Opioid Receptors

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Abstract

Opioids are the oldest and most potent drugs for the treatment of severe pain. Their clinical application is undisputed in acute (e.g., postoperative) and cancer pain, but their long-term use in chronic pain has met increasing scrutiny. This article reviews mechanisms underlying opioid analgesia and other opioid actions. It discusses the structure, function, and plasticity of opioid receptors; the central and peripheral sites of analgesic actions and side effects; endogenous and exogenous opioid receptor ligands; and conventional and novel opioid compounds. Challenging clinical situations, such as the tension between chronic pain and addiction, are also illustrated.

PAIN

Excitatory Mechanisms

Pain may be roughly divided into two broad categories: physiologic and pathologic pain. Physiologic (also called acute or nociceptive) pain is an essential early warning sign that usually elicits reflex withdrawal and thereby promotes survival by protection from (further) injury. This type of pain is not an objective of therapeutic intervention. Pathologic (e.g., neuropathic, chronic) pain is an expression of (mal-) adaptation of the organism to tissue injury. Most painful conditions initially involve the activation of dorsal root ganglion (DRG) neurons, which give rise to high-threshold Aδ- and C-fibers (nociceptors) innervating peripheral tissues (skin, bone, joints, viscera) (1). Primary afferent neurons transduce noxious stimuli into action potentials and conduct them to the spinal cord. Transmission of input from nociceptors to ascending spinal neurons and to the brain is then mediated by monosynaptic contacts and/or through interneurons (reviewed in 2).

Inhibitory Mechanisms

Concurrent with such excitatory events, powerful endogenous mechanisms counteracting pain unfold in the periphery and in the central nervous system. In injured tissue, these include interactions between leukocyte-derived opioid peptides and peripheral nociceptor terminals carrying opioid receptors (3, 4), as well as other antiinflammatory mediators (5). In the spinal cord, inhibition is mediated by the release of endogenous opioids or gamma-aminobutyric acid (GABA) from interneurons, which activate presynaptic opioid and/or GABA receptors on central nociceptor terminals to reduce excitatory transmitter release. The opening of postsynaptic K^+ or Cl^- channels by opioids or GABA evokes hyperpolarizing inhibitory potentials in dorsal horn neurons. During ongoing nociceptive stimulation, spinal interneurons upregulate gene expression and production of opioids (6, 7). Descending inhibitory noradrenergic, serotonergic, and opioid pathways become activated. A key region is the periaqueductal gray matter. It relays to the rostral ventromedial medulla, which then projects along the dorsolateral funiculus to the spinal dorsal horn.

The central integration of signals from excitatory and inhibitory neurotransmitters and from cognitive, emotional, and environmental factors results in the perception of "pain." When the intricate balance between biological (neuronal), psychological (e.g., memory, distraction), and social (e.g., attention, reward) factors becomes disturbed, chronic pain can develop (8).

OPIOIDS

Opioid Receptors, Signal Transduction, Receptor Recycling

Opioid receptors are expressed by central and peripheral neurons and by neuroendocrine (pituitary, adrenal), immune, and ectodermal cells (reviewed in 4, 9). Early binding studies and bioassays defined three main types of opioid receptors in the central nervous system, the mu, delta, and kappa receptors (**Table 1**) (10). Additional receptor types were proposed (e.g., sigma, epsilon, orphanin) but are no longer considered "classical" opioid receptors. The identification and sequence analysis of complementary DNA (cDNA) and the selective deletion of opioid receptor genes in mice confirmed the existence of only three genes (11). Opioid receptors belong to the class A gamma subgroup of seven transmembrane G protein–coupled receptors (GPCRs) and show 50–70% homology between their genes (12). Additional pharmacologic subtypes may result from alternative splicing, posttranslational modifications, and/or receptor oligomerization

Table 1 Opioid receptors and ligands (adapted from 4 with permission)

