Review Article

Opioid receptor systems and the endorphins: a review of their spinal organization

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A review of the spinal organization of opioid receptor systems and endorphins is presented. The review is a consideration of the physiological mechanisms underlying the effect of spinal opioids, the pharmacology of the opioid receptors that moderate a variety of spinal processing systems, and the endorphin systems that act upon the spinal receptors.

KEY WORDS • spinal cord • opioid receptor • endorphin • pain

Morphine delivery limited to the spinal cord will produce a powerful modulation of certain specific aspects of spinal neuronal processing. This action occurs by virtue of specific populations of opioid receptors which are associated with spinal substrates related to sensorimotor and autonomic function. As a result of this fortuitous relationship, spinally administered opiates yield a powerful, selective, and clinically relevant analgesia. These receptors also represent the likely synaptic site of action for local terminals which release endogenous materials that behave as do opioid alkaloids (for example, endorphins). The thesis of this overview is to consider: 1) the physiological mechanisms underlying these effects of spinal opiates; 2) the pharmacology of the opioid receptors that modulate a variety of spinal processing systems; and 3) the endogenous systems that act upon these spinal receptors.

Opioid Receptor Systems

Classification of Opioid Receptors

Before discussing the specific role and action of spinal opiates, it is necessary to consider the general issue of opioid receptors. Although receptors are physical entities located in the cell membrane, current classification of receptors is largely based on the identification of the descriptive pharmacology that associates the actions of a structurally related family of agents to a given physiological endpoint (such as blockade of the stimulated contraction of smooth muscle, analgesia, or respiratory depression). The essential elements of the descriptive pharmacology are: 1) the ordering of the structure activity relationship measured in different assay systems; 2) the characteristics of the antagonist interaction; and 3) cross tolerance (or lack thereof) between different agonists. Based on these considerations, a number of subpopulations of opioid receptors have been identified.

Historically, the current separation of opioid receptors is based on the initial formulation by Martin and colleagues in which they proposed the separation of \( \mu \), \( \kappa \), and \( \sigma \) receptors on the basis of several observations made in chronic spinally transected dogs: 1) distinctive behavioral syndromes (for example, \( \mu \): depression of thermal/pressure reflexes; \( \kappa \): depression of pressure reflexes; \( \sigma \): agitation, delirium); 2) sensitivity to naloxone antagonism (\( \mu > \kappa >> \sigma = 0 \)); and 3) cross tolerance (\( \mu \) agonists would suppress withdrawal in morphine-tolerant animals, \( \kappa \) agonists would suppress withdrawal in \( \kappa \)-tolerant but not \( \mu \)-tolerant animals; \( \sigma \) agonists precipitate withdrawal).

Isolation of the enkephalins (see Endogenous Systems Acting Through Spinal Opioid Receptors, below)
revealed a class of sites (δ) that were distinct from those classified as μ. This separation was based on the differential effects of the μ alkaloid agonists and the pentapeptide enkephalins on the ileum of the guinea pig versus the vas deferens of the mouse, and on the selective sensitivity to naloxone antagonism (μ > δ). 113

Subsequent studies have indicated that a fifth class of receptors may be identified in certain bioassays based on the relative activity of β-endorphin fragments. 157

Table 1 presents a summary of the receptor classes that have been generally proposed. Several points regarding the content of Table 1 should be made. First, as indicated, many of the agonists currently available are not uniformly selective. Observation that a given agent produces an effect may be taken only as an indication that one of the several receptors may be involved. Thus, β-endorphin may exert its actions at a variety of receptors including those designated as μ. The μ versus δ receptor selectivity for D-ala2-D-leu5-enkephalin (DADL) is about 10 to 20:1. The κ versus μ selectivity for ethylketocyclazocine (EKC) may range from around 1:1 to 5:1. The second point is that additional evidence defining a subpopulation of receptors may be adduced by the consideration of the characteristics of the antagonists. As indicated, naloxone has a measurable action at all opioid receptors except that designated as the σ receptor. Importantly, however, estimates of the relative affinity of naloxone for the several receptors have indicated significant differences such that μ > δ ≥ κ, where the ratio of μ/δ affinity is approximately 10:1 to 20:1 (see Lord, et al., 113 and Oka, et al., 133).

Independent support for the above functional subdivision of the opioid receptor has derived from radioligand binding studies. 1) Early investigators pointed to multiphasic Scatchard plots which suggested that the labeled ligand (for example, naloxone) was binding to at least two classes of sites which differed in their affinity for the ligand. 2) The ability to competitively displace opioid alkaloids and peptides noted above varied predictably according to the several families identified by the bioassay. Thus, with regard to the ability to displace several radiolabeled ligands, the results of a variety of studies may be summarized as follows (see Yaksh91,192 for a review):

1H-dihydromorphine (μ): sufentanil >> (DADL, USO488H)
1H-DADL (δ): met-enkephalin >> (morphine, USO487H)
1H-EKC (κ): USO488H > (morphine, DADL)
1H-SKF10047 (σ): SKF10047 >> (DADL, morphine).

3) Changes in the binding condition (such as Na+/Mg++ levels, pH, and presence of cyclic nucleotides) have been shown to exert differential effects upon the binding of the various families of ligands. 6 In early studies, it was shown that elevated Na+ would decrease agonist binding, leaving antagonist binding unaffected. 36 The μ and δ binding is differentially suppressed in the presence of guanine nucleotides. 31 Other investigators have, in fact, proposed that subclasses of opioid binding sites may be uniquely classified as being guanosine triphosphate (GTP)-sensitive and GTP-resistant. 20 4) Receptor protection experiments are carried out where binding with noncompetitive (for example, irreversible) ligands, such as phenoxybenzamine, β-chlonnalntrrexsime, or n-ethylmalaleimide, is performed in the presence of receptor-selective ligands. Such experiments have demonstrated that μ drugs would protect the binding site acted upon by other μ ligands, but not by δ ligands and vice versa. 147,162 5) The anatomical distribution of the binding of the different radiolabeled ligands in brain and spinal cord was found to differ markedly. Thus, for example, the ratio of μ/δ binding was found to be greater than 1 in the substantia nigra and thalamus and less than 1 in the hippocampus and cortices. 127 Quantitative receptor autoradiography has also demonstrated even more marked differences within limited regions. 187 In cerebral cortex, for example, laminae I and IV display elevated μ binding while laminae II, III, and V show elevated δ binding. 69 This differential distribution of binding is found throughout the brain and emphasizes that the differences in binding likely reflect physically distinct sites.

In addition to the above subclasses of receptors, binding studies have led to the suggestion of other

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**TABLE 1**

Summary of generally accepted receptor classes *

<table>
<thead>
<tr>
<th>Receptor Designation</th>
<th>Prototypical Exogenous Agonists</th>
<th>Probable Endogenous Agonists</th>
<th>Receptor Antagonists</th>
<th>Ex Vivo Bioassay System</th>
</tr>
</thead>
<tbody>
<tr>
<td>μ</td>
<td>morphine, 118 sufentanil, 100 morphiceptin</td>
<td>met-enkephalin, 113 β-endorphin</td>
<td>naloxone, 118 β-FNA</td>
<td>mouse GPI</td>
</tr>
<tr>
<td>δ</td>
<td>DADL, 113 DPDPE</td>
<td>met-enkephalin, 113 β-endorphin</td>
<td>naloxone, 118 IC1-174864</td>
<td>mouse GPI</td>
</tr>
<tr>
<td>κ</td>
<td>EKC, 118 USO488H</td>
<td>met-enkephalin, 113 β-endorphin</td>
<td>naloxone, MR-2266</td>
<td>rabbit GPI</td>
</tr>
<tr>
<td>ε</td>
<td>?</td>
<td>dynorphin, 134</td>
<td>naloxone, MR-2266</td>
<td>rat GPI</td>
</tr>
<tr>
<td>σ</td>
<td>SKF10047</td>
<td>β-endorphin</td>
<td>none</td>
<td>?</td>
</tr>
</tbody>
</table>

* Superscript numbers in the table are reference numbers in the bibliography. Abbreviations: ? = not known; DADL = D-ala2-D-leu5-enkephalin; DPDPE = D-pen2-D-pen5-enkephalin; EKC = ethylketocyclazocine; USO488H = (trans-3,4-dichloro-N-methyl-N-[2-(I-pyrrolidinyl)cyclohexyl] benzeneacetamide; SKF10047 = N,N-diallyl-Tyr-Aib-Aib-Phe-Leu-OH; GPI = guinea pig ileum; VD = vas deferens.
distinguishable opioid sites. High levels of 3H-labeled diprenorphine binding are found in the cerebellum (a structure with essentially no other opioid binding) and these are referred to as \( \lambda \) sites.\(^{71} \) Based on the binding of a covalently linked antagonist, naloxazone, it has been proposed that binding sites be separated into high- and low-affinity \( \mu (\mu_1/\mu_2) \) sites and \( \delta \) sites which have pharmacological significance.\(^{112,137,209} \) In summary, independent lines of evidence support the hypothesis that there are distinguishable subpopulations of pharmacologically defined opioid receptors.

