Compelling evidence has accumulated over the last several years from our laboratory, as well as others, indicating that central hyperactive states resulting from neuronal plastic changes within the spinal cord play a critical role in hyperalgesia associated with nerve injury and inflammation. In our laboratory, chronic constrictive injury of the common sciatic nerve, a rat model of neuropathic pain, has been shown to result in activation of central nervous system excitatory amino acid receptors and subsequent intracellular cascades including protein kinase C translocation and activation, nitric oxide production, and nitric oxide-activated poly(ADP ribose) synthetase activation. Similar cellular mechanisms also have been implicated in the development of tolerance to the analgesic effects of morphine. A recently observed phenomenon, the development of “dark neurons,” is associated with both chronic constriction injury and morphine tolerance. A site of action involved in both hyperalgesia and morphine tolerance is in the superficial laminae of the spinal cord dorsal horn. These observations suggest that hyperalgesia and morphine tolerance may be interrelated at the level of the superficial laminae of the dorsal horn by common neural substrates that interact at the level of excitatory amino acid receptor activation and subsequent intracellular events. The demonstration of interrelationships between neural mechanisms underlying hyperalgesia and morphine tolerance may lead to a better understanding of the neurobiology of these two phenomena in particular and pain in general. This knowledge may also provide a scientific basis for improved pain management with opiate analgesics.

A number of studies, both from our laboratory as well as others, indicate that central hyperactive states resulting from neuronal plastic changes within the spinal cord play a critical role in hyperalgesia associated with nerve injury and inflammation. We have recently shown in a rat model of neuropathic pain that chronic constrictive injury (CCI) of the common sciatic nerve can result in activation of central nervous system excitatory amino acid receptors and subsequent intracellular cascades including protein kinase C translocation and activation, nitric oxide (NO) production, and NO-activated poly(ADP ribose) synthetase (PARS) activation. Similar cellular mechanisms also have been implicated in the development of tolerance to the analgesic effects of morphine. Of particular interest is that morphological changes in the spinal cord dorsal horn, the development of so called “dark neurons,” are associated with both CCI and morphine tolerance. A site of action involved in both hyperalgesia and morphine tolerance has also been shown to be in the superficial laminae of the spinal cord dorsal horn. We will first summarize recent evidence indicating central mechanisms of hyperalgesia and morphine tolerance. The main focus of this article will be on the recent development in our understanding of the involvement of PARS activation in central mechanisms of morphine tolerance. A working hypothesis with regard to interactions between hyperalgesia and morphine tolerance within the spinal cord dorsal horn will be discussed. Finally, clinical implications of such interactions will be addressed.

Central Mechanisms Subserving Hyperalgesia. Recent insights into neural mechanisms of hyperalgesia are based on knowledge of the involvement of the N-methyl-D-aspartate (NMDA) receptor and associated intracellular cascades. These insights result from studies of central nervous system (CNS) neuronal plasticity in general and from studies of spinal cord mechanisms of neurogenic and inflammatory hyperalgesia in particular (1–8). Hyperalgesia after tissue injury and inflammation may reflect central sensitization resulting from prolonged and excessive activation of spinal cord excitatory amino acid receptors and subsequent intracellular cascades. Tonic activation of NMDA receptors activates second-messenger systems, an ultimate result of which is phosphorylation and hence sensitization of ion channel complexes, including that of the NMDA receptor (9). These central changes are initiated by abnormal and often tonic input to the spinal cord. This tonic input, in turn, may result from peripheral nerve injury or tissue inflammation. Potential peripheral generators of this input include nerve injury-induced impulse discharges (3), generation of ectopic nerve action potentials (10), aberrant sympathetic influences (11), and/or sensitization of peripheral nociceptors (2, 11). Of the several central changes initiated by tonic nociceptive afferent input, protein kinase C (PKC) translocation/activation and/or NO production are pivotal intracellular events within spinal cord neurons. Translocation/activation of PKC enhances postsynaptic neuronal excitability by means of increasing the efficacy of receptor–ion channel complexes (12–15). Similar changes may also occur at presynaptic sites via activation of presynaptic NMDA receptors localized on primary afferent fibers (16) and/or via the effects of extracellular NO (17). Central hyperactive states reflect the combined effects of these pre- and postsynaptic mechanisms. Direct support of the involvement of PKC in these mechanisms is provided by an experiment in which a phorbol ester (a PKC activator) increased spontaneous and stimulus-evoked activity of dorsal horn spinothalamic tract neurons (18) and by an experiment in which a PKC activator produced a long-lasting increase in the amplitude and duration of excitatory postsynaptic potentials evoked in dorsal horn neurons by orthodromic dorsal root stimulation (19). Both experiments show that activation of PKC can indeed modulate activity of spinal cord neurons.
nociceptive neurons. These experiments are complemented by studies that demonstrate elevated dorsal horn levels of translocated PKC or increased biosynthesis of PKC in neuropathic rats (20, 21).