Receptor	Site of Action	Effects	Agonist[a]	Antagonist
Mu	Systemic	Analgesia, euphoria, constipation, respiratory depression	DAMGO, morphine, fentanyl, endomorphins, beta-endorphin	CTOP, naloxone
	Peripheral	Analgesia, constipation, reduced inflammation	DiPOA, HS731/AS006, loperamide, frakefamide, DALDA, morphine-6-glucuronide, IQMF-4, SS620	Alvimopan, naloxone methiodide, methylnaltrexone
Delta	Systemic	Analgesia, convulsions, anxiolysis	DPDPE, SNC 80, enkephalins, deltorphin, beta-endorphin	Naltrindole; ICI 174,864; naloxone
	Peripheral	Analgesia, constipation	UK-321,130; ADL5747; ADL5859; JNJ-20788560	Naloxone methiodide
Kappa	Systemic	Analgesia, diuresis, dysphoria	U-69593; U50,488; bremazocine; dynorphin	Norbinaltorphimine, naloxone
	Peripheral	Analgesia, reduced inflammation	Asimadoline, FE200665/CR845, ADL10-0101, CJC-1008, ICI204448	Naloxone methiodide

[a]DAMGO, [D-Ala2, N-MePhe4, Gly-ol]-enkephalin; DALDA, Tyr-Arg-Phe-Lys-NH2; DiPOA, [8-(3,3-diphenyl-propyl)-4-oxo-1-phenyl-1,3,8-triazaspiro[4.5]dec-3-yl]-acetic acid; HS-731/AS006, 2-[(4,5alpha-epoxy-3-hydroxy-14beta-methoxy-17-methylmorphinan-6beta-yl)amino]acetic acid; SNC 80, 4-(alpha-(4-allyl-2,5-dimethyl-1-piperazinyl)-3-methoxybenzyl)-N,N-diethylbenzamide; FE200665/CR845, H-D-Phe-D-Phe-D-Nle-D-Arg-NH-4-picolyl; ADL5747, N,N-diethyl-3-hydroxy-4-(spiro[chromene-2,4′-piperidine]-4-yl)benzamide; ADL5859, N,N-diethyl-4-(5-hydroxyspiro[chromene-2,4′-piperidine]-4-yl) benzamide; JNJ-20788560, [9-(8-azabicyclo[3.2.1]oct-3-ylidene)-9H-xanthene-3-carboxylic acid diethylamide]; ICI204448, (R,S)-N-[2-(N-methyl-3,4-dichlorophenylacetamido)-2-(3-carboxyphenyl)-ethyl]pyrrolidine hydrochloride. All others are proprietary names.

(reviewed in 10, 11). Because many of these studies have relied on antibody-based experimental techniques, it is noteworthy that specificities of currently available antibodies against opioid receptors have been questioned and that more rigorous controls have been called for, raising caveats on the interpretation of such reports (13–15). High-resolution crystallized tertiary structures of mu, delta, and kappa opioid receptors have been resolved recently (10).

After binding of a ligand, conformational changes allow intracellular coupling of heterotrimeric $G_{i/o}$ proteins to the C terminus of the receptor. At the G_α subunit, GTP replaces GDP and dissociation of the trimeric G protein complex into G_α and $G_{\beta\gamma}$ subunits ensues. The former inhibit adenylyl cyclases and cAMP production, whereas the latter directly interact with different ion channels in the membrane (**Figure 1**) (16, 17). All three opioid receptors can modulate pre- and postsynaptic Ca^{2+} channels, suppress Ca^{2+} influx, and thereby attenuate the excitability of neurons and/or reduce the release of pronociceptive neuropeptides (14). In addition, opioid receptor activation leads to opening of G protein–coupled inwardly rectifying K^+ (GIRK) channels, thereby preventing neuronal excitation and/or propagation of action potentials (9, 17, 18). Opioids also inhibit Na^+ channels, I_h channels, transient receptor potential vanilloid-1 (TRPV1) channels, and acid-sensing ion channels (ASICs) in DRG neurons (**Figure 2**), as well as excitatory postsynaptic currents evoked by glutamate receptors in the spinal cord (19–23). The result is decreased transmission of nociceptive stimuli at all levels of the neuraxis and profoundly reduced perception of pain.

