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Review

Opioids in chronic pain

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Abstract

The advance in our understanding of the biogenesis of various endogenous opioid peptides, their anatomical distribution, and the characteristics of the multiple receptors with which they interact open a new avenue for understanding the role of opioid peptide systems in chronic pain. The main groups of opioid peptides: enkephalins, dynorphins and β-endorphin derive from proenkephalin, prodynorphin and proopiomelanocortin, respectively. Recently, a novel group of peptides has been discovered in the brain and named endomorphins, endomorphin-1 and -2. They are unique in comparison with other opioid peptides by atypical structure and high selectivity towards the μ-opioid receptor. Another group, which joined the endogenous opioid peptide family in the last few years is the pronociceptin system comprising the peptides derived from this prohormone, acting at ORL1 receptors. Three members of the opioid receptor family were cloned in the early 1990s, beginning with the mouse δ-opioid receptor (DOR1) and followed by cloning of μ-opioid receptor (MOR1) and κ-opioid receptor (KOR1). These three receptors belong to the family of seven transmembrane G-protein coupled receptors, and share extensive structural homologies. These opioid receptor and peptide systems are significantly implicated in antinociceptive processes. They were found to be represented in the regions involved in nociception and pain. The effects of opioids in animal models of inflammatory pain have been studied in great detail. Inflammation in the periphery influences the central sites and changes the opioid action. Inflammation increased spinal potency of various opioid receptor agonists. In general, the antinociceptive potency of opioids is greater against various noxious stimuli in animals with peripheral inflammation than in control animals. Inflammation-induced enhancement of opioid antinociceptive potency is characteristic predominantly for μ opioid receptors, since morphine elicits a greater increase in spinal potency of μ- than of δ- and κ-opioid receptor agonists. Enhancement of the potency of μ-opioid receptor agonists during inflammation could arise from the changes occurring in opioid receptors, predominantly in affinity or number of the μ-opioid receptors. Inflammation has been shown to alter the expression of several genes in the spinal cord dorsal horn. Several studies have demonstrated profound alterations in the spinal PDYN system when there is peripheral inflammation or chronic arthritis. Endogenous dynorphin biosynthesis also increases under various conditions associated with neuropathic pain following damage to the spinal cord and injury of peripheral nerves. Interestingly, morphine lacks potent analgesic efficacy in neuropathic pain. A vast body of clinical evidence suggests that neuropathic pain is not opioid-resistant but only that reduced sensitivity to systemic opioids is observed in this condition, and an increase in their dose is necessary in order to obtain adequate analgesia. Reduction of morphine antinociceptive potency was postulated to be due to the fact that nerve injury reduced the activity of spinal opioid receptors or opioid signal transduction. Our recent study with endogenous ligands of the μ-opioid receptor, endomorphins, further complicates the issue, since endomorphins appear to be effective in neuropathic pain. Identification of the involved differences may be of importance to the understanding of the molecular mechanism of opioid action in neuropathic pain, as well as to the development of better and more effective drugs for the treatment of neuropathic pain in humans.

Keywords: Opioid; Opioid receptor; Endomorphin; Nociceptin; Inflammation; Neuropathic pain

1. Introduction

Over the last three decades, considerable advances have been made in our understanding of the biogenesis of various endogenous opioid peptides, their anatomical distribution, and the characteristics of the multiple receptors with which they interact. It has been shown that opioid peptides are derived from three different precursor proteins: proopiomelanocortin (POMC), prodynorphin (PDYN), and proenkephalin (PENK), which were cloned in the late 1970s and early 1980s (Nakanishi et al., 1979; Kakidani et al., 1982; Noda et al., 1982). The details concerning discovery and progress in endogenous opioid

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peptide research, their body and brain distribution and properties have been reviewed elsewhere (Höllt, 1990). These discoveries open a new avenue for understanding the role of the opioid peptide system in nociceptive transmission and chronic pain control.

2. Multiple opioid peptides and receptors

The main groups of opioid peptides: enkephalins, dynorphins and β-endorphin derive from PENK, PDYN, and POMC, respectively. PENK is the source of [Met^5]- and [Leu^5]-enkephalins and several longer peptides. Endogenous opioid peptides such as dynorphin A, dynorphin B and α- and β-neoendorphin and several larger molecules can be generated from PDYN. POMC is the precursor of β-endorphin, α-endorphin and several non-opioid peptides. The endogenous opioid peptide-containing neurons have been found to be represented in the regions involved in the nociceptive response, e.g. the thalamus, periaqueductal grey, limbic system, cortex and in the spinal cord. Similarly, the autonomic nervous system centers have been shown to be innervated by central and peripheral opioidergic neurons. Recently, a novel group of peptides has been discovered in the brain and named endomorphins, endomorphin-1 (Tyr–Pro–Trp–Phe–NH_2) and endomorphin-2 (Tyr–Pro–Phe–Phe–NH_2). They are unique in comparison with other opioid peptides, having a characteristic atypical structure and high selectivity towards the μ-opioid receptor (Zadina et al., 1997). Anatomical studies demonstrated a distinct anatomical distribution of endomorphins (e.g. endomorphin-1 is present mainly in the brain and endomorphin-2 in the spinal cord) and their synthesis in separate cellular systems. Another group which has joined the endogenous opioid peptide family in the last few years is the pronociceptin system comprising the peptides derived from this prohormone, acting at ORL1 receptors (Meunier, 1997; Reinscheid et al., 1995).

