy, hyp tides are mediated by opioid receptors in the central
nervous system and are usually accompanied by a variety
of naloxone-reversible effects including catalepsy, hyper-
activity, "wet-dog" shakes, and Straub tail (13, 73, nervous system and are usually accompanied by a varie
of naloxone-reversible effects including catalepsy, hype
activity, "wet-dog" shakes, and Straub tail (13, 73, 149
Intracerebrally administered β_h -endorphin causes p of naloxone-reversible effects including catalepsy, hyper
activity, "wet-dog" shakes, and Straub tail (13, 73, 149)
Intracerebrally administered β_h -endorphin causes poten
and relatively long-lasting analgesia in a vari morphine is 18–33 times less potent in different analgesic 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
 mals, including humans (18, 48, 102). In comparison, of morphine is 18–33 times less potent in different analgesic rassays (102), and even high doses of the enkephalins policit only a weak and transient effect when adminis 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 poten assays (102), and even high doses of the enkephalins pelicit only a weak and transient effect when administered altracerebroventricularly (6, 23, 25). The low potency of T
the enkephalins in these assays is certainly due 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 st 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, indi-
cating that selectivity for opioid receptor types and the enkephalins in these assays is
to their rapid degradations in v
analogues have lower potencies the
cating that selectivity for opioid
intrinsic activity is also important
A great many β -endorphin analo their rapid degradations in vivo, but many stable in alogues have lower potencies than β -endorphin, inditing that selectivity for opioid receptor types and/or at trinsic activity is also important. A great many β -en analogues have lower potencies than β -endorphin, indi-
cating that selectivity for opioid receptor types and/or
afferentinsic activity is also important.
A great many β -endorphin analogues have been tested to a
for

cating that selectivity for opioid receptor types and/or
intrinsic activity is also important.
A great many β -endorphin analogues have been tested to
for their analgesic potencies, usually in mice assayed by a
the hotintrinsic activity is also important.
A great many β -endorphin analogues have been test
for their analgesic potencies, usually in mice assayed
the hot-plate method or in rats assayed by the tail-fli
method, after intra A great many β -endorphin analogues have been tested to for their analgesic potencies, usually in mice assayed by als the hot-plate method or in rats assayed by the tail-flick (4) method, after intracerebroventricular a 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 the hot-plate method or in rats assayed by the tail-flick (45 method, after intracerebroventricular administration.
The results indicate that the whole peptide sequence is the involved in determining this activity, but aga 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, a 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 involved in determining this activity, but again the of proteolytic inactivation is difficult to assess, an time courses of the effects are not generally prese so that a detailed interpretation of the results i possible. of proteolytic inactivation is difficult to assess, and the courses of the effects are not generally presente so that a detailed interpretation of the results is n possible. N^o-acetyl- β_h -endorphin (32), [D-Tyr¹]- $\$ time courses of the effects are not generally presented, long
so that a detailed interpretation of the results is not duc
possible. N^o-acetyl- β_h -endorphin (32), [D-Tyr¹]- β_c -en-pot
dorphin (163), [Des-Gly²]- β so that a detailed interpretation of the results is not due possible. N"-acetyl- β_h -endorphin (32), [D-Tyr¹]- β_c -en-
dorphin (163), [Des-Gly²]- β_c -endorphin (98), and β_c -en- [G
dorphin(6-31) (99) all have lit possible. N^o-acetyl- β_h -endorphin (32), [D-Tyr¹]- β_c -en-podorphin (163), [Des-Gly²]- β_c -endorphin (98), and β_c -en-[G dorphin (6-31) (99) all have little or no activity, and a 3.' variety of other modificati dorphin (163), [Des-Gly²]- β_c -endorphin (98), and β_c -en-
dorphin, 2.45 (95); [Trp²⁷]- β_h -endorphin,
dorphin(6–31) (99) all have little or no activity, and a 3.72 (96); [Tyr³¹]- β -endorphin, 1.16 (160); [Phe dorphin(6-31) (99) all have little or no activity, and a 3.72 (S
variety of other modifications of single residues in the β -endor
enkephalin segment of β -endorphin drastically reduce its 2.25 (S
potency, thus establ variety of other modifications of single residues in then the enkephalin segment of β -endorphin drastically reduce i potency, thus establishing the vital importance of the region (161, 163). Residues 6-31 of β -endor 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 oth potency, thus establishing the vital importance of this energion (161, 163). Residues 6-31 of β -endorphin show (16
less sensitivity to single residue changes or other modi-
fications including deletions (9, 10, 94-96, region (161, 163). Residues 6–31 of β -endorphin show
less sensitivity to single residue changes or other modi-
fications including deletions (9, 10, 94–96, 98, 160, 165)
and some such analogues have greater potency tha 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*). Howe fications 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 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,

TAYLOR AND KAISER
(figure 5; (1-5)-(28-31) have only 0.003 and 0.001 times the po-
nt for the tency of β_c -endorphin, respectively (99). D KAISER
(1-5)-(28-31) have only 0.003 and 0.001
tency of β_c -endorphin, respectively (99).
Lee and Smith have proposed a model fo

not degrade β -endorphin so readily. The resistance to the apparent involvement of the whole β -endorphin molinactivation displayed by these model peptides in both ecule in determining its analgesic potency and involv activity, "wet-dog" shakes, and Straub tail (13, 73, 149). potent antagonist (4 times more potent than naloxone)
Intracerebrally administered β_h -endorphin causes potent for β_h -endorphin-induced analgesia (66a). This KAISER
-5)-(28–31) have only 0.003 and 0.001 times the po-
ncy of β_c -endorphin, respectively (99).
Lee and Smith have proposed a model for β -endorphin
teractions with the analgesic receptor, which explains (1-5)-(28-31) have only 0.003 and 0.001 times the po-
tency of $β_c$ -endorphin, respectively (99).
Lee and Smith have proposed a model for $β$ -endorphin
interactions with the analgesic receptor, which explains
the apparen (1-5)-(28-31) have only 0.003 and 0.001 times the po
tency of β_c -endorphin, respectively (99).
Lee and Smith have proposed a model for β -endorphin
interactions with the analgesic receptor, which explains
the apparen tency 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 mol-
ecule 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 mol-
ecule in determining its analgesic potency and invo interactions with the analgesic receptor, which explains
the apparent involvement of the whole β -endorphin mol-
ecule in determining its analgesic potency and involves
 α -helical structure in the carboxy-terminal reg 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 consis 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 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 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 t 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 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(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(terminus of β -endorphin interacts with the lipid particle and β -endorphin-induce analgesia by β_c -endorphin(6-31) and β_c -endorphin(2031), but not by β_h -endorphin(1-15) (88), is cited as ev dence for the carb 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 t 31), but not by β_h -endorphin(1–15) (88), is cited as evi-31), but not by β_h -endorphin (1-15) (88), is cited as evi-
dence for the carboxy-terminal site, and α -helical struc-
ture in this region is suggested in view of the helix-
stabilizing effects of phosphatidyl serine dence 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 ture 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 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 nalox 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 acti 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 bind for β_h -endorphin-induced analgesia (66a). This action 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 -end was predicted on the basis of the relatively tight bindin
of this peptide to ³H- β_h -endorphin-labelled receptors i
rat brain (0.3 times as potent as β_h -endorphin in com
petitive displacement assays), compared to it 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 the carbox perminal site, and α -helical structure in this region is suggested in view of the helix-
ture in this region is suggested in view of the helix-
stabilizing effects of phosphatidy series and cerebroside
sulf petitive 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 algesic 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 recept 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
a 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 o 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 recept (45). rboxy-terminal binding site has apparently been lost
allow even nonproductive binding to these receptors,
so in agreement with the brain receptor binding assays
5).
In recent years, Li and coworkers have investigated
e an

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 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-res (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
l 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 pro-
duc the analgesic potencies of more than so 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 pro-
du modifications in an effort to develop more potent alonger lasting effects. Very few modifications have p duced more potent peptides. These peptides and the potencies relative to β_h -endorphin are as follo [Gln⁸]- β_h longer lasting effects. Very few modifications have pro-
duced more potent peptides. These peptides and their
potencies relative to β_h -endorphin are as follows:
 $[G\ln^8]$ - β_h -endorphin, 2.45 (95); $[Trp^{27}]$ - β_h -endo potencies relative to β_h -endorphin are as follows:

[Gln⁸]- β_h -endorphin, 2.45 (95); [Trp²⁷]- β_h -endorphin,

3.72 (96); [Tyr³¹]- β -endorphin, 1.16 (160); [Phe²⁷,Gly³¹]-
 β -endorphin, 1.19 (165); [Gly [Gln⁸]- β_h -endorphin, 2.45 (95); [Trp²⁷]- β_h -endorphin, 3.72 (96); [Tyr³¹]- β -endorphin, 1.16 (160); [Phe²⁷,Gly³¹]- β -endorphin, 1.19 (165); [Gly³¹]- β_h -endorphin amide, 2.25 (97); [Gly³¹]- β -end 3.72 (96); [Tyr³¹]- β -endorphin, 1.16 (160); [Phe²⁷,Gly³¹]-
 β -endorphin, 1.19 (165); [Gly³¹]- β _h-endorphin amide,

2.25 (97); [Gly³¹]- β -endorphin-Gly, 2.17 (97); [Gly³¹]- β -

endorphin-Gly-Gly a β -endorphin, 1.19 (165); [Gly³¹]- β _h-endorphin amide
2.25 (97); [Gly³¹]- β -endorphin-Gly, 2.17 (97); [Gly³¹]- β
endorphin-Gly-Gly amide, 1.08 (93); [Gln⁸,Gly³¹]- β _h-en
(162); [Ala⁸,Gln³¹]- β 2.25 (97); [Gly³¹]- β -endorphin-Gly, 2.17 (97); [Gly³¹]- β -endorphin-Gly-Gly amide, 1.08 (93); [Gln⁸,Gly³¹]- β_h -en-
(162); [Ala⁸,Gln³¹]- β_h -endorphin, 1.16 (162); and
[Val⁸,Gln³¹]- β_h -endorphin, endorphin-Gly-Gly amide, 1.08 (93); $[Gln^8, Gly^{31}]$ - β_h -
(162); [Ala⁸,Gln³¹]- β_h -endorphin, 1.16 (162); a
[Val⁸,Gln³¹]- β_h -endorphin, 1.21 (162). All of these po
tides differ from β_h -endorphin in one or more (162); [Ala⁸,Gln³¹]- β_h -endorphin, 1.16 (162); an [Val⁸,Gln³¹]- β_h -endorphin, 1.21 (162). All of these pep tides differ from β_h -endorphin in one or more of just three different residue positions: 8, 27, and [Val⁸,Gln³¹]- β_h -endorphin, 1.21 (162). All of these pep-
tides differ from β_h -endorphin in one or more of just
three different residue positions: 8, 27, and 31; modifi-
cations of other residues always resulted tides differ from β_h -endorphin in one or more of just
three different residue positions: 8, 27, and 31; modifi-
cations of other residues always resulted in lower anal-
gesic potencies. Furthermore, the effects of diff three different residue positions: 8, 27, and 31; modifications of other residues always resulted in lower anal-
gesic potencies. Furthermore, the effects of different
modifications were not always additive. For example,
s

PHARMACOLOGICAL REVIEWS

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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 -**STRUCTURAL CHARACTERIZATI**
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 $[D-Thr^2, D-Lys^9, Phe^{27}, Gly^{31}]$ of $[Phe^{27},Gly^{31}]-\beta_h$ -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 $[D\text{-}Thr^2, D\text{-}Lys^9, Phe^{27},Gly^{31}]-\beta_h$ -endorphin was 0.42 t of $[Phe^{27}, Gly³¹] - \beta_h$ -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 $[D\text{-}Thr², D Lys⁹, Phe²⁷, Gly³¹] - \beta_h$ -endo position 9 gave an
algebra of colencies 0.21 times that of ρ_h -
endorphin in each case, but the potency of $[D\text{-}Thr^2]$,
Lys⁹,Phe²⁷,Gly³¹]- β_h -endorphin was 0.42 times that of β_h -
endorphin (9, 165). The inco Equal doses of $[{\rm D-AII}]$. $\beta_{\rm h}$ -endorphin was 0.42 times that of $\beta_{\rm h}$ -endorphin (9, 165). The incorporation of individual D-
amino acid residues into the β -endorphin structure has
not led to more prolonged ana Lys , The , Giy $1-\rho_h$ -endorphin was 0.42 times that of ρ_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 endorphin (5, 165). The incorporation of mulvidual b-
amino acid residues into the β -endorphin structure has
not led to more prolonged analgesic activities either.
Equal doses of $[D-Ala^2]-\beta_c$ -endorphin and β_c -endorph amino acid residues into the *p*-endorphin structure
not led to more prolonged analgesic activities eit
Equal doses of $[D-Ala^2]-\beta_c$ -endorphin and β_c -endorp
have the same potencies and similar lengths of act
and other in mot 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 c mave the same potencies and similar lengths of action, ρ -e
and other inversions of configuration caused a consider-
ges
able loss of analgesic potency with no prolonged activity wo
reported (9, 56, 65, 161, 163, 165).

