THE PEPTIDES

Analysis, Synthesis, Biology

EDITED BY

SIDNEY UDENFRIEND
JOHANNES MEIENHOFER

Volume 6

Opioid Peptides: Biology, Chemistry, and Genetics

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Preface

The Peptides is an open-ended treatise providing comprehensive and critical reviews of important developments in all areas of peptide research, including analysis, synthesis, and biology. These reviews are intended as a reference for the specialist, a guide for the novice, and a forum for all investigators concerned with peptides and proteins.

Volume 6 is the first in this treatise that presents a biological topic of peptide research. Of all areas of peptide and protein research, biology has been growing most rapidly, and it continues to expand at an accelerated rate. This poses a considerable challenge to the production of reviews such as *The Peptides* because parts of the books, up to date at the time production starts, may not include further developments made by the time they are published. On the other hand, many colleagues indicate to us that the treatise has become a convenient way of keeping informed. As the editors we are caught in the middle and have to deal with both situations. However, we believe that Volume 6, entitled *Opioid Peptides: Biology, Chemistry, and Genetics* is both timely and exciting. It contains research results up to early 1984, a fact made possible by a most cooperative and responsive group of authors.

In the first chapter, Shosaku Numa reviews the cloning of cDNAs for opioid peptide precursors, sequencing and assignment of protein sequences, cloning and structural analysis of precursor genes, regulation of gene expression, and the biological significance of multihormone precursors.

Proenkephalin and the products of its processing are discussed by Sidney Udenfriend and Daniel L. Kilpatrick in Chapter 2. Essential microchemical and biological procedures for the isolation and characterization of enkephalin-containing peptides are presented as well as the cloning, sequencing, and regulation of proenkephalin biosynthesis.

In Chapter 3, the role of pro-opiomelanocortin (POMC) as a protein at the interface of the endocrine and nervous systems is examined by Olivier Civelli, James Douglass, and Edward Herbert. The distribution and site of POMC-derived peptides, their transcriptional characteristics and regulation, as well as posttranscriptional regulation are also described.

Avram Goldstein presents in Chapter 4 a comprehensive account of the dynorphin κ opioid receptor, dynorphin structure–activity relationships, and pharmacological and binding selectivities. Possible physiological functions are examined.

The overview of opioid receptors by Stewart J. Paterson, Linda E. Robson, and Hans W. Kosterlitz in Chapter 5 covers the heterogeneity of opioid receptors, the opioid binding sites, characteristics of μ, δ, and κ types, central nervous system and peripheral binding sites, the pharmacology of opioid receptors in isolated tissues, and other related topics.

In Chapter 6, Donald Yamashiro and Choh Hao Li review structure–activity relationships of β-endorphin. The synthesis of homogeneous analogs and their careful biological evaluation are essential for these studies. Naturally occurring sequences, their hybrids, truncated and extended sequences, and substitution analogs are described.

Conformational analysis of enkephalins and conformation–activity relationships are presented by Peter W. Schiller in Chapter 7. Conformational models of enkephalin, theoretical energy calculations, crystal structure determinations, conformations in solution, and conformationally restricted enkephalin analogs provide a mosaic of unprecedented complexity.

Philip E. Hansen and Barry A. Morgan undertake the herculean task, in Chapter 8, of describing a most interesting selection of structure–activity relationships among enkephalin peptides. Minimal structural requirements, structural preferences of receptor subclasses, peptide antagonists of opiate receptors, and structure–activity relationships *in vivo* are delineated.

In the final chapter, Vicky Clement-Jones and G. Besser examine in detail the clinical significance of opioid peptides in humans. The description of the strategies used in this study, the distribution of opioid peptides in humans, and their possible physiological roles (e.g., in pain modulation, narcotic dependence, psychiatric disease, tumors, and many other syndromes) appear to promise potential therapeutic benefits of opioid peptides in certain human diseases.

We wish to thank the authors for their efforts in preparing these chapters on time. We would also like to express our gratitude to the staff of Academic Press for their prompt production of the book.