Three members of the receptor family were cloned in the early 1990s, beginning with the mouse δ-opioid receptor (DOR1) (Evans et al., 1992; Kieffer et al., 1992) and followed by cloning of μ-opioid receptor (MOR1) (Chen et al., 1993a,b; Fukuda et al., 1993; Thompson et al., 1993) and κ-opioid receptor (KOR1) (Li et al., 1993; Meng et al., 1993; Minami et al., 1993; Nishi et al., 1993). These three receptors belong to the family of seven transmembrane G-protein coupled receptors, and share extensive structural homologies. The cloned μ-opioid receptor is a morphine-like receptor, and endomorphins may be its endogenous ligands. The enkephalins bind to the δ-opioid receptor with great affinity, and therefore, are considered to be endogenous δ-opioid receptor agonists. The affinity of β-endorphin binding to μ- and δ-opioid receptors was found to be similar. Dynorphins bind to κ-opioid receptors and therefore appear to function as its endogenous ligands. Opioid peptides do not bind exclusively to one specific receptor type but have some affinity for other opioid receptors as well. In addition to the well-established three types of opioid receptors, an orphan opioid-like receptor (ORL1) was cloned a few years ago. This receptor has nearly 70% sequence homology with the opioid receptors. The endogenous ligand for the ORL1 receptor was isolated and named nociceptin or orphanin FQ (Meunier, 1997; Reinscheid et al., 1995). Naloxone, a non-selective opioid receptor antagonist, has low affinity for the ORL1 receptor.

Several subtypes of the opioid receptors (μ_1, μ_2; δ_1, δ_2; κ_1, κ_2, κ_3) have been postulated on the basis of pharmacological studies. Molecular attempts to identify subtypes of opioid receptors have not been successful so far, although the existence of several variants of opioid receptors has been suggested (Koch et al., 1998; Uhl et al., 1999; Abbadie et al., 2000; Pasternak and Pan, 2000). Cloning of all opioid receptors to date yielded a single receptor type, and the suggested subtypes are possibly alternative splicing products. In addition, oligomerization of various opioid receptors generates unique functional properties (Jordan and Devi, 1999).

3. Opioid peptides and their receptors in the antinociceptive pathways

β-Endorphin and related peptides deriving from POMC are present in the nucleus arcuatus of the mediobasal hypothalamus (Khachaturian et al., 1985; Bugnon et al., 1979; Sofroniew, 1979). An extensive nerve fiber system originating in the arcuate nucleus terminates in many areas of the brain which have been implicated in the pain response, e.g. the hypothalamic nuclei, limbic and raphe nuclei, and some pontine nuclei. In addition, some of the structures conveying nociceptive stimuli might also be innervated by POMC-containing neurons located in the nucleus tractus solitarii of the caudal medulla, which project laterally and which also enter the spinal cord (Bronstein et al., 1992; Maley, 1996).

PENK-containing neurons are widespread throughout the central and peripheral nervous systems. They are localized predominantly in interneurons, some of which form local longer tract projections. These neurons have also been found in the spinal cord nociceptive network. A number of PENK neurons exist in the limbic system structures, e.g. the hippocampus, septum, and bed nucleus of the stria terminalis and might be involved in the emotional response to pain. PENK-containing neurons are also abundant in the striatum, substantia nigra, periaqueductal grey and hypothalamus. A variety of PENK-containing cells are present in the adrenal medulla (Viveros et al., 1979).

PDYN-positive neurons are widely distributed in the brain areas associated with nociception (Watson et al., 1981; Khachaturian et al., 1985). Dynorphin and related peptides are present in the magnocellular neurons of the

paraventricular nucleus of the hypothalamus. In addition, these peptides have been found in the nucleus tractus solitarii. Further, PDYN neurons occur in the limbic system and in areas of the spinal cord involved in the transmission of nociceptive stimuli. Spinal interneurons appear to be the main source of spinal dynorphin although immunohistochemical studies also revealed dynorphin in lamina I projection neurons. Thus, dynorphin may be involved in a local circuit within the spinal cord, as well in supraspinal functions (Lima et al., 1993). Interestingly, dynorphin was also detectable in the cutaneous nerves with a distribution similar to that of calcitonin gene-related peptide, a specific marker for sensory neurons (Hassan et al., 1992).

Endomorphins, which bind to μ-opioid receptor with a high affinity and selectivity (Zadina et al., 1997), are localized in neuronal circuits involved in processing of nociceptive information (Zadina et al., 1999). Endomorphin-2 was found in the central nervous system, in regions associated with nociception and rich in μ-opioid receptors, such as the spinal cord and thalamus. In the spinal cord, endomorphin-2 is localized predominantly in the primary sensory afferents. This peptide may be synthesized in the ganglia of primary sensory neurons and then transported to superficial layers of the dorsal horn of the spinal cord, as has already been demonstrated in the rat and monkey. (Martin-Schild et al., 1998; Pierce et al., 1998). Further, endomorphin-2 was found to be co-localized in a subset of substance P- and μ-opiate receptor-containing fibers in the spinal cord. This indicates that endomorphins may be major endogenous opioid ligands for pre- and postsynaptic spinal μ-opioid receptors, and could be critical regulators of pain perception. In fact, endomorphins inhibit nociceptive transmission in the spinal cord through μ-opioid receptors.

Nociceptin-positive neurons are widespread throughout the brain structures: the cortex, hippocampus, brain stem and to a lesser degree, hypothalamus and thalamus. Nociceptin-containing nerve fibers are dense within the structures conveying nociceptive information: the spinal cord dorsal horn, sensory trigeminal complex, raphe nuclei and periaqueductal gray (Schulz et al., 1996). In the spinal cord, laminae II and III of the dorsal horn contain a high density of nociceptin neurons and a lower density was observed in laminae VIII and IX.