**Localization of Opioid Receptors in the Spinal Cord**

Opioid binding studies have demonstrated that \( \mu, \delta, \) and \( \kappa \) opioid binding is found throughout the spinal gray matter, although the highest levels are observed in the dorsal horn.\(^{2,30,158,161} \) Receptor autoradiography has demonstrated high levels in the substantia gelatinosa in the vicinity of small primary afferent terminals.\(^3 \) In the thoracic cord, significant levels of opioid binding are observed in the intermediolateral cell column where the preganglionic sympathetic neurons are found.\(^{159} \) The anatomical localization of these spinal receptors may be assessed by changes in binding following anatomical or biochemical manipulations and by the characteristics of the effects of the opioid on the electrophysiological response of identified elements. Of particular importance for mechanistic interpretation is the issue of whether opioid effects may be mediated by action on the sensory afferent terminals or on elements postsynaptic to the primary afferent.

**Presynaptic Locus.** Studies have found \( \mu \) and \( \delta \) opioid binding in primary afferents\(^{61} \) and in the neurites of dorsal root ganglion cells in culture.\(^{27} \) Rhizotomies or ganglionectomies leading to a loss of afferent terminals will result in a significant (\( > 40\% \) to 60\%) but subtotal reduction in ligand binding in the dorsal horn.\(^{108} \) Ventral horn levels of opioid ligand binding appear largely unaffected. Capsaicin, a neurotoxin that selectively destroys small (unmyelinated C-fiber) afferents, produces a reduction in opioid binding similar to that of rhizotomies.\(^{64} \) These results thus suggest that a significant proportion of dorsal horn opioid binding is to be found on the terminals of small primary afferents.

In corroboration of the binding studies, physiological investigations also indicate that opioids can act presynaptically on the primary afferent pathway. Recordings from dorsal root ganglion cells in culture have revealed a direct effect of \( \mu, \delta, \) and \( \kappa \) receptor agonists on membrane conductance.\(^{24,181} \) Measurement of primary afferent excitability (a measure of the ability to depolarize spinal primary afferent terminals) has suggested that opioid receptors in the dorsal horn may produce a local hyperpolarization which could block the central propagation of the action potential.\(^{28} \) Further evidence suggesting a presynaptic locus of the opioid receptor in intrinsic dorsal horn neuron activity may be considered in terms of alterations in primary afferent neurotransmitter release. Statistical analysis of the second-order neuron response in dorsal root ganglion-spinal cord culture has indicated that opiates will produce depression of the number of quanta released in cell culture.\(^{114} \) The release by the cord of substance \( P, \) an 11-amino acid peptide known to exist in unmyelinated primary afferents and to excite dorsal horn presynaptic neurons, is depressed by \( \mu \) and \( \delta \) but not by \( \kappa \) opiates in a naloxone-reversible fashion (see Yaksh\(^{195} \) for a review).

**Postsynaptic Locus.** Although the evidence outlined above emphasizes an action on primary afferents, equally convincing data also support a direct action on nonafferent neurons. Thus, as noted, even extensive rhizotomy produces only a subtotal loss of dorsal horn opioid binding, and binding in the ventral horn is little affected. Iontophoretic application of the excitatory amino acid glutamate onto dorsal horn interneurons and spinthalamic cells as well as onto motor horn cells will directly depolarize the neuron by an increase in Na\(^+\) conductance. This depolarizing effect of glutamate is blocked by \( \mu \) and \( \delta \) opiates.\(^{204,210-212} \) In summary, based on electrophysiological effects and ligand binding studies, opioid receptors have been shown to exist on primary afferent terminals and on nonafferent dorsal horn interneurons and projection neurons, as well as on motor horn cells.

**Effects of Spinal Opioid Receptor Systems on Reflex and Single-Unit Activity**

Following systemic administration in spinally transected animals, opioid alkaloids: 1) suppress the afferent-evoked polysynaptic ventral root reflex\(^{183} \) with the suppression being most prominent for those reflexes evoked by C afferents and least for those evoked by A\(\delta\) afferents;\(^{27} \) 2) reduce the response in dorsal horn neurons evoked by the electrical activation of slowly conducting (A\(\delta/C\)) primary afferents, and by somatic and visceral stimuli which evoke pain behavior in the unanesthetized animal;\(^{57,91,108,188} \) 3) will have no effect at low doses on dorsal horn activity evoked by rapidly conducting afferents (A\(\beta\)) or by otherwise innocuous stimuli (such as light touch or joint movement); 4) reduce the size of the cutaneous receptive field of the spinal neuron;\(^{189} \) 5) reduce the slope (gain) of the stimulus intensity-cell response curve;\(^{175,189} \) and 6) block the repetitive activity (wind-up) commonly evoked by C-fiber stimulation. In paraplegic man, systemic morphine at analgesic doses will attenuate the polysynaptic reflex of the tibialis anterior muscle evoked by sural nerve stimulation with no effect on the monosynaptic H reflex.\(^{185} \)

The local microiontophoretic administration of \( \mu \) and \( \delta \) agonists within the substantia gelatinosa (in the vicinity of the dendritic arbors of dorsal horn neurons and terminals of primary afferents)\(^{55,26} \) and, to a lesser degree, in the nervus propius near lamina V cell bodies\(^{26,212} \) will suppress neuronal activity otherwise evoked by stimuli which activate A\(\delta/C\) classes of pri-
While primary afferents. Importantly, both systemically and iontophoretically administered opiates have been shown to depress the activity in spinofugal nociceptive neurons, indicating that the opiates in the dorsal horn interact with neurons projecting extrasegmentally to higher centers.

These effects on dorsal horn neuronal activity outlined above are mediated by an opioid receptor as defined by the following criteria. 1) The ordering of the structure-activity relationship is in accord with the efficacy of these agents in established opiate receptor bioassay systems; for example, etorphine > fentanyl > levorphanol (−isomer) ≥ morphine ≥ d,l-methadone >> U50488H ≥ meperidine ≥ dextrorphan (+isomer) = naloxone = 0 (see above). 2) Consistent with this observation, the intrathecal or epidural administration of opiates onto Renshaw cells has been demonstrated to facilitate their evoked discharge. Repetitive firing of the motor horn cell evoked by high-frequency afferent input leading to posttetanic potentiation is diminished by opiates. As above, these effects are dose-dependently antagonized by opioid antagonists.

In the ventral horn of the acute spinal transected animal, systemically administered opiates will depress monosynaptic flexor reflexes and antagonize the firing of α motor neurons evoked by muscle stretch. Intracellular studies in culture indicate that opiates diminish the opening of voltage-sensitive K⁺ and/or Ca⁺⁺ channels. Such effects will stabilize membrane voltages and diminish the likelihood of depolarization. The apparent selectivity of the effects of opiates on Aβ/δ-C as opposed to Aδ-β-evoked activity may result from two mechanisms. First, as noted above, the binding studies have shown a relatively unique association of presynaptic opioid binding sites on specific subpopulations of small unmyelinated primary afferents. Thus, the presynaptic effects of opiates would be limited to this class of afferents and the information these afferents transmit. Second, intracellular recording has demonstrated that opiates, by an effect upon local ion channels, may effectively reduce the time constant of the membrane. Intracellular recordings have, for example, demonstrated that opiates result in a reduction in the rate of rise of afferent evoked excitatory postsynaptic potentials (EPSP's). As large-fiber signals travel more rapidly than do small-fiber signals, the input from large afferents will tend to arrive synchronously with the input from small afferents. Thus, the slowed rate of rise of the EPSP will affect the safety factor of the small afferent input more significantly than the input from the large afferent. Although the mechanisms have been described for the relationship between primary afferent and the second-order neuron, they likely represent a general paradigm for opioid action in the several spinal systems to be discussed below.

