modern
pharmacology–toxicology
volume 20

endorphins

chemistry, physiology, pharmacology, and clinical relevance

edited by
JEFFREY B. MALICK and ROBERT M.S. BELL

ENDORPHINS

Chemistry, Physiology, Pharmacology, and Clinical Relevance

EDITED BY

Jeffrey B. Malick, Ph.D.
Stuart Pharmaceuticals
Division of ICI Americas Inc.
Wilmington, Delaware

Robert M. S. Bell, M.D.
Rama Medical Clinic
Charlotte, North Carolina

MARCEL DEKKER, INC. New York and Basel

Library of Congress Cataloging in Publication Data
Main entry under title:

Endorphins: chemistry, physiology, pharmacology, and clinical relevance.

(Modern pharmacology-toxicology)
Includes indexes.
1. Endorphins. I. Malick, Jeffrey B. II. Bell, Robert M. S.
[DNLM: 1. Endorphins. W1 MO167T v. 20 / QU68 E55]
QP951.E5E5 612'.814 81-22214
ISBN 0-8247-1687-6 AACR2

Marcel Dekker, Inc.
270 Madison Avenue, New York, New York 10016

Current Printing (last digit):
10 9 8 7 6 5 4 3 2 1

Printed in the United States of America

ENDORPHINS

MODERN PHARMACOLOGY-TOXICOLOGY

A Series of Monographs and Textbooks

COORDINATING EDITOR
William F. Bousquet
Department of Product Research
Searle Laboratories, G. D. Searle & Co.
Chicago, Illinois

ASSOCIATE EDITOR
Roger F. Palmer
University of Miami
School of Medicine
Miami, Florida

1 A Guide to Molecular Pharmacology-Toxicology, Parts I and II *Edited by R. M. Featherstone*
2 Psychopharmacological Treatment: Theory and Practice *Edited by Herman C. B. Denber*
3 Pre- and Postsynaptic Receptors *Edited by Earl Usdin and William E. Bunney, Jr.*
4 Synaptic Receptors: Isolation and Molecular Biology *Eduardo De Robertis*
5 Methods in Narcotics Research *Edited by Seymour Ehrenpreis and Amos Neidle*
6 Horizons in Clinical Pharmacology *Edited by Roger F. Palmer*
7 Synthetic Antidiarrheal Drugs: Synthesis–Preclinical and Clinical Pharmacology *Edited by Willem Van Bever and Harbans Lal*
8 Receptors and Mechanism of Action of Steroid Hormones, Parts I and II *Edited by Jorge R. Pasqualini*
9 Hormone-Receptor Interaction: Molecular Aspects *Edited by Gerald S. Levey*
10 Structure and Function of Monoamine Enzymes *Edited by Earl Usdin, Norman Weiner, and Moussa B. H. Youdin*
11 Receptors in Pharmacology *Edited by John R. Smythies and Ronald J. Bradley*
12 Noncatecholic Phenylethylamines (in two parts) *Edited by Aron D. Mosnaim and Marion E. Wolf*

13 Biochemistry of Mental Disorders: New Vistas *Edited by Earl Usdin and Arnold J. Mandell*
14 Developments in Opiate Research *Edited by Albert Herz*
15 Cadmium Toxicity *Edited by John H. Mennear*
16 Body Temperature: Regulation, Drug Effects, and Therapeutic Implications *Edited by Peter Lomax and Edward Schönbaum*
17 Interferon and Interferon Inducers: Clinical Applications *Edited by Dale A. Stringfellow*
18 The Spinal Cord and Its Reaction to Traumatic Injury: Anatomy–Physiology–Pharmacology–Therapeutics *Edited by William F. Windle*
19 Hypertension Research: Methods and Models *Edited by Frederick M. Radzialowski*
20 Endorphins: Chemistry, Physiology, Pharmacology, and Clinical Relevance *Edited by Jeffrey B. Malick and Robert M. S. Bell*

To Our Wives and Parents

Preface

. . . dreams out of the ivory gate,
and visions before midnight.

Sir Thomas Browne, *On Dreams*

The ancient Sumerians referred to the poppy as the "joy plant." It seems probable that they were aware of the mood-elevating properties of the sticky juices, later called opium, that oozed from the flower seed capsule. Greek and then Roman physicians from 300 B.C. onward were knowledgeable of its actions, prescribing it to their patients for various conditions. Since then opium has been used in most countries down through the ages, not only for its medicinal properties (e.g., pain relief) but also as a social drug that provided a sense of well-being, a dreamlike state, and a calming effect. It was the latter effects that often led to addiction and antisocial drug-seeking behavior. Of all the drugs that have been used, probably none has produced so much benefit and yet, paradoxically, so much despair as opium.

