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Mechanisms of Induction of Persistent Nociception by Dynorphin

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ABSTRACT

The opioid peptide dynorphin has been demonstrated to be both nociceptive and antinociceptive. This article will review the potential mechanisms through which dynorphin contributes to spinally mediated nociception. Specifically, we will examine the interaction of dynorphin with multiple sites on the NMDA receptor complex. Dynorphin-induced opioid activity is generally inhibitory, with a

tendency to impede nociceptive signals and serve in a neuroprotective capacity. In contrast, dynorphin's interaction with multiple sites on the NMDA receptor complex produces excitatory responses resulting in nociceptive and even toxic effects. Thus, it is hypothesized that dynorphin has both physiological and pathological roles in acute and chronic pain states.

Background

The opioid peptide dynorphin A (H-Tyr-Gly-Gly-Phe-Leu-Arg-Arg-Ile-Pro-Lys-Leu-Lys-Trp-Asp-Asn-Gln-OH) was first isolated from the porcine pituitary (Chavkin et al., 1982) and has since been shown to be distributed widely throughout the central nervous system. Although its role in many areas is not clear, an extensive line of investigation suggests that dynorphin located in the spinal cord plays a pivotal role in the sensitization of nociceptive neurons. Whereas relatively low doses of dynorphin produce analgesia, higher doses (3 nmol in mice and 15 nmol in rat) produce hyperalgesia that persists for greater than 60 days after a single intrathecal injection (Vanderah et al., 1996; Laughlin et al., 1997). This protracted effect appears to be independent of activation of opioid receptors (Vanderah et al., 1996; Laughlin et al., 1997). Increasing the dose beyond that necessary for hyperalgesia results in severe motor dysfunction, characterized by flaccid extension and complete loss of muscle tone (Stevens and Yaksh, 1986), demonstrating a pathological action of dynorphin. This review will address the potential mechanisms by which dynorphin contributes to the development of spinal cord sensitization that leads to an apparently irreversible nociceptive state.

The increased levels of dynorphin in the spinal cord associated with several nociceptive models have been suggested to play a role in the nociceptive state (Dubner and Ruda,

1992). Dynorphin immunoreactivity in spinal cord is contained in both interneurons and projection neurons (Nahin et al., 1989), and the dynorphin released from interneurons in persistent nociceptive states is thought to initiate dynorphin's excitotoxic effects (Dubner and Ruda, 1992). Mice with osteolytic tumors in the lumen of the femur show increased nociceptive responsiveness associated with increased incidence and intensity of dynorphin immunoreactivity in spinal cord dorsal horn neurons (Schwei et al., 1999). The induction of a neuropathic state in rats by nerve ligation or constriction is associated with an up-regulation of dynorphin synthesis, reflected by enhanced expression of preprodynorphin protein and mRNA, increased dynorphin immunoreactivity (Kajander et al., 1990; Malan et al., 2000), and a greater percentage of spinal neurons receiving dynorphin-immunoreactive contacts (Nahin et al., 1992). Furthermore, the enhanced dynorphin expression correlates both temporally and spatially with behavioral signs of tactile allodynia (Malan et al., 2000). Thus, the development of segmental pain commonly associated with the neuropathic condition may be due to the resulting extrasegmental increases in spinal dynorphin content. The up-regulation of dynorphin observed in laboratory animals resembles similar effects in humans: the concentration of dynorphin in the cerebrospinal fluid of patients with fibromyalgia, a condition involving widespread mechanical

ABBREVIATIONS: CFA, complete Freund's adjuvant; NMDA, *N*-methyl-D-aspartate; nNOS, neuronal nitric-oxide synthase; iNOS, inducible NOS; IL, interleukin.

pain, is increased compared with that of controls (Vaeroy et al., 1991).

The augmented spinal cord expression of dynorphin in peripheral inflammation must be accompanied by increased spinal release to initiate its excitatory effects. In support of this hypothesis, increased spinal cord levels of dynorphin peptide and mRNA induced by peripheral inflammation (Dubner and Ruda, 1992) are accompanied by increased dynorphin release, detected by dorsal horn microprobes, from spinal cord cells (Riley et al., 1996). Although noxious compression of the hind paw of normal rats does not evoke spinal release of dynorphin, noxious compression of ankles inflamed by complete Freund's adjuvant (CFA) does increase dynorphin release in spinal cord laminae 2 to 5, where it is normally distributed, and evokes an additional novel release in laminae 6 and 7 (Riley et al., 1996). This result suggests that the hyperalgesia associated from CFA-induced inflammation may be the result of enhanced release of dynorphin in the spinal cord.

Correlational studies showing elevated expression and release of dynorphin in nociceptive models do not exclusively prove dynorphin's contribution to nociception. In support of these studies, many groups have demonstrated that manipulation of spinal dynorphin, either addition or removal, alters nociception. Intrathecal administration of dynorphin in rat has been demonstrated to alter C-fiber reflexes (Caudle and Isaac, 1988) and enlarge receptive field size of dorsal horn neurons to hindpaw mechanical stimulation (Hylden et al., 1991), suggesting that dynorphin modulates pain transmission by an action at the spinal cord level. Furthermore, a single intrathecal injection of dynorphin A (1-17) (3 nmol) elicits mechanical allodynia in mice lasting for more than 100 days (Fig. 1) (Laughlin et al., 1997). A single injection (15 nmol) of dynorphin A (1-17), dynorphin A (2-17), or dynorphin (2-13) similarly elicits allodynia in rats lasting more than 60 days (Vanderah et al., 1996). Conversely, removing the endogenous dynorphin from the spinal cord can decrease nociception. For example, the attenuated potency of morphine in various neuropathic pain models is restored by the intrathecal administration of dynorphin antiserum to rats

with spinal nerve ligation, suggesting that the enhanced expression of dynorphin following injury contributes to central sensitization in the spinal cord (Nichols et al., 1997). Together these results argue that dynorphin may contribute to ongoing spontaneous activity as well as the pathology underlying neuropathic pain.

