Opioids have been used medicinally for millennia for their potent effects on nociception. However, the past 20 years have led to important insights into the influences and mechanisms of opioid actions, which are more extensive than merely analgesia, including human synthesis of opioids, critical roles of opioids during development and following nerve injury, and actions of different opiate alkaloids and their receptors. Due to the vast literature on opioids, the scope of this review has been limited to opioid actions in maintaining neuron viability during development, promoting neurological function following nerve injuries, in inflammation, disease and against ischemia; alleviating neuropathic pain; raising and lowering cellular immunity; and mechanisms modifying morphine tolerance.

Key words: Analgesia, Addiction, Drug tolerance.
leading to changes in opioid action by increasing the potency of various opioid receptor agonists (25, 28-30). This is indicated by the antinociceptive potency of opioids being greater against various noxious stimuli in animals with peripheral inflammation than in control animals (28-30). Inflammation-induced enhancement of opioid antinociceptive potency is characteristic of the mu opioid receptors, since morphine elicits a greater increase in spinal potency of mu- than of delta- and kappa-opioid receptor agonists. This enhanced potency of mu-opioid receptor agonists during inflammation appears to be brought about by changes in the affinity or number of the mu-opioid receptors, which is suggested by the increased gene expression for these receptors in the spinal cord dorsal horn (31-32). Similarly, following spinal cord or peripheral nerve damage leading to neuropathic pain, there is an increase in the biosynthesis of endogenous dynorphins (30, 33-35). Enhancement in the synthesis of both opiate receptors and endogenous dynorphins following injury is a straightforward means for ameliorating nociception.

Endorphins, the endogenous ligands of the mu-opioid receptor, but not morphine, are effective against neuropathic pain (30). This results not because neuropathic pain is opioid-resistant, but because of the development of a reduced sensitivity to systemic morphine under this condition (36). This leads to a required increased morphine dose to obtain adequate analgesia. The reduction in morphine antinociceptive potency may result from nerve injury reducing the activity of spinal opioid receptors or of opioid signal transduction (37, 38). Alternatively, opioids may induce long-term changes in the numbers and binding activities of opioid receptors (16). These differences in action and consequences are important to understanding 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.

**Distribution of Opioid Receptors**

MOR are mainly expressed in small-diameter unmyelinated, but also in some large-diameter dorsal root ganglion neurons, and they co-localize with bradykinin B2 receptors (39-40). In addition, some DRG neurons express both MOR and DOR mRNAs, suggesting that the MOR and DOR seen in the spinal dorsal horn originate, at least in part, from DRG neurons (40).

Following nerve injury in mice, MOR expression in DRG neurons undergoes a drastic decrease (41-42). The lower potency of systemic morphine in neuropathic pain is in part caused by a decreased MOR expression in DRG neurons, and the subsequent loss of peripheral morphine analgesia in such a condition (39).

Within 24 hours of ligating the sciatic nerve, there is a transient increase in MOR immuno-reactivity in the cell bodies of DRG and nodose ganglion neurons, and in the sciatic nerve stump proximal to the ligation (43). However, 1 week later, MOR-like immunoreactivity declines in DRG neuron cell bodies of the fourth and fifth lumbar nerves, on the side of the ligation (43). These results indicate that MOR is transported peripherally by axonal flow in the peripheral axons of sensory ganglion neurons, and MOR synthesis in sensory ganglion neurons is influenced by peripheral axon injury.

Two broad classes of nociceptors can be distinguished based on their growth factor requirements and their binding of isoelectric B4 (IB4). Both 1-10 μM of the mu-receptor agonist D-alal2, N-Me-Phe4,Gly-ol5 enkephalin (DAMGO), and 1-10 μM morphine have greater effects on high voltage-activated Ca2+ currents in IB4-negative than in IB4-positive cells (44). However, DAMGO has no significant effect on T-type Ca2+ currents in both groups (45). The N-type Ca2+ current is the major subtype of Ca2+ currents inhibited by DAMGO in both IB4-positive and -negative neurons (44). Although DAMGO has no effect on L-type and R-type Ca2+ currents in both groups, it produces greater inhibition on N-type and P/Q-type Ca2+ currents in IB4-negative than IB4-positive neurons (46). Furthermore, double labeling reveals significantly higher mu opioid receptor immunoreactivity in IB4-negative than IB4-positive cells (44). These results suggest that N-and P/Q-type Ca2+ currents are more sensitive to inhibition by the mu opioids in IB4-negative than IB4-positive DRG neurons.