Opioid receptors are differentially distributed in the neuronal nociceptive system. A moderate density of μ- and κ-opioid receptor binding sites has been seen in the periaqueductal grey, locus coeruleus, substantia nigra, ventral tegmental area, raphe nuclei and nucleus tractus solitarii, while low δ-opioid receptor binding was demonstrated in the substantia nigra and nucleus tractus solitarii. Further, opioid receptor-containing neurons occur in the limbic system where they may mediate the emotional component of pain, and in areas of the spinal cord involved in the transmission of nociceptive stimuli. Opioid receptors have also been found in the peripheral nervous system (Wittert et al., 1996; Hedner and Cassuto, 1987; Bechara and van der Kooy, 1985). In addition, they are expressed by various immune cells (Wybran et al., 1979; Blalock et al., 1985; Sibinga and Goldstein, 1988; Carr et al., 1988; Stein et al., 1990; Gaveriaux et al., 1995; Chuang, 1995; Peterson et al., 1998).

Prominent expression of the ORL1 receptor has been detected in the amygdala septum, hypothalamus and thalamus and in the perikarya in the dorsal root ganglia and spinal cord (Monteillet-Agius et al., 1998). In the spinal cord, the receptor is present in the gray matter of the ventral and dorsal horns. Therefore, the ORL1 receptor is present in neurons distinct from those in which other opioid receptors occur, and no co-localization with μ-opioid receptors could be observed at various levels of the neuroaxis.

4. Opioids in antinociceptive processes

The central POMC system is significantly implicated in antinociceptive processes. Lesions of the arcuate nucleus of the hypothalamus, the main area of POMC synthesis in the brain, weakens post-stress analgesia, and reduces the antinociceptive effect of electrical stimulation of the periaqueductal gray, where β-endorphinergic nerve endings are localized. On the other hand, β-endorphins administered both to the lateral brain ventricle and intrathecally exert a strong antinociceptive action, and these effects are blocked by naloxone.

It appears that β-endorphin and other opioid peptides can elicit a peripheral analgesic action in the hyperalgesia associated with strong inflammation. This effect is probably a result of the local interaction of opioids (also synthesized in immunocytes) with opioidergic receptors localized on peripheral afferent nerve terminals (Stein et al., 1990).

PENK-derived peptides are very susceptible to proteolytic action, so their central antinociceptive action is short-lasting. The augmentation of enkephalin action has been observed after the administration of inhibitors of enzymatic degradation of these peptides (Roques et al., 1980). Moreover, some strong enkephalinase and aminopeptidase inhibitors characterized by low selectivity, exhibit an antinociceptive action. Enkephalin analogues, such as [D-Pen^2,D-Pen^5]enkephalin (DPDPE), resistant to proteolytic enzymes show strong antinociceptive activity. A number of studies have indicated that longer PENK-derived peptides, bearing the enkephalin sequence, evoke stronger an analgesic action than do enkephalins themselves. However, enkephalin analogues penetrate the blood–brain barrier poorly after peripheral administration, which is a serious obstacle to their potential use in pain treatment. A majority of proenkephalin-originating peptides interact with δ-opioid receptors.

A lack of antinociceptive action of dynorphin after its administration to the lateral brain ventricle (Walker et al.,

1982), and some slight antinociceptive activity after intrathecal injection (Przewlocki et al., 1983; Stevens and Yaksh, 1986) have been reported. The observation of antinociceptive actions of dynorphin in the spinal cord is hindered by its neurotoxic effects. These can result from a non-opioidergic mechanism of action of the peptide (Przewlocki et al., 1983; Faden and Jacobs, 1984). Other κ-opioid receptor agonists also possess some antinociceptive activity after their intrathecal administration. However, the effect is much weaker on a molar basis than that evoked by μ- or δ-opioid receptor agonists. On the other hand, in electrophysiological experiments in spinalized rats, μ- and κ-opioid receptor agonists reduced reflexes stimulated by thermal and mechanical nociceptive stimuli to the same extent and in a dose-dependent manner (Parsons and Headley, 1989), but in the visceral nociception induced by colorectal distension only spinal μ-, but not κ-opioid, receptors played a significant role (Harada et al., 1995). It can be concluded that the spinal action of opioids acting at κ-opioid receptors seems to depend on the noxious stimuli used.

Current studies have shown the analgesic activity of endomorphin-1 and -2. These endomorphins have been shown to induce analgesia via μ-opioid receptors (Goldberg et al., 1998; Przewlocka et al., 1999b; Stone et al., 1997; Zadina et al., 1997). Both these peptides showed a potent, dose- and time-dependent antinociceptive effect after their intraventricular and intrathecal injection to mice (Goldberg et al., 1998; Zadina et al., 1997). Several studies demonstrated spinal analgesic activity of endomorphins in rats. Both endomorphin-1 and -2 exerted a potent dose- and time-dependent antinociceptive effect after their intrathecal injection to rats. The antinociceptive effect of endomorphins was observed after the application of acute thermal and mechanical stimuli. The response to thermal stimuli was long-lasting, while the reaction to mechanical stimuli was substantially shorter (Przewlocka et al., 1999b).

The antinociceptive effect of these endomorphins in acute pain models in rats was weaker than that of [D-Ala^2,*N*-MePhe^4,Gly^5-ol]enkephalin (DAMGO), but comparable to the effect of morphine. A spinal antinociceptive effect of endomorphins has recently been demonstrated in mice with a tail-flick test (Stone et al., 1997). In the latter study, endomorphins produced short-term antinociception. In contrast, a prolonged antinociceptive effect was found in rats (Przewlocka et al., 1999b). Interestingly, in our present study, endomorphin-2 was distinctly less potent than endomorphin-1 in both tests used, i.e. tail-flick and paw pressure tests. This observation is in agreement with the recent finding in mice that endomorphin-2 was also significantly less active than endomorphin-1 (Goldberg et al., 1998; Zadina et al., 1997).