The modern impetus to opioid research can probably be dated to 1803, when Setürner isolated the morphine alkaloid from opium. He named the compound after Morpheus, the Greek god of dreams. Since that time, the drug has provided the basis of research for countless scientists. Their quest to conquer pain, to understand the pharmacological profiles of opioids, and to unravel their physiological actions has been relentless. This pursuit was fired not only by the zeal of their calling but also by public pressure to rid society of the problems arising from the abuse of such compounds.

This book takes up the story in the 1970s, when it was demonstrated that morphine receptors were present in the brain of vertebrate animals, and carries it forward into the early 1980s. The discovery of opiate receptors prompted a provocative question: If there were receptors, shouldn't there be endogenous substances which would bind with them? The answer was soon forthcoming with the discovery of the endorphins, which included β-endorphin and methionine- and leucine-enkephalin. With these discoveries, a whole new era of narcotic research began. In these pages we document much of the work undertaken during the exciting early years following the identification of the morphine receptor. Hundreds of scientific papers have been produced in what has been an avalanche of information. With each new paper there has been a general furthering of our understanding of what lies beneath each of nature's veils.

Where this exponential quest for knowledge is taking us is not easy to see. The early hopes of discovering a nonaddictive morphine substitute with fewer side effects have not been fulfilled. It seems that the naturally occurring opioid peptides and their synthetic congeners that have been made to date have properties similar to those of the ancient botanical alkaloid. Many pharmaceutical companies and research institutions are testing synthetic polypeptides in an attempt to find analgesics that are improvements on currently available drugs. We hope that the effort is rewarded. However, although the endorphins have been shown to be analgesics in humans, they do not appear to offer significant advantages over existing poppy derivatives since they exhibit tolerance, physical-dependence liability, and cross-tolerance to morphine, produce respiratory depression, and are self-administered by animals. Thus, unless some advantage other than potency can be discovered, it appears highly unlikely that the endorphins will be used widely as antinociceptive agents.

The function of endorphins in the body and their significance in disease states are slowly unfolding. Their role in psychiatric disorders has been one of the areas more aggressively pursued and many claims are currently being made. Endorphins are distributed widely throughout the body and one can speculate that they may eventually be shown to be involved in many other functions, such as blood pressure control, sleep regulation, and gastrointestinal function, just to mention a few. In addition, they may help solve many of the other enigmas of both medicine and physiology. However, like Morpheus, it is easy to dream.

We would like to thank all the authors who have made this volume possible, our secretaries for making it happen, and our wives for their great patience and for insisting that we persevere.

Robert M. S. Bell
Jeffrey B. Malick

Contributors

*Eugene R. Baizman, Ph. D.** Addiction Research Foundation, Pala Alto, California

Floyd E. Bloom, M. D. A. V. Davis Center for Behavioral Neurobiology, The Salk Institute, San Diego, California

Brian M. Cox, Ph. D.† Addiction Research Foundation, Palo Alto, California

William L. Dewey, Ph. D. Department of Pharmacology, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia

Jeffrey M. Goldstein, Ph. D. Biomedical Research Department, Stuart Pharmaceuticals, Division of ICI Americas Inc., Wilmington, Delaware

John A. H. Lord, Ph. D. Pharmaceutical Division, Reckitt and Colman Ltd., Hull, England

Jeffrey B. Malick, Ph. D. Biomedical Research Department, Stuart Pharmaceuticals, Division of ICI Americas Inc., Wilmington, Delaware

Michael J. Rance, Ph.D. Pharmaceutical Division, Reckitt and Colman Ltd., Hull, England

Present Affiliation:

*Sterling-Winthrop Research Institute, Rensselaer, New York

†Department of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, Maryland

Jean P. Rossier, M. D., D. S. * A. V. Davis Center for Behavioral Neurobiology, The Salk Institute, San Diego, California

Eric J. Simon, Ph. D. Departments of Psychiatry and Pharmacology, New York University Medical Center, New York, New York

Colin F. C. Smith, Ph. D. Pharmaceutical Division, Reckitt and Colman Ltd., Hull, England

Derek G. Smyth, Ph. D. Laboratory of Peptide Chemistry, National Institute for Medical Research, Mill Hill, London, England

Lars Terenius, M. D. Department of Pharmacology, University of Uppsala, Uppsala, Sweden

Present Affiliation:
*Department of Neurophysiologic Research, C. N. R. S. Laboratories, Gif-sur-Yvette, France

Contents

Preface v

Contributors vii

1. HISTORY 1
Eric J. Simon

I. Introduction 1
II. Developments That Led to the Postulation and Discovery of Opiate Receptors 1
III. Discovery of Opiate Receptors 2
IV. Discovery of Endogenous Opioid Peptides 4
V. Nomenclature 6
References 7