Involvement of NMDA Receptors in Dynorphin Nociception

Although dynorphin is an endogenous opioid peptide, the nociceptive actions of dynorphin may require activation of the *N*-methyl-D-aspartate (NMDA) receptor complex (Shukla and Lemaire, 1994). Released during peripheral inflammation, dynorphin induces its own synthesis, an apparently regenerative, feed-forward process via a pathway involving NMDA receptor activity. In support of this, pre-emptive treatment with MK-801 (10 μ g/ml/h), a noncompetitive antagonist of NMDA receptor channels, almost completely blocks increased expression of dynorphin following CFA-induced inflammatory hyperalgesia in rats (Zhang et al., 1998). Up-regulation of dynorphin via an NMDA receptor-mediated pathway appears to be an ongoing process underlying hyperalgesia that accompanies inflammation. This process is evidenced by the observation that pre-emptive administration of MK-801 also attenuates the development of CFA-induced hyperalgesia under these conditions, in part, perhaps, through suppression of dynorphin up-regulation (Zhang et al., 1998). This regenerative cycle of dynorphin production may underlie the persistence of some dynorphin effects. Up-regulation of its own synthesis may also explain the ability of dynorphin to outlast the nociceptive effects of NMDA receptor agonists.

Allodynia is a painful sensation elicited by a stimulus that does not normally provoke pain. When induced by exogenous dynorphin, allodynia, like hyperalgesia, is inhibited in a dose-related fashion by intrathecal pretreatment with NMDA receptor antagonists (MK-801 and LY235959, a competitive NMDA receptor antagonist) but is unaffected by the opioid receptor antagonist naloxone (Vanderah et al., 1996; Laughlin et al., 1997). Importantly, allodynia is similarly induced by intrathecal administration of dynorphin A (2-17) (Vanderah et al., 1996; Laughlin et al., 1997), the N-terminally truncated fragment of dynorphin that has no activity at opioid receptors (Walker et al., 1982). Together these data support the conclusion that exogenous dynorphin produces chronic allodynia by activation of NMDA, rather than opioid receptors.

NMDA receptors are also instrumental in the neurotoxic effects, such as paralysis, observed at higher doses of dynorphin. Dynorphin-induced neurotoxicity is blocked by NMDA receptor antagonists, whereas opioid receptor antagonists are without effect, reinforcing the view that effects induced by higher doses of dynorphin are brought about by mechanisms that are independent of opioid receptor activity (Caudle and Isaac, 1988; Shukla et al., 1997).

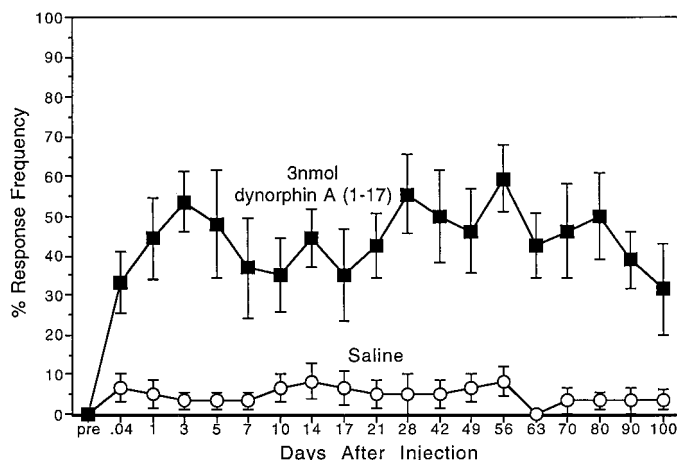


Fig. 1. Time course of mechanical sensitivity after i.t. treatment with 3 nmol of dynorphin A (1-17) (filled squares) compared with saline (open circles). Mechanical allodynia measured with the 0.4 mN von Frey filament lasted for at least 100 days after the dynorphin injection. The mean \pm S.E.M. represents the percentage of response frequency of foot withdrawals to six stimulations. $n = 9$ mice per group.

Binding Studies

The importance of NMDA receptor activity in many of the actions of dynorphin focused extensive research into the possibility that dynorphin interacts directly with one of the

many binding sites on the NMDA receptor complex. The NMDA receptor is composed of a glutamate agonist (ligand) binding site as well as several auxiliary sites providing modulatory input from glycine, protons, zinc, redox states, and polyamines. In addition, [³H]MK-801, a noncompetitive antagonist at the NMDA receptor complex, binds to a site inside the open channel of the NMDA receptor complex. Because of the critical location of [³H]MK-801 binding, glutamate, by virtue of its effect on the ligand binding site, and glycine, by virtue of its positive modulation of the NMDA receptor current, both increase [³H]MK-801 binding by promoting the open channel state. [³H]MK-801 is, therefore, a useful marker, predictive of NMDA receptor channel activity. These interactions suggest that dynorphin interacts with multiple sites on the receptor complex.