Immunostaining with MOR and neurofilament (NF200) antibodies, combined with calcium imaging of MOR function indicates that a larger number of neonatal than adult DRG neurons express functional MOR (57% versus 40%) (40, 47). Further, with age, MOR expression is confined to the large, neurofilament positive sensory neurons, while expression in small, nociceptive, neurofilament negative neurons remains unchanged (40). Sensory threshold testing shows that the analgesic potency of systemic morphine to mechanical stimulation is significantly greater in the neonate and declines with postnatal age. While the analgesic potency to thermal nociceptive stimuli does not change with postnatal age (47). This is consistent with postnatal regulation of MOR expressed by large DRG neurons (47).

**Opioid Modulation of the Immune System**

A functional immune system is important to fight against neurotoxicity in neurodegenerative diseases (48-49). Since the late 19th century, morphine has been used to suppress cellular immunity and lower resistance to bacterial infection.
(50–51). While exogenous opioids mediate immunosuppression, endogenous opiates exert the opposite actions (50). Endogenous opioid peptides are involved in CNS functions from development to immune modulation (52–53). These influences are mediated mostly via specific opioid receptors uniquely localized in different brain regions and cells (52).

Acute and chronic opioid administration have inhibitory effects on humoral and cellular immune responses, including antibody production, natural killer cell activity, cytokine expression, and phagocytic activity. Opiates behave like cytokines, modulating the immune response by interacting with their receptors in the CNS and in the periphery (54–55). Potential mechanisms by which central opiates modulate peripheral immune functions may involve both the hypothalamic-pituitary-adrenal axis, and the autonomic nervous system (56–61). Opioid receptors also exist outside the CNS in peripheral nerves and in immune inflammatory cells (50).

Neurogenic inflammation and related pain can be modulated by opioids inhibiting the function of primary afferent neurons. Inflammatory conditions lead to an increased anterograde axonal transport of opioid receptors from dorsal root ganglia (DRG) toward their peripheral nerve endings (62). The increased number of opioid receptors (among other mechanisms) leads to the improved analgesic effects of exogenously administered ligands (i.e. morphine) and of endogenous leukocyte-derived opioid peptides (i.e. beta-endorphin) (62). This suggests that during inflammatory processes, endogenous opioid peptides can be secreted from immunocytes, occupy peripheral opioid receptors on sensory nerve endings, and thereby produce analgesia by inhibiting the excitability of these nerves, or by the release of pro-inflammatory neuropeptides (62).

When consumed, heroin is rapidly transformed to 6-acetylmorphine (6-AM), then to morphine, which in turn is mainly metabolized to morphine-3-glucuronide (M3G) and, to a lesser extent, to morphine-6-glucuronide (M6G). Unlike M3G, M6G is a potent opioid agonist (63). In the pathogenesis of HIV-1 infection in heroin drug addicts, immunosuppression mediated by opiates, such as morphine, are postulated to promote progression of the virus and development of secondary opportunistic infections (52). Opiates may also promote immunodeficiency virus infection by decreasing the secretion of alpha and beta chemokines, which are important inhibitory cytokines for the expression of HIV (50, 64). Simultaneously, opiates may cause an increased expression of chemoreceptors CCR5 and CCR3, which are co-receptors for the virus, and increases in free radicals (reactive oxygen intermediates (ROI) and nitric oxide (NO)), which are produced by activated glial cells (microglia and astrocytes) (52, 65–66).

Peripheral immunosuppression is mediated at least in part by opioid receptors located in the CNS, and intrathecally administered opioids do not exert the same immunosuppressive effects (67–69). These findings may have important clinical implications for patients receiving long-term opioid therapy for malignant and nonmalignant pain.

**Actions of Morphine at Ultra-low Concentrations on Neurite Outgrowth**

Axon injury leads to prominent cellular responses, including enhanced synthesis of RNA and protein in the neuronal perikaryon, and proliferation of reactive Schwann cells (70). Because morphine significantly depresses cellular metabolism, it also influence these and other neuron/axon responses involved in nerve regeneration (70–71).

An ultra low concentration of morphine (10^{-14}M) has neurotrophic influences, and increases the length of the longest process extended by adult rat spinal and cortical neurons by 24% and 18%, respectively (72). These influences are exerted in 61% of the spinal neurons, and 48% of the cortical neurons (72). Morphine treatment also increases the number of calcitonin gene-related peptide-immunoreactive neurons possessing multiple, long branches (i.e. with at least one branch >0.5 mm) (73). However, at higher concentrations, morphine (10^{-6}M) has no significant effect on neurite length (72).