Endomorphin-induced analgesia at both spinal and supraspinal levels was antagonized by the non-selective opioid receptor antagonist, naloxone, and selective μ-opioid receptor antagonists, β-funaltrexamine and cyprodime (Schmidhammer et al., 1989), and, to a lesser extent, by the μ_1 receptor antagonist, naloxonazine (Goldberg et al., 1998). Neither δ- nor κ-opioid receptor selective antagonists were effective (Przewlocki et al., 1999). Therefore, the results suggest that endomorphins are highly selective ligands for the μ-opioid receptor in binding assays (Zadina et al., 1997). Further, these peptides may be released in response to painful and traumatic stimuli and thus act as endogenous analgesics. This hypothesis is further supported by a recent study which showed the presence of these peptides in nociceptive pathways at both the spinal and supraspinal levels of the neuroaxis (Martin-Schild et al., 1998).

Electrophysiological and behavioral data have indicated that intrathecal administration of nociceptin evoked an analgesic action (Erb et al., 1997; Yamamoto et al., 1997a,b,c; Yamamoto and Nozaki-Taguchi, 1997; Meunier, 1997). However, intrathecal administration of nociceptin at high doses in rats impaired locomotor activity, inhibited spatial learning, increased appetite, influenced the excitation threshold under stress and stimulated pituitary hormone secretion. Furthermore, it stimulated the immune system and differentiation of neurons (Meunier, 1997). Nociceptin given to rats after ligation of the sciatic nerve had a dose-dependent, antiallodynic effect, however, at low doses (0.1 μg i.th.) a tendency to evoke pronociceptive effects was observed in rats and mice (Mika et al., in preparation). In ORL1 receptor knockout mice, the thermal and visceral pain threshold was not changed (Nishi et al., 1997), and the response to morphine remained unaltered, while the development of tolerance was delayed. Elimination of nociceptin resulted in hyperalgesia and abolishment of stress-induced analgesia in mice (Ueda et al., 1997). Intraventricular administration of nociceptin in rats can evoke pronociceptive and anti-opioid actions (it inhibits analgesic effects of opioids) (Okuda-Ashitaka et al., 1998). Similar effects of dynorphin have also been reported (Przewlocki et al., 1983; Stevens and Yaksh, 1986).

5. Effects of opioids in inflammation

The effects of opioids in animal models of inflammatory pain have been studied in great detail. In general, the antinociceptive potency of opioids is greater against various noxious stimuli in animals with peripheral inflammation than in control animals. Thus, inflammation in the periphery influences the central sites and changes the opioid action. Inflammation increases the spinal potency of various opioid receptor agonists. However, this increase in antinociceptive potency is not the same for agonists of different opioid receptor types. Inflammation-induced enhancement of opioid antinociceptive potency is characteristic predominantly for μ-opioid receptors, since morphine elicits a greater increase in spinal potency of μ- than of δ- and κ-opioid receptor agonists (Hylden et al., 1991). Enhancement of the potency of μ-opioid receptor agonists

during inflammation could arise from the changes occurring in opioid receptors, predominantly in affinity or number of μ-opioid receptors. But most binding studies in animals with inflammation of a hind limb showed no significant changes in either opioid receptor density or affinity within the spinal cord (Cesselin et al., 1980; Delay-Goyet et al., 1989; Stanfa and Dickenson, 1993). Another possible explanation was that the enhanced antinociceptive response to intrathecally administered opioids in monoarthritic rats is connected with the increased sensitivity of adenylate cyclase to the inhibitory effects of μ-(D-Ala^2,D-Leu^5-enkephalin, DADLE) and δ-(DPDPE) and κ-(dynorphin) opioid receptor agonists. However, a κ-opioid receptor agonist, (*trans*-(±)-3,4-dichloro-*N*-methyl-*N*-[2-(1-pirrolidinyl)cyclohexyl]benzeneacetamide) (U50,488H), did not evoke similar effect (Przewlocka et al., 1992a). Furthermore, the changes in cholecystokinin (CCK) system activity or increased activity of the noradrenergic descending system have been listed among the reasons for an inflammation-induced increase in effectiveness of μ-opioid receptor agonists. This effect can be related to the decreased activity of the spinal cholecystokinin and/or noradrenergic descending system. Thus, both these systems seem to modulate opiate-induced analgesia during inflammation. In fact, results of several studies support this hypothesis (Stanfa and Dickenson 1993; Ossipov et al., 1990; Wigdor and Wilcox, 1987; Wilcox et al., 1987). On the other hand, endomorphins, endogenous μ-opioid receptor ligands, did not show any increase in potency against the inflammatory formalin-induced pain (Przewlocka et al., 1999b; Przewlocki et al., 1999). In another study, the effect of endomorphin-2 in rats with peripheral inflammation was not significantly different from that in normal animals (Grass et al., 2000). In contrast, the μ-opioid receptor agonists, DAMGO and morphine potently inhibited the pain-related behavior induced by formalin.

Thus, endomorphins appear to be less potent and shorter-acting than other μ-opioid receptor agonists such as morphine in this model of experimental inflammatory pain. This is contrary to the generally accepted view that the analgesic efficacy of μ-opioid receptor agonists is increased in inflammation. The reason for this discrepancy is still unknown.

6. Role of endogenous opioid systems in inflammation

Inflammation has been shown to alter the expression of several genes in the spinal cord dorsal horn. The central β-endorphin system is activated by prolonged noxious stimulation. Injection of formalin into the rat paw causes an increase in β-endorphin levels in periaqueductal grey, thalamus and ventromedial hypothalamus (Porro et al., 1991). On the contrary, intraventricular administration of the antibody against β-endorphin potentiates formalin-evoked hyperalgesia. Most studies found small changes in the PENK mRNA and peptides derived from this prohormone molecule in the spinal cord during peripheral hind limb inflammation (Iadarola et al., 1986; Przewlocka et al., 1992b; Noguchi et al., 1989). In contrast, several studies have demonstrated profound alterations in the spinal PDYN system when there is peripheral inflammation or chronic arthritis. The levels of dynorphin were dramatically increased in the lumbar part of the spinal cord of rats with local inflammation of a hind limb (Przewlocki et al., 1985; Iadarola et al., 1986; Weihe et al., 1989). Furthermore, an increase in PDYN mRNA levels induced by acute and chronic inflammation has also been observed in the spinal cord (Iadarola et al., 1986; Przewlocka et al., 1992b; Noguchi et al., 1989; Ruda et al., 1988; Weihe et al., 1989).

