The Receptors

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The Opiate Receptors

Second Edition

Humana Press
Preface

The growth of the opiate field has been enormous. Early work focused upon the strategic clinical importance of morphine and the attempt to develop non-addicting analogs with fewer side-effects, but the discovery of the receptors and the enkephalins and other endogenous opioid peptides and the recognition of their widespread actions within brain has expanded the field to include investigators in almost all areas of neuroscience and pharmacology. However, this field of research with its vast literature has become progressively more complex. The receptors are no longer limited to opiates, but include many subtypes selective for the opioid peptides. Indeed, they might be better termed opioid, rather than opiate, receptors. Many controversies have emerged and been settled; others remain. Early studies must now be interpreted on the basis of current information. Thousands of papers examining various aspects of opiates and the endogenous opioids present separate pieces of a large puzzle. The goal of this volume is to put the pieces together and attempt to obtain a coherent overview of opiate receptor pharmacology with insights into both the molecular and classical pharmacology of opiates and the opioid peptides. However, many pieces of this immense puzzle remain unknown and will need to be addressed in the future.

The study of opiates and opioid peptides provides a unique research opportunity in the neuropharmacology of drug receptors. The availability of a wide variety of agonist and antagonist ligands has permitted studies not possible in other systems. Second, the close association of opiate drugs with easily measurable pharmacological bioassays and behavioral responses permits the correlation of molecularly defined receptors with pharmacological actions and helps to bridge the gap between molecular and classical pharmacology. In this regard, the opiate system is relatively unique.

Understanding the multiple classes of opiate and opioid peptide receptors at the molecular level and functionally is the major focus of this second edition. Much has happened since the first edition of this volume. The greatest advance has been the cloning of the various classes of opioid receptors. This has opened new areas of investigation and provided greater insight into the biochemical understanding of the receptors and their actions. This second edition has tried to incorporate these new areas and merge them with the earlier studies. Sections of the book cover historical perspectives in the concept of multiple opiate receptors along with a general
overview of the opioid peptides and the molecular and functional characterization of the receptors. Throughout the entire volume, we have attempted to provide an integrated approach that builds on the groundwork set forth in the first edition, pulling together the biochemical, physiological, and pharmacological studies of opiate action. We feel that this volume will be a valuable resource for scientists actively working in the opiate field, as well as others interested in neuroscience and pharmacology in general.

New York, NY

Gavril W. Pasternak
Contents

1  You’ve Come a Long Way Baby! ................................................................. 1
   Solomon H. Snyder

2  The Evolution of Concepts ................................................................. 7
   W.R. Martin

3  Pharmacology of Opioid Drugs ...................................................... 23
   Brian M. Cox

4  Opioid Receptors: The Early Years .............................................. 59
   Gavril W. Pasternak

5  Endogenous Opioids ...................................................................... 93
   Iryna Berezniuk and Lloyd D. Fricker

6  Molecular Biology of Mu Opioid Receptors ................................. 121
   Ying-Xian Pan and Gavril W. Pasternak

7  Delta Opioid Receptors ................................................................. 161
   Wendy Walwyn, Fatima Macho Sánchez-Simon, Raquel Rodríguez, and Christopher J. Evans

8  Kappa Opioid Receptor Gene and the Regulatory Mechanisms of Its Protein Expression ........................................ 181
   Li-Na Wei and Horace H. Loh

9  Opioid Receptor Signal Transduction Mechanisms .................. 195
   Ping-Yee Law

10 Mu Opioid Receptor Mediated Analgesia and Reward .............. 239
    Howard L. Fields
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td><strong>Kappa Opioid Receptor Function</strong></td>
<td>265</td>
</tr>
<tr>
<td></td>
<td>Julia C. Lemos and Charles Chavkin</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td><strong>Delta Opioid Receptor Function</strong></td>
<td>307</td>
</tr>
<tr>
<td></td>
<td>Edita Navratilova, Victor J. Hruby, and Frank Porreca</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td><strong>Genetic Studies of Opioid System Function in Mice</strong></td>
<td>341</td>
</tr>
<tr>
<td></td>
<td>Michael A. Ansonoff, Ting Wen, and John E. Pintar</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td><strong>Opioid Receptor Trafficking</strong></td>
<td>389</td>
</tr>
<tr>
<td></td>
<td>Mark von Zastrow</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td><strong>Opioid Receptor Dimerization</strong></td>
<td>407</td>
</tr>
<tr>
<td></td>
<td>Raphael Rozenfeld, Ivone Gomes, and Lakshmi A. Devi</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td><strong>Molecular Modulation of In Vivo Tolerance</strong></td>
<td>439</td>
</tr>
<tr>
<td></td>
<td>Charles E. Inturrisi and Ann M. Gregus</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td><strong>Genetics of Opioid Actions</strong></td>
<td>457</td>
</tr>
<tr>
<td></td>
<td>Jörn Lötsch and Jeffrey S. Mogil</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Index</td>
<td>499</td>
</tr>
</tbody>
</table>
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Chapter 1
You’ve Come a Long Way Baby!

Solomon H. Snyder

Abstract  Current challenges in opiate pharmacology today are remarkably similar to those almost 40 years ago, when opiate receptors were first identified. There are two key problems to be resolved: (1) We need less-addicting opiates. (2) An understanding of addiction is lacking. Characterization of opiate receptors led to insights into the differentiation of agonists and antagonists. As mixed agonist–antagonists are less addicting, there were hopes for more effective, safer drugs – but these hopes have largely gone unrealized. Appreciation of opioid peptides as endogenous ligands for receptors portended promised further insights into addiction – but we still do not understand the fundamentals of the field. Cloning of receptors and creation of knockouts for them may help answer some of these questions.