able 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 info A study of the analgesic potencies of several of the V
naturally occurring β -endorphin structures proved to be for
much more informative than such assays of analogues la
chosen essentially at random (66). The potencies A study of the analgesic potencies of several of the winductureally occurring β -endorphin structures proved to be for a much more informative than such assays of analogues lar chosen essentially at random (66). The pot much more informative than such assays of analogues
chosen essentially at random (66). The potencies were po
ordered as follows: camel = equine > ostrich = human > fig
salmon I = turkey. However, despite many differences ordered as follows: camel = equine > ostrich = human > figus salmon I = turkey. However, despite many differences in all the amino acid sequences of these peptides, their potencies are all very high and lie within a narro salmon I = turkey. However, despite many differences it 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 times 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 th
times as potent as the salmon I and turkey endorphins. camer 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 The relatively high potency of the salmon peptide
particularly surprising in view of its structure at
carboxy terminus relative to β_h -endorphin residues
31. The critical role of these residues in the analge
activities 31. The critical role of these residues in the analgesic 31. The critical role of these residues in the analges
activities of the human peptide is apparently compe-
sated for by other aspects of the structure of β_s -endorph
I. The high potencies of the ostrich and salmon I pe 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 sated 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 I. The high potencies of the ostrich and salmon I peptides follow indicate that the hydrophilic segments of their structures corresponding to residues $6-12$ of the human peptide do not interact strongly with the receptor

on or peptide Hormones
nonhomologous to the mammalian structures. This sug-
gests a probable role for residues 6–12 as a linker congests a probable role for residues for residues on the mannibologous to the mannibolism structures. This suggests a probable role for residues 6-12 as a linker con-
necting essential binding sites in the β -endorphin st ON OF PEPTIDE HORMONES 30
nonhomologous to the mammalian structures. This su
gests a probable role for residues 6–12 as a linker con-
necting essential binding sites in the β -endorphin structure at the amino-terminal a 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 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 necting 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 ture at the amino-terminal and carboxy-terminal ends,

carboxy terminus relative to β_h -endorphin residues 26-
ide 5), these results provide a very convincing demon-
31. The critical role of these residues in the analgesic stration that the general features of their design 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 developreceptors of the RVD.
The multiple variations in the sequences of the natu
 β -endorphins and their high analgesic potencies s
gested that the approach of studying peptide moc
would be a particularly appropriate method f 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 s β -endorphins and their high analgesic potencies sug-
gested that the approach of studying peptide models
would be a particularly appropriate method for develop-
ing structure-activity relationships with this assay.
Whe gested that the approach of studying peptic
would be a particularly appropriate method for
ing structure-activity relationships with th
When peptides 1–6 were tested by the hot-plat
for analgesic activity in mice after int would be a particularly appropriate method for develop-
ing structure-activity relationships with this assay.
When peptides 1–6 were tested by the hot-plate method
for analgesic activity in mice after intracerebroventricu When peptides 1–6 were tested by the hot-plate method
for analgesic activity in mice after intracerebroventricu-
lar administration, peptides 3, 5, and 6 all produced
potent and long-lasting antinociception, as shown in
fi for analgesic activity in mice after intracerebroventricu-
lar 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
 lar 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
behavio potent and long-lasting antimocreeption, 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 v 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
struct 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
struc In view of the minimal sequence homology compared to
the natural endorphins of the modelled structures in
these active peptides (and especially the nonnatura
structures of the hydrophilic linking region in peptide 6
and t the natural endorphins of the modelled structures
these active peptides (and especially the nonnatu
structures of the hydrophilic linking region in peptid
and the left-handed amphiphilic α -helical region in p
tide 5), 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 pep-
tide 5), these results provide a very convincing d 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 tide 5), these results provide a very convincing demontide 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, fo stration 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 analges 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 poten 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 sinreceptors. Compared to β_h -endorphin, these peptides

analgesic effects of equal doses (3 μ g) of β_h -endorphin (O), peptide 3 (A), peptide 5 (D), or peptide 6 (\bullet) compared to saline controls (\Box), as a function of the time after their intracerebroventricular injection into different groups of mice (11, 126, 146).

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gle residue changes of a conservative nature compared to tide
the natural sequences (9, 10, 93, 94, 98, 165) and indicates of β TAYLOR AN
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 TAYLOR AND 1
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 im
unable to duplicate more specific f 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 n gle residue changes of a conservative nature compared to tid
the natural sequences (9, 10, 93, 94, 98, 165) and indicates of
that, as would be expected, the model structures are im-
unable to duplicate more specific featur 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
r 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 mo 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 could be identified and incorporated. Diffusion to the ing
receptors apparently required longer for the active model resi-
peptides, as they all produced their maximal effects at aro-
about 40-80 min after administration, 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
and 10–20 min for β_h -endorphin. This behavior ma peptides, as they all produced their maximal effects at arous about 40–80 min after administration, compared to about and 10–20 min for β_h -endorphin. This behavior may be a form result of greater nonspecific binding to 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 degradatio 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 ear 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 app 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 pep-
tides as a result of proteolytic degradation, N^{α 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 pep-
tides as a result of proteolytic degradation, N^{α and may be an additional factor reducing their apparent
potency. However, the loss of activity of the model pep-
tides as a result of proteolytic degradation, N^{α} -acetyla-
tion, or other mechanisms was also considerab potency. However, the loss of activity of the model perides as a result of proteolytic degradation, N^{α} -acety
tion, or other mechanisms was also considerably slow
than that of β_h -endorphin, even when equal doses we tides as a result of proteolytic degradation, N^{α} -acetylation, or other mechanisms was also considerably slowe
than that of β_h -endorphin, even when equal doses were
compared. This again corresponds to the relative on, or other mechanisms was also considerably slower
an that of β_h -endorphin, even when equal doses were
mpared. This again corresponds to the relative behav-
is of these peptides in the in vitro assays (table 2).
Pept

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). or Peptides 1, 2, and 4 produced no antinociceptive e compared. This again corresponds to the relative behaviors of these peptides in the in vitro assays (table 2). Other peptides 1, 2, and 4 produced no antinociceptive effects move to the same assay. This was expected for of iors 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
 Peptides 1, 2, and 4 produced no antinociceptive effects molument tested in the same assay. This was expected for of a peptide 4, which may not have been able to bind to the cite necessary opioid receptors and was certainl when tested in the same assay. This was expected for of a peptide 4, which may not have been able to bind to the citenecessary opioid receptors and was certainly more susceptible to inactivation by the endogenous enzymes peptide 4, which may not have been able to bind to the cinesessary opioid receptors and was certainly more sus-
reeptible to inactivation by the endogenous enzymes as a tice
result of its nonamphiphilic structure. However, necessary opioid receptors and was certainly more succeptible to inactivation by the endogenous enzymes as
result of its nonamphiphilic structure. However, the last of activities in peptides 1 and 2 was surprising, sin
th ceptible 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 re 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 repro-
duced the affinities for opioid receptors of β_h of activities in peptides 1 and 2 was surprising, since in
these peptides were resistant to degradation and repro-
duced the affinities for opioid receptors of β_h -endorphin fin
in binding assays, as well as its potent these peptides were resistant to degradation and repro-
duced 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 tha duced 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 a in binding assays, as well as its potent opioid activities c
in the GPI and RVD assays. It is possible that the shape
of the hydrophobic domain in the potential amphiphilic 1
 α helix formed by residues 13-31 of these m 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 inappr 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 ana 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 ass 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 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 tivity. All of the active peptides in this assay were de-
signed to form an amphiphilic α helix with a hydrophobic braidomain that twists around the helix in a clockwise direc-
tion, corresponding more precisely to the signed to form an amphiphilic α helix with a hydrophobic
domain that twists around the helix in a clockwise direc-
tion, corresponding more precisely to the corresponding
feature in β_h -endorphin. Another possibility tion, corresponding more precisely to the corresponding
feature in β_h -endorphin. Another possibility related to
this difference, though, is that the much stronger am-
phiphilic properties that arise from this feature o feature in β_h -endorphin. Another possibility related to cothis difference, though, is that the much stronger ampliphilic properties that arise from this feature of the a design of peptides 1 and 2 resulted in a strong 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 dif phiphilic properties that arise from this feature of the actions design of peptides 1 and 2 resulted in a strong nonspecific than adsorption to cell surfaces that prevented their diffusion vasor to receptors from the site design of peptides 1 and 2 resulted in a strong nonspecific than
adsorption to cell surfaces that prevented their diffusion vasod
to receptors from the site of injection. The marked dif-
simila
ferences in the time courses adsorption to cell surfaces that prev
to receptors from the site of injectiferences in the time courses of the
and 2 compared to peptides 3 and 6
are consistent with this explanation.
 $F_{\rm c}$ Feture Datida Madela of 8 Eas *Ferences in the time courses of the actions*
 F. Future Peptide Models of β *-Endorphin*
 F. Future Peptide Models of β *-Endorphin*

Now that the structural characterization d 2 compared to peptides 3 and 6 in the GPI and R^V
e consistent with this explanation.
Future Peptide Models of β -Endorphin
Now that the structural characterization of β -end
in in terms of the [Met⁵]-enkephalin

are consistent with this explanation. be

F. Future Peptide Models of β -Endorphin

Now that the structural characterization of β -endor-

in phin in terms of the [Met⁵]-enkephalin segment, the

hydrophilic link in F. Future Peptide Models of β -Endorphin
Now that the structural characterization of β -er
phin in terms of the [Met⁵]-enkephalin segment,
hydrophilic link in residues 6-12, and the carboxy
minal amphiphilic α he F. Future Peptide Models of β -Endorphin

Now that the structural characterization of β -endor-

phin in terms of the [Met⁵]-enkephalin segment, the

hydrophilic link in residues 6-12, and the carboxy-ter-

loop

mi Now that the structural characterization of β -endor-
phin in terms of the [Met⁵]-enkephalin segment, the resi
hydrophilic link in residues 6-12, and the carboxy-ter-
loop
minal amphiphilic α helix has been thoroug phin in terms of the [Met⁵]-enkephalin segment, the rest hydrophilic link in residues 6–12, and the carboxy-ter-
loominal amphiphilic α helix has been thoroughly tested by in the peptide modelling approach, it will b hydrophilic link in residues 6–12, and the carboxy-ter-
minal 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 ret

the natural sequences $(9, 10, 93, 94, 98, 165)$ and indicates of β -endorphin to diffuse rapidly to receptors with min-
that, as would be expected, the model structures are imal cell surface adsorption. Other features **D** KAISER
tides 3 and 6, for example, while reproducing the abilities
of β-endorphin to diffuse rapidly to receptors with minof KAISER
tides 3 and 6, for example, while reproducing the abilitie
of β -endorphin to diffuse rapidly to receptors with min
imal cell surface adsorption. Other features of the β D KAISER
tides 3 and 6, for example, while reproducing the abilities
of β -endorphin to diffuse rapidly to receptors with min-
imal cell surface adsorption. Other features of the β -
endorphin structure that still nee tides 3 and 6, for example, while reproducing the abilities
of β -endorphin to diffuse rapidly to receptors with min-
imal cell surface adsorption. Other features of the β -
endorphin structure that still need to be i tides 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 inve 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 imal 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 spec endorphin structure that still need to be investigated
include the length requirements of the hydrophilic link-
ing region and the possible importance of certain specific
residues such as the proline in position 13 and the include the length requirements of the hydrophilic link-
ing 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 ing 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 per-
 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 aromatic side chains on the helix surface in positions 18
and 27. In conjunction with the studies already per-
formed, enough information would then be available to
allow the rational design of peptides having greater poand 27. In conjunction with the studies aformed, enough information would then be allow the rational design of peptides having tencies than β -endorphin and more desira specificities and pharmacokinetic properties France Controller and Table 3-endorphin and more desirable

all pharmacokinetic properties.
 V. Calcitonin and CGRP

domain that twists around the helix in a clockwise direc-
tion, corresponding more precisely to the corresponding in vivo (84). CGRP is produced in specific areas of the
feature in β_h -endorphin. Another possibility rel specificities and pharmacokinetic properties.

