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CENTRAL ANALGETICS

Edited by
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Series Preface

The process of drug development has undergone major changes in the last two decades. To appreciate the magnitude of the change, one needs to think back to the mid-1950s. This was the boom period of pharmaceutical development; better than half the structural classes available to today's clinician had their inception in that era. Yet, in spite of all the demonstrable successes, this was not a period of truly insightful research. Rather, regulations were sufficiently liberal so that novel chemical entities could be—and were—taken to the clinic with only a demonstration of safety and some preliminary animal pharmacology. It is perhaps as a result of this that many of our pharmaceutical mainstays owe their existence to serendipitous clinical findings.

It should, of course, be added that the crude nature of the available pharmacology was a reflection on the state of the art rather than on a desire to skimp on research. A good many of the current concepts in pharmacology postdate the boom era in drug development.

The same applies to medicinal chemistry. With a few notable exceptions, much of the synthesis was aimed at achieving a patentable modification on someone else's drug or consisted in following "interesting chemistry" in the hope of coming up with biological activity. The dialogue between the medicinal chemist and the pharmacologist was in its infancy.

The drug development process in 1982 is an entirely different discipline; though the time and effort involved in taking a drug from the bench to registration has increased enormously, it has, in spite of this, become more intellectually satisfying. The increased knowledge base permits more informed decisions.

The increased stake involved in taking a drug to the clinic means that upper management in drug companies wants greater assurance of suc-

cess before taking that very expensive step. Consequently, compounds are studied pharmacologically in far greater detail than ever. The gap between animal pharmacology and human therapy is being steadily narrowed by the development of ever more sophisticated tests which may more accurately forecast human responses. Much of this has been made possible by enormous strides in pharmacology. Understanding of drug action is approaching the molecular level.

Medicinal chemistry too has acquired a firmer theoretical underpinning. The general desideratum is rational, or directed, or deliberate, drug development. (*Rational* strongly implies that those who do not follow that design are irrational. There is too large an element of luck, serendipity, and informed intuition involved in drug discovery to use the term irrational for those who choose a more intuitive approach.) This approach has in fact achieved its first success: Cimetidine was developed by studying the interaction of histamine and its congeners with its receptors. Captopril came from a research program motivated by a consideration of the role of the renin angiotensin system in the control of blood pressure.

A hallmark of many laboratories involved in drug development is the existence of the project team. All individuals assigned to research on drugs in a given therapeutic area are expected to interact with a greater or lesser degree of formality and to make their own day-to-day research decisions in close consultation. While the makeup of such teams varies considerably, the medicinal chemist and the pharmacologist are almost obligatory members. It becomes incumbent on each to be able to communicate with the other. The pharmacologist will thus profitably be acquainted with the names and, if possible, structures of compounds relevant to the therapeutic area, be these drugs or endogenous agonists and antagonists. While not expected to actually design analogue series, the pharmacologist may find it appropriate to be able to recognize pharmacophoric groups. The chemist, on the other hand, will certainly want at least nodding acquaintance with the pharmacological basis for drug therapy in an assigned area. An understanding of biological screens, tests, and their limitations will help the chemist better understand the biological significance of test results on compounds being studied.

There are today very few convenient sources to which a scientist can turn for such information. As a rule the pharmacology on any therapeutic area will be scattered in original articles and reviews in the biological literature. An individual seeking the medicinal chemistry background will have to choose between consulting superficial reviews, perusing

some sixteen or more volumes of highly condensed periodic reports, or going back to the original literature.

Chemistry and Pharmacology of Drugs is a series of books intended to allow scientists involved in drug development to become familiar with specific therapeutic areas by consulting a single volume devoted specifically to that area.

Each of the volumes of the series envisaged treats a fairly discrete disease entity, or sometimes a therapeutic class. Each of the books treats separately the pharmacology, screening, and development methods and medicinal chemistry relevant to the topic. In each book, the first section deals in some detail with both the normal and diseased physiology of the appropriate organ system; it is in this section that the pharmacology and, if pertinent, biochemistry are discussed. The next section deals with the various primary screens that have been used to discover active compounds. More elaborate tests designed to elucidate mechanism of action and the like are discussed as well. The medicinal chemistry section deals with the chemistry used to prepare active compounds; where available, the SAR of active series; and the rationale that led a particular direction to be chosen. Since such a volume is today beyond the scope of any single author, each book will be written by three or, at the most, four authors.

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DANIEL LEDNICER
Series Editor

Preface

Alleviation of pain constitutes the central theme of any book on analgetic agents. That sensation—pain—is sufficiently complex to merit a lengthy set of definitions in dictionaries. These do, however, finally zero in on the fact that pain involves discomfort. In the teleological sense, pain does serve a vital role in any organism's economy. The sensation is an alerting mechanism that all is not well, that some external or internal injury or conflict with the environment exists. More frequently than not, however, the sensation of pain persists well after it has fulfilled its primary alerting function. It is this nonfunctional pain which is the usual target of analgetic agents.

Though analgetics were among the first classes of medical agents to be incorporated into the pharmacopeia, existing drugs have a sufficient number of deficits so that research aimed at new agents persists today in very active fashion. The so-called peripheral analgetics, exemplified by aspirin, will be discussed in a later volume in this series. Suffice it to say in passing that aspirin and its successors tend to suffer from limited efficacy and a propensity to exacerbate, and occasionally cause, gastric ulcers. The subject of the present volume is the very large class of analgetic drugs that act by way of the central nervous system. As will emerge in this book, the drawbacks of drugs in this category may be due, at least in part, to their mechanism of action.

This is a particularly apt time to be composing a book on central analgetics. Much of the work for the past century was carried out in a purely empirical manner. The medicinal chemist and pharmacologist collaborated on programs which had as the end point a series of responses in rodents which involved development of tolerance to pain. Increased recognition of the dual problems of addiction and abuse potential led to the elaboration of some supplementary elegant, albeit purely

empirical, models to study this liability in animal systems. This pragmatic approach has led to some successful drugs which represent an approach to the ideal analgetic. The receptors hypothesized for these molecules by various workers were largely intellectual constructs built on structure–activity relationships.

The first section of this book deals with the physiology of pain. Mohrland's discussion begins with a description of the neural pathways involved in sensation of pain as well as a description of the involvement of various neurotransmittors and biochemical modulating substances. Interaction of pain stimuli with other central nervous system functions is treated as well.

The chapter on the pharmacological study of pain begins with a discussion of mechanistic sites open to intervention. There follows a description of the various animal screening methods that have been used to detect antinociceptive agents. Von Voigtlander then presents some of the more detailed pharmacological tests used to characterize agents of potential clinical interest. These assays include opiate receptor binding studies and a battery of tests used to define abuse potential. The section closes with a discussion of tests for ancillary pharmacology to ensure safety of candidate compounds in the clinic.

Commercial availability of pure tritium gas was to place receptor theory on a sound footing. As early as 1962, Jensen used this gas to obtain estradiol, which was sufficiently radioactive so that its interaction with tissues thought to contain receptors could be carried out using physiological levels of the hormone. This technique allowed detailed characterization of the estrogen receptor. An analogous technique later led to the identification in mammalian brain homogenates of a high-affinity receptor for morphine. The search for the endogenous agonist which binds to this receptor was rewarded with the discovery of so-called natural opioids—the enkephalins. The past few years have seen the characterization of several additional peptides which show high binding affinity to this class of receptors. The near future will no doubt see these findings used to place the mode of action of the pain-sensing and mediating system on a sound mechanistic footing. The very newness of these results means that their impact on research toward new analgetics is just coming to be felt, with the identification of additional peptides as well as the synthesis of impressive numbers of analogues. This work is reviewed in Morley's section of this book.

A description of the organic and medicinal chemistry of central analgetics forms the last chapter of this book. In this section, I describe in some detail the chemistry and structure activity relationships of the more

important classes of opioids developed to date. The discussion goes beyond agents used in the clinic in order to illustrate salient points on the evolving concept of the SAR of these agents. A critical review of SAR, as used to carry out receptor mapping in the opioid series, has been included.

In sum, this book describes an area of therapy in which the process of drug development is in a state of flux. A series of exciting developments in the understanding of pain and of its transmission and modulation promises to bring about major strides. While it is still too early to forecast the direction future efforts will take, that work will no doubt be far less empirical than it was in the past. It is expected that the work will lean heavily on advances detailed in this volume.

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Columbus, Ohio
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Contents

CENTRAL ANALGETICS

Pain Pathways: Potential Sites for Analgetic Action

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For centuries man has endeavored to unveil the complex network of events preceding the sensation of pain with the hope that an understanding of these events would provide a means by which pain relief could be afforded. Progress toward achieving this goal has occurred at an unprecedented pace in recent years, albeit knowledge of the precise fate of incoming pain impulses remains in the future. Much of the impetus for the current surge of investigations pertaining to pain stems from the recent discovery that the body possesses its own pain-relieving system.

Although a number of early studies alluded to the existence of an endogenous system for the control of pain, the recent progress toward elucidating the components and mechanism of such a system stems from three major discoveries. The first came in 1969, when Reynolds reported that electrically stimulating a specific mesencephalic brain site produces a surgical level of anesthesia.[268] The second was the discovery from the laboratories of Snyder[243] and Terenius[295] that fractions from mammalian brain and intestine can stereospecifically bind opiates. The third discovery, by Hughes[152] and Terenius[296] in 1975, was that endogenous opioid-like peptides (endorphins) are present in mammalian tissues.

Collectively, these discoveries denoted potential neurotransmitters and receptors for an endogenous pain-controlling system which could be recruited by electrical or pharmacological activation. As the anatomical participants are being elucidated, the pharmacologist is provided a better understanding of the site(s) of action of centrally acting analgesics and a more specific target at which to direct future analgesic agents. Thus the discovery of an endogenous analgesic system has served to reinforce the concept that a thorough understanding of the physiology of pain will eventually provide a means by which pain sensation can be specifically eliminated once it has served to warn of impending tissue damage.

The descending component of this pain-modulating system is examined in detail in this chapter. First the physiological aspects of pain transmission are reviewed in a manner oriented toward identifying likely sites at which centrally acting analgesics may exert their pain-obtunding properties.

PAIN THEORIES

Historically, two opposing theories sought to explain the sensation of pain: the *specificity theory* and the *intensive theory*. According to the specificity theory, pain is transmitted from peripheral pain receptors to a pain center in the brain. Thus pain is considered a separate sensory modality conducted via its own distinct anatomical machinery.

The intensive theory proposes that pain results simply from overstimulation of other sensory modalities such as touch, sight, and sound. This theory is sometimes referred to as the *summation theory*, since it attributes pain sensation to a central summation of sensory input.

In 1965 Melzack and Wall[209] proposed a new pain theory called the *gate-control theory* which, in effect, was an attempt to bridge the chasm between the specificity and intensive theories as well as to accommodate some of the psychological aspects of pain. According to the gate-control theory, noxious impulses are conveyed by a specific group of nerve fibers. However, their activity is attenuated by other nerve fibers conveying non-noxious impulses until the stimulus intensity reaches a critical level, at which time the noxious information is projected rostrally and pain is perceived. This modulatory mechanism has been suggested as the basis by which certain stimulation procedures (e.g., transcutaneous nerve stimulation, dorsal column stimulation, and acupuncture) produce an analgesia. The gate-control theory also incorporates the descending projections from supraspinal sites which can further modulate the spinal gating mechanism.

Thus the gate-control theory allows for peripheral specificity, central summation, and psychological alteration of the pain experience. Some of the details of the gate-control theory have been modified or elaborated on in recent years to accommodate new developments in pain research,[2] yet it still falls short of adequately explaining the pain experience (can any theory?). However, it has provided direction for further experimentation which is gradually unraveling the mysteries of pain.

Before examining some of these studies it is necessary to make a few comments on nomenclature (cf. Ref. 36 for recent review of pain taxonomy). As alluded to above, pain has both physiological (sensory-discriminative) and psychological (motivational-affective) components.[208] The sensory-discriminative aspect entails the processing of the properties of a tissue-damaging (noxious) stimulus, such as place and magnitude. The motivational-affective component takes into account the variety of factors that might influence one's response to a given noxious stimulus, such as past experience, ethnic background, and environ-

mental setting. Thus pain can be defined as the sensory phenomena that may occur in reaction to a noxious stimulus. In that context it is difficult to determine to what extent "pain" is actually being evaluated in studies with laboratory animals, since such studies are confined primarily to the examination of the sensory-discriminative component. For that reason the term *nociception* is used in this chapter to refer to studies done on laboratory animals. Similarly, *antinociception* is used instead of analgesia to refer to animal studies.

PERIPHERAL PAIN PATHWAYS

Although the debate about the degree of specificity in neural pathways involved in pain is far from over, the best evidence to support specificity can be found in the periphery. In that regard peripheral receptors specifically activated by noxious stimulation have been described in numerous tissues. These noxious sensitive receptors, aptly termed *nociceptors*, appear as free (undifferentiated) nerve endings. Nociceptors have been most extensively studied by Perl and co-workers (cf. Refs. 47, 241) in the cutaneous tissue of cat[28,48] and monkey.[172,240] In their studies of the responsiveness of primary afferent fibers to peripheral stimuli they identified a group of small-caliber, slowly conducting myelinated (Aδ) and unmyelinated (C) fibers that were activated exclusively by noxious intensities of mechanical and/or thermal cutaneous stimulation. They also observed afferent fibers that were activated by innocuous stimuli but discharged maximally when the stimulus intensity reached the noxious range.

Thus nociceptors were demonstrated to be associated specifically with Aδ and C primary afferent fibers. However, the reverse is not true; that is, not all Aδ and C fibers are nociceptive in nature. The percentage of Aδ and C fibers carrying noxious information varies considerably among species.

In the studies from Perl's laboratory and others (cf. Ref. 47) nociceptive primary afferent fibers were found to exhibit several characteristics consistent with their participation in the transmission of the discriminative aspects of a noxious stimulus. These neurons can convey information about the potential severity of the stimulus since their rate of neuronal discharge is proportional to the intensity of the noxious stimulus. Furthermore, their small receptive fields attest to their capacity to provide information regarding the spatial properties (i.e., the locale) of the stimulus. In addition, these fibers respond to a given noxious stim-

ulus increasingly with iterative stimulation.[241] That is, repeated exposure to the stimulus results in a reduction in the threshold required for activation, an enhanced responsiveness, and an increase in background discharge. This is in striking contrast to the behavior observed for low-threshold receptors, which exhibit fatigue following repetitive stimulation.[241] Such "sensitization" to noxious stimuli has been suggested as a plausible basis for the hyperalgesia observed in some disease states, and may be associated with inflammation.[241]

Primary afferent nociceptors appear to possess some degree of modality specificity. Aδ and C mechanonociceptors, Aδ mechanothermonociceptors, and C-polymodal nociceptors have all been described. C-polymodal nociceptors comprise an interesting group of fibers that respond maximally to noxious mechanical and thermal cutaneous stimulation, as well as to chemical irritants such as those believed to be involved in the inflammatory/pain process.[28] Intense but subnoxious stimulation produces a minor, yet observable activation of C-polymodal nociceptors; their most vigorous discharge is reserved for stimulus intensities that are potentially or actually tissue damaging. The majority of C nociceptors are of the polymodal variety.

Observations in humans generally corroborate the reports from animal studies that Aδ and C fibers are primary afferents that convey nociceptive information. Recordings from peripheral nerves in man indicate that painful stimuli selectively activate Aδ and C fibers.[257,298,305] Likewise, percutaneous electrical stimulation, which selectively activates Aδ and C fibers, produces a sensation that is perceived as painful.[254] It is interesting that the qualitative nature of the pain produced by Aδ-fiber activation differs from that by C-fiber activation. The pain from Aδ-fiber activation is sharp and rapid, whereas the pain from C-fiber activation is dull, burning, and delayed. It has been suggested that this phenomenon relates to *first pain* and *second pain*, respectively, where first pain is an acute intense pain like a pinprick and second pain is the dull, chronic, burning pain characteristic of many pathological conditions.[254,257]

Sensitization of C fibers, such as that reported in animals following repetitive noxious stimulation, has not been observed in humans (cf. Ref. 172). This discrepancy may be a result of the particular protocol employed in the human studies since iterative stimulation of C fibers in man does produce a progressively more intense pain.[254]

Somewhat at odds with the aforementioned findings that nociception is conveyed specifically by small-caliber afferent fibers is the recent report that repetitive stimulation of large-diameter myelinated afferent fibers

(Aα and Aβ) can also evoke pain sensation in man.[318,319] Although the significance of this observation is uncertain at present, the authors suggest that it may explain the persistence of pain in certain neuropathological diseases.

SPINAL PAIN PATHWAYS

A certain degree of specificity for nociceptive pathways is maintained at the level of the dorsal root entry zone. That is, the small-diameter primary afferents (including nociceptive Aδ and C fibers) enter the spinal cord via a route that differs from that of the large-diameter afferent fibers. The large-caliber fibers travel in the medial aspect of the dorsal root, whereas the small-caliber fibers take a more lateral course.[164] As shown in Figure 1, this lateral projection enables the smaller fibers to enter directly into the tract of Lissauer, which puts them in a position to make direct synaptic connections with neurons in the superficial layers of the dorsal horn. This route can be readily differentiated from that taken by the large-diameter fibers, which enter proximal to the dorsal columns and approach the upper layers of the dorsal horn from a ventral direction.

Dorsal Horn

The spinal cord dorsal horn, the site of the first synapse of nociception-relevant primary afferent neurons, is comprised of a complex network of cells forming a rather unique cytoarchitecture that has been described

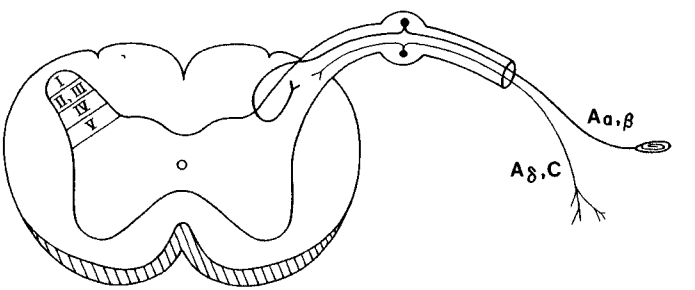

Figure 1 Cross section of the spinal cord depicting Rexed's laminae I-V and the different routes of entry by large ($A_{\alpha,\beta}$) and small (A_{δ},C) primary afferent fibers. Adapted from *Arch. Surg.* **112** (1977) 752.

by Rexed[269] as six distinct laminae (Figure 1). The differentiation of the dorsal horn into various laminae has also been made on a functional basis,[312] which more or less corresponds to Rexed's morphological separation.

Lamina I

Recent evidence based on the horseradish peroxidase-staining technique[189,239] has verified the results of earlier anatomical studies which showed that the lateral division of the dorsal rootlets, that is, the small diameter afferents, terminates extensively in the most superficial layer of the dorsal horn, lamina I (also called the *marginal zone*). Microelectrode recordings of individual neurons in lamina I[62,71,171,211] substantiate this anatomical association since cells activated exclusively by noxious stimuli are observed. Moreover, the excitatory response of one group of lamina I neurons to noxious mechanical stimuli is associated with the activation of Aδ primary afferent fibers. Another group of cells, which responded to noxious mechanical and noxious thermal stimuli, were shown to receive convergent input from both Aδ and C fibers.

Noxious-sensitive lamina I neurons have small receptive fields, although they are somewhat larger than those of primary afferent fibers. These lamina I neurons have also been shown to project rostrally to nociception-relevant brain sites such as the thalamus.[8,56,299,323] Although a significant portion of cells in lamina I are specific for nociceptive input, a number of marginal cells are activated by innocuous stimuli, most notably non-noxious thermal stimulation, indicating that neurons in lamina I are involved in more than nociceptive processes.

Laminae II and III

Because of the lack of clear demarcation, the next two laminae of the dorsal horn, laminae II and III, are typically described together and referred to collectively as the *substantia gelatinosa* (SG). SG cells are presumed to be predominantly small, inhibitory interneurons and are most often ascribed a modulatory role in nociception. Although some spinothalamic fibers have been shown to originate from the SG, such projections are sparse; therefore the SG is considered to be primarily involved in local circuitry (cf. Ref. 61). It is this region of the dorsal horn that is the site of the spinal gating mechanism in Melzack and Wall's[209] gate-control theory, which was discussed earlier, wherein intrinsic spinal interneurons delicately balance input from nociceptive (small-diameter) and non-nociceptive (large-diameter) afferent fibers.

In addition to the modulatory role of the SG, evidence is accumulating

that the cells in these laminae also convey nociceptive information. Small-diameter afferent terminals have been observed in the SG,[189] and it has been suggested that Aδ and C fibers synapse preferentially with dendritic branches in lamina I and lamina II, respectively.[61]

Price and co-workers[256] reported that most cells in the upper layer of the SG have dense arborizations into lamina I. They found afferent input to lamina I quite similar to lamina II, and observed responses of rostral-projecting lamina I cells consistent with a convergence of lamina II neurons onto lamina I cells. These authors proposed that one function of SG interneurons in nociception is to relay primary afferent input to lamina I neurons which project rostrally to the thalamus. It is apparent from studies such as these that it is no longer cogent to portray the SG exclusively as a modulator of nociception. Additional electrophysiological examinations of these small interneurons are needed to better understand their relative contribution to nociception.

Laminae IV–VI

A novel class of nociresponsive neurons, found in large numbers in laminae IV–VI of the dorsal horn, have received considerable attention for their potential role in nociception (cf. Ref. 255). These neurons readily respond to non-noxious mechanical or thermal cutaneous stimuli, however they discharge with increasing frequency as the intensity of the stimulus is increased, so they respond maximally to noxious stimuli.[258,310] They have relatively large receptive fields and exhibit enhanced responsiveness to repetitive C-fiber stimulation.[123,258] Thus neurons in these laminae have several common features with C-fiber polymodal nociceptors.

The neurons in laminae IV–VI described above are often called *wide dynamic range* (WDR) neurons.[258] They are also frequently termed *lamina V type* neurons because of the high degree of localization of neurons of this type in lamina V. It should be remembered, however, that WDR neurons are not found exclusively within the boundaries of lamina V, but also partially extend into laminae IV and VI. Thus the term *lamina V type* is a functional rather than a morphological classification and is indicative of diminished correlation between Rexed's[269] cytoarchitectural description and that derived from functional characteristics for the deeper laminae. In fact, WDR cells have recently been described in more superficial layers of the dorsal horn.[256]

Interestingly, the neuroanatomical studies of primary afferent terminals mentioned earlier failed to observe substantial projections of Aδ and C fibers into the lamina V region. However, it has been pointed

out[165] that the long dendritic branches of cells in the deeper layers of the dorsal horn extend well into the upper layers, which should provide adequate contact with the axon terminals of nociceptive primary afferents. Although it remains to be seen whether the noxious input to lamina V type neurons is direct via primary afferents or indirect via spinal cord interneurons, the rostral projection of lamina V cells is well described. Both anatomical and electrophysiological evidence indicates that lamina V WDR cells project rostrally to the thalamus via the spinothalamic tract.[8,299,300,321,323]

The importance of WDR cells in pain sensation is exemplified by the report of Mayer and co-workers,[201] who found selective electrical stimulation of WDR fibers, traveling in the anterolateral quadrant, capable of producing an identifiable, localized pain in humans. Most interestingly, the stimulation parameters (i.e., frequency, threshold, and refractory period) required to elicit pain sensation in man are nearly those required for activation of WDR neurons in the dorsal horn of monkeys.[258]

Lamina V WDR cells are also quite sensitive to a number of pain-obtunding manipulations and therefore may have particular significance in central analgesic mechanisms.

To summarize in general terms, two basic types of noxious-sensitive neurons have been identified in the dorsal horn of the spinal cord: one localized in the superficial layers, which responds exclusively to stimulation intensities in the noxious range and has small receptive fields, the second localized in the deeper layers, which responds to a wide range of intensities (maximally to noxious stimulus levels) and has broader receptive fields. These neurons have the capacity to convey this information rostrally since they are known to project into the thalamus. Though neurons in the substantia gelatinosa are interneurons which probably modulate nociception, recent evidence indicates that they may also contribute to the transmission of nociceptive information.

Although the discussion of these nociceptive neurons has been limited to the spinal cord dorsal horn, it should be kept in mind that an analogous organization in the trigeminal system mediates oral-facial pain. More specifically, the trigeminal (V) subnucleus caudalis is considered to be the brain stem analogue to the spinal cord dorsal horn, both in a functional (i.e., physiological and pharmacological profile)[90,151] and a cytoarchitectural sense.[114]

Spinal Nocireceptive Neurotransmitters

Numerous studies have attempted to identify the neurotransmitter(s) in primary afferent fibers which mediate(s) nociception at the first pri-

mary afferent synapse in the dorsal horn. From a pharmacological point of view there is immense potential value in elucidating the neurochemical mediators at the first synaptic locus. Namely, identification of the neurotransmitter agonist would provide direction toward the synthesis of an antagonist, with the intention that such a compound would be an analgesic. Furthermore, if it were possible to selectively localize it to the spinal cord, an analgesic acting at the first primary afferent synapse would not be likely to cause the serious central side effects that have plagued the narcotic analgesics.

Substance P

At present the best neurotransmitter candidate for nociceptive primary afferents is the peptide Substance P (SP). In 1931 von Euler and Gaddum[307] first described SP in extracts of mammalian brain and intestine (the P was an abbreviation for preparation, the notation originally used in their laboratory to describe the active component of the extract). Although SP was first proposed as a neurotransmitter of primary afferents by Lembeck[181] as far back as 1953, it was not isolated and identified until 1970–1971, when Leeman and co-workers[64,65] found that it is an undecapeptide with the amino acid sequence H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2.

Immunohistochemical studies have found SP to be highly concentrated in the superficial laminae of the dorsal horn of the spinal cord;[142,144,294] smaller but detectable concentrations of SP are also present deeper in the gray matter around lamina V.[222] Dorsal horn SP originates, at least in part, from the dorsal root ganglia of primary afferent neurons since SP levels in the dorsal horn are dramatically reduced following dorsal root rhizotomy[156,294] or pretreatment with capsaicin[223] (the active constituent of chili peppers that has been shown to deplete SP from small diameter primary afferent fibers[109]). Moreover, activation of Aδ and C primary afferent fibers produces a calcium-dependent release of SP into the dorsal horn of the spinal cord.[235,327]

Most important, in single neuron studies on the cat the microiontophoretic administration of SP onto nociresponsive dorsal horn neurons produces an excitatory response analogous to that following activation by noxious cutaneous stimuli.[138,264] A striking characteristic of the excitatory response of dorsal horn neurons to iontophoresed SP is the slow time course of effect, which has been cited as evidence that SP behaves as a neuromodulator rather than a neurotransmitter in the small-caliber fibers. However, recent studies indicate that the delayed onset of SP's effect can be accounted for on the basis of a slow release of the peptide

from glass micropipettes and thus need not be indicative of its physiological function.[122]

Studies aimed at providing behavioral evidence that SP is a nociceptive neurotransmitter in primary afferent neurons have produced contradictory results. Some reports observed a reduction in nociceptive thresholds following SP administration in rodents,[106,228] while others found that SP elevates nociceptive thresholds.[193,194,218,228,288] SP has also been shown to elicit a behavioral response (scratching) in mice which can be mimicked by topically administered algesic agents.[247]

It has been suggested that the disparity in these findings is related to the dosage and/or the source of SP [106,218] The different behavioral effects of SP have also been used as evidence that SP functions as a neuromodulator of nociception rather than as a neurotransmitter.[228]

SP has received considerable attention in investigations of the spinal cord modulation of pain. The significance of spinal modulatory processes in central analgesic mechanisms is apparent from the profound analgesia produced in animals[324,329] and humans[313] when narcotic analgesics are administered directly into the spinal subarachnoid space (i.e., intrathecally). Furthermore, it has been shown that opiate analgesics can produce an antinociception and inhibit noxious dorsal horn neurons even after the animal has been spinalized.[168,179]

Jessell and Iversen[155] have proposed that this spinal pain modulation is a presynaptic inhibitory process in which small interneurons containing endogenous opioidlike materials decrease both the activity and the corresponding release of SP from small-diameter primary afferents (Figure 2). In support of their proposal it has been shown that the degeneration of primary afferent terminals following dorsal root rhizotomy[156,176] or capsaicin pretreatment[223] reduces not only SP concentration but also the number of opiate receptor binding sites in the dorsal horn. Moreover, opioids can inhibit the release of SP both *in vitro*[155,192] and *in vivo*;[327] such an inhibition could explain the elevated levels of SP in the dorsal horn subsequent to morphine treatment.[304] Furthermore, systemically administered opioids do not inhibit SP-evoked discharges of dorsal horn neurons when SP is administered microiontophoretically,[248] a result that is consistent with a presynaptic inhibitory mechanism.

Nonetheless, spinal pain-modulating mechanisms postsynaptic to primary afferent terminals probably also exist. In that regard both dorsal root rhizotomy[156,176] and capsaicin pretreatment[223] reduce dorsal horn opiate receptor binding by only 40–50%. Also, opiates have been shown to inhibit non-noxious-evoked and spontaneous activity (in addition to

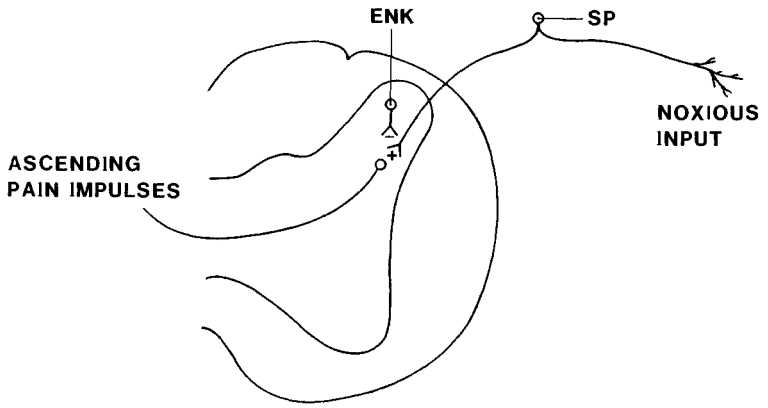

Figure 2 Presynaptic inhibition of Substance P (SP) containing primary afferents by enkephalinergic (ENK) interneurons, proposed by Jessell and Iversen.[155]

noxious-evoked activity) in dorsal horn neurons.[94] Furthermore, a recent electron-microscopic/immunohistochemical study of methionine-enkephalin localization within the superficial layers of the rat spinal cord found that enkephalin terminals form synaptic contacts primarily with dendritic shafts and spines rather than with axons and terminals.[153] It would appear then that both presynaptic and postsynaptic inhibition of nociceptive neurons occur in the dorsal horn of the spinal cord.

Some of the ostensible inconsistencies found in the literature regarding the exact role of SP as a primary afferent neurotransmitter may arise because it is only one of the neuropeptides with such a role. For example, multiple nociceptive neurotransmitters may explain the residual sensation of pain following depletion of SP-containing primary afferents.[223] In that regard there is some evidence that SP is specific for certain forms of noxious cutaneous stimulation.[135] Several neuropeptides (e.g., somatostatin, cholecystokinin, and vasoactive intestinal polypeptide) have been immunohistochemically identified in dorsal root ganglia and thus are potential candidates as nociceptive neurotransmitters.[143] When somatostatin was tested for its effect on nociresponsive dorsal horn neurons, it was found to produce inhibition rather than excitation.[263] The eventual role, if any, of these neuropeptides in nociception awaits further study.

Other sources of confusion surrounding SP arise from trying to separate supraspinal and spinal modulatory mechanisms. Delineation of

the relative contribution of each system may explain why mimicking systemic opiate effects on dorsal horn neurons is difficult to achieve with locally administered drugs,[49,89,93,248] why cells whose noxious-evoked activity is inhibited by iontophoresed opiates are not readily excitable by iontophoresed SP,[91] and why SP may elevate nociceptive thresholds.[106,193,194,218,228,288]

In addition to SP being released from spinal terminals, it is also released from sensory nerve endings in the periphery.[229] However, peripherally released SP does not appear to be activating nociceptors and thereby function as a peripheral chemogenic mediator of pain.[182] There is good evidence, at least for inflammatory pain, that the chemogenic activation of nociceptors results from the release of pain-producing substances such as bradykinin and histamine which act on nociceptors that have been sensitized to chemical stimulation by prostaglandins.[96] Lembeck and co-workers[109,183] have provided evidence that SP peripherally released from sensory nerves is responsible for antidromic vasodilation and neurogenic plasma extravasation. Thus they propose that SP released into the skin produces a vasodilation that promotes repair of the damaged tissue.

ASCENDING PAIN PATHWAYS

There are multiple pathways through which noxious information from spinal cord dorsal horn neurons ascends to influence supraspinal nuclei. The most prominent of these ascending pain pathways is the spinothalamic tract (Figure 3), which in higher mammals is comprised of two divisions, the neospinothalamic tract and the paleospinothalamic tract.[32,206]

Neospinothalamic Tract

The neospinothalamic tract (NST) is considered to be phylogenetically more recent since it is readily observed in primates and, although it may be present, is not as apparent in lower species.[7,166,205,206] Most dorsal horn neurons whose axons travel in the NST cross the midline in the gray matter of the cord before ascending into the lateral aspect of the ventrolateral quadrant [lesions of which have long been known to afford pain relief in chronic pain patients (cf. Ref. 205)]. The NST consists primarily of rapidly conducting myelinated fibers that ascend without

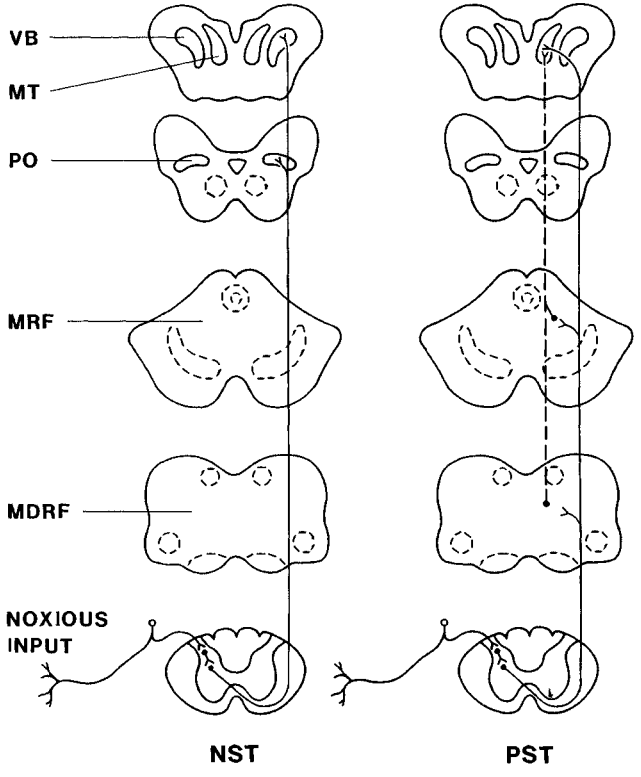

Figure 3 The two divisions of the spinothalamic tract: the neospinothalamic tract (NST) and the paleospinothalamic tract (PST). MDRF: medullary reticular formation, MRF: mesencephalic reticular formation, PO: posterior thalamus, MT: medial thalamus, VB: ventrobasal thalamus. Adapted from Ref. 51.

synaptic interruption to the contralateral thalamus, synapsing there predominantly with cells in the ventroposterolateral nucleus and posterior thalamic group.[166,206,207] Other thalamic destinations have been described (cf. Ref. 80) and found to vary with the species (e.g., in the cat medial thalamic projections are prominent[35,147]).

The cells of origin of spinothalamic tract (presumably NST) fibers have undergone extensive anatomical and electrophysiological examination. Although cells throughout the dorsal horn laminae may give rise to NST fibers, most anatomical studies agree that in primates the largest

number of spinothalamic tract cells originate from lamina I and lamina V;[7,8,299,300,321] in cats they originate from deeper laminae.[56,189,299,301]

Willis and co-workers[13,322,323] who studied the response characteristics of dorsal horn neurons that had been identified as direct thalamopetal neurons, found that most are of the wide dynamic range lamina V type. High-threshold dorsal horn neurons like those found in the superficial laminae have also been observed among NST fibers, as have cells that are clearly non-nociceptive in nature, such as those responding maximally to joint rotation.[323] Furthermore, NST fibers can be activated by electrical stimulation of Aδ- and C-fiber afferents.[20,73,104] Hence the characteristics of NST neurons indicate that this pathway is capable of rapidly transmitting discriminative information regarding a noxious stimulus. In addition, this pathway has the capacity to integrate other sensory modalities that are not noxious but may contribute to the overall perception of pain.

Paleospinothalamic Tract

The more medial division of the spinothalamic tract is the paleospinothalamic tract (PST), which is considered to be phylogenetically older since it is found rather uniformly in all vertebrates.[32,206] PST fibers initially travel a course similar to that of NST fibers; they cross the midline and ascend into the ventrolateral tract, just medial to NST fibers. However, unlike NST neurons PST neurons rarely project directly to the thalamus but instead synapse via collaterals or direct fibers at various levels along the neuraxis of the brain stem reticular core.[206] Information from PST cells is subsequently relayed to the thalamus by reticulothalamic fibers, which terminate primarily in medial thalamic nuclei.[38,39,80]

Thus, unlike the rapid NST with its direct projections, the PST is a multisynaptic pathway innervating rather diffuse supraspinal structures. These characteristics have led to the suggestion that the PST conveys impulses related to pain that is perceived as dull and poorly localized, and is associated with some of the motivational/affective components of pain.[80] If this is true, the PST is paramount in the study of centrally acting analgesics since it is pain of this type that is best relieved by these agents.[286]

Spinoreticular Tract

Although the spinothalamic tract is undoubtedly the major ascending nociceptive pathway, several other ascending systems have been re-

ported to play partial or supportive roles in conveying nociceptive impulses to higher centers. For example, second order dorsal horn neurons responsive to noxious stimuli have been identified in the spinoreticular tract (SRT).[7,101,210] In fact, a recent study[210] which examined dorsal horn neurons that travel in the SRT of the rat found them to have electrophysiological properties that are very similar to those of spinothalamic tract neurons.

Anatomically, SRT fibers ascend both ipsilaterally and contralaterally into the ventrolateral quadrant of the cord alongside spinothalamic tract neurons. SRT terminals are known to synapse at various levels along the medial reticular core. Thus the SRT is a multisynaptic pathway with many anatomical and physiological features that are coincident with those of PST and therefore possibly involved in dull, poorly localized pain. Because of this, differentiating between the PST and the SRT may be difficult; the most distinguishing characteristics of SRT fibers are their predominantly ipsilateral location and their lack of direct connections to the thalamus.[80]

Dorsal Columns

Spinofugal pathways outside the ventrolateral quadrant have also been implicated in the rostral transmission of nociception, although these pathways predominantly transmit sensations other than pain. The dorsal columns, for instance, consist primarily of long, ascending, myelinated primary afferent fibers known to convey impulses pertaining to touch and proprioception. Yet electrophysiological evaluation of the secondary neurons (which comprise only about 10% of the neurons) in the dorsal columns of cats indicates that they may participate in rostral pain transmission.[80] Unlike primary dorsal column neurons a large percentage of these postsynaptic cells respond maximally to noxious stimuli with characteristics similar to wide dynamic range dorsal horn neurons.[12,246] Although it remains to be shown whether this information is transmitted rostrally to the thalamus, dorsal column fibers are known to have a thalamic connection. Dorsal column fibers ascend ipsilaterally and synapse in the lower medulla in the nucleus cuneatus and the nucleus gracilis. These nuclei give rise to decussating fibers that ascend in the medial lemniscus and terminate in the ventroposterolateral nucleus of the thalamus.[51]

In addition to possibly participating in ascending nociceptive transmission, the dorsal columns have been suggested to be a modulator of nociception.[209] In that regard electrical stimulation of the dorsal columns

has been useful in providing pain relief for some chronic pain patients.[97] As mentioned previously, the analgesia produced by dorsal column stimulation likely results from the ability of non-nociceptive fibers to attenuate the activity of fibers conveying noxious impulses. Activation of dorsal column fibers has been shown to inhibit the activity of nociceptive dorsal horn neurons.[105]

Spinocervical Tract

The spinocervical tract (SCT) has also been implicated in ascending nociceptive mechanisms, particularly in the cat. A substantial number of neurons projecting into the feline SCT respond maximally to noxious mechanical and/or thermal stimuli, as well as to stimulation of Aδ and C primary afferent fibers.[41–43] Although the SCT is not nearly as prominent in primates, nociresponsive SCT fibers have been observed in the monkey.[44] Since SCT fibers project rostrally in a manner similar to dorsal column fibers, they too have the anatomical connections necessary for involvement in pain processes. Dorsal horn SCT neurons originate primarily in the region of lamina V, ascend ipsilaterally in the dorsolateral funiculus, and terminate in the lateral cervical nucleus. The latter is known to project to the contralateral thalamus (predominantly to the ventroposterolateral region) via the medial lemniscus.[34,44,45]

Propriospinal Tract

Noxious impulses have also been suggested to ascend via the propriospinal tract.[99,164] Spinal cord neurons responsive to noxious stimuli have been identified within this tract.[102] Since the propriospinal tract is a polysynaptic pathway that both originates and terminates within the spinal cord, it may be involved in the retention of pain responsiveness following cordotomies.[226]

To summarize, although the spinothalamic tract is the major pathway, no single spinofugal pathway is responsible for the rostral transmission of noxious information. Dennis and Melzack[80] have used the anatomical and physiological commonalities of these pathways (excluding the propriospinal tract) to conveniently group them into two basic divisions: a lateral group, consisting of NST, dorsal columns, and SCT, which is capable of rapidly conducting discrete information regarding a nociceptive stimulus, and a medial group, consisting of PST and SRT, which conducts in a slower fashion and is more diffuse. These two groups are

thus involved in transmitting impulses related to acute-intense pain and dull-chronic pain, respectively. Yet even within these two groups subtle yet significant differences can be found. Hence, as these authors rightly point out, the body's utilization of multiple ascending pathways in response to noxious stimuli does not necessarily indicate that the pathways are all mediating the same information. Rather, because the pathways possess unique properties, they can work in concert to elicit an adequate perception of pain, for example, when the stimulus intensity becomes potentially tissue-damaging, without interfering with other ongoing activities of the organism.

SUPRASPINAL PAIN PATHWAYS

Thalamus

Ventrobasal Thalamus

The vast majority of neospinothalamic and medial lemniscal (spinocervical and postsynaptic dorsal column) fibers terminate in the ventroposterolateral region (VPL) of the ventrobasal thalamus, which is a major somatosensory thalamic relay to the somatosensory cortex.[51] A well-defined localized pain has been reported in humans following focal brain stimulation in this region,[127] and lesions in this area have been used to produce analgesia.[29] Therefore it is not surprising that this thalamic region is believed to play an integral role in nociceptive processes.

Surprisingly, many electrophysiological studies have found few or no VPL neurons with nociceptive response properties.[132,133,242] Rather, VPL cells are typically reported to be activated by innocuous tactile and mechanical (e.g., joint rotation) stimulation and to exhibit a high degree of modality and spatial specificity.[249,250] However, some recent single unit studies have found substantial numbers of VPL cells that respond to noxious stimuli.[120,121,159–161,244]

Willis and co-workers[13,159–161] have found that, by selectively biasing the neuronal population sampled to a region known to receive nociceptive spinothalamic fibers, they could find adequate support that neurons in the ventrobasal thalamus receive nociceptive input and subsequently relay this information to the somatosensory cortex. Initially, they identified spinothalamic tract axons which project to VPL, and found that both wide dynamic range and high-threshold dorsal horn neurons reach this nucleus, particularly the caudal aspect, that is, the nucleus ventralis, the posterior lateralis, and the pars caudalis.[13,73] In a later study

they recorded from single neurons within this defined region and identified a substantial number that were maximally or exclusively discharged by noxious mechanical or thermal stimulation.[159–161] Furthermore, these nociresponsive neurons fired at a rate proportional to the intensity of the noxious stimulus, had small receptive fields that were somatotopically organized, and could be antidromically activated from a nociresponsive region of the SI cortex.[160,162] Similar results have since been reported in rats.[244]

The lack of nociresponsive VPL neurons observed in some of the earlier electrophysiological investigations may partially be a result of their being done on cats. As mentioned earlier, spinothalamic tract neurons in the cat do not project primarily to the ventrobasal thalamus but instead to more medial thalamic nuclei.[35,147]

Guilbaud and co-workers,[121] who have recorded from ventrobasal neurons in both the cat and the rat, found several cells in the rat that responded exclusively to noxious mechanical stimulation, whereas no such cells were observed in the cat. The noxious-sensitive cells in the rat responded to noxious radiant heat and intraperitoneal injection of the pain-producing substance bradykinin, and they appeared to receive input from peripheral Aδ and C fibers. Other studies in the rat have shown that ventrobasal neurons are activated by tooth pulp stimulation,[282] an effect that is antagonized by morphine administration.[280]

Species differences cannot account for all the discrepancies among reports on the percentage of noxious-sensitive cells in the ventrobasal thalamus since not all the early studies were done on cats. In that regard Guilbaud and co-workers[120] have recently shown that some of the disparity stems from the type of anesthesia employed. They found that in rats deep chloralose anesthesia decreased the noxious-evoked responses of ventrobasal thalamic neurons that had been observed when the animals were under moderate volatile anesthesia. Moreover, concomitant with the diminution in response to noxious stimuli, an increase in responsiveness to non-noxious stimuli was observed in the deeply anesthetized animals.

In summary, recent electrophysiological investigations have observed response properties of ventrobasal thalamic neurons consistent with this region's anatomical association with nociception-relevant ascending pathways. The disparity between these reports and those that failed to observe nociresponsive cells in the ventrobasal thalamus is at least partially attributable to differences in neuronal sampling bias, species, and anesthesia.