Inhibition of Glutamate Binding. Dynorphin was first shown to interact directly with the NMDA receptor complex at a naloxone-insensitive site (Massardier and Hunt, 1989). Here dynorphin has two opposing actions on NMDA receptor ligands: potentiating binding of competitive antagonists at the glutamate recognition site but inhibiting binding of non-competitive antagonists, such as MK-801, within the ion channel (Shukla et al., 1997). Dynorphin accomplishes the latter by binding preferentially to the NMDA receptor in its closed or desensitized states, thereby promoting the closed state. Consistent with this model, binding of radiolabeled dynorphin is enhanced by competitive NMDA receptor antagonists, strychnine-insensitive glycine-site antagonists, and a polyamine-site antagonist (Tang et al., 1999), all of which enhance the population of receptors easily accessible in the closed state. In contrast, noncompetitive antagonists, like MK-801, that interact only when the channel is open, promote an open but inhibited state, attenuating [³H]dynorphin binding (Tang et al., 1999). Once bound, dynorphin apparently produces its effects by negatively influencing the NMDA ligand-binding site on the receptor complex. Although the binding sites of excitatory amino acid receptors are likely to be very similar, by this indirect action on NMDA receptors, dynorphin exhibits a high degree of selectivity among these sites, potentially displacing binding of [³H]glutamate on the NMDA receptor complex, while sparing [³H]kainate or [³H] α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid sites. Consistent with a nonopioid inhibitory action at low concentrations, dynorphin prevents the potentiating effects of glutamate and glycine on [³H]MK-801 binding (Massardier and Hunt, 1989), suggesting that dynorphin interferes with agonist-induced opening of the channel.

Enhancement of Glutamate Binding. Additional binding studies support inhibitory (Massardier and Hunt, 1989; Shukla et al., 1992; Shukla et al., 1997; Tang et al., 1999) as well as excitatory effects (Dumont and Lemaire, 1994) of dynorphin on NMDA receptor activity. Specifically, dynorphin enhances binding of competitive NMDA receptor antagonists to the glutamate ligand binding site on rat brain membranes. This enhancement is not mediated by an opioid receptor because it is insensitive to a κ -opioid receptor antagonist (norbinaltorphimine), is replicated with the nonopioid peptide dynorphin (2–13), and is unaltered by opioid receptor agonists (U50488H and enkephalin). Glycine and the glycine site antagonist HA-966 block the augmentation of NMDA receptor antagonist binding by dynorphin in rat brain membranes (Dumont and Lemaire, 1994). These *in vitro*

studies predict enhancement of NMDA receptor activity by dynorphin *in vivo*. Consistent with this prediction, 200 pmol of dynorphin coadministered intrathecally with NMDA gradually potentiates mouse nociceptive behavioral responses induced by repeated intrathecal injections of NMDA, an injection schedule that normally results in a gradual desensitization to responses produced by NMDA alone (Skilling et al., 1992). These data suggest that the potentiative effect of dynorphin *in vivo* is most important during the maintenance of sustained NMDA receptor activity.

Glycine Sites. Several groups postulate that augmentation of the NMDA receptor by dynorphin occurs by an interaction at the glycine site (Jarvis et al., 1997; Zhang et al., 1997), perhaps via the glycine amino acid residues in the dynorphin peptide (Zhang et al., 1997). Glycine and glycine site antagonists (HA-966, kynurenic acid) have been reported to alter dynorphin's enhancement of NMDA binding to rat brain membranes (Dumont and Lemaire, 1994) and dynorphin's amplification of NMDA-receptor activation (Zhang et al., 1997), while other studies suggest that glycine site ligands have no influence on the effect of dynorphin on NMDA receptor activity (Chen et al., 1995; Lai et al., 1998). Additional support in favor of this possibility is that a 15-min intrathecal pretreatment with NMDA receptor glycine site antagonists, 5-fluoroindole-2-carboxylic acid or kynurenic acid, attenuates dynorphin-induced (20 nmol, *i.t.*) paralysis and mortality (Bakshi and Faden, 1990). Thus, an interaction of dynorphin with glycine binding sites on the NMDA receptor complex may account for the excitotoxic effect of dynorphin.

Oxidation-Reduction Sites. A 30-min intrathecal pretreatment with a *reducing* agent, dithiothreitol, enhances mechanical allodynia induced by dynorphin (3 nmol, *i.t.*) in mice, whereas a 48-h post-treatment with an *oxidizing* agent, 5,5'-dithio-bis-(2-nitrobenzoic acid), attenuates the dynorphin-induced mechanical allodynia for 5 days (Laughlin et al., 1998a). These results may be interpreted to mean that a reduced NMDA receptor contributes to the development and maintenance of the chronic nociceptive effect of dynorphin. Thus, dynorphin may inhibit NMDA receptor function by an interaction with the redox-modulatory site located on that complex (Chen et al., 1995), which influences the intensity of dynorphin-induced allodynia (Laughlin et al., 1998a).

Polyamine Sites. The positively charged basic amino acids on dynorphin may allow it to bind to the polyamine site of the NMDA receptor complex, thereby enhancing the NMDA receptor current. Dynorphin A (1–17) (100 nM) enhances synaptically evoked NMDA receptor-mediated currents in guinea pig hippocampal slices. This effect was blocked by the NMDA receptor polyamine site antagonist ifenprodil (Caudle and Dubner, 1998).