Various kinases can phosphorylate intracellular regions of opioid receptors, and GPCR kinases promote binding of arrestin molecules (reviewed in 24, 25). The formation of arrestin–opioid

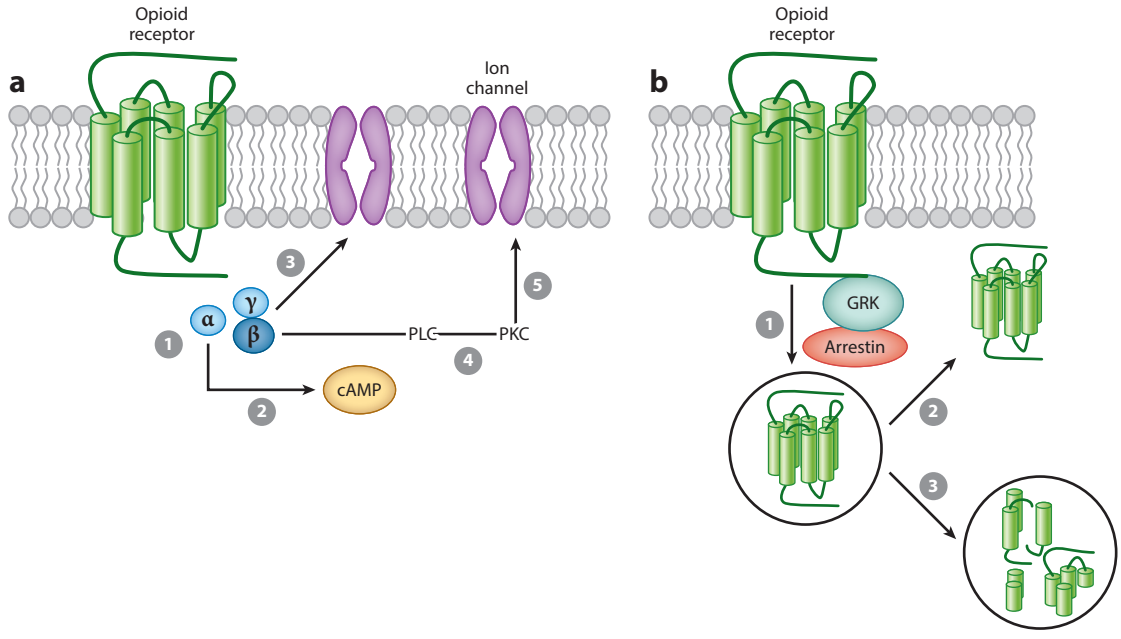

Figure 1

Opioid receptor signaling and recycling (adapted from Reference 9 with permission). (*a*) Opioid receptor ligands induce a conformational change at the receptor that allows coupling of G proteins to the receptor. The heterotrimeric G protein dissociates into active G_{α} and $G_{\beta\gamma}$ subunits ❶, which can inhibit adenylyl cyclase and reduce cAMP ❷, decrease the conductance of voltage-gated Ca^{2+} channels, or open rectifying K^{+} channels ❸. In addition, the phospholipase C/phosphokinase C pathways can be activated ❹ to modulate Ca^{2+} channel activity in the plasma membrane ❺. (*b*) Opioid receptor desensitization and trafficking is activated by G protein–coupled receptor kinase (GRK). After arrestin binding, the receptor is in a desensitized state at the plasma membrane ❶. Arrestin-bound receptors can then be internalized via a clathrin-dependent pathway and either be recycled to the cell surface ❷ or degraded in lysosomes ❸.