Posterior Thalamus

The anatomical association of the posterior nuclear complex (PO) with ascending pathways conveying pain impulses, that is, the neospinothalamic and medial lemniscal tracts, and with the somatosensory cortex[76] implicates the PO as a nociceptive relay station. Electrical stimulation of the tooth pulp in rats has been shown to evoke responses in the PO[282] which can be reduced by morphine administration.[280] In humans electrical stimulation in this general brain region has been reported to elicit pain,[134] while lesions that included this area relieved pain.[205]

Both wide dynamic range and high-threshold cells have been identified in the PO, although the actual percentage of the neuronal population that responds to noxious stimuli varies markedly among reports.[40,242 250] PO neurons are typically reported to exhibit little or no modality or spatial specificity and to have large bilateral receptive fields.[77] In contrast, a recent study on rats found that the response properties of nociresponsive PO neurons are very similar to those of ventrobasal thalamic neurons.[121] This latter finding may indicate a difference in response characteristics of PO neurons in the cat and the rat.

Interestingly, Brinkhus and co-workers[40] found neurons in the PO which are capable of transmitting the intensity of noxious thermal stimuli applied to the skin in cats, but noted that the noxious-evoked response was attenuated as compared to that of spinal dorsal horn neurons, suggesting a modulation of neuronal responsiveness in the PO.

Medial Thalamus

The medial thalamus (MT), in particular the intralaminar nuclei centrum medianum and parafasicularis, has been implicated in rostral pain transmission. As mentioned earlier, the MT receives nociceptive afferent input directly from paleospinothalamic second order neurons and indirectly from reticulothalamic fibers that relay input from both the paleospinothalamic and spinoreticular tracts. Injection of horseradish peroxidase into the MT indicates that the densest spinal projection arises from the deeper laminae of the spinal cord (VI–VIII).[321] However, projections of lamina I neurons to the submedius nucleus of MT have also been observed using this method.[75]

In studies of the response of MT neurons to somatosensory input a high percentage of cells responded maximally or exclusively to noxious levels of stimulation and to electrical stimulation of Aδ and C primary afferent fibers.[6,86,224,242] The response characteristics of nociceptive MT

neurons indicate a lack of modality specificity with little somatotopic organization; receptive fields are often large and bilateral. However, one study has found a topographic organization of MT neurons in response to electrical stimulation of the tooth pulp.[238] The generally diffuse nature of MT neuronal responsiveness is not surprising since this thalamic region is often viewed as the rostral extension of the brain stem reticular formation.

Lesion studies further attest to the importance of the MT in nociception. Lesions in the MT have been shown to elevate nociceptive thresholds in animals, as evidenced by a reduction in escape behavior elicited from electrical shock of the feet and tooth pulp.[9,158,195,212,213] Moreover, lesions in the region of the MT have been used to produce pain relief in man.[302]

Unlike the VPL and the PO thalamic regions the MT appears to participate in central analgesic mechanisms. Seemingly incongruous with the aforementioned findings, some studies report that electrical stimulation of the MT produces an antinociception in animals[198,202] and an analgesia in humans,[149] indicating that the MT obtunds nociceptive input. One study reported an antinociception in rats following microinjection of morphine in the MT.[306] Yet another study found microinjection of morphine in the MT to have no effect on nociceptive thresholds in rats.[334] Rather, this study noted differences in the affective response to the noxious stimulus, such as vocalization, defecation, urination, and fear. In that respect the close association of the MT with reticular and limbic structures has prompted some to suggest that the MT is concerned more with the affective aspect of pain than with the sensory aspect.

A plausible explanation of why both lesions and electrical stimulation in the MT can result in an antinociception may be that different MT nuclei were influenced in those studies. Some studies fail to precisely identify the specific MT nuclei affected by their manipulations, partly because the phylogenetic development of the MT makes it difficult to differentiate the nucleus centrum medianum from the nucleus parafasicularis in lower animals such as the rat. Studies in the cat have shown that significant functional differences exist among MT nuclei. For example, one single unit recording study of feline MT neurons found nociresponsive neurons in the nuclei parafasicularis, subparafasicularis, and centralis lateralis, but not in the nucleus centrum medianum.[86] This finding may relate to anatomical evidence that only the former nuclei receive direct spinothalamic projections.[86] There is also evidence that the nucleus centrum medianum is responsible for the pain-modulating

capacity of the MT, since electrical stimulation in this nucleus in cats suppresses nociceptive neurons in the nucleus parafasicularis[107] and in the nucleus reticularis gigantocellularis of the medullary reticular formation.[237]

To summarize, the ventrobasal, posterior, and medial thalami have all been implicated in nociceptive transmission. Probably the best evidence for such a role is the dense projection of nociceptive afferent neurons to these nuclei and the known efferent connections of the thalamus to the somatosensory cortex. Electrophysiological studies have had mixed success in supporting this role. However, it appears that current studies, using stringent controls and recording from discrete, anatomically defined loci, will further clarify the specific functions of these nuclei in relaying information regarding pain. The multimodal nature of this neuronal population, with its convergent input, strongly suggests a processing of nociceptive and non-nociceptive impulses such that the resultant output is integrated with other sensory functions. Clearly, the best evidence that any thalamic nuclei are directly involved in central analgesic mechanisms exists for the MT.

Brain Stem Reticular Formation

The brain stem reticular core still remains one of the most poorly understood regions in the central nervous system (CNS). The reasons for this lack of understanding are almost as diverse as the variety of tasks in which it presumably participates, including arousal, blood pressure, motor activity, respiration, and pain (cf. Refs. 38 and 283 for recent reviews of reticular formation functions). Nonetheless, the dense innervation of this brain stem region by spinoreticular and paleospinothalamic nociceptive fibers and its efferent connections to the medial thalamus indicate that the region is involved in some aspect of pain, be it autonomic, motor, or sensory.

Medullary Reticular Formation

Most spinofugal reticular nociceptive fibers terminate at the medullary level of the reticular formation. The medullary reticular nucleus that has been most extensively studied for its role in nociception is the nucleus reticularis gigantocellularis (NGC). Examination of neurons in the NGC reveals that, although they are excited by noxious and non-noxious stimuli, they typically respond maximally and sometimes exclusively to noxious somatic stimuli.[57,115,214] Excitation of NGC neurons has also been

observed following intraarterial injection of the pain-producing substance bradykinin[117] as well as after Aδ- and C-fiber activation.[59] A characteristic of nociresponsive neurons in the NGC is their large receptive fields, frequently covering the entire body.[57–59,115,214]

Electrical stimulation in the NGC has been shown to elicit escape behavior in cats at stimulus intensities that excite NGC neurons.[58,59] Furthermore, lesions in this site increase the thresholds for escape.[10,128] The association of neuronal activity in the NGC with escape, in conjunction with the known projection of NGC fibers to the motor-related ventral horn of the spinal cord,[16] has led to the suggestion that the NGC is involved in the affective-motivational or motor response to pain.[58] The available evidence does not indicate to what extent, if any, the NGC is involved in transmitting the sensory-discriminative aspects of a noxious stimulus.

In addition to its function in nociceptive transmission the medullary reticular formation has been the focus of several studies on descending pain-modulating mechanisms; these are discussed later in this chapter.

Mesencephalic Reticular Formation

The mesencephalic reticular formation (MRF) also receives input from high-threshold afferent fibers, though to a lesser degree than the medullary reticular formation (cf. Ref. 38). The likelihood that this nociceptive input is relayed rostrally is great because of the anatomical connections of the MRF with the medial, posterior, and ventral thalamic nuclei and the somatosensory cortex (cf. Ref. 38).

Recent studies from Haigler's laboratory[125,126,148] indicate that the MRF may also be important in central analgesic mechanisms. They found that morphine or methionine-enkephalin applied microiontophoretically directly onto MRF cells in the rat inhibits noxious input to those cells.[125,148] Also, microinjection of small quantities of morphine or an enkephalin analogue in the MRF resulted in an elevation in the nociceptive threshold.[126] In agreement with these results is the identification of enkephalinergic terminals[303] and opiate receptor binding sites[14] in the MRF. Collectively, these findings provide some of the best evidence to date that opiate-induced analgesia may result in part from a direct supraspinal inhibition of noxious input. In addition to this direct modulation of nociceptive input, the MRF may contribute to the endogenous attenuation of nociceptive input at the spinal level by descending inhibitory pathways since electrical stimulation in the MRF in the cat has been shown to inhibit noxious-evoked discharges in the dorsal horn.[53]

Somatosensory Cortex

The projection of afferent axons from the thalamus to the somatosensory area of the cerebral cortex is well documented (cf. Ref. 316). The primary somatosensory cortex receives projections predominantly from the ventroposterolateral thalamus, while the secondary somatosensory cortex is innervated by thalamic neurons originating from the ventroposterolateral and the posterior nuclear groups.[51] In addition to the potential nociceptive input supplied to the cortex by the ventroposterolateral and posterior thalami, less dense but detectable projections from the medial thalamus and the brain stem reticular formation exist (cf. Ref. 38). Thalamocortical axon terminals have been shown to form asymmetric synapses with a variety of cell types in layer IV and lower layer III of the neocortex.[245]

The projection of noxious information through these extensive thalamocortical pathways, though often assumed, is not readily demonstrable. Single unit studies have found that neuronal activity in the somatosensory cortex is primarily involved in processing non-noxious information. Most somatosensory cortical neurons respond to innocuous mechanical stimulation and exhibit both modality and spatial specificity.[27,52,253,317] Yet these studies have reported a small number of neurons responsive to noxious stimuli in the posterior aspect of the second somatosensory cortex; a recent investigation in monkeys[162] found noxious-sensitive cells in the primary somatosensory cortex as well. In the latter study both side dynamic range and high-threshold cortical cells were observed; the intensity of neuronal firing varied proportionally to the intensity of the stimulus.

The results of human studies in which the somatosensory cortex was lesioned or electrically stimulated provide little additional support that the cerebral cortex has a critical role in pain transmission. Electrical stimulation in this region is not typically perceived as painful, and cortical ablation in man has proved disappointing in alleviating pain (cf. Refs. 30 and 157).

The best evidence to support the participation of the cerebral cortex in pain transmission is provided by somatosensory-evoked potential studies. Electrical stimulation of the dental pulp has been shown to evoke cortical potentials in numerous species,[11,30,70,281,309,333] including man.[68] The potentials evoked by tooth pulp stimulation were observed in a rather circumscribed area indicative of a topographically arranged projection, although the exact region involved varies among species.

More recent studies have attempted to use cortical evoked potentials as a quantifiable physiological correlate to the subjective report of pain in human subjects exposed to a noxious stimulus. These studies found that noxious thermal, mechanical, or electrical stimuli consistently evoke a long-latency, high-amplitude waveform.[50,67,69,163] Estimates of the conduction velocities of peripheral nerves suggest that these evoked potentials are generated from activity in Aδ afferent fibers.[163] Furthermore, analgesia-producing measures such as morphine or aspirin treatment[46] or acupuncture[67] have been shown to diminish this noxious-evoked cortical waveform. Although it remains difficult to assess the precise relationship between cortical evoked potentials and the perception of pain, these studies do lend support to the involvement of the cerebral cortex in pain transmission.

There is some evidence that the role of the cerebral cortex in nociception is to modulate noxious information and that it may thus participate in central analgesic mechanisms. In that regard cortical stimulation has been shown to inhibit trigeminal neuronal discharge evoked by tooth pulp stimulation or noxious stimulation of the facial skin in cats, although the inhibitory effect was not entirely specific for noxious input.[308] The descending projections of cortical fibers to the thalamus and the brain stem reticular formation are consonant with this descending inhibition.[51]

Both endogenous opioid substances[283] and opiate receptor binding sites[243] have been observed in the cerebral cortex. Furthermore, both morphine and enkephalin have been reported to inhibit the spontaneous and glutamate-evoked activity of cortical neurons.[275,276,335] However, noxious-evoked discharges were not tested in these studies; thus the opioid-induced inhibition may not have been related to the analgesic activity of the compounds. Even so, the somatosensory cortex is unlikely to be either a "pain" or "analgesia" center, but rather (like the thalamus) is more apt to be involved in integrating exposure to a noxious stimulus with other somatosensory input.

DESCENDING MODULATION OF NOCICEPTION

As alluded to throughout this chapter, the modulation of nociception can occur at various levels along the neuraxis. Thus the neuronal activity of nociresponsive dorsal horn neurons may be influenced by other neurons whose cell bodies are as proximal as neighboring spinal cord cells[81] or as distal as those in the cerebral cortex.[74] The most intriguing mod-

ulatory system originates from the medial brain stem. When activated, this system can produce effects so dramatic that it has been considered to be an endogenous analgesic system. Also, there is substantial evidence that the analgesia produced by the narcotic analgesics arises in part from the activation of this descending system.

Periaqueductal Gray Region

Although the complexity of pain makes it unlikely that a single brain locus is uniquely associated with an endogenous analgesic system, the site most commonly implicated as the nodal point is the periaqueductal gray (PAG) region of the mesencephalon (Figure 4). Focal electrical stimulation[202,268] in the PAG has been shown to produce a profound analgesia in a number of species, including man.[150] A sizable mass of evidence indicates that the analgesia produced by PAG stimulation results from the activation of a descending inhibitory system rather than from diminution of nociceptive impulses passing through the PAG. Though nociceptive impulses are known to ascend through the PAG, inactivation of this locus by microinjection of local anesthetics[330] or electrolytic lesions[88,187] fails to alter the thresholds required for a reaction to a noxious stimulus. Moreover, electrical stimulation in the PAG results in blockade of spinal nociceptive reflexes[198,202] and inhibition of the response of lamina V dorsal horn neurons to noxious stimuli.[26,230]

Morphine microinjected into the PAG has also been shown to produce an analgesia in several species.[154,279] Consistent with the analgesic efficacy of morphine when administered in the PAG are the moderate levels of opiate receptors[15] and endogenous opioidlike substances[284] in this region. It has been suggested that the analgesias produced by focal brain stimulation and morphine administration in the PAG utilize a common neural mechanism;[26,200] that is, morphine's analgesic action results from the activation of a PAG-originated endogenous pain-attenuating system. This hypothesis is predicated on the numerous parallels between stimulation-produced and morphine-produced analgesias: (1) both exhibit tolerance as well as cross-tolerance to each other,[197,199] (2) both inhibit specific nociresponsive neurons,[26,55,188,230] (3) both can be at least partially antagonized by naloxone,[5,150] and (4) both are attenuated by destruction of specific anatomical regions.[16,19,221] There is certainly some degree of overlap in the analgesias produced by opiates and focal brain stimulation, however one can also find evidence that these analgesic manipulations recruit unique descending neuronal systems, or that possibly they affect the same system differently.[214] There is also limited evidence

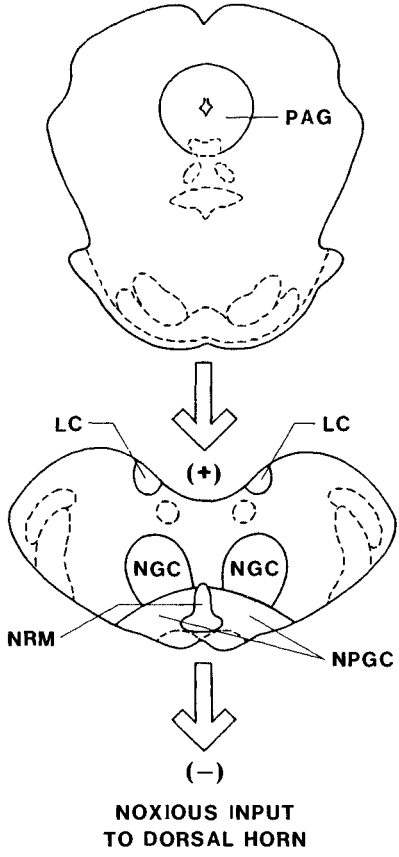

Figure 4 Cross sections of the mesencephalon and medulla indicating key structures of the descending antinociceptive pathway. Periaqueductal gray (PAG) afferents activate spinopetal fibers originating from nucleus raphe magnus (NRM), nucleus reticularis gigantocellularis (NGC), nucleus reticularis paragigantocellularis (NPGC), and locus coeruleus (LC), which results in the inhibition of noxious input in the spinal cord dorsal horn.

that opiate-induced analgesia may not be mediated by activation of a descending inhibitory pathway.[92,178]

Although PAG-induced analgesias appear to be mediated via a spinopetal pathway, there is a paucity of evidence for a direct anatomical projection from the PAG to the spinal cord, suggesting that more caudal brain stem nuclei are involved, for example, as a relay, in this pathway.

The nuclei for which there is sufficient evidence to implicate their participation in this pathway are located in and around the medullary reticular formation, approximately at the level of the facial nucleus (Figure 4). Of these nuclei the most extensively studied is the nucleus raphe magnus, which lies on the midline just dorsal to the pyramids.

Nucleus Raphe Magnus

Efferent projections from the PAG to the nucleus raphe magnus (NRM) have been demonstrated with both autoradiographic[271] and retrograde tracer[108] techniques. Physiological evidence corroborates these anatomical findings; electrical stimulation in the PAG results in synaptic activation of NRM neurons.[98,190,251] Similarly, microinjection of opiates in the PAG has an excitatory effect on NRM units.[22,98,217]

Unlike the PAG a raphespinal projection is well established. Axonal fibers from NRM cells descend the spinal cord via the dorsolateral funiculus and terminate in the medullary trigeminal nucleus caudalis and spinal cord dorsal horn.[16,18,180,297] Activation of these raphespinal neurons by electrical stimulation in the NRM has been shown to inhibit the noxious-evoked activity of trigeminal subnucleus caudalis[191,278] and spinal cord dorsal horn[21,100,112,118,204,270,320] cells in several species. The inhibition of dorsal horn neurons following stimulation in the NRM is conveyed spinopetally via the dorsolateral funiculus, since the effect is antagonized by dorsolateral funiculus lesions.[16,100]

The inhibitory effect of NRM stimulation on dorsal horn neurons is greater on neurons excited by cutaneous C fibers than on those excited by Aδ fibers,[112] and greater on Aδ fibers than on the large-diameter myelinated afferent neurons.[320] However, it remains unclear whether the depressant effect of NRM stimulation is specific for activity evoked by noxious stimuli;[87,100,112] most reports indicate that innocuous input is also suppressed. Electrical stimulation in the NRM has also been reported to produce an antinociception in the cat which is at least as effective as stimulation in the PAG.[231,233,234]

These findings, in conjunction with known nociceptive input into the NRM,[25,82,119] have led to the proposal that the NRM participates in a negative feedback loop that modulates nociceptive transmission.[17] That is, an increase in nociceptive input excites the NRM both directly and indirectly via the PAG, which then activates descending fibers that inhibit the incoming noxious input at the spinal level. This system may be tonically active, since some investigators have found that electrolytic

lesions of the NRM lower nociceptive thresholds below baseline levels.[261,328]

As mentioned earlier, opiate-induced analgesia appears to be mediated, at least to some degree, by engaging this spinopetal system. It remains uncertain whether utilization of this pathway by opiates requires activation at the level of the PAG, or whether it affects the more caudally located NRM directly. Microinjection of opiates in the NRM has been reported to produce an antinociception.[83,84,177,184] However, rather than inhibiting the response of dorsal horn cells to noxious stimuli, microinjection of opiates in the NRM excites nociresponsive spinal cord neurons.[177]

Numerous studies have attempted to identify the particular neurotransmitters that may mediate the descending antinociceptive pathway described above. In regard to the neurotransmitters at the PAG the most efficacious site for stimulation-produced analgesia is the ventrolateral aspect,[111,185] which borders the dorsal paraventricular bundle, dorsal tegmental bundle, and nucleus raphe dorsalis. Thus noradrenergic, dopaminergic, and serotonergic systems have all been implicated. However, those pathways primarily project rostrally, and little is known about the neurochemical nature of the projection from the PAG to the bulbar nuclei relevant to the descending antinociceptive system. Microiontophoretic studies have indicated that the neurotransmitter involved in the interaction between the PAG and NRM is not norepinephrine[23] or substance P.[252]

Serotonin

Most neurochemical investigations have dealt with the subsequent step in the pathway, that is, the medullary-spinopetal pathways. In that regard NRM falls within the boundaries of the serotonin-rich B-3 cell group described by Dahlstrom and Fuxe,[78] and is considered a major source of serotonin in the spinal cord. The importance of spinal serotonergic mechanisms in antinociception is well documented. Both the antinociception[3,4,136] and the inhibition of noxious-evoked activity of dorsal horn neurons[53,262] from electrical stimulation in the PAG or the NRM are antagonized by reducing serotonergic tone with serotonin depletors or receptor-blocking agents. Moreover, electrical stimulation in the NRM accelerates serotonin synthesis in the spinal cord.[37] Also, administration of 5-hydroxytryptophan, a serotonin precursor,[232] may reverse the tolerance to stimulation-produced analgesia in animals made tolerant by iterative NRM stimulation.

Serotonin itself has been shown to produce a dose-dependent antin-

ociception if administered directly onto the spinal cord.[332] Furthermore, microiontophoretic application of serotonin can mimic the effects of stimulation in the NRM on dorsal horn neurons[137,265] and on intraspinal C-fiber terminals.[54,139]

Additional evidence for serotonergic involvement in an endogenous descending antinociceptive pathway stems from studies of the activation of this pathway by opiates. The analgesia produced by microinjection of morphine in the PAG can be antagonized by systemically administered antiserotonergic agents such as methysergide or cinanserin.[326] This antagonism likely involves blockade of serotonergic receptors associated with raphespinal fibers, since it also occurs when the antiserotonergic agent is administered directly onto the spinal cord.[325] The most compelling evidence that serotonin is a neurotransmitter in the descending antinociceptive pathway activated by opiates are the studies from Yaksh's laboratory, which demonstrated that the antinociception produced by microinjection of morphine in the PAG can be antagonized by intraspinal administration of the serotonin antagonist methysergide,[325] and that it evokes the release of serotonin in the spinal cord.[331]

If opiate-induced analgesia results from the activation of a spinopetal pathway that is relayed by medullary raphespinal serotonergic neurons, then destruction of these neurons should attenuate the analgesia produced by systemic opiate administration. Indeed, destruction of the NRM by electrolytic lesions[113,259,261,328] or selective destruction of the serotonergic cells in the NRM with the serotonin neurotoxin 5,7-dihydroxytryptamine[216] significantly attenuates morphine-induced antinociception in the rat. However, these studies have been somewhat disappointing in that the degree of attenuation observed following destruction of the NRM is less than what would be expected on the basis of other results implicating the NRM in descending inhibition of nociceptive input. In fact, it has been suggested that the effects of lesions in the NRM on morphine-induced antinociception result from neuronal inactivation of sites other than the NRM, since the effect is delayed in onset and cannot be reproduced by temporary inactivation of the NRM by microinjection of the local anesthetic tetracaine.[260] However, it is also plausible that the NRM participates in opiate-induced analgesia, but in the absence of an intact NRM opiates can recruit other descending systems that do not require participation of NRM and/or can circumvent all descending pathways and exert an effect directly at the spinal level.

An interesting observation that can be made when one juxtaposes the studies using electrolytic lesions with those using a neurotoxin to specifically destroy serotonergic cells in NRM is that electrolytic[259,261] but not

neurotoxin lesions[216] produce hyperalgesia, that is, a lowering of baseline nociceptive threshold. The hyperalgesia appears to be primary to NRM inactivation, since it does not have a delayed onset and is also observed following microinjection of tetracaine in the NRM.[260] The disparity between neurotoxic lesions and electrolytic/tetracaine inactivation of NRM may result from the destruction of cell bodies other than those containing serotonin by the latter procedures. In that regard conduction velocity measurements of NRM efferent fibers indicate that a large percentage of the cells in the NRM are nonserotonergic.[315] Collectively, these studies argue against the participation of the serotonergic population of neurons in the NRM in the "tonically active" component of the descending inhibitory system.

Additional confusion regarding the extent that serotonin is involved in descending inhibition stems from a recent study which found that neither serotonergic enhancement nor depletion alters the response of dorsal horn cells to noxious stimuli, regardless of whether the spinal cord is intact or cold blocked.[287] Another recent study reported that administration of a serotonergic antagonist on dorsal horn neurons failed to reduce supraspinal descending inhibition.[116] Thus, although serotonin certainly plays some role in the descending antinociceptive system, early descriptions of this system probably overestimated the relative importance of serotonergic NRM spinopetal fibers to the system as a whole.

Possibly the nonserotonergic component of NRM-mediated effects involves raphespinal substance P or enkephalinergic fibers or both, since recent immunohistochemical studies showed that the NRM is a rich source of spinal substance P (SP)[66,145,146] and enkephalin[146] terminals. In this regard enkephalin is well known to inhibit nociresponsive dorsal horn neurons. Although SP excites dorsal horn cells, whether administered systemically or intracranially, SP has been reported to produce an antinociception.[106,193,194,218,288] This unexpected behavior of SP may be a function of whether it is acting predominantly on receptors associated with ascending pain-signaling fibers or on receptors associated with spinopetal inhibitory neurons.[218] A recent report from Lembeck's group[110] supports differences in endogenous SP systems. They found that the SP-neurotoxin capsaicin preferentially releases SP from the spinal cord, leaving supraspinal SP unchanged. SP has also been reported to coexist with serotonin in raphespinal neurons, suggesting that SP may behave as a neuromodulator.[66,145,146] It is worthy of note that nonserotonergic NRM-mediated effects on dorsal horn neurons need not be a direct NRM-spinal cord phenomenon, but may instead result from NRM activation of a neighboring bulbar nucleus with nonserotonergic descending fibers.

It is generally accepted that the descending inhibitory influences of raphespinal neurons are exerted via presynaptic inhibition of primary afferent neurons,[100,139] although there is limited evidence for postsynaptic inhibitory mechanisms.[113] Evidence to support presynaptic mechanisms stems from the comparable effects exerted by NRM stimulation[139] and iontophoretically administered serotonin[54] on intraspinal C-fiber terminals.[53] Also consistent with presynaptic inhibition is that iontophoresed serotonin inhibits noxious-evoked activity, yet enhances spontaneous activity of dorsal horn neurons.[24] An NRM spinopetal pathway involving an enkephalinergic spinal interneuron presynaptically inhibiting primary afferent terminals has been proposed.[17] It was primarily predicated on the overlap between the regional distribution of opiate receptors and enkephalin, and the location of axon terminals from the NRM (particularly in the dorsal horn and trigeminal system), and the ability of naloxone to antagonize the analgesia produced by electrical stimulation in the NRM.[223]

Yet some studies fail to support the existence of a dorsal horn enkephalinergic interneuronal link in this pathway. For example, Carstens and co-workers[55] found that naloxone does not antagonize the inhibitory effect of stimulation in the PAG on the response of dorsal horn neurons to noxious heating of the skin. Additional evidence, albeit indirect, is the inability of naloxone to antagonize the analgesia produced by intrathecally administered serotonin.[332] Naloxone does, however, antagonize the analgesia produced by electrical stimulation in the PAG,[5,150,230] which indicates that an enkephalinergic link must exist somewhere along this pathway.

Medullary Reticular Formation

The studies cited *vide supra* provide evidence for only a partial role for the NRM in the descending inhibitory effects produced by activation of the PAG. Therefore other bulbar nuclei must be involved. Ample evidence now exists that the area of the medullary reticular formation adjacent to the NRM also participates in this descending system. This region of the reticular formation consists of two nuclei, the dorsally located nucleus reticularis gigantocellularis (NGC) and the more ventral nucleus reticularis paragigantocellularis (NPGC)[2,103] (Figure 4). The NPGC corresponds to the nucleus reticularis magnocellularis in the cat.[16,18]

Many studies have failed to make a distinction between these two nuclei and refer to the entire region as the NGC. This has led to some confusion regarding the relative contributions of the dorsal-ventral as-

pects of this area to descending inhibition. To avoid additional confusion this review refers to the area as the NGC/NPGC except where distinctions between the two nuclei have been clearly indicated by the investigators.

There is both anatomical and pharmacological evidence that indicates that the NGC/NPGC participates in the descending inhibitory effects of PAG activation. Projection of PAG efferents to the NGC/NPGC have been described.[108,271] Moreover, activation of these fibers, either by electrical stimulation[214,220] or by microinjection of opiates[214,215] in the PAG, has been shown to alter the spontaneous firing of NGC/NPGC neurons. Although both excitation and inhibition of spontaneous neuronal activity were observed in these studies, an excitatory effect appears to be the more pharmacologically specific, since it was more commonly produced by morphine administered in the PAG, never occurred in animals in which the PAG manipulations failed to produce an antinociception, and was more readily reversed by naloxone.[214] These studies found little evidence to support a difference between the NGC and the NPGC in PAG-mediated antinociception.

Spinal projections from the NGC and the NPGC have been described using tracer transport techniques, and the pathways the two nuclei take appear to differ. NPGC fibers descend the dorsolateral and the ventrolateral funiculi to the dorsal and ventral horns, respectively, whereas fibers from the NGC project exclusively to the ventral horn via the ventrolateral funiculus.[16,18,180,314]

The projection of NGC neurons to only the motor-related ventral horn, in conjunction with the aversive aspects of stimulation in the NGC,[57,59] has led to the suggestion that the NGC is not involved in descending antinociceptive mechanisms, as are the NRM and the NPGC. However, stimulation in the PAG, though certainly analgesia producing, has also been reported to be aversive/noxious.[167,186,225] Furthermore, electrical stimulation of either the NGC or the NPGC is efficacious in suppressing the response of dorsal horn neurons to noxious cutaneous stimuli.[112,123,124,203,204,231] In fact, one study found that stimulation in the NGC and the NPGC and stimulation in the NRM affected nociresponsive dorsal horn neurons similarly, both qualitatively and quantitatively.[124]

Stimulation in the NGC/NPGC also excites some spinothalamic tract dorsal horn neurons, suggesting that this medullary reticular formation locus participates in both positive and negative feedback loops in nociceptive transmission[124] by facilitating and suppressing nociception. Since dorsolateral funiculus tractotomies fail to disrupt the effects of stimulation in the NGC/NPGC, they are probably mediated via the ven-

trolateral funiculus, which indicates that the effects may be conveyed to spinothalamic tract dorsal horn neurons via spinal cord interneurons.[124] As stimulation in the NGC/NPGC also produces primary afferent depolarization, a presynaptic mechanism for these effects has been suggested.[196]

Additional support for the participation of both the NGC and the NPGC in a descending antinociceptive system comes from studies on opiate-induced analgesia. For example, bilateral electrolytic lesions in the NGC prevent the expression of the antinociception produced by the microinjection of morphine in the PAG of rats.[219] Opiates also appear to be capable of activating the NGC and the NPGC directly, since their microinjection into either nucleus produces an antinociception.[2,290,291,292] Ostensibly, this effect is mediated by descending inhibition, since it is accompanied by a suppression of dorsal horn nociresponsive neurons.[290] The fact that the NGC and the NPGC are efficacious loci for opioid-induced antinociception is consistent with the significant concentrations of opiate-binding sites[15] and endogenous opioidlike peptides[95,141] in this region and with the predominant excitatory effect of microiontophoresed morphine on NGC/NPGC neurons.[79,215,273] Morphine also excites neurons in the NGC/NPGC when the drug is administered in the PAG, and morphine has an antinociceptive effect when administered in either the PAG or the NGC/NPGC. These results indicate that systemically administered opiates may activate NGC/NPGC-linked descending pathways directly at the NGC/NPGC in addition to indirectly at the PAG. Opiates do not, however, appear to directly (i.e., supraspinally) inhibit nociceptive input into the NGC/NPGC, as has been shown for the mesencephalic reticular formation,[125,148] since iontophoresed morphine does not alter the response of neurons in the NGC/NPGC to a noxious somatic stimulus.[215]

Norepinephrine

There is good evidence that noradrenergic systems are involved in the descending inhibitory effects mediated through the NGC/NPGC. The antinociception produced by microinjection of opiates in the PAG[324,325] or the NGC/NPGC[173] can be antagonized by blockade of spinal α-adrenoreceptors with intrathecally administered phenoxybenzamine. Moreover, noradrenergic depletion antagonizes the inhibitory effect of stimulation in the NGC/NPGC on nociresponsive dorsal horn neurons, and the antagonism can be overridden by subsequent L-DOPA administration.[291]

Neurochemical studies by Takagi and co-workers[175,293] also implicate

descending noradrenergic systems in this pathway. They found that analgesia-producing microinjections of morphine or [met]enkephalin in the NGC/NPGC markedly increase the concentration of normetanephrine, a norepinephrine metabolite, in the dorsal half of the spinal cord, indicative of accelerated activity in the spinal noradrenergic system.[175] Noxious stimuli also elevate normetanephrine levels in the dorsal spinal cord but not by as much as morphine.[293] These findings suggest that these noradrenergic fibers function in a negative feedback system similar to the one described earlier for the NRM. That is, pain activates descending noradrenergic systems, which attenuate transmission of the incoming nociceptive impulse in the spinal cord. However, the ability of pain to activate this system is considerably smaller than that of narcotic analgesics.

If indeed noradrenergic pathways were integral components in antinociceptive mechanisms, noradrenergic agonists would be expected to produce an analgesia. Although norepinephrine is not an analgesic when administered peripherally, presumably as a result of its inability to traverse the blood-brain barrier, norepinephrine does produce a dose-dependent antinociception when administered directly on the spinal cord.[174,266,267] In addition, norepinephrine, microiontophoresed directly onto dorsal horn neurons, reduces their response to noxious cutaneous stimulation.[24,137,274]

Despite the overwhelming evidence that the regulation of nociceptive input in the spinal cord by activation of the NGC/NPGC involves a noradrenergic system, NGC/NPGC neurons themselves do not appear to be noradrenergic. Noradrenergic cell bodies were not found in the NGC/NGPC in the classic work of Dahlstrom and Fuxe,[78] nor in more recent histofluorescence studies.[236] A dearth of spinopetal noradrenergic cells in the NGC/NPGC is further supported by a report that microinjection of the noradrenergic neurotoxin 6-hydroxydopamine in this locus does not affect norepinephrine levels in the spinal cord.[175] Moreover, electrolytic lesions in the NGC which significantly attenuated opiate-induced antinociception did not affect the content of norepinephrine or serotonin in the spinal cord.[219]

Collectively, these findings suggest that norepinephrine's contribution to NGC/NPGC-mediated antinociception arises from norepinephrine-containing spinopetal neurons to which NGC/NPGC fibers project and recruit on activation of the NGC/NPGC.[289] Thus the NGC/NPGC may inhibit spinal cord neurons receiving noxious input through a direct spinopetal projection and through an indirect pathway involving a relay to a spinopetal noradrenergic nucleus.

It is unclear whether the descending influences of the NGC/NPGC

on dorsal horn neurons require an enkephalinergic interneuron at the primary afferent level. Antinociception produced by electrical stimulation in the NPGC can be blocked by naloxone,[289] but this antagonism can occur anywhere along the neuraxis. The norepinephrine-mediated inhibition produced by activation of the NGC/NPGC probably does not involve such an enkephalinergic link, since α-adrenergic receptor blockers antagonize the antinociception produced by intrathecally administered norepinephrine,[173] although naloxone does not.

Locus Coeruleus

Another nucleus at this level of the medulla that may be involved in the descending inhibition of nociception is the noradrenergic A6 cell group, the locus coeruleus (Figure 4). Neurons in the locus coeruleus (LC) are readily affected by noxious somatic stimuli.[169] Moreover, the diffuse projections of LC fibers include substantial innervation of both the ventral and dorsal aspects of the spinal cord.[131,227]

The role of the LC in nociceptive modulation is somewhat confusing, since the evidence suggests that the LC can obtund and exacerbate noxious stimuli. Electrical stimulation in the LC has been shown to produce an antinociception[277] and to inhibit the response of dorsal horn neurons to noxious cutaneous stimuli.[140] However, analgesia-producing agents such as morphine[1,31,169] and noradrenergic agonists[1,60] profoundly inhibit neurons in the LC, suggesting that an inhibition of neuronal activity in the LC accompanies analgesia.

Lesion studies have been the source of much of the confusion surrounding the LC's involvement in pain and analgesia. Destruction of the LC with electrolytic[33] or radiofrequency[272] lesions has been reported to elevate nociceptive thresholds,[33] whereas nociceptive thresholds are not affected when monosodium-L-glutamate (MSG) lesions are used to destroy the LC.[130] Since presumably only MSG lesions spare axons of passage, these results suggest that the effect of electrolytic and radiofrequency lesions on nociceptive thresholds is not primarily due to the elimination of LC cell bodies. Electrolytic, radiofrequency, and MSG lesions of LC have all been shown to attenuate opiate-induced antinociception,[130,170,272] an interesting result in light of the dense concentration of opiate-binding sites[14] and endogenous opiatelike substances[285,303] found in the LC.

From a speculative standpoint a disinhibition of LC to produce attenuation of nociceptive transmission is intriguing, since recent studies indicate that the NRM is under a tonic inhibition by noradrenergic neurons,[129] and an LC to NRM projection has been described.[72] Thus an

inhibition of LC neurons, for example, by opiates, may result in a disinhibition of NRM cells which could serve to activate descending inhibitory systems originating from the NRM. It is also possible that the LC is involved in the noradrenergic relay described earlier for NGC/NPGC-mediated effects. Whatever the case, it is likely that the LC has a multifunctional role in nociceptive/antinociceptive processes.

To summarize, spinal cord dorsal horn neurons, which transmit noxious information from the periphery to the brain, undergo modulation by descending inhibitory pathways. The major pathway described thus far is the spinal cord via the medullary nuclei NGC, NPGC, and NRM. It appears that this system may be activated at the medullary level as well. There is also evidence for a descending pathway that stems from the locus coeruleus, although it is less well understood. These descending pathways are considered to be an endogenous analgesic system, since they can be activated by incoming noxious impulses.

The neurotransmitters that mediate this inhibition of nociception have been partly elucidated. The descending fibers from the NRM are in part serotonergic, whereas those from the locus coeruleus are noradrenergic. Noradrenergic systems are also involved in descending influences from the NGC and the NPGC. However, this latter pathway requires a relay to a spinopetal noradrenergic nucleus. Other neurotransmitters surely participate in this system, but they remain to be identified. Also, many of the details of this descending system, such as the location of enkephalinergic interneurons and whether the inhibition of dorsal horn neurons is presynaptic or postsynaptic, are yet to be clearly understood. It is clear, however, that the activation of these pathways by electrical stimulation and opiates results in a profound analgesia. Furthermore, it appears that this aspect of opiate action contributes significantly to its overall analgesic effect.

In conclusion. the dramatic progress that has been made in unveiling the mysteries associated with pain has helped us to better understand analgesic mechanisms. More importantly, however, these discoveries have provided new, more intricate questions regarding these systems which will spur more pertinent research. Although it will be some time before the precise mechanism(s) by which central analgesics exert their effects is (are) elucidated, we now have new targets at which to direct the agents currently in development, with the hope that they will adequately relieve pain, with fewer adverse effects, in a greater percentage of the population of patients suffering from pain.

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Pharmacological Alteration of Pain: The Discovery and Evaluation of Analgetics in Animals

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SITES AT WHICH, AND MECHANISMS WHEREBY, PAIN MAY BE ALTERED

From the preceding chapter on the physiology of pain it is apparent that there are several sites, within the CNS as well as the periphery, that may be sensitive to analgesic drugs. Some of these sites have been more clearly implicated in the mechanisms of action of known analgesics, whereas others, though not known to be altered by presently available analgesic agents, may prove to be sensitive to analgesics of as yet unknown mechanisms and analgesics that are yet to be discovered. To pursue the latter it is useful to have a knowledge of the plausible sites of action of analgesic agents.

In the relief of pain the site of origin of the painful sensation is an obvious site of action for analgesia. Clearly the avoidance of painful injury is ideal for the survival of the organism, and pain itself may be viewed in an operant sense as the conditioning stimulus to train the organism not to injure itself. However, once injury is inevitable or has occurred, pharmacological intervention targeted at the site of injury can be very effective. Such treatment, as it is now understood, is largely anti-inflammatory in nature. The steroidal and nonsteroidal anti-inflammatory agents have their primary pain relieving actions at this site. The steroid glucocorticoid anti-inflammatory drugs interfere with the inflammatory processes and coincident pain by blocking the release of the mediators of inflammation.[1] The nonsteroidal anti-inflammatory agents, such as aspirin and ibuprofen, interfere with the synthesis of prostaglandins and thromboxanes by blocking the cyclo-oxygenase step in their synthesis.[2] The cyclo-oxygenase products, particularly the E prostaglandins, potentiate the pain induced by the algesic mediator, bradykinin.[3,4] Thus aspirin and mechanistically related compounds exert anti-inflammatory and analgesic effects by virtue of a decrease in the prostaglandin enhancement of bradykinin. This hyperalgesic effect of prostaglandins may be mediated by cyclic adenosine monophosphate and elevations in intracellular calcium.[5] On this basis compounds that interfere with cAMP metabolism or Ca^{2+} permeability may be expected

to have local anti-inflammatory or analgesic effects. Bradykinin, in turn, may function as a sensory mediator at the nerve endings of pain fibers.[6] This suggests that effective analgesia could also be induced with bradykinin antagonists. The discovery of such compounds is necessary to test this hypothesis.

Pain may also, of course, be blocked between the sensory nerve ending and the CNS by local nerve blocks. The local anesthetic agents are widely used for reversible activity of this sort. Sensory nerves involved in pain impulse conduction are most sensitive to these agents due to their small diameter and/or unmyelinated nature. Extensive development of local anesthetic agents has continued since the discovery of procaine as a synthetic substitute for the local anesthetic cocaine.[7]

After conduction of the pain impulse from the periphery to the spinal cord or brain stem, the sensory nerve forms synapses with other nerve cells. Neurotransmission is required at this synapse to convey the impulse from the primary afferent pain fiber to the cells in the CNS. The nature and identity of the neurotransmitter at this site have been a matter of controversy. However, there is accumulating evidence that the undecapeptide Substance P may function in this capacity. This peptide is present in the terminal area of some primary afferent neurons,[8] and it disappears on destruction of these neurons.[9] In support of the neurotransmitter function of Substance P, it has been localized in synaptic vesicles,[10] found to be released from the spinal cord by electrical stimulation,[11] and found to excite spinal neurons.[12]

Interference with the proposed primary afferent neurotransmitter function of Substance P would be expected to yield analgesic effects. Such interference could be by way of depleting Substance P, blocking its release, or blocking the receptor at which it exerts its excitatory effects. There is now evidence that a depletor of Substance P has analgesic properties. Capsaicin, the pungent principle of red pepper, when injected systemically or directly into the fluid bathing the spinal cord, causes a long-lasting depletion of Substance P[13] and analgesia.[14,15] Since capsaicin is, however, a very toxic compound with a low therapeutic index (median lethal dose/analgesic median effective dose), it seems unsuitable for therapeutic use. When given to neonatal rats, capsaicin causes not only a long-lasting depletion of Substance P, but also ultrastructural evidence of damage to sensory nerves.[16] It is not clear that such damage is necessary for the analgesic effects in adults. Other less toxic Substance P depletors may be expected to be unique and useful analgesic agents. The search for such compounds is technically feasible

and may yield an important therapeutic advance, particularly in the treatment of chronic, severe pain.

Inhibition of Substance P release may be an important mechanism of action of existing analgesic agents. In addition to other supraspinal analgesic mechanisms, opioids (both endorphins and synthetic compounds) have been shown to inhibit the release of Substance P from sensory neurons.[17,18] This effect probably accounts for the inhibition of the dorsal root potential by opioids *in vitro*[19] and the analgesic effects of opioids applied directly to the spinal cord in animals[20] and man.[21] The relative contribution of this spinal mechanism to the analgesic effects of systemically administered opioids is controversial. However, it appears to be subordinate to the supraspinal mechanisms as determined by lesion studies[22] and local injections of narcotic antagonists[23] in conjunction with systemic morphine. This being the case, compounds with more potent and selective effects on the release of Substance P at the spinal level may prove to have advantages over the presently available opioid analgesics.

A third, though more speculative, analgesic mechanism involving spinal Substance P is the blockade of Substance P receptors. The test of this hypothetical mechanism awaits the development of an effective Substance P receptor blocker. However, evidence that depletion of Substance P and inhibition of its release result in analgesia provides sufficient evidence to encourage the search for Substance P receptor blockers as analgesic agents.

In addition to spinal analgesia Substance P blockers may also exert local anti-inflammatory effects. Substance P is released from sensory neurons in the periphery[24] and may be involved in neurogenic inflammation or the so-called *axon reflex*.[25] In this regard capsaicin has been shown to deplete Substance P and block neurogenic plasma extravasation.[26] Whether this mechanism will prove therapeutically useful awaits the development of effective Substance P blockers or less toxic Substance P depletors. One has to wonder, however, if the traditional use of capsaicin-containing liniments[27] would have involved this mechanism.