Thus, many forms of peripheral inflammation induce a dramatic up-regulation of PDYN biosynthesis in nociceptive neurons of the spinal dorsal horn, which parallels the behavioral hyperalgesia associated with the inflammation. Interestingly, levels of the PDYN-derived peptides, dynorphin and α-neoendorphin, were also increased in the spinal cord of rats subjected to repeated inescapable footshock (Przewlocki et al., 1987). This profound increase was visible in spinal cord segments corresponding to the regions receiving nociceptive information from forelimbs and hindlimbs. The most likely interpretation of these results is the fact that the spinal cord opioid systems react to profound prolonged as well as recurrent noxious stimulation by enhancement of their activity. These changes probably reflect a functional response to pain.

Nociceptin is an effective analgesic against inflammatory pain (Taylor and Dickenson, 1998). Inflammation enhances nociceptin expression in the spinal root ganglia (Andoh et al., 1997) and nociceptin receptor expression in the dorsal spinal cord (Jia et al., 1998). Thus, inflammation activates nociceptin biosynthesis, and possibly release from primary afferents, as well as enhances sensitivity of nociceptin receptors within the spinal cord.

Experimental data clearly demonstrate that opioids are able to inhibit nociception arising in inflamed tissue by exerting a local peripheral action, presumably via the terminal region of the sensory nerves. Similar effects are elicited by endogenous opioid peptides such as β-endorphin and enkephalins released under pain or stress from immune cells present in an inflamed tissue (Stein et al., 1990; Przewlocki et al., 1992; Herz, 1995, 1996). Although the mechanism of release of opioid peptides from the immunocytes is not fully understood, there are indications that this process involves cytokines and corticotropin-releasing factor (CRF) (Machelska and Stein, 2000).

7. Effects of opioids in neuropathic pain

Several clinical studies showed that opioids, particularly morphine, lack potent analgesic efficacy in neuropathic

pain in humans (Arner and Meyerson, 1988). On the other hand, a few studies showed that morphine could be effective in some patients suffering from neuropathic pain (Rowbotham et al., 1991; Portenoy et al., 1990). These authors suggest that neuropathic pain is not opioid-resistant but only reduced sensitivity to systemic opioids is observed in this condition, and an increase in their dose is necessary in order to obtain adequate analgesia (Portenoy and Hagen, 1990).

It is well known that the antinociceptive efficacy of intrathecally administered morphine is decreased in rats with nerve injury (Ossipov et al., 1995). Bian et al. (1995) demonstrated that intrathecal morphine injection failed to alleviate mechanical allodynia even at doses up to 100 μg in a L5/L6 ligation model of neuropathic pain. It has been suggested that the ineffectiveness of morphine in neuropathic pain is due to the reduced number of presynaptic opioid receptors as a result of degeneration of primary afferent neurons caused by nerve damage (Ossipov et al., 1995). In fact, such a reduction in the number of μ-opioid receptors may be an important factor in diminishing the efficacy of morphine and other μ-opioid receptor agonists. However, several recent studies have yielded a more complicated picture of opioid action in neuropathic pain depending on modalities of nociceptive stimuli (thermal or tactile) and route of opioid administration (intrathecal, intracerebroventricular or systemic). Morphine, when applied intrathecally at a very high dose (up to 100 μg), had no effect on tactile allodynia (Lee et al., 1995; Bian et al., 1995) while it inhibited in a dose-dependent manner the thermal hyperalgesia induced by heat (Wegert et al., 1997; Mao et al., 1995) or cold (Przewocka et al., 1999b), although its potency was very low. Noxious thermal stimuli appear to be conveyed via C-fibers, while non-noxious tactile stimuli are believed to be transmitted to the spinal cord through myelinated Aβ fibers. Therefore, a simple interpretation was that morphine affected nociceptive input-transmitting pathways with different efficacy, since most opioid receptors appear to be localized on C-fibers. Further, the reduction of morphine antinociceptive potency was postulated to be due to the fact that nerve injury reduced the activity of spinal opioid receptors or opioid signal transduction. However, recent data have shown only minor differences, if any, in opioid receptor binding characteristics as well in signal transduction pathways (Robertson et al., 1999), although some decrease in μ-opioid receptor immunoreactivity was demonstrated after axotomy (Zhang et al., 1998). Therefore, a deficiency of opioid receptor function did not appear to fully explain the lower efficacy of morphine in injured animals. Our recent results with endogenous ligands of the μ-opioid receptor, endomorphins, further complicate the issue, since endomorphins appear to be effective in neuropathic pain (Przewlocka et al., 1999b). However, Grass et al. (2000) found a reduced efficacy of endomorphin-2 administered i.th. in rats that developed autotomy-related behavior after axotomy of the sciatic nerve. Thus, sensitivity to endomorphin-2 after peripheral nerve injury may be reduced in rats with nerve axotomy. However, in line with our findings, the reflex depressive effect of intrathecal endomorphin-2 treatment was unchanged in axotomized rats without autotomy and remained similar to that in control rats.