Keywords  Naloxone • Nicotinic receptor • Receptor multiplicity • Pro-opiome-lanocortin • Methionine enkephalin

The field of opiate receptors and associated endogenous ligands, memorialized in the second edition of this now classic volume, has indeed “come a long way.” Our sophistication in understanding how opiates act is far greater than it was decades ago. The tools of molecular biology have greatly augmented insights into receptors and their ligands. And yet, some of the fundamental questions have not yet been satisfactorily addressed. What is the nature of the addictive process? Is there a rational approach to the design of less-addicting opiates based on differential influences upon subtypes of opiate receptors? Just what are the unique functions of the different opioid peptides, and which is physiologically associated with which receptors? I have not personally been involved in opiate research for many years and so will not address recent findings in detail. Instead let me attempt to shed
historical perspective that may facilitate efforts of the new generations of opiate researchers.

In founding the American Philosophical Society, Benjamin Franklin established its mission as “the promotion of new and useful knowledge.” Of all the biomedical sciences, pharmacology best epitomizes this motto. Our goal is to understand the workings of the body, but we always bear in mind the need to apply our understanding to the development of novel therapies.

As a medical student, I adored pharmacology, even learning both the generic and brand names for major drugs. Working with Julie Axelrod at the NIH afforded many lessons on how neurotransmitters act and interface with drugs. When I came to Johns Hopkins for a psychiatry residency and then joined the faculty in pharmacology, my initial focus was upon how psychotropic drugs act via the biogenic amines. I knew nothing of opiates and could barely distinguish morphine from marijuana.

My entry into the opiate field stemmed from the pressures of the day related to the epidemics of heroin addiction in American cities and among our soldiers in Vietnam. My friend Jerry Jaffe, President Nixon’s “czar” of drug abuse, pressured me to “do something.” He put his money where his mouth was by creating a series of drug abuse research centers. Johns Hopkins was one of the initial grantees. I could have merely continued with our work on amphetamines and catecholamines, but chose instead to heed Jerry’s admonition and address the challenges of opiate research.

In those days – 1971–1972 – we knew much about the biosynthesis of neurotransmitters, their degradation, and their inactivation by reuptake or other mechanisms. However, how they acted at receptors had never been defined in molecular terms. Drug development typically utilized screens in intact rats. The paradigm derived from anti-convulsant development – hooking a rat to the house current and screening drugs for their ability to prevent convulsions. This was the means whereby Houston Merritt discovered the classic anti-convulsant phenytoin (Dilantin).

For such screens one needed veritable chemical engineering to come up with 25 g of material for screening. Structure-activity analysis was feeble, because one drug might be more active than another because of lesser metabolism or more efficient penetration to the target organ rather than greater potency at receptors. In terms of mechanism of action, pharmacologists administered opiates to rats and measured diverse biochemical markers with no way of knowing whether they were dealing with an initial receptor-related event, something secondary, or something unrelated to pharmacologic actions. Considering this morass, it was evident that identifying molecular receptors for opiates, other drugs and neurotransmitters could have a major impact.

### 1.1 Initial Findings

In 1970, several groups had identified the nicotinic cholinergic receptor in the electric organ of electric fish by the binding of the extremely potent, pseudo-irreversible toxin α-bungarotoxin. Despite the fact that cholinergic receptors constitute up to
20% of weight by the electric organ, success depended on this unique toxin. The great pharmacologist Vincent Dole had already done armchair calculations indicating that presumed opiate receptors would amount to only about one-millionth by weight of mammalian brain. Hence, the very success of the nicotinic receptor work implied that conventional neurotransmitter receptors would remain inaccessible for the foreseeable future.

Accordingly, identification of opiate receptor binding in early 1973 utilizing reversible ligands was unexpected. The use of drugs labeled with high specific radioactivity, coupled with rapid but exhaustive washing to eliminate non-specific binding, made the system work. Radioligands based on diverse drugs soon led to the identification of receptors for most of the principal neurotransmitters, such as those for dopamine, norepinephrine, serotonin, glycine, and GABA. Differences in the binding properties of various ligands implied the existence of receptor subtypes. Receptor binding was rapidly adapted to high throughput analysis in the drug industry so that one could easily screen thousands of drugs a day with the chemists needing to synthesize only microgram quantities of drug candidates.

Major insights into how opiates mediate their pharmacologic actions came from the rather simple dissection of small regions of monkey brain in which opiate receptor binding was measured biochemically. Such studies were soon followed by autoradiographic microscopic localization of opiate receptors. Receptors were selectively enriched in areas of the brain mediating the “subjective” sense of pain which that is classically affected by opiates, e.g., the lateral thalamus. Multiple targets for euphoric actions of opiates emerged such as very high densities of receptors in the locus coeruleus, the source of most norepinephrine cell bodies, as well as areas of the limbic system.

Even the miotic actions of opiates can be explained by dense concentrations of receptors in the pretectal nuclei, which regulate pupillary diameter. Opiate analgesia at the spinal level could be explained by a very high density of opiate receptors in the substantia gelatinosa of the spinal cord on terminals of afferent pain fibers. Opiates act by inhibiting the release of pain-reducing peptide neurotransmitters from these neurons.

Work on opiate receptors evolved so rapidly that by May 1974, at a meeting of a Neurosciences Research Program, reports from diverse investigators provided insights into some of the biggest questions facing the field. Let me summarize some of the issues.

Vincent Dole opened the meeting by questioning the term “the opiate receptor.” He was concerned about each word. He noted that “the” suggested there was only a single opiate receptor, while differential effects of various opiates in animals and humans implied the possibility of multiple receptors. The word “opiate” referred to an agent of plant origin that presumably did not exist in mammals, raising the question of an endogenous ligand. The term “receptor” needed elucidation in terms of intracellular messengers that transform opiate recognition by a binding site into altered cellular function. The field moved so rapidly that when proceedings of the meeting were published a year later, a number of Dole’s prescient concerns had been addressed.
At the meeting in 1974, John Hughes and Hans Kosterlitz had presented their preliminary observations that brain extracts elicited influences on contractions of the guinea pig ileum and mouse vas deferens that mimicked morphine and were blocked by the opiate antagonist naloxone. In December 1975, they published the structures of the two enkephalin pentapeptides, the first endogenous opioid ligands.