V. Calcitonin and CGRP

The calcitonin and calcitonin gene-related peptide

(CGRP) amino acid sequences are encoded in the same 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 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 o 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 o 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 o (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 al 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 calother through the enzymatic processing of precumolecules occurs in a tissue-specific manner as a reof alternative processing of the mRNA. Mammalian citonin is produced in the thyroid and is release response to elevations i molecules occurs in a tissue-specific manner as a result
of alternative processing of the mRNA. Mammalian cal-
citonin is produced in the thyroid and is released in
response to elevations in the serum calcium concentra-
ti 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 (osteo citonin 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 response to elevations in the serum calcium concent
tion. By inhibiting the activity of bone-resorbing ce
(osteoclasts) and reabsorption of calcium and phospha
in the renal tubule, the hormone reduces calcium lev
to normal tion. 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 ident (osteoclasts) and reabsorption of calcium and phosphate
in the renal tubule, the hormone reduces calcium levels
to normal (148). Calcitonin receptors have been identi-
fied in cell membrane preparations of bone, kidney, t in the renal tubule, the hormone reduces calcium levels
to normal (148). Calcitonin receptors have been identi-
fied in cell membrane preparations of bone, kidney, the
central nervous system, and human cancer cell lines by to normal (148). Calcitonin receptors have been identi-
fied 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 fied 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 an 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
bindin 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 ac 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 and its analogues have been assessed in competit
binding assays to these receptors, as well as by measur
activation of the adenylate cyclase second messen
system in the same membrane preparations (except
brain) in vitro (1 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 hypocal-
ce activation of the adenylate cyclase second messenger
system in the same membrane preparations (except the
brain) in vitro (106), and by determining the hypocal-
cemic response to s.c. or i.v. injections of the hormone
in v 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 process brain) in vitro (106), and by determining the hypocal-
cemic 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
po cemic 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
ac 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 relea 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 poten 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 th actions are likely to be more related to its point
than the circulatory calcitonin. These include
vasodilatory effects and presynaptic actions on
similar to those of β -endorphin, as well as poter
in nociception and oth an the circulatory calcitonin. These include potent sodilatory effects and presynaptic actions on the RVD milar to those of β -endorphin, as well as potential roles nociception and other behaviors (19, 132). Calcitonin

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
bee 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
a 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 re-
sulting fr 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 re-
sulting from a disulfide bridge between cysteine residue been characterized (figure 7; ref. 104). All are 32 amin acid residues long, contain an amino-terminal loop r sulting from a disulfide bridge between cysteine residu
in positions 1 and 7, and have an amidated prolinesidue 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 sulting 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
i 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
o 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 calcit 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

STRUCTURAL CHARACTERIZATION OF PEPTIDE HORMONES

	$(s-11)$	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 (1

reduction of the disulfide bridge in human calcitonin 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 sul 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 sul of activity. A cyclic structure is nevertheless required
reduction of the disulfide bridge in human calcito
with or without methylation of the cysteine sulfhydi
decreases activity dramatically (129). The carboxy t
minus is reduction of the disulfide bridge in human calcitonin computed with or without methylation of the cysteine sulfhydryls in decreases activity dramatically (129). The carboxy terminus is also essential, and deletion of the c with or without methylation of the cysteine sulfhy
decreases activity dramatically (129). The carboxy
minus is also essential, and deletion of the cark
terminal proline amide, leaving either an amide o
free acid in positio creases activity dramatically (129). The carboxy ter-
inus is also essential, and deletion of the carboxy-
grained proline amide, leaving either an amide or the
level and position 31, diminishes activity (142).
Studies of

minus 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). n
Studies of deletion analogues of porcine calcitonin (
have terminal proline amide, leaving either an amide or there acid in position 31, diminishes activity (142) .
Studies of deletion analogues of porcine calcitonic have demonstrated that partial structures are generall devoid free acid in position 31, diminishes activity (142). na
Studies of deletion analogues of porcine calcitonin (6
have demonstrated that partial structures are generally pl
devoid of activity (142). One exception to this is Studies of deletion analogues of porcine calcitoni
have demonstrated that partial structures are generall
devoid of activity (142). One exception to this is [Dee
Tyr²²]-salmon calcitonin I which has the same activit
as have demonstrated that partial structures are generally p
devoid of activity (142). One exception to this is [Des-
Tyr²²]-salmon calcitonin I which has the same activity re
as the unmodified peptide (47). Nevertheless, devoid of activity (142). One exception to this is [Des-
Tyr²²]-salmon calcitonin I which has the same activity res
as the unmodified peptide (47). Nevertheless, the natu-
rally occurring calcitonins display a considerab Tyr²²]-salmon calcitonin I which has the same activity reas the unmodified peptide (47) . Nevertheless, the naturally occurring calcitonins display a considerable degree reasidue considerable degree reasidue considerab as the unmodified peptide (47). Nevertheless, the naturally occurring calcitonins display a considerable degre
of sequence variation between the disulfide loop at the
amino terminus and the carboxy-terminal proline amid
re rally 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 p of sequence variation between the disulfide loop at the has
amino terminus and the carboxy-terminal proline amide labo
residue. In this region, only the leucine residue in posi-
tion 9 and the glycine in position 28 are in amino terminus and the carboxy-terminal proline amide
residue. In this region, only the leucine residue in posi-
tion 9 and the glycine in position 28 are invariant, yet
all of these peptides are highly active according to 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 ye tion 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 t all of these peptides are highly active according to the vivo assay of hypocalcemic response. Several years a
it was recognized that the central portions of the natural
citonins could form amphiphilic α -helical structu vivo assay of hypocalcemic response. Several years ago, the it was recognized that the central portions of the natural the calcitonins could form amphiphilic α -helical structures line that were similar to those formed 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 al

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 appeared likely to extend from residue 8 immediately
of activity. A cycl ogues. The entire human calcitonin sequence is shown, as well as the
species (104).
appeared likely to extend from residue 8 immediately
after the amino-terminal loop, to residue 22 which is
commonly followed by a helix-br 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-formi appeared likely to extend from residue 8 immediat
after the amino-terminal loop, to residue 22 which
commonly followed by a helix-breaking proline resid
in position 23 as well as several residues of low hel
forming propens 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 aspara-
 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 aspara-
gine, in positions 24-31. Furthermore, Fukushima in ou in position 23 as well as several residues of low helix-
forming propensity, such as glycine, serine, and aspara-
gine, in positions 24–31. Furthermore, Fukushima in our
laboratory (51) was able to correlate the potencies gine, 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 gine, 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 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 amphilic α helicity of these structures that took in natural calcitonins, as well as several synthetic analogues (63), with a parameter describing the relative amphilic α helicity of these structures that took into accoun the helix-forming propensity (29) of their consti (63), with a parameter describing the relative amphi-
philic 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 hydrop philic 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 f 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 al 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 Eisenb (39). cently, a similar correlation with amphiphilicity alone
is also been made (44), using the method of Eisenberg's
boratory to calculate relative hydrophobic moments
9).
Two peptide models of calcitonin (peptides 7 and 8;
gur

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
 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 int (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 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 li 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
 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 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

308 TAYLOR AND KAISER
112). In both peptide models, the amphiphilic α -helical associatio
structure only was modelled, using leucine, lysine, and vesicles, v 308 TAYLOR AND K
112). In both peptide models, the amphiphilic α -helical ass
structure only was modelled, using leucine, lysine, and ves
glutamine residues to maximize the α -helix-forming po- apo 308 TAYLOR A
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 po-
tential of the structure, and in su 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 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 structure only was modelled, using leucine, lysine, and ve glutamine residues to maximize the α -helix-forming po-
tential of the structure, and in such a way as to minimize resonology with any of corresponding natural tential of the structure, and in such a way as to minimize res
homology with any of corresponding natural sequences. hel
In each case, all of the general characteristics of the hel
natural structures were retained, includi homology with any of corresponding natural sequences. he
In each case, all of the general characteristics of the
natural structures were retained, including the length
photomorphic of the helix; the size, shape, and
reprin 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 neu-
tral natural structures were retained, including the length and amphiphilicity of the helix; the size, shape, a orientation relative to the rest of the peptide of hydrophobic domain; and the occurrence of mostly not ral or basi and amphiphilicity of the helix; the size, shape, and residue
orientation relative to the rest of the peptide of its nativel
hydrophobic domain; and the occurrence of mostly neu-
tral or basic residues on its hydrophilic s orientation relative to the rest of the peptide of its nature hydrophobic domain; and the occurrence of mostly neutral or basic residues on its hydrophilic side. The amphiphilic helical segments of peptides 7 and 8 differe hydrophobic domain; and the occurrence of mostly neu-
tral or basic residues on its hydrophilic side. The amphi-
philic helical segments of peptides 7 and 8 differed from
sech other and the natural structures in certain mo tral or basic residues on its hydrophilic side. The amphi-
philic 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 philic 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 posit 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 le 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 leu-
cine residues in the model peptides to idealize the of the hydrophobic domain commonly occurs in position
15 of the calcitonin structures and was replaced by leu-
cine residues in the model peptides to idealize the am-
phiphilicity; peptide 7 incorporated a tryptophan resid 15 of the calcitonin structures and was replaced by leu-
cine residues in the model peptides to idealize the am-
phiphilicity; peptide 7 incorporated a tryptophan residue
pro
in the middle of the hydrophobic domain, wherea cine residues in the model peptides to idealize the am-
phiphilicity; peptide 7 incorporated a tryptophan residue
in the middle of the hydrophobic domain, whereas pep-
tide 8 did not have this aromatic residue but did have phiphilicity; peptide 7 incorporated a tryptophan residue
in the middle of the hydrophobic domain, whereas pep-
tide 8 did not have this aromatic residue but did have a
tyrosine residue in position 22, where an aromatic re in the middle of the hydrophobic domain, whereas pep-
tide 8 did not have this aromatic residue but did have a
contyrosine residue in position 22, where an aromatic residue
expression to all of the natural structures. The tide 8 did not have this aromatic residue but did have a control tyrosine residue in position 22, where an aromatic residue calcitonin is common to all of the natural structures. The model we structures in peptides 7 and tyrosine residue in position 22, where an aromatic residue cent
is common to all of the natural structures. The model wer
structures in peptides 7 and 8 are compared to the form
 α -helical structure postulated for salmo is common
structures
 $α$ -helical st
residues 8-
figure 8.
In aqueo ructures in peptides 7 and 8 are compared to the
helical structure postulated for salmon calcitonin I
sidues 8–22 by means of helical wheel diagrams in
ure 8.
In aqueous, salt solutions at neutral pH, salmon cal-
onin I ha α -helical structure postulated for salmon calcitonin I p
residues 8–22 by means of helical wheel diagrams in w
figure 8. In aqueous, salt solutions at neutral pH, salmon cal-
citonin I has a CD spectrum typical of mixt

residues 8–22 by means of helical wheel diagrams in
figure 8.
In aqueous, salt solutions at neutral pH, salmon cal-
citonin I has a CD spectrum typical of mixtures of
 α -helical and random structures. No concentration d figure 8. ural
In aqueous, salt solutions at neutral pH, salmon cal-
citonin I has a CD spectrum typical of mixtures of disr
 α -helical and random structures. No concentration de-
lipid
pendency has been observed, and t 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 monom citonin I has a CD spectrum typical of mixtures of disrupt α -helical and random structures. No concentration de-
pendency has been observed, and the peptide appears to ences in
be monomeric according to analysis by equ α -helical and random structures. No concentration de-
pendency has been observed, and the peptide appears to
be monomeric according to analysis by equilibrium cen-
trifugation at concentrations near 1 mM (111, 112). A pendency has been observed, and the peptide appears to ence the monomeric according to analysis by equilibrium centrifugation at concentrations near 1 mM (111, 112). A true low amount of helical structure is normally prese trifugation at concentrations near 1 mM (111, 112)
low amount of helical structure is normally presen-
aqueous solutions of calcitonins, but this increases u
interactions with phospholipids and helix-promoting
vents (21, 4 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 phos aqueous solutions of calcitonins, but this increases upon
interactions with phospholipids and helix-promoting sol-
vents (21, 43, 44). Despite its interactions with phospho-
lipid micelles, salmon calcitonin I does not bin 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 vents (21, 43, 44). Despite its interactions with phospholographic lipid micelles, salmon calcitonin I does not bind to unilamellar egg lecithin vesicles (111). It does, however, the form stable monolayers of moderately h lipid micelles, salmon calcitonin I does not bind to unlamellar egg lecithin vesicles (111) . It does, howeve form stable monolayers of moderately high collapse pre sure (12 dyn/cm) at the air-water interface, indicatine lamellar 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 s

tential of the structure, and in such a way as to minimize result of the shorter length of the potential amphiphilic
homology with any of corresponding natural sequences. helix in residues $8-22$ of the calcitonins (four association and inability to interact with phospholipid D KAISER
association and inability to interact with phospholipid
vesicles, when compared to those properties of the serum
apolipoproteins and related model peptides, may be a D KAISER
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 a results 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 am 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 amphiphili vesicles, when compared to those properties of the servapolipoproteins and related model peptides, may be result of the shorter length of the potential amphiphibility in residues 8–22 of the calcitonins (four turns helix s 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 hy result of the shorter length of the potential amphiphii
helix in residues 8–22 of the calcitonins (four turns
helix structure compared to at least six) or to the hydr
philic elements on its hydrophobic face, such as the ac helix in residues 8–22 of the calcitonins (four turns of
helix structure compared to at least six) or to the hydro-
philic elements on its hydrophobic face, such as the acidic
residue in position 15 or tyrosine hydroxyl gr 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. Alter-
natively, the hydrophobic domain of the helix 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 str natively, the hydrophobic domain of the helix may be mains in the peptide through formation of some tertiary calcitonin are in close proximity (24). structure, as is suggested by immunological studies in-
dicating that the amino and carboxy termini of human
calcitonin are in close proximity (24).
Compared to salmon calcitonin I, the physicochemical
properties of peptid

be monomeric according to analysis by equilibrium cen-
trifugation at concentrations near 1 mM (111, 112). A trum given by peptide 8 showed increasing α helix con-
low amount of helical structure is normally present in Compared to salmon calcitonin I, the physicochemical dicating 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 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 e 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
p 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
c 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 c 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 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
for 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
pre 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 m 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 nat-
ural st 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 nat-
ural structure in this environment; peptides 7 and 8 both
inte pressures greater than 20 dyn/cm, and these peptides
were more compact and less compressible than the nat-
ural structure in this environment; peptides 7 and 8 both
interacted strongly with unilamellar egg lecithin vesicle were more compact and less compressible than the nural structure in this environment; peptides 7 and 8 bot interacted strongly with unilamellar egg lecithin vesicle disrupting their structure and forming mixed peptide a li ural 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 pronounce interacted strongly with unilamellar egg lecithin vesicles
disrupting their structure and forming mixed peptide an
lipid aggregates (111, 112). The most pronounced differ
ences in the behavior of these model peptides comp disrupting their structure and forming mixed peptide and
lipid aggregates (111, 112). The most pronounced differ-
ences in the behavior of these model peptides comparec
to each other were seen in their CD spectra. The spe lipid aggregates (111, 112). The most pronounced diffences in the behavior of these model peptides compator to each other were seen in their CD spectra. The spectra run given by peptide 8 showed increasing α helix cont ences in the behavior of these model peptides compared
to each other were seen in their CD spectra. The spec-
trum given by peptide 8 showed increasing α helix con-
tent above 1 μ M concentrations, indicative of self to each other were seen in their CD spectra. The spec-
trum given by peptide 8 showed increasing α helix con-
tent above 1 μ M concentrations, indicative of self-asso-
ciation and concomitant stabilization of the amp trum given by peptide 8 showed increasing α helix content above 1 μ M concentrations, indicative of self-association and concomitant stabilization of the amphiphilic helical structure in that peptide, and consistent tent above 1 μ M concentrations, indicative of self-ass
ciation and concomitant stabilization of the amphiphil
helical structure in that peptide, and consistent with tl
observed trimerization at a concentration of 1 mM. ciation 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 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 observed trimerization at a concentration of 1 mM. In
contrast, peptide 7 gave concentration-independent spec-
tra in the concentration range between 100 nM and 100
 μ M, indicating that the observed self-association oc-
 contrast, peptide 7 gave concentration-independent spec-
tra in the concentration range between 100 nM and 100
 μ M, indicating that the observed self-association oc-
curred at higher concentrations or without significan tra in the concentration range between 100 nM and 100 μ M, indicating that the observed self-association oc-
curred at higher concentrations or without significant
stabilization of helical structure. In either case, the