receptor complexes leads to opioid receptor desensitization by preventing G protein coupling and promotes receptor internalization via clathrin-dependent pathways. Recycling of dephosphorylated opioid receptors and their reintegration into the plasma membrane reinstates signal transduction, whereas targeting to lysosomes leads to receptor degradation (**Figure 1**). GPCR-associated sorting proteins modulate lysosomal sorting and functional downregulation (reviewed in 24, 26). In vitro studies showed a good correlation between G protein activation, arrestin recruitment, phosphorylation, and internalization of mu opioid receptors (27). In DRG neurons, beta arrestin was shown to promote the inhibition of Ca^{2+} channels by mu agonists, to increase constitutive recycling, and to decrease cell-surface localization of mu receptors (28). Additional opioid-modulated pathways involve mitogen-activated protein kinase and phospholipase C (25).

Intense research efforts are currently directed toward elucidating the functional consequences of heteromerization and allosteric modulation of opioid receptors (11, 29), as well as ligand-biased activation of different signaling pathways (11, 26). In DRG neurons, interactions between different opioid receptor types, possibly via hetero- or oligomerization, can facilitate coupling to Ca^{2+} channels (30), and allosteric interactions as well as delta/kappa receptor heteromers were shown (31). A caveat is that such phenomena were mostly studied in vitro and may be species- or tissue-specific in vivo.