As for the concept of multiple receptors, when the first batches of [3H]enkephalins were available, Kosterlitz noted that their binding properties to opiate receptors showed a different peptide/drug specificity than [3H]opiates. The enkephalin-prefering receptors were dubbed delta, because they were enriched in the vas deferens, while the morphine-prefering receptors were designated μ, for morphine. A year later Kosterlitz identified a third class of receptors, called κ, because they showed selective high affinity for ketocyclazocine. Remarkably, though the ligand techniques employed were relatively crude, molecular cloning has revealed that Kosterlitz was right on the mark, with the principal opiate receptor genes being indeed μ, δ, and κ.

What about connections to second messengers? Early insights emerged from efforts to differentiate agonists and antagonists. In initial receptor binding studies matched pairs of agonists and antagonists, e.g., morphine/nalorphine or oxymorphone/naloxone, displayed identical binding curves. A routine examination of ionic effects in our laboratory revealed that sodium ions selectively decreased the binding affinity of agonists with negligible effects upon pure antagonists and intermediate influences on mixed agonist–antagonist drugs.

When G proteins were subsequently characterized with GTP influencing ligand binding, it became evident that GTP and sodium synergistically differentiated agonists from antagonists, pointing to the notion that opiate receptors are G protein-coupled receptors. Workers in Marshall Nirenberg’s laboratory first characterized effects of opiates upon adenyl cyclase.

Addiction is the bête noire of all psychoactive agents. The formal properties of the addictive process – tolerance, physical dependence, and compulsive drug-seeking behavior – are held in common by agents as diverse as alcohol, cocaine, and opiates. As opiates have been the paradigm for addiction research, all of us had hoped that conquest of the opiate receptor would resolve the riddles of addiction. No matter how hard we and other groups tried, however, we never found any meaningful alteration in opiate receptors as a product of the addictive process. Once we could measure enkephalins by radioimmunoassay, we hoped that alterations in their levels would explain it all, but nothing meaningful emerged.

1.2 Recent Advances

That is where matters stood in the mid- to late 1970s. What have we learned in the ensuing 30 years? The principal advances, largely at the molecular level, illustrate the complexity of opioid systems.
Cloning of $\mu$, $\delta$, and $\kappa$ receptors was an important step forward. Mice with genetic deletion of each of these receptors as well as mice lacking two or three of the receptors have been generated, which we hoped would answer some fundamental questions. Which receptor subtypes mediate which forms of analgesia? Which receptor subtypes underlie addiction as well as lethal side effects such as respiratory depression?

Some answers have emerged, but conflicting and ambiguous findings abound. Difficulties of interpretation seem to rest in part on the surprisingly large variations among strains in opiate responsiveness. Many of the published studies have used mice with mixed genetic backgrounds with findings sometimes contaminated by differential strain influences. Hopefully, the uniform use of congenics will clarify such discrepancies.

There are too many conflicting findings to warrant an attempt to summarize the overall picture. The most extensive investigations have involved $\mu$ knockouts and have established that these receptors are primarily responsible for analgesia elicited by morphine and a variety of other opiates. Knockout of the $\mu$ receptors also abolishes tolerance and physical dependence to morphine. Hence, we are comforted that the binding sites all of us have been addressing for nearly 40 years are pharmacologically relevant.

However, the pharmacologic alterations observed with the three different receptor subtypes have failed to indicate that an agonist at only one of these subtypes will provide the long-hoped for less less-addicting, side-effect-free opiate analgesic. This conclusion is buttressed by the extraordinary effort of the pharmaceutical industry to develop drugs selective for receptor subtypes with negligible success.

Since the first identification of the enkephalins, substantial advances have been made in the biochemistry of opioid peptides, which are best differentiated by their precursors, pro-enkephalin, pro-opiomelanocortin (POMC), pro-dynorphin, and pronociceptin. Mice with targeted deletion of genes for the precursors of these peptides have been generated. In several cases the precursors give rise to multiple peptides. For instance, POMC is cleaved to yield ACTH as well as $\beta$-endorphin. Knock-in mice have been designed permitting selective deletion of one or another peptide. These mutant mice have permitted investigations of the role of the various peptides in mediating analgesia and diverse behaviors.

Studies of mutants for peptide precursors as well for opiate receptor subtypes have asked which receptors are the “physiologic” targets for which peptides. As with opiate receptor knockouts, early studies utilizing mixed strains have produced ambiguous results, while few studies have employed congenic strains. Enkephalin deletion does lead to enhanced pain responses, while $\beta$-endorphin loss seems to do the opposite, and very few effects are observed with dynorphin disruption. Studies comparing peptide and receptor mutants have so far failed to establish definitively the physiologic receptor for each of the peptides.

What about our understanding of addiction? Most pharmacologists have long assumed that tolerance and physical dependence reflect the following general model. Opiates exert some sort of effect on target cells, e.g., inhibition of adenyl cyclase.
To compensate, the target cell synthesizes more adenyl cyclase. When opiates are withdrawn one sees augmented cyclase responses. This model was demonstrated experimentally in early studies by Marshall Nirenberg by utilizing neuroblastoma cell lines expressing δ receptors. In recent years Eric Nestler has extended this approach to nuclear events involving acetylation of CREB and histones which that are influenced by addiction to cocaine and other drugs as well as opiates.

Some patterns have emerged. For instance, chronic cocaine administration represses the histone-methylating enzyme G9a in the nucleus accumbens, a dopamine-enriched cocaine target, leading to reduced levels of histone H3K9 dimethylation. Moreover, G9a downregulation increases preference for cocaine. Though the sequence of signaling steps that impacts histone alterations is not yet clear, elucidation of this pathway may lead to ways of manipulating the addictive process. The similarity in actions of cocaine and opiates fits with the view that addiction to most drugs is regulated by common mechanisms.

What is the bottom line? We know far more now than we did 35 years ago. However, we have not yet solved fundamental questions such as the nature of addiction, the functional roles of the different peptides or their relationship to receptor subtypes. In terms of Benjamin Franklin’s challenge that we should come up with “useful knowledge,” we must plead failure. There have been no major breakthroughs in the development of opiate drugs that can be attributed to our enhanced molecular understanding.