In our study, morphine administered intrathecally produced a slight antiallodynic effect only at a dose up to 50 μg in cold allodynia and mechanical allodynia test in rats with sciatic nerve injury. In contrast, both endomorphin-1 and endomorphin-2, administered intrathecally at increasing doses (2.5–5 μg), dose-dependently inhibited cold and mechanical allodynia. The antiallodynic effect of these endomorphins was antagonized by the μ-opioid receptor-selective antagonist, cyprodime, indicating the involvement of μ-opioid receptors (Przewlocka et al., 1999b). Thus, that study clearly showed that endomorphins possess antinociceptive properties in neuropathic pain in rats and display a marked antiallodynic action via μ-opioid receptors. This difference in morphine and endomorphin activity in neuropathic pain is in contrast to acute nociception, in which the potency of endomorphins is similar to that of morphine. The reason for this discrepancy is unknown, since both morphine and endomorphins appear to act via the same μ-opioid receptors. Identification of the differences involved would help in the understanding of the molecular mechanism of opioid action in neuropathic pain, as well as with the development of better and more effective drugs for the treatment of neuropathic pain in humans. It is possible that different μ-opioid receptor subtypes mediate the effects of morphine and endomorphins in neuropathic pain, or that molecular characteristics of μ-opioid receptors are modified following nerve injury. Furthermore, it is also possible that some non-opioid receptors or unknown mechanisms in addition to μ-opioid receptor activation are involved in the analgesic effects of endomorphins in neuropathic pain.

Several recent studies have pointed to a great similarity in the molecular effects of morphine and of endomorphins, but, interestingly, several effects of endomorphins have been found to differ from those of morphine in various behavioral and molecular assays. Increased locomotor activity was observed after intraventricular administration of morphine, but not after endomorphin-1 or endomorphin-2 treatment by the same route (Soignier et al., 2000). Morphine induces analgesia and has rewarding properties as well. However, endomorphin-1 produces potent analgesia without eliciting reward-related behaviors (Wilson et al., 2000). Intracerebroventricular morphine injection increases plasma corticosterone levels within 30 min, whereas endomorphin-1 and endomorphin-2 does not induce this effect. Similar to morphine, endomorphin-1 produces antinociception but in contrast to morphine, does not induce immunomodulatory effects in the rat (Carrigan et al., 2000). Further, endomorphin-1 and endomorphin-2 do not displace [^{3}H]dihydromorphine binding from immunocytes, as

does morphine. Thus, these opioid peptides do not appear to interact with the putative μ_3-opioid receptor subtype characterized in immunocytes (Rialas et al., 2000). Furthermore, endomorphin-1 potentiates the expression of human immunodeficiency virus type 1 in microglial cells via the activation of an atypical μ-selective opioid receptor, while the classical μ-opioid receptor agonists, morphine and DAMGO, have no effect on viral expression (Peterson et al., 1999). It is interesting to note that, in contrast to morphine, both endomorphin-1 and DAMGO caused internalization of the μ-opioid receptor in human embryonic kidney (HEK) cells (Burford et al., 1998). Therefore, these data suggest that endomorphins and morphine may act on a different subset of the μ-opioid receptors (Coventry et al., 2001), but the molecular mechanisms of these different activities of endomorphins and morphine are unknown.

In conclusion, the results of our studies showed that analgesic efficacy of endomorphins appeared to be preserved in a majority of rat neuropathic pain models. This observation is in contrast with the fact that the analgesic potency of morphine is reduced in all neuropathic pain states. Undoubtedly, further studies are necessary to better understand the effects of these agonists of μ-opioid receptors in neuropathic pain.

A vast body of evidence suggests an important role of δ-opioid receptor agonists in antinociception at the level of the spinal cord (Stewart and Hammond, 1993; Misicka et al., 1991; Mattia et al., 1992). Our recent study was undertaken to analyze spinal analgesic and antiallodynic effects of δ_1- and δ_2-opioid receptor agonists and antagonists after their acute and chronic i.th. administration in a neuropathic pain model in the rat (Mika et al., in preparation). In rats with a crushed sciatic nerve, DPDPE and deltorphin II dose-dependently antagonized the cold water allodynia, which developed after sciatic nerve injury. These effects of DPDPE were antagonized by 7-benzylidenenaltrexon, while the effects of deltorphin II were antagonized by 5′ naltrindole izotiocyanate. Both agonists had a dose-dependent, statistically significant effect on tail-flick latency in two tests, utilizing focused light and cold water as noxious stimuli. Chronic administration of DPDPE and deltorphin II resulted in significant prolongation of the reaction time determined on days 2, 4, and 6 post-injury. In conclusion, our results have shown an antiallodynic and analgesic action of DPDPE and deltorphin II at the spinal cord level, which suggests that both δ-opioid receptor subtypes play a similar role in neuropathic pain. Thus, both δ_1- and δ_2-opioid receptor agonists can be regarded as potential drugs for the therapy of the neuropathic pain.

It has been suggested that hyperactivity in the spinal CCK system is responsible for the lower efficacy of opioids in neuropathic pain. Small doses of CCK reduce (Faris et al., 1983), while CCK-receptor antagonists enhance morphine analgesia. The mechanism of CCK interaction with opioidergic transmission is not known but it was suggested that CCK might modify opioid receptors (Wang and Han, 1990), for example, via heterologous interaction or by receptor dimerization. Alternatively, CCK may inhibit the release or synthesis of enkephalins, which act synergistically with morphine to evoke antinociception (Ossipov et al., 1994). However, this interesting hypothesis appears to apply to certain kinds of neuropathic pain but not to others. Interestingly, a recent study has shown that the combination of CCK-B antagonist and morphine had a superadditive interaction in diabetic, but not in mononeuropathic rats (Coudore-Civiale et al., 2000). Furthermore, in the partial sciatic nerve injury model, the effect of morphine on the injured paw was less potent than that on the uninjured paw, and CCK-B antagonist potentiated the morphine analgesia in the uninjured paw and had only a minor effect on morphine analgesia in the injured paw (Yamamoto and Sakashita, 1999).