In summary, opioid receptors in the dorsal horn selectively modulate the responsivity of certain classes of spinal neurons to small afferent (nociceptive) input. In the dorsal and ventral horn, opiates diminish the tendency of interneurons, projection neurons, and motor neurons to display repetitive activity upon depolarization. These effects occur as a result of the specific localization of the opioid receptor on specific subpopulations of primary afferent terminals and by the effect on the excitability of the nonafferent cell, brought about (in the absence of changes in resting membrane potential) by changes in the conductance to voltage-sensitive ion channels.

**Spinal Opioid Receptor-Mediated Effects**

**Effects of Spinal Opioid Receptor Systems on Sensory Processing**

Effects of Spinally Administered Opioids. As was noted, classic studies have demonstrated that opiates with an action on spinal cord could inhibit nociceptive reflexes and depress the firing of spinal neurons evoked by noxious stimuli. An important issue is whether these spinal systems, the activity of which is altered by opiates, play a role in the generation of the pain state following high-threshold somatic and visceral stimuli. Direct assessment of this issue may be approached by the administration of opiates into the intrathecal or epidural space in unanesthetized and spinally intact animals. Simple animal models, which permit the atraumatic chronic catheterization of these species, have proven of utility in assessing these issues. Table 2 presents a summary of the experimental pain

**Table 2**

<table>
<thead>
<tr>
<th>Experimental Pain Endpoint</th>
<th>Species</th>
<th>References</th>
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<tbody>
<tr>
<td>thermal-spinal reflex</td>
<td>mouse</td>
<td>139</td>
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<tr>
<td>skin twitch</td>
<td>cat</td>
<td>156</td>
</tr>
<tr>
<td>thermal-supraspinal: hot plate</td>
<td>mouse</td>
<td>139</td>
</tr>
<tr>
<td>cold-supraspinal: limb immersion electrical</td>
<td>human</td>
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<tr>
<td>shock vocalization</td>
<td>rat</td>
<td>168</td>
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<tr>
<td>shock titration</td>
<td>rat</td>
<td>205</td>
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<tr>
<td>pressure</td>
<td>primate</td>
<td>190</td>
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<tr>
<td>pinch</td>
<td>rat</td>
<td>102</td>
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<tr>
<td>tourniquet pressure</td>
<td>human</td>
<td>171</td>
</tr>
<tr>
<td>inflammatory</td>
<td>mouse</td>
<td>146</td>
</tr>
<tr>
<td>writhing</td>
<td>rat</td>
<td>156</td>
</tr>
</tbody>
</table>

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models and species that have been examined, and Table 3 summarizes the effects of morphine in certain clinical situations. As indicated, in virtually every experimental pain model available and in man under a variety of acute and chronic pain conditions, morphine given intrathecally or epidurally will evoke a powerful suppression of pain behavior, as evidenced by escape response or verbal report.

Systematic consideration of the types of clinical pain affected has indicated that, following spinal morphine, the characteristic sensation most readily relieved is that described as continuous and originating from deep somatic and visceral structures. Less predictably managed are intermittent somatic and visceral (intestinal obstruction) pain. Cutaneous (incisional) pain is poorly modified by spinal morphine at "analgesic" concentrations. Neurogenic pain, such as associated with deafferentation, is often little modified, even by high doses of spinal opiates.

This differential effectiveness of spinal opiates against different types of pain demonstrated in man and animals reflects upon the spinal substrates whereby the information from these stimulus modalities is coded and with which the opiate receptor is associated. As noted in the preceding sections, the postsynaptic safety factor of rapidly conducting fibers is thought to be relatively unaffected by opiates. Thus, incisional or sharp ("first") pain, which may reflect the activation of A6-fibers, is less likely to be altered than is the sensation evoked by the activation of cutaneous C-fibers, which has been reported to evoke the so-called "second" pain. Failure to reliably effect a response at even high doses of opiates suggests that "second pain" sensations (such as dysesthesia) may be encoded differently from sensations that are readily suppressed by spinal opiates.

A factor that is relevant to the sensory effects of spinal opiates is that, at analgesic doses, the behavioral syndrome reflects a selective inhibition of the noxious aspects of the somatic stimuli. Consistent with the lack of effect on large-afferent evoked activity, neurological examination in man and animals routinely fails to demonstrate loss of the two-point threshold or limb position sense. Importantly, at analgesic doses there is no effect on motor function, strength, coordination, or tone.

**Mechanism of Analgesic Action of Spinal Opiates.** Ample evidence suggests that spinally administered opiates exert their effects by a movement into the spinal gray matter. As discussed above, opiates have been shown to have direct effects upon dorsal root ganglia in cell culture. In adult systems, however, opiates have not been shown to alter transmission through the ganglion or through primary afferents. It should be noted that opioid binding sites do exist within the axons of afferents. These sites, however, likely reflect the axonal transport of binding material between the site of synthesis (cell body) and the terminals. The failure of opiates to alter transmission through the axon suggests that these binding sites are not coupled to membrane systems such as ion channels.

Topical administration of opiates onto the spinal cord results in a time-dependent suppression of the discharge of nociceptive neurons. Joint consideration of three factors (1: the time course of the onset of behaviorally defined analgesia; 2: the distance of diffusion by the topically applied radiolabeled agent; and 3: the time of onset of the inhibition of the second-order spinal neuron) suggests that topically applied opiates exert their anti-nociceptive effects by diffusion into the dorsal gray matter of the spinal cord (see Tung and Yaksh). Importantly, agents with high lipid partition coefficients are known to diffuse rapidly in tissue after topical application to brain and to show a correspondingly rapid onset as regards analgesia and the correlated inhibition of evoked neuronal activity.

It should be appreciated that, as with other routes of administration, the physical chemical properties of the drug as well as its receptor affinity and efficacy will define the properties of the drug effect. Thus, as noted, the time of onset after intrathecal administration varies directly with the lipid partition coefficient of the drug; for example, fentanyl acts more rapidly than morphine. This difference will be exaggerated by epidural administration, where agents such as morphine display an even greater delay in onset of analgesia and require up to 10 times higher doses to achieve analgesic effects equal to those achieved via the intrathecal route of administration. In contrast, agents with high lipid partition coefficients are cleared rapidly from the tissue and show correspondingly higher blood levels and shorter durations of action. Parenthetically, this rapid absorption means that relatively smaller amounts of a lipid-soluble drug will remain in the cerebrospinal fluid (CSF). Thus, it is not surprising that, in man, cisternal CSF levels of intrathecally administered drugs such as methadone are lower than levels after corresponding

**TABLE 3**

<table>
<thead>
<tr>
<th>Clinical Pain Endpoint</th>
<th>References</th>
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<tr>
<td>postoperative: thoracic/upper abdominal</td>
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<td>visual analogue scale</td>
<td>146</td>
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<tr>
<td>modified McGill pain questionnaire</td>
<td>23, 129, 173</td>
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<tr>
<td>mobilization scale</td>
<td>23, 129</td>
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<tr>
<td>duration or time to first analgesic (&gt; 10 hrs)</td>
<td>23, 129, 173</td>
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<tr>
<td>cumulative consumption of additional analgesics</td>
<td>23, 129</td>
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<tr>
<td>respiratory function</td>
<td>146, 174</td>
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<tr>
<td>peak expiratory flow rate</td>
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<tr>
<td>vital capacity</td>
<td>23, 146, 154</td>
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<tr>
<td>forced expiratory volume at 1 sec</td>
<td>80</td>
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<tr>
<td>CO2 response curves</td>
<td>146, 154</td>
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<tr>
<td>pulmonary x-ray film changes</td>
<td>42, 43</td>
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<td>terminal cancer</td>
<td>42, 125, 135</td>
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<tr>
<td>visual analogue scale</td>
<td>42, 125, 208</td>
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</table>