However, we need not feel discouraged. The same criticisms can be made of the cancer field. Our understanding of molecular features of cancer today vastly exceeds what was known 35 years ago. Therapeutic breakthroughs based on fundamental science, such as inhibitors of specific tyrosine kinases, are heartening. Nonetheless, the death rate from cancer has not changed notably. My suspicion is that the large body of seemingly conflicting opiate data that has accumulated in recent years will begin to form a pattern – a portrait which that will unexpectedly, and perhaps suddenly, afford the therapeutic advances we all seek.

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Chapter 2
The Evolution of Concepts

W.R. Martin

Abstract κ Opioid receptors were first proposed by the author based on the actions of benzomorphans, such as ketocyclazocine. κ receptors are involved with a wide range of actions, providing novel targets for drug development. This chapter will explore the pharmacology of κ receptors in a range of behavioral effects and their functions at the biological level.

Keywords κ Receptor • KOR • Electrophysiology • Dynorphin • Epilepsy • Stress • Depression • Learning • LTP • DRG • Ion channel • Kinase

2.1 Introduction

The early evolution of concepts of endogenous opioids and multiple receptors had its inception in a concerted program to develop safe, nonaddicting substitutes for opiates [1]. This endeavor was initiated but the Bureau of Social Hygiene and subsequently supported by the US Public Health Service under the auspices of the National Research Council. An empiric approach was taken in which a large number of chemicals, synthesized by University-based chemists and the pharmaceutical industry, were examined for their pharmacologic effects, particularly their analgesic activity and their abuse potential.

Although heroin and morphine addition were the initial driving force of this endeavor, the economic gains associated with the marketing of a less-addicting analgesic became the most important factor of the pharmaceutical industry’s synthetic effort. From a societal perspective, however, the economics of drug abuse is by far the most important economic factor, since drug abuse costs the United States well over $100 billion dollars a year. The search for safer and less-abusable
analgesics has not been entirely successful. The evolution of ideas concerning multiple opioid receptors and endogenous opioid transmitters is still active.

The critical opioids in the pharmacologic dissection of multiple opioid receptors were $N$-allylnorcodeine, $N$-allylnormorphine, naloxone, cyclazocine, ethylketazocine, $N$-allylnormetazocine, and buprenorphine. Proceeding on the concept that allyl substitutions functioned as respiratory stimulants, Von Braun [2] synthesized $N$-allylnorcodeine and Pohl [3] studied the interactions between $N$-allylnorcodeine and morphine and first demonstrated that $N$-allylnorcodeine was capable of antagonizing the respiratory depressant effects of morphine.

Although Pohl’s important observations were published in 1915 and were confirmed by Meissner [4], these findings lay dormant until they were resurrected again by Chauncy Leak. Dr. Leake, then of the University of California in San Francisco, stimulated efforts to synthesize $N$-allylnorcodeine and allylnormorphine. These endeavors have been briefly recounted [5], and, like Pohl’s concept, were based on the hypothesis that allyl groups are respiratory stimulants.

The initial synthesis of $N$-allylnormorphine was controversial. As a consequence of resolving the synthetic issues, $N$-allylnormorphine (nalorphine) was independently synthesized by Weijlard and Erickson at Merck Laboratories [6] and by Hart and McCauley at the University of California [7]. Both Unna [8] and Hart and McCauley [7] also studied the pharmacology of this interesting compound and extended the observations of Pohl by showing that nalorphine antagonized other actions of morphine.

The issue of respiratory stimulant action can be clearly differentiated from its morphine antagonistic effect and from the respiratory stimulant actions of dinitrophenol. The observations of nalorphine’s antagonistic effects were reluctantly accepted, as were speculations concerning its mechanism of action (Unna, personal communication). The clinical use of nalorphine for the treatment of acute morphinism was not pursued despite Unna’s urging and was not demonstrated until Eckenhoff et al. [9] conducted the critical experiment in man.

To further elaborate on the importance of opiate addiction in stimulating research on opioid drugs, a Committee on Drug Addiction was formed by the Committee on Social Hygiene in 1920 to increase the understanding of addiction processes. Following the passage of the Harrison Narcotic Act and on the recommendation of the American Medical Association, clinics that provided narcotics to addicts were closed, leaving most addicts without a legitimate source for their narcotics. As part of the Committee’s activities, they proposed a strategy for identifying new analgesics that would be devoid of the toxic and dependence-producing actions of the opium analgesics. Subsequently, the Federal government assumed the responsibility of continuing the activities of this committee.

Among the important activities were the initiation and continuation of a synthetic program, the development of an animal screening program, and finally assessment of new analgesics in humans for their ability to produce or sustain physical dependence. Many compounds were synthesized and evaluated by Dr. Eddy’s laboratory at the National Institutes of Health, by Dr. Severs’ laboratory at the University of Michigan, and by investigators of human subjects at the Addiction Research Center.
Most of the drugs studied were sufficiently like morphine that they were judged not to have any marked advantage. In retrospect, there may have been significant differences between the drugs; these were either not detected using the methods at hand, or differences were not pursued. Two examples of compounds that had unique pharmacological properties in human subjects were meperidine and normorphine. It was much more than difficult to produce physical dependence on these drugs than it was to produce physical dependence on morphine.

Doctor Harris Isbell has developed an interest in the use of N-allylnormorphine as an analgesic. He obtained the drug, however, at a time when he was very much involved in conducting his studies on alcohol and barbiturate dependence. Lasagna and Beecher [10] did study the analgesic actions of nalorphine and found it to be nearly as potent as morphine. Dr. Abraham Wikler attempted to substitute nalorphine for morphine in a dependent subject and observed that it precipitated a violent abstinence syndrome that could not be antidoted by morphine. He subsequently characterized precipitated abstinence in humans and in the spinal dog [11, 12]. These observations provided an important clue in the development of nonaddicting, safer analgesics and were pursued by several pharmaceutical firms that synthesized a number of compounds with antagonistic effects.