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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 surfac FIG. 8. Helical wheel diagrams comparing the distributions of the amino acid residue side chains in positions 8–22 of salmon calcitoni
(*SCT-I*), peptide 7 (*MCT-I*), and peptide 8 (*MCT-II*) on the surface of a regular (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*.

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CAL REVIEW

ARMACOLOGI

STRUCTURAL CHARACTERIZATION OF PEPTIDE HORMONES ³⁰⁹

STRUCTURAL CHARACTERIZATIC
peptide 2 compared to peptide 1, and it is attributed
likewise to the presence of a tryptophan residue on the STRUCTURAL CHARACTERIZATION
peptide 2 compared to peptide 1, and it is attributed ph
likewise to the presence of a tryptophan residue on the sal-
hydrophobic face of the helical structure having a dis-STRUCTURAL CHARACTERIZA
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 dis-
ruptive effect on potential heli 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) provide 2 compared to peptide 1, and it is attributed provide to the presence of a tryptophan residue on the subprophobic face of the helical structure having a distintive effect on potential helix-helix interactions (111)

likewise to the presence of a tryptophan residue on the
hydrophobic face of the helical structure having a dis-
ruptive effect on potential helix-helix interactions (111). if
The peptide models of calcitonin were both com hydrophobic face of the helical structure having a dis-
ruptive effect on potential helix-helix interactions (111). if the
The peptide models of calcitonin were both compared are
to salmon calcitonin I in assays of bindin ruptive 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 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 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 hypocalcemic effect in rats 1 h after s.c. injection (111, and 112). Similar results were obtained in each assay. Salmon it calcitonin I was slightly more potent than peptide 8 in bothe binding assay, and these two peptide 112). Similar results were obtained in each assay. Salmon ity calciton in I was slightly more potent than peptide 8 in bothe binding assay, and these two peptides were approxi-
the binding assay, and these two peptides we calciton in I was slightly more potent than peptide 8 in but the binding assay, and these two peptides were approxi-
mately equipotent in the in vivo assay. Peptide 7 was the about 10-20 times less potent in each assay th 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 por 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 phar-
macological behavior of peptide 8 to that of salmon
 about 10-20 times less potent in each assay than the st
salmon peptide, which corresponds to a similar potency co
to porcine calcitonin, the most active of the mammalian ca
structures tested (119). The close similarity of 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 phar-
macological behavior of peptide 8 to that of salmon
 to porcine calcitonin, the most active of the mammalian
structures tested (119). The close similarity of the phar-
macological behavior of peptide 8 to that of salmon
calcitonin I was further demonstrated in binding assays structures tested (119). The close similarity of the pharmacological behavior of peptide 8 to that of salmon nealcitonin I was further demonstrated in binding assays 6, to rat kidney cortical membranes and assays of adenyl macological behavior of peptide 8 to that of salmon relationin I was further demonstrated in binding assays 6 to rat kidney cortical membranes and assays of adenylate revoluse activation in the same tissue (111) . In bot calcitonin I was further demonstrated in binding assa
to rat kidney cortical membranes and assays of adenyla
cyclase activation in the same tissue (111). In both
these assays, the model peptide and the salmon peptii
were e 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 pharmacologic cyclase activation in the same tissue (111). In both of these assays, the model peptide and the salmon peptide by were equipotent. Overall, therefore, the pharmacological fassays of these model peptides provide a convinci these assays, the model peptide and the salmon peptide blue were equipotent. Overall, therefore, the pharmacological face assays of these model peptides provide a convincing podemonstration that peptides designed to have were equipotent. Overall, therefore, the pharmacological face assays of these model peptides provide a convincing posed
emonstration that peptides designed to have idealized the amphiphilic α -helical structures in resi assays of these model peptides provide a convincing podemonstration that peptides designed to have idealized the amphiphilic α -helical structures in residues 8–22 of the geolationin structure can reproduce the most pot demonstration that peptides designed to have idealize
amphiphilic α -helical structures in residues 8–22 of the
calcitonin structure can reproduce the most potent ar
specific actions of the natural hormones. As alreace
 amphiphilic α -helical structures in residues 8–22 of the gested (78), and a study of peptide models with con-
calcitonin structure can reproduce the most potent and strained conformations could determine the importance 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 str specific actions of the natural hormones. As discussed, the minimal homology of these mode tures to the corresponding natural sequences is al strong evidence that residues 8–22 are in the α conformation on the calciton scussed, the minimal homology of these model struc-
res to the corresponding natural sequences is also very litt
rong evidence that residues 8–22 are in the α -helical Horomation on the calcitonin receptor surface.
A fu

tures to the corresponding natural sequences is also very little r
strong evidence that residues 8–22 are in the α -helical Howev
conformation on the calcitonin receptor surface. genera
A further test to establish that strong evidence that residues 8–22 are in the α -heli
conformation on the calcitonin receptor surface.
A further test to establish that amphiphilicity in
helical structure is essential for high activity may n
be attempt conformation on the calcitonin receptor surface. get

A further test to establish that amphiphilicity in the

helical structure is essential for high activity may now

bridge attempted through the study of appropriate non 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 nonam-
phiphilic calcitonin model peptides corresponding to the helical structure is essential for high activity may now
be attempted through the study of appropriate nonam-
phiphilic calcitonin model peptides corresponding to the
 β -endorphin model peptide 4. However, the lower act be attempted through the study of appropriate nonam-

phiphilic calcitonin model peptides corresponding to the

ila
 β -endorphin model peptide 4. However, the lower activity (fig

of peptide 7 compared to salmon calcit phiphilic 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 interacti β -endorphin model peptide 4. However, the lower activity (figure of peptide 7 compared to salmon calcitonin I and peptide ms 8 has already indicated that receptor interactions are an sensitive to changes in the hydroph 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 st 8 has already indicated that receptor interactions are and
sensitive to changes in the hydrophobic face of the am-
phiphilic helix involving aromatic residues. Again, the construption residue in position 12 of peptide 7 is sensitive to changes in the hydrophobic face of the am-
phiphilic helix involving aromatic residues. Again, the
tryptophan residue in position 12 of peptide 7 is most
strongly implicated, in view of its disruptive effect o phiphilic helix involving aromatic residues. Again, the contryptophan residue in position 12 of peptide 7 is most rest strongly implicated, in view of its disruptive effect on of its self-association as well as the nonesse 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 a 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 previ self-association as well as the nonessential nature (47) cof the tyrosine residue in position 22 of peptide 8 and side salmon calcitonin I. This effect is similar to previous for observations of Maier et al., who suggested of the tyrosine residue in position 22 of peptide 8 and susalmon calcitonin I. This effect is similar to previous full observations of Maier et al., who suggested that aromatic of residues in positions 12, 16, and 19 were salmon calcitonin I. This effect is similar to previous observations of Maier et al., who suggested that aroma
residues in positions 12, 16, and 19 were responsible :
the lower activities of the mammalian calcitonins in t
 observations of Maier et al., who suggested that aromatic or residues in positions 12, 16, and 19 were responsible for left the lower activities of the mammalian calcitonins in the chypocalcemic assay, and showed that sequ residues in positions 12, 16, and 19 were responsible the lower activities of the mammalian calcitonins in thy
pocalcemic assay, and showed that sequential substution of leucine residues in all three positions increase
th the lower activities of the mammalian calcitonins in the composed the hypocalcemic assay, and showed that sequential substitution of leucine residues in all three positions increased to the potency of human calcitonin in t 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 o tution of leucine residues in all three positions increased
the potency of human calcitonin in this assay signifi-
cantly (105). Sensitivities of this sort to the nature of
the residues on the hydrophobic face are clearly structure. the residues on the hydrophobic face are clearly in sup-

membranes using ¹²⁵I-labelled salmon calcitonin I to and 6 in vivo relative to the less structured and less radiolabel calcitonin receptors, and in assays of their amphiphilic β_h -endorphin. Since the assays of peptid radiolabel calcitonin receptors, and in assays of their amphiphilic β_h -endorphin. Since the assays of peptides 7 hypocalcemic effect in rats 1 h after s.c. injection (111, and 8 were performed under conditions of optim STRUCTURAL CHARACTERIZATION OF PEPTIDE HORMONES 309
btide 1, and it is attributed philic properties of peptide 8 in particular compared to
a tryptophan residue on the salmon calcitonin I, it would be interesting to determi on OF PEPTIDE HORMONES 309
philic 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 on OF PEPTIDE HORMONES 309

philic 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 philic 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 philic 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 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 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 peptid 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 l are slower relative to the salmon peptide, just as wa
observed for the antinociceptive effects of peptides 3,
and 6 in vivo relative to the less structured and lea
amphiphilic β_h -endorphin. Since the assays of peptides 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 optima 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 activ-
ity of salmon calcitonin I (84), it may well be th amphiphilic β_h -endorphin. Since the assays of peptides 7
and 8 were performed under conditions of optimal activ-
ity of salmon calcitonin I (84), it may well be that one or
both of the model peptides are more active an 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 rol both of the model peptides are more active and/or longer
the casting than the salmon peptide. The functional role of
the carboxy-terminal residues 32-31 also remains to be
studied. If these residues simply serve a linking 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 ami 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 ami 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 de 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 no carboxy-terminal residue, it should be possible to design
a simple model structure, perhaps consisting of non-
natural amino acid residues such as were used in peptide
6, which would adequately perform the same role. The
m carboxy-terminal residue, it should be possible to design
a simple model structure, perhaps consisting of non-
natural amino acid residues such as were used in peptide
6, which would adequately perform the same role. The
m a simple model structure, perhaps consisting of not
natural amino acid residues such as were used in peptic
6, which would adequately perform the same role. The
multiple amino acid substitutions seen in this region
the nat natural 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 possi 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 surf multiple amino acid substitutions seen in this region of
the natural sequences suggest that this should be possi-
ble, and that no specific interactions with receptor sur-
faces are required of these residues. One alternat ble, 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 sug ble, 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 sug faces 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 constrai possibility, that these residues l
the helix in salmon calcitonin l
gested (78), and a study of pe
strained conformations could de
of this type of tertiary structure.
The primary structures of m e helix in salmon calcitonin I, has already been sug-
sted (78), and a study of peptide models with con-
rained conformations could determine the importance
this type of tertiary structure.
The primary structures of mammal

gested (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 strained 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 striki of this type of tertiary structure.
The primary structures of mammalian CGRPs be
little resemblance to those of the calcitonins (113, 13).
However, there is a striking homology in terms of the
general structures (78). The 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 follow-
in 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 follow-
ing the amino-terminal loop formed by the disulf 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 CGRF are ing 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 sim-
ilar characteristics to the one formed by the calcitonins ing 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 sim-
ilar characteristics to the one formed by the calcitonins 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 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 su ilar 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 (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 main 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 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. 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 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 i 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 f 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 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 residu sumed 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, 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 thi 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
te 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 t 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-
re 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
 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 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 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 wou

distribution of the amino acid side chains on the surface of a regular α helix is shown. The hydrophobic residues are *circled*. helix is shown. The hydrophobic residues 8-28. The particular distribution of the amino acid side chains on the surface of a regular α fishelix is shown. The hydrophobic residues are *circled*. (a) que hydrophilic side