Electrophysiological studies on nociceptive DRG neurons in vitro show that extremely low (FM-nM) concentrations of morphine, and many other bimodally-acting mu, delta and kappa opioid agonists, elicit direct excitatory opioid receptor-mediated effects, whereas higher (μM) opioid concentrations evoke inhibitory effects (72, 74). These findings indicate that doses of morphine far below those currently required for clinical treatment of pain may become effective when opioid hyperalgesic effects are blocked by co-administration of appropriately low doses of opioid antagonists (74). This low-dose-morphine co-treatment procedure should markedly attenuate morphine tolerance, dependence, and other aversive side effects.

Morphine can also inhibit axon regeneration. Acute administration of morphine into lesioned rat facial nerve axons slows the rate of axon regeneration, and results in axons with smaller diameters (70). Chronic morphine treatment for 2 weeks results in significantly fewer axons regenerating, although the axons have normal diameters (70). Further, chronic morphine exposure causes Schwann cell hypertrophy, proliferation, and inhibition of myelin.
Morphine Induced Neuroprotection and Neuron Death

Exogenous opioids influence and modulate neuronal and glial cell function via an opioid receptor mediated mechanism leading to brain protection or damage (52). Kappa opioid receptor (KOR) ligands appear to play a neuroprotective role (52, 75-77). However, more studies are needed to determine how opioids exert their effects on glial cells and neurons.

Morphine Induced Neuroprotection and Neuron Death

Morphine (0.1-10 μM) provides neuroprotection against ischemia (20 minutes) of cerebellar Purkinje, there is an 18% increase (39 to 57%) in the yield of viable neurons if these neurons are pretreated with morphine prior to ischemic insult (78-81). This influence is via activation of delta1-opioid receptors, possibly involving mitochondrial adenosine triphosphate-sensitive potassium channel activation, and free radical production (78,81). Thus, opioids and agents that specifically work on signaling molecules for opioid preconditioning-induced protection may prove to be useful in inducing protection against ischemia in clinical practice.

Neuron death during development and neurodegenerative diseases, is induced by apoptosis (triggered cell death) and necrosis (death due to lack of neurotrophic nurture). Opiates promote apoptotic death in cells of both the immune and nervous systems, but can also potentiate neuron survival during development (82-84). In multiple inflammatory disease models, such as Parkinson’s disease, traditional anti-inflammatory drugs have limited therapeutic use because of their narrow spectrum and severe side effects after long-term use (85). However, morphinans, a class of compounds containing the basic morphine structure, provide neuroprotection (85-88).

Morphometric analysis of the brains of individuals who died of opiate overdose, show that the largest number of dead neurons are those with a small diameter (89). This neuron death is attributed to morphine toxicity of small morphine-sensitive neurons (90).

Caspases are intracellular cysteine proteases that induce cell death and inflammation. Caspase-3 activation is a major mediator of apoptotic and necrotic cell death (91-92). Morphine administration induces the up regulation of the caspase-3 and Bax proteins, and the down regulation of the antiapoptotic Bcl-2 protein in the spinal cord dorsal horn (93-94). Thus, morphine also induces neuron death.

Morphine-induced neuronal apoptosis can be blocked by the co-administration of morphine with caspase inhibitors (83, 93). Naloxone, the nonselective DOR antagonist, also blocks morphine-induced apoptosis (83). These findings indicate the involvement of activated opiate receptors and caspase-3 in morphine-induced apoptosis. Thus, caspase inhibitors may provide a therapeutic protection against morphine induced apoptosis.

The blockade of spinal caspase-like activity also partially prevents morphine tolerance and the associated increase in nociceptive sensitivity. These results indicate an opioid-induced neurotoxic consequence regulated by the NMDAR-caspase pathway, which may have clinical implications in opioid therapy and substance abuse (93).

Daily administration of exogenous morphine to chick embryos increases the number ciliary ganglion, but not spinal neurons that survive the normal death phase during development (95). Conversely, the chronic application of naltrexone (long-lasting opiate antagonist), significantly increases the number of ganglion neurons that die (96). These findings indicate the critical role of endogenous opioids in regulating neuron survival and death during development, and that the elimination of endogenous opioids, or blocking their receptors, influences neurons during development.

Recent observations of dissociated adult frog and human, but not rat, DRG neurons, indicate that they have six different subpopulations that can be characterized at the light microscopic level based upon neuron, soma diameter, and the presence of cytoplasmic granules with unique morphologies (Kuffler, unpublished results). Current experiments are examining whether these apparent different neuron subpopulations correspond to distinct physiological subpopulations of DRG neurons. If so, this simple morphological characterization of subpopulations will allow opiate responding neurons to be selected from a large mixed population of sensory neurons and studied with much greater reliability than presently possible.