It has been well established that dynorphin interacts with the opioid receptor, but now multiple lines of evidence from binding studies indicate that dynorphin interacts with multiple sites on the NMDA receptor complex. Many studies have demonstrated that the excitatory amino acids and the NMDA receptor are key components involved in the processing of nociception (for review, see Dubner and Ruda, 1992). It is believed that dynorphin's interactions with NMDA receptors produce the excitatory and thus toxic/nociceptive effects observed in many animal models of pain, and possibly the enhanced release of dynorphin leads to its binding to NMDA

receptors, thus resulting in a the development and maintenance of a chronic nociceptive state. Many of the details of this process remain to be worked out; as described above, dynorphin has been shown to interact both negatively and positively with multiple sites on the NMDA receptor. However, the multiple splice variants and subunits of the NMDA receptor may provide multiple regulatory sites with which dynorphin can interact. Diverse effects of dynorphin on NMDA receptor activity may be elicited based on the variability of subunit composition in tissues.

Electrophysiological Studies

Traditionally, the endogenous opioid peptides have been shown to inhibit neuronal transmission (for review, see Simmons and Chavkin, 1996); however, recently dynorphin has been demonstrated to have multiple effects on neuronal transmission. Consistent with binding studies described above, dynorphin is able to enhance (Jarvis et al., 1997; Zhang et al., 1997; Lai et al., 1998), inhibit (Chen et al., 1995; Brauneis et al., 1996), or produce mixed (Caudle et al., 1994; Caudle and Dubner, 1998) effects on NMDA receptor-induced depolarization. Based on these *in vitro* studies, it would be predicted that responses to NMDA *in vivo* might be influenced by dynorphin in a fashion that reflects the concentration of dynorphin available to the receptor population, the subunit composition of NMDA receptors in the area, the presence of other ligands at the NMDA receptor complex, and the open/closed state of the receptors. These variables provide room for an array of responses which, as indicated below, have been documented in a variety of systems.

Inhibition. Dynorphin inhibits all NMDA-activated currents in *Xenopus* oocytes expressing NMDA receptors (NR1 and NR2A, -2B, -2C, or -2D) in a nonopioid receptor-mediated fashion because inhibition is neither blocked by opioid receptor antagonists nor mimicked by other opioid receptor agonists (Brauneis et al., 1996). Dynorphin-induced inhibition of NMDA receptor function does not alter the EC_{50} value of NMDA, indicating that dynorphin is neither competing with the NMDA receptor agonist at the binding site nor decreasing the affinity of agonist binding by an allosteric interaction (Chen et al., 1995; Brauneis et al., 1996). Inhibition of NMDA receptor-induced depolarization is voltage-independent in isolated trigeminal neurons (Chen et al., 1995), isolated periaqueductal gray neurons (Lai et al., 1998), and *Xenopus* oocytes (Brauneis et al., 1996), suggesting that dynorphin does not act as an open-channel blocker. The ability of dynorphin to block NMDA-activated currents in isolated rat trigeminal neurons is dependent on chain length with the following potency sequence: dynorphin (1–32) > dynorphin (1–17) > dynorphin (1–13) \gg dynorphin (1–10) = dynorphin (1–8) (which have no effect) (Chen and Huang, 1998). Amidation of the dynorphin peptide reduces (~ 25 fold) the IC_{50} value for dynorphin blockade of NMDA receptor-mediated currents in isolated rat trigeminal neurons, suggesting that the negative charge at the C terminus of dynorphin profoundly influences the affinity of dynorphin on the NMDA receptor complex (Chen and Huang, 1998).

Enhancement. Dynorphin rapidly and reversibly augments NMDA-activated currents in a subpopulation of isolated rat periaqueductal gray neurons (Lai et al., 1998). This enhancement is mimicked by the nonopioid peptide dynor-

phin (2–17) but not by the κ -opioid receptor agonist U50,488. Furthermore, the opioid receptor antagonist naloxone and κ -opioid-selective antagonist norbinaltorphimine do not compromise this potentiation (Lai et al., 1998). In one study, dynorphin both inhibited (>300 nM) and enhanced (<300 nM) NMDA receptor-induced currents in the CA3 region of the guinea pig hippocampus. The inhibitory effects were attributed to κ -opioid receptors (because the κ -opioid agonist bremazocine mimicked the inhibitory effects) (Caudle et al., 1994), and the excitatory effects were attributed to an interaction at the polyamine site of the NMDA receptor (Caudle and Dubner, 1998). Taken together, these electrophysiological studies are in agreement with the binding studies described above. It is believed that dynorphin's diverse effects on the NMDA receptor activity may be the result of the multiple splice variants and subunits of the NMDA receptor producing assorted responses.