8. Role of endogenous opioid systems in neuropathic pain

Little is known about the role and involvement of the POMC system in neuropathic pain. Tsigos and his colleagues demonstrated that β-endorphin levels in cerebrospinal fluid were reduced in patients with diabetic polyneuropathy but that this was not related to the presence of neuropathic pain (Tsigos et al., 1995).

Also, no significant variation was found in patients suffering from deafferentation pain (Salar et al., 1991). Interestingly, melanocortins, which derive from POMC, appear to be involved in neuropathic pain since melanocortin receptor antagonists, when applied intra cisterna magna (Vrinten et al., 2000) or intrathecally (Starowicz et. al, in preparation), have a strong antiallodynic effect in neuropathic rats.

There is much evidence that nerve injury is associated with elevated levels of spinal dynorphin (Dubner and Ruda, 1992). The changes are ipsilateral to the injury in the superficial laminae I and II, as well as in deeper laminae V–VII in CCI rats. Rats with spinal nerve L5/L6 ligation showed an increase in dynorphin level in the ipsilateral dorsal parts of L4–L6 segments (Bian et al., 1998). The increase in PDYN-derived peptides was found in the local spinal interneurons as well as in neuronal projections (Lima et al., 1993).

Spinal injury, which may cause neuropathic pain, also increases the dynorphin level in the spinal cord (Faden et al., 1985; Przewlocki et al., 1988). Both PDYN mRNA and dynorphin levels were significantly increased, whereas those of PENK mRNA and Met-enkephalin remained unchanged 3 days after injury. The increase in the spinal levels of PDYN mRNA was highest in the areas close to the side of transection. Thus, endogenous dynorphin levels increase under various conditions associated with neuropathic pain following damage to the spinal cord and injury of peripheral nerves.

Interestingly, the increase in dynorphin content occurs across multiple spinal segments adjacent to the site of nerve injury. Moreover, ligation of lumbar spinal nerves increased the level of dynorphin in the ipsilateral lumbar part but also in the sacral spinal cord. Therefore, a decrease in the antiallodynic activity of spinal morphine treatment in neuropathic pain may to be due to the elevation of dynorphin across multiple spinal segments following nerve injury (Malan et al., 2000).

In fact, the site and time of increase of the dynorphin content in the spinal cord after peripheral nerve injury appear to correlate with the appearance of neuropathic pain. Furthermore, a single intrathecal injection of dynorphin A to mice induced mechanical allodynia, and cold allodynia (acetone applied to the dorsal hind paw) lasting several days (Laughlin et al., 1997). Interestingly, dynorphin A_{2-17}, a non-opioid peptide, induced cold and tactile allodynia analogous to that induced by dynorphin A_{1-17}, indicating the involvement of non-opioid receptors. Furthermore, dynorphin A has non-opioid activity and may damage the spinal cord when given in high doses and may produce hind limb paralysis when administered intrathecally to rats. This effect is mediated at least partly via non-opioid receptors, since the des–Tyr fragment of dynorphin can induce a similar effect (Przewlocki et al., 1983; Faden and Jacobs, 1984). Furthermore, a recent study has shown that the injection of anti-dynorphin antiserum has no effect in sham-operated rats while it reverses the thermal hyperalgesia following nerve injury, further indicating an involvement of spinal dynorphin in the phenomenon.

Some of the observed effects of dynorphin appear to be mediated by NMDA receptors since pretreatment with the NMDA receptor antagonists, dizocilpine (MK-801) and [3S-(3α,4aα,6β,8aα)]-Decahydro-6-(phosphonomethyl)-3-isoquinolinecarboxylic acid (LY235959), but not the opioid receptor antagonist, naloxone, blocks the induction of allodynia (Laughlin et al., 1997). A model has been proposed in which dynorphin enhances neuronal excitability via the action of NMDA receptor sites, leading first to dorsal horn hyperexcitability and then to excessive depolarization and excitotoxicity. The peptide applied to the spinal cord also induces an enlargement of receptive fields and facilitates C-fiber-evoked reflexes.

These findings suggest that the elevated spinal dynorphin content, which is a consequence of peripheral nerve injury, may govern sensitization of the spinal cord, partly through its direct or indirect action on the NMDA receptor complex (Bian et al., 1999). Thus, dynorphin might be responsible for sensitization and for expanding the receptive sites in neuropathic pain.

Recent study has demonstrated that spinal treatment with DAMGO elicits an increase in lumbar dynorphin content and a decrease in μ-opioid receptor immunoreactivity in the spinal dorsal horn, signs also observed after nerve injury. Therefore, results of these studies further support the view that an elevated spinal dynorphin content has important functional significance in neuropathic pain. However, a recent study with dynorphin knockout mice showed that dynorphin was not essential for induction of neuropathic pain but might be important for maintenance of neuropathy (Wang et al., 2001).