**Contributions of Central Excitement to Hyperalgesia.** It has been proposed that central sensitization may be manifested as increases in spontaneous and stimulus-evoked neuronal activity within the spinal cord, which, in turn, contribute to the development and maintenance of neurogenic and inflammatory pain syndromes. There may be an increase in spontaneous neural activity of pain transmission pathways and hence spontaneous pain. This increased spontaneous action potential activity includes that of spinal cord dorsal horn neurons (22, 23), and that of pain-related thalamic neurons (24) of CCI rats with neuropathic hyperalgesia (25). These extensive elevations in neural activity, which have also been mapped by using the 2-deoxyglucose metabolic technique (26, 27), occur within the spinal cord and a variety of pain-related brain regions even in the absence of overt somatic stimulation. Furthermore, responses of central pain-related neurons of CCI rats show exaggerated responses to innocuous and noxious peripheral stimulation. This is indicated by increased responses of spinothalamic neurons to mechanical or thermal stimulation (23). An exaggerated response to innocuous stimulation may contribute to allodynia, whereas the enhanced response to noxious stimulation is likely related to hyperalgesia. Peripheral receptive fields also expand after tissue inflammation (28). Given the role of receptive field size in neuronal recruitment, expanded receptive fields would mean that a nociceptive stimulus would activate more central neurons than would normally occur, leading to exaggerated pain and exaggerated spatial radiation of the painful sensation. Taken together, elevated spontaneous discharges, increased stimulus-evoked impulse frequencies, and expanded receptive fields are likely to operate in concert to cause persistent hyperalgesia, spontaneous pain, allodynia, and radiation of pain.

**Contributions of Central Disinhibition to Hyperalgesia.** Excitatory amino acid-induced PKC translocation/activation and NO production may also result in disinhibitory processes associated with excitotoxic consequences including neuronal death within the CNS. The involvement of such excitotoxic processes in mechanisms of injury-induced pain syndromes has been suggested by several experimental observations. There is histological evidence showing that peripheral nerve injury induces excitotoxic transsynaptic morphological changes of superficial dorsal horn (laminae I–II) neurons (dark neurons), which have been proposed to be inhibitory interneurons (29). Importantly, PARS activation may include alterations in cell morphology, whereas dark neurons do not exhibit this characteristic. Neurons that exhibited all three of the above-mentioned characteristics were counted as dark neurons, whereas other cells were excluded.