Other Nociceptive Mechanisms of Dynorphin

Dynorphin at submicromolar concentrations reduces calcium influx via κ -opioid receptor activity, but higher concentrations (10–100 μ M) enhance depolarization-enhanced NMDA receptor-mediated current in mouse spinal cord cultures (Hauser et al., 1999). Although activity involving spinal NMDA receptors is *necessary* for dynorphin-induced allodynia, such action is not *sufficient* for its induction. For example, when injected intrathecally to mice, relatively high doses (1 and 3 nmol) of NMDA induce allodynia that lasts for only 3 days compared with over 100 days of allodynia (see Fig. 1) induced by dynorphin (3 nmol) administered intrathecally (Laughlin et al., 1997). Thus, other mechanisms must contribute to dynorphin-induced nociception. Not only does spinal application of dynorphin increase the concentration of glutamate and aspartate in the extracellular fluid of the rat dorsal spinal cord (Skilling et al., 1992), but neuronal activation appears to release dynorphin, along with excitatory amino acids from primary afferent sensory fibers in mouse spinal cord (Arts et al., 1992) and mossy fibers in rat hippocampus (Conner-Kerr et al., 1993). Besides glutamate, dynorphin also increases the release of substance P from primary afferent C-nociceptors in an NMDA receptor-dependent and opioid receptor-independent manner (Arcaya et al., 1999). Very recent work suggests that dynorphin may utilize a nonopioid and non-NMDA mechanism to enhance intracellular calcium in cultured rat cortex neurons (Tang et al., 2000). This group speculates that such an action could enhance excitatory transmission indirectly.

In addition to the direct depolarizing effect of calcium influx, the increase in intracellular calcium can activate nitric-oxide synthase (NOS) activity. Dynorphin-induced mechanical allodynia and spinal cord injury both involve activation of NOS. In a rat model of spinal cord injury induced by dynorphin A 1–17 (20 nmol, *i.t.*), the expression of neuronal NOS (nNOS) protein and mRNA as well as enzymatic activity is increased as early as 30 min, persisting for 1 to 4 h after induction of paralysis. On the other hand, inducible NOS (iNOS) mRNA expression is increased at 2 h, and enzymatic activity is increased at 4 h, persisting for 24 to 48 h after induction of paralysis (Hu et al., 2000). These results suggest that nNOS is predominantly involved in the early stages of toxicity, whereas iNOS perpetuates the later stages of dynor-

phin-induced rat spinal cord injury (Hu et al., 2000). Furthermore, nitric oxide produced by iNOS (as evidenced by aminoguanidine antagonism) is required for the development of dynorphin-induced allodynia in mice, whereas that generated by nNOS (as evidenced by 7-nitro indazole antagonism) maintains the ongoing nociceptive signal (Laughlin et al., 1998b).

Intrathecal administration of high doses (25 and 50 nmol) of dynorphin causes dose-related cell loss in the rat spinal cord gray matter (Long et al., 1988), which corresponds to the onset of paralysis. Dynorphin inhibits presynaptic inhibitory mechanisms, resulting in disinhibition and, thus, excitation (Stewart and Isaac, 1991; Randic et al., 1995). Dynorphin, applied topically to the exposed spinal cord, increases the excitability of rat lumbar spinal cord dorsal horn neurons (Knox and Dickenson, 1987; Hylden et al., 1991), enhancing their utilization of oxygen, yet decreasing spinal blood flow (Long et al., 1987). This action probably contributes to ischemia and related mechanisms of toxicity (Long et al., 1987a).

Finally, dynorphin may modulate nociception and toxicity by an action on immune function, as demonstrated by observation of opioid receptor-independent actions on macrophage/glia cell cultures (Ichinose et al., 1995; Kong et al., 1997). A 30-min intrathecal pretreatment with the anti-inflammatory cytokines IL-10 and IL-1ra impedes the development of dynorphin-induced allodynia in mice (Laughlin et al., 2000). Because inhibition of protein synthesis prevents dynorphin-induced allodynia (Laughlin et al., 2000), we speculate that the pro-inflammatory cytokine cascade (i.e., synthesis of IL- β together with subsequent activation of the nuclear transcription factor NF- κ B), as well as de novo synthesis of dynorphin, may be necessary for these effects.

Presynaptic or Postsynaptic Mechanism of Dynorphin Action

Whether dynorphin interacts presynaptically or postsynaptically relative to primary afferent fibers in spinal cord is unclear. Several studies suggest that dynorphin interacts presynaptically toward the production of a hyperalgesic state. First, exogenously applied dynorphin increases the release of neurotransmitters (glutamate, aspartate, substance P) in the rat spinal cord (Skilling et al., 1992) and trigeminal nucleus slices (Arcaya et al., 1999). Second, dynorphin (6 nmol, i.t.) facilitates, via a nonopioid mechanism, cutaneous C-fiber-evoked responses of rat spinal cord dorsal horn neurons (Knox and Dickenson, 1987). Third, dynorphin (10 nM) increases A δ - and C-fiber-evoked rat spinal cord dorsal horn neuronal excitatory postsynaptic potentials, an action that is associated with no change in resting membrane resistance or input resistance, suggesting a presynaptic site of dynorphin-induced excitation (Randic et al., 1995). However, this dynorphin action is likely postsynaptic relative to the primary afferent because it involves inhibition of GABAergic or glycinergic interneurons, enhancing postsynaptic depolarization of spinal cord neurons (Stewart and Isaac, 1991; Randic et al., 1995). In addition to presynaptic mechanisms, dynorphin may interact postsynaptically to produce nociception. Dynorphin (10 μ M) potentiates α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid- and kainate-induced current responses in cultured rat spinal cord neurons by

modifying postsynaptic membrane resistance as well as activating protein kinase A (Kolaj et al., 1995). Thus, it would appear that dynorphin produces its effects through both pre- and postsynaptic mechanisms.