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). helix is shown. The hydrophobic residues are *circled*. The computation of a considered in this putative CGRP structure, suggesting that a computer helix might be favored (78). Another difference asseme this putative CGRP hydrophilic side of the structure, suggesting the shorter helix might be favored (78). Another differe in this putative CGRP structure is the presence (hydrophilic serine residue in the middle of the hydrophic domain. This 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 5-residue in the middle of the hydrophobic domain. This res 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 hydropho-
bic domain. This residue is also part of a 5-residue
se in this putative CGRP structure is the presence of hydrophilic serine residue in the middle of the hydrophic domain. This residue is also part of a 5-resid sequence in positions $17-21$ that includes two glycir and is exp hydrophilic serine residue in the middle of the hydropho-
bic 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 forma bic 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 forma-
tion of helical structure (29). Given the capacity of ce sequence in positions 17-21 that includes two glycines only h
and is expected to have very little propensity for forma-
peptide
tion of helical structure (29). Given the capacity of cell
surfaces to adsorb peptides contain and is expected to have very little propensity for formation of helical structure (29) . Given the capacity of cell id surfaces to adsorb peptides containing this type of amphiphilic structure in large quantities at rela surfaces to adsorb peptides containing this type of am-
phiphilic structure in large quantities at relatively low
concentrations, it is reasonable to speculate that some bina
type of helix destabilization may be more impor phiphilic structure in large quantities at relatively low glu
concentrations, it is reasonable to speculate that some bin
type of helix destabilization may be more important in res
the longer amphiphilic structure potentia 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
hyd the longer amphiphilic structure potentially formed by glucage CGRP compared to the calcitonins, in order that it may antago function in vivo. The presence of a certain number of tial for hydrophilic residues on the hydrop CGRP compared to the calcitonins, in order that it may
function in vivo. The presence of a certain number of
hydrophilic residues on the hydropholic face of an am-
phiphilic helix does not prevent such structures from
form 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, hydrophilic residues on the hydrophobic face of an am-
phiphilic helix does not prevent such structures from
forming at the air-water interface or phospholipid sur-
faces, as has been demonstrated previously for CRF,
sauva phiphilic helix does not prevent such structures from has
forming at the air-water interface or phospholipid sur-
faces, as has been demonstrated previously for CRF, dia
sauvagine, and GRF (*vide infra*), as well as a pept 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 faces, 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 sauvagine, and GRF (*vide infra*), as well as a peptid model of the serum apolipoproteins (53) . In keeping wit the above analysis, the formation of stable monolayer at the air-water interface has been demonstrated for r model of the serum apolipoproteins (53). In keeping with 1'
the above analysis, the formation of stable monolayers (f
at the air-water interface has been demonstrated for rat w
CGRP, and these monolayers collapse at a sur 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 pres-
sure similar to the monolayer collapse pressure of sal at the air-water interface has been demonstrated for rcGRP, and these monolayers collapse at a surface presure similar to the monolayer collapse pressure of salmo calcitonin I (78). Analysis of the role of this potential CGRP, and these monolayers collapse at a surface pres-
sure α helix in residues 5-29. Interactions with an amphiphilic
sure similar to the monolayer collapse pressure of salmon
calcitonin I (78). Analysis of the role o sure similar to the monolayer collapse pressure of salmon calcitonin I (78). Analysis of the r
amphiphilic α -helical structure in de
icochemical and pharmacological prove
the peptide modelling method is un
ratory and should prove informative
NJ Changana and Palate Internal and pharmacological properties of CC

ide modelling method is under way in ou
 VI. Glucagon and Related Peptides
 VI. Glucagon and Related Peptides
 von is produced by the A cells of the pan e peptide modelling method is under way in our labotory and should prove informative.

VI. Glucagon and Related Peptides

Glucagon is produced by the A cells of the pancreatic

ets of Langerhans (125), and its release from

ratory and should prove informative. The same of the Marine Changer of Chucagon is produced by the A cells of the pancreatic whislets of Langerhans (125), and its release from the Repancreas is regulated by a variety of fa **Paramel VI. Glucagon and Related Peptides**

Glucagon is produced by the A cells of the pancreatic vislets of Langerhans (125), and its release from the I

pancreas is regulated by a variety of factors including r

) KAISER
glucose, insulin, and somatostatin, which inhibit release,
and fatty acids, fasting, stress and exercise, which in-) KAISER
glucose, insulin, and somatostatin, which inhibit release,
and fatty acids, fasting, stress and exercise, which in-
crease plasma levels (58). Glucagon acts primarily at cell D KAISER
glucose, insulin, and somatostatin, which inhibit release,
and fatty acids, fasting, stress and exercise, which in-
crease plasma levels (58). Glucagon acts primarily at cell
surface receptors in the liver where b surface in sulin, and somatostatin, which inhibit release,
and fatty acids, fasting, stress and exercise, which in-
crease plasma levels (58). Glucagon acts primarily at cell
surface receptors in the liver where binding re glucose, insulin, and somatostatin, which inhibit release,
and fatty acids, fasting, stress and exercise, which in-
crease plasma levels (58). Glucagon acts primarily at cell
surface receptors in the liver where binding re 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 messenge crease plasma levels (58). Glucagon acts primarily at surface receptors in the liver where binding results stimulation of the adenylate cyclase second messen system and increased rates of glycogenolysis and lipoly (131). 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 hepa stimulation of the adenylate cyclase second messenger
system and increased rates of glycogenolysis and lipolysis
(131). Analysis of glucagon binding to isolated hepato-
cytes and hepatic plasma membranes using ¹²⁵I-label system and increased rates of glycogenolysis and lipolys (131). Analysis of glucagon binding to isolated hepat cytes and hepatic plasma membranes using 125 I-labell glucagon as a radioligand indicates that there are tu (131). Analysis of glucagon
cytes and hepatic plasma m
glucagon as a radioligand i
distinct populations of recep
ties for the hormone (15).
Mammalian glucagon ha glucagon as a radioligand indicates that there are two
distinct populations of receptors having different affini-
ties for the hormone (15).
Mammalian glucagon has a highly conserved amino
acid sequence consisting of 29 re

Fig. 9. Helical net diagram of human CGRP residues 8-28. The pared to the invariant mammalian structure, avian and $\begin{bmatrix} \text{Phe} \\ \text{Fe} \\ \text{$ concentrations, it is reasonable to speculate that some binding potency, although it did not elicit a biological
type of helix destabilization may be more important in response, indicating that it might be possible to desi glucagon as a radioligand indicates that there are two
distinct populations of receptors having different affini-
ties for the hormone (15).
Mammalian glucagon has a highly conserved amino
acid sequence consisting of 29 re distinct populations of receptors having different affini-
ties for the hormone (15).
Mammalian glucagon has a highly conserved aminc
acid sequence consisting of 29 residues. Significant se-
quence homology with several ot ties for the hormone (15).
Mammalian glucagon has a highly conserved amino
acid sequence consisting of 29 residues. Significant se-
quence homology with several other peptide hormones,
including secretin, vasoactive intest Mammalian glucagon has a highly conserved amino
acid sequence consisting of 29 residues. Significant se-
quence homology with several other peptide hormones,
including secretin, vasoactive intestinal peptide (VIP),
peptide 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 including secretin, vasoactive intestinal peptide (VIP), 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). peptide histidine isoleucine (PHI), gastrin, and GRF, has
been noted (5, 143, 143b), suggesting that these peptides
might have similar active structures (figure 10). Com-
pared to the invariant mammalian structure, avian a been noted (5, 143, 143b), suggesting that these peptides
might have similar active structures (figure 10). Com-
pared to the invariant mammalian structure, avian and
fish glucagon homologues have a small number of se-
que 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 hydrop pared to the invariant mammalian structure, avian and
fish glucagon homologues have a small number of se-
quence differences involving conservative substitutions
of hydrophilic residues only (70). Receptor binding, ad-
eny quence differences involving conservative substitutions
of hydrophilic residues only (70). Receptor binding, ad-
enylate cyclase activation, glycogenolysis, and lipolysis
assays of a variety of deletion analogues indicate of hydrophilic residues only (70). Receptor binding, adof hydrophilic residues only (70). Receptor binding, acenylate cyclase activation, glycogenolysis, and lipolys assays of a variety of deletion analogues indicate the residues throughout the mammalian peptide are essentiati enylate 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 [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-
His¹]-glucagon (70) and [Des-Asn²⁸, Thr²⁹]-glu residues throughout the mammalian peptide are essential
for high activity (20, 41, 50, 70, 152, 153). Even [Des-
His¹]-glucagon (70) and [Des-Asn²⁸,Thr²⁹]-glucagon (41)
only have a small fraction of the activity of for high activity (20, 41, 50, 70, 152, 153). Even [Des-
His¹]-glucagon (70) and [Des-Asn²⁸,Thr²⁹]-glucagon (41)
only have a small fraction of the activity of the intact
peptide, and removing residues 5–9, residues 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 only have a small fraction of the activity of the intact
peptide, and removing residues 5–9, residues 10–15, res-
idues 16–21, or residues 22–26 from the structure results
in even lower potencies (50). Perhaps more interes 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 rec 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 glucagon(5–29) was found to retain considerable recep
binding potency, although it did not elicit a biologic
response, indicating that it might be possible to desi
glucagon antagonists (50). Several partial agonists a
anta 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 pot response, indicating that it might be possible to designal glucagon antagonists (50). Several partial agonists and antagonists have indeed been prepared, and their potential for clinical use in the treatment of diabetes, a glucagon antagonists (50). Several partial agonists and
antagonists have indeed been prepared, and their poten-
tial for clinical use in the treatment of diabetes, as well
as their uses as probes for glucagon signal transd

Analysis of the glucagon structure using helical net tial for clinical use in the treatment of diabetes, as we
as their uses as probes for glucagon signal transduction
has been extensively discussed elsewhere (20a, 70, 99a)
Analysis of the glucagon structure using helical ne 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 sep has been extensively discussed elsewhere (20a, 70, 99a).

Analysis of the glucagon structure using helical net

diagrams indicates the potential formation of two sepa-

rate hydrophobic domains by residues 5–16 and residu Analysis of the glucagon structure using helical net
diagrams indicates the potential formation of two sepa-
rate hydrophobic domains by residues 5–16 and residues
17–29, if these segments adopt an α -helical conformati 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 wo rate 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 continuo 17–29, if these segments adopt an α -helical conformation (figure 11). However, the two hydrophobic doma would be oriented on opposite sides of a single continue α helix in residues 5–29. Interactions with an amphiph (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 would be oriented on opposite sides of a single continuo α helix in residues 5–29. Interactions with an amphiphiliterface might, therefore, be expected to favor a discortinuity in the hydrophilic segment of the peptide α neitx in residues 5–29. Interactions with an amphiphilic
interface might, therefore, be expected to favor a discon-
tinuity in the hydrophilic segment of the peptide that
links the two amphiphilic structures, so that tinuity in the hydrophilic segment of the peptide that
links the two amphiphilic structures, so that the hydro-
phobic domains would be aligned on the same side of the
molecule. However, each helix would then consist of on links the two amphiphilic structures, so that the hydro-
phobic 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 onl phobic 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. Th 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 struc 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 (1 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

ARMACOLO

STRUCTURAL CHARACTERIZATION OF PEPTIDE HORMONES ³¹¹

(Gastrin) Lys-Ile-Arg-Gln-Gln-Asp-Phe-Val-Asn-Trp-Leu-Leu-Ala-Gln-Lys-
(GRF) Gln-Leu-Ser-Ala-Arg-Lys-Leu-Ceu-Gln-Asp-Ile-Met-Ser-Arg-Gln-
GRF(1-30), which is the human structure (143). VIP, vasoactive intestinal peptide; P $GRF(1-30)$, which is the human structure (143). VIP, vasoactive intestinal peptide; PHI, peptide histidine isoleucine; GRF, growth hormone releasing factor. For gradings of the porcine hormones are shown (143b), except for the peride; *PHI*, peptide histidine isoleucine; GRF, growth hormon of glucagon with a variety of lipids and other surfactants in whose presence glucagon ca

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 (c 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 (ci 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 residu

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 form, but at high concentration were adopted.