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injections of polar agents such as morphine or the peptide DADL.123

Pharmacology of Spinal Opioid Analgesia. The spinal action of opiates in animals and man is hypothesized to be mediated by an action on local opioid receptors. This likelihood is generally supported by the observation that: 1) these effects are antagonized by naloxone; and 2) an equivalent behavioral syndrome is produced by opioid alkaloids with a structure activity profile which resembles that observed in various opioid bioassay systems (for example: fentanyl > morphine > meperidine). An essential issue is: which of the several designated receptor types mediates this spinal action? Characterization of the receptors in spinal cord involved in modifying pain transmission may be considered in terms of the structure activity relationship and of the quantitative effects of receptor antagonists and cross tolerance between families of spinally administered opiate receptor agonists.

a. Structure activity profiles. Table 4 summarizes the pharmacological characteristics of spinally administered opiates in a rat nociceptive model. Comparable observations have been made in the primate.190,203 Several issues may be considered, based on the large quantity of information summarized in Table 4. First, the rank ordering of potency suggests the possible relevance of spinal μ, δ, κ, and ε receptors in modulating nociceptive transmission. In the experiments testing spinal neuronal activity, κ- and ε-type agonists have little or no activity. In certain visceral chemical measures, δ and σ agonists are not active at doses that do not produce motor dysfunction. Beta-endorphin, a putative δ receptor agonist, is active on both thermal and visceral chemical nociceptive measures. The dual implication is that drugs like DADL and U50488H act on different circuits and that the spinal pathways through which these stimuli modalities are processed are anatomically discrete.

b. Antagonism. These changes in the animal’s response evoked by intrathecal opioid agonists are uniformly antagonized by intrathecal or systemically administered naloxone or naltrexone, but not by a variety of other receptor antagonists.

Two types of quantitative studies have been carried out to examine the ability of systemic naloxone to shift the dose-response curves of intrathecally administered opioid agonists. The first is the quantitative dose-ratio analysis. This approach (a dose-ratio or Schild analysis) permits one to estimate the pA2, which is a measure of the affinity of the agonist for the receptor acted upon by the spinal agonists to produce analgesia (pA2 = negative logarithm of the dose of agonist in moles per kg, which doubles the ED50 (50% effective dose) of the agonist). A pA2 of 7 indicates that a dose of 10^-7 M/kg of the antagonist will shift the agonist dose-response curve by twice. Thus, with naloxone, this dose is approximately 30 μg/kg for morphine and 300 μg/kg for DADL.200 Under these conditions, agonists for which the antagonist pA2 are similar likely act upon a common receptor. Different pA2 values reflect different receptor interactions. The pA2 values for β-endorphin, morphine, and sufentanil are identical (near 7) while those for DADL and D-Pen2-D-Pen5-enkephalin (DPDPE) are approximately 1 unit less. This bimodal grouping indicates that these two groups of agents act upon two classes of receptor for which naloxone displays a distinguishable affinity. These observations are consistent with the results obtained in the ex vivo bioassays and binding studies when the affinity of naloxone for the μ receptor is approximately 10 times that of the δ receptor.13

The second approach is the use of receptor-selective antagonists. Beta-funanalxetamine is an irreversible antagonist of the μ receptor. Pretreatment with the agent will block the effects of intrathecal μ but not δ receptor agonists. Certain peptide analogues (such as ICI174864) have been shown to have a relative affinity for the δ and not the μ receptor. This agent has been reported to antagonize the intrathecal effects of δ but not μ agonists.76

c. Tolerance. Prolonged exposure of an opioid receptor to an opiate agonist will result in a diminution of the effect produced by that agonist. Tolerance development has been clearly demonstrated following chronic spinal administration of a variety of opioid ligands, including those classified as μ (morphine,190,203 sufentanil, 202 and β-endorphin197) or δ (DADL190) receptor agonists. The cellular mechanism of tolerance is not known. Changes in receptor number, affinity, and cou-

<table>
<thead>
<tr>
<th>Activity</th>
<th>Opiates</th>
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<tbody>
<tr>
<td>structure-activity relationship</td>
<td>β-endorphin &gt; DPDPE = DADL = sufentanil = morphine &gt; U50488H = bremazocine = SKF10047 = (+)morphine = 0</td>
</tr>
<tr>
<td>thermal tests</td>
<td>β-endorphin = sufentanil = morphine = U50488H = bremazocine &gt; SKF10047 = (+)morphine = 0</td>
</tr>
<tr>
<td>visceral chemical tests</td>
<td>L-naloxone = naltrexone = D-naloxone = phentolamine = propranolol = atropine = methysergide = 0</td>
</tr>
<tr>
<td>antagonist</td>
<td>morphine = β-FNA &gt; ICI-174864 = ICI-1748647, β-FNA</td>
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<tr>
<td>DADL = DPDPE</td>
<td>[β-endorphin = sufentanil = morphine]</td>
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<tr>
<td>naloxone affinity for spinal receptor</td>
<td>[morphine = sufentanil = β-endorphin]</td>
</tr>
<tr>
<td>cross tolerance</td>
<td>= = [DADL = DPDPE]</td>
</tr>
</tbody>
</table>

* Abbreviations: DADL = D-Ala2-D-Leu5-enkephalin; DPDPE = D-Pen2-D-Pen5-enkephalin; β-FNA = β-funanalxetamine. See also Table 1. Data are from references 98, 143, 156, 191, 192, 201, 202, and unpublished observations.
† Hot-plate and tail-flick tests.
‡ Intraperitoneal irritant.

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The animal models are virtually identical to those presently defined in effect by action on a population of opioid receptors to sufentanil and β-endorphin, but only a minimal loss of response to DADL. 176,197,202 This phenomenon has indeed been demonstrated for a variety of opioid agonists such that animals made tolerant to spinal morphine show a relative loss of response to sufentanil and β-endorphin, but only a minimal loss of response to DADL. 176,197,202

The analgesic effects of epidural morphine are antagonized in a dose-dependent fashion (5 to 10 µg/kg) by naloxone. 145 Systematic studies have not, however, been carried out to compare the relative reversibility of the several agonists.

Patients tolerant to systemic morphine, such as those undergoing long-term treatment for metastatic disease, show a reduction in the response to spinal morphine (tolerance). 125 This suggests that the receptor acted upon by the spinal drug is also part of the population of receptors acted upon by the systemically administered drugs. Similarly, continued spinal administration is frequently accompanied by the need for increasing concentrations to achieve adequate pain relief. 42,141 Although the necessity to increase the dose may reflect in part a worsening clinical state, 187,208 the reversibility of the phenomenon 125 argues that at least a portion of the dose augmentation reflects an underlying substrate similar to that observed in the animal studies.

It should be stressed that the identification of the receptor acted upon by the spinal opioid is not a trivial issue. High concentrations of drugs are achieved after spinal administration, and the possibility of a non-opiate receptor interaction must be considered. Thus, intrathecal meperidine has been reported to have local anesthetic actions. 42 This is likely the result of the concentrations required because of the limited receptor affinity. 42 In other studies, it has been shown that high concentrations of morphine (30 to 50 mg/ml) in animal models yield a prominent allodynia which is not antagonized by naloxone. 199 This may be due to the strychnine-like effect that pharmacologists have long known to be possessed by many morphine-like alkaloids. Thus, the issue of the receptor acted upon by the spinal agent must be considered, particularly as one approaches the accepted limits of the dose-response function of even common agents such as morphine or meperidine.