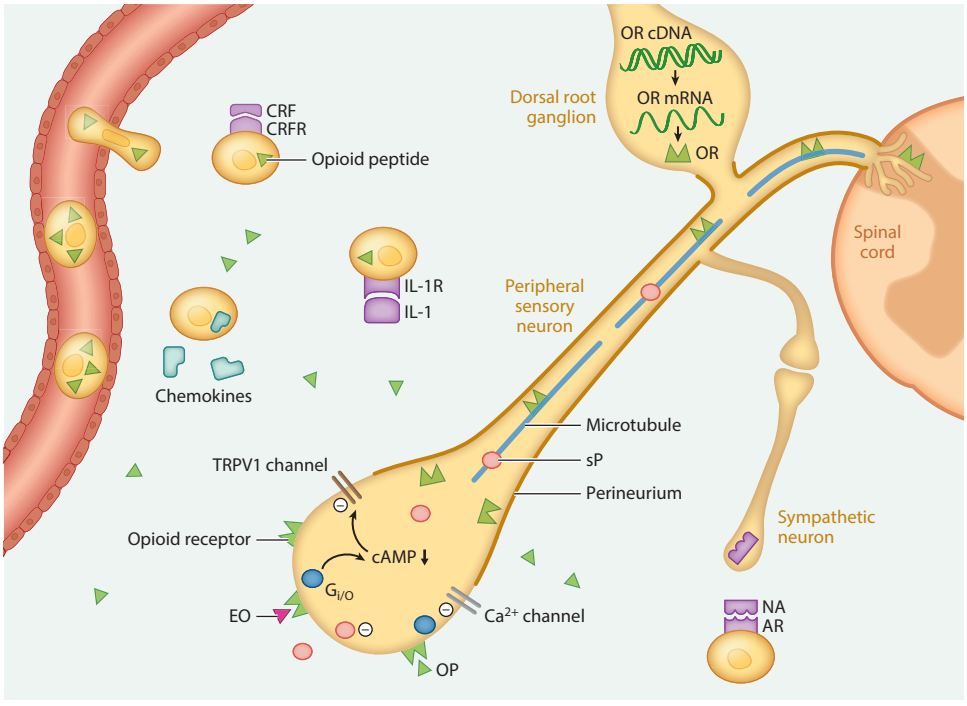

Figure 2

Endogenous antinociceptive mechanisms within peripheral injured tissue (adapted from Reference 4 with permission). Opioid peptide-containing circulating leukocytes extravasate upon activation of adhesion molecules and chemotaxis by chemokines. Subsequently, these leukocytes are stimulated by stress or releasing agents to secrete opioid peptides. For example, corticotropin-releasing factor (CRF), interleukin-1β (IL-1), and noradrenaline (NA, released from postganglionic sympathetic neurons) can elicit opioid release by activating their respective CRF receptors (CRFR), IL-1 receptors (IL-1R), and adrenergic receptors (AR) on leukocytes. Exogenous opioids (EOs) or endogenous opioid peptides (OPs, *green triangles*) bind to opioid receptors (ORs) that are synthesized in dorsal root ganglia and transported along intraaxonal microtubules to peripheral (and central) terminals of sensory neurons. The subsequent inhibition of ion channels (e.g., TRPV1, Ca^{2+}) and of substance P (sP) release results in antinociceptive effects.

Plasticity of Opioid Receptors and Signaling Pathways

Pathologic pain is associated with multiple adaptations in the nervous, endocrine, and immune systems (5, 8). With regard to opioid receptors, extensive investigations were conducted on the influence of painful peripheral inflammation and nerve damage. This is in keeping with the notion that inflammation is an essential component of a large group of painful syndromes including arthritis, neuropathic pain, cancer, wounds, and postoperative pain (32) and the consideration that chronic diseases with an inflammatory component are among our greatest health threats (33). Besides the characterization of plasticity in the central nervous system (6, 7), particular attention was directed toward the periphery. In the late 1980s, evidence began to accumulate that significant antinociceptive effects can be mediated by opioid receptors localized on peripheral sensory neurons (3, 34) (**Figure 2**). Initial studies showed that the systemic or local application of mu-, delta-, and kappa-receptor agonists elicited significantly more pronounced analgesic effects in inflamed than in noninflamed tissue of animals and humans (reviewed in 35). This intriguing finding stimulated extensive research into the underlying mechanisms.

Alterations in opioid receptor expression occur at the mRNA and protein levels and can be subject to epigenetic regulation (10). With regard to opioid receptor synthesis, it was found that peripheral tissue inflammation can induce differential regulation of opioid receptor types and their mRNAs in DRG neurons. For example, in a rat model of unilateral paw inflammation, mu-receptor mRNA displayed a biphasic upregulation, whereas delta-receptor mRNA remained unchanged, and kappa-receptor mRNA showed only one peak. In parallel, mu- and kappa-receptor binding was upregulated. This upregulation was dependent on neuronal electrical activity and on cytokine production in the inflamed tissue, and it may be related to cytokine-induced binding of transcription factors to opioid receptor gene promoters. Mu receptors were most extensively studied and were consistently shown to be upregulated (reviewed in 4, 36).

With regard to opioid receptor trafficking, it was shown that the peripherally directed axonal transport of opioid receptors was augmented, and opioid receptor expression on DRG membranes was enhanced (37, 38). The axonal transport was stimulated by cytokines and nerve growth factor produced within the inflamed tissue (39, 40), and it resulted in increased density and antinociceptive functionality of opioid receptors on peripheral nerve terminals (3, 41). The upregulation of opioid binding sites was due to an increase in the number of neurons expressing receptors and the number of receptors per neuron, while receptor affinity remained unchanged (38). However, brief inflammatory stimuli did not significantly alter the number of opioid receptors on peripheral sensory nerve terminals (reviewed in 4). Inflammation was also accompanied by a disrupted perineural barrier facilitating the access of opioid agonists to their receptors (42, 43).

In cultured sensory neurons, bradykinin was found to stimulate the trafficking of intracellular delta-opioid receptors to the plasma membrane (44). Furthermore, priming with bradykinin, prostaglandin E_2 (PGE_2), or a protease-activated receptor agonist led to more potent inhibition of neuropeptide release and of cyclic adenosine monophosphate (cAMP) accumulation by opioid agonists (44, 45). Also, painful paw inflammation and activation of sensory neurons by capsaicin or purinergic 2Y (P2Y) receptor agonists enhanced membrane recruitment of delta receptors (46, 47). In contrast, pretreatment of cultured DRG neurons with proinflammatory chemokines resulted in internalization and functional inhibition of mu receptors (48). In vivo, local pretreatment with bradykinin or arachidonic acid enabled (49), whereas the genetic ablation of bradykinin receptors reduced (50), peripheral opioid antinociception (reviewed in 4).