Dynorphin at NMDA Receptor Sites Is Toxic; Dynorphin at Opioid Receptors Protects

As described above, dynorphin exerts both inhibitory and excitatory actions on neurons. Dynorphin both inhibits and enhances guinea pig hippocampal NMDA receptor-induced currents (Caudle et al., 1994; Caudle and Dubner, 1998) and C-fiber-evoked potentials in rat spinal cord dorsal horn neurons (Randic et al., 1995); the inhibitory effect of dynorphin results from action at κ -opioid receptors (Caudle et al., 1994), and the excitatory effect results from action at NMDA receptors (Vanderah et al., 1996). Furthermore, intracerebroventricular administration of a synthetic dynorphin peptide in mice evokes analgesia through the opioid receptor and motor dysfunction through NMDA receptors (Shukla et al., 1992). The concentration- and calcium-dependent production of cell death in mouse spinal cord neuron cultures is prevented by NMDA receptor antagonists (MK801, AP5, and 7-chlorokynurenic acid) but enhanced by opioid receptor antagonists (naloxone and the κ -selective antagonist norbinaltorphimine) (Hauser et al., 1999). Lastly, application of dynorphin (1 nM–3 μ M) with naloxone is neurotoxic to guinea pig hippocampal neurons, although cells are stable in the presence of dynorphin or naloxone alone. Neurotoxicity is blocked by the addition of the NMDA polyamine site receptor antagonist ifenprodil (Caudle and Dubner, 1998). These results suggest that the opioid site protects cells from the toxic effects of dynorphin. Therefore, it is hypothesized that dynorphin's interaction at opioid receptors produces the inhibitory and thus protective effects, whereas dynorphin's interactions with the NMDA receptors produce the excitatory and thus toxic/nociceptive effects (Fig. 2). Thus, opioid receptors impede the nociceptive signal. Consequently, if dynorphin's action at NMDA receptors outweighs that at opioid receptors,

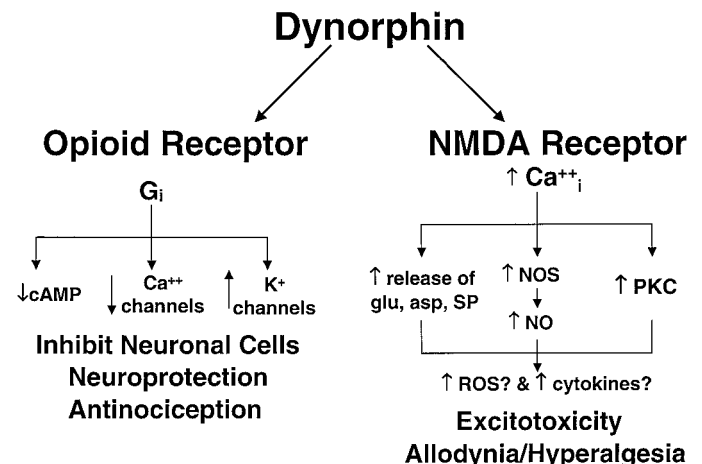


Fig. 2. Dynorphin acting at NMDA receptors elevates intracellular calcium, which elicits neurotoxicity via inter cellular (NOS \rightarrow NO) or intracellular [protein kinase C (PKC)] signaling together with increased transmitter release, whereas its action at opioid receptors would likely be neuroprotective via G $_i$ -coupled signaling. glu, glutamate; asp, aspartate; SP, substance P; ROS, reactive oxygen species.

there will be an excitatory outcome, and vice versa for a stronger action at the opioid receptors.

In conclusion, this review has explored several different potential mechanisms of dynorphin's contribution to the development and maintenance of spinal cord sensitization and nociception. It is believed that dynorphin may have both a physiological (through opioid receptors) and a pathological (through NMDA receptors) role in acute and chronic pain states. The increased spinal cord levels of endogenous dynorphin observed in many nociceptive models may participate in the development and maintenance of prolonged nociception. Inflammation results in a greater than 400% increase in dynorphin peptide expression (Iadarola et al., 1988), levels that may reach the nociceptive and toxic range.