Although this discussion of analgesic mechanisms involving primary afferent neurotransmission has focused on Substance P, other putative neurotransmitters and modulators are also present in these neurons.[28] A better understanding of the function of these mediators may lead to the discovery of additional potential analgesic mechanisms. Some of these compounds, namely neurotensin[29] and cholecystokinin octapeptide,[30] do display analgesic properties, but not specifically at the spinal

cord level. Interpretation of the functional importance of these peptides is difficult based solely on their analgesic effects when one considers that Substance P, which in all certainty is involved in the pain pathway, has been shown to be analgesic[31-33] or hyperalgesic.[34-36]

After transmission to the cells in the spinal cord, the processing of pain impulses, their perception as painful information, and the organism's response to the perceived pain are highly complex and incompletely understood neurophysiological events. However, we do know that these portions of the pain pathway are amenable to pharmacological manipulation. Indeed, much of our knowledge of the central processing of pain inputs is derived from studies utilizing drugs as pharmacological tools. For example, the analgesic effects of opioids in the brain stem, particularly the periaqueductal gray area and nucleus reticularis paragigantocellularis, are mediated in part by descending neural pathways to the dorsal horn of the spinal cord. These pathways are in part serotonin[37] and in part norepinephrine[38] mediated. Thus serotonergic and/or noradrenergic agonist or potentiating compounds might be expected to have analgesic properties. Both serotonin[39] and norepinephrine,[40] when injected into the spinal column, exert analgesic effects, as do synthetic alpha-adrenergic agonists such as clonidine.[41] The latter compound and its congeners could have practical utility as analgesics because they also exert this action on parenteral administration.[42] This utility is hampered primarily by their profound hypotensive effects. The potentiation of serotonergic and noradrenergic effects at this spinal site by tricyclic antidepressants and the nonopioid analgesic nefopam[43] may be causally related in part to their analgesic properties.[44,45] Similarly, alterations in serotonin concentration caused by precursor loading or precursor restriction cause analgesia[46] and hyperalgesia, respectively.[47]

Analgesia mediated by a drug acting directly on higher centers has already been alluded to (i.e., the opioids' analgesic sites of action in the periaqueductal gray area and nucleus reticularis paragigantocellularis). Interestingly, the effects of morphine at these supraspinal areas may be antagonized by Ca^{2+}, related divalent cations, and Ca^{2+} ionophores, and enhanced by chelating agents.[48] Thus, as in the periphery, compounds that interfere with Ca^{2+} mechanisms would be expected to have analgesic effects. Of course, direct alteration of Ca^{2+} distribution in neural tissue would result in innumerable other effects as well.

In addition to the opioids, which act at endorphin receptor sites, other endogenous mediators may exert fairly selective analgesic effects when administered into the brain. These effects may in some cases be mimicked, though less selectively, by parenteral administration of stable

analogues. For example, intracerebroventricular injection of acetylcholine results in analgesia in mice.[49] This effect is dependent on muscarinic receptors in the brain as it is blocked by atropine. Cholinergic agonists that penetrate the blood-brain barrier exert similar analgesia when given parenterally.[50] Guanosine 3,5′-monophosphate[51] and its dibutyryl derivative[52] also exert analgesic effects of a selective nature when injected directly into the brain. The injection of many other neuroactive substances directly into the brain may also alter an animal's motor responses to painful stimuli, however these effects must always be carefully assessed as to whether they are sufficiently selective to be considered analgesic or merely indicative of a more general behavioral disruption. Depending on their site of administration, even analgesics such as morphine may be shown to elicit either hyperalgesia[53] or motor inhibitory effects,[54] the latter of which might be confused with antinociception.

TESTING FOR ANALGESIC ACTIVITY IN ANIMALS

Testing for analgesia in animals involves the presentation of a painful stimulus and an appropriate measurement of the animal's response to it. Obviously, the design and interpretation of such assays must take into account the ability of nonanalgesic drug effects to disrupt the measured response; that is, severe behavioral disruption or drug-induced motor deficits should not be interpreted as analgesia. Table 1 lists several types of stimuli that have been used in analgesic tests.

The type of response measured depends on the type of stimulus presented and on the behavioral repertoire of the species tested. Most of the endpoints measured, however, fall into one of the categories listed in Table 2.

Table 3 briefly describes a number of analgetic assays insofar as the stimulus used, the response measured, and the sensitivity of the assay to various classes of analgesics. Some of the more commonly used clas-

Table 1 Stimuli Used in Analgesic Testing

Thermal	Hot plate, focused light
Mechanical pressure	Pinch, mesenteric stretching
Chemical or physical irritation	HCl, EDTA, phenylquinone
Electrical	Tail, foot shock, tooth pulp
Specific pain mediator	Bradykinin
Ultrasonic	Tail, digital stimulation

Table 2 Types of Responses Measured in Analgesic Assays

Spinal reflex	Tail flick, flinch
Complex unconditioned motor	Paw lick, jump, writhe
Vocalization	Squeak, screech
Autonomic	Blood pressure, pupillary diameter
Emotional	Vocalization after discharge
Operant	Lever press, avoidance

sical assays are the tail flick,[55] hot plate[56] or writhing[57] assays, and their more recent modifications, the tail immersion,[58] hot plate–warm plate,[59] air-induced writhing,[60] and EDTA-induced vocalization[61] tests. In the tail flick assay a high-intensity light is focused on the animal's tail and the time required for the spinal reflex flick of the tail out of the light path is measured by a photoelectric relay system. In response to analgesic drugs the response is delayed or even completely blocked. The hot plate assay also utilizes a thermal stimulus, but one that is applied to the feet. Rats or mice placed on a high specific heat surface such as a copper plate heated to a carefully regulated temperature in excess of 45°C will respond by licking their paws. The higher the plate temperature, the shorter is the latency required for the animals to respond. Again, in this assay lengthening of the latency to respond is the index of analgetic activity. The writhing assays utilize the intraperitoneal administration of a chemical or physical irritant. Mice or rats so treated display a stereotyped abdominal stretching, or in the case of rats also back arching or lateral torsion. These behaviors are readily observed and quantified. A compound that decreases their frequency may be acting as an analgetic. There are other less commonly used assays. Among these are assays using bradykinin as an analgesic stimulus. When administered intraperitoneally to dogs,[62] or intraarterially in rodents,[63] bradykinin causes reproducible behavioral syndromes that are blocked by various analgesic agents. This stimulus has the advantage that it is likely to be similar to pathological pain mediated by bradykinin. Another stimulus that differs subjectively from the classical stimuli when tested on human subjects is ultrasonic stimulation.[64] Ultrasound generates a pain of a different character than that arising from thermal, mechanical, or electrical shock stimulation.[65] Whether this stimulus more closely resembles pathological pain is unclear; however, when applied to the tails of rodents, it causes a reliable response that is sensitive to known analgesics.[66]

The problem of developing analgesic assays that perfectly mimic hu-

Table 3 Summary of Various Analgesic Assays

Assay	Stimulus	Response	Drug Sensitivity					
			μ[a] Agonists	μ Agonist-Antagonist	Non-μ-opioids	α Agonists	Amine Potentiators	Anti-inflammatories
Rat tail flick	Thermal	Spinal reflex	+	−	−	−	−	−
Mouse tail flick	Thermal	Spinal reflex	+	+	+	+	−	−
Rat tail immersion	Thermal	Spinal reflex	+	+	+	+	−	−
Rat hot plate	Thermal	Complex motor	+	−	+	+	+	−
Rat warm plate	Thermal	Complex motor	+	+	+	+	+	−
Mouse writhing	Chemical irritation	Complex motor	+	+	+	+	+	+
Rat air writhing	Physical irritation	Complex motor	+	+	+	+	+	+
Guinea pig EDTA test	Chemical irritation	Vocalization	+	+				−
Mouse tail clamp	Mechanical pressure	Complex motor	+	+	+	+	−	−
Rat inflammed paw	Mechanical pressure	Complex motor	+	+	+	+		+
Rabbit tooth pulp	Electrical	Complex motor	+	+	+	+	+	−
Monkey shock titration	Electrical	Lever press	+	+	+		+	−
Rat shock titration	Electrical	Vocalization	+	+	−	+	−	−
Dog bradykinin	Pain mediator	Vocalization and complex motor	+	+			+	+
Guinea pig bradykinin	Pain mediator	Vocalization	+	+				+
Mouse ultrasonic	Ultrasonic	Complex motor	+	+				+

[a] Here μ refers to morphinelike opioids.

man clinical pain does not seem to be solvable. Thus of the animal assays in Table 3 none are perfect predictors of clinical efficacy. However, by the use of the appropriate combination of assays involving different stimuli, responses, and species, one may gain considerable confidence in the likelihood of a compound displaying analgesic efficacy in man. For example, for the initial screening of opioids the mouse tail flick procedure is useful because of its sensitivity to a wide range of opioids. For a wider range of sensitivity to nonopioids at the cost of a corresponding loss of selectivity for analgesics, the mouse-writhing procedures may be relied on as preliminary screens for analgesic activity. Actives from these tests may then be confirmed in other more laborious and costly assays with the purpose of determining species specificity, stimulus, and response specificity and generating some estimate of the degree of analgesic efficacy. For the latter purpose measuring response latencies in the hot plate test, or maximally tolerated intensities of electrical stimulation in the tail shock and tooth pulp stimulation procedures, is particularly useful. Stronger analgesics elevate these thresholds to higher levels than do weak or moderately strong analgesics.

The problem of separating drug-induced motor deficits from analgesia interferes more or less with the interpretation of most of these tests. Commonly, the selectivity of the analgesic effect is determined separately, by measuring the animal's motor performance on other tests such as the rotorod[67] or inclined screen test.[68] As an alternative, analgesia could be measured in such a way that the determination of the presence or absence of analgesic activity is independent of motor performance. One such technique involves the use of an analgesic drug as a descriminative stimulus in rats with chronic arthritis.[69] In this paradigm the rats are trained to press one of two levers subsequent to treatment with an effective analgesic and to press the other lever if treated with saline. After training, the rats are tested with drugs of unknown activity. They may respond in one of three ways: at the saline lever, at the active drug lever, or not at all. Responses at the active drug lever indicate that the drug is subjectively similar to the active training drug. One must assume that this similarity is caused by the common ability of the drugs to alter the animal's internal environment by altering the perception of pain from the arthritic joints. Regardless of this, motor deficits do not result in false-positive responses in this test; animals so affected do not respond at all.

Although the aforementioned animal analgesic tests are generally reasonably simple to conduct and evaluate, other *in vitro* methods may be used to rapidly prescreen compounds and to define their mechanisms

of action. These tests may be based on the biochemical actions of the drugs on *in vitro* systems or, more commonly, on the displacement of therapeutically effective analgesics from membrane binding sites. The latter type of studies, referred to as *receptor binding assays*, are useful additions to analgesic testing methodology.

RECEPTOR BINDING ASSAYS

Early studies[70] of *in vitro* ^{3}H-ligand receptor binding to brain receptors utilized ^{3}H-levorphanol, a potent narcotic agonist. The principle of these, and later more successful,[71] studies was the observation that a significant percentage of drug exposed to brain membrane preparations is loosely bound to the membrane in a stereospecific and saturatable fashion. The rank ordering of the affinities of opioid analgesics for these binding sites, that is, their ability to displace the ^{3}H-labeled opioid ligand, agree well with their relative analgesic potency. Thus the membrane binding sites displayed important characteristics of opioid receptors, and these assays became widely used to predict opioid receptor activity. Because both agonists and antagonists bind at the same receptor, simple displacement of one or the other cannot be assumed to indicate specifically either agonist or antagonist activity. However, differentiation of agonist and antagonist binding can be affected utilizing one of two methods. Sodium ion was found[72] to decrease agonist and increase antagonist binding affinity. Similarly, the nucleotide guanosine triphosphate enhances the dissociation of agonists, but not antagonists, from membrane binding sites.[73] Thus by determining the degree of sodium or GTP effect on binding affinity, the agonist : antagonist ratio of the opioid may be estimated.

Opioid binding assays utilizing ^{3}H-agonists,[74] ^{3}H-antagonists,[72] and ^{3}H-opioid peptides[75,76] have all been utilized. However, with the use of these different ligands comes the possibility that different subpopulations of opioid receptors may be preferentially labeled. Indeed, pharmacological studies[77] have suggested the existence of functionally distinct subpopulations of opioid receptors, mu(μ), kappa (κ), and sigma (σ) all blocked by the antagonist, naloxone, but differing in regard to agonist specificity. Thus the use of ^{3}H-naloxone as a ligand could result in the labeling of all subclasses of opioid receptors. Greater specificity of receptor labeling might be gained by the use of selective agonists. This may be successful in the case of μ (morphine) receptors, but the available ligands for the other putative receptors (κ and σ) may not be

sufficiently selective to specifically label their respective subpopulations.[78,79] Yet another subpopulation of opioid receptor may be more specifically labeled with the enkephalin analog ^{3}H-D-Ala^{2}-DLeu^{5} enkephalin.[80] This latter subclass, termed *delta (δ) receptors*,[81] has been pharmacologically characterized in peripheral tissues, but again, as with the κ and σ receptors, a clear understanding of its functional significance in the brain awaits further study. In this regard the development of highly selective agonists of these various receptors is critical. Regardless, it is clear that the μ receptors are of primary importance to opioid analgesia and abuse potential. Thus receptor binding assays utilizing this type of ligand have considerable predictive utility.

Since the pioneering work on opioid receptor binding, similar methods have been used to study the receptors for many other drugs and neurotransmitters. Because some of these other receptors may also be involved in nonopioid analgesic mechanisms, such binding assays may be useful in analgesic drug discovery and evaluation. For example, the α-adrenergic agonist clonidine, which has analgesic properties, can be used as a ligand for receptor binding studies.[82] Compounds thus identified as clonidine-like might well have similar analgesic properties. The search for Substance P antagonist analgesics would be greatly enhanced by the development of a Substance P receptor binding assay. This would allow rapid screening of compounds for this activity. Such assays have been reported[83,84] but have not been widely evaluated. However, a recent report[85] describes a Substance P binding assay that apparently circumvents a number of problems associated with the earlier assays.

METHODS FOR PREDICTING ABUSE LIABILITY

Despite the multiple sites of action of potential analgesic agents, most, if not all, of the presently available central analgesic drugs exert their effects through opioid receptors. This is a reflection of a past emphasis on the study of opioid analgesics and, in light of the imperfection of these agents, the basis of continued research for better opioid analgesics. The use of classical opioids of the morphine type is associated with euphorogenic activity, physical dependence, and tolerance. All these properties contribute more or less to the abuse liability of the opiates. Thus it is important to quantitate these activities in the process of selecting new opioid analgesic drugs. New agents exerting analgesia through other mechanisms should also be tested for physical dependence, self-administration, and tolerance development to verify that their

mechanistic differences are associated with significant advantages over opiates.

Psychic dependence related to euphorogenic activity is probably the most important determinant of abuse potential. This leads to compulsive drug-seeking behavior with the concomitant neglect of other activities important to the survival of the individual and the maintenance of his place in society. In animals self-administration of drugs serves as an indicator of such euphorogenic activity and drug-seeking behavior. Early experiments[86] demonstrated that rats equipped with intravenous catheters will lever press to receive small doses of morphine. With additional refinements in the drug delivery hardware and the paradigm for controlling the doses of drug and the fixed ratio of responses to injections,[87] this technique of intravenous self-administration in the rat has proven to be quite useful in predicting abuse potential in man. Because of the importance of rapid reinforcement (the so-called rush) to the abuse potential of opiates, the use of such an intravenous model of self-administration is particularly important in the study of parenterally administerable analgesics. For compounds that are formulated only for oral administration models of abuse potential based on this route may also be appropriate. One such model is based on lever press–activated intragastric injection[88] in analogy to the intravenous methods. Rats may also demonstrate their preference for opiates by selectively working (lever pressing) to receive morphine-containing food pellets rather than nondrugged food.[89] However, such experiments add another variable, namely the aversive taste of the drug to confound the interpretation of the experiment. Even in the case of morphine, the rats must overcome an apparent reluctance to consume adulterated food to express their preference for the drug. Thus for drugs that can be administered via solutions the intragastric and intravenous routes are preferable.

Not surprisingly, different species have somewhat different preferences of agents for self-administration. The aforementioned rat models may be viewed as overly sensitive, and thus capable of generating false positive results considering the willingness of this species to self-administer apomorphine[90] and to maintain high rates of pentazocine self-administration.[87] On the other hand, rats avidly lever press for phencyclidine injections,[91] a preference also demonstrated by sociopathic humans, but difficult to predict from the effects of the drug in "normals."[92] Regardless of the interpretations that may be placed on this observation, it would seem wise to use more that one species to predict abuse potential in humans. Intuitively, subhuman primates appear to be an excellent choice due to their greater similarity to man. Indeed, rhesus

monkeys were shown to intravenously self-administer morphine[93] in experiments similar to those earlier done in rats, and now a large number of compounds have been studied in monkeys for their ability to support self-administration.[94] These data support the validity of this model to predict human abuse of analgesics and psychotropic agents. Because of the economic as well as moral expense of using large numbers of monkeys in the laboratory, streamlined methods have been developed and demonstrated to be useful[95] for rapidly determining whether a compound has the ability to maintain self-administration in animals already trained on a standard. In analogy to the rat self-administration paradigms intragastric self-administration may also be used in monkeys[96] to test compounds intended only for oral use.

Just as it is desirable to attempt to predict the ability of drugs to support drug-seeking behavior in man from self-administration studies in animals, it is also useful to investigate the basis of this effect, namely the subjective properties of the drug in animals, to predict what types of subjective effects it will exert in man. Surprisingly, despite the obvious impossibility of directly querying animals as to how a drug makes them feel, animal methods are available to determine whether an experimental compound is subjectively similar to known standard agents.[97] These behavioral methods, referred to as *discriminative stimulus properties generalization,* involve training animals to respond for reward in a distinctive fashion (e.g., at one of two levers) when under the influence of the standard drug or saline injection. When retested with an unknown, the animals may indicate by the lever they choose whether the drug is more similar to the training drug or to saline. By carefully selecting the training drug dose, one may apparently cause the animals to cue more or less specifically on the basis of the pertinent subjective effects of the drugs. Such discriminative stimulus generalization studies have proven capable of identifying morphine-like opiates in both rats[98] and monkeys,[99] as well as pigeons.[100] Thus they may be used to predict euphorogenic activity. These methods are capable of discriminating the stimulus properties of opioid agonists and partial agonists (agonist-antagonists).[101] The latter compounds have detectable stimulus properties of their own which may be related to their dysphoric and/or psychotomimetic properties in humans. Thus generalization to the discriminative stimulus properties of cyclazocine[102,103] and pentazocine or nalorphine[104] in animals may be of use in predicting the likelihood that new compounds will cause similar dysphoric effects in man.

Ideally, an analgesic should have neither euphoric nor dysphoric properties. In fact, it would be preferable for the compound to exert no

detectable subjective effects in normals, and in pain patients only to remove the subjective symptoms of pain. Such an ideal is a more realistic goal for a peripherally acting analgesic than for one with a CNS site of action. Nevertheless, a highly desirable goal is an analgesic combining the high efficacy of centrally acting agents and minimal subjective effects. To develop such a compound, an animal assay capable of identifying the presence or absence of subjective effects, regardless of their quality, would be useful. Food aversion learning[105] shows some potential in this regard. These procedures take advantage of rodents' conditioned avoidance of distinctively flavored food, the intake of which has been temporally associated with a detectable internal stimulus (subjective effect). With the use of such procedures one could select compounds with relatively few detectable subjective effects at analgesic doses.

The abuse potential of opioids involves not only their subjective effects and consequent self-administration, but also their ability to induce physical dependence. The dysphoric effects of withdrawal in the physically dependent individual are a strong stimulus to maintain compulsive drug-seeking behavior and self-administration. These facts were recognized early in the modern search for opiate analgesics of lowered abuse potential and led to the development of a number of animal models of opioid physical dependence. These assays rely on the detection of the signs and symptoms of abstinence or withdrawal in animals treated chronically with the test compound. In rats, for example, early investigators[106] noted an increased irritability on morphine withdrawal. Weight loss on abstinence is a more objective measurement,[107] although it is mediated largely by gastrointestinal mechanisms. Wei and co-workers[108] have emphasized the importance of using several endpoints, including weight loss, diarrhea, ear blanching, and so-called wet dog shakes, to quantitate the full range of opiate withdrawal signs. This allows the investigator to not only quantify withdrawal, but also to compare it qualitatively to that induced by morphine abstinence.

Other species have also been extensively used for predicting the physical dependence–inducing effects of opioids. Rhesus monkeys were utilized in the pioneering studies at the University of Michigan,[109] and subsequently have been extensively used in a government supported effort to find less addictive analgesics.[110] Such testing in monkeys is not generally used as a drug discovery or selection tool, but more often to support observations made in rodents. The rapid screening of compounds for physical dependence liability may now be done with mice. After chronic exposure to morphine by means of morphine pellet implantation[111] or multiple injections,[112] mice display an abstinence syn-

drome characterized by a stereotyped jumping behavior. This single endpoint, induced by naloxone treatment, provides the basis for a rapid screen for opioid physical dependence in mice treated chronically with the test compound. It is also apparent on cessation of opioid treatment, but its appearance is more temporally variable because of differences in drug half-life. In addition to jumping, abstinent mice also display weight loss, hypothermia, and increases in motor activities.[113] Although these other endpoints are less robust, it may be argued that, as in the rat, measuring the full range of withdrawal signs allows one to make qualitative comparisons between the dependence-inducing properties of different drugs. The utility of such qualitative comparisons in animals as the foundation of useful predictions of the nature of human physical dependence is not however, clear.

Physical dependence is generally associated with repeated exposures to opiates; however, there is evidence that a mild physical dependence can occur after a single dose of morphine in man.[114] Likewise, in mice a single dose of morphine followed by naloxone results in withdrawal-type jumping behavior.[115] The onset and peak of this effect are delayed as compared to the analgesic and motor stimulant effects of morphine. This suggests that it is not an acute effect of morphine, but indeed a manifestation of physical dependence. Since a number of other opioids have been shown to be active in this single dose physical dependence test,[116] it appears to have predictive utility. Although the single dose technique is less sensitive than the multiple dose methods, it has special utility in studies of the mechanisms of physical dependence and its possible suppression.

By necessity, the aforementioned tests of physical dependence rely on the observations of withdrawal signs, that is, outwardly observable changes in behavior. In humans, however, it is the unpleasant subjective effects or symptoms of withdrawal which are of the greatest consequence. Although we tacitly assume that the signs and symptoms of opiate withdrawal go hand in hand, this need not be the case, particularly for atypical opioids or other analgesics differing in mechanism from morphine. Thus to ultimately answer the question of whether a drug causes physical dependence, it would be much more useful to be able to measure the general subjective adversiveness of withdrawal in animals than merely to monitor the physical signs of a particular type of abstinence. Behavioral methodology again offers some promise to ward this end. The subjective adversiveness of morphine physical dependence has been measured in rats by the saccharin taste adversion method,[117] in which the taste of saccharin is paried with cessation of morphine treatment. The aversion is measured by the subsequent avoid-

ance of saccharin. The general utility of such techniques remains to be established for measuring physical dependence to other opioids and drugs of abuse.

The development of opiate tolerance *per se* contributes in only a minor way to the overall problem of opiate abuse. It is, however, a major problem in the maintenance of effective analgesia in chronic, severe pain. Here the relatively rapid and profound tolerance to opiates greatly limits their usefulness. In animals the ability to induce tolerance is associated with the development of physical dependence,[118] and may be studied with similar chronic (and acute) dosing schedules followed by the determination of the analgesic effect of acutely administered doses. By chronically administering the test compound at maximally tolerated doses in such an experiment, the question of whether an experimental compound is capable of inducing tolerance is readily answered. The more important question of whether the drug is likely to induce tolerance when administered only at the dose and dose interval so as to obtund pain is more difficult to answer with animal studies. For agents that do not in themselves support self-administration models in which animals with chronic pain control their analgesic intake[119] could prove useful. Another approach to the study of tolerance, at least for opioids, involves quantitating changes in receptors after chronic treatment. *In vivo* chronic morphine treatment causes an enhanced sensitivity to naloxone[120] coincident with the decreased sensitivity to morphine. Thus the former qualitative change in opiate receptors may be used to determine tolerance development. Unfortunately, *in vitro* changes in ^{3}H-naloxone binding do not reflect tolerance development in chronically treated animals.[121] However, changes in other biochemical parameters, such as endorphin release, receptor microenvironment, and receptor-effector linkage, may prove to be important to tolerance development and useful as a means of quantitating tolerance. Of course, with any analgesic the possibility must be considered that tolerance is mediated by pharmacokinetic changes (absorption, distribution, metabolism, and excretion).

One of the strategies employed to develop opioid analgesics with lower abuse potential has been to reduce the morphine-agonist efficacy by the introduction of narcotic-antagonist structural modifications. This effort was based on the early observation that the narcotic antagonist nalorphine, which has weak agonist properties, has analgesic activity in man.[122] Subsequent similar structural modifications of other known opiate compounds led to the development of so-called agonist-antagonist analgesics (pentazocine,[123] butorphanol,[124] and nalbuphine[125]) which, like nalorphine, have a significantly lower abuse potential.

The pharmacological discovery of agonist-antagonist analgesics has

relied on the identification of their weak analgesic activity and their ability to antagonize the effects of morphine agonists in a number of tests. For example, these compounds decrease the analgesic effects of morphine in the mouse tail-flick assay[126] and rat tail-shock vocalization test.[127] Since these antagonism models are based on blocking the agonist property of interest, namely analgesia, they are less sensitive to agonist-antagonists with a high ratio of analgesic agonist/antagonist activity and consequent high analgesic efficacy. Other antagonism tests are based on blockade of some of the side effects of morphine agonists. The Straub[128] tail-response and locomotor stimulant[129] effects of morphine are both sensitive to antagonism by narcotic antagonists and agonist-antagonists. Although these are more sensitive tests for narcotic antagonism, because of the nature of the measured endpoint, they are less specific. Behaviorally depressant agents also block these responses.[130] The antagonism of the depressant effects of morphine provides a somewhat more specific model of narcotic-antagonist activity. For example, the antagonism of morphine-induced respiratory depression in the rabbit provides a sensitive and fairly selective test[131] for this activity. However, the most sensitive tests are based on the precipitation of abstinence in morphine-dependent animals. The simplest of these involves the measurement of jumping behavior in mice following morphine pellet implantation and subsequent injection of the test compound.[132] In addition, the aforementioned physical dependence-related endpoints in rats and monkeys may also be used to measure precipitated abstinence by putative antagonists.

In vitro tests may also be of some utility in predicting narcotic-antagonist activity. Smooth muscle assays, such as reversal of inhibition of the guinea pig ileum by morphine, may serve as the basis for detecting narcotic antagonists.[133] Both agonist activity and the ability to block morphine (antagonist activity) may thus be determined in the same tissue. *In vitro* binding studies may also be useful in predicting narcotic-antagonist activity, at least in certain series of heterocyclic compounds. Generally, binding studies only measure affinity, not efficacy of binding, but certain ions and cofactors have been shown to differentially alter the affinity of agonists and antagonists. Thus Na^+ increases the binding of antagonists and markedly decreases the binding of agonists.[72] Similarly, guanine nucleotides decrease the binding of agonists.[73] Ratios of binding affinity in the presence or absence of Na^+ or GTP correlate generally well with other *in vivo* measurements of narcotic antagonists. These *in vitro* tests do not, however, always predict agonist-antagonist ratios of peptides and structurally novel opioids. The reason for this is

not entirely clear, but it may be due to the interactions of the latter compounds with subtypes of opioid receptors with different Na^+ and GTP sensitivities.

Another, and in some ways more recent, strategy for the development of opioid analgesics of lower abuse potential is to make the drugs more specific for a particular subclass of opioid receptors. In a broad sense even the historical efforts to develop improved opiates were based on this concept, that is, that different opioid receptors are involved in analgesia and side effects (euphoria, respiratory depression, etc.) and that selective agents could thus be developed. More recent pharmacological experiments[77] with some of the many structurally and pharmacologically novel synthetic opioids have provided a theoretical construct in which to categorize the various opioids. This work of Martin and his collaborators indicated the existence of at least three subtypes of opioid receptors named after protypical agonists: mu (μ, morphine), kappa (κ, ketocyclazocine), and sigma (σ, SKF 10047). To some degree the multireceptor hypothesis supplants the agonist-antagonist explanation for the analgesia but limited abuse potential compounds such as nalorphine and pentazocine. In fact, Martin[134] had earlier advanced a two receptor hypothesis to explain the paradox of the morphine antagonist yet analgesic effects of nalorphine. This suggested that nalorphine is an antagonist at the μ receptor and an agonist at a second receptor. Both receptors mediate analgesia, though of an apparently different type, as indicated by the differing activities of these compounds in various analgesic tests. As explained by the three receptor hypothesis, the analgesic activities of nalorphine and pentazocine are apparently caused by agonist actions at the κ receptor. Other compounds, notably cyclazocine and ketocyclazocine, were also identified as strong agonists of the κ receptor, but antagonists and partial agonists, respectively, at the μ receptor. In addition, the agonist activity of cyclazocine at the σ receptor was suggested to be related to the stimulant and psychotomimetic effects of this compound. Thus if this theory is correct, compounds with agonist effects primarily at the κ receptor may prove to be novel analgesics lacking the abuse potential (μ-receptor related) and dysphoric-psychotomimetic actions (σ-receptor related) of other analgesics. However, the verification of this postulate requires the development of a relatively pure κ agonist.

The pharmacological search for a κ-receptor analgesic is difficult considering the lack of a more selective prototype than ketocyclazocine and the consequent imperfect knowledge of κ receptor–mediated pharmacological effects. However, certain basic guidelines are clear. Paramount among these is the selection of compounds lacking μ properties but

retaining naloxone-antagonizable strong analgesic effects.[135,136] On the preliminary screening level the absence of morphine-like behavioral signs (motor stimulation and Straub tail) may be taken as an indication of reduced μ-receptor activity. Further confirmation of the reduced μ activity may be gained through cross-tolerance and cross-dependence assays. If the putative κ agonist does not rely on μ receptors for analgesic effects, it should display no analgesic cross-tolerance in morphine-tolerant animals.[136,137] Similarly, if it does not suppress or precipitate abstinence in morphine-dependent mice[138] or rats,[139] the compound lacks major μ-agonist or -antagonist properties.[140] On the positive side the pharmacological spectrum of κ agonists includes analgesia that is antagonized by naloxone, although higher doses of naloxone are required than for equianalgesic doses of morphine.[136] This difference in naloxone's apparent affinity for the analgesic receptors may be quantitated by the pA_2 method as adapted for *in vivo* studies; κ-agonist receptors display a lower pA_2 (lower naloxone affinity) than do μ-agonist receptors in naloxone interaction studies. In addition to analgesia κ agonists cause several characteristic side effects. In dogs,[77] rats,[135] and mice[136] κ agonists cause a dose-related naloxone-antagonizable sedation. Rats also display a water-diuretic response to these compounds,[141] which is in contrast to the antidiuretic effects of morphine.[142] Diuresis is also seen with butorphanol and other so-called agonist-antagonists.[143] This probably is an indication of their κ-agonist, rather than μ-antagonist, properties, as narcotic antagonists do not cause a similar diuresis. They do, however, block the diuretic effects of putative κ agonists, thus indicating the opioid receptor–related mechanism of this action.[141] These naloxone-antagonizable side effects (sedation and diuresis) may be used to pharmacologically identify κ agonists. In addition, cross-tolerance and cross-dependence may be useful in classifying compounds as κ agonists. Tolerance develops to the analgesic effects of these compounds in rodents with minimal cross-tolerance to morphine.[135,136] In spinal dogs a cyclazocine and ketocyclazocine physically dependent state can be induced by chronic treatment with these compounds.[144] Presumably, cross-dependence to some of the newer κ agonists could be demonstrated in such a model.

If κ opioid receptors do indeed exist as separate physical as well as functional entities distinct from μ receptors, receptor binding studies should be capable of differentiating them. As mentioned previously, this has not been successful using ^{3}H-ethylketocyclazocine as a receptor ligand. This may well arise from the nonspecificity (κ and μ) of this agent. The use of more selective κ agonists as ligands may allow a discrimination of these receptors *in vitro*. However, it is also possible that such

in vitro techniques will not be useful in differentiating these receptors: μ and κ agonists are structurally very similar. The corresponding subtle differences in their receptors could be artifactually lost in the course of the membrane preparation for the binding studies.

Although the biochemical evidence for the existence of δ receptors is better than that for κ receptors,[145] it is questionable whether these receptors mediate analgesia. In smooth muscle tolerance develops to δ-receptor agonists and there is no cross-tolerance with μ agonists.[146] This further substantiates the distinction between these receptors and provides another model system to measure δ receptor–agonist properties.

METHODS FOR PREDICTING SIDE EFFECTS

In addition to abuse liability there are other side effects of opioid analgesics that are also extensions of their pharmacological actions. For the μ or morphine-like agonists these include depression of respiration, constipation, and altered levels of circulating hormones. Narcotic-antagonist analgesics and non-μ opioids may have a whole spectrum of their own side effects of this sort, but the most troublesome of these is their propensity to cause unpleasant subjective (dysphoric) and hallucinatory (psychotomimetic) effects. Any new analgesic proposed for clinical use, regardless of its mechanism of action, should be characterized by an advantageous ratio of these side effects and analgesic effects. In addition, other general side effect properties of the compound should be considered. For example, effects on blood pressure, cardiac parameters, EKG, immunological, hematopoietic, and mutagenic test systems should be investigated.

Among the μ agonist–related side effects, respiratory depression is the most important. In man[147] narcotic-induced depression of respiration is the primary mechanism of morphine toxicity. Animal models are fairly unambiguous in predicting this effect. On a simple screening level measurement of respiratory rate is useful. In mice such measurements may be made using a rapidly responding thermister and appropriate electronics.[148] Rabbits, due to their slow respiratory rate, may be used with direct recording of respiratory movements.[131] Their size also makes them suitable for the measurement of expired CO_2. This parameter, as well as blood pCO_2, pO_2, and pH, are more sensitive indicators of opiate-induced respiratory depression than are simple measurements of respiratory rate. Any species, including man, may be used for these former parameters, but the rat[149] is a useful model for blood gases and pH

changes, insomuch as analgesic measurements may be readily performed in the same species.

Although clinically useful in their own right, the antidiarrheal or constipating effects of opiates[150] are generally unwelcome to those seeking analgesia, particularly postoperatively, when other factors are also conspiring to interfere with intestinal function. Thus in the development of new analgesic agents methods for predicting and eliminating this activity are important. *In vitro* measurements of the effects of the drug on intestinal smooth muscle may give some insight into the direct effect of the drug on the gut. Surprisingly, however, the inhibition of intestinal motility by opioids is in part mediated by a primary effect on the CNS.[151] Thus measurement of intestinal transit in the intact animal provides the most suitable model for measuring the constipating properties of opioids. The so-called charcoal meal method in mice[152] provides a simple, reliable screen for the effects on intestinal motility. Again, such an *in vivo* model in a rodent has the added advantage that analgesic data may be readily generated in the same species, thus allowing the potency as an intestinal inhibitory agent to be compared to analgesic potency.

The alterations in circulating hormones induced by analgesics are not generally considered a major side effect problem, particularly not as concerns their acute usage. However, with chronic use these effects may lead to unwanted physiologic and psychic changes. Since a number of these hormone alterations are observed in both rats and humans, the former serve as an appropriate laboratory model for quantitating these effects. For example, μ opioids lower luteinizing hormone and testosterone levels[153] in both species, whereas narcotic antagonists elevate luteinizing hormone.[154] These effects may in part account for the decreased libido characteristics of chronic opiate abusers. In addition μ agonists elevate the prolactin level in rats[155] and man,[156] a property that is common to other psychotropic drugs as well.[157] Alterations in growth hormone levels are not as clearly mu receptor related; in rats μ agonists and mixed agonist-antagonists both elevate growth hormone.[155] In humans β-endorphin lowers the plasma level of this hormone.[156] Undoubtedly, the release and circulating levels of other hormones are also altered by opioids a reflection of the involvement of endorphins and their respective receptors in the physiological control of hormonal homostatis. An additional example of such is the previously mentioned diametrically opposed effects of μ and κ agonists on water diuresis, an observation that suggests opposing effects of different subclasses of opioid receptors on antidiuretic hormone release.

Non-μ opioid analgesics have side effects that distinguish them from

μ or morphinelike analgesics. The above-mentioned diuretic and sedative actions are characteristic of the κ agonists. The diuretic action may be readily quantitated by measuring the urine output of water-loaded rats. The sedative effects are readily measured with standard locomotor activity cages, direct observation, or operant behavior (lever pressing) techniques. The more disturbing side effects of non-μ opioids, namely dysphoric and psychotomimetic reactions, are associated with the σ agonists in the classification of Martin and co-workers. These effects are serious enough to preclude the use of nalorphine and cyclazocine as analgesics and to restrict the useful dose range of pentazocine. The prediction of such side effects from animal models would seem difficult, at best. In fact, one may question whether animals are capable of hallucinations. Nevertheless, several animal techniques are available which identify subjective and biochemical alterations characteristic of compounds with σ-agonist properties. The technique of stimulus generalization, as previously described in this chapter, may be used to identify a phencyclidine-like component in the subjective effects of certain opioids (cyclazocine and *n*-allylnormetazocine).[158] Thus the dysphoric-psychotomimetic actions of these so-called σ agonists may be quite similar to those of phencyclidine. Clearly, compounds with subjective properties similar to phencyclidine are undesirable as analgesic agents if one wishes to most selectively disrupt the sense of pain, not the general sensorium. This stimulus discrimination model may be useful in differentiating such activity.

If, in fact, animals such as rats do experience hallucinations, or at least some type of perceptual distortion when treated with σ agonists or other known hallucinogens, one might expect them to have demonstrable deficits in their perceptual discrimination. Recent results[159] with rats suggest that this may be the case. When rats were trained and tested for brightness discrimination in a Y-maze, cyclazocine, *n*-allylnormetazocine, phencyclidine, and 5-methoxy-N,N-dimethyltryptamine were found to impair acquisition of this discrimination. All of these compounds are known to have hallucinogenic activity in man; their activity in this assay may be the result of similar perceptual distortions in rats. Such assays may prove useful in the prediction of hallucinogenic activity.

The dysphoric effect of pharmacological agents may also be studied by measuring stress-related biochemical changes in the animal. One might expect, for example, that compounds such as cyclazocine, if they are dysphoric in animals as they are in man, would be stressful to the animal, and that the reaction to such stress might be measurable. The corticosteroid levels in rats have been noted to be sensitive to environ-

mental[160] and internal stress.[161] Recently, cyclazocine, nalorphine, and other opioids have been shown also to elevate plasma corticosteroids.[162] In rats these drug-induced corticosteroid elevations correlate well with a lack of, or lowered rates of, self-administration of these compounds, in contrast to other opioids with a lower propensity to elevate corticosteroids[162] that are self-administered. Thus the stressful or dysphoric effect of σ-agonist compounds may in part be responsible for their low self-administration potential. If these dysphoric compounds are indeed punishing to rats, they may also effectively block the self-administration of reinforcing compounds such as morphine when coadministered. The interpretation of such an experiment would be difficult due to the narcotic antagonist activity of cyclazocine or nalorphine, but with the use of a different type of reinforcer, such a technique might also be useful to detect dysphoric properties.

In developing mechanistically novel analgesics special attention should be directed to the investigation in animals of potential side effects or toxicities that are extensions of the agent's mechanism of action. For example, α-adrenergic agonist analgesics must be carefully evaluated for sedative and hypotensive properties. Similarly, a Substance P antagonist analgesic may have characteristic side effects whose nature is only a matter of speculation at this point. They might, however, involve decreases in gastrointestinal motility and salivation and extrapyramidal motor signs, owing to the excitatory nature of Substance P in these systems. Such studies of potential side effects in animal models prior to clinical development are all the more important today; the great expense and time necessary for the development of new therapeutic agents do not allow the luxury of making these discoveries in clinical studies.

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The Potential of Centrally Acting Regulatory Peptides as Analgetics

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An exciting development in recent years has been the realization that most, if not all, of the peptides isolated previously from endocrine tissues, and classically conceived as *hormones*, also exist extensively in neurons.[1] Their function is to relay a biological message (encoded in their sequence) from one cell to another. The originating cell may be an endocrine cell or a neuron, and the target cell may be one of a great variety of cell types, including another endocrine cell or neuron. The mode of transport to the target cell may be via blood, axons, dendrites, somas, axonlike processes of endocrine cells, or simple extracellular diffusion. In fulfilling this function the peptide is a chemical messenger or *hormone*. However, some still prefer to restrict the use of this term to the special circumstances in which the messenger is transported via blood. To avoid confusion the alternative term *regulatory peptide* is used to cover all types of transport, including that involved in nervous transmission and regulation. Other names suggested include *cybernin*[2] and *regulin*.[3]

In the transmission of central pain signals we are concerned with interneuronal communication. Although the role of regulatory peptides in this communication is still poorly understood, it is certainly not limited to that of neurotransmitters in classical synaptic transmission. Based on results with the enkephalins and Substance P (SP) using cultured mouse spinal neurons, Barker distinguishes three effects.[4] First, alteration of membrane conductance over a wide range of membrane potential, an effect characteristic of conventional transmitterlike actions. Second, modulation of postsynaptic transmitter function (neuromodulation was defined as the alteration of synaptic receptor-coupled conductances without direct activation of such conductances). Third, reversible elevation in spike threshold, which effectively depresses excitability. The position is further complicated by the coexistence, revealed now in several cases, of regulatory peptide and biogenic amine in the same neuron.[1] Thus a diverse range of molecules mediate apparently similar signals. How these signals, which alter chemical and/or electrical excitability in neurons, are integrated in the perception of pain, and in the autonomic, endocrine, motor, and sensory responses that may accompany pain, is even less understood.

Nevertheless, knowledge has already accrued that provides a basis for speculation in the design of new types of analgetics. SP, cholecystokinin (CCK), vasoactive intestinal peptide (VIP), and somatostatin (SS) have been identified in nociceptive primary afferent fibers; enkephalins, neurotensin (NT), and SP have been identified in spinal interneurons; concentrations of several regulatory peptides are high in brain loci thought to be involved in pain perception or control; and enkephalin and SP and SP-5HT descending fibers have been identified. SP is a strong neurotransmitter candidate for nociceptive primary afferents. There is evidence that enkephalins or other opioid peptides modulate pain at the spinal level via presynaptic inhibition of SP release. When administered centrally in standard analgesic tests, opioid peptides, CCK (as octapeptide, CCK-8), NT, and SP produce analgesia or apparent analgesia. We shall be concerned with attempts to apply this knowledge in analgetic drug design.

Dominating the scene and this chapter is work with opioid peptides, a class of regulatory peptides that includes [Met]enkephalin and [Leu]enkephalin, a variety of enkephalin precursors, and endorphins. The isolation and characterization of the enkephalins[5] precipitated intensive research aimed at analogues with more favorable pharmacological profiles. Over 1000 tetra- or pentapeptide analogues have been described in the general or patent literature, and many have been subjected to detailed pharmacological analysis. A main objective has been to overcome problems inherent in the metabolism, transport, and absorption of the enkephalins by molecular manipulation. One of the most gratifying outcomes of these endeavors has been the emergence of analogues of high potency (equal to or greater than that of morphine) in all standard *in vivo* tests of analgesia following intravenous, subcutaneous, or oral administration of the compounds.[6] However, we shall see that it is doubtful that dissociation of analgesic effects from physical dependence has been achieved. Other opportunities, based on enkephalins, have been foreseen, and these are now being pursued more actively. In particular, we discuss attempts to control the release of endogenous enkephalins and their degradation by specific enkephalinases. Our knowledge of the structures of the several possible enkephalin precursors that have now been isolated is also summarized.

In addition to the work with opioids, several groups are now actively engaged in attempts to design SP antagonists. The rationale for believing that SP antagonists might be antinociceptive agents follows from the postulated transmitter role for SP. Very little of the work is published, but the first reports of success are discussed.

The high potency of CCK-8 and caerulein (an analogue naturally occurring in frog skin) in analgesic tests, as well as the first clinical reports of the effectiveness of caerulein in alleviating renal and biliary colic and cancer pain, are also mentioned.

Finally, brief mention is made of other regulatory peptides that may be involved in pain mechanisms.

ENKEPHALINS: STRUCTURE-ACTIVITY RELATIONSHIPS (SAR)

Modulation of the biological activity of all regulatory peptides may be achieved by structural modification of the parent peptide, offering great potential in the design of analogues with improved or altered properties. In the case of the enkephalins the initial target was the design of analogues with improved analgesic activity, and this attracted attention on an unprecedented scale. As a result there is an enormous amount of structure-activity data for enkephalins in a variety of *in vitro* and *in vivo* tests.

Guinea pig ileum and mouse vas deferens *in vitro* tests, mouse or rat hot plate and tail flick *in vivo* tests, and receptor binding tests have been most studied. The effect of single substitution (any one of the five amino acid residues varied, but not two) on biological activity in these tests has been reviewed recently.[6] Selected results for the guinea pig ileum test, for which the most data are available, are summarized in Table 1, in which [Leu]enkephalin methyl ester is the reference compound. Tables 2 and 3 summarize similar results using [D-Ala^2, Leu^5] enkephalin methyl ester and [D-Met^2, Pro-NH$_2^5$]enkephalin as the reference compound, respectively; in these tables, since the reference compounds are singly or doubly substituted enkephalins, we are dealing with multiply substituted enkephalins. It is hoped that the three summary tables will prove useful in presenting the voluminous data in an interrelatable form. A clear appreciation of the format is essential, however.

The structure of the appropriate reference compound is shown across the second horizontal column of Tables 1–3. All analogues described are derived (formally) by change of the structure of the reference compound at one of the five amino acid positions, which correspond with the vertical columns. Individual analogues are identified by an entry in one of these vertical columns indicating the structural change involved; for example, in Table 1 D-Ser in the column corresponding to Gly^2 indicates results with the analogue Tyr-D-Ser-Gly-Gly-Leu-OMe. Since the boxes

Table 1 Effect on the *In Vitro* Activity (Guinea Pig Ileum Test) of [Leu]enkephalin Methyl Ester Resulting from Single Changes of the Amino Acid Residues (Results of ICI Group[7a]

More active	Me Lys(Boc) Lys Arg Gly Dab		D-Ser D-Met D-Ala Azala D-Thr D-Lys(Boc) D-Phe D-Leu D-Asp	Azgly		Pro-NHEt Met Azleu Nle Me-Leu	NH_2 NHEt
	H	Tyr	Gly	Gly	Phe	Leu	OMe
50–100%	Glu–Gly | Lys–		D-Lys Aib			NH_2 | $(CH_2)_3$ | $-NH-CH_2$ $CHMe_2$ | CH_2 | $-NH-NH$	OH
10–49%	Dab(Boc)– Me_2 HN || H_2N $C-NH-CH_2-CO-$		D-Trp Ala Azgly	Azala	Me-Phe Azphe		
Slightly Active	Phe D-Asp D-Lys(Boc) Boc Glu Des-NH_2 Ac	OH $-NH-CH-CO-$	Sar β-Ala $-NH-(CH_2)_2-CO-$ $-NH-(CH_2)_4-CO-$ D-Pro	D-Ala		$CHMe_2$ | CH_2 | $-NH-CH_2$ des-Leu	
Inactive		Aztyr His des-Tyr	Pro D-Pro	Sar D-Pro β-Ala des-Gly D-Pro D-Pro	D-Pro D-Pro		

[a] See text for general explanation. Abbreviations follow IUPAC/IUB recommendations; Azgly = NH-NH-CO; Azala = NH—NMe-CO; Azleu = NH-N(CH_2-$CHMe_2$)-CO; Azphe = NH-N(CH_2-C_6H_5)-CO; Aztyr = NH-N(CH_2-C_6H_4-*p*-OH)-CO.

within columns grade potency, the general effect of structural change is evident.