Exposure of microglia and neurons in culture to 10^{-4} M morphine induces a 4-fold increase in apoptosis of these cells (83, 97-98). In contrast, astrocytes are completely resistant to morphine-induced apoptosis (83, 99). Due to the extensive glia-neuron interactions, morphine-triggered glial death will influence the neurons associated with these glial cells.

Neuropathic Pain

Neuropathic pain is often a consequence of nerve injury due to surgery, bone compression, diabetes, or infection, and is generally resistant to currently available treatments. Morphine is the first-line strong opioid of choice for patients with extreme pain. While most patients achieve adequate analgesia with morphine, a significant minority
suffer either intolerable side effects, inadequate pain relief, or both (100-102). For these patients, switching to an alternative opioid is an established clinical practice (101, 103-104). However, there is no good evidence for the effectiveness of opioid switching (102, 105). Further, the treatment of neuropathic pain is an area of largely unmet medical need.

Microglia are activated by CNS trauma, apoptosis, ischemia, inflammation, and infection (106-108). When activated, microglia show a progressive series of changes in morphology, gene expression, function, and number; and produce and release various chemical mediators, including proinflammatory cytokines that can produce immunological actions and act on neurons to alter their function (109).

Extracellular adenosine 5'-triphosphate (ATP) and activated microglia play important roles in neuropathic pain hypersensitivity (109). Activated spinal microglia enhance their expression of the ATP receptors subtype P2X4; and the presence of ATP leads to neuropathic pain. ATP facilitates spinal pain transmission via ionotropic P2X nucleotide receptors (110). This can be demonstrated by blocking the P2X4 receptor pharmacologically, which reduces the neuropathic pain (109). It appears that neuropathic pain results from the activation of the ATP receptors on activated microglia, which triggers them to release several cytokines, such as interleukin-1beta (IL-1beta), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-alpha) leading to neuropathic pain (109).

The reduction in analgesic potency against neuropathic pain of systemic administration of morphine over time appears as a result from neurochemical changes in DRG and spinal cord neurons, a loss of morphine analgesia at the periphery (39), and a reduction in the retrograde transport of nerve growth factor (NGF) (111).

Chronic intrathecal infusion of NGF reverses neuropathic pain symptoms and restores morphine’s effectiveness in an animal model of neuropathic pain (111). Spinal infusion of NGF does not affect the expression of tactile allodynia or thermal (hot) hyperalgesia in neuropathic rats, although it significantly increases cold water response by day 14 (111). Morphine also substantially attenuates neuropathy-induced warm and cold hyperalgesia, as well as tactile allodynia in neuropathic rats chronically infused with NGF (111). Thus, NGF appears critical for maintaining the neurochemical homeostasis in nociceptive neurons, and its administration may be beneficial in restoring and/or maintaining opioid analgesia in chronic pain conditions resulting from traumatic nerve injury.

Following nerve injury in mice, the dose-response curves for morphine analgesia is unchanged (111). This indicates there is no decrease in morphine potency at the supraspinal level. However, the dose-dependent analgesia produced by intraplantar morphine in sham-operated mice almost completely disappears in nerve-injured mice. Using a sensitive algogenic-induced nociceptive flexion test, one sees a significant reduction in the analgesic potency of systemic morphine for bradykinin (BK) nociception in nerve-injured mice, and the analgesic effect of morphine against BK nociception in sham-operated mice disappears in nerve-injured mice (111).

The functional down regulation of mu-opioid receptors on sensory neurons following a nerve lesion contributes to the poor efficacy of opioids in neuropathic pain (18, 112, 113). Also, the increased effectiveness of nociceptin after axotomy supports the hypothesis that its actions are mediated via a non-opioid receptor (112). Despite this, spinal administration of nociceptin, or agonists that activate ORL1 (opioid-like orphan receptor), may prove to be of clinical interest in the management of neuropathic pain.

**Morphine Tolerance**

Tolerance to opiate analgesia reduces its effectiveness in the treatment of severe pain. Tolerance appears to result for a number of reasons. One is raising level of pain, resulting from increasing nociceptive input as disease or injury progresses (114). Another is neuronal adaptation to continuous opiate administration, leading to over activity of pro-nociceptive systems (115-116). The development of morphine tolerance (with the dose required to relieve pain increasing from 0.5, 1, and 5 to 10 μM morphine over 6 days) is correlated with a significant increase in calcitonin-gene-related-peptide-like immunoreactivity (CGRP-IR) in primary sensory afferents of the dorsal horn (117-119). This suggests the involvement of changes in pain-related neuropeptides in DRG neurons (118). Morphine treatment also increases the number of calcitonin gene-related peptide-immunoreactive neurons possessing multiple, long branches (i.e. with at least one branch >0.5 mm) (73). The development of tolerance to spinally infused morphine is modulated by the blockade of dorsal horn CGRP receptors using the potent CGRP antagonist hCGRP (8-37, 118). Data also show that the phosphorylation of MAP kinases and CREB plays a role in the morphine-induced increase in spinal CGRP in primary sensory afferents (116). The presence of other spinally located peptides such as substance P, galanin, and neuropeptide Y are unaffected (118). These findings suggest that CGRP receptor antagonists could be useful adjuncts in the treatment of pain and tolerance to the antinociceptive effects of morphine.