In summary, in a variety of animal models and man it appears, on the basis of distinguishable structure activity profiles, differential sensitivity to receptor antagonists, and cross tolerance (or lack thereof), that three distinct populations of receptors that are relevant to pain processing may be defined within the spinal

### TABLE 5

<table>
<thead>
<tr>
<th>Drug</th>
<th>Operative Location or Clinical Situation</th>
<th>Dose Range (mg)</th>
<th>Activity Ratio to Morphine†</th>
<th>Molar activity ratio relative to a 6-mg dose of morphine.</th>
</tr>
</thead>
<tbody>
<tr>
<td>morphine</td>
<td>upper abdominal, thoracic, Caesarean section, gynecological, lower abdominal, orthopedic, terminal cancer pain</td>
<td>4-10^6,125,142,174</td>
<td>1</td>
<td>2-4^7,116,132</td>
</tr>
<tr>
<td>lornetanil</td>
<td>abdominal</td>
<td>0.005</td>
<td>1500</td>
<td></td>
</tr>
<tr>
<td>fentanyl</td>
<td>upper abdominal, thoracic</td>
<td>0.06</td>
<td>88</td>
<td></td>
</tr>
<tr>
<td>alfentanil</td>
<td>orthopedic, abdominal</td>
<td>0.015-0.3^73</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>buprenorphine</td>
<td>major abdominal, orthopedic</td>
<td>0.06-0.3^150</td>
<td>27</td>
<td></td>
</tr>
<tr>
<td>β-endorphin</td>
<td>lumbar laminectomy, major abdominal, vascular surgery</td>
<td>3^36</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td>diamorphine</td>
<td>terminal cancer pain</td>
<td>5^46,113</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>methadone</td>
<td>upper abdominal, thoracic</td>
<td>9^33</td>
<td>0.6</td>
<td></td>
</tr>
<tr>
<td>meperidine</td>
<td>upper abdominal, thoracic</td>
<td>50-60^13,131</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>pentazocine</td>
<td>gynecological, abdominal</td>
<td>10-50^9,121</td>
<td>0.1</td>
<td></td>
</tr>
</tbody>
</table>

* Superscript numbers are reference numbers in the bibliography.† Molar activity ratio relative to a 6-mg dose of morphine.‡ Doses required for terminal cancer pain are variable, depending upon state of tolerance.

The analgesic effects of epidural morphine are antagonized in a dose-dependent fashion (5 to 10 µg/kg) by naloxone. 145 Systematic studies have not, however, been carried out to compare the relative reversibility of the several agonists. **Table 5** presents the range of doses which have been shown effective following epidural administration in man for various pain requirements. Although diverse, the ordering of activity is very close to that reported in the animal model. Thus, μ agonists such as fentanyl, morphine, methadone, and meperidine have an ordering of activity compatible with an action on a μ receptor. If given spinally to terminal cancer patients, DADL produces a prominent long-lasting degree of pain modification. 123,134 The activity of epidural nalbuphine and butorphanol appears to implicate a receptor interaction which is comparable to that observed in the animal models on visceral chemical tests.
cord: \( \mu \), \( \delta \), and \( \kappa \). Other receptors may exist (for example, \( \epsilon \)), but the lack of a distinguishable pharmacology prevents their description. These receptors will result in a prominent and selective modulation of the response of the organism to an otherwise painful somatic or visceral stimulus.

**Effects of Spinal Opioid Receptors in Motor Function**

As noted previously, physiological studies have shown that opiates can exert significant receptor-selective effects on spinal motor neurons. Yet, one of the prominent findings associated with intrathecal or epidural morphine is the lack of any apparent effect on motor function either at maximum analgesic doses in man or at up to several times that concentration in animals. At extreme concentrations, \( \delta \) agonists (DADL, DPDPE) and dynorphin result in a caudal paralysis of the hindlimb by a rigidity that is not a local anesthetic action.\(^{155,164} \) Importantly, those effects are generally not antagonized by even high doses of naloxone, and are produced by these agents when their action is limited to the spinal cord. High doses of \( \mu \) agonists will also produce a marked rigidity, but this appears to occur as a result of redistribution to brain-stem centers.\(^{16} \)

In spite of the lack of effect of these agents at low (and presumably opioid receptor-selective) concentrations on normal motor function, it has been demonstrated that where abnormal motor tone is present (as in spasticity following spinal injury or in multiple sclerosis) opiates (morphine and fentanyl) will produce evident relaxation.\(^{58,165} \) Although the pharmacology of this effect has not been investigated, the phenomenon is achieved at a concentration which in man is commonly associated with an opioid receptor interaction (1 to 2 mg), and is reversed by naloxone. As noted, in animals with chronic spinal transection, morphine will depress monosynaptic flexor reflexes\(^{90,93} \) and reduce the activity on \( \alpha \)-motor neurons evoked by muscle stretch.\(^{101} \) Other evidence has shown that opioid receptor systems can reduce \( \gamma \) motor activity, perhaps by a local (spinal) inhibition of the descending facilitatory drive which exists on \( \gamma \) motor neurons.\(^{90,93} \)

Recent studies have suggested that motor dysfunction following spinal cord injury may be attenuated by opioid antagonism.\(^{60} \) The mechanism underlying this report is not known, although the actions of an endogenous peptide dynorphin (see below) have been implicated by virtue of its effects following spinal administration.\(^{59} \) The findings that the motor dysfunction is not reversed by naloxone and that other \( \kappa \) receptor ligands do not yield this effect\(^{151} \) suggest, however, that the ameliorating effects of naloxone may be mediated by other mechanisms.

In summary, opioid receptor systems play a minor role in modulating the coordinated outflow from the motor horn except in the presence of elevated motor tone as occurs in spasticity. These effects are consistent with electrophysiological studies showing suppressive effects on \( \gamma \) motor and facilitatory effects on Renshaw cell activity.

**Effects of Spinal Opioid Receptors and Autonomic Function**

Opioid ligand binding is found in the intermediolateral cell column.\(^{159} \) With regard to cardiovascular function, spinaly administered \( \mu \) or \( \delta \) ligands have little effect upon a variety of resting measures in man\(^{117,35,38,46,120} \) or animals,\(^{6,201,207} \) including heart rate, blood pressure, peripheral resistance, or plasma catecholamine or \( \beta \)-endorphin levels. Similarly, intrathecal or epidural administration of \( \mu \) agonists has no effect on sudomotor activity or skin temperature. The Valsalva response is unaffected and orthostatic hypotension is not observed following receipt of spinal opiates.\(^{24} \) In spite of the lack of effect on resting cardiovascular/sympathetic parameters, spinal opiates can modulate a number of evoked autonomic reflexes. Thus, in dogs, exercise-induced vasodilation is diminished by intrathecal morphine.\(^{140} \) Following spinal administration of opioid ligands, sympathetic responses (blood pressure changes, plasma catecholamine secretion) to noxious stimuli in man and animals are variably diminished.\(^{45} \) This effect of spinal opioids on the intraoperative sympathetic-hormonal responses noted above may reflect the differential effect of opiates on A\( \varepsilon \)- versus C-fiber evoked activity which manifests itself in the failure of opiates to block the pain of incision.

Gastrointestinal effects (ileus or constipation) following receipt of spinal opiates have not been reported in man, although systematic studies have not been carried out. In animal studies, spinal \( \mu \) and \( \delta \) receptor ligands have been shown to produce a slowing of intestinal transit.\(^{161} \)

An important effect of spinally administered opiates is the suppression of the micturition reflex. Cystometricgrams in man and in animal models show an increase in bladder capacity with no change in urethral pressures,\(^{144} \) but with a slight increase in the tone of the external sphincter.\(^{2} \) In man, spinal morphine has been shown to diminish tone during bladder spasm.\(^{8} \) In cats with chronic spinal transection, naloxone has been observed to augment the micturition reflex.\(^{172} \) Systematic studies of the pharmacology of this spinal effect on micturition reveal the role of populations of spinal opioid receptors which, by virtue of their structure activity relationship, sensitivity to naloxone antagonism, and cross tolerance, are classified as \( \mu \) and \( \delta \), but not \( \kappa \) or \( \epsilon \).\(^{21,52} \)

In summary, the spinal \( \mu \) and \( \delta \) opioid receptor agonists have little effect on the resting autonomic outflow, but appear to modulate certain classes of autonomic responses evoked by afferent input, such as pain-evoked changes in cardiovascular activity, the volume-evoked micturition reflex, and gastrointestinal motility.
Spinal organization of opioid receptors and endorphins

Endogenous Systems Acting Through Spinal Opioid Receptors

Effects of Opioid Receptor Antagonism on Spinal Activity

The presence of opioid receptors associated with specific spinal systems, the potent modulating effects of these receptor systems on sensory, motor, and autonomic processing, and the effect of various forms of afferent stimulation on the release of spinal endorphins with μ, δ, and κ receptor affinities clearly suggest a role of these spinal systems in ongoing spinal processing. The role of these endogenous systems may be directly examined by assessing the effect of a receptor antagonist on the physiological endpoint evoked by a particular stimulus.