Johannes Meienhofer

Introduction

The opioid peptides were discovered just as the new biotechnologies were appearing. In the area of protein and peptide chemistry, high-performance liquid chromatography (HPLC), coupled with fluorescence or ultraviolet detection systems, provided heretofore unattainable resolution and sensitivity. Purification of trace substances became a science rather than an art. The introduction of microsequencing made it possible to characterize the small amounts of peptide or protein attainable by these procedures. The coupling of solid-phase peptide synthesis to HPLC solved the problem of purification and made synthetic peptides more readily available. Of equal or even greater importance was the introduction of recombinant DNA technology. This revolution in biochemical technology made it possible to identify and determine the structures of three distinct genes that code for proteins that contain one or more enkephalin sequences and to isolate and characterize over 20 products of processing of the three gene products. All of this information was accumulated within just a few years. It is of interest that we now know far more about the chemistry and genetics of the enkephalin-containing peptides than we do about their physiological roles. It is likely that the same technologies will soon permit the full characterization of the various opiate receptors at the molecular level. At the moment, however, it appears that the application of good "old fashioned" physiology and pharmacology are still required to elucidate the role(s) of the opiate peptides in health and disease.

The articles in this volume present the fascinating story of the "enkephalins" by investigators who played key roles in the unfolding saga. It is hoped that this volume, coming out just 9 years after the discovery of the enkephalins, will serve as a reference source and help stimulate other investigators in this and related fields.

Sidney Udenfriend

Nomenclature and Abbreviations*

Abbreviations

A	adenylic acid
AcOH	acetic acid
ACTH	corticotropin
ACTH-β-LPH	pro-opiomelanocortin, corticotropin-β-lipotropin precursor
ADH	antidiuretic hormone (vasopressin)
Aib	α-aminoisobutyric acid
AL	anterior lobe of pituitary gland
Alu	*Arthrotobacter luteus* restriction site
cAMP	cyclic adenosine monophosphate
ATG	start codon, translation initiator
AtT-20-D_{16v}	anterior pituitary cell line
AUG	start codon, translation initiator
b	bovine species
Bam	bovine adrenal medulla (peptide)
Boc	*tert*-butyloxycarbonyl
bp	base pair
pBR322	*Eschericia coli* expression plasmid
*n*BuTyr	*N*-*n*butyltyrosine
BzlGly	*N*-benzylglycine
C	cytidylic acid
c	canine species

*All symbols and abbreviations used in this volume are listed except the three-letter symbols of the common amino acids. For peptide size nomenclature, abbreviation policy, and oxazolone designation see Volumes 1–3. The one-letter symbols for amino acids are as follows:

A alanine	G glycine	M methionine	S serine
C cysteine	H histidine	N asparagine	T threonine
D aspartic acid	I isoleucine	P proline	V valine
E glutamic acid	K lysine	Q glutamine	W tryptophan
F phenylalanine	L leucine	R arginine	Y tyrosine

ca	camel species
CAAT	RNA polymerase binding site
CHAPS	3-[3-(cholamidopropyl)dimethylamino]-1-propanesulfonate
Cit	citraconyl
CLIP	corticotropin-like intermediate lobe peptide
(4Cl)Phe	(4-chloro)phenylalanine
CD	circular dichroic spectroscopy
CNS	central nervous system
nC8Phe	*N*-*n*octylphenylalanine
Cpe	cyclopentyl
Cpm	cyclopropylmethyl
CRF	corticotropin-releasing factor
CSF	cerebrospinal fluid
δ	delta receptor
Dbu	α,γ-diaminobutyric acid (A_2bu)
ΔAla	α,β-dehydroalanine
ΔLeu	α,β-dehydroleucine
ΔPhe	α,β-dehydrophenylalanine
Δ^3Pro	3,4-dehydroproline
DEX	dexamethasone
DHM	dihydromorphine
[$^2H_6$]-DMSO	[$^2H_6$]dimethyl sulfoxide
*c*DNA	complementary deoxyribonucleic acid
DNA	deoxyribonucleic acid
Dns	5-dimethylamino-1-naphthalene sulfonyl (dansyl)
Dpr	α,β-diaminopropionic acid
Dyn	dynorphin
e	equine species
EC	enkephalin-containing
ECEPP	empirical conformational energy program for peptides
ECP	enkephalin-containing peptide
EKC	ethylketazocine or ethylketocyclazocine
β-END	β-endorphin
ENK	enkephalin
epr	electron paramagnetic spin resonance
EtPhe	*N*-ethylphenylalanine
EtTyr	*N*-ethyltyrosine
f	feline species
Fmoc	9-fluorenylmethyloxycarbonyl