2. STRUCTURAL RELATIONSHIPS OF THE POTENT ENDORPHINS 9
Derek G. Smyth

I. Number and Variety of Opioid Peptides 9
II. Isolating Endorphins 12
III. Physiology and Differentiation of Endorphins 14
IV. Address Sequences: A Hypothesis 18
V. Conclusions 19
References 20

3. STRUCTURE-ACTIVITY RELATIONSHIPS 23
William L. Dewey

I. Introduction 23
II. Peptides with Fewer Than Five Amino Acids 25
III. Substitutions for Amino Acid 61 in LPH 61-65 28
IV. Substitutions for Amino Acid 62 or 63 in LPH 61-65 30
V. Substitutions for Amino Acid 64 in LPH 61-65 32
VI. Substitutions for Amino Acid 65 in LPH 61-65 32
VII. Substitutions for More Than One Amino Acid in LPH 61-65 44
VIII. Peptides with More Than Five Amino Acids 45
IX. Summary and Conclusions 51
References 53

4. ASSAY OF THE ENDORPHINS AND RELATED ANALOGS 57
John A. H. Lord, Michael J. Rance, and Colin F. C. Smith

I. Introduction 57
II. Isolated Tissue Assays 58
III. Binding Assays 66
IV. Radioimmunoassay 71
V. Neuroblastoma X Glioma Hybrid Cells 73
VI. Opiate Receptor Multiplicity 76
Addendum 83
References 84

5. DISTRIBUTION OF OPIOID PEPTIDES 89
Jean P. Rossier and Floyd E. Bloom

I. Introduction 89
II. Preparation of Antisera 89
III. Distribution of Enkephalins 90
IV. Distribution of β-Endorphin 101
V. Conclusions 108
References 108

6. PHYSIOLOGICAL FUNCTIONS OF ENDORPHINS 113
Brian M. Cox and Eugene R. Baizman

I. Introduction 113
II. Enkephalins as Neurotransmitters or Neuromodulators 114
III. β-Endorphin as a Neurotransmitter or Neuromodulator 136
IV. Endorphins as Hormones 144

V. Functional Studies Indicating a Physiological Role for Endorphins 160
VI. Endorphin Function in Opiate Tolerance and Dependence 175
VII. Overview 177
References 179

7. ANIMAL PHARMACOLOGY OF ENDORPHINS 197
Jeffrey B. Malick and Jeffrey M. Goldstein

I. Introduction 197
II. Role of Endorphins in Pain Perception 198
III. Physical Dependence and Abuse Potential 215
IV. Effects of Endorphins on Behavior 217
V. Other Pharmacological Effects of Endorphins 222
VI. Summary and Conclusions 223
References 223

8. CLINICAL ASPECTS 229
Lars Terenius

I. Introduction 229
II. Occurrence and Distribution in Humans 230
III. Physiologic Function of Endorphins in Humans 231
IV. Endorphins and Stimulation-Produced Analgesia 236
V. Involvement of Endorphins in Disease 238
VI. Trials of Narcotic Antagonists in Disease 244
VII. Effects of Endorphin Administration in Humans 246
VIII. Conclusions 248
References 249

Author Index 257
Subject Index 291

chapter 1

History

Eric J. Simon

New York University Medical Center
New York, New York

I. INTRODUCTION

The editors have asked me to be the historian for this volume. I consider this invitation to be both an honor and a challenge. After some (but probably not enough) reflection I agreed to take on this task, aware of the not inconsiderable hazards involved in it, not to mention the obvious problem that other chapters contain certain aspects of the history of this field. Since the editors felt the need for a separate chapter covering the historical development of this rapidly moving and exciting field, I shall attempt to perform this task with all the objectivity and accuracy at my disposal.

II. DEVELOPMENTS THAT LED TO THE POSTULATION AND DISCOVERY OF OPIATE RECEPTORS

The modern era of opiate research is usually considered to have begun in 1973 with the demonstration of stereospecfic opiate receptors in the central nervous system of animals and humans. This discovery, like all discoveries, was not made in a vacuum. This section deals with developments that led to the recognition that such receptor sites must exist and to their ultimate discovery.

Opium is probably the oldest known medication and morphine has been known since the nineteenth century to be the major alkaloid responsible for most of its pharmacological and medicinal effects. The roots of the recent revolutionary developments in the opiate field are contained in the large body of research that was carried out over the last 50 years on the mode of action of

morphine and related drugs and the large-scale efforts in many laboratories to synthesize a nonaddictive analgesic. Although this has not yet been achieved, thousands of compounds, more or less structurally related to morphine and many of them very useful, have been synthesized. A considerable amount of information on the structural requirements for analgesic action came out of this work. It was recognized that analgesic action resides in only one of the enantiomers of a racemic mixture, usually the levorotatory isomer. Furthermore, it became quickly apparent that parts of the morphine molecule could be altered drastically or dispensed with entirely without major changes in pharmacological potency. On the other hand, even small changes in certain parts of the molecule resulted in profound effects on its pharmacology. The best studied and most interesting such change is the substitution of the methyl group on the tertiary nitrogen by an allyl or cyclopropylmethyl group, which causes the resulting molecule to become a potent specific antagonist against many of the pharmacological effects of morphine and related opiates. Some of these antagonists retain some of their "agonist" properties (e.g., nalorphine, cyclazocine), whereas others are "pure" antagonists (e.g., naloxone and naltrexone).