After systemic administration of doses of naloxone sufficient to antagonize μ/δ or κ receptors (30 to 300 μg/kg276), single units in the spinal dorsal horn display only mild increases, if any, in their spontaneous firing.62,89,193 This observation suggests that, in anesthetized or unanesthetized (decerebrate-spinally transected) animals, endogenous opioid activity is minimal. In contrast, it is apparent that naloxone can frequently increase the magnitude of the response of a variety of spinal sensory and motor systems activated by afferent excitation. Thus, naloxone will enhance monosynaptic and polysynaptic ventral root reflexes evoked by myelinated (Group I muscle afferents: Aβ cutaneous) afferents and unmyelinated (C cutaneous) afferents in animals10,39,54,68 and monosynaptic extensor reflexes in man.19 This general augmentation of motor horn outflow argues for a postsynaptic mechanism whereby enkephalinergic neurons that depress motor horn excitability are activated by all classes of afferents. In contrast, while ventral root reflexes evoked by both nociceptive and non-nociceptive afferents are equally facilitated, recording from dorsal horn neurons or spinofugal pathways reveals that naloxone augments only the ascending volley that is evoked by C-fibers.14,54

Classification of the Endogenous Opioids

In the preceding section it was emphasized that the local action of opioids in the spinal cord exerted potent effects on various aspects of spinal cord function and these effects are mediated by several classes of well-defined receptors. The existence of such receptors presents the issue as to what normally acts upon these membrane sites. Agents derived from the body, which act upon an opioid receptor (for instance, agents with a pharmacology comparable to that of the opioid alkaloids, which exert physiological effects) are referred to by the generic term "endorphins." As will be discussed below, the endorphins represent a complex family with at least three major subdivisions. An appreciation of several concepts simplifies the understanding of their organization.

First, each principal family derives from a prohormone which has been identified by virtue of distinct gene codes necessary for its ribosomal synthesis. Currently, three major prohormone families have been identified and sequenced: 1) proenkephalin (263 amino acids128); 2) pro-opiomelanocortin (265 amino acids126); and 3) prodynorphin (256 amino acids144). These prohormones are synthesized at ribosomes, packaged in the cell body, and transported to the site of release. Figure 1 gives a schematic representation of the preprohormone sequences for these three principal families. As indicated, each preprohormone possesses an initial amino acid sequence which serves in the initial ribosomal synthetic process to mark the prohormone sequence.

Second, the prohormone form generally has no biological (opioid) activity, but after translation becomes exposed to a variety of enzymes which may add elements to reactive groups (such as methylation or acetylation) and more importantly begin to cleave the prohormone into smaller fragments. Frequently, as emphasized in Fig. 1, an active form of the peptide (such as met-enkephalin) is cryptically embedded between pairs of basic amino acids (for example, arginine and lysine) which are sensitive to a variety of trypsin-like enzymes that expose the amino terminus. Further exposure to carboxypeptidases leads to subsequent cleavages that expose the carboxyterminus. Table 6 presents a summary listing of common sequences with opioid activity that are encrypted in the several prohormone sequences.

Third, in many prohormones, a number of copies of the same molecule may appear as well as sequences of other biologically active hormones. Thus, as shown in Fig. 1 in proenkephalin, there are four copies of the sequence of met-enkephalin, as well as forms of met-enkephalin that are extended by one or two amino acids. In the pro-opiomelanocortin sequence, adrenocorticotropic hormone (ACTH) and β-lipotropin are present; neither possess opioid receptor activity. It should be stressed that a given peptide which contains an encrypted sequence may not always undergo complete cleavage to expose all encrypted fragments. Thus, β-endorphin-containing cells do not contain free met-enkephalin, the amino terminus fragment of β-endorphin. The reason for this limitation may be the absence of relevant enzymes or posttranslational changes (such as methylation) which alter the conformation of the molecule, rendering the cleavage sites inaccessible. An important implication of the prohormone synthesis cycle is that a cell that possesses the gene for one of the prohormones may likely possess multiple encrypted fragments.

Fourth, in addition to the principal families of endorphins outlined above, there are reports of other endogenous agents with an opioid pharmacology which have novel sequences or structures. These are: β-casomorphin, which has been identified in casein hydrolysates74; dermorphins, isolated from the skin of South American frogs and which uniquely contain a d-alanine.
group;²² and a morphine-like substance which has been isolated in small quantities from brain and identified as virtually identical to the alkaloid.¹⁶³

Finally, given the multiple forms of the opioid peptides, it is not surprising that they possess actions at the several classes of receptors which have been identified. What is not so evident, however, is that for a given series of fragments derived from a single prohormone, the spectrum of receptor activity may vary. Table 6 summarizes the relative receptor affinities for several peptides derived from each prohormone. Thus, while met-enkephalin is largely δ in its action, metorphamide is largely μ. Leu-enkephalin is δ, but carboxy-extended fragments are principally κ in nature. Given that these several forms may exist within a single neuron, it is clear that the postsynaptic event will depend upon which of the fragments are released.

In summary, based on prohormone sequences, three principal classes of endogenous opioids may be described: proenkephalin, prodynorphin, and pro-opiomelanocortin. Each prohormone possesses multiple cryptically embedded opioid receptor active forms which may be freed by posttranslational enzymatic cleavages and stored. The several fragments deriving from a single prohormone may possess different opioid receptor affinities.

Organization of the Spinal Endogenous Opioid Systems

Both radioimmunoassays and immunohistochemistry have revealed significant levels of opioid peptides deriving from the proenkephalin and prodynorphin sequences. Beta-endorphin has not been identified in spinal cord.

Table 7 summarizes the overall distribution by laminae of several endorphin products. Prominent levels of the measured agents have been identified in dorsal horn neurons, particularly those in lamina I (nonprojecting: marginal neurons), lamina II and III (islet and stalked cells¹⁄₄), and pyramidal cells in laminae IV and V and comparable cells in lamina X.¹ⁱ⁹ Within the substantia gelatinosa (laminae II and III), enkephalin levels are highest in the inner portion of lamina II (IIι⁴). In the ventral horn, the presence of endorphin-containing neurons declines and endorphins are not contained in motor horn cells. Enkephalin- and dynorphin-containing terminals are, however, prominently observed around motor horn cells. Comparable distributions of enkephalin have been observed in human spinal cord.⁴⁹

Although the principal source of endorphins appears to be in the cells and terminals of intrinsic neurons, investigators have found cell bodies in the brain stem which project to the spinal cord and contain immunoreactive enkephalin.⁷⁸ With regard to primary afferents, dynorphin- but not enkephalin-like immunoreactivity has been demonstrated in a small population of dorsal root ganglion cells.¹⁸

The organization and possible functional role of the spinal opioid peptide systems may be considered in terms of the laminar distribution of cells and terminals.

![Diagram](image)

**FIG. 1.** Schematic display of the organization of the principal peptide fragments found in pro-opiomelanocortin, proenkephalin, and prodynorphin (dynorphin). **Arrows** indicate the presence of lysine (†) and arginine (†). Abbreviations: MS = melanocyte-stimulating hormone; Clip = corticotrophin-like intermediate lobe peptide; ACTH = adrenocorticotropic hormone; END = endorphin; LPH = lipotrophin; M = met-enkephalin; L = leu-enkephalin; M/RGL = met-enkephalin-arginine-glycine and leucine; M/RF = met-enkephalin-arginine-phenylalanine; αN = α-neoendorphin; D = dynorphin.
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Marginal Zone (Lamina I). As indicated schematically in Fig. 2, enkephalin-containing axodendritic synapses have been identified on lamina I neurons (marginal cells). As the majority of these dendritic connections derive from local neurons,66 this enkephalin input probably derives also from enkephalin-containing lamina I cells. As many lamina I neurons give rise to spinofugal projections, not surprisingly lamina I enkephalin-containing neurons have been shown to synapse on spinothalamic tract cells.12 Lamina I neurons which project to the thalamus do not, however, appear to contain enkephalin.7 Electrophysiologically, large populations of lamina I neurons have been shown to receive input from afferents which are excited by high-threshold noxious stimuli and which conduct at a velocity consistent with Aδ- and C-fibers.10,11 Several lines of evidence support the thesis that these cells may be an important element in the nociceptive pathway. Whether the enkephalin-containing marginal neurons receive Aδ, Aδ, and/or C input specifically is not known. In any case, this connectivity suggests that local endorphin feedback activated by afferent input might regulate the output of the marginal lamina I nociceptors.198