The spinal cord sections were divided into three zones for examination by microscope. They were divided, by using Rexed’s laminar system, into laminae I–II, III–IV, and V–VI. Sections were examined with the experimenter blind to the treatment regimen. Dark neurons were counted in each zone mentioned above under medium-power magnification. If there was a dark neuron that was in question, it was then viewed under high-power magnification to discern if it was indeed a dark neuron. At least three 1-μm spinal cord sections were viewed for each rat. This yielded an average number of dark neurons for each subdivision. The tail-flick data were analyzed by using two-way ANOVA to discern differences resulting treatment groups. When main effects were seen, a Waller–Duncan D ratio t test was performed to determine the source of variations between the groups. Dark neurons were counted for the left and right sides of the dorsal horn in laminae I–VI. The numbers were then averaged and analyzed by using a two-way ANOVA. The total number of dark neurons from a sampled region was analyzed to determine (i) differences in the number of dark neurons between the left and right side of the dorsal horn; (ii) differences in numbers of dark neurons among sampled dorsal horn regions (i.e., laminae I–II);
and (iii) differences in the number of dark neurons among treatment groups.

An initial experiment was performed to determine the effect of the chronic administration of morphine on the development of dark neurons. Three groups of rats (n = 5–9 per group) were used: (i) rats treated with saline i.t. once a day; (ii) rats treated with 10 \( \mu \)g of morphine i.t. once daily; and (iii) rats treated with 20 \( \mu \)g of morphine i.t. once daily. The doses of morphine given have previously been shown to induce the development of tolerance to the antinociceptive effects of morphine (34). All of the agents were given once daily for 8 days.

On day 8, those rats receiving morphine and saline showed the development of tolerance to the analgesic effects of morphine. Rats that were made tolerant to morphine exhibited a reliable increase in the number of dark neurons in the dorsal horn of the lumbar spinal cord (\( P < 0.01 \)). Several features characterized this increase in dark neurons. Dark neurons were primarily located in laminae I–II and to a much lesser degree to laminae III–IV. Also, there was no statistical difference in the number of dark neurons observed on the left and right sides of the spinal cord (\( P > 0.05 \)). Because chronic administration of morphine induced tolerance and the development of dark neurons, we examined the effect of the selective PARS inhibitor benzamide, which has been shown to prevent the development of hyperalgesia in the CCI model (30), on the development of morphine tolerance and dark neurons resulting from chronic morphine administration. For this experiment, seven groups of rats were used. They included rats receiving 100, 200, or 400 nmol benzamide and 20 \( \mu \)g of morphine on days 1–8, rats receiving 400 nmol benzamide and saline on days 1–8, rats receiving 20 \( \mu \)g morphine and saline on days 1–8, rats receiving only saline on days 1–8, and rats receiving saline on days 1–7 and 20 \( \mu \)g of morphine on day 8.

As shown in Fig. 1, coadministration of 20 \( \mu \)g of morphine with 200 or 400 nmol (not 100 nmol) benzamide for 7 days reliably attenuated the development of tolerance (\( P < 0.01 \)). Neither baseline tail-flick latency nor the response to a single injection of 20 \( \mu \)g of morphine changed after repeated saline treatment for 7 days. Co-administration of 20 \( \mu \)g of morphine with benzamide (100–400 nmol) for 7 days also reliably prevented the increase in dark neurons (\( P < 0.01 \); Fig. 2). Neither repeated benzamide (400 nmol) treatment alone nor a single injection of 20 \( \mu \)g of morphine on day 8 (the 20*/0 group) affected the occurrence of dark neurons as compared with the saline group.

The data from the previous experiment showed that benzamide was effective in inhibiting the development of morphine tolerance and dark neurons. The specificity of this effect to PARS inhibition was examined by utilizing other PARS inhibitors. For this experiment, four groups of rats were used; they included rats receiving 400 nmol benzamide and 20 \( \mu \)g of morphine on days 1–8, rats receiving 200 nmol 3-aminobenzamide and 20 \( \mu \)g of morphine on days 1–8, rats receiving 1 \( \mu \)mol niacinamide (nicotinamide) and 20 \( \mu \)g of morphine on days 1–8, and rats receiving 20 \( \mu \)g of morphine and saline on days 1–8. Co-administration of 20 \( \mu \)g of morphine with either 200 nmol 3-aminobenzamide or 1 \( \mu \)mol niacinamide (nicotinamide) for 7 days reliably (\( P < 0.01 \)) attenuated the development of tolerance as compared with that of day 1 in the same group (Fig. 3). As shown in Fig. 4, coadministration of 20 \( \mu \)g of morphine with either 200 nmol 3-aminobenzamide or 1 \( \mu \)mol niacinamide for 7 days also reliably (\( P < 0.05 \) and \( P < 0.01 \) for the drugs respectively) prevented the increase in dark neurons as compared with the morphine + saline group.