With regard to signaling pathways, G protein coupling of opioid receptors was augmented during paw inflammation (38). Earlier studies had shown that low extracellular pH (as seen in inflammation) increased opioid agonist efficacy, possibly by altering the interaction of opioid receptors with G proteins and adenylyl cyclase (51, 52). This may augment inhibition of cAMP, Ca^{2+}, Na^{+}, TRPV1, and/or ASIC currents, as well as neuronal hyperpolarization via the opening of GIRK channels (4, 18–21). Under conditions of elevated cAMP/protein kinase A activity (as seen in inflammation), morphine can inhibit TRPV1 translocation to the plasma membrane (53). Furthermore, extracellular recordings from sensory nerve fibers supplying injured tissue demonstrated opioid inhibition of spontaneous and stimulus-evoked action potentials (54–57).

Nerve injury resulting in neuropathic pain is another condition influencing opioid receptor expression in peripheral sensory neurons. Studies in animal models have used nerve transsection, nerve constriction, and chemical or metabolic impairments (reviewed in 4, 58). The mRNA levels of all three opioid receptors were usually unchanged or downregulated in the DRG, regardless of the type of nerve injury. This was not always correlated with protein expression. Mu receptors were most often studied and were found to be downregulated or unchanged in diabetes, nerve transsection, and sciatic nerve ligation; decreased or increased after partial sciatic nerve ligation; and unchanged or increased following chronic constriction injury (4, 59, 60). However, the net protein level in DRG cell bodies might depend on injury-induced receptor relocation along the

peripheral neuronal processes. For example, an upregulation of opioid receptors was shown at the nerve injury site and in hind paws innervated by damaged saphenous nerves (reviewed in 4, 58). In the nerve constriction model, this was accompanied by enhanced antinociceptive activity of opioid agonists administered at the injury site (61) or at the peripheral nerve terminal (57). Thus, regardless of changes in the DRG, opioid receptor protein expression was enhanced in mechanically lesioned nerves and in paw skin in various models.

Besides plasticity in the context of injury, the nuclear export and local translation of kappa-opioid receptor mRNA into receptor protein in distal neuronal compartments of noninjured DRG neurons has been studied extensively. These studies uncovered speedy transport systems utilizing RNA-containing granules and their role in neuronal survival and function (reviewed in 62).

In summary, the expression, axonal transport, signaling, and accessibility of opioid receptors on DRG neurons depends on receptor type and duration of injury, and these mechanisms apparently contribute to enhanced analgesia and/or antiinflammatory effects (4, 63). This is consistent with the notion that tissue or nerve injury is required to "unmask" peripheral opioid effects, while the local application of opioids along uninjured nerves or in normal tissue does not reliably produce analgesia (34, 64).