Inspection of Table 1 reveals that the structural changes in [Leu]enkephalin methyl ester which cause increased potency in the ileum assay are (*a*) methylation of the amino (Tyr) terminus, (*b*) acylation of the amino terminus by certain L-amino acid residues, such as Lys,

Table 2 Effect on *In Vitro* Activity (Guinea Pig Ileum Test) of [D-Ala^2,Leu^5]enkephalin Methyl Ester Resulting from Single Changes of the Amino Acid Residues (Results of ICI Group[9])[a]

More active	Me- Phe-		D-Ser D-Met Azala				- $O(CH_2)_2NH_2$ - N O
	H	Tyr	D-Ala	Gly	Phe	Leu	OMe
50 - 100%	Lys- Lys(Boc)- Asp- Gly_3 Lys- Asp Lys-		D-Thr			- Hse (L) - Nle - - NH NH O - Hse (D) - Met - CH_2CHMe_2 -NH - CH- N-N N-N	- OCH_2 - $CH{=}CH_2$ - Thr - OH - NH_2 - $NH(CH)_2$ - NHMe - $O(CH_2)_2$ - NMe_2 - Me - $NH(CH_2)_2$ - NMe_2 - $O(CH_2)_2$ - NHZ - NEt_2 - N
10 - 49%	Lys Lys - Glu - Gly Lys - Gly_3- Pro_2- Leu-Gly_2- Glu-		D-Lys (Boc) D-Phe D-Leu D-Asp D-Ser(Bu^t)		- Phe(6H) -	- NH NH O - Azleu - NH_2 - Hse-OH - Pro-NHEt - NH NH O - Pro-NH_2	- NHEt - OH - Gly - OMe - N - NHC_6H_{11} - Ala - OMe - D - Thr - OH - D - Ala - OMe
1 - 9%	Lys - Gly_3 - Gly_4 - Asp - Gly_3 - Pro_4 - Gly_6 - D - Lys	OH	Gly D-Lys Aib D-Trp				
v	β - Ala- D - Lys (Boc)- MeCO - Phe des - NH_2 MeCO- EtCO-	NH_2 - CH - CO - His des - Tyr	Ala Azgly Sar B-Ala D-Pro	D - Ala D - Pro			

[a] For explanation see text and footnotes to Table 1. The reference compound (structure across second horizontal column) is 28 times more potent than [Leu]enkephalin or [Leu]enkephalin methyl ester. Hse = homoserine; Dab , Orn , Lys = lactams derived from Dab, Orn, and Lys; Hse = homoserine lactone; LeuT = tetrazole analogue of L-leucine.

Table 3 Effect on *In Vitro* Activity (Guinea Pig Ileum test) of [D-Met², Pro-NH₂⁵]enkephalin Resulting from Single Changes of the Amino Acid Residues (Results of ICI Group⁹)[a]

More active						D-Hse⌋ Hse⌋	
	H —	TYR —	D-MET —	GLY —	PHE —	PRO —	NH_2
50 - 100 %	Gly–					Leu D-Pro	$-O(CH_2)_2OH$
10 - 49 %	Lys–		D-Ala D-Asp Azala			-N — NH Azpro	-Thr-OH
1 - 9 %	Gly_4– Gly_5– Gly_3– Gly_2– Gly_6–		D-Ser		Phe(6H)		-D-Thr-OH
<1 %			Gly Sar $-NH-(CH_2)_2-CO-$ $-NH-(CH_2)_4-$ $-NH-CH_2-CH=CH-CH_2-CO-$				

[a] For explanation see text and footnotes to Tables 1 and 2. The reference compound (structure across second horizontal column) is 59 times more potent than [Leu]enkephalin.

providing hexapeptide analogues, (*c*) substitution of Gly^2 by a variety of, but not all, D-amino acid residues, (*d*) substitution of Gly^3 by α-azaglycine (-NH-NH-CO-), and (*e*) certain changes at the C-terminal position. [Leu]enkephalin methyl ester and [Leu]enkephalin are equipotent in the assay, and the same conclusions generally apply with [Leu]enkephalin as reference compound.

Inspection of Table 2, where [D-Ala^2,Leu^5]enkephalin methyl ester is the reference compound, shows that the effects are not always additive; for example, Met in place of Leu in [Leu]enkephalin or in its methyl ester, results in an increase in potency (two- to fivefold) whereas similar

substitution in [D-Ala^2,Leu^5]enkephalin methyl ester results in decreased potency.

Table 3 shows the effect of structural change in an analogue, [D-Met^2,Pro-NH$_2^5$]enkephalin, which has potent analgesic activity *in vivo*. Change at the 2 position may now have opposite effects to those observed with [Leu]enkephalin methyl ester as reference compound. For example, Tyr-D-Ser-Gly-Phe-Leu-OMe (55 times more potent than [Leu]enkephalin) is more potent than Tyr-D-Ala-Gly-Phe-Leu-OMe (28 times), whereas Tyr-D-Ser-Gly-Phe-Pro-NH$_2$ (4 times) is much less potent than Tyr-D-Ala-Gly-Phe-Pro-NH$_2$ (59 times).

Results from the guinea pig ileum and mouse vas deferens assays are in broad agreement, and lead to the following conclusions about SA relationships in these tests.[6] Of the five amino acid residues in the enkephalins, the structural and configurational requirements at Tyr^1, Gly^3, and Phe^4 (in this order) are most stringent. The only changes at Tyr^1 that provide more potent analogues are N-methylation and, in some cases, N-extension by amino acid residues. Most analogues are inactive. The requirements at Gly^3 and Phe^4 are also precise. A little more configurational freedom is permitted (Azgly^3 and Azphe^4 analogues active), but the substitution of D-amino acid residues is not. N-Methylation of Phe^4 (but not of Gly^3) causes only a small drop in potency, the aromatic ring of Phe^4 is not sacrosanct, and the Trp^4 analogue has activity, but most substitutions lead to inactive or weakly active analogues. Much more latitude is allowed at the remaining two positions (Gly^2 and Met/Leu^5). The dominant feature at the Gly^2 position is the large increase in potency (both assays) resulting from substitution of certain D-amino acid residues (D-Ala, D-Met, D-Ser). The Met/Leu^5 position can be varied widely without associated elimination of activity; however, the resulting analogues are usually less potent than the parent compound, the exceptions being amides (GPI assay) and D-Leu^5 analogues (MVD assay), which are often more potent.

The results from *in vitro* tests have also enabled broad conclusions about SA relationships at the opiate receptor but, unlike the situation with classical opiate agonists, they have proved of limited value in predicting analgesic activity in *in vivo* tests. One distinction has emerged, however. Analogues with weak analgesic potency generally resemble [Leu]enkephalin in being more potent in the vas than in the ileum assay, whereas analogues with high analgesic potency tend to be equipotent in both assays (usually through increased potency in the ileum). This has led to speculation that analgesia is mediated via distinct opioid receptors (μ) of postulated greater relative abundance in the ileum than in the vas (see the section "Multiple Opiate Receptors").

One of the main problems in the interpretation of results from the ileum and vas assays is the extent to which analogues are degraded by peptidases (or other enzymes) present in the preparations. There is no doubt that both preparations do contain enzymes that are capable of degrading [Leu/Met]enkephalin and many of the analogues. Until recently, attention has been mainly focused on those enzymes (arising from broken cells and plasma, and present in both the tissue and the surrounding organ bath) that degrade by cleavage of the N-terminal Tyr residue. These "released" amino peptidases may account for the majority of enzymic activity in the preparations, but they are probably not specific enkephalin degrading enzymes. The argument[10] that enkephalin degradation is "not a source of errors" in the MVD and GPI assays because (*a*) the depressant effects of agonists is achieved in less than 1 minute, and (*b*) the organ bath serves as an inexhaustible reservoir of exogenously added agonist, may be valid for degradation by "released" enzymes, but does not hold good for degradation by membrane-bound enzymes. It seems likely (see the section "Enkephalinase Inhibitors") that membrane-bound enzymes do exist in the vicinity of opioid receptors, and that their specific function is to inactivate the enkephalins. The effective concentration of agonist at the receptor is then determined by the equilibrium between agonist and inactivated agonist, controlled by such enzymes, as well as by the concentration of agonist that arrives in the vicinity of the receptor (receptor compartment). In agreement with this model, the potency of [Met]enkephalin in both assays was increased 2- to 10-fold by the addition of various enkephalinase inhibitors (which had no effect on the potency of normorphine, β-endorphin, or "stabilized" enkephalins) to the organ bath.[11]

The S-A relations discussed above lend strong support to the conclusion that in both assays aminopeptidase, carboxypeptidase, and probably endopeptidase activity play a role in determining observed activity. It may be further speculated that aminopeptidase activity is of major importance in the GPI assay, that the endopeptidase activity is mainly cleavage of the Gly-Phe bond, and that the main controlling enzymes are membrane bound. This does not preclude the possibility that certain structural changes (e.g., D-amino acids for Gly^2 in both assays, and for Met/Leu^5 in the MVD assay) also favor involvement in a receptor interaction (e.g., affinity). However, in these circumstances only the following conclusions about the nature of the receptor interaction(s) seem justified. The *minimal fragment* (if such a term has meaning) of enkephalin for interaction may be defined as the descarboxy tetrapeptide Tyr-Gly-Gly-$NH(CH_2)_2Ph$. The presence of N-terminal amino (or alkylamino) and

tyrosine hydroxyl, their correct spacial disposition, and the correct spacial disposition of the Tyr and phenyl rings are essential. The peptide backbone serves to promote correct spacing, but the peptide bonds themselves are not involved. Receptor binding assays confirm these general conclusions but provide little further information, since interpretation of results is again complicated by metabolic and other factors (for a discussion of the data from receptor binding studies, see reviews 12–14).

ENKEPHALINS AS ANALGETICS

The mouse tail flick or hot plate tests have been commonly used as models in assessing analgesia. The potencies of some of the most active analogues **(1–10)**, and of dermorphin, β-endorphin, and morphine **(11–13)**, in these two tests are given in Table **4**. It is instructive to see how they evolved. The apparent failure of *in vitro* results (from GPI, MVD, and opiate receptor binding studies) to predict *in vivo* analgesic potency after central administration was first attributed to rapid degradation of the peptides by brain enzymes (brain extracts were indeed found to contain enzymes that caused rapid degradation of [Met/Leu]enkephalin). The finding of potency approaching that of morphine in enzyme-resistant analogues supported this conclusion. However, such analogues, derived by D-Ala^2 substitution, are not active at relatively high doses after intravenous administration, and this was attributed to failure of the peptides to cross the blood-brain barrier. Entry from blood into the CNS is, of course, one important parameter to be considered, but it is now clear that many other parameters are of equal importance in determining potency by this route. There are also further factors (e.g., entry into blood, absorption from the GI tract, diffusion from subcutaneous sites) to be considered in arriving at subcutaneously or orally active analogues.

The groups of Pless and Roemer, Bajusz and Ronai, Morgan and Metcalf, and Li and Kiso have been most successful in resolving these problems. Their major findings are as follows. Stability to enzymes in body fluids and tissues is, of course, an essential factor (but the relevant enzymes in tissues may be membrane bound; therefore the results of *in vitro* experiments where analogues are incubated with tissue extracts must be treated cautiously). This consideration seems to have dominated the work of Roemer and Pless,[18] who looked for structural changes that resulted in "the formation of longer acting and orally active (i.e., *more stable*) analogues" (author's italics). Their conclusions were that "the

Table 4 Molar Potencies of Enkephalin Analogues Relative to [Met]enkephalin (*In Vitro* Tests) or Morphine (*In Vivo* Tests)[a]

Analogue[b]	*In vitro* Results ([Met]enkephalin = 1)			*In vivo* Results (morphine = 1)								
				Tail Flick Test				Hot Plate Test				
	GPI	MVD	Ref.	icv	sc	iv	po	icv	sc	iv	po	Ref.
1. D-Ala^2, MePhe^4, Met(O)-ol^5	21.2	0.94	(15)	10^3	3.2	6.4	.32		3.1		.45	(16,17)
2. MeTyr^1, D-Ala^2, MePhe^4, Met(O)-ol^5					16.4	4.9	1.6					(18)
3. DMet^2, Pro-NH_2^{5}	9.3	0.33	(19)	78	1.6	5.9		25				(20)
	29.2	0.97	(21)							1.7		(21)
4. MeTyr^1, D-Met^2, Pro-NH_2^{5}	45.2	2.2	(21)							2.2		(21)
5. D-Thr^2, Thz-NH_2^{5}				27	1.5	4.8	1.7					(22,23)
6. Tyr-D-Ala-Gly-MePhe-NH$(CH_2)_2$-N(0)Me_2	6.5	0.18	(24)			7						(24)
7. D-Ala^2, MeMet-NH_2^{5}		5.0	(25)					238	0.3[c]			(26)
		2.6	(26)					144	4.0[d]			(26)
8. D-Ala^2, Met-NH_2^{5}	8.0	1.9	(27)	~1		<0.03						(28)
	7.7	3.8	(21)	0.16		<0.08						(29)
9. Tyr-D-Met(O)-Gly-MePhe-ol	14,562.0		(30)		9.1							(30)
10. Tyr-D-Met(O)-Gly-NMe-$(CH_2)_2$Ph					1.1							(31)
11. Dermorphin	57.0	0.92	(32)	750				2170		11.0		(32)
12. β-Endorphin	3.5	0.35	(33)	30								(17)
				31.5				17				(34)
						4.2						(35)
13. Morphine	2.2	0.03	(33)									

[a] Reference numbers are listed in parentheses.

[b] Thz = L-thiazolidine-4-carboxylic acid.

[c] Licking was used as an end point in determining reaction times.

[d] An attempt to jump off the hot plate was used as the end point.

substitution of Gly in position 2 by D-Ala, N-methylation of Tyr and Phe in position 1 and **4** respectively, and the conversion of Met in position 5 to the corresponding alcohol results in stable, highly potent analogues." However, in an elegant study by Bajusz and co-workers,[36] analogues of similar resistance to enzymic degradation by human serum, rat brain extracts, or aminopeptidase varied markedly in their analgesic potency. A particularly interesting example is the comparison of data for [Met]enkephalin and [Pro^5]enkephalin; the latter was found to be rather more vulnerable to enzymes (and it is less potent in *in vitro* assays), yet it was much more potent (ED_{50} 64 μM/kg) after intravenous injection in the tail flick test (when [Met]enkephalin is virtually inactive). They concluded that the significance of increased enzyme resistance is overstated in the literature and that there are three other requirements which must be satisfied for high potency, i.e., "favourable transport properties, ability to cross the blood-brain barrier, and enhanced or improved binding capacity." Contrary to previously expressed opinions, overall results in all studies with enkephalin analogues indicate that lipophilicity *per se* is not of importance in the penetration of the blood-brain barrier by peptides; more probably, active transport mechanisms exist, and it is surprising that these have not yet been studied more systematically. To Bajusz's list may be added the ability to permeate other "barriers," and favorable binding to plasma proteins (the significance of which may extend beyond "transport"). It may be speculated that the conclusions of Roemer and Pless with respect to the 1–4 positions of enkephalins accurately reflect the requirements for enzymic stability and the receptor interaction, that is, that the 1–4 tetrapeptide is the unit of structure which is concerned with the receptor interaction, and that it must be stabilized to enzymic attack by a structural change that does not impede this interaction. But modification of position 5 may be the additional key to optimization of other factors. From the results in Table **4** it would appear that 5-position modifications that have most successfully achieved this are substitutions by Pro-NH_2, Met-ol, 2-amino-thiazolidine-5-carboxamide, and N,N-dimethyl-N-oxyammonioethylamino.

Deletion of the residue at position 5, accompanied by certain modifications of the Phe^4 residue, may also result in considerably increased potency in *in vitro* and *in vivo* tests. One of the most spectacular of recent findings concerns the tripeptide analogue **9** (Table **4**), in which the 5-residue is deleted, and the Phe^4 residue is changed to N-methylphenylalaninol [NH-CH(CH_2Ph)-CO- changed to NMe-CH(CH_2Ph)-CH_2OH]. This analogue is 3 orders of magnitude more potent than any previous enkephalin analogue in the guinea pig ileum test, and is 9 times more

potent than morphine in the mouse tail flick test (subcutaneous administration).[30] Further contraction of the molecule (replacement of the C-terminal CH_2OH group by hydrogen) provided the analogue **10**, which is as potent as morphine in the tail flick test.[31]

Dermorphin (Figure 1), isolated by Ersparmer and his colleagues[32] from frog skin, has interesting structural similarities to analgesic enkephalins. Uniquely in peptides isolated from vertebrate tissue, this heptapeptide contains a D-Ala residue, and the residue follows N-terminal Tyr. Thus the N-terminus of dermorphin (Tyr-D-Ala-) is that characteristic of many analgesic enkephalins. Residues 3 and 4 of the enkephalins (Gly-Phe) are then reversed in dermorphin, and Phe^5 is changed to Tyr. Dermorphin is probably the most potent enkephalin analogue in the hot plate test, and has the highest vas/ileum IC_{50} ratio.

Despite the considerable achievement in transforming [Leu/Met]enkephalin, whose analgesic effects can only be demonstrated under special conditions, into analogues considerably more potent than morphine following intravenous, subcutaneous, or oral administration in common tests for analgesia, the consensus of present opinion is that the analgesia they produce has not been dissociated from physical dependence. In a carefully devised study Wei[37] examined eight analogues, all active after systemic administration but of differing potencies, in common analgesic tests. The analogues (varying doses) were infused continuously for 3 days into the brain (periaqueductal gray region) of rats by means of osmotic minipumps, after which a quantifiable withdrawal syndrome was produced in each case following administration of an opiate antagonist. There was good correlation between the ability of analogues to produce physical dependence and their antinociceptive activity. It had been implied[38] that enkephalin analogues containing a hexahydrophenylalanine residue at position 4 can give rise to analgesia without associated physical dependence, but such hopes were not realized,[39] and claims[40] that [D-Ala^2,MeMet-$NH_2{}^5$]enkephalin (= metkephamid) (compound **7** in Table 4) has similar properties must be viewed with caution. There were little or no withdrawal symptoms after chronic administration of metkephamid to rats in increasing subcutaneous doses (10–160 mg/kg), whereas morphine in similar doses produces a high level of dependence.[25,40] However, the analgesic potency of metkephamid administered via this route is less than that of morphine, and

Tyr-D-Ala-Phe-Gly-Tyr-Pro-Ser-NH_2

Figure 1 Structure of dermorphin.

its biological half-life was not determined. It may follow that all enkephalin analogues with potent analgesic activity are also potent in producing physical dependence and, if this conclusion is correct, their potential as analgetics is limited.

CORRELATION OF ENKEPHALIN- AND MORPHINELIKE STRUCTURES

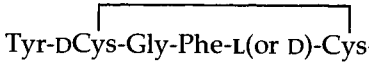
The isolation and identification of the enkephalins, endowed with all the pharmacological actions of morphine, highlighted an important aspect of drug design. Morphine and other opiates had been used as drugs for many years but, like most drugs, they are foreign to the body. It may be presumed that an accident of molecular shape permits these foreign compounds to act as drugs by fitting into receptors for endogenous compounds that play a normal role in human or animal physiology, thus activating or blocking the receptors. For opiate drugs evidence was soon forthcoming that the enkephalins were indeed endogenous opiates. Could the resemblance in molecular shape between the opiate drugs and the enkephalins now be expressed in rational terms, and could knowledge be forthcoming which, when applied to this and other regulatory peptides, would enable design of new nonpeptide drugs?

Progress in answering these questions has been disappointingly slow. One of the main reasons is that the enkephalins, like most small peptides, are very flexible molecules. Much work has been directed toward deducing preferred conformations of the enkephalins in the solid state or in solution,[41] but these do not correlate well with SAR findings, and thus seem unrelated to the conformation that the enkephalins adopt at a receptor. The general principle that the receptor bound conformation of a drug is the same as its more probable solution conformation finally seems to have been discarded. All that can be claimed is that the receptor bound conformation is not an energetically disfavored solution conformation.

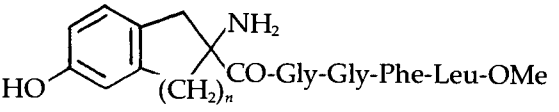
Marshall's approach[42] to the receptor bound state, in which a series of conformationally restrained analogues are examined, looks more promising. By comparing the conformational restraints and the resulting effect on biological activity, one can deduce whether the restraint is consistent with the requirements of the receptor for recognition and activation. Applied to angiotensin II, somatostatin, and thyroliberin, the results confirm, in each case, that the receptor bound conformer is different from the conformer observable in solution for the native peptide.[43]

Progress with the enkephalins is impeded by the limited availability of conformationally restrained analogues. Results within a restricted range have already led to a proposed conformation for receptor bound enkephalin,[44] and we can expect further progress as the range is extended.

Of particular interest is the high potency recently reported[45] for the analogues **14** in which a disulfide bridge connects the 2 and 5 positions of enkephalin (prepared by substituting D-cysteine at position 2, L- or D-cysteine at position 5, and oxidative disulfide bond formation). Other active conformationally restrained analogues of interest are the tyrosine-



14



15

modified analogue **15** (n = 2) [the analogue in which n = 1 is only weakly active],[46] and certain double bond[47,48] or other carba isosteres[49] of the amide bonds.

MULTIPLE OPIATE RECEPTORS

The behavior of the enkephalins, morphine, and their analogues in displacing various radioligands in receptor binding assays has provided powerful evidence that more than one type of opiate binding site exists.[50] This and other evidence are now generally interpreted as implying the existence of more than one type of opiate receptor. Mu (μ), delta (δ), kappa (κ), and sigma (σ) types have been proposed; of these the μ and δ types are best characterized.

The general view also is that the analgesic effects of opioid peptides are mediated through μ receptors.[15,51–53] Within a series of analogues it therefore follows that analgesic potency, measured under conditions in which all members of the series are delivered equally effectively to the receptor, should correlate with affinity for the μ receptor, or potency in

the guinea pig ileum assay (which is established as being related to μ-type binding). It will be recalled that Kosterlitz and Waterfield[54] did indeed demonstrate good correlation between the relative inhibitory potencies in the ileum assay and the relative analgesic efficacies in man for a series of morphine-related compounds. No similar correlation is found when analgesia is assessed after peripheral administration of enkephalin analogues, but this is not surprising, since under these conditions they stand little chance (unlike morphine-related compounds) of being delivered "equally effectively" to the receptor. What if they are administered centrally? Audigier's group[52] examined 10 analogues, all amidated at the C terminus, measured analgesia after administering them intracerebroventricularly, and found correlation between analgesic potency and μ-type binding. Ronai and co-workers[55] studied 48 analogues of more diverse structural types, assessed analgesia under the same conditions, and found no such correlation.

In challenging accepted views, Ronai and colleagues[55] point out that it remains an open question why the enkephalins are poor analgesics when given either centrally or peripherally. Common explanations (their susceptibility to enzymic degradation, and poor penetration through the blood-brain barrier) must not be the complete story. Under certain circumstances the enkephalins may lower instead of raising the pain threshold, so the authors suggest that activation of these pathways may hinder the expression of analgesic activity. In support of this view they present data that lead them to the conclusion that significant analgesic potency is present in analogues which, as compared with the enkephalins, have suffered loss of δ-agonist potency, rather than gain in μ-agonist potency. In contrast there have been reports (e.g., Ref. 56) that the weak analgesia seen following central administration of the enkephalins is strongly potentiated by simultaneous administration of inhibitors of enkephalin degradation.

ENKEPHALINASE INHIBITORS

As with other neuropeptides the problems involved with unambiguous identification of enzymes responsible for the inactivation of synaptically released enkephalin are considerably more difficult than with classical neurotransmitters. A common structural denominator in all neuropeptides is the presence of peptide bonds, and nature has devised an armory of enzymes, the peptidases, to cleave these bonds. None of the peptidases has substrate specificity comparable to that for acetylcholinester-

ase, for example, so any given neuropeptide can generally act as a substrate for many different peptidases. In facing these problems Schwartz and co-workers[57] have listed six criteria that they feel should all be satisfied before the candidacy of a putative neuropeptidase is accepted. First, cleavage by the peptidase should result in products that are biologically inactive. Second, the peptidase should be strategically located to exert its assumed role, for example, to hydrolyze the enkephalins immediately after their synaptic release. Third, substrate specificity for the peptidase should account for structure-activity relations in synthetic analogues which cannot be explained at the receptor level. Fourth, adaptive changes in the peptidase activity might occur following sustained changes in neurotransmission or neuromodulation elicited by the associated neuropeptide. Fifth, selective inhibition of the peptidase activity should protect the synaptically released associated neuropeptide from degradation. Sixth, selective inhibition of the peptidase activity should result in biological responses similar to those elicited by stimulation of the receptors for the associated neuropeptide.

Over the past few years several enzymes satisfying the first criterion (ability to cleave enkephalins to products that are inactive at opiate receptors) have been described as enkephalinases. All belong to one of three general types, aminopeptidases, dipeptidylaminopeptidases, or dipeptidylcarboxypeptidases, whose actions on [Met]enkephalin are shown in Figure 2.

The only candidate satisfying all six criteria is of the dipeptidylcarboxypeptidase type; that is, it cleaves [Met]enkephalin to Tyr-Gly-Gly and Phe-Met. This enzyme was first identified in an extensively washed particulate fraction of mouse striatum,[58] and has been isolated and partially purified from rat and rabbit brain in two laboratories.[59–61] It is

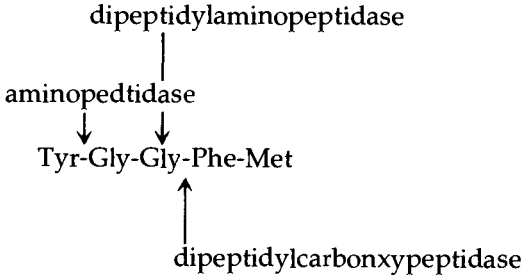


Figure 2 Modes of breakdown of [Met]enkephalin, and the enzyme activities involved.

membrane bound, and is now generally recognized as *enkephalinase* (see Schwartz[57] for a summary of the evidence). Though it is probably a metallopeptidase containing zinc,[62] it is distinct from angiotensin converting enzyme (ACE).[60,63–65]

Existing knowledge suggests that enkephalinase is localized in the vicinity of opiate receptors. For example, the regional[66,67] and subcellular[68] distributions of enkephalins and enkephalinase are parallel, and amounts of the enzyme increase following chronic treatment with morphine.[69] Inhibitors of enkephalinase action may therefore be a basis for design of new types of antinociceptive agents. The most potent inhibitor to date is (DL-3-mercapto-2-benzylpropanoyl)glycine, named *thiorphan* (Figure 3) which protects the enkephalins from the action of enkephalinase *in vitro* in nanomolar concentration.[70] Thiorphan was designed on the assumption that binding of enkephalins to the active site of enkephalinase occurs in a manner analogous to that of substrates for carboxypeptidase A.

In the tail withdrawal test thiorphan potentiates the antinociceptive activity of ICV administered [D-Ala^2,Met^5]enkephalin when coadministered ICV or systemically.[70] In the absence of thiorphan the analgesia caused by the enkephalin analogue (20 μg) is short and hardly significant, whereas in the presence of high doses of thiorphan (30 μg ICV, or 100 mg/kg IV) nearly maximal analgesia is observed for up to 4 hours. In accordance with the assumption that this effect arises from protection of the analogue from degradation by enkephalinase, the effect elicited by another analogue, [D-Ala^2,Met-NH$_2^5$]enkephalin, which is poorly recognized by enkephalinase, is not significantly modified by thiorphan. Interestingly, thiorphan alone (and also naloxone) has no effect in this test, but high doses of the compound are claimed to give rise to naloxone-reversible antinociception in the mouse hot plate test (jump response, hot plate at 50 or 55°C). Naloxone alone causes hyperalgesia in the hot plate test. As to why thiorphan causes analgesia (and naloxone hyperalgesia) in the hot plate test but not in the tail withdrawal test, the authors suggest that differing noxious stimuli may provoke release of endogenous opioid peptides to varying extents.[70]

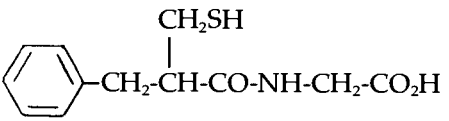


Figure 3 Structure of thiorphan.

In summary, a new approach to analgetics, based on inhibition of an enzyme that is probably involved in the inactivation of synaptically released enkephalin, is still in its infancy, but the prospect of its leading to success looks good.

ENKEPHALIN RELEASERS

Some regulatory peptides, such as CCK,[71] cause release of opioid peptides when they are administered centrally or peripherally, but this effect is probably a secondary consequence of their main actions. There might, however, be specific regulatory mechanisms for the release of opioid peptides, and knowledge of these could also lead to new types of analgetics.

A dipeptide named *kyotorphin*, isolated from bovine brain and having the structure Tyr-Arg, is a likely candidate as a specific releaser of [Met]enkephalin (it now is unclear whether other opioid peptides are also released).[72–74] Kyotorphin is inactive at opiate receptors, but causes calcium-dependent release of [Met]enkephalin from guinea pig striatal slices, which is abolished by tetrodotoxine. The release of enkephalin is markedly enhanced when the slices are field stimulated at 10 Hz. The results indicate that kyotorphin depolarizes enkephalinergic nerve terminals and releases [Met]enkephalin from its storage sites.[72,73] Support for its role in pain regulation is provided by the regional distribution of kyotorphin in the rat CNS (the highest concentrations are found in the midbrain, the pons and medulla oblongata, and the dorsal part of the spinal cord),[73] and from its analgesic properties.[72,74] Injected intracisternally, kyotorphin causes dose-related antinociceptive effects (increased latency of biting response) in the mouse tail pinch test. The ED_{50} is 11.7 μg/mouse, and the effect, about 4.2 times greater than that evoked by [Met]enkephalin, is totally abolished by naloxone. Analgesic effects are also seen in the mouse hot plate test, where the ED_{50} is 5.3 μg/mouse. The analgesia was attributed mainly to release of [Met]enkephalin by kyotorphin, with some augmentation from the inhibitory effect of kyotorphin on enkephalin degradation.[72,74] The latter effect is seen when [Met]enkephalin is incubated with mouse brain homogenate,[72] but the protection of the enkephalin to degradation is weak, probably arising via interference with aminopeptidase action.

One analogue, Tyr-D-Arg, has so far been described and tested as an analgetic.[73] In the tail pinch test it is about 5 times more potent than kyotorphin, and the antinociceptive effect is of longer duration.

ENKEPHALIN PRECURSORS

Mechanisms controlling the biosynthesis and distribution of the enkephalins are of direct relevance to the role of enkephalins in pain pathways. The biosynthetic pathway is complex, but knowledge of the precursors and their processing is increasing rapidly. What follows is an attempt to summarize the present position.

Although it is established that β-endorphin is formed from the cleavage of pro-opiocortin, which also serves as a common precursor for corticotrophin, melanotropin, and β-lipotropin,[75] the enkephalins do not appear to arise via this route but, rather, from a different but common precursor (or precursors).[76,77] The main features of the structure of a 50K dalton adrenal proenkephalin and of several smaller but related proteins, as well as the full structures of several enkephalin-containing peptides in extracts of bovine adrenal medulla, are now known[78] through the work of the groups of Udenfriend and Matsus (see Figure 4 for details). Most if not all of the smaller proteins and peptides, as well as [Met]- and [Leu]enkephalin, are probably derived from the 50K proenkephalin. Within its sequence it contains both [Met]- and [Leu]enkephalin, flanked by pairs of Lys or Arg residues, and there are 6–7 copies of [Met]- to one copy of [Leu]enkephalin. Thus the [Met] : [Leu]enkephalin ratio found in whole brain (5 : 1 to 7 : 1)[86,87] is explained, and the occurrence of separate neurones for the two enkephalins is questioned. [Met]enkephalinyl-Arg-Phe, recently isolated from striatum,[89,90] may also arise from this 50K proenkephalin, but the 50K structure does not accommodate the various C-terminally extended forms of [Leu]enkephalin (e.g., α-neo-endorphin, Figure 5,[91,92] and dynorphin, Figure 6[93,94]) isolated in recent years. Either the 50K protein is not the primary product of translation of the enkephalin gene or there is more than one primary precursor. In the former case the primary precursor must be a protein of >50K daltons in which the 50K protein is extended at its C terminus. Since the [Leu]enkephalin sequence is N-terminal in the neoendorphins and dynorphin, and they have different structures, the > 50K protein must contain at least two more [Leu]enkephalin sequences. The largest brain proenkephalin yet isolated has a molecular weight of 90 Kdaltons and generates roughly equal amounts of [Met]- and [Leu]enkephalin after digestion with trypsin and carboxypeptidase B.[95] It therefore differs from the 50K adrenal protein, though the possibility still remains that the 50K adrenal protein is contained within its sequence.

Enkephalin precursors having at least one N-terminal enkephalin residue may themselves have interesting and distinct biological properties.

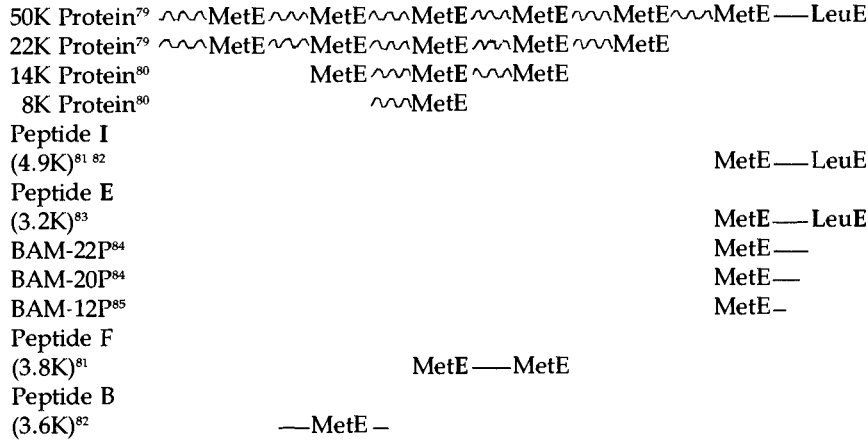


Figure 4 Schematic structures of 50K adrenal proenkephalin and the smaller proteins and peptides isolated from bovine adrenal medulla. The 50K and 22K proteins have not yet been isolated in pure form; the numbers of methionenkephalin (MetE) and leucinenkephalin (LeuE) residues were determined after sequential treatment with trypsin and carboxypeptidase B. Wavy lines indicate that the connecting sequences are unknown. The 14K and 8K proteins, and all the peptides shown, have been isolated in pure form; full structures of the peptides have been deduced, and their probable correspondence with parts of the structure of 50K protein is indicated by vertical alignment. Peptide I is an N-terminally extended form of Peptide E, and BAM-22, -20, and -12P are progressively C-terminally shortened forms of Peptide E. The nucleotide sequence of cDNA copies of the mRNAs for bovine adrenal proenkephalin has now been deduced, enabling unambiguous assignment of the protein structure.[231,232] The schematic structure shown in Figure 4 is confirmed, except that one of the MetE residues is to the right of the LeuE residue. As judged by the flanking with two basic residues, there are four copies of MetE, and one each of LeuE, MetE-Arg-Phe, and a hitherto undetected opioid peptide, MetE-Arg-Gly-Leu. The structure of human phaeochromocytoma proenkephalin has also been deduced, and shown to have a similar distribution of enkephalin sequences.[233]

Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Arg-Pro-Lys-Leu-Lys-Asn-Gln

Figure 5 Structure of dynorphin.

For example, Peptide E[83] and dynorphin (as the 1–13 sequence)[93] are 30–1000 times more potent than the enkephalins in the guinea pig ileum assay.

One complication that has just been revealed is the presence of [Tyr(SO_3H)^1,Leu^5] enkephalin in brain extracts.[96] It seems probable that some if not all of the [Leu]enkephalin in the 90 K protein is also O-sulfated, and it remains to be seen whether a similar situation applies to the 50K adrenal protein. Sulfated [Leu]enkephalin is inactive or only weakly active at the opiate receptor, but [Leu]enkephalin is rapidly generated by the action of arylsulfatases (which are widely distributed throughout the body). At this stage we can only speculate about the role of sulfation in the case of the enkephalins. It could perhaps control proteolytic cleavage of proenkephalins and thereby account for the differing [Met] : [Leu]enkephalin ratios (1 : 1 to 10 : 1) in different brain areas.[97] Or it might be a mechanism whereby the endogenous opiates are stored in an inactive form for generation, as required, by arylsulfatases.

Inactivation of opioid peptides can also be achieved by acetylation of the amino group of N-terminal tyrosine residues. Smyth[98] has demonstrated the existence of N-acetylated forms of β-endorphin and β-endorphin fragments in both the pituitary and brain. More recently, N-acetylated [Leu]enkephalin has been found in the neurointermediate pituitary but not in the brain.[99]

Sulfation, acetylation, and other posttranslational processing (e.g., amidation) may thus confer a much greater flexibility on neuropeptide systems than is seen with biogenic amines, by providing the ability to regulate the quantity of material available and its overall biological properties.

ENDORPHINS

The term *endorphin* is descriptive of any *end*ogenous substance with m*orphine*like activity, but is probably best applied in a more restricted

Tyr-Gly-Gly-Phe-Leu-Arg-Lys-Tyr-Pro-Lys

Figure 6 Structure of α-neo-endorphin.

sense to fragments of β-lipotropin which exhibit such activity.[100] Five, as free peptides or sometimes as N-terminally acetylated forms, have so far been identified in tissue extracts, the 61–65, 61–76, 61–77, 61–87, and 61–91 fragments, generally referred to, respectively, as [Met]enkephalin, α-, γ-, δ-, and β-endorphin (see Figure 7 for structures). There is a strong case to further restrict the definition to fragments derived from β-lipotropin by regulatory biochemical pathways. By this definition [Met]enkephalin is excluded, as well as [Leu]enkephalin and enkephalin precursors. We are left with β-endorphin and possibly α-, γ-, and δ-endorphin and certain of their N-acetylated derivatives.

β-Endorphin is the most potent species in all tests of analgesia. Unlike the situation with [Met] and [Leu]enkephalin, analgesia can be observed in animal models after peripheral administration of β-endorphin.[35] This was recognized soon after the isolation of β-endorphin,[101,102] and was followed by considerable effort to establish its presumed role in pain pathways.

We now know that a brain β-endorphinergic system exists, that it is not affected by hypophysectomy (indicating its independence from pituitary endorphins), and that it is distributed differently from the enkephalinergic system.[103] The precursor of β-endorphin is pro-opiocortin, a 31,000 (31K) protein.[75] As in the intermediate lobe of the pituitary, CNS neurones process 31K to β-endorphin and α-MSH (via ACTH). A 31K system probably concerned in pain pathways is centered within the arcuate nucleus; fibers project throughout the hypothalamus, amygdala, nucleus accumbens, ventral-lateral septum, periventricular thalamus, periaqueductal gray, and caudally to the level of the locus coeruleus.[104,105] Receptor binding studies have established that β-endorphin is recognized by brain opiate receptors,[103] and suggest that it interacts selectively with μ rather than δ types.[33] We are still far from understanding the relevance of endorphins in clinical pain, but significant correlations are

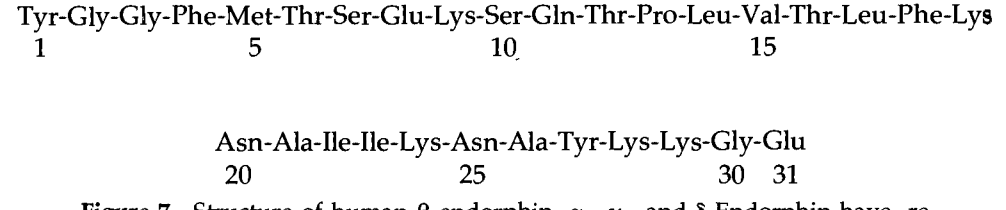


Figure 7 Structure of human β-endorphin. α-, γ-, and δ-Endorphin have, respectively, the 1–16, 1–17, and 1–27 sequences. Note that Tyr^{61} of β-lipotropin is now at the N-terminus of these peptides, hence is numbered Tyr^{1}.

emerging. In postoperative pain patients with low endorphin levels require more analgetic for pain relief than those with high levels.[106] There is also evidence to suggest that endorphin deficiency is of pathogenetic importance in chronic pain syndromes of neurogenic origin.[106] Electrically stimulated analgesia in humans is accompanied by a large increase in β-endorphinlike immunoreactivity in cerebrospinal fluid.[107–109]

The attention paid to analogues of β-endorphin has been modest. About 80 have been synthesized and tested in the guinea pig ileum (GPI), mouse vas deferens (MVD), mouse tail flick assays, or opiate binding tests. The results and structure-activity relations have been reviewed recently.[100] Human $_h$, porcine $_p$, and sheep $_s$ [≡ camel $_c$] β-endorphins appear to be equipotent in the GPI assay, and 4–4.5 times more potent than [Met]enkephalin (the structures of the pig and sheep species are similar to that of the human species except that β_p-endorphin has Val[23], His[27], and Gln[31], and β_s-endorphin has His[27] and Gln[31]). β_h-endorphin is about one third less potent than [Met]enkephalin in the MVD assay. Therefore the selectivity for the vas seen in the enkephalins is not a property of β-endorphin, since it is approximately equipotent in the two assays.

In the ensuing discussion of β-endorphin structure-activity relations reference to the original literature is generally omitted, but may be found in the review cited.[100]

The N-terminal pentapeptide sequence plays a crucial role in the activity of all endorphins, but there are interesting similarities and differences in SAR within this sequence as compared with the enkephalins. The similarities are seen at the 1 and 4 positions. Thus, as in the case of the enkephalins, an N-terminal residue of L-configuration seems essential for activity, as evidenced by the inactivity of the des-Tyr[1] and D-Tyr[1] analogues in the GPI assay, and there are also exact requirements at the Phe[4] position (the D-Phe[4] analogue has very low potency). The differences are most marked at the 2 position. Thus, whereas D-Ala[2] substitution in the enkephalins causes a marked increase in potency in both assays, similar substitution in β-endorphin causes increased potency only in the MVD assay and a small fall in potency in the GPI assay. Furthermore, it is inferred that the configurational requirements are different. [L-Ala[2]]β_c-endorphin has appreciable potency in the GPI assay, whereas [L-Ala[2],Met[5]]enkephalin is almost inactive. At the 5 position the change of Met to Leu in β_h- and β_p-endorphin decreases potency in the GPI, but has differing effects in the MVD assay. Thus Coy's group[110] reported that [Leu[5]]β_h-endorphin has about twice the MVD potency of β_h-endorphin, whereas Waterfield and co-workers[33] reported that

[Leu^5]β_p-endorphin has rather less than half the potency of β_p-endorphin. In contrast to the decreased potency of [Leu^5]β_h-endorphin in the GPI assay, the Nle^5 analogue of [Phe^{27}, Gly^{31}]β_h-endorphin (itself 1.28 times more potent than β_h-endorphin) has approximately the same potency as the Met^5 analogue. The D-Leu^5 and Pro^5 analogues of β_h-, and the D-Met^5 analogue of β_c-endorphin have very weak activity in the GPI assay; the change from Met^5 to D-Met^5, D-Leu^5, or Pro^5 in these analogues probably results in a sharper decrease in potency than do corresponding changes in [Met]enkephalin. Composite changes [e.g., D-Ala^2, Me-Phe^4, Met(O)^5] that provide enkephalin analogues of very high potency in the GPI assay, as well as unchanged or weaker potency in the MVD assay, have a reverse effect in the β-endorphin series; [D-Ala^2,Me-Phe^4,Met(O)^5]β_s-endorphin, for example, has one tenth the potency of β_s-endorphin in the GPI assay, but it is 2–3 times more potent in the rat vas deferens assay.

The effect of substitutions at other than the 1–5 positions has been little studied, and what limited knowledge we have is the result of work by C. H. Li and his collaborators using the GPI assay.[111] Their main findings are as follows. C-Terminal substitutions in β_h-endorphin may cause an increase in potency. Thus replacement of the C-terminal glutamic acid residue by glycine or glycinamide (Gly^{31} or Gly-NH$_2^{31}$ analogues) provides analogues that are, respectively, 1.68 and 2.0 times more potent than β_h-endorphin.[112] In the Gly^{31} analogue further replacement of the tyrosine residue at position 27 by phenylalanine causes only a small decrease in potency.[113] The resulting analogue, [Phe^{27},Gly^{31}]β_h-endorphin, is 1.28 times more potent than β_h-endorphin, and has been used to study the effect of other structural changes. In one study[113] the hydroxy groups of the threonine and serine residues at positions 6 and 7 were shown not to be of significance, since the [Ala^{6,7},Phe^{27}, Gly^{31}]analogue, in which both residues are replaced by alanine, is almost equipotent. Another study[114] examined the effect of reducing the conformation flexibility of the peptide chain by internal cystine bridges. For this purpose the alanine residue at position 26 and, in turn, the serine, glutamine, and leucine residues at positions 7, 11, and 17 were replaced by cysteine; oxidation of the resulting three dicysteine analogues provided analogues with internal cystine (disulfide) bridging. The 11–26 and 17–26 bridged analogues are respectively more potent and equipotent as compared with the parent analogue (1.86 and 1.28 times β_h-endorphin). However, the 7–26 bridged analogue has very low potency. It was concluded that the 7–26 bridging brings the C-terminal region of β_h-endorphin close to the 1–5 region, thereby impeding interaction of the latter with the opiate receptor.