To confirm that the development of tolerance and dark neurons in morphine-treated rats is associated with the activation of opioid receptors, we examined the effect of the opioid receptor antagonist, naltrexone, on the ability of morphine to produce tolerance and dark neurons. For this experiment, two groups of rats were used; they included rats receiving 10 mg/kg naltrexone intraperitoneally 5 min before 20 \( \mu \)g of morphine i.t. on days 1–8 and rats receiving 20 \( \mu \)g of morphine and saline on days 1–8. Co-administration of 20 \( \mu \)g of morphine with 10 mg/kg naltrexone for 7 days reliably prevented both the development of the antinociceptive tolerance (\( P < 0.01 \)) and the increase in dark neurons (\( P < 0.01 \)) as compared with the morphine + saline group.

The major findings of this series of studies are (i) the incidence of dark neurons increased significantly within the spinal cord dorsal horn, particularly the superficial laminae I-II, of rats injected daily for 8 days with i.t. morphine; (ii) benzamide and other PARS inhibitors reduced or prevented the development of
of this is that the excitotoxicity from neuropathic pain may, under some circumstances, reduce the response to opioids.

Several perplexing observations about neuropathic pain, opioid tolerance, and the interactions between them led us to propose an early model of the events involved (34) and now lead us to propose a revised version of this model here (Fig. 5). These observations include the following. (i) Neuropathic pain syndromes often present with symptoms indicative of both hyperexcitability (e.g., hyperalgesia) and disinhibition (e.g., spontaneous pain, allodynia). Some of these symptoms, such as hyperalgesia, can be at least partially reversed in animal models by NMDA receptor antagonists (35), whereas others, such as allodynia, cannot (36). (ii) NMDA receptor antagonists prevent, but do not acutely reverse, tolerance to opioids (37). (iii) Our model assumes, for numerous reasons (34), that postsynaptic opioid and NMDA receptors are on the same neurons, at least in the spinal cord. In fact, we have reported direct immunohistochemical evidence of this (38). Opioids are known to produce hyperpolarization via an inwardly rectifying K⁺ channel. Despite the hyperpolarized state in these cells, voltage/ligand-gated NMDA receptors must be activated during the process of tolerance development, because NMDA receptor antagonists block the development of tolerance. (iv) At the spinal cord level, it is likely, except in the presence of nociceptive input, that presynaptic elements release only small amounts of glutamate onto postsynaptic elements in the superficial laminae of the dorsal horn that contain NMDA receptors related to opioid tolerance, yet tolerance to opioids occurs in the absence of nociceptive input. (v) PKC translocation to the cell membrane is greatly increased by chronic as compared with acute morphine treatment (34). (vi) Chronic opioid administration produces hyperalgesia, which can be reversed by NMDA receptor antagonists (27). (vii) CCI which produces hyperalgesia causes a rightward shift in the dose–response curve to morphine (39). (viii) NO is involved in opioid tolerance (40). (ix) CCI-induced hyperalgesia (30) and opioid tolerance (39) are associated with the development of dark neurons (30). As reviewed in this article, the development of hyperalgesia, morphine tolerance, and dark neurons can be prevented by inhibiting the nuclear repair enzyme PARS.