Substantia Gelatinosa (Laminae II and III) and Nucleus Proprius (Laminae IV to VI). In lamina II, local enkephalin-containing interneurons have been identified on the basis of morphology as islet cells and stalked cells.13,104,105 One population of stalked cell collateralizes into lamina I and has been described, on the basis of terminal morphology, as a possible excitatory interneuron.67 Cells lying in the outer layer of lamina II (lamina IIo) receive prominent nociceptive input, while cells in lamina II (inner) receive principally non-noxious drive.13,111 As presented schematically in Fig. 2, this raises the possibility that enkephalin-containing interneurons may attenuate an indirect excitatory drive on marginal projection neurons. Given the presence of Aδ- and C-fiber input into lamina IIo, the lamina IIο enkephalin-containing neurons would be driven by high-threshold input, while the enkephalinergic release from lamina II cells would be driven by low-threshold stimuli. Importantly, small enkephalin-containing interneurons in lamina III resemble cells that receive only low-threshold mechanoreceptor input.12

| TABLE 6
<p>| Common functional sequences of opioid peptides derived from identified prohormone sequences and their receptor selectivity |</p>
<table>
<thead>
<tr>
<th>Opioid Peptides</th>
<th>Sequence*</th>
<th>Relative Receptor Selectivity†</th>
</tr>
</thead>
<tbody>
<tr>
<td>pro-enkephalin</td>
<td>YGGFM</td>
<td>δ &gt; μ = κ</td>
</tr>
<tr>
<td>met-enkephalin</td>
<td>YGGFL</td>
<td>δ &gt; μ = κ</td>
</tr>
<tr>
<td>leu-enkephalin</td>
<td>YGGFRF</td>
<td>δ &gt; μ = κ</td>
</tr>
<tr>
<td>met-enk-arg-phe</td>
<td>YGGFMRGL</td>
<td>δ &gt; μ = κ</td>
</tr>
<tr>
<td>met-enk-arg-gly-leu</td>
<td>YGGFMVVRV-NH2</td>
<td>μ ≥ κ &gt; δ</td>
</tr>
<tr>
<td>pro-dynorphin</td>
<td>YGGFL</td>
<td>δ &gt; μ = δ</td>
</tr>
<tr>
<td>dynorphinI,5</td>
<td>YGGFLRRI</td>
<td>κ ≥ μ = δ</td>
</tr>
<tr>
<td>dynorphinI,8</td>
<td>YGGFLRRPKLKDWNQ</td>
<td>κ &gt; μ = δ</td>
</tr>
<tr>
<td>dynorphinI,17</td>
<td>YGGFLRRQQFKVVT</td>
<td>κ &gt; μ = δ</td>
</tr>
<tr>
<td>pro-opiomelanocortin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-endorphin</td>
<td>TGGFMTSKEQTPVL</td>
<td>μ = δ &gt; κ = ε</td>
</tr>
<tr>
<td>β-endorphin</td>
<td>YGGFMTSKGQTPVL</td>
<td>ε = μ &gt; δ ≥ δ</td>
</tr>
<tr>
<td>γ-endorphin</td>
<td>TGGFMTSGQTPVL</td>
<td>μ = δ &gt; κ = ε</td>
</tr>
<tr>
<td>other unique sequences</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-casomorphin</td>
<td>YPFPGP</td>
<td>μ = δ = κ</td>
</tr>
<tr>
<td>dermorphin</td>
<td>Yd-AFGYPS</td>
<td>μ = δ = κ</td>
</tr>
<tr>
<td>morphine</td>
<td></td>
<td>μ &gt; δ = δ</td>
</tr>
</tbody>
</table>

* Abbreviations: A = alanine; R = arginine; N = asparagine; D = aspartic acid; C = cysteine; Q = glutamine; G = glycine; H = histidine; I = isoleucine; L = leucine; K = lysine; M = methionine; F = phenylalanine; P = proline; S = serine; T = threonine; W = tryptophan; Y = tyrosine; V = valine.
† Relative ordering of reported activity frequently may vary somewhat in different organ systems: see references 47, 98, 191, 192.

| TABLE 7
<p>| Summary of endorphin activity in the spinal cord * |</p>
<table>
<thead>
<tr>
<th>Region</th>
<th>Structure</th>
<th>Leu-Enkephalin65</th>
<th>Met-Enkephalin41</th>
<th>Met-Enk-RGL42</th>
<th>Dynorphin A1–8†19</th>
</tr>
</thead>
<tbody>
<tr>
<td>marginal zone (lamina I)</td>
<td>cell bodies</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>substantia gelatinosa (laminae II &amp; III)</td>
<td>fibers/terminals</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>nucleus proprius (laminae IV–VI)</td>
<td>cell bodies</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>ventral horn (laminae VII–IX)</td>
<td>fibers/terminals</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>central canal (lamina X)</td>
<td>cell bodies</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>primary afferents (dorsal root ganglion)</td>
<td>terminals</td>
<td>0</td>
<td>0</td>
<td>?</td>
<td>+</td>
</tr>
</tbody>
</table>

* Superscript numbers refer to reference numbers in the bibliography.

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Dynorphin-like immunoreactivity has been demonstrated in cells in laminae II and III. Significantly, in recent studies dynorphin has also been identified in large lamina V cells.119

**Primary Afferents.** The ample physiological and biochemical evidence that primary afferent terminals possess opioid receptors is in distinct contrast to the paucity of axoaxonic relationships between enkephalin-containing terminals and primary afferents.66 Figure 2 summarizes the principal configurations (as identified by ultrastructural analysis) that exist between primary afferent terminals and enkephalin-containing spinal elements: 1) enkephalin-containing dendrites or soma receive afferent input; 2) enkephalin-containing terminals are separated from an afferent terminal by a non-afferent/non-enkephalinergic element.65,166 Thus, the expected anatomical relationship between afferent and enkephalin terminals as predicted by the localization of opioid binding has not been observed. Such observations raise the possibility that: 1) endorphins as yet unidentified may be relevant to the presynaptic effect; and 2) neurotransmitter release in some systems may not necessarily depend upon sites of synaptic specialization. Such a situation has been hypothesized in other systems.9

**Ventral Horn (Laminae IX and X).** Enkephalinergic fibers have been identified in the ventral horns lying proximal to motor horn cells. The source of these fibers is not known, but, in view of the paucity of enkephalin-containing cells in the ventral laminae, they likely originate from cells in the overlying lamina or from bulbospinal pathways.

**Intermediolateral Cell Column.** The intermediolateral cell column displays dense accumulations of enkephalin-containing fibers in the vicinity of the several nuclear groups of preganglionic sympathetic neurons in the thoracic cord.149 These fibers were arrayed to correspond with the ladder-like network which has
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previously been described by Laruelle\textsuperscript{107} in the vicinity of preganglionic sympathetic cells. Spinal transections have been shown to diminish met-enkephalin-like immunoreactivity around intermediolateral cell column axons.\textsuperscript{79} Thus, in addition to local enkephalin-containing neurons, opioid peptide activity in this region may largely derive from a bulbospinal pathway.\textsuperscript{78} A similar dense distribution of enkephalin-containing fibers has been identified in the vicinity of the sacral preganglionic parasympathetic nucleus.\textsuperscript{65}

Summary. Enkephalin- and dynorphin-containing neurons are found in high levels among the intrinsic neurons in laminae I, II, III, V, and X. Many of these cells resemble morphologically identified marginal cells, stalked cells, and islet cells. Complex systematic arrangements have been described, many of which are postsynaptic to primary afferents. Little evidence exists to support the existence of an axoaxonal opioid synapse at primary afferent terminals.