Form, but at high concentrations a concentration-dender throw and the mondom, glucagon has little structure in the mondom. the the increase in the sidues 5–9 and residues 26–29. In aqueous tion
solution, glucagon has little structure in the monomeric 9 and
form, but at high concentrations a concentration-de-
pendent increase in helical structu 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-de-
pendent increase in helical structure is observed in the
CD s solution, glucagon has little structure in the monomeric
form, but at high concentrations a concentration-de-
pendent increase in helical structure is observed in the
CD spectra which is consistent with formation of trimer

nal peptide; PHI, peptide histidine isoleucine; GRF, growth hormone
of glucagon with a variety of lipids and other surfactants,
in whose presence glucagon can often form mixed mi-
celles (16, 43, 136, 156). An analysis of of glucagon with a variety of lipids and other surfactants,
in whose presence glucagon can often form mixed mi-
celles (16, 43, 136, 156). An analysis of partial glucagon
sequences suggests that the helical structure stabi of glucagon with a variety of lipids and other surfactants,
in whose presence glucagon can often form mixed mi-
celles (16, 43, 136, 156). An analysis of partial glucagon
sequences suggests that the helical structure stabi of glucagon with a variety of lipids and other surfactants,
in whose presence glucagon can often form mixed mi-
celles (16, 43, 136, 156). An analysis of partial glucagon
sequences suggests that the helical structure stabi in whose presence glucagon can often form mixed m
celles (16, 43, 136, 156). An analysis of partial glucage
sequences suggests that the helical structure stabilize
in this way lies in residues $19-29$ (156), and nucle
mag celles (16, 43, 136, 156
sequences suggests thin this way lies in remagnetic resonance (
ment with this (22).
Two glucagon pept: quences suggests that the helical structure stabilized
this way lies in residues 19–29 (156), and nuclear
agnetic resonance (NMR) experiments are in agree-
ent with this (22).
Two glucagon peptide models (peptides 9 and 1

Ash Methema and the set of peptides in the natural peptide

FIG. 11. Helical net diagram of mammalian glucagon residues

5-29. The distribution of the amino acid side chains on the surface of peptides, including an acidic in this way lies in residues $19-29$ (156), and nucle
magnetic resonance (NMR) experiments are in agreement with this (22).
Two glucagon peptide models (peptides 9 and 10
figure 2) with multiple changes in the carboxy-ter 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 ment 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 $\$ 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 (11 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 (11 region designed to enhance the helix-forming propensit
and amphiphilicity of the potential α -helical structure is
residues 19–29 were synthesized and studied (115, 117)
These model peptides differed from one another on 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 i 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 posi-
tions 22 (phenylalanine) and 25 (tryptophan) that are
 These model peptides differed from one another only in
that peptide 10 retained the aromatic residues in posi-
tions 22 (phenylalanine) and 25 (tryptophan) that are
present in the natural sequence in this region. Comparec 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, tions 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 des 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 pep 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 vi 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 aromat 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 pept have been substituted by hydrophilic reversa, and specific features in addition residues in peptide 10 have been con peptides, including an acidic residue in the carboxy-terminal threonine residue.
As expected from their d rsa, and specific features in addition to the aromatic
sidues in peptide 10 have been conserved in both
ptides, including an acidic residue in position 21 and
e carboxy-terminal threonine residue.
As expected from their de

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 mod peptides, including an acidic residue in position 21
the carboxy-terminal threonine residue.
As expected from their design, CD spectra indic
that these peptide models had more helical struc
than glucagon and self-associate 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 concentra-
tions. At the air-water As expected from their design, CD spectra indicated
that these peptide models had more helical structure
than glucagon and self-associated at lower concentra-
tions. At the air-water interface, glucagon and peptides
9 and that these peptide models had more helical structure
than glucagon and self-associated at lower concentra-
tions. At the air-water interface, glucagon and peptides
9 and 10 all formed compact, incompressible monolayers
of than glucagon and self-associated at lower concent
tions. At the air-water interface, glucagon and peptic
9 and 10 all formed compact, incompressible monolay
of similar moderate stabilities (collapse pressures wa
round 10 tions. At the air-water interface, glucagon and peptic
9 and 10 all formed compact, incompressible monolay
of similar moderate stabilities (collapse pressures we
around 10 dyn/cm) that indicated formation of amp
philic hel 9 and 10 all formed compact, incompressible monolayers
of similar moderate stabilities (collapse pressures were
around 10 dyn/cm) that indicated formation of amphi-
philic helical structures (116). However, when the poten-

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ing assays using isolated rat hepatocytes and purified de
¹²⁵I-glucagon as a radiolabel, considerable differences h TAYLOR AN

ing assays using isolated rat hepatocytes and purified

¹²⁵I-glucagon as a radiolabel, considerable differences

were observed (115, 117). Glucagon itself displaced the TAYLOR AND 1
ing assays using isolated rat hepatocytes and purified de
¹²⁵I-glucagon as a radiolabel, considerable differences hy
were observed (115, 117). Glucagon itself displaced the ine
¹²⁵I-glucagon binding over ²⁵¹²

ing assays using isolated rat hepatocytes and purified

¹²⁵I-glucagon as a radiolabel, considerable differences

were observed (115, 117). Glucagon itself displaced the

¹²⁵I-glucagon binding over a wide range ing assays using isolated rat hepatocytes and purified
¹²⁵I-glucagon as a radiolabel, considerable differences
were observed (115, 117). Glucagon itself displaced the
¹²⁵I-glucagon binding over a wide range of concent ¹²⁵I-glucagon as a radiolabel, considerable differences here observed (115, 117). Glucagon itself displaced the i¹²⁵I-glucagon binding over a wide range of concentrations cand in a manner consistent with binding of th were observed (115, 117). Glucagon itself displaced the indi¹²⁵I-glucagon binding over a wide range of concentrations of a and in a manner consistent with binding of the hormone limito two noninteracting types of recept ¹²⁵I-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 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} 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 an 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 ¹²⁵I-
glucagon binding even at a concentration of pM and 41 nM, and the receptor populations (B_{max} values) were similar. Peptide 9 did not displace any of the ¹²⁵I-
glucagon binding even at a concentration of 10 μ M, but s
peptide 10 showed more interesting behavio were similar. Peptide 9 did not displace any of the ¹²⁵I- in receptor binding and adenylate cyclase activation as-
glucagon binding even at a concentration of 10 μ M, but says using liver plasma membranes, and was equ glucagon binding even at a concentration of $10 \mu M$, but seeptide 10 showed more interesting behavior, displacing was about half of the 125 I-glucagon binding over a relatively reprive narrow concentration range. Further peptide 10 showed more interesting behavior, displace about half of the ^{125}I -glucagon binding over a relatively narrow concentration range. Further analysis revear that peptide 10 was able to bind to the high affinity about half of the ¹²⁵I-glucagon binding over a relatively results a narrow concentration range. Further analysis revealed the pote that peptide 10 was able to bind to the high affinity region of binding sites of glucago narrow concentration range. Further analysis revealed that peptide 10 was able to bind to the high affinity relation displacement of 1.2 μ M. Thus, in the presence of 10 μ M the peptide 10, the concentration-dependent that peptide 10 was able to bind to the high affinit
binding sites of glucagon very selectively with a dissociation constant of 1.2 μ M. Thus, in the presence of 10 μ l
peptide 10, the concentration-dependent displace binding sites of glucagon very selectively with a dissociation constant of 1.2 μ M. Thus, in the presence of 10 μ M the peptide 10, the concentration-dependent displacement of natural peptide to a single population of

tion constant of 1.2 μ M. Thus, in the presence of 10 μ M this
peptide 10, the concentration-dependent displacement of mon
¹²⁵I-glucagon by glucagon itself was consistent with bind-
ing of the natural peptide to a s peptide 10, the concentration-dependent displacement of
¹²⁵I-glucagon by glucagon itself was consistent with bind-
ing of the natural peptide to a single population of sites
with a dissociation constant of 46 nM, corresp ¹²⁵I-glucagon by glucagon itself was consistent with bind-
ing of the natural peptide to a single population of sites gluce
with a dissociation constant of 46 nM, corresponding to phip
the value observed for its binding with a dissociation constant of 46 nM, corresponding t
the value observed for its binding to the low affinity site
only (115). In assays of the abilities of these peptides t
stimulate glycogenolysis, their inhibitory effec 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 only (115). In assays of the abilities of these peptides to (a stimulate glycogenolysis, their inhibitory effects on the cincorporation of radiolabelled carbohydrate into hepa-
incorporation of radiolabelled carbohydrate i stimulate glycogenolysis, their inhibitory effects on the calincorporation of radiolabelled carbohydrate into hepatocyte glycogen were measured and found to correspond gato the observed binding to the high affinity glucag 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 s 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 ha sites (115, 117). Both glucagon and peptide 10 were able obsto stimulate glycogenolysis with half-maximal effects at α about 18 pM and 10 nM, respectively, and peptide 9 had exessentially no effect, even at a concentra to stimulate glycogenolysis with half-maximal effects at a about 18 pM and 10 nM, respectively, and peptide 9 had exessentially no effect, even at a concentration of 1 μ M. of Peptide 10 was, however, considerably more about 18 pM and 10 nM, respectively, and peptide 9 had
essentially no effect, even at a concentration of 1 μ M.
Peptide 10 was, however, considerably more potent than
expected on the basis of the binding assay. This is Peptide 10 was, however, considerably more potent than hyperpected on the basis of the binding assay. This is the wopposite behavior to that expected for a partial agonist for antagonist and suggests that this peptide has 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 bindi 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 con or antagonist and suggests that this peptide has greater
intrinsic activity than glucagon, although its binding
affinity is considerably lower. These results are consist-
ent with the notion expressed elsewhere (20a, 50, 9 intrinsic activity than glucagon, although its binding wi
affinity is considerably lower. These results are consist-
ent with the notion expressed elsewhere (20a, 50, 99a) and
that the carboxy-terminal region of glucagon affinity is considerably lower. These results are consient with the notion expressed elsewhere (20a, 50, 99, that the carboxy-terminal region of glucagon serves p
marily to enhance receptor binding affinity, whereas t
amin tion. at the carboxy-terminal region of glucagon serves pri-
arily to enhance receptor binding affinity, whereas the
nino-terminal region is important for receptor activa-
n.
Assays of peptide 10 in particular have therefore pro

marily to enhance receptor binding affinity, whereas the α amino-terminal region is important for receptor activa-
tion.
Assays of peptide 10 in particular have therefore provided good evidence that it is the high affi amino-terminal region is important for receptor activa-
tion.
Assays of peptide 10 in particular have therefore pro-
vided good evidence that it is the high affinity glucagon
tureceptor identified in binding assays that me ion.

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 to

physiological response to glucagon. The peptide 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 model-
 vided good evidence that it is the high affinity glucagon
receptor identified in binding assays that mediates the
physiological response to glucagon. The peptide model-
ling approach has, so far, been less useful in identi 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 responsibl physiological response to glucagon. The peptide model-
lic domain, and no hydrophobic residues would occur on
ling approach has, so far, been less useful in identifying
the hydrophilic side of the helix. In keeping with t ling 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 peptid for initiating this response, since the relatively small ist
number of conservative amino acid substitutions (six) an
made in the natural sequence of glucagon to give peptide
potential structure as
nitude lower. This does number of conservative amino acid substitutions (six) and made in the natural sequence of glucagon to give peptide potentions in the individual side chain situate lower. This does not rule out helical structure as be a pos made in the natural sequence of glucagon to give peptide
10 has resulted in potencies about three orders of mag-
initude lower. This does not rule out helical structure as
be a possibility, however, since the individual si 10 has resulted in potencies about three orders of magnitude lower. This does not rule out helical structure as be a possibility, however, since the individual side chain infunctionalities on its surface could easily prod mitude lower. This does not rule out helical structure as be possibility, however, since the individual side chain in functionalities on its surface could easily produce such the specificity. Should this be the case, the a possibility, however, since the individual side chain interactionalities on its surface could easily produce such the specificity. Should this be the case, the hydrophobic face we of the helix is likely to provide most

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d purified design of peptide models in which only residues on the
ifferences hydrophilic face were substituted might provide a better indication of the importance of that structure. This type In KAISER
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 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 t 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 t 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 desig 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). T of approach has recently been successfully applied to a
imited extent by Krstenansky et al. in their design of
the analogue [Lys¹⁷,Lys¹⁸,Glu²¹]-glucagon (83a). This an-
alogue was more helical in aqueous salt solutio limited extent by Krstenansky et al. in their design of
the analogue [Lys¹⁷,Lys¹⁸,Glu²¹]-glucagon (83a). This an-
alogue was more helical in aqueous salt solutions (100
 μ M peptide at pH 9.2), was more potent than alogue was more helical in aqueous salt solutions (100) μ M peptide at pH 9.2), was more potent than glucagon μ M peptide at pH 9.2), was more potent than glucagon
in receptor binding and adenylate cyclase activation as-
says using liver plasma membranes, and was equipotent
with glucagon in an in vivo glucose release assay. The in receptor binding and adenylate cyclase activation as ays using liver plasma membranes, and was equipote with glucagon in an in vivo glucose release assay. The results are clearly very supportive of a functional role if says 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 carbo 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 wort 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 the potential am
region of glucage
more extensive
this region of the
more rigorously.
When the pep gion of glucagon, and it would seem worthwhile making
ore extensive substitutions of hydrophilic residues in
is region of the molecule in order to test this hypothesis
ore rigorously.
When the peptide hormones that are hom 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

ing of the natural peptide to a single population of sites glucagon (figure 10) are also analyzed for potential am-
with a dissociation constant of 46 nM, corresponding to phiphilic α -helical structure, helical net dia to the observed binding to the high affinity glucagon discontinuity in the two hydrophobic domains is also
sites (115, 117). Both glucagon and peptide 10 were able observed in each case. The amino-terminal amphiphilic
to 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 am-
phiphilic α -helical structure, hel more rigorously.