Thus, the driving force for the enormous commitment for development of opioid antagonists as analgesics was that they had analgesic activity and did not appear to substitute for morphine in morphine-dependent subjects. It is important to recognize the importance of the substitution technique for identifying morphine-like drugs devised by Himmelsbach [13]. Although Himmelsbach did not couch his concepts in receptor theory, his work was one of the first critical pieces of evidence that strongly indicated that opioids were exerting their effects by acting through a common mechanism. Himmelsbach [14] attributes the development of this technique to the observations of Eddy [15], who demonstrated cross-tolerance between morphine, codeine, and heroin in the dog.

Himmelsbach reasoned that cross-dependence could also exist, and that dependence was a major determinant of the addictiveness of analgesics. He demonstrated that a number of morphine congeners substituted for morphine in morphine-dependent subjects. These studies had several major implications. One of the drugs studied by Dr. Himmelsbach was desomorphine. Desomorphine did not produce dependence in the monkey; it substituted for morphine in morphine-dependent subjects, however. This was to be only the first of several drugs with the ability to sustain dependence that was much greater in humans than in the monkey. These observations, and others, led to the suggestion that opioid receptors differed in the intimate details of their configuration from one species to another [16, 17].

Another important innovation was the application of bioassay statistical techniques to not only suppression and precipitation data but also to subjective effects data as assessed by questionnaires. Harris Isbell introduced this technique to help strengthen the conclusions that had been reached concerning the abuse potentiality of phenazocine, the first of a series of benzomorphans that had a critical role in the formulation of concepts concerning multiple opioid receptors. Phenazocine was much less potent than morphine as an analgesic in humans. In humans, however,
phenazocine was three to four times more potent than morphine in constricting pupils and producing subjective effects and was eight times more potent in suppressing abstinence [18].

In addition to emphasizing the large differences in response to opioids among species, several other important lessons were learned through this quantitative comparison between drug measure and species. (1) Different experimental variables (e.g., pupillary diameter vs. subjective effects) that were measured using different scales (e.g., ordinal, nominal, or ratio) yield potency estimates that were not only equivalent, but had similar confidence limits. (2) The use of dose–response relationships became an important criterion for identifying changes in subjective states that were relevant to the drug effects. (3) The concomitant use of both a physiologic and behavioral measure provided an internal validation of the behavioral measures [19]. The effects of opioids on subjective states became an important criterion for differentiating the receptor subtypes.

Isbell’s use of bioassay statistics and techniques to compare the relative potencies of opioids to suppress abstinence, to alter subjective states and to induce physiologic changes provided a powerful tool for quantitatively characterizing the pharmacologic profiles of drugs [18]. The use of crossover designs allowed for simultaneous and efficient assessment of relative potencies on several experimental parameters. Thus, valid assays could be obtained on studies employing four to six subjects using a four-point assay [20, 21]. This design allowed for the partitioning out of the between-subjects variance, and the error term for calculating the confidence limits of potency estimate was the residual part of the between-doses variance.

The seminal approach, however, had a major statistical problem in that different pharmacological effects were measured with different types of scales. For example, pupils were photographed and measured with a ruler (ratio scale). Some subjective states were measured using a nominal scale; others using an ordinal scale. Isbell’s first effort (Table 2.1) revealed that the confidence limits of the potency estimated for the different types of measurement scales were similar despite differences in the inherent properties of the scales.

From a practical and empirical perspective, potency estimates obtained from dose–response relationships employing data bearing on the frequency of occurrence of signs and symptoms using nominal scales, data bearing on the subjectively estimated intensity of feeling states using ordinal scales, and the measurement of pupillary diameter from Polaroid photographs were in close agreement and had similar confidence limits [19].

| Table 2.1 | The relative potency of phenazocine and levophenacylmorphan in comparison to morphine in constricting pupils (interval scale), altering signs and symptoms (nominal scale), and suppressing abstinence (mixed scale)* |
|-----------|-------------------------------------------------|-----------------|-----------------|
|           | Pupils (miosis) | Questions | Suppression |
| Phenazocine | 3.8 (1.3–5.6) | 3.2 (2.3–5.0) | 8.2 (4.2–17.2) |
| Levophenacylmorphan (NIH-7525) | 5.2 (2.7–8.0) | 6.11 (5.0–7.5) | 9.1 (4.8–20.0) |

*From Fraser and Isbell [18]
These potency estimates further agreed with estimates of analgesic potency obtained in patients suffering from both acute and chronic pain. These observations were taken to mean that (1) the miotic phenomenon and changes in subjective states were probably the consequence of the drugs acting through a similar mechanism and that measures of subjective states were valid measures of drug effect; and (2) any lack of additivity among signs and symptoms, and deviations from linearity for nominal and ordinal scales, was probably small compared to between-subjects and across-time variance.

These latter issues were pursued experimentally. Thus, the frequency of occurrence of various signs or the intensity of symptoms and the degree of miosis produced by both morphine and heroin were found to be linearly related to the logarithm of dose. Hence, we knew that the principle of additivity was applicable to data obtained using nominal and ordinal ratio scales. We began to apply the criterion of dose responsiveness for the selection of questionnaire items [19, 22], yet another approach that enhances the rigor of additivity for our behavioral scales. Different signs were weighted such that the signs that exhibited lesser sensitivity were given greater weight. Thus, by weighting, different responses could be equated (e.g., pupils and liking).

The Himmelsbach method for scoring the intensity of opioid abstinence is composed of data derived from nominal, interval, and ratio scales that have different weighing values that are related to the severity of abstinence. A similar system for assessing abstinence was developed for precipitation and suppression studies in the dog [23, 24] that was composed of changes that were suppressed or precipitated in a dose-related way by agonists and antagonists and that were measured using nominal, ordinal, interval, and ratio scales. Those signs of abstinence, the frequency or intensity of which were related to the dose of the agonists in suppression studies and the dose of antagonists in the precipitation studies, were selected for measuring the intensity of abstinence, and each sign was weighted such that all signs made an approximately equal contribution to the abstinence syndrome score. Thus, the criterion of additivity and linearity were fulfilled.

By establishing linearity and additivity for items of subjective effects questionnaires, through a weighting and dosing relationship, a report of the two effects can be added. In a similar manner, the abstinence signs – yawning, piloerection, and body temperature – can be added. Through the technique of mapping, we have shown that there is a linear relationship between dose-related changes in score on the nominal, ordinal, interval, and ratio scales. This relationship is implicit when valid parallel line assays are obtained for different measures and effects. This is illustrated in Table 2.1.