The initial effects of opioid administration in most individuals are analgesia, sedation, nausea/vomiting,
respiratory depression, pupillary constriction, constipation, and euphoria or dysphoria (114). However, tolerance to different opioid effects develops at different rates, called selective tolerance. While tolerance to nausea, vomiting, sedation, euphoria and respiratory depression occur rapidly, there is minimal development of tolerance to constipation and miosis (114). Such diversity suggests receptor-related differences in the speed of tolerance development. Administration of compounds such as benzodiazepines together with opioids seems to increase the rate of tolerance development to opiates.

Morphine tolerance may also be due to a reduction in the activity of the descending inhibitory neuronal connections (120). Thus, the higher the intrinsic activity of an opioid at one receptor site, the fewer receptors needed to induce a potent analgesic effect. As a net result, the incidence of tolerance is less likely to become clinically apparent when potent opioid ligands, such as fentanyl or sufentanil, are administered (114).

Changes in metabolism appear to have little effect on the rate of development of morphine tolerance (121-122). In chronic pain treatment with morphine, an increased ratio of the metabolite morphine-3-glucuronide, with antiopioid effects, to morphine-6-glucuronide, is associated with staggering doses of the analgesic. Opioids that interact with mu- and/or kappa-binding sites, demonstrate an adaptation process called desensitization, which is due to a reduced interaction of the receptors with the internal second messenger G-protein system (114). This influence is only brief following ligand binding. Another mechanism underlying tolerance development is internalization (endocytosis) of the opioid receptors. This short-lived phenomenon leads to the availability of fewer receptor-binding sites for mediating the analgesia. A mechanism of long-term opioid binding is subsequent protein kinase C (PKC), phospholipase C (PLC) translocation, and activation of nitric oxide synthetase (NOS). These lead to activation of the N-methyl-D-aspartate (NMDA) receptor leading to its anti-opioid effect and opioid tolerance (114).

Conclusions

In addition to their roles as analgesics, exogenously administered, and endogenous released opiates have clear and extensive influences on many human physiological functions. Some of these are similar and some opposite. Opiates play important roles during fetal brain development, recovery following neurological injuries, and on the course of certain neurodegenerative diseases. Further studies are needed to characterize the effects and mechanisms by which opioids exert their effects on glial cells, Schwann cells. Additionally, it is critical to gain great understanding of the influences of opioids on neurons, and how these effects alter the interactions between glia, Schwann cells and neurons, and whether it may be possible to use opioids clinically to promote axon regeneration and neuroprotection following CNS trauma. Additional studies are also required to determine how the development of opioid tolerance can be slowed or prevented. Finally, it is vital to develop more potent opioid receptor agonists and to understand how additional factors or drugs can be used to potentiate the effects of exogenous and endogenous opiates.

Resumen

Por milenios, los opioides se han utilizado medicinalmente por su efecto potente y selectivo sobre la nocicepción, y por su pequeño efecto sobre otras modalidades sensoriales. Los pasados 20 años han llevado al conocimiento de las influencias de los opioides y sus mecanismos de acción, incluidos los opioides peptídicos endógenos, al conocimiento de su expresión genética, su metabolismo enzimático y la caracterización de los receptores opioides a nivel molecular. Estos intereses surgieron como resultado de la realización que los opioides ejercen efectos más extensos que la analgesia sola. Los humanos sintetizan opioides y los opioides juegan un papel crítico durante el desarrollo y luego de un daño al nervio en el adulto. Este repaso examina algunas de las acciones de los opioides, incluido su crítico rol durante el desarrollo del sistema nervioso en mantener las neuronas vivas, que de otra manera morirían, proveyendo neuroprotección en contra de la isquemia que puede surgir luego de una lesión, aliviando el dolor neuropático, proveyendo mecanismos para modificar la tolerancia a la morfina, aumentando o disminuyendo la inmunidad celular, y su rol en la promoción de la función neurológica luego de daños a los nervios y durante la inflamación y la enfermedad.

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