A model of our current working hypothesis concerning the development of neuropathic pain, opioid tolerance, and their interactions that is consistent with these observations is presented in Fig. 5. In this model, excessive release of glutamate resulting from peripheral events, such as those occurring in the CCI model, initiates a series of intracellular events, which, via different messenger systems, leads to (i) at least partially NMDA antagonist reversible hyperexcitability that results from a PKC-mediated alteration of NMDA receptors (pathway 1, Fig. 5); (ii) events such as allodynia, which are not reversible with NMDA antagonists (36) but are prevented by inhibition of the NO/ARS pathway (30) and thus may be mediated by cellular dysfunction resulting from depletion of cellular energy stores (pathway 2, Fig. 5). This cellular dysfunction may be morphologically manifested by the appearance of dark neurons, which can also be prevented by inhibition of the NO/ARS pathway (30); and (iii) the rightward shift of the morphine dose–response curve resulting from CCI (39), which has been hypothesized to result from relatively long duration, PKC-mediated alterations in gene expression (pathway 3, Fig. 5), which may result in PKC-mediated opioid receptor/K⁺ channel uncoupling (41). With regard to opioid tolerance, in this model, activation of the μ-opioid receptor may initiate PKC translocation to the membrane (42). This PKC translocation allows the NMDA receptor to function as a ligand-gated channel by removal of the voltage-dependent Mg²⁺ blockade (15). The removal of the Mg²⁺ blockade from the NMDA receptor allows for an increased influx of Ca²⁺ despite membrane hyperpolarization by μ-opioids and low levels of presynaptic glutamate release. This influx of Ca²⁺ has two effects. It activates either a separate pool of PKC (PKC₂) or much greater amounts of the original pool of PKC₁. The other
pool of PKC may be translocated directly to the membrane, modifying various excitatory amino acid and/or other receptors (pathway 1), and/or it may modify nuclear transcription (pathway 3), the products of which result in delayed and persistent changes in cellular function such as opioid receptor/K+ channel uncoupling (41). This consequence cannot be reversed by acute administration of NMDA antagonists. A second effect of the influx of Ca2+ is that it activates NO synthase, which increases the production of NO. In addition, the influx of Ca2+ results in the production of superoxide from mitochondria. The simultaneous generation of these two molecules favors the production of peroxynitrite (ONOO−), a very potent initiator of DNA strand breakage, which, in turn, initiates the production of the nuclear repair enzyme, PARS. Pronounced activation of PARS can result in cell dysfunction and eventually cell death because of inhibition of mitochondrial respiration and depletion of cellular energy stores, which in turn may lead to the formation of dark neurons, perhaps by way of programmed cell death. PKCα, various pools of protein kinase C; GPro, heterotrimeric guanine nucleotide binding protein; NOS, nitric oxide synthase.

Clinical Implications. Recent progress in investigating neural mechanisms subserving neuropathic and inflammatory pain as well as opioid tolerance has significantly advanced our knowledge about pain and pain modulation. These studies represent two important frontiers in pain research. First, these studies have led to the concept that pathological pain may reflect a disease process with both dynamic and progressive changes during its course (1, 2, 9). A key feature of this process is that neuronal plastic changes occur within the CNS in association with the progress of pathological pain states. This concept provides, at least in part, a basis for explaining pathological pain that often persists long after the initial insults. Second, mechanisms of opioid tolerance may involve neuroplastic changes within the CNS as well (9, 39). Neuroplastic changes in relation to opioid tolerance have much in common with those of pathological pain, both of which begin with the activation of NMDA receptors (9). Evidence also exists indicating that interactions do indeed occur between cellular and intracellular mechanisms of pathological pain and opioid tolerance, and such interactions are likely to be a contributing factor to a generally weak analgesic effect of opioids in pathological pain states (39).