Regarding shortened sequences, Ling and co-workers[115] examined various C-truncated analogues of β-endorphin, ranging from 1–5 (i.e. [Met]enkephalin) to 1–27, in the GPI assay and found a small progressive decrease in the potency. In the 1–27 transition (lacking the C-terminal tetrapeptide) to β-endorphin there was a 10-fold increase in potency. Li's group[116] confirmed this trend, but observed high potency in 1–26 and 1–21. Clearly, features at the C-terminus of β-endorphin make important contributions to the high potency of β-endorphin in the GPI assay, but the effect cannot be entirely attributed to the C-terminal tetrapeptide. The cutoff was even more pronounced in the tail flick test (compounds administered intracerebroventricularly); as compared with β_h-endorphin (=1), the potencies of 1–30, 1–29, and 1–28 were, respectively, 0.72, 0.20, and 0.06.[117] The role of the C-terminal feature, which accounts for the high ileal and *in vivo* potency of β-endorphin, is not known. It could be involved in additional binding at the receptor site, it could induce a more rigid conformation in the molecule which is reflected in improved affinity at the receptor site for residues 1–4, it could increase stability towards peptidases, or it could favorably affect transport of the molecule to the receptor compartment. The little evidence available suggests that it may function primarily in increasing the stability to peptidases.[100]

Opportunities similar to those for the enkephalins, but presently unexplored, exist for agents that stimulate the release of β-endorphin or prevent its deactivation. No rationale for such approaches has yet emerged, however. β-Endorphin is released from 31K by the action of trypsin, but this type of processing is not specific to 31K. It seems that N-terminal acetylation is a mechanism by which β-endorphin and certain of its fragments are deactivated,[98] but the physiological relevance of the mechanism is uncertain.

SUBSTANCE P

In addition to the work on opioid peptides, a second major area of current research, directed toward analgetics based on regulatory peptides, centers around antagonists of Substance P (Figure 8).

Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2
1 2 3 4 5 6 7 8 9 10 11

Figure 8 Structure of Substance P.

Substance P (SP) was discovered in 1931 by von Euler and Gaddam in alcoholic extracts of intestine and brain.[118] Attempts during the 1960s to purify the peptide were only partly successful,[119] and it was only after the realization[120,121] that it might be identical with Leeman and Hammerschlag's sialogogic peptide[122] (from bovine hypothalamic extracts) that pure material[123] became available. Structural elucidation[122,124] and synthesis[125] followed rapidly, ensuring adequate supplies for subsequent research.

We now know that SP from bovine hypothalamus, superior and inferior colliculi, dorsal roots, and equine intestine are structurally identical.[124,126] Indeed, the same SP may be present in all organs and species.[127] It is widely distributed in central and peripheral nerves, the GI tract, and many other tissues. Within the CNS the highest concentrations are found in the superficial layers of the dorsal horn in the spinal cord, in the trigeminal nerve nucleus, and in the substantia nigra, and the lowest concentrations are found in the cortex and white matter. There are numerous small SP-containing cell bodies in spinal ganglia at all levels and in trigeminal ganglia, and fine SP-containing nerve fibers are present in most peripheral nerves and tissues. Though the role of SP in the nervous system is still poorly understood and is likely to be complex, there is little doubt that it is involved in sensory transmission and pain perception. Current interest in antinociceptive agents based on SP evolves around the hypothesis that it acts as a transmitter of nociceptive sensory afferents. Evidence in support of this hypothesis has been presented in the first chapter, and has also been covered in several recent reviews (see Refs. 128–131). If the hypothesis is correct, several opportunities are presented for the design of new types of analgetics. For example, antagonists of the action of SP, depleters of SP synthesis, inhibitors of SP release, or promoters of SP degradation, may all be expected to exhibit analgesic activity. Most work is being directed toward antagonists of SP.

It should be appreciated that attempts to design antagonists of regulatory peptides have generally not been very successful. Since such endeavors are likely to be a major feature of future work with SP and other regulatory peptides, the general background and underlying principles are discussed separately at the end of this chapter. Against this background immediate successes in arriving at specific SP antagonists are encouraging.

The specific SP antagonists, of which three have so far been described,[132–134] are derived structurally from SP by multiple D-residue substitution (see Figure 9 for structures).

Pharmacological details remain sparse, but all three analogues are

Arg-D-Pro-Lys-Pro-Gln-Gln-D-Phe-Phe-DTrp-Leu-Met-NH_2

Arg-D-Pro-Lys-Pro-Gln-Gln-D-Trp-Phe-DTrp-Leu-Met-NH_2

DArg-D-Pro-Lys-Pro-Gln-Gln-D-Phe-Phe-DTrp-Leu-Met-NH_2

Figure 9 Structures of three antagonists of the action of Substance P: [D-Pro^2,D-Phe^7,D-Trp^9]substance P (**16**); [D-Pro^2,D-$Trp^{7,9}$]substance P (**17**); [D-Arg^1,D-Pro^2,D-Phe^7,D-Trp^9]substance P (**18**).

said to specifically and competitively antagonize the action of SP in the guinea pig ileum preparation even though they themselves do not have agonist effects; the ED_{50} dose of SP was increased 5 times in the presence of the D-Phe^7 analogues (**16** and **18**) and 30 times in the presence of the D-Trp^7 analogue (**17**) (all 10^{-4} M).[134] The D-Phe^7 analogues (**16** and **18**) (1–2 mg/kg IV) inhibited SP-induced salivary secretion in chloralose-anesthetised rats,[132,134] but the D-Trp^7 analogue (**17**) did not inhibit this response. The D-Phe^7 analogue (**16**) also blocked the vasodilator effects of SP and the vasodilation induced by antidromic nerve stimulation, without itself being vasoactive.[135] Do they block the CNS effects of SP and, in particular, do they have the expected antinociceptive properties? It seems so. The D-Trp^7 analogue (**17**) is reported to be an effective and specific antagonist of SP-induced excitation of noradrenaline-containing neurones of the rat locus caeruleus,[133,136] and there are also preliminary reports[137,230] that analogues **16** and **17** display antinociceptive effects in common tests for analgetics. We can expect rapid developments during the coming months as new and more potent antagonists are described.

Though the guiding rationale for this work is interference with SP's postulated role as a transmitter of nociceptive sensory afferents, it should be recognized that modulation of other SP pathways by SP analogues could occur. SP-habenulo-interpenduncular[138] and striato-nigral[139–141] pathways are now well-characterized, and proposed SP pathways include an intrinsic projection within the amygdala,[142] a diffuse ascending projection from the dorsal and median raphe nuclei to the telencephalon,[143] a descending projection from the raphe pallidus and raphe magnus to the spinal cord,[144] and a projection to the medial preoptic area from the interstial nucleus of the stria terminals.[145]

Apart from opiate-mediated inhibition of SP release, other opportunities remain unexplored. The analgesia resulting from intrathecal capsaicin may be the consequence of a series of events in which capsaicin

Table 5 Responses Following Central or Peripheral Administration of Substance P

	Tail Flick			
	Rat		Mouse	
	Central	Peripheral	Central	Peripheral
Stewart et al.[153]				
Oehme et al.[225]				
Malick and Goldstein[155]	↑ 0.3–10 μg IC (PAG)			
Starr et al.[226]				
M-Sørenssen et al.[227]	0 40 μg ICV or IC (PAG)			
Frederickson et al.[154]				
Scott-Mohrland and Gebhart[228]	↑ 1 μg IC (PAG)	↑ 100–400 μg IP		
Growcroft and Shaw[160]				
Hayes and Tyers[151]				
Oehme et al.[152]				

[a] ↑ Prolongation of reaction time or reduced writhing, i.e., antinociceptive effect.
↓ Decrease of reaction time or increased writhing, i.e., increased nociception.
○ No effect.

in Common Analgesic Tests[a]

Hot Plate				Mouse Writhing Test (acetic acid)		
Rat		Mouse				
Central	Peripheral	Central	Peripheral	Central	Peripheral	Notes
		↑ 2 ng IC	↑ 5 ng + 1 μg IP ↓ ~10 μg IV			Centrally administered into PAG (?). But see later paper[152]
						Administered onto the dorsal nucleus of raphe in midbrain PAG. Effect rapid (1 min), peaking at 3 min.
			↑ 1 + 5 μg IP			At 1 μg no effect at 30 min, but effect at 60 min. At 5 μg effect at 30 min, peaked at 60 min. Slight ↑ by PAG route only.
		↑ 1–5 ng ICV ↓ >50 ng ICV				Effect blocked by naloxone. Hyperalgesia when combined with naloxone. Analgesia when combined with baclofen.
↓ 1 μg IC (PAG)	○ 40–400 μg IP					Centrally administered into PAG. Conclude mouse more sensitive to SP than rat.
			○ ~1 + 5 μg IP			
↓ 10 ng–10 μg intrathecal		○ 2 ng–2 μg ICV				Algesic effect seen at 3 min, but no effect at 10 or 30 min (rat only). Effects seen by others due to sedative effects of SP.
			↓ 10–500 ng IV ↑ 1 + 10 μg IV		↑ 20–200 ng IV ○ ~1 μg IV	Although biphasic, responses at low doses in hot plate are the *reverse* of those seen by Frederickson. The difference may be due to use of animals with different control latencies.

Key to route administration: IC = intracerebral, usually into PAG (periaqueductal gray); ICV = intracerebroventricular; IV = intravenous; IP = intraperitoneal. Doses given are per whole animal (mouse usually ~25 g, rat 100–400 g or unstated).

Table 5 (Continued)

	Tail Flick			
	Rat		Mouse	
	Central	Peripheral	Central	Peripheral
Del Rio et al.[229]	↑* 0.1–10 μg ICV			
Lembeck et al.[230]			↓* 50 ng intraspinal	
Rosell[137]				
Doi and Jurna[156]	↑ 0.1–100 μg intrathecal			
Kotani et al.[157]				
Mészáros et al.[158]	↑* 0.5 μg IC			

rapidly liberates the majority of releasable stores of SP from primary afferent terminals and subsequently induces a prolonged permanent depletion of SP from primary, nociceptive, sensory neurons. However, there have been no systematic attempts to explore capsaicin analogues. It could also be that the analgesia is related to nonspecific damage to the spinal cord.[146] The degradation of SP in brain and other tissues, and the enzymic mechanisms involved have been reviewed.[147,148] An interesting recent development has been the isolation, from human brain, of a membrane-bound enzyme with properties that suggest it may be involved in the physiological inactivation of SP by neural tissues.[149]

A very recent development has been the finding that SP and CCK (see the following section) immunoreactivity coexist in a small population of central SP and CCK neurones (in the midline of the rostral, ventral periaqueductal gray).[223] It may also be that SP and CCK coexist in primary sensory neurons (SP and CCK fibers in the dorsal horn of the spinal cord show a marked degree of overlap, and both disappear after capsaicin treatment).[224] However, it should be appreciated that the vast majority of SP neurons in the rat CNS do not appear to contain CCK-like immunoreactivity.

Hot Plate				Mouse Writhing Test (acetic acid)		
Rat		Mouse				
Central	Peripheral	Central	Peripheral	Central	Peripheral	Notes
						*Using electrical stimulation of tail. Analgesia due to release of MetE? MetE antibodies but not β-endorphin antibodies inhibit effect. *Tail withdrawal.
↓ intrathecal						
		↑ 5 ng ICV	○ 10 mg IP	↑ 5 ng ICV	↑ 10 mg IP	
			↑ 0.25 + 0.5 mg IP			*Tail compression (squeak endpoint).

Finally, reference should be made to an apparent paradox. In mice or rats the same dose of SP can elicit either hyperalgesia[137,150–152,225,230] or analgesia.[152–158,228,229] Furthermore, since the analgesic effects have not been universally reproduced, considerable (and often heated!) argument has developed. For an objective consideration of this matter, the salient results are given in Table 5. It does not seem to be generally appreciated that, if care is taken to compare results in the same test, with the same species, and with the same route of administration of SP, the disagreements are not great (see Table 5). In the tail flick test, where responses are the result mainly of spinal events, analgesia has usually been observed following administration of SP to rats by all routes (including intrathecal), whereas hyperalgesia has been observed following intrathecal administration to mice. In the hot plate test, where responses are the result of more complex events, only hyperalgesia has so far been observed in rats, whereas analgesia or hyperalgesia (depending on the dose and method of administration) has been observed in mice. On the occasions when SP has been administered directly into the spine, hyperalgesia has been observed by Lembeck's group[230] (mouse tail flick test), Hayes and Tyers,[151] and Rossel[137] (rat hot plate test), and analgesia

by Doi and Jurna[156] (rat tail flick test). The main disagreements are (a) in the mouse hot plate test, after intraventricular administration of SP, the negative results (at all doses) of Hayes and Tyers,[151] in comparison with the analgesia (low doses) and hyperalgesia (high doses) observed by Frederickson's group,[154] and (b) in the mouse hot plate test after intraperitoneal administration of SP, the negative results of Growcroft and Shaw[160] in comparison with the analgesia observed by Stewart's group[153] and by Starr and co-workers[226]. The overall situation is clearly complex, and observed results are dependent on the species of animal and nociceptive reaction employed in testing, and the dose and route of administration of SP. Oehme and colleagues[161] suggest, with supporting evidence, that the action of SP on nociception depends on the condition of individual test animals, that is, the individual control response latency before SP treatment. Hyperalgesia would arise by direct excitation of nociceptive activity according to the mechanisms already discussed, whereas analgesia might arise indirectly via the tranquilizing effects of SP. Alternative possibilities are that analgesia might arise by excitation of descending SP pathways or via SP release of opioids, and that hyperalgesia might partly arise via SP release of histamine.

CHOLECYSTOKININ (CCK)

Vanderhaeghen and co-workers first described gastrinlike immunoreactivity in the brain,[162] but this was later identified as CCK-like immunoreactivity.[163,164] The confusion arose because CCK and the gastrins have a common C-terminal tetrapeptide sequence (CCK-4 or G-4, Figure 10), and Vanderhaeghen's antiserum, raised against gastrin, was directed against this tetrapeptide sequence. His antiserum hence cross-reacted with CCK.

There are, in fact, small amounts of true gastrins in the CNS,[164] but most of the immunoreactivity described as gastrinlike in Vanderhaeghen's work and subsequent papers is now known to be CCK-like.

CCK contains 33 amino acids. The 33-peptide species, CCK-precursors, and at least four truncated species contribute to CCK-like immu-

Trp-Met-Asp-Phe-NH_2

Figure 10 Structure of C-terminal tetrapeptide of CCK and the gastrins (CCK-4 or G-4).

Asp-Tyr(SO_3H)-Met-Gly-Trp-Met-Asp-Phe-NH_2

Figure 11 C-terminal octapeptide of CCK (CCK-8).

noreactivity,[163,165] but the most abundant species in the CNS is the C-terminal octapeptide (CCK-8, Figure 11).
An even smaller species may exist in large amounts, but it is debated whether this is CCK-4.[166,167] With this reservation CCK-8 immunoreactivity is interpreted as arising from CCK-8 in the subsequent discussion. As yet, CCK-8 is the only species that has been isolated from brain extracts.[168]

In the brain the total amount of CCK-8 is much higher than that reported for other peptides, and its regional distribution is unique in that the cortex contains the highest concentrations.[164,169] There is increasing evidence that CCK-8 is involved in central pain mechanisms:

1 CCK-8 is found in nerve terminals of primary afferent fibers in the dorsal horn,[170] and is present in several of the brain loci involved in nociception.[171,172] Specifically, the periaqueductal gray contains the most densely packed collection of CCK-8 cells, especially at the level of the exit of cranial nerve III.[171] The occurrence is primarily in synaptosomal vesicular fractions of these tissues.[173,174]
2 This distribution is generally matched by specific CCK receptors.[175,176]
3 CCK (as precursor) can be synthesized rapidly and in large amounts by brain tissue.[177,178]
4 During superfusion of brain slices and synaptosomal preparations, depolarization induces a calcium-dependent release of CCK.[179–181]
5 Application of fmol amounts of CCK-8 (and CCK-4) to the postsynaptic membrane of hippocampal neurons causes marked excitation within a few seconds.[182] CCK-8 also excites neurons of the cortex, spinal cord, and hypothalamus.[183,184]
6 Of the regulatory peptides that decrease the reflex response of the rat to noxious stimuli, after injection into the PAG or subarachnoid space, CCK-8 is by far the most potent (400–700 times more than morphine). Caerulein (ceruletide), which structurally may be regarded as a simple derivative of CCK-8, is even more potent (4000–7000 times more than morphine).[185]
7 Caerulein is analgesic in man in biliary and renal colic[186] and in cancer pain[187] after intravenous injection.

Altogether this is convincing evidence that CCK-8 pathways are involved in nociception and nociceptive responses at both spinal and supraspinal sites.

A feature of CCK/caerulein-induced antinociception is that it is naloxone reversible,[185] suggesting the involvement of opiate receptors. Could, therefore, the analgesia arise via CCK-induced release of an endogenous opioid peptide? β-Endorphin levels do indeed rise after intravenous infusion of caerulein in man,[188] but in mice there is little or no cross-tolerance between the analgesic actions of CCK-8 or caerulein and morphine.[189] Thus it is unlikely that the analgesic effects of CCK/caerulein are mediated via direct interaction with known opiate receptors, or by release of endogenous opioids.

Zetler[190] suggests that the CCK receptor and the opiate receptor are located in adjacent parts of the same protein molecule, permitting allosteric interactions when either is occupied. This speculation arises from analysis of *in vitro* results using the guinea pig ileum preparation. CCK-like peptides are thought to stimulate the ileum by release of acetyl choline on intramural postganglionic neurons. Morphine and opioids, which are known to inhibit release of acetyl choline, antagonize this effect in a way that suggests competitive interaction. Naloxone blocks the antagonistic effect of morphine and opioids, but has no effect on the CCK response. So, as originally conceived,[190] the hypothesis implies that occupation of the opiate receptor by morphine or opioids, but not by naloxone, causes an allosteric change in the adjacent CCK receptor. To explain naloxone-reversible, CCK-induced analgesia,[185,189] we must postulate that interaction of the corresponding CNS receptors is also possible after occupation of the opiate receptor by naloxone; thereby occupation of central opiate receptors by naloxone (but not morphine?) prevents activation of adjacent CCK receptors by CCK. The speculation remains interesting, but uncorroborated.

Zetler also describes analgesic effects after subcutaneous administration of CCK-8 or caerulein to mice.[189] The molar potencies of caerulein in the hot plate (53°C, jump response as endpoint) and writhing tests (acetic acid as noxious agent) were, respectively, 114 and 15 times those of morphine, and the effects peaked at 15 minutes and lasted for 1 hour. CCK-8 was 3–10 times less potent than caerulein. The dose of naloxone required to antagonize the effects was considerably lower than that required to antagonize the analgesic effect of enkephalins and other neuropeptides. Surprisingly, since the antinociceptive effects described previously after central administration of CCK-8/caerulein were obtained using the rat tail flick test, neither CCK-8 nor caerulein was active in the mouse tail flick test.

Table 6 Central Effects[a] of CCK-8, Caerulein, and Analogues in Mice[191]

Caerulein = Glp-Gln-Asp-T yr(SO$_3$H)-Thr-Gly-Trp-Met-Asp-Phe-NH$_2$
1 2 3 4 5 6 7 8 9 10

CER-(4-10) = H-Tyr(SO$_3$H)-Thr-Gly-Trp-Met-Asp-Phe-NH$_2$
1 2 3 4 5 6 7

Tyr(SO$_3$H)-CCK-4 H-Tyr(SO$_3$H)-Trp-Met-Asp-Phe-NH$_2$

	Analgesia[b]	Ptosis[c]	REA[d]	PIC[e]	MIG[f]
Caerulein (CER)	100	100	100	100	100
CCK-8	30	56	6	25	30
Desulfated-CER	<3	0	0	0	0
[Nle]^{8}CER	36	200	78	173	409
[Val^5,Nle^8]CER	64	80	29	100	94
[Met(O)^8]CER	8	4	7	46	94
[β-Asp^9]CER	4	6	7	21	68
Des-amide^{10}-CER	0	0	0	0	0
Boc-[Leu^5]CER-(4-10)	28	14	14	45	55
Boc-[Nle^5]CER-(4-10)	23	27	33	166	298
[Nle]^{5}CER-(4-10)	26	11	5	8	254
Tyr(SO$_3$H)-CCK-4	0	0	0	0	0

[a] Potencies relative to caerulein = 100 in the five tests specified following subcutaneous administration (calculated from ED_{50}s).

[b] Delayed reaction to nociception in the hot plate test.

[c] Production of ptosis (eyes half-closed).

[d] Inhibition to exploratory rearing activity.

[e] Increase in picrotoxin convulsive threshold dose.

[f] Inhibition of methylphenidate-induced gnawing.

A major concern in the development of new central analgetics based on CCK-8 is that it may not be possible to dissociate analgesic from other central and peripheral effects of CCK-8. There are, however, grounds for believing that such dissociation is achievable in analogues. Zetler[191] examined CCK-8, caerulein, and 10 analogues in five tests of central activity (see Table 6). All the tests were on mice, and all test compounds were administered subcutaneously. It was concluded that the general

features of structure-activity relations deduced for peripheral CCK-like effects also apply to central effects. Thus desulfation (desulfated-CER analogues), deamidation (des-amide^{10}-CER analogue), and switch of the tyrosine-O-sulfate residue two places nearer to the C terminus [Tyr(SO_3H)-CCK-4 pentapeptide analogue] cause loss of, or greatly reduced, activity in all tests. However, these broad conclusions may poorly reflect structure-activity relations at the receptor level. When administered centrally in rats, desulfated caerulein is an exceedingly potent analgetic (potency one fifth that of caerulein, i.e., equal to that of CCK-8). Could, therefore, the low analgesic potency of the desulfated species after peripheral administration arise from its poor ability to gain access to central receptors? This implies that sulfated species are more able than desulfated species to gain access to central receptors after peripheral administration, but that the two species differ little in their ability to interact with central receptors. Receptor binding studies should provide an answer to this question. Alternative explanations of the activity of centrally applied desulfated caerulein, for example, that it is metabolized to caerulein by the CNS, seem less attractive.

Returning to the results given in Table 6, we see that an interesting dissociation of central effects is seen even with the limited number of analogues yet examined. Thus, as compared with caerulein, the analogue in which Met is replaced by norleucine, [Nle^8]caerulein, has one third analgesic potency, but 2–4 times potency in the ptosis, antipicrotoxin, and antimethylphenidate tests. Selectivity toward analgesic action is most pronounced with CCK-8 and [Nle^5]CER-(4-10), but even these two compounds, which have almost the same analgesic potency, show a dissimilar profile of activity in the other four tests. Thus within CCK-like peptides there seems considerable scope for modulation of different types of central activity by structural modification.

OTHER REGULATORY PEPTIDES

Other regulatory peptides are also implicated in pain pathways, but this knowledge has not yet stimulated specific research toward new analgetics.

Neurotensin (Figure 12), a tridecapeptide isolated from bovine hypothalamus, is one.[192] Studies of apparently pre- and postsynaptic neurotensin systems in the brain have given increasing support to a neurotransmitter or neuromodulatory role for neurotensin in the CNS. The presence in the brain of functional neurotensin receptors is supported

Glp-Leu-Tyr-Glu-Asn-Lys-Pro-Arg-Arg-Pro-Tyr-Ile-Leu

Figure 12 Structure of neurotensin.

by receptor binding studies,[193-195] the changes in local neuronal firing patterns[196] and neurochemistries,[197] and the global physiological and behavioral effects[198,199] that accompany central administration of neurotensin. Neurotensin immunoreactivity is localized to neuronal perikarya, fibers, and nerve terminals,[200] and is released from rat hypothalamic slices by potassium depolarization.[201]

Progress is also being made in tracing neurotensin circuitry across the neuroaxis. Dense neurotensin immunoreactivity is found in the substantia gelatinosa zones of both the spinal cord and the trigeminal nuclear complex, suggesting that neurotensin-containing systems might have a modulatory influence on nociceptive input.[202] In support of this role centrally administered neurotensin has antinociceptive activity in the mouse hot plate test (minimum effective dose, 25 ng), and acetic acid writhing test (minimum effective dose, 0.25 ng).[203] In the hot plate test the potency was approximately the same as that of [D-Ala^2,Met-NH_2^{5}]enkephalin, and doses of 250 ng gave responses lasting for about 1 hour. These effects were not seen after intravenous injection of neurotensin.

Most of what has been said in support of a neurotransmitter/neuromodulatory role for neurotensin can also be said of *somatostatin* (Figure 13), a tetradecapeptide isolated from sheep hypothalami.[204] The criteria of Barchas and co-workers[205] are, however, not fully satisfied, notably because (*a*) we have little evidence of the mechanisms of biosynthesis or inactivation of either peptide in the nervous system, and (*b*) the release of somatostatin from nerves following stimulation of afferents, and the correlation of endogenous release and exogenous application with biological effects have not yet been studied. A major problem is the lack of specific agonists, synthesis inhibitors, or receptor antagonists. Elegant chemical work with somatostatin analogues has enabled a good understanding of structure-activity relations, however, and has provided a conformationally restrained hexapeptide analogue of high potency.[206] In contrast to pathways originating from the hypothalamus, other somato-

Ala-Gly-Cys-Lys-Asn-Phe-Phe-Trp-Lys-Thr-Phe-Thr-Ser-Cys
1 2 3 4 5 6 7 8 9 10 11 12 13 14

Figure 13 Structure of somatostatin.

statin-containing pathways in the CNS remain poorly defined at present. Of main interest to the present discussion is a system of primary sensory neurons containing immunoreactive somatostatin. Dorsal root ganglia contain small somatostatin cells, distinct from those containing Substance P, and somatostatin fibers are visible in the dorsal horn of the spinal cord (with the highest concentration in lamina II).[207] There are also somatostatin fibers in the gray matter around the central canal (lamina X), the intermedio-medial nucleus, and the lateral column (lamina VI and VII).[208] Somatostatin is active in the (electrically stimulated) guinea pig ileum test,[209] and interacts weakly with opiate receptors.[210,211] There have been no reports of antinociceptive effects following central administration of somatostatin, but several of the behavioral effects elicited have been described as similar to those of morphine.[212]

GENERAL OPPORTUNITIES AND THE DESIGN OF ANTAGONISTS

The general opportunities for design of new analgetics based on regulatory peptides are now clear. If it is established or surmised that a peptide is a neurotransmitter or neuromodulator of inhibitory pathways in pain mechanisms, (e.g., the enkephalins), new analgetics may result from the design of analogues that are more potent agonists, more selective in action, metabolically stable, or have other desirable pharmacokinetic properties. If the status is established or surmised in sensory or other recognition pain pathways (e.g., SP), new analgetics may result from the design of antagonists of the action of the peptide. Stimulators/inhibitors, as the case may be, of the synthesis, release, processing, activation, or deactivation of such peptides may also lead to analgetics.

In the first circumstance the considerable progress in the design of more potent, metabolically stable analogues of peptides is well illustrated by the work with enkephalins. There has also been good progress in designing "selective" analogues, that is, analogues which, compared with the parent peptide, have different relative potencies in various tests characteristic of multiple actions of the parent (see Refs. 213–215, and preceding sections on multiple opiate receptors and CCK). In the second circumstance, when the peptide is involved in recognition pathways, less success has attended attempts to design antagonists of the receptor interaction of peptides (see Refs. 213–215). Because such endeavors are likely to be a major feature of future work with SP and other regulatory peptides, the general background and underlying principles are now discussed.

When judged by acceptable pharmacological criteria, successes in attempts to design receptor antagonists of regulatory peptides are limited to gonadoliberin (GnRH), angiotensin II, oxytocin (oxytocic responses), [Lys]- or [Arg]vasopressin (pressor and behavioral responses), and SP. For structures of the antagonists that have evolved, see recent reviews[213–215] and the section of this chapter on SP antagonists. Failures include the cases of gastrin, CCK, the enkephalins, somatostatin, neurotensin, and bradykinin. What have been the underlying concepts in the attempts? Are they valid, or do they need modification?

There are two principal models of the action of regulatory peptides at the receptor level. In one, the *participation model*,[216,217] it is envisaged that the peptide agonist occupies binding sites at the receptor, and also supplies a chemical group essential for a chemical event associated with activation of the receptor (proposed, e.g., in the case of gastrin/CCK). In the other, the more popular *allosteric model*,[218] it is envisaged that agonist-receptor binding alone can cause activation by inducing a critical displacement of the normal conformation of the receptor. Intrinsic activity (or efficacy) is therefore related to the presence of a specific chemical group in the agonist in the participation model, and to the ability of the agonist to induce favorable conformational changes in the allosteric model.

With few exceptions the search for antagonists of regulatory peptides has been directed toward analogues, compounds that bear an obvious chemical relationship to the agonist. It is argued that the agonist-receptor interaction involves a number of subsites, and it is possible to arrive at analogues that still possess affinity for all or some of these subsites, but lack intrinsic activity. Such analogues would be competitive antagonists of the action of the parent agonist. Lack of intrinsic activity may arise if the analogue lacks a chemical group essential for the action of the agonist (participation model), if the analogue-receptor binding interaction causes unfavorable conformational changes in the receptor molecule (allosteric model), or if the interaction prevents access of the agonist to at least one of its binding sites (applicable to both models).

Consider in this context the receptor, multisubsite model of De Lean, Munson, and Rodbard,[219] which satisfactorily explains the normal and abnormal dose response curves seen with agonists and antagonists, as well as many phenomena observed in receptology. Ligand-receptor binding is considered to involve interaction of discrete regions of the ligand molecule with complementary receptor "subsites." In the simplified case of a divalent ligand interacting with two subsites (Figure 14), binding to the two subsites may involve one ligand molecule (represented by the three states P1, P2, and P3) or two (represented by the

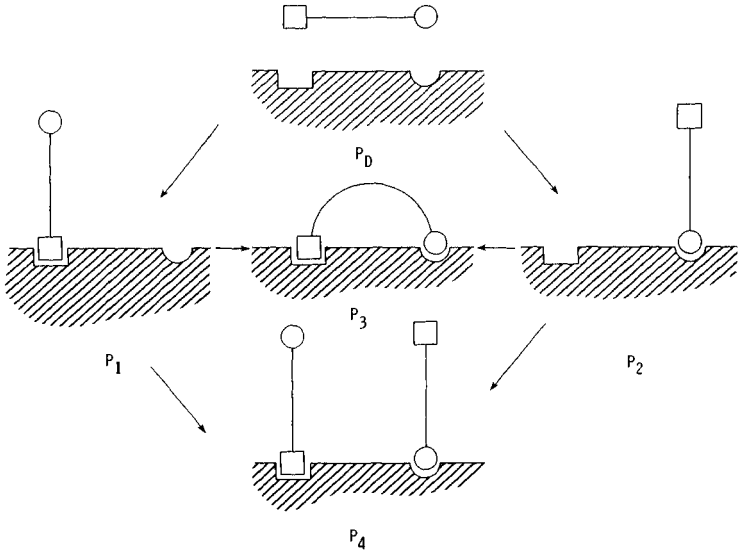


Figure 14 Binding reaction scheme of De Lean and co-workers[219] for a divalent ligand interacting with two subsites. In this and subsequent figures the two binding sites of an agonist are shown as an open circle or square.

state P4). Only those ligands able to form the "active state" P3 serve as agonists. We are concerned with circumstances in which ligand binding prevents formation of this active state. The previous comments about participation and allosterism relate to this model as follows. If participation pertains, analogues of an agonist which are also capable of binding to both subsites may be antagonists if they are unable to supply (i.e., lack) the missing chemical group (Figure 15). The most explored case based on participation is that of gastrin, where from structure-function studies the carboxyl group of the penultimate aspartyl residue (and therefore present in CCK-8) was identified as a candidate for such a role. Analogues lacking this carboxyl group were not agonists, but they were also not antagonists! The imidazole group of the His^2 residue of GnRH may also participate in the same sense as gastrin's carboxyl group, in which case the action of GnRH antagonists (which all lack His^2)[220] is explained. If allosterism pertains, analogues utilizing both subsites may be antagonists if they induce conformational changes in the receptor molecule which are only marginally different from those induced by the agonist (Figure 16). However, inspection of the structure of known SP

Figure 15 Possible mode of action of antagonists utilizing two agonist subsites (participation model). The agonist (*a*), in occupying both subsites, supplies a chemical group (carboxyl shown) which is essential for a chemical event associated with activation. Hypothetically, if analogues can still occupy both subsites but lack the activating chemical group, they may act as antagonists (*b*).

antagonists, for example, leads one to the conclusion that it is difficult to imagine them acting in this way.

More interesting circumstances arise when only one of the subsites is utilized by an analogue. Here the state is represented by P4 (Figure 14), but instead of both subsites being occupied by agonist molecules, one is occupied by agonist and the other by antagonist (Figure 17). Clearly, if this state is favored, formation of the active state P3 (Figure

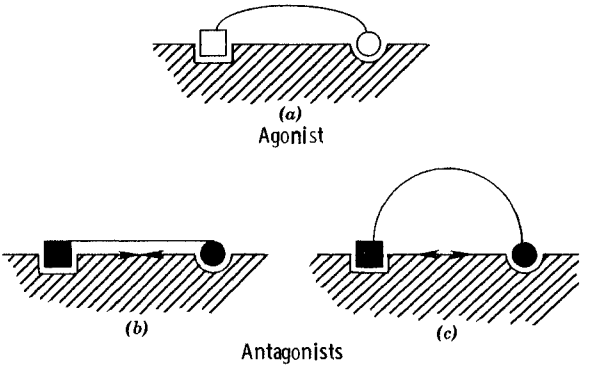


Figure 16 Possible mode of action of antagonists utilizing two agonist subsites (allosteric model). The agonist (*a*), in occupying both subsites, causes a critical displacement of the conformation of the receptor, which is necessary for activation. Analogues that occupy the same subsites, but cause less displacement (*b*) or more displacement (*c*), may act as antagonists.

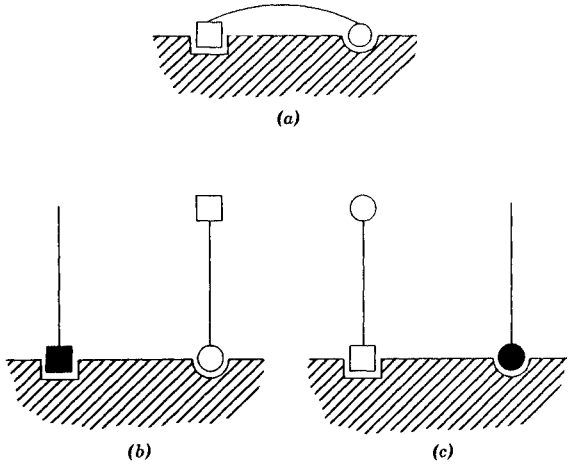


Figure 17 Possible mode of action of antagonists utilizing one agonist subsite. In the P4 states of De Lean and co-workers'[219] model (Figure 14), one of the subsites is occupied by the agonist (the open circle and square represent its two binding sites), and the other by an analogue *(b,c)*. The analogue is capable of binding to one subsite but not to the other; therefore it cannot form the active P3 state *(a)*. If a P4 state is stabilized, the agonist is prevented or hindered from forming the P3 state, and antagonism results.

14) is prevented, and antagonism results. The crucial question is *how* the P4 state can be favored, bearing in mind the strong forces driving the agonist to displace the analogue with generation of state P3. Rodbard's group[219] suggests that it occurs by means of *cooperativity*, which they define as "any mechanism by which the affinity of a ligand for some of the subsites is changed by occupancy of other subsites." Cooperativity in the P4 state is positive if sites of the agonist and analogue molecules *other* than those involved in receptor subsite binding can mutually interact (see Figure 18). This corresponds with the "side-side" interactions discussed by Lindeberg and colleagues.[221] For example, using the simplified two subsite model, we can hypothesise that the two subsites for SP involve binding to N- and C-terminal features of the SP molecule. If SP antagonists utilize only the subsite for N-terminal binding, then antagonism may result from interaction of lipophilic regions of SP and antagonist molecules, with resulting stabilization of state P4.

A neglected aspect of peptide antagonist design deserves final comment. There have been few attempts to utilize binding subsites *other*

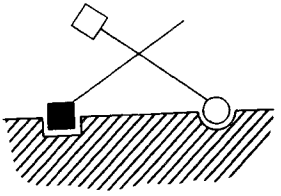


Figure 18 Stabilization of a P4 state by interaction of agonist and analogue at sites other than those involved in receptor subsite binding (e.g., lipophilic regions of the two molecules).

than those involved in agonist binding, despite evidence[222] that antagonists of monoamine transmitters act in this way. Again referring to the two subsite models, binding to only one of the agonist subsites may be stabilized not by cooperativity, as defined, but by additional binding to a third (accessory) subsite. This third subsite may be part of the receptor molecule different from that involved in agonist binding, or part of a different molecule (Figure 19). Furthermore, antagonism is possible with compounds that do not utilize any of the agonist subsites. Binding of such compounds to accessory subsites only could effectively block access of the agonist to its own subsites (Figure 20). Antagonists acting by these mechanisms will chemically resemble corresponding agonists only if at least one of the agonist subsites is being utilized. Even then the resemblance will apply only to parts of the molecules. The high lipophilicity of monoamine transmitter antagonists, and their greatly increased af-

Figure 19 Stabilization of a P4 state by utilization of a third, accessory subsite. Binding of the analogue only is shown. The analogue is capable of binding to one of the agonist subsites, and also to a third subsite represented as a V cleft. The third subsite may be located in an adjacent part of the receptor (*a*) or in a different associated molecule (*b*).

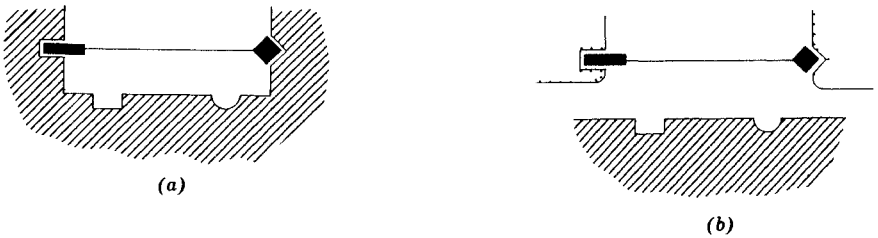


Figure 20 Possible mode of action of antagonists not utilizing agonist subsites. The antagonist shown is incapable of binding to either of the agonist subsites (represented, as in preceding figures, by half-square and half-circle clefts), but can bind to third and fourth accessory subsites (represented as V and half-rectangle clefts). The latter may be located in adjacent parts of the receptor (*a*) or in a different, associated molecule (*b*). It is postulated that access of agonist to its subsites is effectively blocked by the interactions shown.

finity as compared with corresponding agonists, suggests that the accessory subsites are located in lipid molecules closely associated with membrane receptor proteins. Their identification would provide a new basis for antagonist design. Accessory subsites could be nonspecific, that is, defined by physical properties as distinct from structure. However, it seems probable that the lipid molecules we are concerned with will adopt conformations determined by the neighboring receptor protein, leading to subsites with high specificity.

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Medicinal Chemistry of Central Analgetics

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HISTORICAL PERSPECTIVE

The chemistry involved in the search for an effective analgetic agent that is free of addiction potential or other side effects in many ways epitomizes the history of much of medicinal chemistry. As is often the case, the original lead came from folk usage. Sometime in antiquity the sedative and euphoriant properties of the dried sap from the immature seed pod of the opium poppy *(Papaver somniferum)* were discovered. By the time history came to be recorded, this natural product, now known as *opium*, was well established as a therapeutic and recreational drug. This substance was usually taken either orally or by smoking in a pipe. It was recognized much later that the compounds in opium responsible for its biological effects are in fact poorly absorbed by either of these routes. The net result was that sedative activity predominated and that at a low level of efficacy; the poor absorption also limited the addiction liability of the mixture. The invention in the eighteenth century of primitive means of parenterally administering compounds permitted application of the drug by a route that allowed a far better expression of activity. It may thus be speculated that the analgetic and sharp euphoriant activities of opium depended on the invention of the hypodermic syringe for their discovery. Recognition of the drug's propensity to cause profound physical dependence cannot have lagged too far behind.

Though the principal active compound from opium was isolated as early as 1803 (Sertuner), organic chemistry was in far too embryonic a form for the event to have any therapeutic impact. It is of interest to note that the name given to the compound—morphine—emphasizes its sedative activity rather than its analgetic properties. The complexity of the structure delayed the assignment of the correct structure for well over a century after its isolation. It is of note, however, that two drugs were produced by chemical manipulation of morphine before its structure was known. Thus diacetyl morphine—known today as heroin—was synthesized in 1884; reduction of naturally occurring codeine (morphine 3-methyl ether) afforded dihyrocodeine, a drug used for some time as an antitussive agent.[1]

The now accepted structure for morphine was proposed by Gulland and Robinson in 1925[2] and independently by Schopf in 1927.[3] Prior to today's availability of a plethora of spectroscopic methods for structural elucidation and confirmation of structure, it took a total synthesis to confirm the structure of a natural product possessing any degree of complexity. This was accomplished for morphine by Gates and Tschudi in 1952;[4,5] 2 years later Elad and Ginsburg announced the preparation of an intermediate that had been converted to morphine.[6]

The accumulated knowledge of that time on the chemistry and biological effects of morphine and codeine suggested that chemical modification might lead to a drug with better oral activity and decreased addiction potential. An experimental program was then set up in 1929 under the auspices of the U.S. National Research Council at the Universities of Michigan (N. B. Eddy) and Virginia (L. F. Small, A. Burger, and E. Mosettig) to pursue just that goal. This group and its successors established the SAR of systematic modification of the morphine molecule.

The serendipitous discovery that a compound designed as an antispasmodic agent (meperdine) in fact showed potent analgetic activity in the clinic showed the way to the preparation of synthetic antinociceptive agents with simplified structures. This discovery of Eisleb's[7] on the eve of World War II was to profoundly influence the direction of research in this field. During that war research in Germany resulted in the ultimate simplication of the morphine structure with the preparation of an acyclic analgetic, methadone.[8]

Research toward syntheses patterned after biogenetic routes to the morphine carbon skeleton led Grewe to develop the elegant method that bears his name.[9] This scheme and others that evolved from it permitted relatively easy synthetic access to molecules lacking only the furan ring of morphine (morphinans) or lacking that ring as well as one of the alicyclic rings (benzomorphans). A number of clinically useful drugs have been obtained subsequently by appropriate modification of these nuclei.

Systematic study of the SAR of these various series suggested that the structural element required for analgetic activity could be stated rather succinctly. As noted by Beckett and Casy, all active compounds had in common an aromatic ring attached to a quaternary center that was substituted by the equivalent of an ethylene chain bearing a basic amine.[10] This generalization withstood the test of time quite well. Several recent reports of very potent analgetics that depart from the rule suggest that the SAR requirements may be in need of refinement.

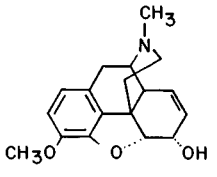
Early work on analgetics was quite successful in modifying the potency of various compounds. The goal of oral activity too could be met by appropriate chemical modification. Abolition of addiction potential proved to be a more elusive goal. Most of the compounds obtained showed only slight, if any, improvement in this liability over morphine itself. The empirical observation that a drug which was an antagonist to morphine, and would thus be expected to be nonaddicting, nalorphine (N-allylnormorphine), showed analgetic activity in the clinic,[11] opened the way to the preparation of compounds with reduced addiction potential. A concerted effort was thus mounted in several laboratories to design a drug that would show in a single molecule the apparently paradoxical properties of analgetic activity and morphine antagonism. This approach recorded its first success in pentazocine.[12] The somewhat limited analgetic efficacy shown by that drug has acted as a spur to continued research aimed at the development of nonaddicting central analgetic agents. The bulk of current research is in fact aimed at the development of molecules that offer the proper combination of antinociceptive and narcotic antagonist activity.

It has been accepted for almost 50 years that analgetics interact with some specific receptor, more on inference from observed changes in biological activity by structural modification than on any direct evidence of drug-receptor interaction. The emergence of the powerful technique of receptor binding assays has culminated in recent demonstrations that the original premise was based on fact. More recent work in the area has led to the suggestion that there are actually distinct populations of opiate receptors; each of these has been shown to exhibit different affinities for distinct opioid structural classes. It would be very illuminating if the following discussion could have been cast in terms of γ, μ or κ receptors. Since the bulk of the work antedates these very recently posited concepts, no attempt has been made to recast conclusions in light of recent findings. Instead, we have chosen to express biological activity strictly in terms of the classical whole animal analgetic endpoints.