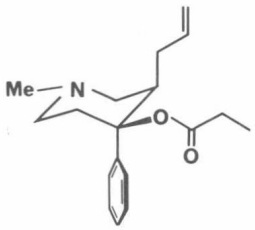
G	guanylic acid
GABA	γ-aminobutyric acid
GH	growth hormone
Glyol	glycinol
GnRH	gonadoliberin
GPA	2,9-dimethyl-3′-hydroxy-5-phenyl-6,7-benzomorphane
GPI	guinea pig ileum (assay, activity)
GTP	guanosine triphosphate
h	human species
Hfe	L-homophenylalanine (*S*-2-aminobenzenebutanoic acid)
HOSu	*N*-hydroxysuccinimide
H_6Phe	L-3-(cyclohexyl)alanine
HPLC	high-performance liquid chromatography
Ia	isoamyl
IC_{50}	concentration to inhibit assay response by 50%
ICC	immunocytochemistry
icv	intracerebroventricular (injection)
it.	intrathecal (injection)
IR	immunoreactive
ir	infrared spectroscopy
iv	intravenous (injection)
κ	kappa receptor
kb	kilobase
kD	kilodalton
K_e	negative log of molar concentration that reduces agonist activity by 50%
LH	lutropin
LH/FSH	lutropin/follitropin
LHRH	gonadoliberin (luliberin)
LPH	lipotropin
β-LPH	β-lipotropin [lipotropin (1–91)]
γ-LPH	γ-lipotropin [lipotropin (1–58)]
LVP	lysine-vasopressin
m	murine species
MeAla	*N*-methylalanine
MeLeu	*N*-methylleucine

MeOH	methanol
MeMet	*N*-methylmethionine
MePhe	*N*-methylphenylalanine
MePheol	*N*-methylphenylalaninol
Met(O)	methionine sulfoxide
Met(O_2)	methionine sulfone
Met(O)ol	methioninol sulfoxide
MeTrp	*N*-methyltryptophan
MeTyr	*N*-methyltyrosine
M_r	relative molecular weight
α-MSH	α-melanotropin
β-MSH	β-melanotropin
γ-MSH	γ-melanotropin
γ1-MSH	γ1-melanotropin
γ2-MSH	γ2-melanotropin
γ3-MSH	γ3-melanotropin
μ	mu receptor
MVD	mouse vas deferens (assay, activity)
NG108-15	cell line
NIL	neurointermediate lobe of pituitary gland
Nle	norleucine
n*M*	nanomole
nmr	nuclear magnetic resonance spectroscopy
NOE	nuclear Overhauser enhancement
(4NO_2)Phe	(4-nitro)phenylalanine
Nps	2-nitrophenylsulfenyl
Nva	norvaline
o	ovine species
ODS	octadecyl (reversed phase HPLC column)
os	ostrich species
p	porcine species
pA_2	negative log of molar concentration that reduces agonist activity by 50%
PAG	periaqueductal gray
PC	partition chromatography
Pe	phenylethyl
Pen	penicillamine, β,β-dimethylcysteine
Ph	phenyl

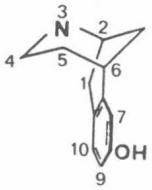
Pheol	phenylalaninol
Phg	C-phenylglycine
PHI-27	porcine N^{α}-His-c^{α}Ile intestinal *27* peptide
PL	posterior (neural) lobe of pituitary gland
POMC	pro-opiomelanocortin
Δ^3Pro	3,4-dehydroproline
*n*PrPhe	*N-n*propylphenylalanine
*i*PrTyr	*N-iso*propyltyrosine
*n*PrTyr	*N-n*propyltyrosine
Pya	3-(2-pyrazinyl)alanine
QSAR	quantitative structure–activity relationship
RIA	radioimmunoassay
*m*RNA	messenger ribonucleic acid
RP	reversed phase
RP-18	(octadecyl reversed phase HPLC column)
RRA	radioreceptor assay
sa	salmon species
SAR	structure–activity relationship
Sar	sarkosin, *N*-methylglycine
sc	subcutaneous (injection)
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEM	standard error of the means
SPS	solid phase peptide synthesis
T	thymidilic acid
TATA box	RNA polymerase binding site
Thz	L-thiazolidine-4-carboxylic acid
Tmp	thiomethylpropyl
TRH	thyroliberin
tris-HCl	tris[hydroxymethyl]aminomethane hydrochloride
TSH	thyrotropin
tu	turkey species
vp	vasopressin