These issues of measures and statistics have been discussed by Stevens [25]. Two important principles emerged. (1) Deviations from additivity and linearity for nominal and ordinal data are small compared to the unaccounted-for variance and (2) the frequency of occurrence of intensity of report are linearly related to the dose (logarithm) of the drug.

The use of pharmacologic syndromes has played a critical role in identifying receptor subtypes and in identifying specific drugs. In detailed studies of cyclazocine...
in humans, it was apparent that cyclazocine produced effects that were not produced by morphine [26]. Although cyclazocine was a potent miotic (10–15 times or more potent than morphine and nalorphine), valid potency assays of this activity were not obtained. Further, cyclazocine in higher doses produced overt ataxia and subjects reported that they were sleepy and felt drunk. These signs and symptoms were not commonly observed in, or reported by, post addicts who had been administered morphine or heroin.

Cyclazocine and nalorphine produced feelings of well-being in some subjects, but not in others. They also produced feelings of dysphoric in more subjects when the dose was sufficient. The dysphoric effects of cyclazocine and nalorphine are complex. The most commonly reported symptom, with minimally dysphoric doses, is recall of disturbing memories. The patient can be distracted but has difficulty suppressing these thoughts. With larger doses, delusions, hallucinations, sleep with disturbing dreams, and anxiety states may be reported.

Cyclazocine was found to be 10–20 times more potent than morphine in equivalent measures. An attempt was made to make patients dependent on an equivalent dose of cyclazocine based on single-dose relative potency. A daily dose of 13.2 mg/70 kg was attained in six subjects. Some subjects found the dysphoric effects of cyclazocine especially disturbing and the dose of cyclazocine was incremented slowly. At the time these studies were initiated, we did not realize that cyclazocine had much longer duration of action than morphine, and hence our estimates of the equipotent dose of cyclazocine may have been high.

Regardless, when the administration of cyclazocine was terminated, we were presented with several surprises. The first was a long latency to onset of signs of abstinence. In fact, signs were not perceptible until the third day of withdrawal. Second, the abstinence syndrome was not associated with drug need. Most subjects were glad the study was over and none sought medication for relief of their symptoms. The third issue was the nature of abstinence syndrome.

Doctors Eddy and Isbell took the position that the cyclazocine abstinence syndrome was just mild abstinence. To help resolve this issue, Dr. Isbell provided me with unpublished data of E.G. Williams [27] who had studied the abstinence syndrome of subjects dependant on different stabilization doses of morphine in an attempt to determine the smallest dose of morphine that produced a clinically significant degree of physical dependence. A sign analysis of Williams’ data and the cyclazocine abstinence data was done. The analysis indicated that the relative magnitude of the signs of cyclazocine abstinence was different from that of morphine abstinence, regardless of the level of dependence [26, 27].

The effects of cyclazocine shared certain characteristics with those of nalorphine [26], except that nalorphine was less potent and the maximum degree of ataxia was less. Whereas cyclazocine could produce overt drunkenness, nalorphine produced liminal ataxia that was only demonstrable with tandem gate walking. The latter difference was subsequently explained when studies were conducted in the chronic spinal dog, in which it was shown that nalorphine showed partial agonistic activity [24, 28]. When nalorphine was administered chronically in doses of 240 mg/kg/
day and then withdrawn, an abstinence syndrome emerged within 24 h and was qualitatively different from the morphine abstinence syndrome and similar to the cyclazocine abstinence syndrome.

Several investigators studied mixtures of morphine and nalorphine in human subjects and in animals, administered acutely and chronically [29]. Of particular importance were the observations of Houde and Wallenstein [30], who found that low doses of nalorphine antagonized the effects of 10 mg of morphine, whereas higher doses produced a lesser antagonism. The nalorphine biphasic dose response antagonism of morphine’s analgesic action could not be explained by assuming that nalorphine was a competitive antagonist or a partial agonist of morphine. Houde and Wallenstein’s observation [30] stimulated a mathematical formulation of receptor dualism [29, 31].

Naloxone antagonized the actions of cyclazocine in the chronic spinal dog [28] and in human subjects [32]. Naloxone in a high dose (15 mg/70 kg) antagonized miotic, respiratory depressant, and subjective effects produced by 1 mg/70 kg of cyclazocine in human subjects. Naloxone (0.2 mg/kg) partially antagonized the depressant effects of cyclazocine (0.063 mg/kg) on the flexor reflex of the chronic spinal dog. The same dose of naloxone completely antagonized the effects of 1.0 mg/kg of morphine. Blumberg showed that naloxone antagonized the analgesic effects of cyclazocine, nalorphine and pentazocine in mice [33].

Thus, four lines of evidence suggested that nalorphine and cyclazocine differed from morphine in their actions. (1) The nature of the subjective effects that they produced were different; (2) they produced different types of dependence; (3) interaction studies between morphine and nalorphine yielded biphasic dose response curves; and (4) the effects of several agonist–antagonists could be antagonized by large doses of naloxone.

These observations lead to the suggestions that there were two opioid receptors an M (morphine) and N (nalorphine). Further, the M and the N receptors operated in concert in some, but not all, physiologic systems. The process of a concerted action was “pharmacologic dualism.” It was suggested that morphine acted as an agonist and nalorphine as a competitive antagonists at the M receptor. Further nalorphine acted as a partial agonist at the N receptor [29]. This concept (pharmacologic dualism) was an elaboration on my concept of pharmacologic redundancy, which postulated parallel neuronal pathways employing different transmitter, as well as co-transmitters and co-receptors as alternative mechanisms for the conduct of function [34].

The hypothesis that there are two opioid receptors that exhibit the principle of receptor dualism reconciled many observations. It was soon apparent, however, that it left other observations unexplained in terms of receptor theory.