References

- Arceya JL, Cano G, Gomez G, Maixner W, and Suarez-Roca H (1999) Dynorphin A increases substance P release from trigeminal primary afferent C-fibers. *Eur J Pharmacol* **366**:27–34.
- Arts KS, Fujimoto JM, and Tseng LF (1992) Involvement of dynorphin A and not substance P in the spinal antianalgesic action of capsaicin against morphine-induced antinociception in mice. *J Pharmacol Exp Ther* **261**:643–651.
- Bakshi R and Faden AI (1990) Competitive and non-competitive NMDA antagonists limit dynorphin A-induced rat hindlimb paralysis. *Brain Res* **507**:1–5.
- Brauneis U, Oz M, Peoples RW, Weight FF, and Zhang L (1996) Differential sensitivity of recombinant N-methyl-D-aspartate receptor subunits to inhibition by dynorphin. *J Pharmacol Exp Ther* **279**:1063–1068.
- Caulle RM, Chavkin C, and Dubner R (1994) Kappa 2 opioid receptors inhibit NMDA receptor-mediated synaptic currents in guinea pig CA3 pyramidal cells. *J Neurosci* **14**:5580–5589.
- Caulle RM and Dubner R (1998) Ifenprodil blocks the excitatory effects of the opioid peptide dynorphin 1–17 on NMDA receptor-mediated currents in the CA3 region of the guinea pig hippocampus. *Neuropeptides* **32**:87–95.
- Caulle RM and Isaac L (1988) A novel interaction between dynorphin (1–13) and an N-methyl-D-aspartate site. *Brain Res* **443**:329–332.
- Chavkin C, James IF, and Goldstein A (1982) Dynorphin is a specific endogenous ligand of the kappa opioid receptor. *Science (Wash DC)* **215**:413–415.
- Chen L, Gu Y, and Huang LY (1995) The mechanism of action for the block of NMDA receptor channels by the opioid peptide dynorphin. *J Neurosci* **15**:4602–4611.
- Chen L and Huang LY (1998) Dynorphin block of N-methyl-D-aspartate channels increases with the peptide length. *J Pharmacol Exp Ther* **284**:826–831.
- Conner-Kerr TA, Simmons DR, Peterson GM, and Terrian DM (1993) Evidence for the corelease of dynorphin and glutamate from rat hippocampal mossy fiber terminals. *J Neurochem* **61**:627–636.
- Dubner R and Ruda MA (1992) Activity-dependent neuronal plasticity following tissue injury and inflammation. *Trends Neurosci* **15**:96–103.
- Dumont M and Lemaire S (1994) Dynorphin potentiation of [³H]CGP-39653 binding to rat brain membranes. *Eur J Pharmacol* **271**:241–244.
- Hauser KF, Folds JK, and Turbek CS (1999) Dynorphin A (1–13) neurotoxicity in vitro: opioid and non-opioid mechanisms in mouse spinal cord neurons. *Exp Neurol* **160**:361–375.
- Hu WH, Qiang WA, Li F, Liu N, Wang GQ, Wang HY, Wan XS, Liao WH, Liu JS, and Jen MF (2000) Constitutive and inducible nitric oxide synthases after dynorphin-induced spinal cord injury. *J Chem Neuroanat* **17**:183–197.
- Hylden JL, Nahin RL, Traub RJ, and Dubner R (1991) Effects of spinal kappa-opioid receptor agonists on the responsiveness of nociceptive superficial dorsal horn neurons. *Pain* **44**:187–193.
- Iadarola MJ, Brady LS, Draisci G, and Dubner R (1988) Enhancement of dynorphin gene expression in spinal cord following experimental inflammation: stimulus specificity, behavioral parameters and opioid receptor binding. *Pain* **35**:313–326.
- Ichinose M, Asai M, and Sawada M (1995) Enhancement of phagocytosis by dynorphin A in mouse peritoneal macrophages. *J Neuroimmunol* **60**:37–43.
- Jarvis CR, Xiong ZG, Plant JR, Churchill D, Lu WY, MacVicar BA, and MacDonald JF (1997) Neurotrophin modulation of NMDA receptors in cultured murine and isolated rat neurons. *J Neurophysiol* **78**:2363–2371.
- Kajander KC, Sahara Y, Iadarola MJ, and Bennett GJ (1990) Dynorphin increases in the dorsal spinal cord in rats with a painful peripheral neuropathy. *Peptides* **11**:719–728.
- Knox RJ and Dickenson AH (1987) Effects of selective and non-selective kappa-opioid receptor agonists on cutaneous C-fibre-evoked responses of rat dorsal horn neurons. *Brain Res* **415**:21–29.
- Kolaj M, Cerne R, and Randic M (1995) The opioid peptide dynorphin modulates AMPA and kainate responses in acutely isolated neurons from the dorsal horn. *Brain Res* **671**:227–244.
- Kong JQ, Leedham JA, Taylor DA, and Fleming WW (1997) Evidence that tolerance and dependence of guinea pig myenteric neurons to opioids is a function of altered electrogenic sodium-potassium pumping. *J Pharmacol Exp Ther* **280**:593–599.
- Lai SL, Gu Y, and Huang LY (1998) Dynorphin uses a non-opioid mechanism to potentiate N-methyl-D-aspartate currents in single rat periaqueductal gray neurons. *Neurosci Lett* **247**:115–118.
- Laughlin TM, Bethea JR, Yezierski RP, and Wilcox GL (2000) Cytokine involvement in dynorphin-induced allodynia. *Pain* **84**:159–167.
- Laughlin TM, Kitto KF, and Wilcox GL (1998a) Redox manipulation of NMDA receptors in vivo: alteration of acute pain transmission and dynorphin-induced allodynia. *Pain* **80**:37–43.
- Laughlin TM, Stone LS, Schreiber KL, and Wilcox GL (1998b) Role of inducible nitric oxide synthase in dynorphin-induced allodynia: a central model of neuropathic pain. *Soc Neurosci Abstr* **24**:638.
- Laughlin TM, Vanderah T, Lashbrook JM, Nichols ML, Ossipov MH, Porreca F, and Wilcox GL (1997) Spinally administered dynorphin A produces long-lasting allodynia: involvement of NMDA but not opioid receptors. *Pain* **72**:253–260.