When the peptide hormones that are homologous to

glucagon (figure 10) are also analyzed for potential am-

phiphilic α -helical structure, helical net diagrams reveal

a similar pattern to that found When the peptide hormones that are homologous to
glucagon (figure 10) are also analyzed for potential am-
phiphilic α -helical structure, helical net diagrams reveal
a similar pattern to that found for glucagon in each 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 cha a similar pattern to that found for glucagon in each case 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 exc (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 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
obser 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 he 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
exa 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
o 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 relativel 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 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 c **Solution** I state in the state of the helix as whereas residues 1–18 of PHI (figure 12) or GRF commeters are form five complete turns of amphiphilic α helix with somewhat larger hydrophobic domain that twists clockies 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 clock-
wise around the helix surface.
In the case of GRF, the $\$ 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

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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 inte wise 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 structu 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 resi 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 domai 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 ax 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 compl this hormone would form a hydrophobic domain ly
along one side of the helix, parallel to its axis,
covering half of its surface for almost seven comp
turns. Throughout this structure, only two hydropl
serine residues would 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 hydropho-
 covering half of its surface for almost seven complete
turns. Throughout this structure, only two hydrophilic
serine residues would be positioned within the hydropho-
bic domain, and no hydrophobic residues would occur on
 turns. Throughout this structure, only two hydropleserine residues would be positioned within the hydroploic domain, and no hydrophobic residues would occu
the hydrophilic side of the helix. In keeping with
formation of a 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 character-
 bic 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 character-
istics, GRF was found to form very stable monolayers
an 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 wa 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 istics, 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 conformat 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 o 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. F 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 thermore, a GRF analogue in which several substitutions

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

phobic 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 determi side of this structure, if it is formed.

similar to that of the natural hormone (150). Therefore, (all

although the studies performed thus far do not determine de

whether α -helical or π -helical structure is invol 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 pep 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 pep 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 str whether α -helical or π -helical structure is involved in the α function of GRF, they do suggest that peptide models the designed on the basis of the π -helical structure might callow useful structure-function re function of GRF, they do suggest that peptide models the designed on the basis of the π -helical structure might condlow useful structure-function relationships to be develant oped. A similar analysis of the other membe designed on the basis of the π -helical structure might collow useful structure-function relationships to be devel-
oped. A similar analysis of the other members of this surfamily of homologous hormones shows that they allow useful structure-function relationships to be devel-
oped. A similar analysis of the other members of this
family of homologous hormones shows that they too can
prom extended amphiphilic π helices, although again oped. A similar analysis of the other members of this stamily of homologous hormones shows that they too can
form extended amphiphilic π helices, although again p
there are always one or two hydrophilic residues locate family of homologous hormones shows that they too can
form extended amphiphilic π helices, although again phi
there are always one or two hydrophilic residues located oth
in the hydrophobic domain formed. The approach form extended amphiphilic π helices, although again philter are always one or two hydrophilic residues located oth in the hydrophobic domain formed. The approach of the idealizing this type of amphiphilic structure mig there are always one or two hydrophilic residues loc
in the hydrophobic domain formed. The approac
idealizing this type of amphiphilic structure might
be fruitful for these peptides, including glucagon, a
alternative to th in the hydrophobic domain formed. The approach of tidealizing this type of amphiphilic structure might also c
be fruitful for these peptides, including glucagon, as an alternative to the possibility of limiting the nonhom idealizing this type of amphiphilic
be fruitful for these peptides, inclu
alternative to the possibility of limi
gous residues in peptide models to
of the potential α -helical structures 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. Oth

Regions of potential amphiphilic secondary structure mones and neurotransmitters or neuromodulators. However, the evidence that these conformations are actually adopted at amphiphilic interfaces and that they might **Figure 3.1 VII. Other Amphiphilic Peptide Hormones**
Regions of potential amphiphilic secondary structure thave been identified in a number of other peptide hormones and neurotransmitters or neuromodulators. How-
ever, the Regions of potential amphiphilic secondary structure
have been identified in a number of other peptide hor-
mones and neurotransmitters or neuromodulators. How-
ever, the evidence that these conformations are actually
adop mones and neurotransmitters or neuromodulators. However, the evidence that these conformations are actually wise adopted at amphiphilic interfaces and that they might type of functional importance varies from case to case, ever, the evidence that these conformations a
adopted at amphiphilic interfaces and that the of functional importance varies from case t
no studies of peptide models have yet been
Some of these peptides are described below opted at amphiphilic interfaces and that they might
of functional importance varies from case to case, and
is studies of peptide models have yet been reported.
me of these peptides are described below.
The hypothalamic hor be of functional importance varies from case to case, and
no studies of peptide models have yet been reported. an
Some of these peptides are described below. que
The hypothalamic hormone, CRF, which stimulates les
secretio

FIG. 12. Helical net diagram of porcine PHI. The distribution of
the situation in the putative π -helical
the amino acid side chains of the entire peptide on the surface of a
the amino acid side chains of the entire pep phobic residues *(circled)* are segregated in a domain lying along one the carboxy-terminal CRF residues in positions 24-41, side of this structure, if it is formed. on OF PEPTIDE HORMONES 313
structurally homologous peptide, sauvagine, isolated
from frog skin, both have strong amphiphilic properties FROM OF PEPTIDE HORMONES 313
Structurally homologous peptide, sauvagine, isolated
from frog skin, both have strong amphiphilic properties
(85). Both peptides bind very tightly to unilamellar egg (85) ON OF PEPTIDE HORMONES

Structurally homologous peptide, sauvagine, isolated

from frog skin, both have strong amphiphilic properties

(85). Both peptides bind very tightly to unilamellar egg

lecithin vesicles withou 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, 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, 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 -he (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 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 collap property shared by the strongly amphiphilic α -helical
serum apolipoproteins, and both peptides form very sta-
ble monolayers at the air-water interface (the collapse
pressures are about 19 dyn/cm) with properties typic 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
pep ble 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
 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 μ M to form tetra-
m 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 μ M to form tetra-
m peptides indicate little secondary structure in aqueous
solution at low concentrations, but CRF was found to
aggregate at concentrations above $1 \mu M$ to form tetra-
meric micelles and larger aggregates all having increase solution at low concentrations, but CRF was found to aggregate at concentrations above $1 \mu M$ to form tetra-
meric micelles and larger aggregates all having increased
helical structure. An examination of the amino acid
se aggregate at concentrations above 1 μ M to form tetra-
meric micelles and larger aggregates all having increased
helical structure. An examination of the amino acid
sequences of CRF and sauvagine on helical wheel dia-
g meric micelles and larger aggregates all having increased
helical structure. An examination of the amino acid
sequences of CRF and sauvagine on helical wheel dia-
grams indicated that these properties probably result
from helical structure. An examination of the amino acid
sequences of CRF and sauvagine on helical wheel dia-
grams indicated that these properties probably result
from formation of amphiphilic α -helical structures by
resid 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 a grams 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 do-
ma 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 do-
main covering over half of the helix surface that lies
p 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
hydro 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, w main 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 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 r 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. Ano 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 p 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 cur on the hydrophilic side of the helix. Another, less
drophobic, amphiphilic α helix has been postulated for
e carboxy-terminal CRF residues in positions 24–41,
t no equivalent structure can be formed by sauvagine.
T

gous residues in peptide models to the hydrophilic sides more, residues 1-8 form an extended helical structure of
of the potential α -helical structures.
VII. Other Amphiphilic Peptide Hormones
Regions of potential amph 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 polypepti 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 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 amphiphi 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 th (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 f 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 s 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 o the hydrophobic domain lies along the helix for over f
complete turns, but twists around the helix slightly in
anticlockwise direction. Again, over half of the he
surface is hydrophobic. The crystal structure of aPP a
prov 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 amp 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 surface is hydrophobic. The crystal structure of aPP also
provides an interesting illustration of how an amphi-
philic structure in a peptide hormone might interact with
other less structured parts of the sequence to prot provides an interesting illustration of how an amphi-
philic structure in a peptide hormone might interact with
other less structured parts of the sequence to protect
them from enzymatic modification or hydrolysis. In thi philic 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 other less structured parts of the sequence to prot
them from enzymatic modification or hydrolysis. In t
case, residues 9–12 of aPP form a β bend so that
amino-terminal residues are folded back across the
drophobic sur 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. Further 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. Further more, residues 1–8 form an extended helical structure o amino-terminal residues are folded back across the hy-
drophobic surface of the amphiphilic α helix. Further-
more, residues 1–8 form an extended helical structure of
the type found in collagen, and proline residues in drophobic 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 more, 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 h 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 hydro-
philic, the extended collagen-like helix in residue terminus to form a hydroph
residues in this segment of aP
philic, the extended collagen
with prolines in every third p
type of amphiphilic structure.
The aPP amino acid sequer sidues in this segment of aPP are all essentially hydro-
illic, the extended collagen-like helix in residues 1–8
th prolines in every third position represents another
pe of amphiphilic structure.
The aPP amino acid sequen philic, 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 pancre

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) se-
quences in about half of the po type of amphiphilic structure.
The aPP amino acid sequence differs from the human
and other mammalian pancreatic polypeptide (PP) se-
quences in about half of the positions (110). Neverthe-
less, the general features of th The aPP amino acid sequence differs from the human
and other mammalian pancreatic polypeptide (PP) se-
quences in about half of the positions (110). Neverthe-
less, the general features of the amphiphilic structures
appear

likely to have similar conformational properties. In par-TAYLOR 1
11 TAYLOR 1
11 Tave similar conformational properties. In par-
11 ticular, the hydrophobic surfaces of the amino- and car-
11 boxy-terminal helical structures might bind to separate TAYLOR ANI
likely to have similar conformational properties. In par-
ticular, the hydrophobic surfaces of the amino- and car-
boxy-terminal helical structures might bind to separate
sites on receptors or other cellular sur sitely to have similar conformational properties. In particular, the hydrophobic surfaces of the amino- and car-
boxy-terminal helical structures might bind to separate
sites on receptors or other cellular surfaces having ticular, the hydrophobic surfaces of the amino- and car-
boxy-terminal helical structures might bind to separate
sites on receptors or other cellular surfaces having am-
phiphilic interfaces as well as interacting with eac in solution. The magnetic state of the amino and can
boxy-terminal helical structures might bind to separate
sites on receptors or other cellular surfaces having am-
phiphilic interfaces as well as interacting with each ot boxy-terminal helical structures might bind to separate
sites on receptors or other cellular surfaces having am-
phiphilic interfaces as well as interacting with each other
in solution. At present, the physiological functi sites on receptors or other centuar 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% ho phiphilic 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 amphip 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 b 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, 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 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 ty 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 both in the central nervous system and in the peripher (110, 143a, 40). These peptides, peptide tyrosine tyrosine (PYY) and neuropeptide tyrosine (NPY), retain the pre-
lines in positions 2, 5, and 8 of the PP sequences, a (110, 143a, 40). These peptides, peptide tyrosine tyrosine (PYY) and neuropeptide tyrosine (NPY), retain the pro-
lines in positions 2, 5, and 8 of the PP sequences, and
the intervening residues are hydrophilic, so that a (PYY) and neuropeptide tyrosine (NPY), retain the pro-
lines in positions 2, 5, and 8 of the PP sequences, and
the intervening residues are hydrophilic, so that a colla-
gen-like amphiphilic helix might again be formed. T the intervening residues are hydrophilic, so that a colla-
gen-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 the intervening residues are hydrophilic, so that a colla-
gen-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 gen-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 13–3 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 struc 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 pro tion 9 of the PP sequences is preserved. Residues 13-
of NPY or 14-32 of PYY can form an amphiphi
structure in the α -helical conformation with genes
properties identical to that observed in the aPP cryss
structure, alt 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 differ-
ences structure in the α -helical conformation with general
properties identical to that observed in the aPP crystal
structure, although there are multiple sequence differ-
ences in this region (144). The carboxy terminus is
 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 seq 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 fam ences in this region (144). The carboxy terminus is
amidated in each case and contains a basic hydrophilic mai
sequence that is highly conserved throughout this peptide form
family in residues 32-36. This structural organ 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 a 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
s 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 (figure 13) is reminiscent of that in the calcitonin and CGRP peptide family, where the amphiphilic helical by segment might serve to connect "active sites" at either end of the molecule and position them in the correct o CGRP peptide family, where the amphiphilic helical ^{oy i}tail 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 surf orientation for agonist activity on a receptor surface. The
functional relationship between PYY and NPY is also vivo stability of these peptides. Their structural homol-
similar to that between calcitonin and CGRP: PYY is 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 ver 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 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 nerv 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 prope peptide present in both central and peripheral nervous ptide present in both central and peripheral nervous
stems (40, 61, 110). This analogy, therefore, suggests
at the amphiphilic structures involved have properties
itable for both types of function.
Since NPY and PYY share systems (40, 61, 110). This analogy, therefore, sugges
that the amphiphilic structures involved have propertie
suitable for both types of function.
Since NPY and PYY share a variety of actions the
are not reproduced by the

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 inter-
esting to identify t 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 inter-
esting to identify the specific features of the apparently
similar architectu Since NPY and PYY share a variety of actions that minal
are not reproduced by the PP structure, it will be inter-
esting to identify the specific features of the apparently forms
similar architecture of these peptides tha are not reproduced by the PP structure, it will be inter-
esting to identify the specific features of the apparently forms a
similar architecture of these peptides that determine idue wi
these differences. Our preliminary esting 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
r 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
(col 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 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 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).
Dime (collapse pressures of about 15 dyn/cm were observed) order that have properties consistent with helical structure for being adopted in this amphiphilic environment (144). con Dimer formation has also been observed in aqu 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 s 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
 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 heli solutions of the pancreatic polypeptides, consistent with of the self-association of amphiphilic α -helical structures segreu (26). It is very likely, therefore, that similar helical hydertructures will be involved in c

mains of porcine PYY (143a). A helical net diagram illustrates the formation of a hydrophobic domain by residues 14-32 in the α -helical conformation (14), and a β turn formed by residues 9-12 connects these two stru 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 res 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 1–8 in a collagen-l:
by residues 9–12 c
"tail" is formed by r
residues are *circled* by residues 9–12 connects these two structures. A basic, hydroph "tail" is formed by residues 33–36 at the carboxy terminus. Hydrophe residues are *circled*.
vivo stability of these peptides. Their structural homogy, combi residues are circled.
vivo stability of these peptides. Their structural homol-

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 apvivo 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 app proach. y, combined with the variety in their amino acid se-
nences, suggests that this family of peptides is ideally
ited to characterization by the peptide modelling ap-
oach.
Yet another peptide hormone for which a potential
n

quences, suggests that this family of peptides is ideally
suited to characterization by the peptide modelling ap-
proach.
Yet another peptide hormone for which a potential
amphiphilic α -helical structure has been postu suited to characterization by the peptide modelling approach.