MORPHINE AND ITS DERIVATIVES

The major alkaloid present in opium is the product of benzylisoquinoline cyclization, morphine **(1)**; this compound is accompanied by a lesser amount of its 3-methyl ether, codeine **(2)**. One of the minor constituents of opium, thebaine **(3)**, is of considerable importance as a starting material for some of the more recent analgetic drugs based on the full

morphine nucleus. Demand for this compound has led to the identification of sources far richer in this alkaloid than in opium. Not surprisingly, assignment of the absolute configuration trailed the assignment of the structure by almost a quarter century.[13] As noted earlier in this volume, morphine has been shown to exhibit high affinity binding for a receptor found in mammalian tissue. It is usually assumed that such binding will show stereochemical preference for one isomer of a chiral pair. It has indeed been demonstrated that the epimer of morphine, obtained in a multistep synthesis starting from sinomenine, is essentially devoid of activity.[14]



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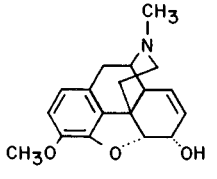

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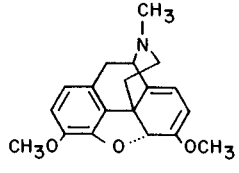

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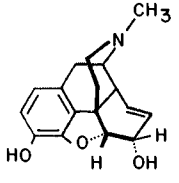

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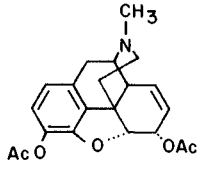


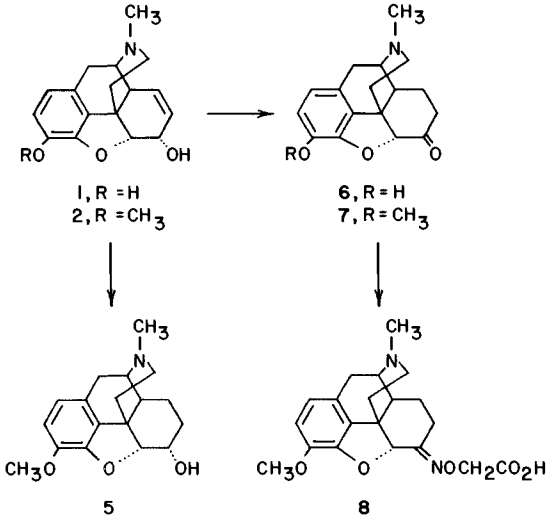
4

Most early attempts to prepare central analgetics superior in their therapeutic properties to morphine involved modification of the natural product, an approach dictated by the complexity of the molecule as well as its relatively ready availability. As noted above, acetylation affords the diacetyl derivative. This compound **(4)**, now far better known as heroin, has proven if anything to be a step backward from morphine as far as its therapeutic utility is concerned. Its extensive use on the street derives at least in part to the sharp feeling of euphoria (rush) experienced on intravenous administration.

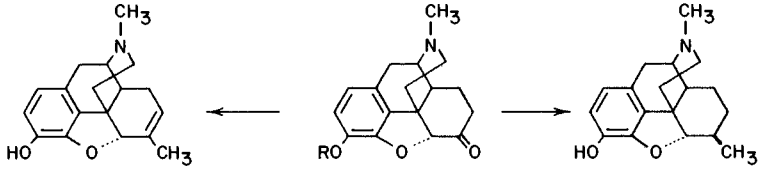
Codeine is used extensively in various applications that require an orally effective, relatively low potency central analgetic. (Its potency in man is about one tenth that of morphine when both compounds are

given by injection.) Though the compound is a natural product found in opium, it is present in relatively low concentration compared to morphine. It has thus proven more practical to obtain this drug by methylation of the phenolic hydroxyl of morphine.

Formal reduction of the double bond in codeine leads to dihydrocodeine **(5)**, a compound that has found some use as an antitussive agent. Rearrangement of the allyl alcohol function present in codeine to the vinyl alcohol, and thus in effect a ketone, in strong acid gives the formal oxidation product **(6)** of dihydrocodeinone. Demethylation of the phenolic ether in the product, hydrocodone **(6)**, affords the corresponding morphine derivative, hydromorphone **(5)**.[15] Both these compounds show a three- to fourfold gain in analgetic potency in man over the respective natural products. This gain in potency is paralleled by an increase in addiction liability, however. Derivatization of the ketone with O-(Carboxyethyl)hydroxylamine affords codoxime **(7)**, a compound said to have more specific antitussive activity.[16]

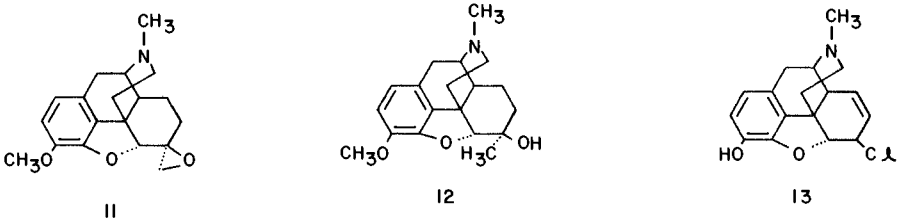


The observation that oxygen in the allycyclic ring is not absolutely essential for good activity foreshadows the SAR of the benzomorphans. Thus both saturated **(9)**[17] and unsaturated **(10)**[18] 6-deoxy-6-methylmorphine derivatives show good analgetic activity.



10 9

Later work confirmed that modification of the substituent at position 6 has relatively minor effects on biological activity. Thus oxirane **11** (obtained by reaction of codone with dimsyl sodium) and the carbinol obtained on reduction show analgetic potency in the range of codeine.[19] Interestingly, replacement of the hydroxyl by chlorine **(13)** leads to a compound that is an order of magnitude more potent than morphine.[20] The products of allylic substitution by halogen **(14–16)** on the other hand show potency in the range of morphine.[20] Displacement of the hydroxyl (as its tosylate or mesylate) in dihydromorphine with azide affords the so-called azidomorphine.[21] This agent **(17)** exhibits potency about 13 times that of morphine in animal models and is reported to have reduced addiction liability. This agent is said to be 40–50 times more potent than morphine in man.[22]

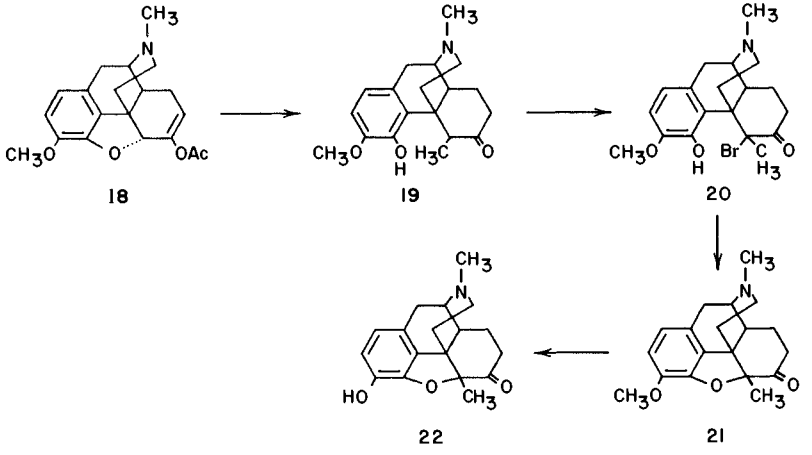


11 12 13

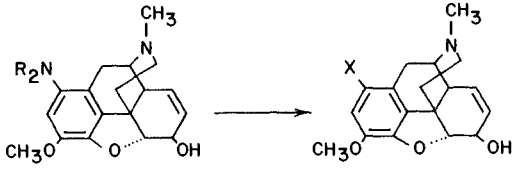
14, X = Cl
15, X = Br
16, X = I

17

Quite early in the research on the morphine molecule it was found that relatively deep-seated changes are consistent with good biological activity. Nuclear alkylation affords methyldihydromorphinone (**22**, metopon), a drug noted for reduced emetic and respiratory depressant side effects. In addition this molecule is more bioavailable on oral administration than is morphine. Key to the sequence that leads to this compound is reaction of the enol acetate of dihydrocodone (**18**) with methylmagnesium iodide. On workup this affords the product of displacement of an allylic phenol ether (**19**). The stability of the latter as a leaving group may provide the driving force for the reaction. Bromination (**20**) followed by base-catalyzed cyclization restores the furan ring (**21**). Demethylation of the phenolic ether affords metopon.[23,24]



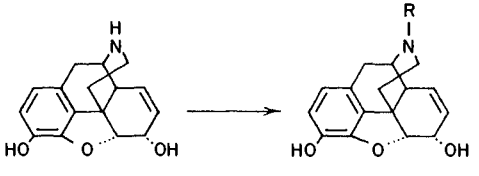
Introduction of additional substituents onto the aromatic ring has not proven particularly fruitful in modifying activity in the morphine series. Thus the 1-bromo (**25**) and 1-chloro (**26**) derivatives of codeine show about half the potency of the parent compound; binding assays show the 1-fluoro derivative (**27**) to have *in vitro* potency and activity comparable to codeine.[25] These compounds are available in a relatively straightforward manner from the amino derivative (**24**), which in turn is obtained by reduction of the nitration product of codeine (**23**).[26]



23, R = O
24, R = H

25, X = Br
26, X = Cl
27, X = F

Modification of the substituent on nitrogen proved to have a very pronounced effect on both quantitative and qualitative activity of morphine. Alkylation of normorphine (**28**; obtained by the von Braun BrCN process or more recently by treatment with chloroformates) with allyl bromide affords the corresponding N-allyl derivative (**29**), nalorphine.[27] This apparently small modification gives a compound that in animal models acts as an antagonist to the action of morphine. The finding that this drug showed analgetic activity in humans set the stage for the development of mixed agonist-antagonists. Substitution by tetrahydrofurylmethyl also leads to a compound with antagonist activity (**30**). In this case the diastereomer substituted by the S heterocycle is a pure antagonist whereas the product from its R epimer shows mixed agonist-antagonist activity.[28] Substitution by phenethyl on the other hand affords an agent (**31**) that is a good deal more potent than morphine, but with qualitatively similar activity.[29]



28

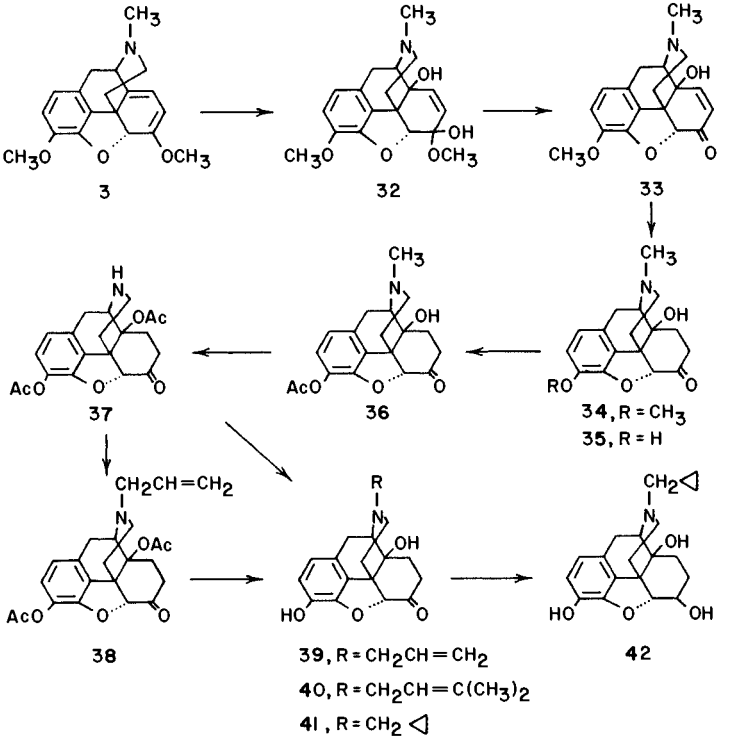
29, R = $CH_2CH{=}CH_2$

30, R = CH_2 

31, R = $CH_2CH_2C_6H_5$

The reactive conjugated diene system in thebaine provides access to compounds that represent marked structural departures from morphine. The fact that several of these have become important clinical drugs has led to a search for plant sources more abundant in this alkaloid than *Papaver somniferum*. Several such strains have been identified, including *Papaver bracteum*, which is reported to contain 26% thebaine in the dried latex.[30]

Oxidation of thebaine with hydrogen peroxide can be viewed at least formally as a 1,4 addition of two hydroxyl groups. Hydrolysis of the intermediate hemiacetal **(32)** affords the hydroxyenone **(33)**. Reduction of the double bond leads to oxycodone **(34)**, an analgetic with modest clinical importance. Demethylation of the phenolic ether leads to oxymorphone **(35)**,[15] one of the most potent clinical analgetics among close relatives of morphine. Again, however, dependence liability parallels potency.

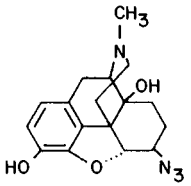


Preparation of the N-allyl analogue is accomplished by first protecting the hydroxyl groups as their acetates. Demethylation (BrCN), followed by alkylation with allyl bromide **(38)** and then removal of the protecting groups, gives naloxone **(39).**[31] This agent is a potent narcotic antagonist similar to its deoxy counterpart. In contrast to nalorphine, however, the 14-hydroxylated compound fails to show analgetic activity, even in man. This property has led to its use as a specific antidote for the reversal of toxic manifestations of narcotics, particularly in cases of overdoses. This highly specific antagonism, which is manifested both *in vivo* and *in vitro*, has led to extensive use of naloxone as a pharmacological tool in analgetic research. It is of note that the epimeric (+) naloxone shows at best 10^{-3}–10^{-4} the activity of the natural isomer.[32]

Analogous schemes using dimethylallyl bromide and cyclopropylmethyl bromide afford nalmexone **(40)**[33] and naltrexone **(41),**[34] respectively, a pair of agents that show a mixture of agonist and antagonist activities. Acylation of secondary amine **37** with cyclobutyl carbonyl chloride, followed by reduction of the resulting amide with hydride, gives the drug nalbuphine **(42).**[33] This has recently been approved for sale in the U.S. as an analgetic with reduced addiction liability.

The potentiating effect of 6-azido and 14-hydroxyl are apparently not additive. The compound incorporating both these features **(43)** exhibits analgetic activity in the range of its desoxy parent in animal models.[34]

Acylation of the alcohol at position 14 by cinnamoyl leads to a major increase in potency. This product **(44)** shows 70–100 times the potency of morphine even though the oxygen atom at 3 is present as the methyl ether.[35] This often overlooked observation may be of particular significance in developing a model of the opiate receptor.



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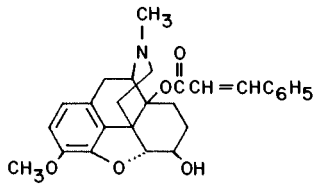
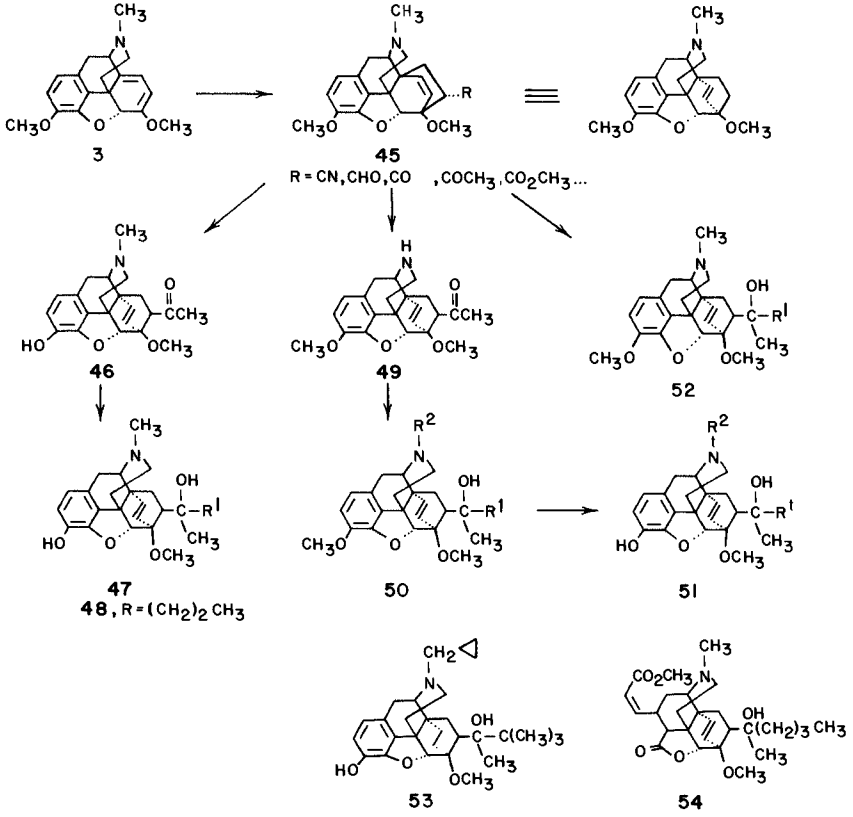


44

Further exploitation of the conjugated diene function in thebaine **(3)** leads to some of the most potent opioids reported to date, as well as an analgetic with low addiction potential. Thus thebaine was found to undergo Diels-Alder condensation with a variety of dienophiles.[37,38] Approach of the reagent from the more open face of the molecule leads to the stereochemistry shown for **45.** The side chain assumes what is in effect the endo configuration.

The observation that some of the adducts show enhanced analgetic activity compared to morphine led Bentley and his colleagues to undertake an extensive program on modification of these *endo*-ethenothebaines. The carbonyl group in the side chain (**45**; R = $COCH_3$) was both reduced and condensed with organometallics to afford carbinols **(57)**.[39] Asymmetric induction leads to high stereoselectivity in products from Grignard reactions. Demethylation of the phenolic ethers gives the corresponding oripavines **(45)**.[40] In the *endo*-ethenothebaine series side chain oxygen was removed entirely.[41] Treatment with cyanogen bromide gives the normorphine analogue;[48] this was alkylated directly to allyl derivatives or alternately acylated and the resulting amides reduced to afford N-cycloalkymethyl derivatives.[37] Oxygen at the 3 position was removed by reduction[42] and the aromatic ring finally dispensed with altogether by ozonolysis.[43]



The SAR of this series is summarized in an extensive review.[44] Briefly, as in the morphine series, phenols show some 10–15 times the potency of their ether counterparts. In the agonist series ($N–CH_3$) the greatest increases in potency are achieved by changes in substitution on the side chain carbinol. Analgetic potency increases to a maximum in both the thebaine and oripavine series for R′ = H to R′ = *n*-propyl; a fall is observed on further extension of the side chain. (An additional increase in potency is observed when this group is phenethyl or cyclohehexylmethyl.) The propyl compound in the phenol series (**48,** etorphine) is one of the most potent opioids to have been studied in great detail pharmacologically. This agent shows antinociceptive potency in animals somewhere between 1000 and 10,000 times that of morphine.[45] Though efficacy and high potency have been demonstrated in man, presumed addiction potential based on animal pharmacology has precluded its clinical commercialization. This high milligram potency has, however, led to extensive application of etorphine in missiles used to temporarily knock down big game. (The ready reversal of its narcotic action by naloxone can be used to ensure that the drugs effect can be halted before toxic effects set in.)

Interestingly, compounds in which the phenolic oxygen has been removed lie between the phenol and its methyl ether in potency. In the series substituted in the side chain by phenethyl, the desoxy analogue shows better potency than even the phenol (2000 times morphine).[42] Oxidative opening of the aromatic ring **(54)** results in a compound that retains a surprising analgetic potency comparable to that of morphine.

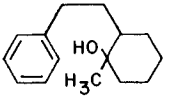
Effects of modification of the substituent on nitrogen follows a pattern quite similar to those of analogous changes in the morphine series; alkylation with groups such as allyl and cyclopropylmethyl affords narcotic antagonists in the O-methylated thebaine series **(50).** The SAR of the free phenols in the oripavine series on the other hand is somewhat more complex; as a rule inclusion of a group on the side chain (**56;** R′) larger than methyl eliminates antagonist activity. Such compounds show only narcotic agonist activity. Thus **50,** where R is propyl and R^2 is allyl, exhibits only agonist activity.[46] Its close relative, in which the group on nitrogen is cyclopropylmethyl and the side chain substituent (R′) is methyl, is described as a strong, pure, narcotic antagonist.[47]

Since the advent of pentazocine it is widely believed that an analgetic agent which incorporates a balance of agonist and antagonist activity would show markedly diminished addiction liability. A compound with such a combination of activities was obtained in the oripavine series by substitution on nitrogen by cyclopropylmethyl, incorporation of tertiary

butyl as the side chain substituent, and reduction of the double bond in the bridge.[48] This drug,[53] buprenorphine, is in clinical use abroad as an analgetic.

MORPHINANS

The structural complexity of the morphine molecule, particularly the presence of the nitrogen-containing bridging ring, frustrated early attempts at total synthesis of this molecule and its relatives. Much of the work in fact concentrated on the preparation of model hydrophenanthrenes containing a quaternary carbon at the **4** position.[49] A cyclodehydration reaction developed in the course of some of this research provided a necessary tool for much of the subsequent work.[50,51] Thus treatment of the tertiary carbinol **55** with strong acid leads to phenanthrene **56,** a compound that contains the better part of the morphine carbon skeleton.



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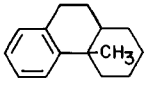
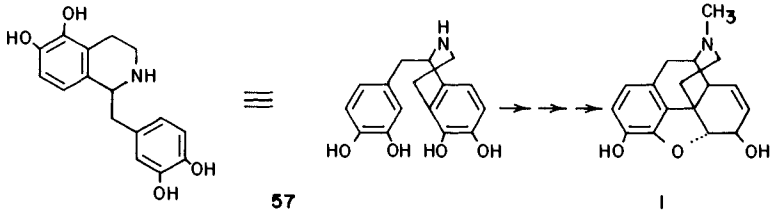


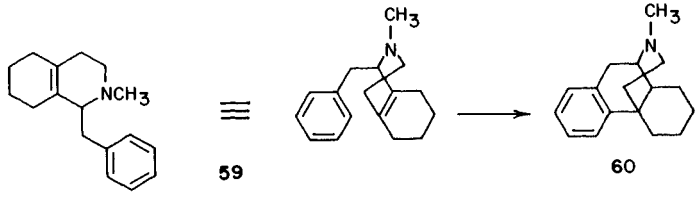
56

A speculative scheme for the biogenetic orgin of morphine is generally held to have provided inspiration for a successful synthetic scheme for the preparation of compounds that contain the full morphine nucleus. Thus Robinson[52] and Schöpf[53] proposed independently that morphine is formed in nature by cyclization of a benzylisoquinoline alkaloid. [This postulate was eventually confirmed when it was shown that norlaudanosoline **(59)** is transformed to morphine in plant tissue.[54,55]]



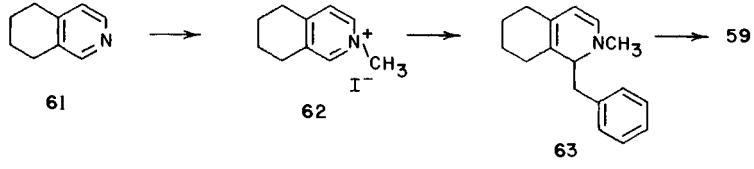
57 I

Using that analogy, Grewe prepared the octahydroisoquinoline **59**. He found that this compound cyclized to the bridged phenanthene **60** in strong acid.[56] (It should be noted that the analogy to the biogenetic reaction is more formal than real; cyclization of **57** represents an oxidative phenol coupling reaction, whereas ring closure of **59** is a carbonium ion process.) This approach to the carbon skeleton of morphine—known as the *morphinan nucleus*—was later shown to be quite general.



59 60

The scheme used for preparation of **59** included a carbon-carbon bond forming reaction which has proven crucial to much subsequent work on both morphinans and benzomorphans. Alkylation of tetrahydroisoquinoline **61** with methyl iodide gives the intermediate salt **62.** It should be noted that this compound now incorporates a ternary imminium group, a function known to add nucleophiles. Indeed, reaction with benzylmagnesium chloride leads to condensation product **62**. Catalytic hydrogenation selectively reduced the enamine double bond to yield **63.** (In more current work the last step is usually carried out by means of sodium borohydride.)



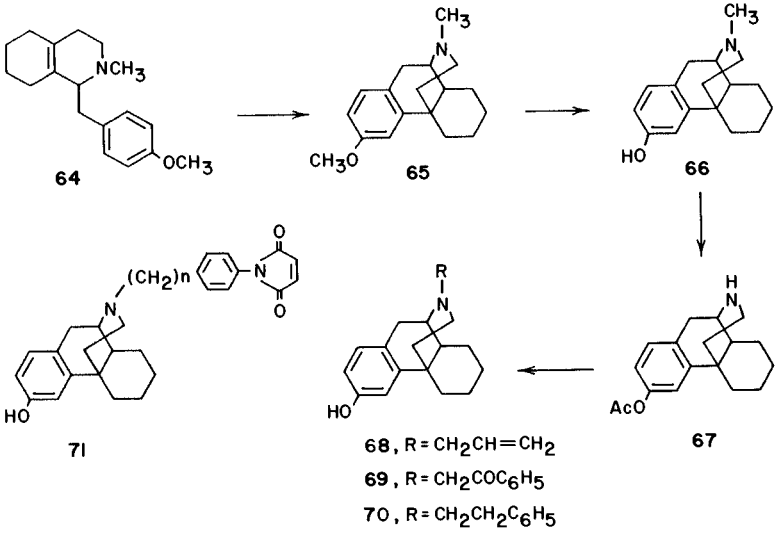
61 62 63

The observation that **60** exhibits considerable analgetic activity spurred intensive investigation of the SAR of morphinans. Initial efforts involved modifications known to change the activity of morphine itself.

Use of *p*-methoxybenzylmagnesium chloride in the condensation reaction **(62 → 63)** leads, after reduction and cyclization of the initial product, to the codeine analogue **65;** demethylation leads to phenol **66,** racemorphan, a potent analgetic drug.[58] Resolution of the product reveals

that analgetic activity is due to the (−) isomer.[59] This compound, levorphanol, is 6–8 times as potent as morphine in man. The methyl ether of the (+) antipode on the other hand shows little analgetic activity, though it retains much of the antitussive action of the racemate. This agent, known as *dextromorphan*, has as a result found extensive use in cough preparations.

Modification of the substituent on nitrogen, as in morphine proper, has a significant effect on the quantitative and qualitative aspects of the pharmacological action of analogues. Access to the key intermediate is provided by first protecting the phenol as the acetate, followed by N-demethylation by the von Braun procedure. The resulting secondary amine **(67)** has been used in the preparation of scores of derivatives.[49]

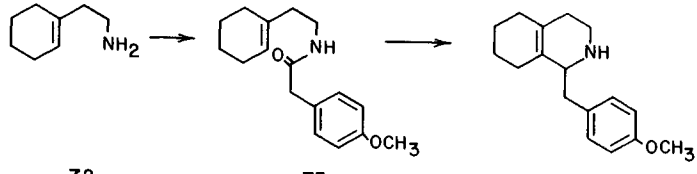


Alkylation of resolved (−) **67** with allyl bromide followed by deprotection of the phenol gives the narcotic antagonist levallorphan **68)**, a compound with little analgetic activity. The propargyl analogue has been reported to exhibit a ratio of agonist to antagonist activities similar to that of nalorphine.[60] The same sequence using phenethyl bromide or phenacy bromide as alkylating agents leads to phenomorphan **(70)** and levophenacylmorphan **(69)**, respectively, a pair of potent analgesics.

A series of phenylalkyl morphinans were recently prepared in an effort to develop an agent that would bind covalently to narcotic receptors by acting as a Michael acceptor. One of these, **71** (n = 2), showed

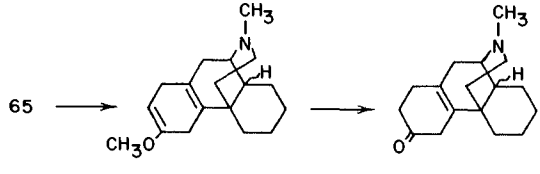
5 times the analgetic potency of morphine *in vivo*. The additional heterocycle thus diminishes potency by only a factor of 2 compared to phenazocine. Though the agent did shift the dose response of morphine, other data were inconsistent with its exerting competitive or noncompetitive inhibition of that agent.[62]

The commercial importance of morphinans led to the development of an alternative synthetic route that avoids the N-demethylation reaction. Acylation of amine **72** with *p*-methoxyphenylacetyl chloride gives amide **73.** Cyclization under Bischler-Napieralsky conditions, followed by reduction of the first-formed imine, leads to secondary amine **74.** This can then be alkylated with the desired side chain prior to cyclization to a morphinan.[63]



72 73 74

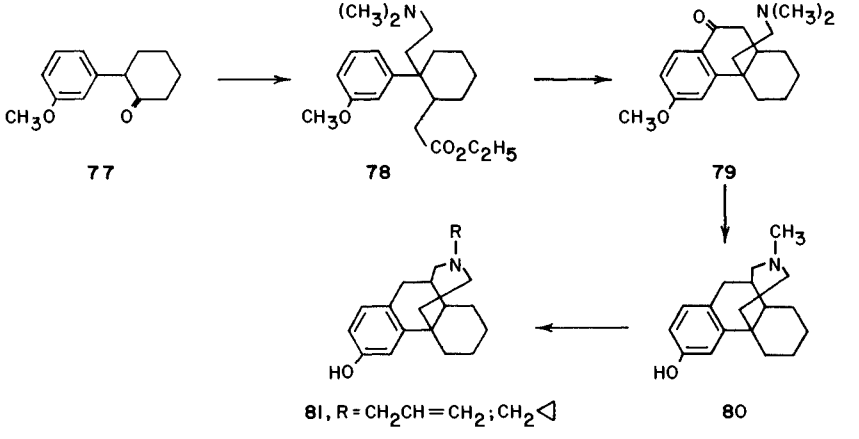
In contrast to the oripavines (see **54**) the morphinans do require an aromatic ring for analgetic activity. The product of Birch reduction of **65** gives unconjugated ketone **76** (B/C cis) on hydrolysis.[64] This product, as well as the isomer containing the trans fusion, shows little if any analgetic activity in standard tests.



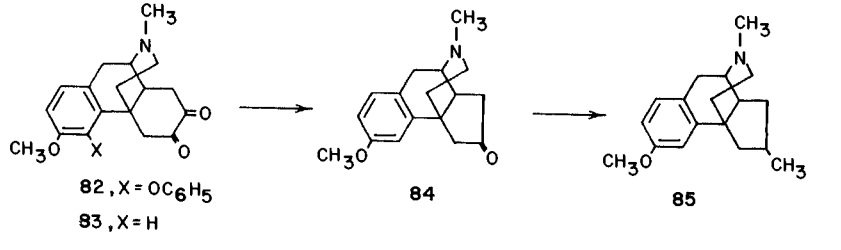
75 76

Expansion of the bridging ring to seven members is consistent with analgetic activity. In rough outline arylcyclohexanone **77** is first alkylated with the nitrogen-containing side chain. Reformatsky reaction on the ketone, followed by dehydration of the resulting carbinol and then reduction, gives ester **78.** This is then cyclized to a phenanthrone and N-demethylated **(79).** Mannich reaction followed by reduction of the carbonyl group leads to the B/C cis homomorphinan **(80).** A similar sequence leads to the B/C trans isomer.[65] Both compounds show analgetic

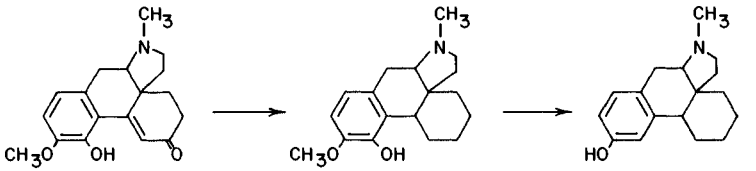
potency comparable to morphine as the free phenols. The N-allyl and N-cyclopropylmethyl derivatives, prepared by the usual sequences, show reduced analgetic potency but little if any antagonist activity.[66]



Contraction of ring C has little effect on analgetic potency. Dissolving metal reduction of diketone **82**, available in several steps from thebaine, gives the corresponding 4-deoxy compound. This was ring contracted by scission of the diketone to an open chain diester followed by recyclization. Reaction of the resulting ketone **(84)** with methylene Wittig reagent followed by reduction gives the C-nor analogue **85**; the free phenol showed about 10 times the analgetic potency of morphine.[67]

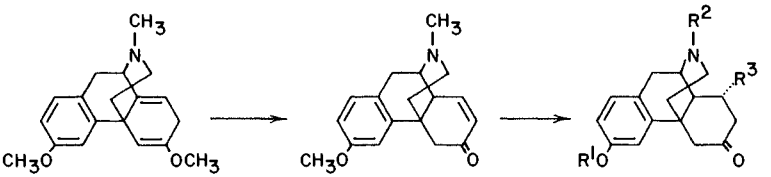


Moving the carbon terminus of the heterocyclic bridge to the 14 position effectively abolishes activity. Sequential reduction of the double bond and carbonyl groups in thebaine rearrangement product **86** leads to the analogue with the fully reduced ring C. Removal of the extra oxygen on ring A by reduction of the corresponding dinitrophenyl ether and finally O-demethylation gives the morphinan regioisomer **88**.[68]



86 87 88

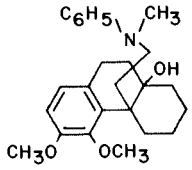
Alkylation at the 7 position in ring C has relatively little effect on activity. Access to these compounds hinges on the ready availability in several steps of enol ether **89** from thebaine. The enol ether can be hydrolyzed to the corresponding ketone under conditions that lead selectively to compounds with either cis or trans fused B/C rings. Alkyl groups are incorporated at the 7 position by conjugate addition of lithium dialkyl cuprates. These adducts are then converted to the phenols; substitution on nitrogen is modified by the usual reaction sequence. In the agonist N-methyl series these analogues are generally less potent than the corresponding desalkyl analogues. Replacement of N-methyl by allyl and cyclopropylmethyl leads to agents with mixed agonist and antagonist activities. Removal of oxygen at 6 leads to diminution of activity. No major differences in potency were found between the cis and trans isomers.[69]



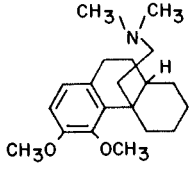
89 90 91

A compound that shares only the octahydrophenanthrene moiety with morphinans brings into question the need for the rigid bicyclic

bridge. This analogue **(92)**, obtained by a multistep degradation of oxycodone, shows about 3 times the potency of morphine.[69] The corresponding analogue lacking oxygen at the 14 position is but one-third as potent as morphine. This observation parallels similar findings on compounds in the morphine series.



92



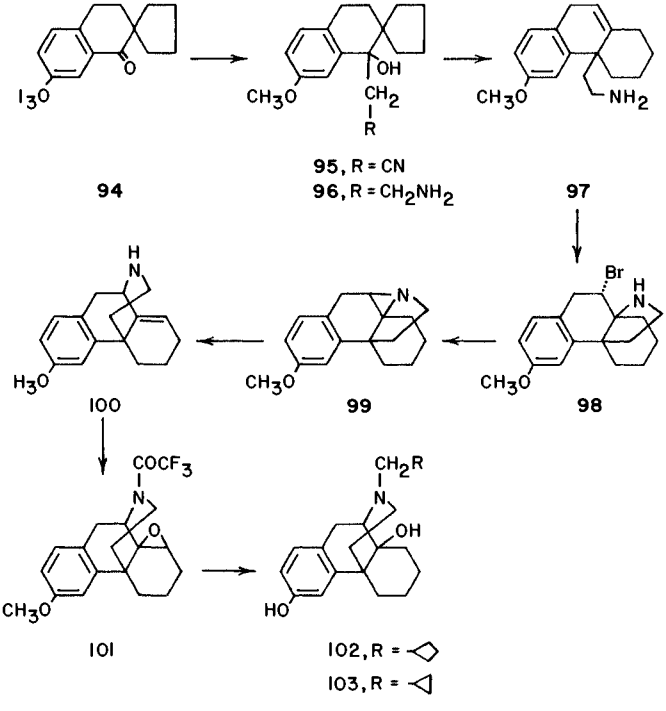
93

Incorporation of 14-hydroxyl into a compound that retains the full morphinan skeleton leads to an analgetic with antagonist activity comparable to that of naloxone and almost equally effective by oral as parenteral administration. Since the classical Grewe synthesis does not lend itself to introduction of the new function, a route was chosen in which the hydrophenanthrene is established by Wagner-Meerwein rearrangement.

Addition of the anion from acetonitrile to tetralone **94** (from alkylation of 7-methoxytetralone with dibromobutane) gives tertiary alcohol **95;** the nitrile is then reduced to the primary amine. Treatment with strong acid gives hydrophenanthrene **97,** presumably via the carbonium ion formed from the tertiary benzylic alcohol. Treatment with bromine leads to the cyclized amine **98.** Stereochemistry suggests that cyclization proceeds by attack of nitrogen on the initial bromonium ion. Simple neutralization of **98** gives morphinan **100.** The intermediacy of aziridine **99** (from displacement of the remaining bromine) is indicated by its isolation when the reaction is run at lower temperature. Oxygen is introduced by epoxidation of the olefin after protection of the amine. (The oxide shown is

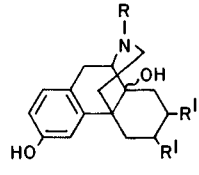
the major product). Reduction of the epoxide affords the desired 14-hydroxy morphinan. Introduction of the N-cyclobutylmethyl group and O-demethylation afford butorphanol **(102)**.[71,72] This drug has recently been released for sale in the U.S. as an analgetic with low abuse potential.

The same sequence was used to prepare the cyclopropylmethyl analogue oxylorphan **(103)**.[71,72] This analogue apparently shows a greater measure of antagonist activity than does **102**. Both compounds show strong binding to opiate receptors in an *in vitro* assay using rat brain homogenates.[73]

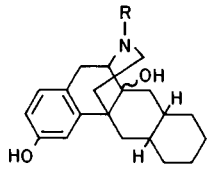


A series of hydroxymorphinans bearing additional substitution on ring C was prepared by a modification of the above synthetic scheme. Careful separation of the epoxides in this case permitted the preparation of analogues bearing both α and β hydroxyl groups. Those analogues incorporating an additional ring **(105** and **106)** are both pure narcotic antagonists whether R is cyclobutymethyl or cyclopropylmethyl and regardless of the orientation of the hydroxyl group.[74]

Inclusion of oxygen directly in the framework of ring C of morphinans also leads to potent analgetic agents that exhibit considerable antagonist

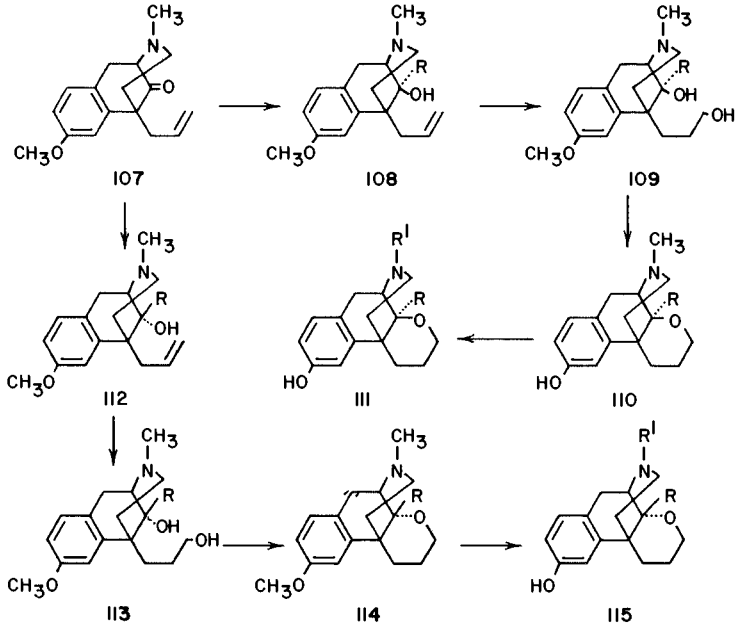


104



105, α-OH
106, β-OH

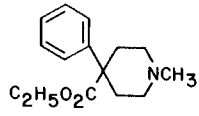
activity when appropriately substituted. Starting material for these molecules is the benzomorphan **107** (preparation of this intermediate is detailed in the section on benzomorphans). Metal hydride reduction or addition of organometallics to the carbonyl in conventional ether solvents affords alcohols **108**. The allyl group is then converted to an alcohol by hydroboration followed by oxidation. The glycols are converted to the tetrahydropyrans by way of the primary mesylates. The phenols and analogues bearing modified alkyl groups on nitrogen are obtained by the usual sequence.[75] Reduction of **107** by means of diisobutyl aluminum hydride in hexane or addition of Grignard reagents in hydrocarbon solvents leads to alcohols with reversed stereochemistry **(112)**.[76] These were taken on to the isomeric oxamorphinan analogues.[77]



Briefly, compounds containing ring oxygen in the α orientation (i.e., **114**) are universally more potent analgetics than the β isomers. The oxamorphinans are quite as effective in animal models as the 14-hydroxy counterparts. Substitution by cyclopropylmethyl leads to compounds that show analgetic activity as well as narcotic antagonist activity. It is interesting that alkylation on the ring B carbon bearing the oxygen atom selectively increases the narcotic antagonist activity.[77]

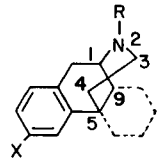
6,7-BENZOMORPHANS

It is tempting to describe research on narcotic analgetics as an effort to eversimplify the morphine structure so as to extract from it the minimum pharmacophore. Such an account would, however, do violence to chronology; the adventitious discovery of the phenylpiperidine analgetics (**116;** meperidine) in 1939 launched an extensive synthetic program on related structures. These molecules, interestingly, were found to be almost indistinguishable from morphine in their pharmacology. Early attempts were unsuccessful in improving the clinical spectrum of these agents by incorporation of some measure of antagonist activity. It was not until comparatively recently that phenylpiperidines have been prepared which showed narcotic antagonist activity (see below).



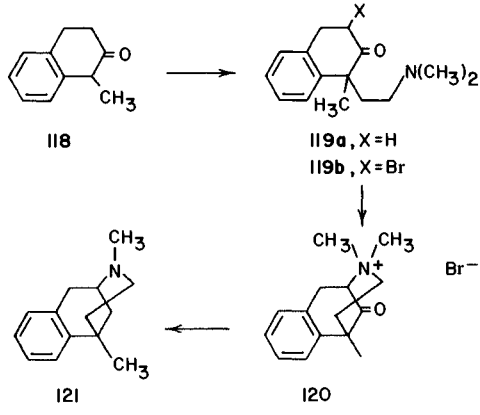
116

After the morphinans the next systematic attempt to improve on morphine involved molecules that incorporate a greater part of the structure of the natural product than do the phenylpiperidines. These molecules, the benzomorphans **(117),** incorporate the tetralin moiety as well as the bridged heterocycle of the morphinans.



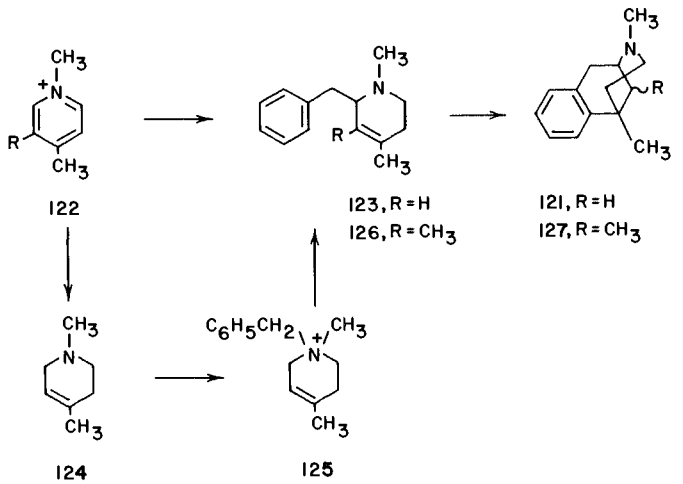
117

Synthetic routes to these molecules as well as their chemistry have recently been described in an extensive review.[78] The original synthetic scheme[79] starts with an appropriately substituted 2-tetralone; alkylation by means of an N,N-dialkyl 2-chloroethylamine affords the chain required for formation of the bridge **(119)**. Bromination of the ketone followed by internal quaternization gives the bridged structure **(120)**. The superfluous methyl group is removed pyrrolytically to afford a benzomorphan **(121)**. This route is particularly useful for preparing 9-hydroxylated derivatives.

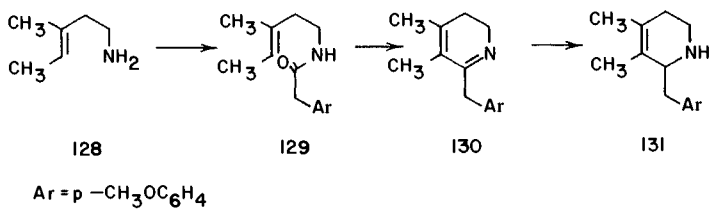


118 119a, X=H 119b, X=Br 120 121

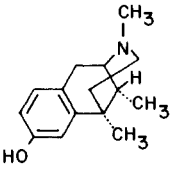
The Grewe synthesis provided a flexible alternative route to benzomorphans. The requisite intermediate tetrahydropyridines are prepared by condensation of substituted pyridinium salts **(122)** with benzylmagnesium halides followed by reduction (catalytic; $NaBH_4$) of the enamine function.[80] An interesting variation consists in alkylation of the tetrahydropyridine **124** with a benzyl halide to give the quaternary salt **125.** Treatment with strong base leads to **123** by a Stevens rearrangement.[81] Acid-catalyzed cyclization of the tetrahydropyridine **(123)** gives the benzomorphan skeleton **(121)**.