- Long JB, Kinney RC, Malcolm DS, Graeber GM, and Holaday JW (1987) Intrathecal dynorphin A_{1–13} and dynorphin A_{2–13} reduce rat spinal cord blood flow by non-opioid mechanisms. *Brain Res* **436**:374–379.
- Long JB, Petras JM, Mobley WC, and Holaday JW (1988) Neurological dysfunction after intrathecal injection of dynorphin A (1–13) in the rat. II. Nonopioid mechanisms mediate loss of motor, sensory and autonomic function. *J Pharmacol Exp Ther* **246**:1167–1174.
- Malan TP, Ossipov MH, Gardell LR, Ibrahim M, Bian D, Lai J, and Porreca F (2000) Extrateritorial neuropathic pain correlates with multisegmental elevation of spinal dynorphin in nerve-injured rats. *Pain* **86**:185–194.
- Massardier D and Hunt PF (1989) A direct non-opiate interaction of dynorphin-(1–13) with the N-methyl-D-aspartate (NMDA) receptor. *Eur J Pharmacol* **170**:125–126.
- Nahin RL, Hylden JL, and Humphrey E (1992) Demonstration of dynorphin A 1–8 immunoreactive axons contacting spinal cord projection neurons in a rat model of peripheral inflammation and hyperalgesia. *Pain* **51**:135–143.
- Nahin RL, Hylden JL, Iadarola MJ, and Dubner R (1989) Peripheral inflammation is associated with increased dynorphin immunoreactivity in both projection and local circuit neurons in the superficial dorsal horn of the rat lumbar spinal cord. *Neurosci Lett* **96**:247–252.
- Nichols ML, Lopez Y, Ossipov MH, Bian D, and Porreca F (1997) Enhancement of the antiallodynic and antinociceptive efficacy of spinal morphine by antisera to dynorphin A (1–13) or MK-801 in a nerve-ligation model of peripheral neuropathy. *Pain* **69**:317–322.
- Randic M, Cheng G, and Kojic L (1995) Kappa-opioid receptor agonists modulate excitatory transmission in substantia gelatinosa neurons of the rat spinal cord. *J Neurosci* **15**:6809–6826.
- Riley RC, Zhao ZQ, and Duggan AW (1996) Spinal release of immunoreactive dynorphin A (1–8) with the development of peripheral inflammation in the rat. *Brain Res* **710**:131–142.
- Schwei MJ, Honore P, Rogers SD, Salak-Johnson JL, Finke MP, Ramnaraine ML, Clohisey DR, and Mantyh PW (1999) Neurochemical and cellular reorganization of the spinal cord in a murine model of bone cancer pain. *J Neurosci* **19**:10886–10897.
- Shukla VK, Bansinath M, Dumont M, and Lemaire S (1992) Selective involvement of kappa opioid and phencyclidine receptors in the analgesic and motor effects of dynorphin-A (1–13)-Tyr-Leu-Phe-Asn-Gly-Pro. *Brain Res* **591**:176–180.
- Shukla VK and Lemaire S (1994) Non-opioid effects of dynorphins: possible role of the NMDA receptor. *Trends Pharmacol Sci* **15**:420–424.
- Shukla VK, Prasad JA, and Lemaire S (1997) Nonopioid motor effects of dynorphin A and related peptides: structure dependence and role of the N-methyl-D-aspartate receptor. *J Pharmacol Exp Ther* **283**:604–610.
- Simmons ML and Chavkin C (1996) Endogenous opioid regulation of hippocampal function. *Int Rev Neurobiol* **39**:145–196.
- Skilling SR, Sun X, Kurtz HJ, and Larson AA (1992) Selective potentiation of NMDA-induced activity and release of excitatory amino acids by dynorphin: possible roles in paralysis and neurotoxicity. *Brain Res* **575**:272–278.
- Stevens CW and Yaksh TL (1986) Dynorphin A and related peptides administered intrathecally in the rat: a search for putative kappa opiate receptor activity. *J Pharmacol Exp Ther* **238**:833–838.
- Stewart P and Isaac L (1991) Dynorphin-induced depression of the dorsal root potential in rat spinal cord: a possible mechanism for potentiation of the C-fiber reflex. *J Pharmacol Exp Ther* **259**:608–613.
- Tang Q, Gandhoke R, Burritt A, Hrubby VJ, Porreca F, and Lai J (1999) High-affinity interaction of (des-Tyrosyl)dynorphin A(2–17) with NMDA receptors. *J Pharmacol Exp Ther* **291**:760–765.
- Tang Q, Lynch RM, Porreca F, and Lai J (2000) Dynorphin A elicits an increase in intracellular calcium in cultured neurons via a non-opioid, non-NMDA mechanism. *J Neurophysiol* **83**:2610–2615.
- Vaeroy H, Nyberg F, and Terenius L (1991) No evidence for endorphin deficiency in fibromyalgia following investigation of cerebrospinal fluid (CSF) dynorphin A and Met-enkephalin-Arg6-Phe7. *Pain* **46**:139–143.
- Vanderah T, Laughlin T, Lashbrook J, Nichols M, Wilcox G, Ossipov M, Malan T, and Porreca F (1996) Single intrathecal injections of dynorphin A or des-Tyr-dynorphins produce long-lasting allodynia in rats: blockade by MK-801 but not naloxone. *Pain* **68**:275–281.
- Walker JM, Moises HC, Coy DH, Baldrighi G, and Akil H (1982) Nonopioid effects of dynorphin and des-Tyr-dynorphin. *Science (Wash DC)* **218**:1136–1138.
- Zhang L, Peoples RW, Oz M, Harvey-White J, Weight FF, and Brauneis U (1997) Potentiation of NMDA receptor-mediated responses by dynorphin at low extracellular glycine concentrations. *J Neurophysiol* **78**:582–590.
- Zhang RX, Ruda MA, and Qiao JT (1998) Pre-emptive intrathecal MK-801, a non-competitive N-methyl-D-aspartate receptor antagonist, inhibits the up-regulation of spinal dynorphin mRNA and hyperalgesia in a rat model of chronic inflammation. *Neurosci Lett* **241**:57–60.

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