Release of Endorphins from the Spinal Cord

Given the presence of the neurons containing spinal opioid peptide as outlined above, an important issue is what activates these systems? A direct method of assessing this issue is to determine the identity and levels of the endorphins that are released into the spinal extracellular space. Early studies indeed demonstrated the presence of \( \beta \)-endorphin and the enkephalin pentapeptides in human lumbar CSF.\textsuperscript{40,62} The relative absence of \( \beta \)-endorphin in spinal cord tissue suggests that these levels result from diffusion from supraspinal structures. As noted previously, each prohormone gives rise to several fragments. Thus, while several forms of endorphins have been extracted from tissue, the question at issue is whether these extended forms play a role as a neurohormone: they may, for instance, only be processing intermediaries. In early studies, Tetenius\textsuperscript{79} and others\textsuperscript{80} examined human CSF using column-coupled opioid radioassay and found that the majority of radioassay activity migrated not with the pentapeptides but as two principal fractions. These appeared to be carboxy-extended forms of the pentapeptides. Similar results have been reported in \textit{in vivo} studies on the release of opioid activity from rat and cat spinal cord using a spinal superfusion technique.\textsuperscript{106}

The release of extended fragments such as Phe\textsuperscript{2}-Arg\textsuperscript{6}, met-enkephalin and Leu\textsuperscript{2}-Gly\textsuperscript{2}-Arg\textsuperscript{6}-met-enkephalin has been confirmed by the use of specific radioimmunoassays and by enzyme cleavage experiments;\textsuperscript{\textsuperscript{17,84}} it has been shown in such experiments that met-enkephalin levels assayed in CSF\textsuperscript{80,86} or in spinal superfusates in cat and rat may rise by a factor of three or four following treatment of the fragments with trypsin and carboxypeptidase B (unpublished observations). This suggests that the release of opioid peptides from spinal neurons is not limited to small molecular fragments, but may also involve the co-secretion of larger amino and carboxyterminus extended forms. A comparable complexity has been observed in the adrenal medulla, where secretion of enkephalin pentapeptide as well as larger forms (up to several thousand molecular weight) have been demonstrated.\textsuperscript{100}

The prevailing evidence strongly suggests that, while the resting levels of spinal enkephalin release are low, they can be significantly augmented by afferent input. Thus, electrical activation of A\( \delta \) and C but not A\( \beta \) somatic nerves results in a prominent increase in the secretion of spinal met-enkephalin-like immunoreactivity and radioimmunoassay assayable levels of several extended enkephalin fragments.\textsuperscript{13,200} Noxious pressure and chemical stimulation also increase spinal enkephalin release.\textsuperscript{29} The observations strongly suggest that small high-threshold afferents may be exerting an excitatory effect on enkephalin-releasing neurons. Further support for this linkage is provided by the observation that the addition to the spinal perfusate of substance P and glutamate, or capsaicin, will increase in a concentration-dependent fashion the levels of enkephalin in rat and cat spinal superfusates.\textsuperscript{84,109,193,196} Substance P, an 11-amino acid excitatory peptide found in unmyelinated primary afferents, is hypothesized to serve in part as a neurotransmitter in high-threshold primary afferents.\textsuperscript{82} Capsaicin is known to depolarize small afferents and release substance P.\textsuperscript{88} In this regard, it is interesting to note that, given the effect of opiates on primary afferent activity, morphine has been shown to suppress the somatic evoked release of met-enkephalin from the spinal cord.\textsuperscript{87} Jointly, these data thus support the hypothesis that small afferents which release substance P glutamate may directly or indirectly depolarize opioid peptide-containing neurons, and those systems themselves are under the regulatory influence of local opioid receptors.

In summary, consistent with the presence of multiple peptide fragments cleaved from opioid prohormones, it appears that several forms (including met-enkephalin, extended sequences of met-enkephalin, and leu-enkephalin) are secreted, and this secretion is maximally enhanced by small high-threshold afferent stimulation.

The Role of the Spinal Endorphin Systems

The preceding cursory analysis of the endorphin systems within the spinal cord reflects the existence of multiple systems, each with a complex hodology, multiple neurotransmitters, and several functionally discrete populations of receptors. Several general points may be considered.

1. The spinal opioid systems appear to demonstrate little spontaneous activity (for example, their low levels of resting release, and the mild effect of naloxone in quiescent spinal cord).

2. The studies on release and the effects of opioid antagonism indicate that these systems may be activated in a selective fashion by a variety of afferent inputs. These observations jointly suggest that the opioid systems in general serve to modulate the responsivity of
the cell to its excitatory drive.

3. The diverse anatomical localization of the endorphin neurons and their receptors makes it certain that these spinal opioid systems are fundamental to many aspects of spinal processing and are not limited to the trivial role of an endogenous "analgesic" system. Thus, simply considering the effects of spinal opiate agents on somatosensory processing, we may assume that several opioid peptide-releasing systems may: 1) regulate the response modality of a cell that responds to many modalities (for example, the wide dynamic range of neurons responding to Aβ, Aδ, and C afferents before morphine administration respond largely to Aβ activity after morphine); 2) alter the gain of the stimulus-response function to enhance the dynamic range of the cell by decreasing the sensitivity of the cell and thereby increasing the range of stimulus intensity to which the cell responds; 3) control the size and nature of the somatic receptive field which normally drives a dorsal or ventral horn cell; and 4) control the postsynaptic response to excitatory drive on motor horn cells and preganglionic sympathetic neurons.

4. Interpretation of the manner in which these opioid peptide-releasing systems function is rendered more complex by virtue of the fact that it appears likely that within a given cell, the several forms of the endorphin (such as met-enkephalin, met-enkephalin-Arg-Phe, and metorphamide) may be subject to differential release as function of characteristics of the afferent excitation. This phenomenon has been observed in the well-characterized adrenal chromaffin cell system. The importance of the identity of the endorphin released from a given cell rests upon the fact that these several forms have a distinguishable synaptic pharmacology (µ vs. δ vs. κ). Thus, a given enkephalin-synthesizing neuron may principally exert a postsynaptic δ effect under one set of conditions and a postsynaptic κ effect under other stimulus conditions. The full significance of this complexity is not known; but, for example, in the intermediolateral cell columns, δ receptors profoundly modify the bladder-spinal-bladder reflex arc, while κ receptors in that region are without a currently detectable effect. Thus, examination of the effects of receptor-selective antagonists on discrete aspects of spinal physiology and attention to the characteristics of the stimulus that releases a given endorphin should reveal an even more complex organization of the several spinal opioid systems.

These functions displayed by spinal opioid receptor systems indicate a high degree of functional specialization and suggest the possibility of a selective pharmacological intervention in a variety of spinally mediated functions. Thus, the use of spinal opiates to produce selective modulation of pain transmission arises from the nature of the substrate with which the opioid receptor is associated. The effects of spinal morphine on bladder function, although in general an unappreciated side effect, reveal for the first time a portion of the central pharmacology of the substrate modulating the bladder-spinal-bladder reflex arc. The failure of opioid receptors to detectably alter normal motor function while they alter spasticity in spinal injury not only offers a possible adjunct to clinical management but also provides a handle on the pharmacology of the reorganization that motor systems undergo following spinal lesions. It is likely that comparable insights will develop for other situations where other spinal cord systems undergo reorganization such as in autonomic hyperreflexia and spastic bladder.

Finally, it should be noted that the focus of this overview has been directed at the spinal endorphin systems. An equally detailed analysis could be directed at the modulatory role of a variety of pharmacologically defined intrinsic systems (gamma-aminobutyric acid, glycine, neurtensin, bombesin), descending systems (norepinephrine, serotonin, dopamine, tryptamine, oxytocin, vasopressin, acetylcholine), and afferent systems (substance P, vasoactive intestinal peptide, cholecystokinin, somatostatin, bombesin, calcitonin-gene-related peptide). Fundamental advances in neurochemistry, pharmacology, and physiology during the past 15 years have revealed the enormous functional complexity of the spinal gray matter. It is as if the complex anatomical renderings of Ramon y Cajal were augmented by colors corresponding to the diverse neurotransmitter pharmacology of the region. Such complexity indicates the degree of pharmacological differentiation and argues that a fundamental understanding of spinal cord pharmacology and hodology will yield significant insight for the common interest of both the clinician and the scientist.

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