Structures of Opiates and Related Nonpeptide Receptor Ligands Discussed in the Text*

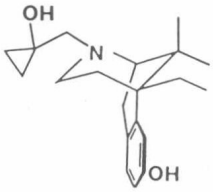
α-Allylprodine



6,7-Benzomorphan



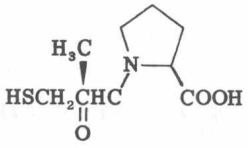
Bremazocine



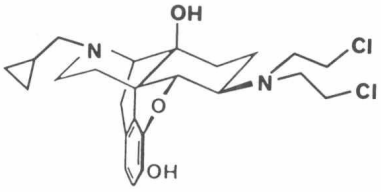
14β-Bromoacetamidomorphine *see* Morphine

*Structures of opiates were prepared by Dr. Barry A. Morgan.

Captopril



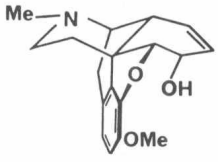
Chlornaltrexamine



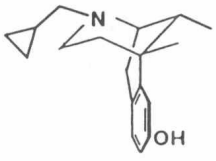
α-Chlornaltrexamine deriv. *see* Naltrexone

β-Chlornaltrexamine deriv. *see* Naltrexone

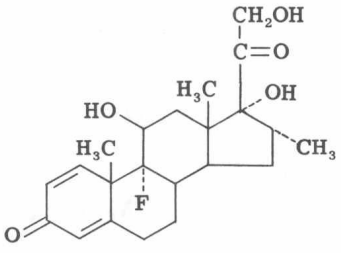
Codeine



Cyclazocine

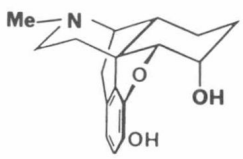


Dexamethasone

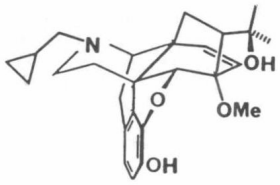


Dextorphan (+)-isomer of levorphanol — *see* Levorphanol

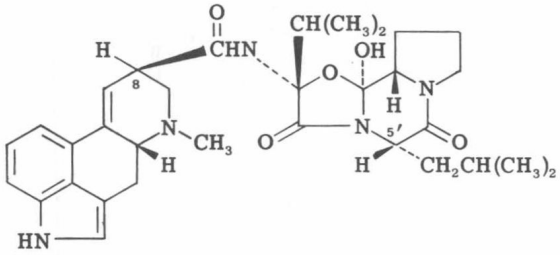
Dihydromorphine



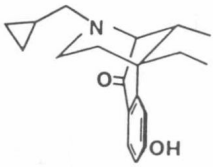
Diprenorphine



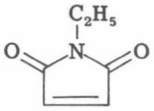
α-Ergocryptine



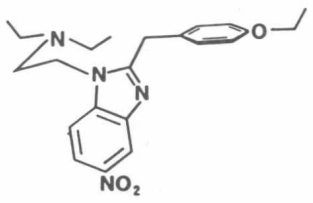
Ethylketazocine



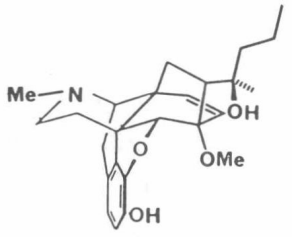
N-Ethylmaleimide



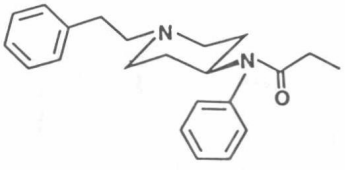
Etonitazene



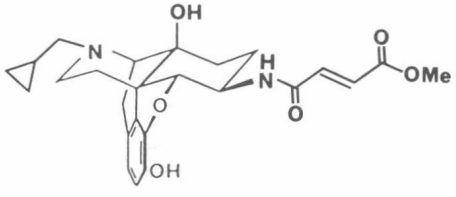
Etorphine



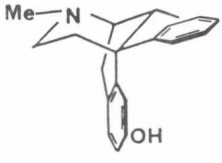
Fentanyl



Funaltrexamine



GPA 1657



Haloperidol