Yet another peptide hormone for which a potential

amphiphilic α -helical structure has been postulated is

PTH (42). In an α -helical conformation, the e 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-ter 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-ter 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 wh 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 residu fragment of this hormone consisting of the amino-ter-
minal 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 res-
i minal 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 res-
idue wide. This domain lies along the complete length row of mostly leucine, valine, and methionine residues
forms a hydrophobic domain that is essentially one res-
idue wide. This domain lies along the complete length of
the helix (a total of nine turns) and, in the regular 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 aroun 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, 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, there-
fore, it is likely that this amphiphilic struct the helix surface once in an anticlockwise direction. In
order to interact with most amphiphilic interfaces, there-
fore, it is likely that this amphiphilic structure would
contain a discontinuity or other irregularities t 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 fore, 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 sug 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 w 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 paral 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 P

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ARMACOLOGI

STRUCTURAL CHARACTERI
in helicity, indicating that amphiphilic secondary stru
ture-might-contribute-to-cell surface-binding of th STRUCTURAL CHARACTERIZATIO
in helicity, indicating that amphiphilic secondary struc-
ture might contribute to cell surface binding of this
hormone also (42, 43). in helicity, indicating the
ture might contribute t
hormone also (42, 43).
An extensive search o helicity, indicating that amphiphilic secondary struc-
re might contribute to cell surface binding of this or
primone also (42, 43).
An extensive search of the amino acid sequences of di
ptide hormones reveals that segment

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 ture might contribute to cell surface binding of this orienta
hormone also (42, 43). Order t
An extensive search of the amino acid sequences of diffusion
peptide hormones reveals that segments of alternating bilizing
hydr hormone also (42, 43). The common section of the amino acid sequences of differential periode hormones reveals that segments of alternating bill hydrophobic and hydrophilic residues, which could form interpresenting amphi 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 amphiphil peptide hormones reveals that segments of alternating bilizing protease-sensitive parts of these molecules to hydrophobic and hydrophilic residues, which could form increase the duration of the messages they convey.
amphi 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 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 structur 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 ex-
tremely hydrophobic surfaces (123, 124). It may properties that such sequences exhibit as a result of their
extensive aggregation into β -sheet structures with ex-
tremely hydrophobic surfaces (123, 124). It may be that
this type of structure would be too lipophilic extensive aggregation into β -sheet structures with ex-
tremely hydrophobic surfaces (123, 124). It may be that
this type of structure would be too lipophilic and might
meven cause the disruption of many biological stru tremely 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 stru this type of structure would be too lipophilic and might mineven cause the disruption of many biological structures to v
just as the hyrophobic amphiphilic α -helical structure of and
melittin, the bee venom peptide, ly even cause the disruption of many biological structures to w
just as the hyrophobic amphiphilic α -helical structure of and
melittin, the bee venom peptide, lyses erythrocytes (33, enzy
35). An earlier proposal that LHR just as the hyrophobic amphiphilic α -helical structure of and
melittin, the bee venom peptide, lyses erythrocytes (33, enz
35). An earlier proposal that LHRH might form such an amp
amphiphilic β -strand structure (77 melittin, the bee venom peptide, lyses erythrocytes (33, e 35). An earlier proposal that LHRH might form such an amphiphilic β -strand structure (77) has been tested in cour laboratory by attempting to spread the peptid 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 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
b our laboratory by attempting to spread the peptide on p
the surface of aqueous solutions containing increasing c
concentrations of KCl. Thus far, however, it has not
been possible to induce this peptide to form monolayers
 the surface of aqueous solutions containing increasing chair
concentrations of KCl. Thus far, however, it has not struce
been possible to induce this peptide to form monolayers midd
at this interface, indicating that there 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 LHR 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, at this interface, indicating that there must be a soluble
folded conformation that is strongly preferred by LHRH of
over the extended amphiphilic structure (82). In contrast,
we have found that the κ -opiate receptor we have found that the *k*-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). over the extended amphiphilic structure (82). In contrast,
we have found that the *k*-opiate receptor selective pep-
tide, dynorphin A(1-17), does form monolayers at the
air-water interface (144). These monolayers have a 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). 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 environ-
men air-water interface (144). These monolayers have a sim-
ilar stability to those formed by β_h -endorphin (126). spectral
Furthermore, the tendency of the dynorphin A(1–17) glue
molecules to form extensive aggregates in t ilar stability to those formed by β_h -endorphin (126).
Furthermore, the tendency of the dynorphin A(1–17) g
molecules to form extensive aggregates in this environ-
ment is typical of the type of behavior expected for an Furthermore, the tendency of the dynorphin A(1-1
molecules to form extensive aggregates in this enviro
ment is typical of the type of behavior expected for
extended amphiphilic β strand structure (34, 123, 12
and, inde molecules to form extensive aggregates in this envinent is typical of the type of behavior expected for extended amphiphilic β strand structure (34, 123, 1 and, indeed, residues 7–15 of this peptide do have hydphobic a ment is typical of the type of behavior expected for a
extended amphiphilic β strand structure (34, 123, 124
and, indeed, residues 7–15 of this peptide do have hydro
phobic and hydrophilic residues arranged in an alter extended amphiphilic β strand structure (34, 123, 124) and, indeed, residues 7-15 of this peptide do have hydro-
phobic and hydrophilic residues arranged in an alternating fashion. Strong nonspecific membrane binding p and, indeed, residues 7–15 of this peptide do have hydro-
phobic and hydrophilic residues arranged in an alternat-
ing fashion. Strong nonspecific membrane binding prop-
erties of dynorphin $A(1-17)$ have also been descri phobic and hydrophilic residues arranged in an alternat-
ing fashion. Strong nonspecific membrane binding prop-
erties of dynorphin A(1-17) have also been described
(67), and it has been demonstrated that the complete 17-
 ing fashion. Strong nonspecific membrane binding prop-
erties of dynorphin $A(1-17)$ have also been described for
(67), and it has been demonstrated that the complete 17 -
residue structure has a much greater resistance erties 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 pro-
teolytic degradation than its carboxy-terminal deleti (67), and it has been demonstrated that the complete 17- ever, the modelling approach has proven useful in the residue structure has a much greater resistance to pro-
technical characterization of glucagon binding sites, residue structure has a much greater resistance to proteolytic degradation than its carboxy-terminal deletianalogues (30), suggesting that this potential amphiphi β -strand structure might determine the properties dynorphin A in much the same way as the correspondinelical s 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 β -strand struct
dynorphin A in
helical structure
ling approach t
pears attractive Following the same way as the extreponding

inelical structure does in β -endorphin. A peptide model-

ing approach to investigating this possibility also ap-
 VIII. Summary and Prospects for Future Studies

Medium-si In approach to investigating this possibility also appears attractive.
VIII. Summary and Prospects for Future Studies
Medium-sized peptide hormones and neurotransmit-

pears attractive.
 VIII. Summary and Prospects for Future Studies

Medium-sized peptide hormones and neurotransmit-

ters that have little or no apparent secondary or tertiary

structure in aqueous solution often have th Form amphiphilic secondary structures which may be
the 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 spec ters that have little or no apparent secondary or tertia
structure in aqueous solution often have the potential
form amphiphilic secondary structures which may
stabilized in the biological milieu in general, or at speci
in 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 From amphiphilic secondary structures which may be
stabilized in the biological milieu in general, or at specific
interfaces that provide a suitable complementary amphi-
philic environment. We have postulated here and else interraces that provide a suitable complementary amphi-
philic environment. We have postulated here and else-
where that such structures may serve multiple functions
in these peptides, including (a) limiting their receptor

on or PEPTIDE HORMONES
are important for agonist activity are held in the correct
orientation; (b) promoting adsorption to cell surfaces in on OF PEPTIDE HORMONES 315
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 ON OF PEPTIDE HORMONES 315
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 con are important for agonist activity are held in the correc
orientation; (*b*) promoting adsorption to cell surfaces i
order to enhance the efficiency of receptor location, limi
diffusion, or control proteolytic processing; 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 orientation; (b) promoting adsorption to cell surface order to enhance the efficiency of receptor location, diffusion, or control proteolytic processing; and (c) bilizing protease-sensitive parts of these molecule increase der to enhance the efficiency of receptor location, liftusion, or control proteolytic processing; and (c) s
lizing protease-sensitive parts of these molecules
crease the duration of the messages they convey.
The approach o

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 bilizing 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 prove 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 fun The approach of studying synthetic peptides incorp
rating models of the potential amphiphilic structures h
proven extremely useful in assessing their importan
and functions. In the case of β -endorphin, an amphiphil
hel rating 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 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 deter-
mine the receptor specificity of the enkephalin str helix at the carboxy terminus has been shown to deter-
mine the receptor specificity of the enkephalin structure
mine the receptor specificity of the message from
and to protect this essential part of the message from
and mine 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 mine 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 tha 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 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 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 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 st 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 parently 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 struc 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, 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 -he 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 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 amphiphilic 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 spe 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
s 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
mos 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
mo 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
amphi 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
aci 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
cons most of this peptide hormone is potentially involved
amphiphilic helical structure, and the multiple amin
acid substitutions made in peptide models resulted
considerable losses in potency. At present, therefore,
is not cle 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 recepconsiderable losses in potency. At present, therefore, it
is not clear what conformation glucagon adopts on recep-
tor surfaces, and it is possible that more than one conconsiderable losses in potency. At present, therefore, it
is not clear what conformation glucagon adopts on recep-
tor surfaces, and it is possible that more than one con-
formation may be involved. Even in this situation, is not clear what conformation glucagon adopts on recep-
tor surfaces, and it is possible that more than one con-
formation may be involved. Even in this situation, how-
ever, the modelling approach has proven useful in th tor 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 formation may be involved. Even in this situation, how-
ever, the modelling approach has proven useful in the
functional characterization of glucagon binding sites, and
it may still be possible to identify the receptor-bou ever, 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 desig nctional characterization of glucagon binding sites, and
may still be possible to identify the receptor-bound
nformation of the natural hormone through the design
more conservative peptide models, as discussed.
The modelli

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
plac 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 am
phiphi 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 am-
phiphilicity of the helical structures studied and on min-
i The modelling strategy, as it has developed so far, has
placed an emphasis on optimizing the stability and am-
phiphilicity of the helical structures studied and on min-
imizing homology to the natural sequences. This ap-
 placed an emphasis on optimizing the stability and am-
phiphilicity of the helical structures studied and on min-
imizing homology to the natural sequences. This ap-
proach was adopted in an attempt to simultaneously
creat imizing homology to the natural sequences. This a proach was adopted in an attempt to simultaneous create hormone analogues that were more potent the natural structures and identify their receptor-bous conformations. Thes proach was adopted in an attempt to simultaneou
create hormone analogues that were more potent th
the natural structures and identify their receptor-bou
conformations. These goals may sometimes be inco
patible, however, as create hormone analogues that were more potent than
the natural structures and identify their receptor-bound
conformations. These goals may sometimes be incom-
patible, however, as minimizing homology in an amphi-
philic s the natural structures and identify their receptor-bound
conformations. These goals may sometimes be incom-
patible, however, as minimizing homology in an amphi-
philic structure might necessitate altering parts of a
hormo philic 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, enhancpatible, however, as minimizing homology in an amp
philic structure might necessitate altering parts of
hormone that interact somewhat specifically with
receptor surface and, in these circumstances, a lov
binding affinity philic 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, enhanc-
 normone that interact somewhat specifically with its
receptor surface and, in these circumstances, a lower
binding affinity is likely to result. Nevertheless, enhanc-
ing the amphiphilic properties of β -endorphin has p

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onstrating that this is a powerful approach to the design

of peptides with the potential for therapeutic use. Freed TAYLOR AND

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 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 th 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 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
stru believe, been completely validated through the design and study of the physicochemical domains in the β -endorphin model has, we believe, been completely validated through the design and study of the physicochemical and higher potencies could also be attained. On the oth
hand, our original hypothesis for the organization
structural domains in the β -endorphin model has, v
believe, been completely validated through the designal
and stud mand, our original hypothesis for the original
structural domains in the β -endorphin model has, we
believe, been completely validated through the design
and study of the physicochemical and pharmacological
properties o 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
fir 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 sim 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 the power of model design with minimal nomology 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
 multiple analogues with single residue changes can be avoided. It is also expected that, given the number of $\frac{2!}{3!}$ and $\frac{2!}{3!}$ analogues with single residue changes can be avoided. It is also expected that, give 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
am multiple analogues with single residue changes can be 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 avoided. It is also expected that, given the amphiphilic structures—particularly helices-
been identified so far, some general rules rel
structures to their functions in peptide hor
neurotransmitters will soon become evide

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