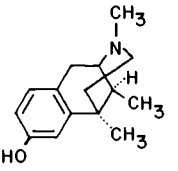
An alternative method for construction of the intermediate dihydropyridines starts by acylation of substituted allylamine **128** with a phenylacetyl chloride **(129)**. Cyclization under Bischler-Napieralski conditions **(130)** followed by reduction ($NaBH_4$) leads to the required intermediate **(131)**.[82]



Cyclization of tetrahydropyridines carrying substituents at the 3 position **(124** and **131)** proceeds to give largely but not exclusively the thermodynamically favored isomer **132** (methyl equatorial to the piperidine ring).[83] The stereochemistry was first established by rates of methiodide formation of the so-called α-isomer **(132)** compared to the β-isomer **(133)**, as well as by comparison of the NMR chemical shifts of the 9-methyl group.[84]

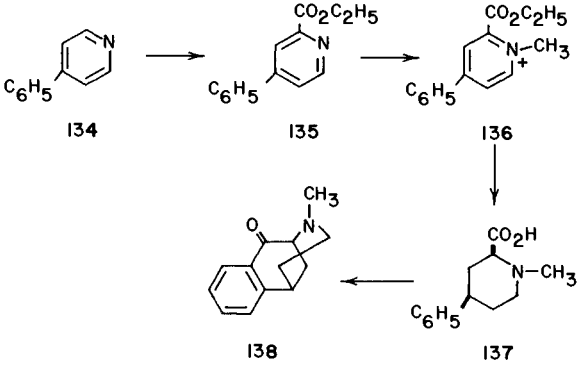


132



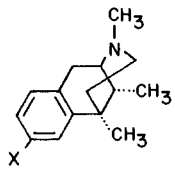
133

A different approach to assembly of the benzomorphan nucleus starts with 4-phenylpyridines **(134)**. Addition of cyanide to the 2 position of the N-methyl derivative, followed by saponification, gives the ester **135**. Catalytic hydrogenation of the methiodide **(136)** affords acid **137** on saponification, probably as the cis isomer. Friedel-Crafts cyclization leads to benzomorphanone **138**.[85]

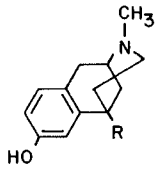


134 135 136 138 137

The SAR of the benzomorphans in many ways parallels that of morphine and the morphinans. Thus hydroxylation in the aromatic ring at the 2′ position—equivalent to the 3 position in morphine—results in increased potency. In the 5,9-dimethyl series the phenol **(132)** shows potency in the same range as morphine,[86] while the methyl ether **(140)** shows but one third that potency;[87] the unsubstituted analogue **(139)** exhibits an ED_{50} 10 times that of the phenol.[88] The bulk of the work in this series has thus involved the oxygenated compounds.



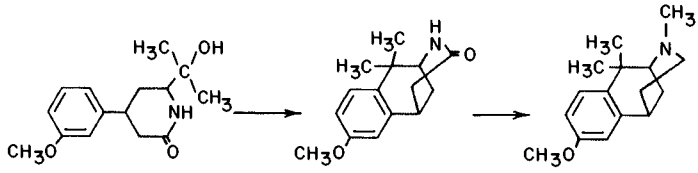
139, X = H
132, X = OH
140, X = OCH_3



141, R = H
142, R = CH_3

The methyl groups were originally thought to contribute to activity by providing bulk similar to that of the deleted alicyclic ring. It is thus of interest that the desmethyl compound **141** shows one third the analgetic potency of morphine.[89] This observation is of particular interest, since **141** is devoid of the quaternary carbon atom, at one time thought essential for analgetic activity (see the section on the Beckett Casy rule). Inclusion of that methyl group at the 5 position **(142)** leads to little change in potency. As noted above, the α-isomer in the 5,9-dimethyl compound shows potency in the morphine range.

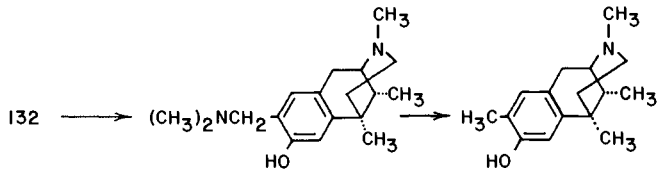
Stereochemistry exerts a major effect on potency; the β-isomer **(133)**, which carries an "axial" 9-methyl group, shows some 15 times the potency of morphine.[86]



143 144 145

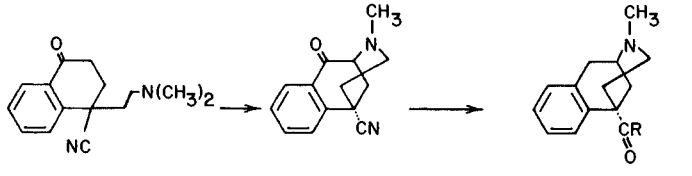
Alkylation of the benzylic methylene group has only a small effect. Preparation of this analogue first involves construction of piperidone **143.** Acid-catalyzed cyclization **(144)**, followed by reduction of the lactam and methylation of the resulting amine, gives a compound **(145)** that is about twice as potent as codeine.[90]

Alkylation of the aromatic ring via the Mannich product **146** affords the *o*-methylated analogue **147**, a compound somewhat less active than morphine.[90]



146 147

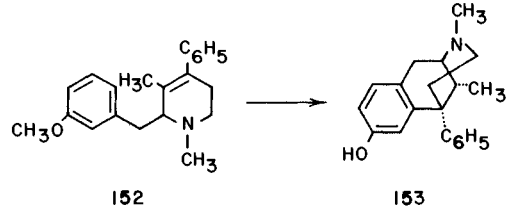
The necessity for a polar function on the carbon atom bearing the phenyl ring is well established in the phenyl-piperidine analgetic series (i.e., **116**). The analogous benzomorphans are prepared by a modification of the Barltrop synthesis, starting with the cyano-substituted tetralone **148.** The final products **(150** and **151)** show analgetic potency in the same range as that of the analogous methyl-substituted compounds.



148 149 150, R = OC_2H_5

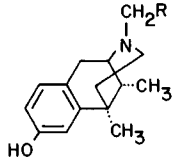
151, R = $N(CH_3)_2$

Placement of a phenyl ring at the 5 position affords compounds that possess many of the structural elements of the open chain opioids such as methadon. Cyclization of the phenyl-substituted tetrahydropyridine **152** leads to the corresponding benzomorphan. These agents, one of which, GPA 1657 **(153),** was studied in some detail, show good analgetic activity.[93]



152 153

Modification of the substituent on nitrogen results in major changes in both the potency and the pharmacological profile of benzomorphans. Arylethyl substitution results in marked increases in potency, as in the case of morphine and the morphinans. The phenethyl analogue **154,** for example, is a decade order of magnitude more potent than the parent N-methyl compound;[94] the thienyl analogue **155** shows a further fivefold increase in potency.[95] The former, phenazocine, underwent fairly extensive clinical trials as an analgetic. Substitution by the *p*-fluorobutyrophenone moiety characteristic of neuroleptic compounds affords an agent **(156)** reported to exhibit both analgetic and tranquilizing activities.[96]



154, R = $CH_2C_6H_5$

155, R = CH_2–(thienyl)

156, R = CH_2CH_2CO–(fluorophenyl)–F

157, R = $CH{=}CH_2$

158, R = $CH{=}C(CH_3)_2$

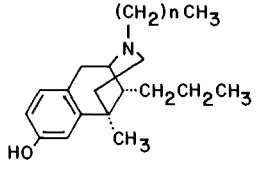
159, R = (cyclopropyl)

160, R = (tetrahydrofuryl)

As might be predicted from a consideration of the SAR of morphine and the morphinans, substitution on nitrogen by allyl affords a compound that shows only narcotic antagonist activity in animal assays.[95] Systematic modification of that grouping led to a series of compounds that antagonize the action of opioids and show little activity in classical analgetic tests.[97] The 3,3-dimethylallyl analogue **(158)** showed demonstrable analgetic activity in the clinic, with little evidence of addiction liability. This compound, pentazocine, is now marketed as a nonaddicting central analgetic. The cyclopropylmethyl analogue, cyclazocine **(159),** though more potent as an antagonist, also shows clinically useful analgetic activity. Administration of cyclazocine is, however, associated with the development of dysphoria and occasional hallucinatory episodes, perhaps as a consequence of its strong antagonist activity.

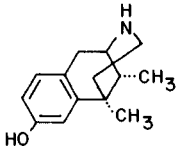
Alkylation with tetrahydrofurylmethyl halide similarly affords compounds **(160)** with mixed agonist and antagonist activities; in this case the workers prepared all possible diastereomers by starting with the resolved norbenzomorphans and tetrahydrofurans.[98] Lengthening the alkyl group at the 9 position affords compounds that give some hints

as to the separation of analgetic and addictive activities.[99] Alkylation of the 9-propyl analogue with long chain alkyl groups gives compounds that are pure, long lasting narcotic antagonists **(161)**.[100]

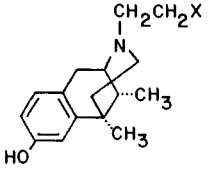


161, n = 1–5

A benzomorphan bearing an alkylating side chain on nitrogen was prepared from secondary amine **162** by condensation with ethylene oxide **(163)** followed by conversion of the alcohol to the bromide. Though the product **(164)** had only very weak analgetic activity, the compound's prolonged depressant action may have been the result of irreversible binding to CNS receptors.[101]

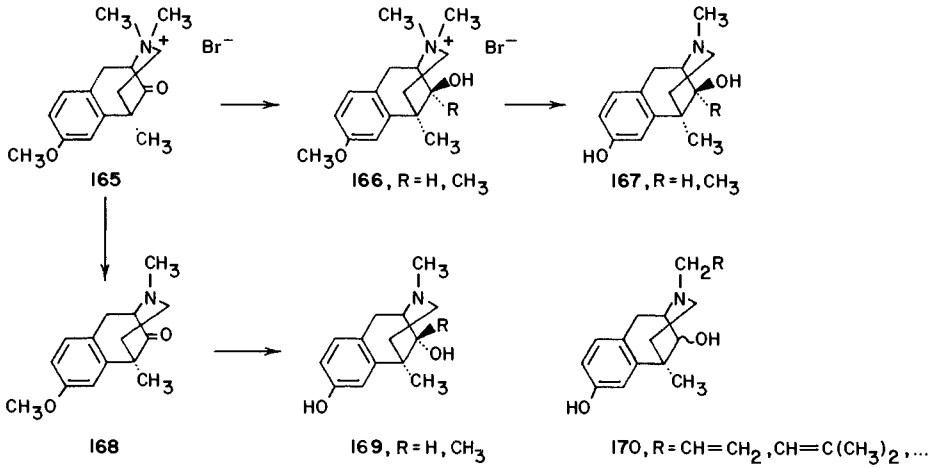


162



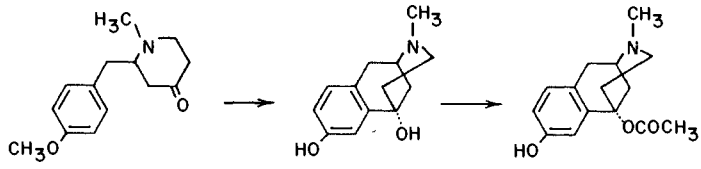
163, X = OH
164, X = Br

As noted above, replacement of hydrogen at the 14 position by hydroxyl leads to a marked increase in potency in both the morphine and morphinan series. Benzomorphans hydroxylated in the analogous position can be prepared from a tetralone intermediate. Reaction of the quaternary salt **(165)** with methylmagnesium bromide gives largely the carbinol from equatorial attack of reagent, after pyrrolytic elimination of methyl followed by O-demethylation. Addition of the Grignard reagent to the free base **(168)**, on the other hand, gives, following O-demethylation, the product **(169)** resulting from axial attack.[102,103] Catalytic reduction of the ketones to the secondary alcohols gives similar stereochemical results. Inclusion of hydroxyl does not give the expected potentiation of analgetic activity in the benzomorphan series.



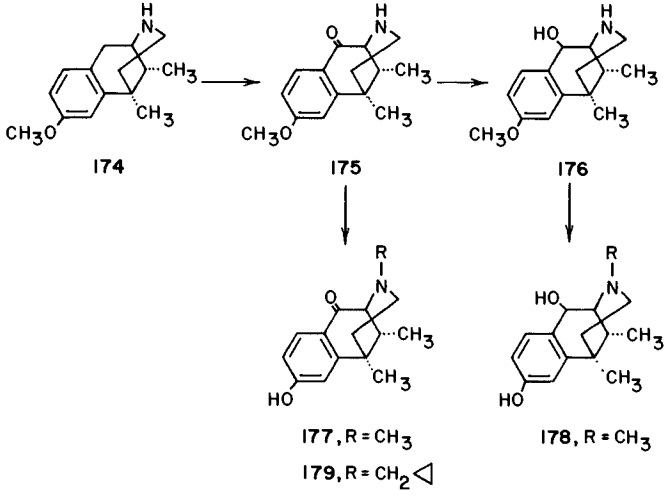
A recent report includes results of a systematic examination of the biological properties of isomeric 9-hydroxybenzomorphans substituted on nitrogen by traditional antagonist side chains. It was concluded that hydroxylation generally decreases analgetic activity. Introduction of hydroxyl oriented away from nitrogen has little effect on antagonist activity; hydroxyl oriented toward nitrogen enhances this activity.[104]

When the polar function on meperidine is reversed (i.e., carbonyl-ester oxygen interchange), the analogue shows increased analgetic potency over the parent compound. The corresponding analogue of **150** was prepared by treatment of piperidone **(171)** with 48% HBr **(172)** followed by acylation. Of a series of esters only the acetyl compound **(173)** showed appreciable analgetic activity.[105]

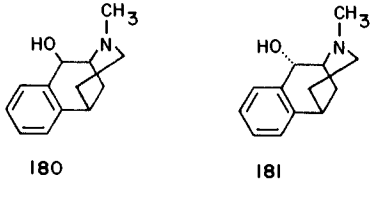


Analogues oxidized at the benzylic methylene group show good analgetic activity. Oxidation of norbenzomorphan **(174)** with chromium trioxide gives the key intermediate **(175)**. Reduction of the carbonyl leads

to alcohol **176.** Alkylation on nitrogen followed by O-demethylation leads to a series of compounds **(177** and **178)** that show varying mixtures of analgetic and narcotic antagonist activities.[106] One of these, ketazocine **(179),** has been studied in some detail as an analgetic with low addiction potential.

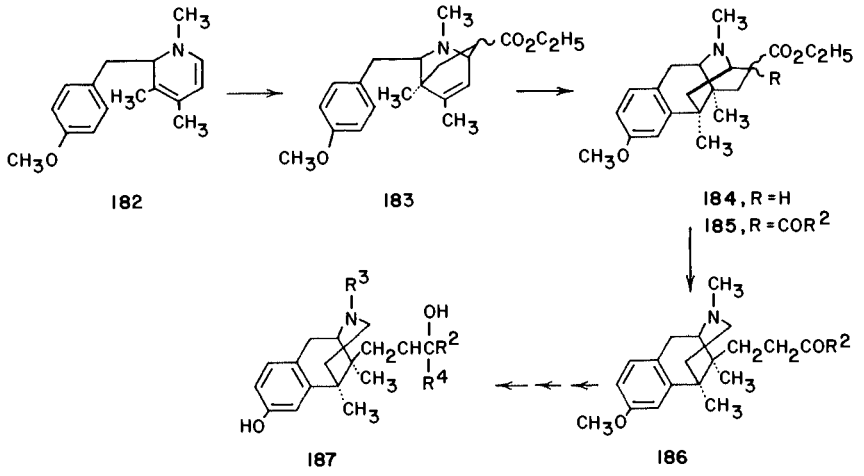


The corresponding alcohols lacking oxygen in the aromatic ring as well as the 5,9-alkyl groups show only weak analgetic potency. Thus **180** and **181** (obtained from the former by an inversion sequence) show, respectively, one half and one sixth the potency of codeine.[107]

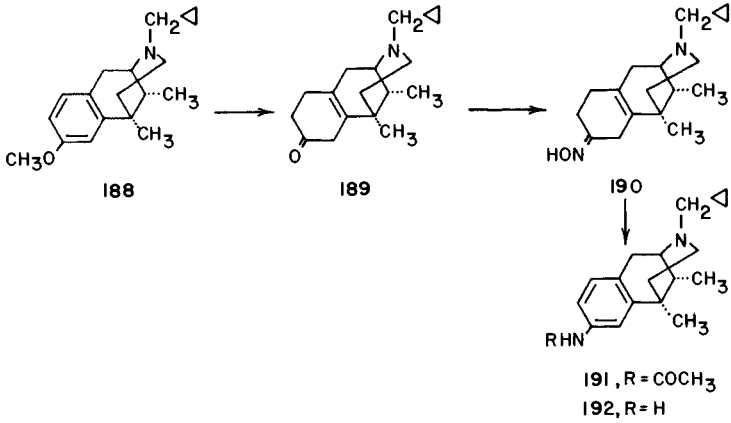


An important structural feature of the high-potency narcotics based on the oripavine skeleton is the presence of the side chain bearing a tertiary alcohol (e.g., **48**). Preparation of the analogous benzomorphans starts with the 2+4 cycloaddition of ethyl acrylate to the extended en-

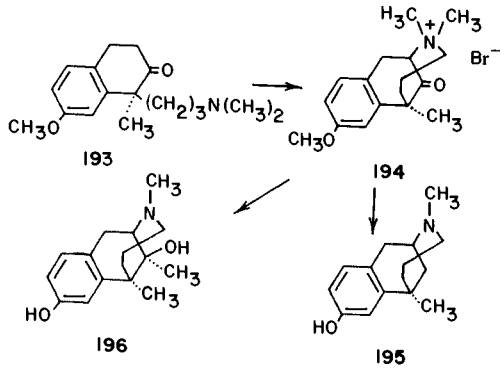
amine **(182)**. Cyclization gives benzomorphan **(184)**. The β-dicarbonyl compound obtained by acylation of the ester undergoes ring scission in formic acid to give the benzomorphan **(186)** alkylated geminally at the 9 position.[109] The ketone is condensed with a series of organometallic reagents to give the tertiary carbinols.[108,110] N-Methylated compounds constitute a class of moderately potent analgetics. Substitution by cyclopropylmethyl leads in many cases to potent narcotic antagonists; **187** ($R^2 = t-Bu$; $R^4 = i-Bu$), for example, is 3–5 times more potent than nalorphine. Selected agents show analgetic activity and antagonize the action of narcotics.



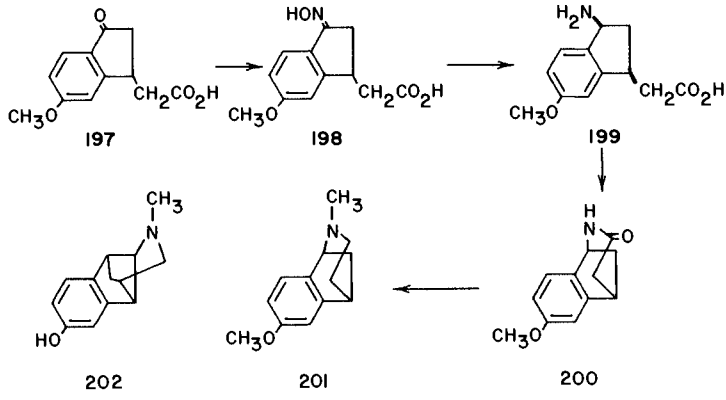
A phenolic hydroxyl group is known to make an important contribution to analgetic activity in the morphine, morphinan, and benzomorphan series. It is thus of some interest that this function can be replaced by the weakly basic amino group with little loss in activity. Birch reduction of cyclazocine methyl ether **(188)** gives enone **189** on hydrolysis of the intermediate. Treatment of the oxime **(190)** with acetic anhydride in acetic acid and HCl leads to acetanilid **(191)**; the corresponding aniline **(192)** shows pharmacological activity and potency comparable to that of cyclazocine.[111]



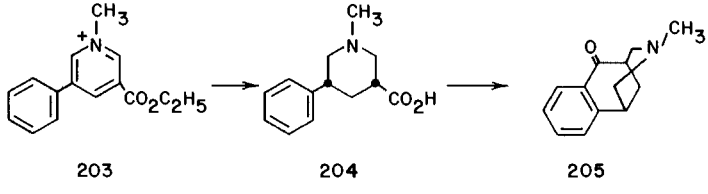
Alteration of the alicyclic part of the nucleus generally leads to loss of potency. Repetition of the Barltrop scheme using a chloropropyl instead of a chloroethyl amine gives homologous benzomorphans **(195)**. These show about half the analgetic potency of codeine. Methyl carbinol **(196)** similarly shows relatively weak potency.[112]



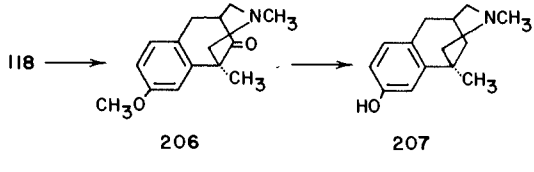
Preparation of a ring contracted analogue starts from indanone **(197)**, obtained by cyclization of the corresponding 3-arylglutaric acid. Cyclization of the cis amino acid from reduction of the oxime **(198)** establishes the bicyclic nucleus **(200)**. Reduction of the lactam followed by N-methylation completes the synthesis. The methyl ether **(201)** shows the same analgetic potency as codeine.[113] The highly rigid analogue **(202)** on the other hand shows only marginal analgetic activity.[114]



In compounds in which nitrogen is interchanged with one of the bridge methylene groups activity depends on substitution in the aromatic ring. Preparation of the unsubstituted compound starts with reduction of the substituted nicotinic acid derivative **(203)**. The cylization product **(204)** shows little if any analgetic activity.[115]

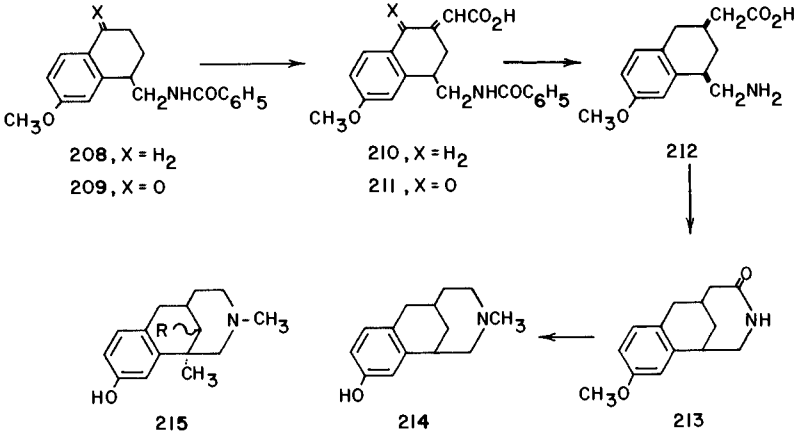


Mannich reaction (CH_2O, $MeNH_2$) on tetralone **118** leads directly to the isomeric benzomorphanone **(206)**. Reduction of the carbonyl group followed by O-demethylation affords **207**, a compound with analgetic activity in the range of morphine.[116]



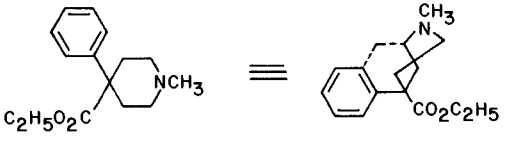
Homologation of the heterocyclic ring, interestingly, increases potency in the iso series, perhaps by providing the ring with greater flexibility. Oxidation of aminotetralin **(208)** leads to aminoketone **(209)**. This

is then converted to amino acid **211** by successive aldol condensation and reduction. The amine is then deprotected and the amino acid cyclized to the bicyclic lactam **(213).** Successive reduction and N-methylation give an agent **(214)** with half the potency of morphine.[117] The 5,9-dimethyl analogue **(215),** prepared by a similar scheme, shows potency equivalent to morphine.[118]



PHENYLPIPERIDINES AND RELATED COMPOUNDS

Analgetics discussed to this point all contain recognizable, significant structural elements of the naturally occurring opioids. At first sight meperidine **(116),** also known as *pethidine,* looks like a molecule designed to test the effect on biological activity of drastic simplification of the narcotic structure. The drug is in fact said to have been intended as an antispasmodic agent;[119] the agent's analgetic activity was discovered in the course of clinical trials.

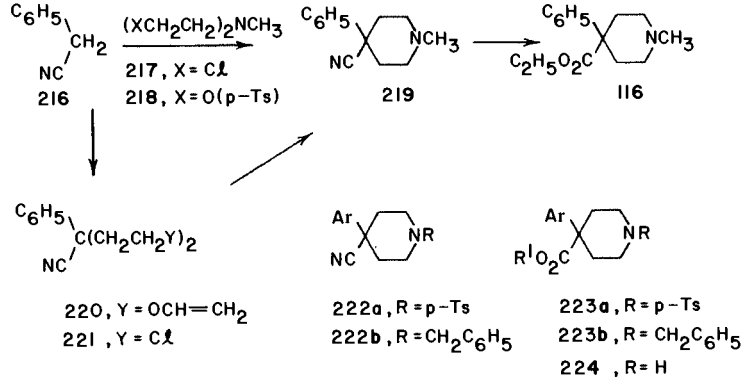


116

Meperidine quickly found a place in the clinic because of its efficacy as an analgetic. It was, however, soon recognized that the compound, which shows about one fifth the potency of morphine, offers little advantage over the natural product, as it showed the same set of drawbacks, including addiction liability. This, together with the relatively straightforward chemistry involved in this class of compounds, led to extensive synthetic programs in many laboratories aimed at producing superior drugs. It has, for example, been estimated that some 4000 analogues had been prepared by 1965.[120] Publications subsequent to that date suggest that phenylpiperidines are still not a dead issue.

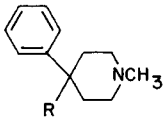
One of the original syntheses of meperidine and its analogues involves alkylation of a phenylacetonitrile **(216)** with nitrogen mustard **(217)** to form piperidine **219.**[121] The highly lachrymatory—and toxic—nature of this alkylating agent led to the development of alternate intermediates such as tosylate **(218).** Use of the mustard can also be avoided by closing the ring in the other sense. Thus alkylation of phenylacetonitrile with 2 moles of 2-chloroethyl vinyl ether gives **220.** This is then converted stepwise to the chloride **(221)** and reacted with methylamine to give **219.**[122] Saponification of the nitrile followed by esterification gives meperidine and its analogues.

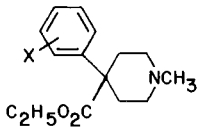
The potency of analgetics in the phenylpiperidine series depends heavily on the nature of the substituent on nitrogen. The normeperidine **(224)** required for manipulation of this substituent can be prepared by the standard route using amines containing readily removable protecting groups such as tosyl or benzyl. The resulting nitriles **(222)** are then taken on to the esters **(223);** deprotection leads to the secondary amines.[123–125]



Modification of the ester alkyl group has little effect on potency, ethyl being about the optimal group; the ester with isopropanol **(226)**

properidine[122] has been investigated in some detail as a drug with longer duration of action. Replacement of carbethoxy by cyano **(219)** leads to virtual loss of activity; an acetyl group in that position leads to compounds with analgetic activity **(228).** The methyl analogue **(227)** shows little activity in the absence of appropriate substitution in the aromatic ring (see below).





226, R = $CO_2CH(CH_3)_2$

227, R = CH_3

228, R = $COCH_3$

229, X = o − CH_3

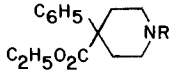
230, X = m − OH

Substitution on the aromatic ring generally reduces analgetic potency; the *o*-tolyl analogue **(229)** seems to be an exception to this rule. As in other structural series, the presence of a hydroxyl group in the meta position leads to compounds with good potency. Thus bemidone **(230)** shows some 15 times the potency of meperidine.

Again, as in previous structural series, replacement of N-methyl by phenethyl leads to increased potency; this analogue pheneridine **(231)** shows an ED_{50} of one half to one third that of meperidine.[126] Substitution on the pendant phenyl group is consistent with good potency **(232;** anileridine),[127] as is hydroxylation in the side chain **(233).**[126]

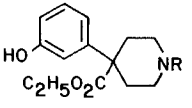
Increasing the distance between the side chain phenyl and nitrogen gies a further increase in potency. Cinnamyl analogues **(234)** are some 10–30 times more potent than meperidine.[128] The product of Mannich condensation between acetophenone and normeperidine **(235,** is 100 times more potent than the prototype.[129] The carbonyl reduction product **(236;** phenoperidine) is 150 times more potent.[130]

Replacement of side chain aryl by pyridyl **(237)** leads to a compound with 5 times the potency of meperidine.[128] A twofold increase is maintained when the ring is saturated, as in morpheridine **(238).**[131] Placement of a dioxolane in that position gives a compound **(239)** with the same potency as the N-methyl analogue.[132] A systematic investigation of a large selection of N-alkyl substituents concluded that, for straight chain alkyl, ω-hydroxyalkyl, ω-ethoxyalkyl, and ω-furylalkyl analogues, optimal potency is obtained at a chain length from 7 to 9 Å.[133]



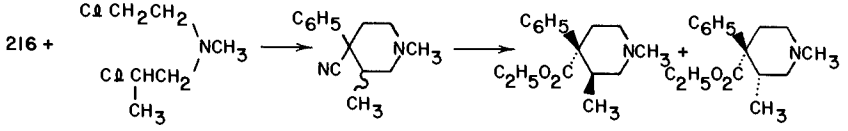
231, R = $CH_2CH_2C_6H_5$
232, R = $CH_2CH_2(p\text{-}C_6H_4NH_2)$
233, R = $CH_2CHOH\ C_6H_5$
234, R = $CH_2CH{=}CH\ C_6H_5$
235, R = $CH_2CH_2COC_6H_5$
236, R = $CH_2CH_2CHOHC_6H_5$
237, R = CH_2CH_2(2-pyridyl)
238, R = CH_2CH_2
239, R = CH_2CH_2CH
240, R = $CH_2CH{=}CH_2$
241, R = $CH_2CH{=}C(CH_3)_2$

The N-allyl derivatives **(240** and **241)** show analgetic potency in the range of the N-methyl compound and apparently fail to antagonize the action of narcotics.[134] By way of contrast the phenylbutyl analogue of bemidone **(242)** has been reported to show the same analgetic potency and to also exhibit antagonist activity.[135] A similar pharmacological profile has been reported for the cyclopropylmethyl derivative **(243).**[136]



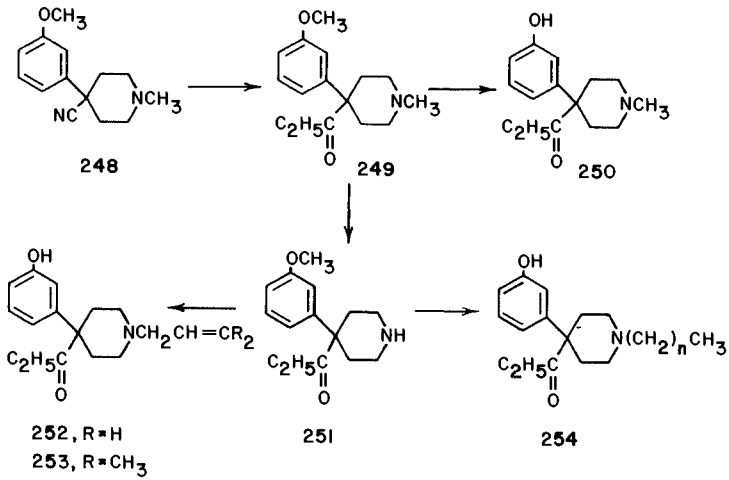
242, R = $(CH_2)_4C_6H_5$
243, R = CH_2

The effect of methylation of the heterocyclic ring on analgetic activity depends markedly on stereochemistry. Alkylation of phenylacetonitrile **(216)** with unsymmetrical nitrogen mustard **(244)** gives a mixture of cis and trans methylated piperidines. These were separated and converted to the respective meperidine analogues. The cis isomer **(246),** which is presumed to carry an axial methyl group, shows some 10 times the potency of its isomer.[137] The latter is about equal in activity to the desmethyl compound.

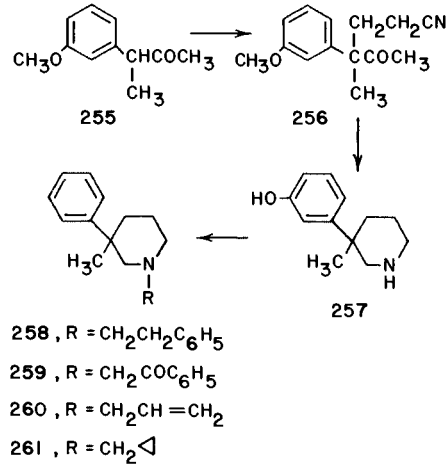


244 **245** **246** **247**

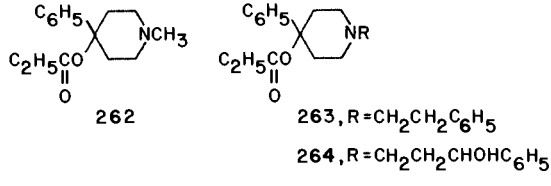
Condensation of nitrile **248** with ethylmagnesium bromide followed by O-demethylation affords the clinically useful analgetic drug ketobemidone **(250).**[138] In this subseries too replacement of N-methyl by allyl groups leads to analgetics **(252** and **253)** devoid of narcotic antagonist activity.[134,139] Surprisingly, substitution with long straight chain alkyl groups affords molecules that show both analgetic activity and narcotic antagonist action. Thus ketobemidone analogues bearing N-substituents from *n*-pentyl to *n*-heptyl **(254;** n = 4–6) show mixed activity *in vivo,*[139] in isolated tissue preparations,[140] and in opiate receptor binding assays.[141]



Analogues in which basic nitrogen is shifted to the 3 position show good analgetic activity in the presence of a methyl group on that ring. The synthetic scheme first involves cyanoethylation of substituted arylacetone **255.** Internal reductive alkylation leads to piperidine **(257).** Alkylation on nitrogen followed by O-demethylation gives the phenols. Both the N-phenethyl and N-phenacyl analogues show about twice the analgetic potency of morphine.[142,143] In this case traditional antagonist side chains have their usual effect. The N-allyl **(260)** and N-cyclopropylmethyl **(261)** analogues have recently been reported to be pure narcotic antagonists devoid of analgetic activity.[144] (Note compound **277** in this connection.)

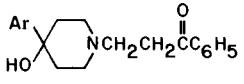


An analogue of meperidine in which the ester is reversed (**262** prodine) showed analgetic activity at one-fifth the dose of the parent compound,[145,146] and this spurred extensive investigations of these relatively easily accessible compounds. Assembly of the molecules usually starts with construction of a 4-piperidone by one of a large number of methods; nitrogen is carried through in some easily deprotected form if this substituent is to be subsequently varied. Reaction with arylmetallic reagents followed by acylation of the carbinol completes the sequence.



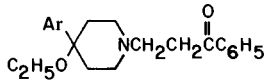
The SAR of substitution on nitrogen follows a pattern quite similar to that of meperidine. The phenethyl compound **(263)** thus shows about 4 times the potency of the N-methyl compound (20 times meperidine).[147] The reduced Mannich product **(264)** is one of the most potent phenylpiperidines, at 3200 times meperidine.[148]

Replacement of the ester by an ether group, obtained by acid-catalyzed alcoholysis of the carbinol, affords compounds that show reduced analgetic activity. The phenyl analogue **(267)** shows **4** times the potency of meperidine,[149] while the furyl analogue **(268)** is only 2.5 times as potent.[150]



265, Ar = C_6H_5

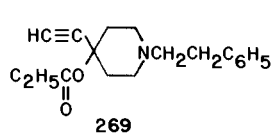
266, Ar = 2-Furyl



267, Ar = C_6H_5

268, Ar = 2-Furyl

Some degree of analgetic activity is retained when some other electron rich moiety, such as acetylene **(269),**[151] replaces the aromatic ring. The methallyl analogue **(270)** is reported to be more potent than its ethynyl analogue.[152]



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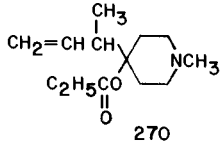
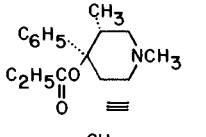


270

As in the case of meperidine itself, alkylation on a ring carbon has a marked effect on the potency of prodine analogues. The magnitude of the effect is closely linked to stereochemistry and chirality. The 3-methylated prodine in which phenyl and alkyl are cis **(271)** shows potency in the range of morphine; the trans isomer (**272;** alphaprodine) by contrast shows only one fifth that potency.[146] A study of a selection of 3-alkyl substituents showed that in the β (cis) series methyl and ethyl increase potency, while propyl depresses activity; in the α (trans) series, methyl has little or no effect, while ethyl and propyl depress activity.[153] The potency rank order was later confirmed by receptor binding assays.[154]



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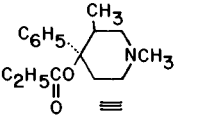
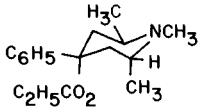


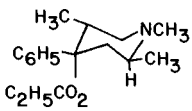
272

The SAR for the corresponding 3-allyl series is reversed; the trans isomer, in which the allyl is presumably equatorial, shows 34 times the potency of morphine.[155] This has been rationalized in terms of interaction of π cloud with a specific site on the receptor.[160]

Activity of compounds that contain two ring methyl groups depend on both relative stereochemistry and the conformation of the ring substituents. Thus a study of 2,6-dimethylated analogues showed that only isomer **273** exhibits appreciable analgetic activity.[156] In the 3,6-dimethyl series isomer **274** shows about the same potency as morphine.[157] Diastereomer **275**, promedol, shows a 20-fold increase in potency.



273



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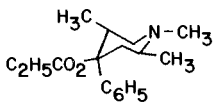
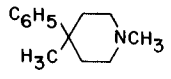


275

SAR derived from morphine, morphinans, and benzomorphans pointed to the necessity for an axial phenyl group on the piperidine ring.[158] Conformational studies on both meperidines and prodines indicated that these molecules are more stable in conformations bearing the aromatic ring in an equatorial position. It has been proposed that these agents act in a conformationally disfavored form or, alternatively, that they interact with alternate sites on the receptor. The very high potency of promedol has been attributed to the fact that the confirmed more stable conformation contains an axial phenyl.[159]

In an elegant series of papers Portoghese has reported on the use of asymmetric prodine analogues as opioid receptor probes.[160] A study of the relative analgetic potencies of resolved, variously alkylated prodines permitted the construction of a quite detailed theory on the mode of interaction of these compounds with receptors. Some of the results of this work suggest that compounds that exist predominantly with phenyl in an equatorial position may interact with loci on the receptor different from those in which phenyl is mainly axial.

It has recently been noted that incorporation of methyl at the 3 position can change pharmacological activity qualitatively as well. Thus the 3-methyl analogue **(277)** of a compound that itself shows only analgetic activity[161] exhibits activity as a pure narcotic antagonist. Replacement of N-methyl by phenethyl and propiophenone leads to marked potentiation of analgetic activity in most series; it is thus of interest that these very modifications in the series at hand lead to an increase in antagonist potency. Thus **279** is comparable to naloxone in potency.[162]



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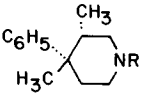
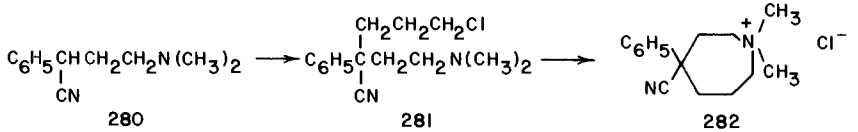
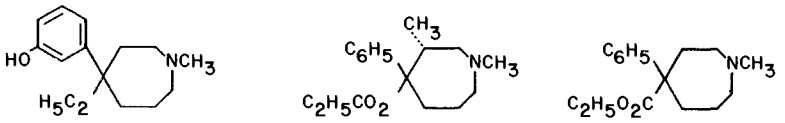


277, R = CH_3
278, R = $CH_2CH_2C_6H_5$
279, R = $CH_2CH_2COC_6H_5$

Expansion of the heterocyclic ring in meperidine is consistent with analgetic activity. The direct homologue, ethoheptazine **(283)**, shows about half the potency of the parent compound. The product **(280)** from alkylation of phenylacetonitrile with (chloroethyl)dimethylamine is first alkylated with 1,3-bromochloropropane. The quaternary salt **(282)** from internal reaction is then demethylated pyrrolytically and the nitrile converted to an ester.[163,164] Compounds containing an additional methyl group at the 2 or 3 position show an approximate twofold increase in potency.

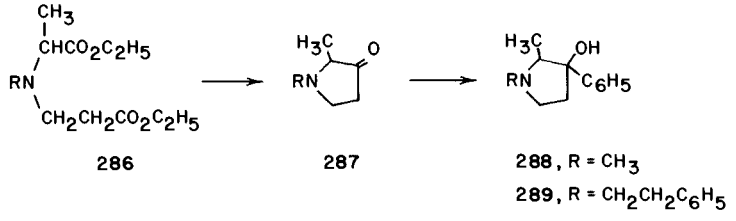


280 281 282



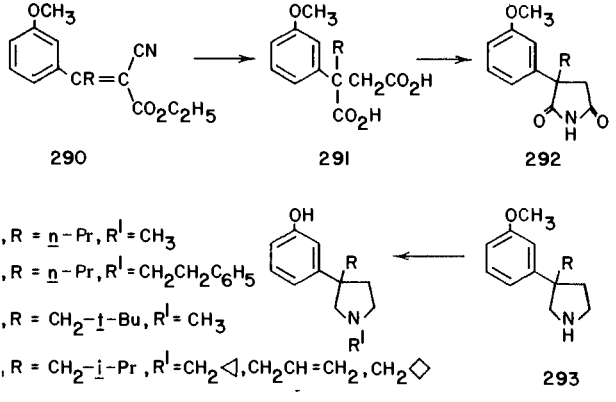
285 284 283

The reversed ester analogue of ethoheptazine alkylated at the 3 position **(284)** shows a 10-fold increase in potency over meperidine.[165] An *m*-hydroxylated analogue **(285)** is reported to be equal to pentazocine as an analgetic and as a narcotic antagonist.[166] It is interesting that ring contracted direct analogues of meperidine show little if any analgetic activity.[167,168] The analogue containing the reversed ester methylated at the 2 position prodilidene **(288)**, exhibits about half the potency of meperidine.[169,170] In this case replacement of N-methyl by phenethyl actually results in a drop in potency.[171] The starting 2-pyrrolidones **(287)** are available by Dieckmann cyclization of the corresponding aminodiesters **(286)**.



286 287 288, R = CH_3
289, R = $CH_2CH_2C_6H_5$

In the *m*-hydroxyphenyl series on the other hand good activity is associated with the presence of an alkyl group on the quaternary carbon. The N-methyl compound, profadol **(294)**, shows 2.5 times the potency of codeine;[172] the corresponding N-phenethyl derivative **(295)** shows 4.9 times the potency of codeine.[173] Investigation of the SAR of the geminal substituent indicates pivaloyl to be the most desirable alkyl group.[174] Preparation of this series first involves condensation of an acetophenone with ethyl cyanoacetate to give the unsaturated ester **290.** Conjugate addition of cyanide, followed by saponification and decarboxylation, gives acid **291,** which is then converted to the succinimide **292.** Reduction to a pyrrolidine, followed by alkylation and O-demethylation, gives the target compounds.



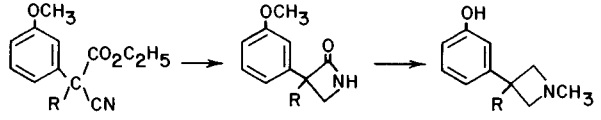
290 291 292

294, R = n-Pr, $R^1 = CH_3$
295, R = n-Pr, $R^1 = CH_2CH_2C_6H_5$
296, R = CH_2-t-Bu, $R^1 = CH_3$
297, R = CH_2-i-Pr, $R^1 = CH_2$◁, $CH_2CH=CH_2$, CH_2◇

293

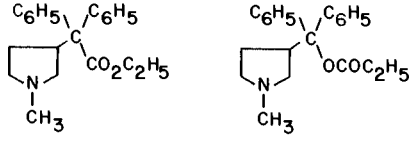
In marked contrast to the meperidine series alkylation on nitrogen with traditional antagonist side chains affords a series of compounds **(297)** that show various combinations of analgetic and narcotic antagonist activity.[175] Yet further contraction of the heterocyclic ring to an azetidine affords analogues that retain a measure of analgetic activity. The best of these **(300)** shows twice the potency of codeine. Reduction of cy-

anoacetate **298,** followed by cyclization by means of methylmagnesium bromide, gives the β-lactam **(299).** This is then further reduced, alkylated on nitrogen, and O-demethylated.[176]



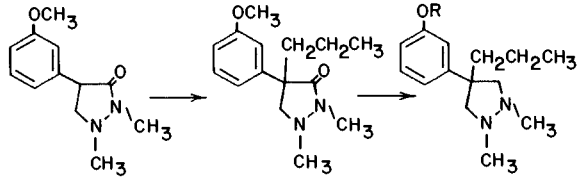
298 299 300, R = H, CH_3

Placement of the quaternary carbon at a position removed from the ring by one bond from the heterocycle affords analgetics with relatively low potency. Both the ester **(301)**[176] and the reversed ester **(302)**[177] show about half the analgetic potency of meperidine.



301 302

Perhaps most surprising is the observation that analgetic activity is retained when one of the ring methylene groups is replaced by basic nitrogen. Alkylation of pyrrazolone **(303)** (from 2-arylacrylic ester and *sym*-dimethylhydrazine) gives the propylated derivative **(304).** Reduction of the carbonyl group gives an agent **(305)** that shows half the potency of codeine as an analgetic. Another unusual feature in this series is the fact that the methyl ether and phenol **(306)** show essentially the same potency.[179]

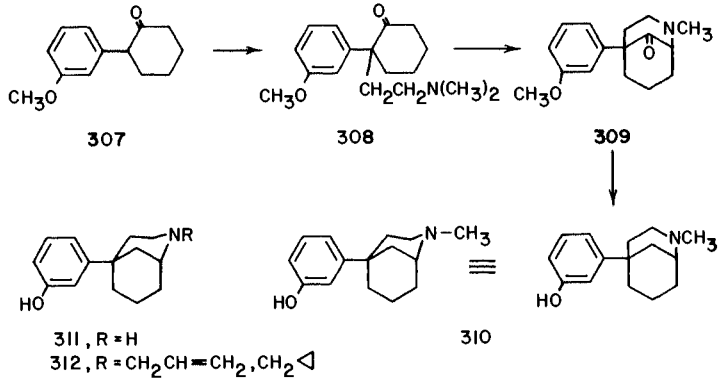


303 304 305, R = CH_3
306, R = H

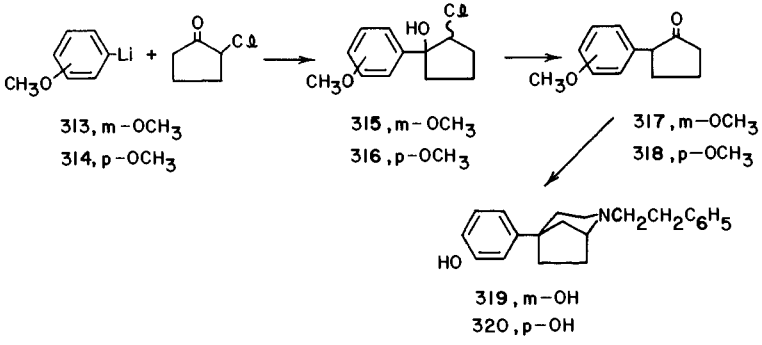
As noted above, phenylpiperidines can be formally derived from naturally occurring opioids by breaking the bridging bonds which render

these molecules rigid. The reverse exercise, that is, locking phenylpiperidines conformationally by adding unnatural bridging rings, is, interestingly, consistent with biological activity.