The first analgesic with predominantly N agonistic activity to be marketed was pentazocine. It was not scheduled as a narcotic because studies at the Addition Research Center indicated that it did not produce as much euphoria as morphine, did not substitute for morphine in morphine-dependent subjects, did not appear to produce physical dependence, and was not liked by post-addict subjects when administered chronically [35].
There were sporadic case reports of abuse of pentazocine. For this reason, and because we had developed new concepts, we decided to reinvestigate the abuse potentiality of pentazocine. One of the important developments in opioid pharmacology was the synthesis of naloxone and the elucidation of its pharmacology. Blumberg had encouraged the synthesis of naloxone with the end of obtaining a more potent antagonist with fewer side effects (e.g., respiratory depression and psychotomimetic effects) [36]. Foldes [37] conducted extensive studies with naloxone showing that it antagonized the respiratory actions of opiate analgesics. Lasagna found that naloxone produced a modest degree of both analgesia and hyperalgesia in patients with pain.

Our task was to assess the abuse potentiality of naloxone [38]. We found that it did not induce subjective changes, did not produce miosis when administered chronically, did not produce physical dependence, and, when administered to morphine-dependent subjects, was seven times more potent than nalorphine in precipitating abstinence [39]. We concluded that naloxone was an opioid antagonist that was devoid of agonistic activity. Naloxone was of great importance in further clarifying the mechanism of action of pentazocine and provided critical proof that morphine was acting as an agonist.

In our reinvestigations of pentazocine, we confirmed several of the observations of Fraser and Rosenberg [35]. Low doses of pentazocine produced a subjective state similar to that produced by low doses of morphine characterized by elevations of MBG scale scores, which measure feelings of well-being. In this regard, pentazocine was about one-fourth as potent as morphine [40]. Further, doses above 40 mg produced dose-related elevation on the LSD and PCAG scale scores, which measure, respectively hallucinations, delusions, and anxiety (LSD) and apathetic sedation (PCAG), and a decrease in the MBG scale scores.

The fact that lower doses of pentazocine produced elevations of MBG scale scores raised the question of whether pentazocine could be a weak partial agonist at the M receptor and a less potent but strong agonist, at the N receptor. To test this hypothesis, subjects were made dependant on decreasingly lower doses of morphine and the ability of pentazocine to suppress the morphine and abstinence syndrome was assessed. In short, pentazocine did not clearly suppress abstinence in subjects dependent on morphine in doses as low as 30 mg/day and as high as 240 mg/day.

When subjects who were dependent and stabilized on 240 mg/day of morphine were administered pentazocine, it precipitated an abstinence syndrome and in this regard was 1/50 as potent as nalorphine. Thus the doses of pentazocine that were necessary to precipitate abstinence were greater than those necessary to cause miosis, analgesia, and subjective effects. When subjects were administered pentazocine in doses of 522–684 mg/day and then abruptly withdrawn, a mild abstinence syndrome emerged that was quantitatively similar to that seen in cyclazocine- and nalorphine-dependent subjects.

Further, an abstinence syndrome could be precipitated in pentazocine-dependent subjects with naloxone in doses approximately ten times larger than necessary to precipitate an abstinence syndrome in morphine-dependent subjects. At this
The Evolution of Concepts

We had been aware that cyclazocine and nalorphine produced a subjective syndrome consisting of dysphoria and an apathetic sedation. The fact that pentazocine produced more feeling of well-being than did nalorphine and cyclazocine, and yet resembled them in many other ways, was a problem – “the pentazocine problem.”

To determine if this problem had a receptor-based explanation, an extensive group of studies was initiated in the chronic spinal dog [23, 41–43]. These studies developed methods that yielded data in the dog that provided potency estimates on a variety of physiologic parameters (pupillary diameter, pulse rate, respiratory rate, body temperature, amplitude of the flexor reflex, and the latent of the skin twitch reflex). In addition, procedures were developed for conducting valid assays of the potency of drugs in suppressing signs of abstinence in the maximally abstinent chronic spinal dog.

A large group of dogs was made dependent on morphine; a prototypic “M” agonist. Another group was made dependant on cyclazocine; a prototypic “N” agonist. Over 20 prototypic drugs were studied. Morphine-like drugs (see Table 2.2) by and large produced a similar pattern of effects suppressing the flexor and skin twitch reflexes, constricting pupils, lowering body temperature, and slowing pulse rate. Further these agents as well as other that resembled morphine suppressed the morphine abstinence syndrome in a dose-related way. Of some importance were the observations that neither meperidine nor normorphine produced morphine-like effects or suppressed the morphine abstinence syndrome. Hence, although they are morphine-like drugs in other species, they do not appear to be morphine-like in the dog.

Buprenorphine in single doses also produced a morphine-like pattern of effects, but differed from morphine in that it produced a lesser maximal effect. Further it suppressed abstinence signs; the slope of its suppression dose–response line was less than that of morphine, however. Buprenorphine also precipitated abstinence in stabilized morphine-dependent dogs; the slope of the precipitation dose-response line, however, was less than that of naloxone and naltrexone. These data were consistent with the hypothesis that the buprenorphine was a partial agonist of the morphine-type.

In contrast to morphine-like drugs, cyclazocine, nalorphine, and pentazocine were relatively ineffective in suppressing the thermally evoked skin twitch reflex, but produced a profound depression of the pressure-evoked flexor reflex. They also, especially in longer doses, dilated pupils and increased heart and respiratory rate, but did not depress body temperature to the degree morphine did.

Keats and Telford [44] in their study of the analgesic properties of a series of N-substituted benzomorphans, had observed that N-allylnormetazocine (NANM; SKF 10,047) produced severe dysphoria and little analgesia. NANM was selected as a prototypic and relatively selective dysphoriant and was studied in the chronic dog. It produced less depression of the flexor reflex than morphine or ethylketazocine, did not depress the skin twitch reflex, increased pupillary diameter, pulse rate, and respiratory rate, and produced a canine delirium.
NANM’s respiratory stimulant action probably has a different mechanism of action than morphine’s in the dog. Morphine causes panting by resetting hypothalamic thermoregulatory center that downregulated the set point and thus body temperature. In contrast, NANM stimulated respiration, while producing a modest hyperthermic reaction. In all probability the respiratory stimulant actions of nalorphine, which are seen in relatively high doses, are a consequence of nalorphine’s $\sigma$ activity [41].