9. Molecular mechanisms of chronic pain

Reorganization of the spinal neuronal systems is known to occur as a result of peripheral injury. Recent evidence has pointed to the involvement of glutamatergic pathways and the NMDA receptors in the plastic changes. Neuropathic and inflammatory pain induce neuronal plasticity via NMDA receptors, therefore, antagonists of glutamate receptors subclass $NMDA_1$ receptor prevent the nervous system plastic alterations. Tissue injury and inflammation induce prolonged activation of excitatory amino acid systems followed by excessive activation of an intracellular cascade of second messengers, protein phosphorylation and activation of transcription factors and gene expression. Excitatory amino acids induce an increase in Ca^{2+} influx, protein kinase C translocation and enhance the production of nitric oxide (NO). The changes in NO synthase (NOS) activity in different models of chronic pain have been analyzed (Solodkin et al., 1992; Hao and Xu, 1996). Goff reported that the number of brain NO synthase-positive neuronal cells decreased by ca. 50% at 7 days in animals with chronic constriction injury or tight nerve ligature (Goff et al., 1998). This decline in immunolabeled brain NOS-containing cells in the dorsal horn ipsilateral to an injury persisted until day 28. In contrast, formalin-induced inflammation resulted in an increase in the number of NOS-labeled neurons in the dorsal horn (Przewlocka et al., 1999a). Persistent pain is believed to activate NMDA receptors, enhance NO production and subsequently, to increase the level of cyclic guanosine 3′,5′-monophosphate (cGMP). Results of several studies indicated that the increased spinal cGMP levels parallel the thermal and mechanical hyperalgesia and tactile allodynia caused by chronic constriction injury of the sciatic nerve in rats (Siegan et al., 1996). Recent reports have defined the role of NO in spinal nociceptive signal processing. The evidence suggests that NO, produced in the spinal cord neurons, plays a pivotal role in multisynaptic local circuit nociceptive processing in the spinal cord (Meller and Gebhart, 1993). The mechanisms responsible for hyperalgesia in chronic pain may involve not only NO itself, but also peroxynitrite, the product of its reaction with the superoxide radical $O2^-$, that can lead to the formation of the free radicals, OH and NO^{2-} (Tal, 1996). Consequently, the generation of NO by neuronal NOS may be critically involved in the maintenance of this abnormal pain-related sensation.

In patients with chronic spinal injury, pain cannot be relieved by a number of conventional analgesics used for the treatment of chronic neuropathic pain, therefore, the possibility of using NOS inhibitors as potential novel analgesics is taken into consideration. In animal models of chronic pain, systemic treatment with NOS inhibitors, *N*(G)-nitro-L-arginine methyl ester (L-NAME) and 7-nitroindazole relieved the mechanical allodynia-like response, dose-dependently in a stereospecific and L-arginine-reversible manner (Hao and Xu, 1996). This suggested that the blockade of NO synthase by L-NAME relieved the chronic allodynia-like behavior in spinally injured rats. This effect was likely to have been mediated by a blockade of neuronal isoforms of NOS, as 7-nitroindazole relieved the allodynia in a L-arginine-reversible manner. Agmatine, which is an intermediate in polyamine biosynthesis which has recently been proposed as a novel neurotransmitter in the brain (Reis and Regunathan, 2000), administered to rodents, also normalized the mechanical hypersensitivity (allodynia/hyperalgesia) produced by chemical or mechanical nerve injury, and reduced autotomy-like behavior after excitotoxic spinal cord injury (Fairbanks et al., 2000). It was reported that agmatine had the activity of both NMDA receptor antagonist and NOS inhibitor. There is also a clinical study on the role of NO synthase inhibitors in chronic pain. The efficacy of the inhibitor of NOS, *N*G-monomethyl-L-arginine hydrochloride (L-NMMA), was tested in 16 patients with tension-type chronic headache. The study demonstrated that the inhibition of NOS had an analgesic effect in these patients, which supports the conclusions drawn on the basis of animal experiments and suggests the possibility of clinical use of NOS inhibitors (Ashina et al., 2000). In laboratory animals, the injection of L-NAME at doses which are not themselves antinociceptive, potentiated the analgesic effect of low doses of morphine in rats (Przewlocki et al., 1993). The effects were reversed by injection of the NO donor, 3-morpholinosydnonimine chloride (SIN-1). Opioid receptor activation by morphine may initiate protein kinase C translocation (Chen and Yu, 1994), activation of NMDA receptors and intracellular influx of Ca^{2+} ions, in spite of membrane hyperpolarization, and finally, activation of the NO/superoxide pathway. We previously showed that the antinociceptive effects of various opioid receptor agonists, including morphine, were clearly enhanced by inhibition of NOS (Machelska et al., 1997a). Interestingly, the antinociceptive effect of intrathecally administered endomorphins was not changed. This may indicate that morphine and endomorphins influence NO pathway in the spinal cord differently. It is likely that endomorphins are potent analgesis in neuropathic rats because they do not significantly influence the NO pathway.

NO is involved not only in the antinociceptive activity of morphine but a growing body of evidence also suggests participation of NO in the development of opiate tolerance and dependence (Majeed et al., 1994). Moreover, it was found that the repeated administration of morphine results in an increase in both NOS mRNA and protein spinal levels, the latter parameter evaluated as the number of NOS-positive cells and optical density (Machelska et al., 1997b). It has been suggested that the increased NOS biosynthesis following chronic morphine treatment may be related to the development of opiate tolerance, a possibility which was confirmed by results of behavioral studies showing the attenuation of tolerance to the antinociceptive action of opioids, as well as of the naloxone-precipitated withdrawal syndrome in morphine-dependent rodents by administration of NOS inhibitors (Majeed et al., 1994). Since chronic pain cannot be relieved by a number of conventional analgesics used for treating chronic neuropathic pain, especially morphine, we suggested that the increased activity of NO in chronic nociceptive stimulation may be responsible for the lower efficacy of morphine in neuropathic pain. In fact, the attenuated antiallodynic effect of morphine in an animal model of neuropathic pain (chronic constriction injury of the sciatic nerve) was restored or even potentiated by systemic injection of L-NAME (Sieja and Przewlocka, in preparation). Mayer et al. (1999) have recently demonstrated that chronic pain may activate an intracellular cascade, production of NO and superoxide, and formation of peroxynitrate ($NOOO^-$), that can eventually lead to initiation of DNA strand break and finally to activation of the nuclear repair enzyme, poly(ADP ribose) synthase. Poly(ADP ribose) synthase activation may lead to cell dysfunction due to inhibition of mitochondrial respiration, depletion of cellular energy stores, and may initiate an excitotoxic pathway and finally cell death, most likely via apoptosis. This results in cell loss and plastic changes within the spinal cord. Interestingly, similar changes may occur upon chronic opiate treatment and opioid tolerance. These events may lead further to plastic changes in spinal neurons similar to those occurring in neuropathic pain.

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