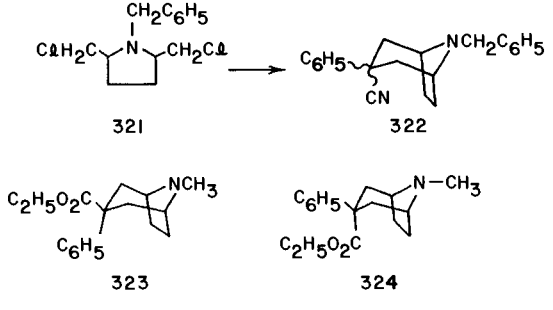
The original preparation of such bridged compounds was closely modeled on the Battersby benzomorphan synthesis. Thus alkylation of 2-(*m*-methoxy)cyclohexanone with 2-choro-N,N-dimethylethylamine gives aminoketone **(308).** This in turn is brominated, cyclized to the quaternary ammonium salt, and demethylated pyrrolytically to **309.** Successive reduction of the ketone and lactam functions, followed by O-demethylation, give the target compound **310.** The racemate proved to show analgetic potency equivalent to that of morphine.[180] Resolution reveals that most of the activity resides in the (+) isomer (4 times morphine); the (−) enantiomer retains about half the activity of morphine. Availability of an alternative synthetic scheme[182] provides ready access to the N-demethyl compound **(311).** Alkylation on nitrogen with a series of groups classically associated with antagonist activity give the analogues **312;** these agents show only weak analgetic activity and only a trace of antagonist activity.[183]



Contraction of the briding ring to cyclopentane leads to slight diminution in potency. The key arylcyclopentanones were obtained by condensation of the appropriate methoxyphenyl lithium reagents **(313** and **314)** with 2-chlorocyclopentanone; thermal rearrangement of the intermediate chlorohydrins **(315** and **316)** gives arylcyclopentanones **317** and **318,** respectively. These were then converted to the bridged piperidines by the scheme depicted here. The most active compound in the *m*-hydroxy series **(319)** shows analgetic potency in the range of morphine.[184] The corresponding *p*-hydroxy analogue is but one third as potent. Substitution of antagonist groups on nitrogen leads to inactive compounds.



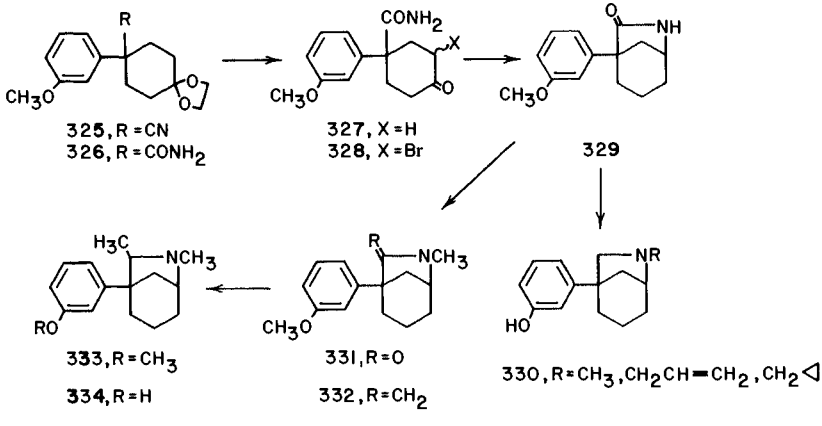
The analgetic activity of direct meperidine analogues bridged across the position adjacent to nitrogen shows surprisingly low sensitivity to stereochemistry. These molecules are constructed by a scheme very reminiscent of that used for meperidine itself. Condensation of dichloromethyl piperidine **321** with phenylacetonitrile gives the bicyclic piperidines **(322)**. These are then converted to the isomeric meperidine analogues **(323** and **324)** by standard manipulations. As might have been predicted, the more potent isomer is that in which the aromatic ring is axial **(323)**, this compound showing about the same analgetic potency as meperidine. It is somewhat surprising that the isomer possessing an equatorial phenyl group **(324)** still shows about one third the potency of meperidine.[186]



Addition of a propylene bridge to pyrrolidine analgetics affords yet another analgetic nucleus. Preparation of this series starts by stepwise conversion of nitrile **325** to the corresponding aminoketone **(327)**. Bromination adjacent to the carbonyl, followed by cyclization by means of methoxide, gives the desired nucleus **(329)**. This intermediate is reduced

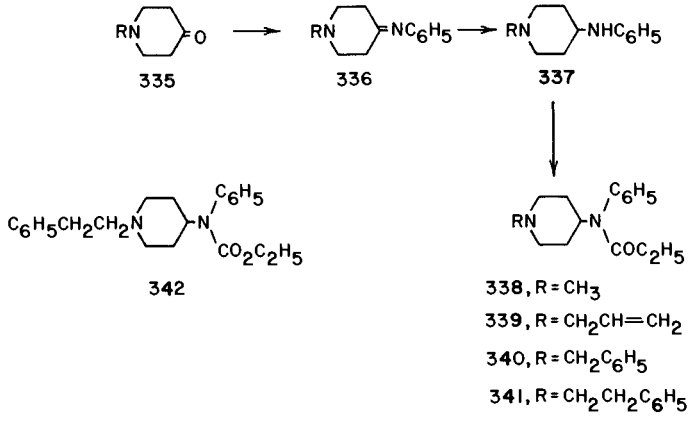
exhaustively, N-alkylated, and finally O-demethylated to give the series of analogues **(330)**.

Alternatively, **329** is first converted to lactam **331**, which is then N-methylated. Treatment of the lactam with methyl lithium leads to exocyclic enamine **332**. Reduction by means of sodium borohydride gives the analogue methylated on the bridge **(333)** as a single isomer. O-Demethylation completes the sequence.[187] The (+) isomer of resolved **333** shows analgetic potency equivalent to morphine in the mouse.[187] The agent is reported to exhibit nalorphinelike antagonism in morphine-dependent monkeys.[188]



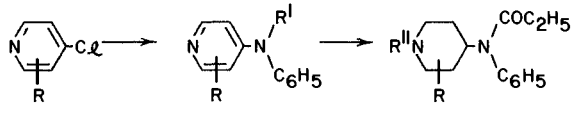
Modified piperidines have provided some of the most potent opioids ever produced. Some of these compounds in fact rival the antinociceptive potency of etorphine **(48)** in some of the small rodent assays. The extraordinary potency shown by these compounds is particularly noteworthy because they represent marked departures from the Beckett-Casy generalizations on structural requirements for analgetic activity.

The prototype, fentanyl **(341)**, can be obtained by one of several schemes starting from 4-piperidone.[189] The most straightforward involves formation of the Shiff base **(336)** of an N-alkylated piperidone with aniline **336**. Reduction, followed by acylation of the products with propionic anhydride, gives the desired products **(338–341)**. As might have been anticipated from the SAR in the meperidine series, the most potent analogue is that bearing an N-phenethyl group. The potency of that compound (300 times morphine in the rat), as well as the large increase in potency over the N-benzyl compound **(340)**, is quite unexpected.



As noted above, N-alkyl derivatives generally show only slight analgetic activity in this series. The N-allyl derivative is thus also a weak analgetic that shows no antagonist activity. The unacylated diamine **(337)** exhibits some analgetic activity[190] as does the carbamate.

The potency-enhancing effect of alkylation on the 3 position of the piperidine ring, first observed in the prodine series, has its counterpart in the fentanyl series. One approach to ring methylated derivatives starts by displacement of halogen by aniline in 4-chloropyridines. Acylation of the product **(344)**, followed by catalytic reduction of the heterocyclic ring, gives the norfentanyls **(346)**. Reductive alkylations with formaldehyde, benzaldehyde, and phenylacetaldehyde, respectively, give the corresponding N-alkylated derivatives **(347–349)**. It was noted that the 2-methyl and 2,5-dimethyl analogues carrying an N-methyl derivative (of unspecified configuration) show a 10-fold increase in potency over the parent compound. The N-methyl and N-benzyl derivatives show only weak activity.

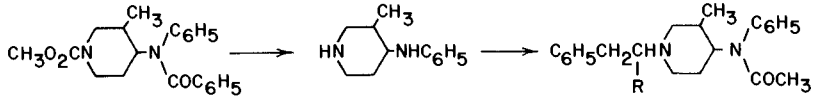


343, R = 2-CH_3; 3-CH_3; 2,5-$(CH_3)_2$

344, R^I = H
345, R^I = COC_2H_5

346, R^{II} = H
347, R^{II} = CH_3
348, R^{II} = $CH_2C_6H_5$
349, R^{II} = $CH_2CH_2C_6H_5$

A more detailed investigation of the effects of alkylation involved separation of isomeric carbamates **(350)** (obtained by a modification of the Shiff base route). The amines obtained on deprotection **(351)** are then alkylated with phenethyl as well as phenylpropyl bromides and then reacylated **(352 and 353).** The phenethyl analogue carrying trans methyl and amide nitrogen **(352b)** is somewhat more potent than fentanyl; the cis isomer **(352a)** on the other hand shows an eightfold increase in potency over the parent molecule. Optical resolution of this analogue showed that most of the activity resided in the (-) isomer, this compound showing 120 times the potency of fentanyl.

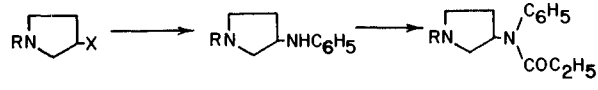


350 351a, cis; 351b, trans

352a, cis, R=H
352b, trans, R=H
353a, cis, R=CH_3
353b, trans, R=CH_3

The presence of a methyl on the side chain was found to have little effect on potency. These compounds did, however, show prolonged action, possibly because of retardation of metabolic N-dealkylation.[192]

In contrast to the meperidine series contraction of piperidine in fentanyl markedly reduces potency. Direct displacement of halogen on pyrrolidines **(354),** followed by acylation of the secondary amines, gives the products **(356).** The most potent compound in this series **(357)** shows about twice the activity of morphine; the phenethyl analogue shows an ED_{50} in the same range as morphine.[193]

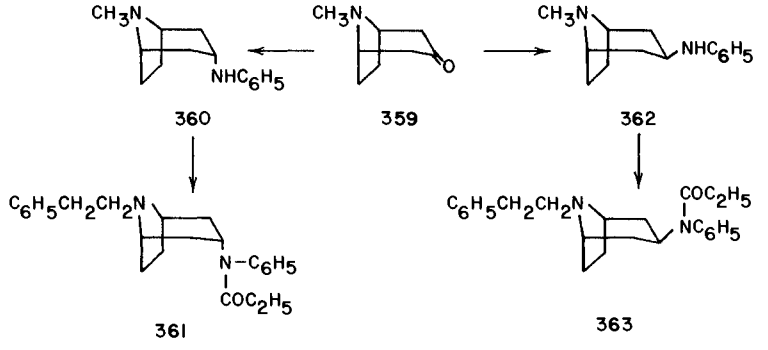


354, X=Br, OSO_2CH_3 355

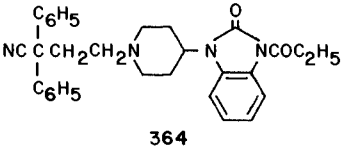
356, R=CH_3, C_2H_5, ...
357, R=$CH_2CH_2COC_6H_5$
358, R=$CH_2CH_2C_6H_5$

Analogues in which the piperidine is conformationally constrained in a bicyclic ring have been used to define the steric requirements for maximal activity. The Shiff base of tropanone **(359)** is converted to amines

(360 and **362)** by stereoselective reductions (catalytic hydrogenation; borohydride). Each is then in turn acylated, N-demethylated, and alkylated to a series of analogues including the phenethyl derivatives **(361** and **363).** The transoid isomer **(361)** shows about the same analgetic potency as morphine; the cisoid isomer is far more potent, showing about half the potency of fentanyl.[194]



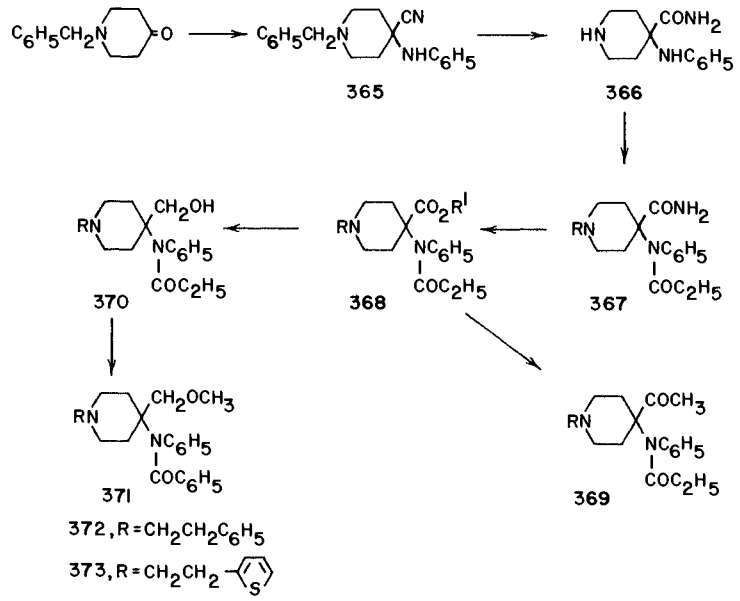
Formal cyclization of the anilide benzene ring with the acyl group affords an analgetic with good oral activity. This product, bezitramide **(364),** exhibits 5–9 times the analgetic potency of morphine in rats on oral administration.[245]



364

Additional substitution of the fentanyl ring carbon bearing the anilide nitrogen results in further increases in analgetic potency. The key intermediate in this series is the α-aminonitrile **(365)** obtained by reaction of N-benzyl-4-piperidone with aniline and hydrogen cyanide. Debenzylation, followed by hydrolysis of the nitrile, gives amide **366.** In a very extensive investigation of the SAR of this structural type, the amine was then alkylated with alkyl and aryl alkyl groups as well as with those groups associated with antagonist activity. The amides were then converted to esters and the corresponding methyl ketone. The esters in turn

were reduced to carbinols, and these were alkylated to their methyl ethers. To summarize an enormous amount of data, in this series, too, maximum potency is observed when nitrogen carries an aryl alkyl substituent. Highest potency is observed in the ester **(368)**, ketone **(369)**, and ether **(371)** series.[246] More detailed studies on the most potent compounds showed the phenethyl compound **(372)** to have about 10,000 times the potency of morphine (ED_{50} = 0.00078 mg/kg) in the rat, while the thienylethyl compound **(373)** is only slightly less potent (ED_{50} = 0.00079 mg/kg).[247] The latter analogue, sulfentanyl, shows 2400 times the potency of morphine when administered IV to dogs; the agent is a classic narcotic readily antagonized by naloxone.[248]



ACYCLIC COMPOUNDS

The relatively rigid structure of the analgetics discussed thus far may well contribute to the activity of these molecules by spatially fixing the

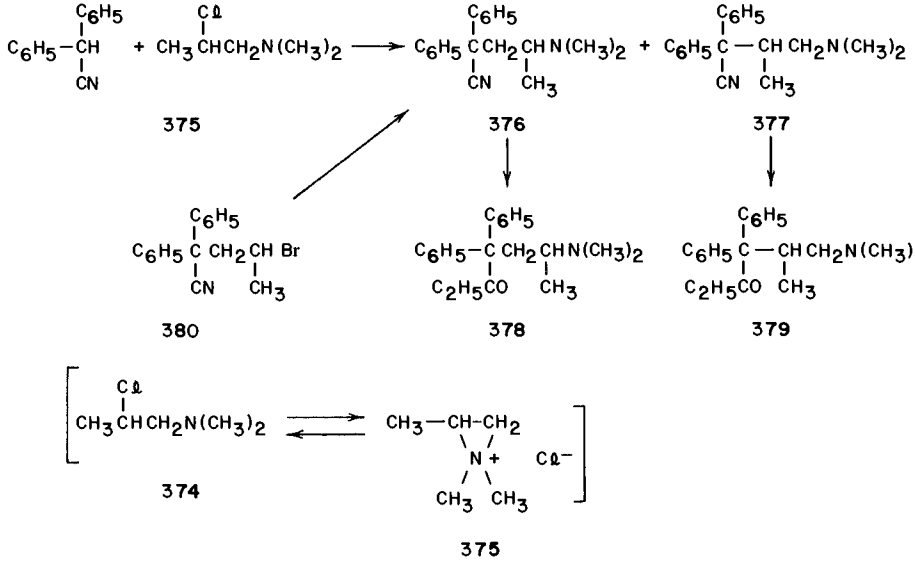
appropriate moieties in positions that maximize receptor interaction. A conformationally unrestrained molecule on the other hand requires some prior arrangement—and expenditure of entropy—to achieve a similar interaction. This conformational freedom can, however, lead to closer interaction than is possible in a less than ideally constituted rigid molecule. Some support for such a duality comes from the observation that a number of acyclic compounds show analgetic potency equivalent or superior to morphine.

The discovery of the first compound in this series, methadone **(378)**, in the mid-1940s occasioned an impressive amount of synthetic work on related molecules.[195] Despite the discovery of a large number of quite potent analgetic compounds, including several that showed good oral activity, few of these found their way into clinical practice. This circumstance was due at least in part to the finding that drugs in this series show a spectrum of pharmacological properties almost indistinguishable from morphine; in addition the compounds exhibit the same addiction liability as the older narcotics. Since this work is today largely of historic interest, only the salient features are presented here. The reader interested in a fuller account is referred to a definitive and exhaustive monograph published by Janssen in 1960.[195]

The lead compound in this series, methadone **(378)**, was discovered in Germany during World War II, a time when scientific publication was at a particularly low ebb. Details as to the structure and synthesis of this new analgetic were thus at first available to the outside world only in the form of intelligence reports.[196] Repetition of the putative synthesis in independent laboratories revealed that ambiguity existed as to the structure of the product of a key reaction. A pivotal step in the preparation of methadone involves alkylation of diphenylacetonitrile with N-(2-chloropropyl)dimethylamine **(374)**. The formation of two regioisomers in this reaction implicated the involvement of aziridinium ion **(375)** in the alkylation reaction; since this heterocycle can open in two ways, it was not clear which of the pathways led to the compound whose properties corresponded to methadone. The structure of the nitrile that is the precursor of methadone **(376)** was established by unambiguous synthesis via displacement of halogen from **380** by means of dimethylamine.[197,198]

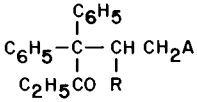
The isomeric nitriles obtained from the original synthesis can be separated; on reaction with ethylmagnesium bromide the product of "abnormal" alkylation **(376)** affords methadone **(37)**. The product of "normal" alkylation **(377)** leads to isomethadone **(379)**.[199] It is of note that the

two isomers exhibit almost equal analgetic potency, both isomers showing ED_{50}s in the range of morphine. It has been shown that the activity of methadone resides almost exclusively in the (-) isomer.[200]



Omission of the methyl group on the nitrogen bearing side chain leads to normethadone **(381)**, a compound with slightly reduced analgetic activity.[201]

Considerable latitude seems to obtain as to the nature of substitution on nitrogen. The diethyl compound **(382)** thus shows about the same potency as the parent drug.[202] Cyclization of the substituents on nitrogen leads to somewhat increased potency; the piperidine derivatives (**383**, hexalgon; **384**, dipipanone)[201,202] and the morpholine analogues (**385**; **386**, phenadoxone)[203,204] all show potency in the same range as methadone. Larger substituents on the carbonyl group **(387)** or abbreviation to an aldehyde **(388)** reduces potency to one fifth and one third of the parent compound,[203] respectively.



381, R = H , A = $N(CH_3)_2$

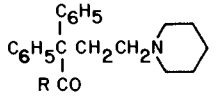
382, R = CH_3, A = $N(C_2H_5)_2$

383, R = H , A = 

384, R = CH_3, A = 

385, R = H , A = 

386, R = CH_3, A = 

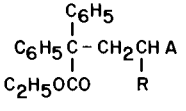


387, R = $CH(CH_3)_2$

388, R = H

Reduction of the carbonyl function of methadone and isomethadone affords in each case a mixture of diastereomers.[204,205] Though the alcohols are generally less active than the parent compounds, their acetylated counterparts show potency in the range of methadone. Activity is surprisingly insensitive to stereochemistry—the disastereomeric acetylmethadols show 1.5 and 2.3 times the potency of methadone, respectively. Recent extensive use of methadone in addiction control programs has led to some reawakening of interest in methadols, as these have been identified among the metabolites of the parent drug.

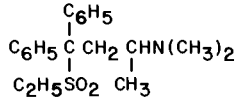
It is difficult to draw firm conclusions on SAR of the methadols, since these drugs can in principle be readily metabolized back to the ketones. A number of analogues substituted at that position by functions not convertible to ketones show good activity; this suggests that this position, too, can tolerate some change.



387, R = CH_3, A = $N(CH_3)_2$

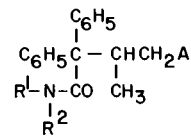
388, R = H, A = 

389, R = H, A = 



400

Whereas the direct analogue of methadone in which ketone is replaced by an ester **(387)** shows little analgetic activity;[201] the corresponding morpholine derivative **(388)** shows one fourth the potency of the parent. The corresponding piperidyl derivative norpipanone **(389)** has found some use in the clinic.[206] The sometime bioisosterism of carbonyl and sulfone finds one of its most striking realizations in the observation that **400** shows the same potency as methadone itself.[207]



401, $R^1 = R^2 = H$, $A = N(CH_3)_2$

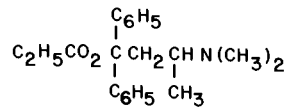
402, $R^1 = H$, $R^2 = C_2H_5$, $A = N\square O$

403, $R^1 = R^2 = CH_3$, $A = N\square O$

404, $R^1 = R^2 = -(CH_2)_4-$, $A = N\square O$

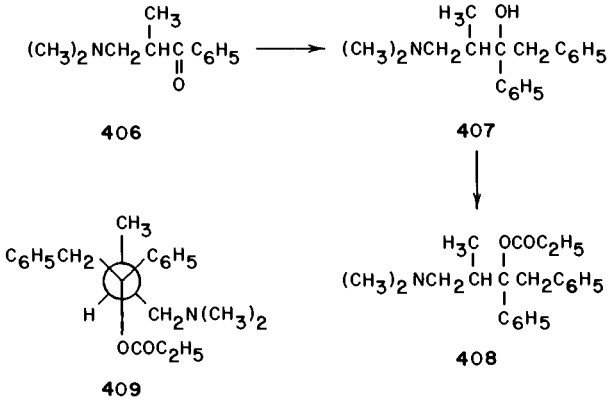
Appropriately substituted amides have also proven to be useful surrogates for ketone carbonyl in this series. The presence of hydrogen on the amide nitrogen apparently greatly diminishes analgetic activity; primary amide **401** shows mainly anticholinergic activity, while the secondary amide **(402)** shows only weak analgetic activity. Tertiary amide **(403)** on the other hand shows close to 4 times the analgetic potency of methadone. The pyrrolidine amide **(404)**, racemoramide, exhibits about the same potency. Resolution reveals that the analgetic activity in this case resides almost entirely in the (+) isomer.[208,209] This drug, dextromoramide, has been used extensively in the clinic, as it has the properties of a classical narcotic analgetic.

The SAR of the methadones also diverges markedly from that of the meperidines with respect to reversed esters. In the latter series prodines are essentially equipotent to pethidines; the corresponding reversed methadone **(405)** shows but one fifth the potency of the parent.[210]



405

It is interesting that insertion of a methylene group between an aromatic ring and the quaternary center serves to restore potency in this reversed ester series. Reaction of the Mannich product **(406)** from propiophenone with benzylmagnesium chloride gives predominantly a single aminoalcohol **(407),** designated α. Acylation of this racemate with propionic anhydride gives the widely used analgetic *d,l*-propoxyphene **(408).**[211] This orally effective drug displays about half the antinociceptive potency of meperidine in a range of animal models. The diastereomeric compound derived from the β-aminoalcohol shows little if any analgetic potency. The relative configuration of the active α-isomer has been demonstrated to be that shown for **409.**

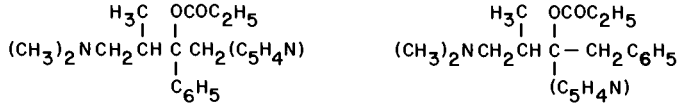


406 **407**

409 **408**

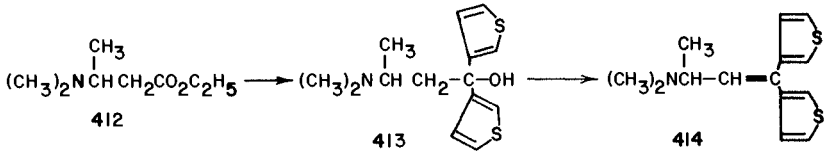
Resolution of carbinol **407** (α-isomer), followed by conversion of each optical isomer to its corresponding ester **408,** showed that analgetic activity resides largely in the (+) isomer.[212] This was shown subsequently, on the basis of degradation studies, to have the (2S:3R) absolute configuration. Resolution of **406,** followed by reactions of the (-) antipode of this ketone with benzyl Grignard and subsequent acylaction, affords a stereoselective route to dextropropoxyphene.[214]

Replacement of the more distant aromatic ring by pyridyl **(410)** doubles potency; paradoxically, replacement of the other ring **(411)** leads to an inactive compound.[215]

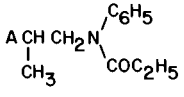


410, (C_5H_4N) = 2-pyridyl **411**

Replacement of both aromatic rings in the open chain series by 2-thienyl completely dispenses with the need for a quaternary carbon atom. One of the most potent compounds in this series, dimethylthiambutene **(414)**, shows about the same analgetic ED_{50} in animals as morphine. Ancillary pharmacological properties mark this drug as a classic narcotic.[216,217] This agent is available in a straightforward manner by reaction of Michael the adduct **412** with excess 2-thienyl lithium, followed by dehydration.[218]



The high-potency piperidine analgetics typified by fentanyl **(341)** depart from the classical opioid structure in replacement of the aryl bearing quaternary center by an acylated anilide. The finding that this structural interchange is consistent with analgetic activity actually came from an observation in an open chain series.



415, A = $N(CH_3)_2$

416, A = $CH_3NCH_2C_6H_5$

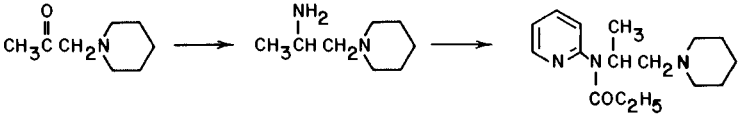
417, A = $CH_3NCH_2CH_2C_6H_5$

418, A = N⬡

The parent compound in this series **(415)**, shows analgetic potency close to that of meperidine. Omission of the methyl group on the carbon bearing the basic substituent leads to a loss of potency. Potentiation by phenethyl in this series is not particularly marked; **417** shows 4 times the potency of meperidine. Substitution on the phenethyl aromatic group generally decreases activity, though some small increase in potency is shown in the case of the analogue bearing an *m*-methyl group.[219–221] A subsequent investigation confirmed this order of potency.[222]

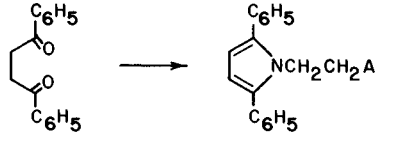
Replacement of aniline by 2-aminopyridine gives a series of orally active analgetics, some of which show some narcotic antagonist activity.

The general approach to this series is typified by the preparation of propiram **(421)**. Reductive alkylation of β-aminoketone **419** gives the diamine **420**. This is then reacted with 2-bromopyridine and the resulting diamine acylated by means of propionic anhydride.[223] Propiram, which shows about the same ED_{50} in antinociceptive screens as meperidine, has recently been introduced as a commercial product.



419 **420** **421**

The nonbasic nitrogen can itself be included in a heterocyclic ring. The most potent **(423)** of a series of products from reaction of ethylene diamines with ketone **422** shows antinociceptive activity equivalent to that of meperidine.[234]



422

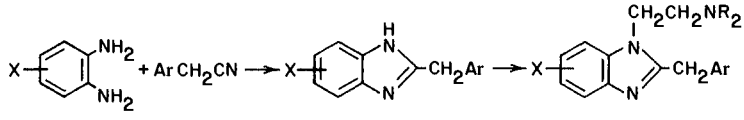
423, A = $N(CH_3)_2$

424, A = N⟨O⟩

425, A = N⟨NCH_3⟩

This observation was foreshadowed by an earlier series that bears a somewhat more distant structural relation to the anilide series. The finding that benzimidazole **428** shows one tenth the analgetic potency of morphine led to an extensive synthetic effort, by the general scheme given here, to define the SAR of this structural class. Biological activity in this series proved exquisitely sensitive to small structural modifications. These compounds are possibly unique among analgetics in that a diethylamino group **(428)** gives higher potency than a dimethylamino **(429)**. Addition of a nitro group at the 5 position of the fused aromatic ring leads to an order of magnitude increase of potency; substitution of ether oxygen on the para position of the pendant aromatic ring also contributes enormously to analgetic potency (the corresponding ortho and meta isomers show little improvement over the parent compound).

The most potent of these benzimidazoles, etonitazene, has roughly 1000 times the analgetic potency of morphine.[225–227] Clinical trials were disappointing in that they revealed that this drug has, if anything, an even smaller therapeutic index than morphine.



426 427

428, X = H, Ar = C_6H_5, R = C_2H_5

429, X = H, Ar = C_6H_5, R = CH_3

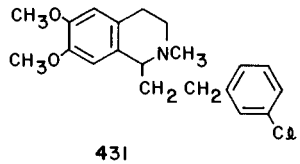
430, X = 5-NO_2, Ar = p-$CH_3O_6H_4$, R = C_2H_5

MISCELLANEOUS COMPOUNDS

Though the analgetics described above vary quite widely in their structural characteristics, they can by and large be related to the prototype, morphine. As noted on several occasions, these real or fancied relationships have led to a number of formalized postulates on structural requirements for activity, as well as speculations on the topology of the opioid receptor.

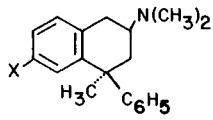
Most of the compounds discussed below cannot be quite so readily classified. It is thus of note that some of these agents show extremely high analgetic potency, an observation that suggests that various SAR postulates are in need of reformulation.

Tetrahydroisoquinolines such as those below embody many of the structural features posited by the Beckett-Casy rule, though it should be noted that basic nitrogen is located on a tertiary rather than a quaternary carbon. A fairly extensive investigation in this series led to the observation that highest potency is associated with dimethoxy (or dimethyl) substitution on the fused ring; the presence of halogen or nitro on the side chain ring is a requisite for activity. Modification of the substituent on nitrogen leads to loss of activity; the N-phenethyl derivative atypically shows no analgetic activity.[228] The optimum compound of this series, methopholine **(431)**, shows analgetic potency in the range of codeine on parenteral or oral administration. This drug was introduced into clinical practice.

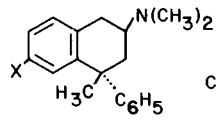


431

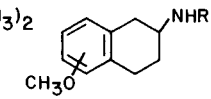
Aminotetralins such as **432** can in some ways be regarded as cyclized methadone analogues (after allowing for replacement of acyl by methyl), though it can be questioned whether the open chain molecule would in fact assume such an internally crowded configuration. In the event it was noted that the desmethoxy compounds **(432** and **433)** show analgetic potency equivalent to maperidine; interestingly, both isomers show virtually the same ED_{50}. The cis phenol **(434)** shows essentially the same activity; in this case potency is doubled in the trans isomer **(435).**[229] In this connection it is of note that several of a large series of simple 2-aminotetralins show some quite modest analgetic activity.[230]



432, X = H
434, X = OH

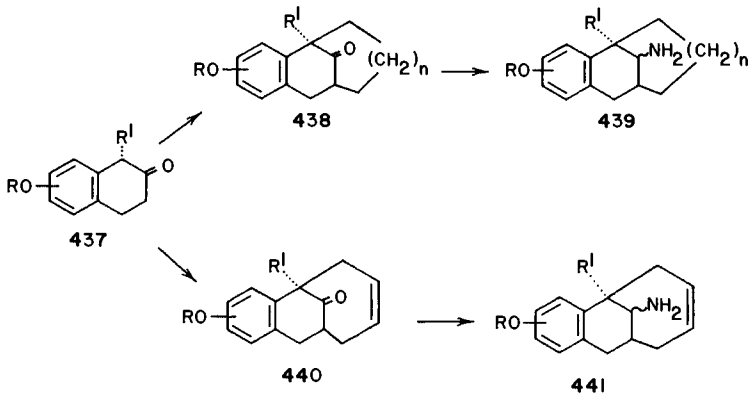


433, X = H
435, X = OH

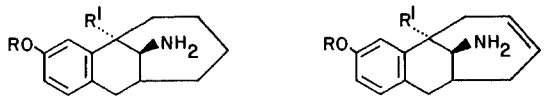


436

Incorporation of a bridge in the hydroaromatic ring of the aminotetralins has a dramatic effect on biological activity, leading to agents with potency in the range of morphine. These compounds are obtained by dialkylation of a 2-tetralone **(437)** with α,ω-dihalides, followed by a two step conversion of the ketone to an amine.[231,232] Activity was found to reside largely in the isomer carrying an equatorial amine group. In this series the primary amines show much better activity than the secondary or tertiary analogues, in contrast to almost all other analgetics. The most active compounds **(442–444)** show analgetic potency about equal to that of morphine; oral activity is particularly notable, **445** showing 2.5 times the potency of morphine.



Resolution of these three compounds showed that in this series too analgetic activity is associated largely with a single optical antipode. Though the sign of optical rotation of the most active compound of a pair varies, intraconversion makes it likely that all active compounds have the same absolute configuration.[233]

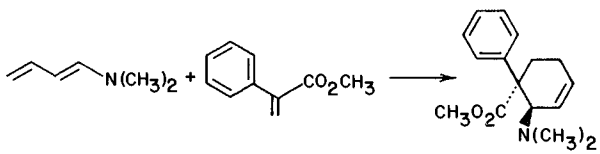


442, $R = R^1 = CH_3$

443, $R = CH_3$, $R^1 = C_2H_5$ 444, $R = CH_3$, $R^1 = C_2H_5$

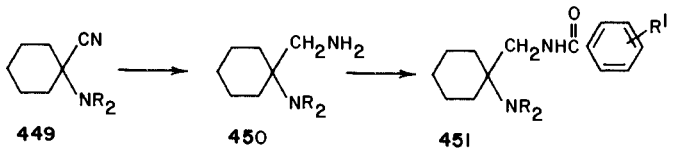
445, $R^1 = H$, $R^1 = C_2H_5$

Yet another compound that would seem to possess the wrong connectivity for central analgetic activity is the major product from the cycloaddition of the enamine **(446)** from crotonaldehyde with acrylate **447.** This compound, tilidine **(448)**, is a potent central analgetic.[234] The minor stereoisomer, as might be expected, shows little analgetic activity. Though originally thought to have little addiction potential, widespread use in the clinic has shown this drug to be subject to abuse.

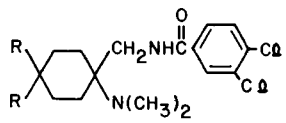


446 447 448

A hybrid between the cyclic and open chain analgetics constitutes another series that cannot readily be classified. Thus anilides from reduction products **(450)** from α-amino nitriles **(449)** show surprisingly good analgetic activity.[235] For the most part these analogues **(450)** show potency in the range of codeine. Surprisingly, the 3,4-dichlorophenyl derivative **(454)** shows virtually the same potency as morphine. In related work from another laboratory it was shown that incorporation of oxygen at the 4 position of the cyclohexyl ring **(453)** leads to a sixfold increase in potency.[236]



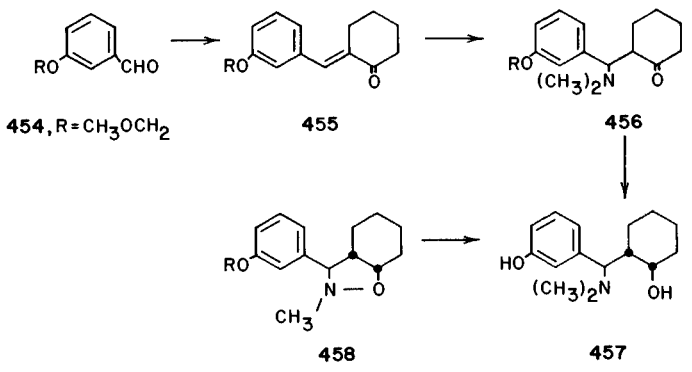
449 **450** **451**



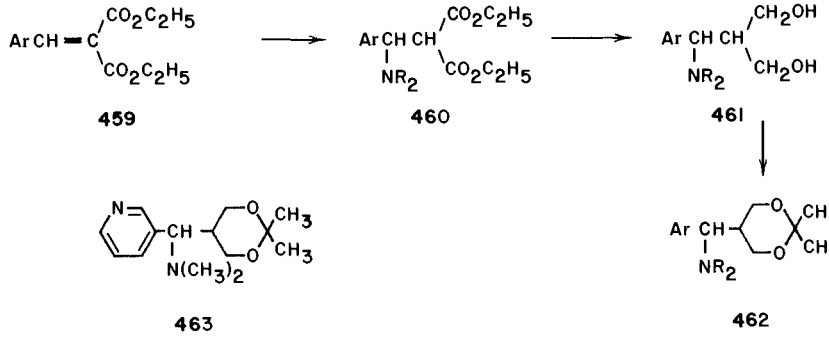
452, R = H
453, R = OCH_2CH_2O

The majority of agents noted above carry basic nitrogen at a distance of at least two carbons from the aromatic ring. This generalization too can be violated. The three recent series described below all carry that basic center on the benzylic carbon attached directly to the aromatic ring.

Conjugate addition of dimethylamine to the condensation product from cyclohexanone and benzaldehyde **454** leads to aminoketone **455.** Reduction, followed by deprotection of the phenol, gives the racemic product **457.** The cis relation of hydroxyl and the side chain follows from the observation that the same product is obtained by elaboration of the adduct **458** from 1,3 dipolar addition of the nitrone from **454** and cyclohexene. Resolution showed that the activity resides in the (-) isomer. This compound, ciramandole, is a mixed agonist-antagonist with about twice the analgetic activity of morphine in the rat.[237]



Work from another laboratory showed that the oxygen can be incorporated into the ring. A series of such acetals were prepared by conjugate addition of amines to the malonate **459**, followed by reduction **(460)** and acetal formation with the resulting 1,3-glycols **(461)**.

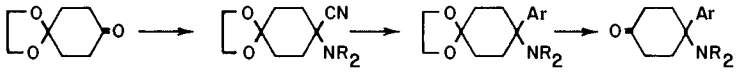


As in the case of more conventional structures, highest potency is obtained in those analogues where the amine is a dimethylamino group. The effect on potency of substitution on the aromatic ring, on the other hand, does not follow the usual pattern. One of the more potent compounds in this series, doxpicomine **(463)**, shows one sixth the analgetic potency of morphine. The phenols on the other hand (**462**; Ar = *p*-C_6H_4OH, *m*-C_6H_4OH) show little differentiation in potency, each showing an ED_{50} roughly a decade higher than that of morphine. Resolution of **463** and the corresponding phenyl analogue showed that the (-) isomer is more potent in each case.[238]

Yet another series of analogues showed that the saturated ring can

be attached directly to the aromatic ring and nitrogen placed on the resulting tertiary center.

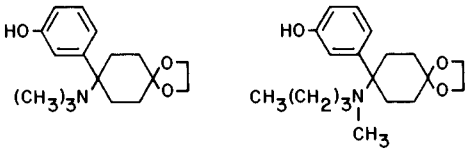
The prototypes were most conveniently prepared by a scheme that involves reaction of the α-aminonitrile **(465)** from the ketal of cyclohexane-1,4-dione **(464)** with arylmagnesium halides.[241]



464 **465** **466** **467**

In this series too the classic dimethylamino compounds show the highest potency; as a rule acetals **(466)** show slightly higher potency than the ketones. Omission of oxygen at the 4 position results in loss of analgetic potency. (These products are in fact phencyclidine derivatives.) The most potent compound in this series (Ar = p-BrC_6H_4, R = CH_3) shows about one third the nociceptive activity of morphine.[240]

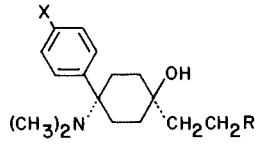
Introduction of the traditional m-hydroxyl group into the basic structure leads to an agent **(468)** that shows predominantly antagonist activity. Replacement of one of the methyl groups by some of the traditional antagonist moieties generally leads to loss of activity. Replacement of one of these groups by N-butyl **(469)** (but not isobutyl) gives a mixed agonist-antagonist.[241]



468 **469**

Addition of organometallics to the ketone in **467** leads to major increases in milligram potency. These nonstereospecific reactions give the isomeric aminoalcohols in equal proportions. In every case the isomer in which hydroxyl and nitrogen are trans shows higher potency. Systematic modification of the side chain showed that highest potency is obtained with the phenethyl group **(470)**. This compound shows about 1000 times the potency of morphine (ED_{50} = 1.4 μg/kg). That the terminal group must be a flat lipophilic moiety rather than specifically a benzene ring was shown by the fact that **471** shows fully half the potency of **470**.[242] Simple additivity obtains in this series; that is, putting together the best potentiating groups at each position leads to a compound **(472)**

with 12,000 times the potency of morphine. This agent displays an *in vitro* binding affinity 30 times that of morphine.



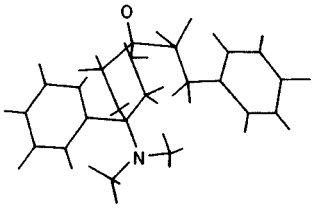
470, X = Cl, R = C_6H_5
471, X = Cl, R = c-C_5H_9
472, X = Br, R = C_6H_5

Receptor mapping by examination of SAR in any given series is an exercise that can present serious pitfalls. If the variety of structures is too narrow, it is almost inevitable that the resulting map will be of limited validity. In the opioid analgetic series, fortunately, there is available a wide variety of structures that have shown some analgetic activity.

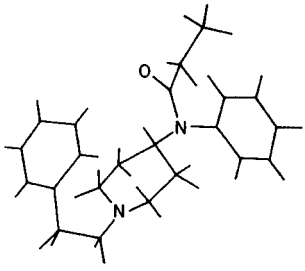
All efforts to deduce the topology of a receptor rest on implicit assumptions, most of which are open to some criticism. The most important of these is the assumption that relative potency is a measure of agonist-receptor fit. This can be readily attacked on the grounds that almost all the potency data come from *in vivo* experiments and thus include all the errors introduced by divergencies in absorption, transport, and disposition. Most of the compounds being considered probably have comparable lipophilicities and might be expected to be handled in comparable manners, somewhat mitigating this criticism.

Receptors are almost certainly not static cavities in a biopolymer awaiting the arrival of an agonist. Despite this it is often assumed that they can be treated as such operationally in describing the binding process. It is thus assumed that valid information on receptor topology can be derived from taking common spatial features of a variety of highly potent agonists.

Thus it has been shown that a model of **472** can be overlaid on that of fentanyl so that an exact correspondence is achieved between the two aromatic rings and the basic nitrogen. The hydroxyl of **472** lies in the same general vicinity as the amide. A similar fit is expected from some of the very recent, superpotent fentanyl analogues (i.e., **372**), since structural changes from the prototype do not affect the fit.

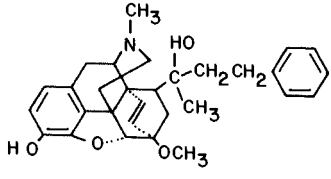


472



FENTANYL

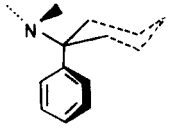
A very similar fit can be obtained by overlaying **472** on the superpotent oripavine **473.**



473

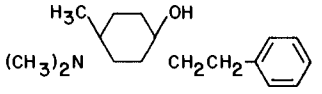
These overlays would seem to include the following requirements for receptor fit necessary for antinociceptive activity:

1. An aromatic ring that bears a relationship to a basic center definable in terms of axial aryl on cyclohexyl and the amine on the benzylic carbon. (It is often overlooked that the original Beckett-Casy generalization included axial phenyl, though they did not use that term[158].)



2. A polar function 5.8 Å from that amine.
3. The results of several systematic SAR studies all lead to the conclusion that an accessory binding site exists at a distance equivalent to an ethyl chain from the polar site.[243] The existence of that site was in fact first proposed by Bentley as an outcome of his studies on the SAR of the oripavanes.[44] Preliminary results indicate that **474,** which

would bind to only the fourth site, the polar center, and the amine site, does in fact show analgetic activity, albeit at very high doses [244]



474

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