Other prototypic drugs studied were ketazocine and ethylketazocine, which depressed the flexor reflex, had little effect on the latency of the skin twitch reflex, produced sedation, and were potent miotics.

Studies in the morphine- and cyclazocine-dependent dog are summarized in Table 2.2. Several points are of importance. Nalorphine precipitated abstinence in both

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<th>Cyclazocine-dependent dogs</th>
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<td>Suppression potency</td>
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<td>Codeine</td>
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<td>Morphine</td>
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<td>Ketobemidone</td>
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PA nalorphine is a partial agonist.

Potency estimates for suppression studies are expressed as milligrams of morphine or cyclazocine that are necessary to produce the same degree of suppression as the experimental drug. Naloxone is used as a standard drug in precipitation studies in morphine-dependent dogs and naltrexone in cyclazocine-dependent dogs.
the morphine- and cyclazocine-dependent dog. In the cyclazocine-dependent spinal dog, however, it exhibited a ceiling effect. These observations were in keeping with the observations in the nondependent dog, namely that nalorphine’s agonistic effects exhibited a ceiling and that it was probably a partial agonist of the $\kappa$ type (see below).

Of great importance were the observations that three groups of drugs suppressed the cyclazocine abstinence: (1) morphine; (2) cyclazocine, nalorphine, and pentazocine, which exhibited excitation effects such as mydriasis and tachycardia; and (3) ethylketazocine and ketazocine, which constricted pupils, but did not suppress the morphine abstinence syndrome. The excitatory effects of cyclazocine, nalorphine, and (to some extent) pentazocine resemble the effects of NANM.

To further compare the pharmacologic properties of NANM with those of the prototypic drugs, morphine and cyclazocine dogs were made dependent on 10 mg/kg/day of NANM administered in equally divided i.v. doses six times a day. This proved to be a difficult experiment to execute. As the dose levels were increased, dogs exhibited canine delirium and loss of appetite and weight. By slowly escalation the dose, a stabilization dose of 10 mg/kg was eventually obtained, and precipitation and withdrawal studies were conducted.

This study showed that chronic administration of NANM induced tolerance to its ability to produce canine delirium, tachypnea, and anorexia. The withdrawal abstinence was mild, consisting of a decrease in body temperature, miosis, brachycardia, tachypnea, and an increase in the amplitude of the flexor reflex. This syndrome was unlike that seen in either morphine- or cyclazocine-dependent animals. The naltrexone-precipitated abstinence syndrome was yet different, consisting of hyperthermia, tachycardia, tachypnea, and an increase in the amplitude of the flexor reflex. These data further showed that some of the effects of chronically administered NANM could be antagonized by naltrexone, whereas others could not. These observations led to the suggestion that NANM might have multiple modes of action.

These and other observations could be reconciled by the hypothesis that (1) there were three opioid-related receptors, $\mu$, $\kappa$, and $\sigma$; (2) these receptors could exert their effects on several physiologic systems through different but converting pathways (receptors dualism and pharmacologic redundancy); and (3) drugs that interact with opioid receptors could act as competitive antagonists partial agonists and strong agonist.

## 2.2 Reflections

In the relatively brief time – two decades – since these hypotheses were proposed, an enormous body of data has been generated that supports them. Further, they have been extended in two major directions: (1) additional types of opioid-related receptors have been identified and (2) endogenous opioid transmitter substances have been discovered. These observations have had, and will continue to have, an enormous impact on neurochemistry, physiology, neuropsychopharmacology, and psychology, as well as on mental health.
2.2.1 Pharmacologic Implications

The first clues concerning the existence of multiple opioids came from studies in humans that were subsequently elaborated on using the chronic spinal dog. The conclusions were drawn from analyses of the patterns of pharmacologic effects using agonists and antagonists of different specificities and differed from other classic analyses of receptor subtypes only in that these comparisons used signs derived from changes in central nervous function for the comparisons. For these pattern comparisons to become meaningful, valid bioassay techniques had to be developed for the various central nervous system functions under study such as subjective effects, pupillary diameter, function of homeostats, and reflex activity. The second element was the use of receptor theory in the design of experiments and conceptualization of hypotheses. The third major ingredient in this endeavor was the very large synthetic effort that yielded a rich diversity of structural modification of important drugs. In this regard the synthetic efforts of Sidney Archer, William Michne, Jack Fishman, John Lewis, and Everett May were particularly important.

In a relatively short time, it was demonstrated that relatively minor structural modifications of opioid drugs could change the specificities for μ, κ, σ, and δ receptors and could alter their activity, yielding agonists, partial agonists, and competitive antagonists. It was also apparent that opioid ligands had a number of reactive sites that could interact with a variety of moieties on opioid receptors. These general observations lead to the formulation of the steric theory of multiple opioid receptors, which offers a theoretical basis for explaining not only the multiplicity of opioid receptors but also differences in their efficacy and activity [17].

The steric theory has several components:

1. It assumes that the opioid receptor has nuclear sites that are responsible for initiating the pharmacologic action of the drug or transmitter, as well as satellite sites that play two roles: (a) determination of the affinity of the drug for the receptor and (b) the orientation of the drug on the receptors.

2. Changes in the configuration of these two components of the receptors may have several effects on drug receptor interactions. The following terms are coined to designate the possible types of changes. **Allomorphism** is a change in the position of the active moieties of the nuclear part of the receptor. Such changes will result in a change in the specificity of drugs for the receptor. **Allosterism** is a change in the positions of moieties of the satellite sites. These result in changes of affinity of the drug for the receptor and in the orienting properties of the receptor toward the drug. **Allotaxia** is the property whereby the drug can occupy the receptor in several positions.

These types of changes in the receptors can hypothetically be interactive. Clearly, changes in the relative positions of satellite moieties could alter both the affinity and the allotaxic properties of a family of drugs and hence alter both the $K_d$ values and the activity of the drug. On the other hand, allomorphic changes will result in a change in the number of receptors of different specificities. These types of changes may result in complicated dose–response curves.