The recognition of the remarkable stereospecificity and structural constraints placed on analgesic and other actions of opiates led to the receptor hypothesis. This hypothesis, held by investigators in this area for several decades, postulates that opiates must bind to specific sites in or on brain cells and that this binding triggers the physical and/or chemical steps that result in the responses observed. These sites or receptors, as they were termed in analogy to the hormone receptors, would permit only drugs with suitable structures and stereochemistry to bind—by far the easiest way to explain the observed specificities of opiate action.

Another important development in the opiate field was the development of in vitro bioassays for analgesic potency. The electrically evoked contractions of the isolated guinea pig ileum were shown by Paton (1) and Schaumann (2) to be inhibited by opiates at low concentrations. This system was studied in great detail in the laboratory of Hans Kosterlitz (3, 4). Remarkable correlation was found between the ability of a drug to inhibit contractions of the guinea pig ileum and its analgesic potency in intact animals. The system also permitted assessment of the antagonist potency of a drug. Kosterlitz and his collaborators (5) also discovered and developed a second extremely useful bioassay system, the vas deferens of the mouse. There was again excellent correlation between inhibition of contraction and the analgesic potency of a drug. Both systems seem to contain receptors with specificities much like those postulated to exist in the brain.

III. DISCOVERY OF OPIATE RECEPTORS

The direct demonstration of the existence of the putative opiate receptors proved difficult. Van Praag and Simon (6) were the first to try to detect opiate receptor

binding in brain homogenate. It was relatively easy to demonstrate binding of an opiate ([^{3}H] dihydromorphine) but much more difficult to distinguish between specific and nonspecific binding. An attempt to detect specific binding by its sensitivity to the opiate antagonist nalorphine was unsuccessful.

Ingoglia and Dole (7) were the first to use the property of stereospecificity in the search for the opiate receptor. They injected l- and d-methadone into the lateral ventricle of rats, but found no difference in the rate of diffusion of the enantiomers into brain tissue.

Goldstein et al. (8) first used stereospecificity as the criterion for receptor binding in mouse brain homogenates. They incubated the homogenates with radioactive levorphanol in the presence of a large excess of either unlabeled dextrorphan or levorphanol. Free and bound radioactivity were separated by centrifugation. Stereospecific binding was defined as that portion of the binding that was prevented by excess levorphanol but not by an equally large excess of dextrorphan. In these experiments only 2% of total binding was found to be stereospecific. Moreover, in retrospect, this stereospecific binding had very different properties and distribution from what we now believe to be opiate receptors. Purification and characterization of the substance responsible for the stereospecific binding by the Goldstein group (9) showed it to be cerebroside sulfate, in agreement with results obtained in Horace Loh's laboratory (10).

Evidence for the existence in animal brain of stereospecific opiate binding that represents a major portion of the total binding came early in 1973, independently from three laboratories, in remarkably simultaneous reports* by Simon et al. (11), Pert and Snyder (12), and Terenius (13). These investigators all utilized very similar modifications of the procedure used by Goldstein et al. (8). Potent opiates, labeled with tritium at very high specific activity, permitted the use of the very low drug concentrations, minimizing nonspecific binding. The introduction of rapid washes with cold buffers of sediments after centrifugation or filters after filtration drastically reduced contamination by unbound or loosely bound radioactivity. In such experiments 50-90% of bound opiate was found to be bound stereospecifically (i.e., replaceable by unlabeled opiates but not by their inactive enantiomers).

Between 1973 and 1975 a great deal of work in the laboratories already mentioned, as well as in others, was devoted to a characterization of the stereospecific binding sites and accumulation of evidence as to their identity with the postulated and long-sought opiate receptors. Space does not permit a detailed discussion of these studies in a chapter on history (for a detailed review, see Ref. 14). Suffice it to say that the properties of the binding sites studied to date, their confinement to the nervous system, and their distribution within the nervous

*The first oral presentation on the "opiate receptor" was made by the author at the Symposium on Current Status of Pharmacological Receptors, at the annual spring meeting of the American Society for Pharmacology and Experimental Therapeutics (FASEB) in Atlantic City, N.J., on April 18, 1973.