Thus far, little information exists with regard to interactions between hyperalgesia and analgesic tolerance in man. It is conceivable that reduced morphine analgesia after repeated administration to patients with chronic pain could result from the development of both pharmacological tolerance to morphine analgesia and tolerance-associated hyperalgesia. Because of the coincidental development of morphine tolerance and tolerance-associated hyperalgesia, progressively higher morphine doses may be needed to overcome both conditions. In turn, a vicious cycle may be initiated involving higher opiate doses, more tolerance, and greater hyperalgesia. Thus, the need for higher opiate doses in a clinical setting of opiate treatment could be partly due to the
development of hyperalgesia that may result from repeated opiate administration. Conceivably, the development of tolerance-associated hyperalgesic states may also contribute to withdrawal signs of opioid dependence. This possibility also suggests that an inappropriate opiate treatment schedule in pain management may precipitate unexpected hyperalgesic responses to pre-existing pain conditions and could be a source of the clinical complexity with respect to the responsiveness to opiate treatment.

On the other hand, evidence indicating hyperalgesia-associated reduction of morphine antinociception (27) has bearing on the controversy concerning opiate effects or lack of effects on neuropathic pain states in man. It is conceivable that the diversity of clinical response patterns to opiate treatment in neuropathic pain patients may result from varying degrees of CNS neuronal plastic changes initiated by nerve injury or injury to other tissues. Such neuronal plastic changes can underlie the development of neuropathic pain syndromes and result in reduced morphine analgesia even before opiate treatment starts (9). To complicate matters further, neuropathic pain syndromes as well as other chronic pain states (such as cancer pain) often present a dynamic and progressive course that demands increased opiate doses for adequate pain relief.

The complexity of opioid tolerance, hyperalgesia, and their interactions calls for a new look into some clinical issues of pain management. Because the development of pathological pain states often involves the activation of NMDA receptors and because there exists an intimate relationship between the NMDA and opioid receptor systems that may lead to changes in responsiveness to opioid analgesics, early recognition of clinical conditions that may lead to the development of pathological pain states would be of utmost importance. Such clinical conditions may include, but may not be limited to, nerve injury, tissue inflammation, and prolonged and ongoing peripheral nociceptive input (such as those seen in persistent postoperative pain). Some of these conditions (e.g., postoperative pain) may be treated preemptively to produce a favorable outcome. However, nerve injury and inflammatory tissue diseases often occur in an unpredictable manner, thereby obviating the possibility of preemptive treatment. Thus, effective early interruption of ongoing nociceptive input from the injured site (e.g., using nerve block or field block), thereby reducing CNS activation of NMDA receptors, could be the key to preventing or minimizing changes in NMDA and opioid systems that may eventually lead to persistent pain and reduced opioid effectiveness for treating such pain states.

As discussed above, interactions between NMDA and opioid receptors could occur in both directions (9). Thus, any condition that results in activation of NMDA receptors within the CNS could modulate opioid receptors, causing reduced efficacy of opioid analgesia; conversely, repeated treatment with opioids could set up a condition mimicking ongoing nociceptive input through interactions between opioid and NMDA receptors (9, 39). This concept is the basis for recommending a combined use of opioids and clinically available NMDA receptor antagonists (9). Importantly, such a strategy should be integrated into the treatment regimen not only for chronic pain management (treating an existing pain condition) but also for preventing an evolving pain condition, such as that after nerve injury. By the same token, effective nerve block or field block should be part of an integrated therapeutic regimen for treating clinical conditions that may later lead to the development of intractable chronic pain states. It should be noted that, although early treatment of pain after tissue injury and inflammation with opioids often provides satisfactory clinical pain relief, opioids alone offer little help for stopping the process of an evolving pathological pain state, because evidence presented in this article and elsewhere suggests that opioids alone could actually contribute to the development of neuronal plastic changes via interactions with NMDA receptors. It can be anticipated that our further understanding of neural mechanisms subserving hyperalgesia, opioid tolerance, and their interactions would advance and improve clinical management of debilitating and intractable pain syndromes.

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