Endogenous Opioid Receptor Ligands

Endogenous opioid receptor ligands are derived from the precursors proopiomelanocortin (encoding beta endorphin), proenkephalin (encoding Met-enkephalin and Leu-enkephalin), and prodynorphin (encoding dynorphins). These peptides contain the common Tyr-Gly-Gly-Phe-Met/Leu sequence at their amino terminals, known as the opioid motif. Beta endorphin and the enkephalins are antinociceptive agents acting at mu- and delta-opioid receptors. Dynorphins can elicit both pro- and antinociceptive effects via N-methyl-D-aspartate (NMDA) receptors and kappa opioid receptors, respectively. A fourth group of tetrapeptides (endomorphins) with unknown precursors do not contain the opioid motif but bind to mu receptors with high selectivity. Opioid peptides and receptors are expressed throughout the central and peripheral nervous system, in neuroendocrine tissues, and in immune cells (9). Interactions between immune cell–derived opioid peptides and peripheral opioid receptors have been examined extensively, particularly with regard to the generation of analgesia (reviewed in 4) (**Figure 2**). Of note, endogenous opioid peptides from immune cells within inflamed tissue appear to produce additive/synergistic interactions rather than tolerance at peripheral opioid receptors (65–67). Extracellular opioid peptides are susceptible to rapid enzymatic inactivation by aminopeptidase N and neutral endopeptidase ("enkephalinases"). Both are expressed in the central nervous system, peripheral nerves, and leukocytes. Preventing the extracellular degradation of endogenous opioid peptides by enkephalinase inhibitors, both in central and peripheral compartments, has been shown to produce analgesic effects in many animal models and in some human trials (68, 69). This strategy avoids unphysiologically high concentrations of exogenous agonists at the receptor and, thus, diminishes the risk for development of receptor downregulation, tolerance, desensitization, and off-site or paradoxical excitatory effects.

CLINICAL IMPLICATIONS

Basic research on pain continues at a rapid pace, but translation into clinical applications has been difficult (70, 71). Obstacles include overinterpretation of data, reporting bias leading to neglect of negative results, and flawed design of experimental and clinical studies (71–73). Animal studies

are indispensable, continue to be improved, and have successfully predicted adverse side effects of drug candidates (70, 73). However, for ethical reasons, many are restricted to days or weeks, whereas human chronic pain can last for months or years. Therefore, animal models do not mirror the truly chronic clinical situation and should be more cautiously termed as reflecting "persistent" pain (70, 72–74). Brain imaging in patients with various pain syndromes is an area of intense research, but such studies have not yet provided reproducible findings specific for a disease or a pathophysiologic basis for individual syndromes (74). Similarly, imaging opioid mechanisms in the human brain has been limited mostly to single-dose studies in healthy volunteers and has not substantially advanced our understanding of pain relief or opioid use in patients (75). Neuroimaging can detect only those alterations associated with nociceptive processes, whereas clinical pain encompasses a much more complex subjective experience that critically relies on self-evaluation. Thus, imaging cannot provide an objective proxy, biomarker, or predictor for pain (76). The genetics of pain is another budding field, but although basic research has produced some evidence for genetic control of pain, such findings are not expected to serve as a guide to individualized ("personalized") clinical pain therapy any time soon (74, 77).

Plasticity of the Nervous and Immune Systems under Pathologic Conditions

When peripheral tissue is damaged, primary afferent neurons are sensitized and/or directly activated by thermal, mechanical, and/or chemical stimuli (e.g., protons, prostanoids, bradykinin, cytokines) (2, 5, 78). Many of these agents lead to opening of cation channels in the neuronal membrane (e.g., TRPV1). This produces inward currents of Na^+ and Ca^{2+} ions into the peripheral nociceptor terminal. If this depolarizing current is sufficient to activate voltage-gated Na^+ channels (e.g., $Na_v1.8$), they will open, further depolarizing the membrane and initiating action potentials that are then conducted along the sensory axon to the dorsal horn of the spinal cord. The central terminals of nociceptors contain excitatory transmitters such as glutamate and substance P. These activate postsynaptic NMDA, neurokinin (NK), and other receptors. Repeated nociceptor stimulation can sensitize both peripheral and central neurons (activity-dependent plasticity; "wind-up") (1). Later, sensitization can be sustained by changes in the expression of genes encoding neuropeptides, transmitters, ion channels, receptors, and signaling molecules (transcription-dependent plasticity) in nociceptors, spinal neurons, and glial cells (2). In addition, physical rearrangement of neuronal circuits by apoptosis, nerve growth, and sprouting occurs in the peripheral and central nervous system (reviewed in 8). Both induction and maintenance of central sensitization are considered critically dependent on the peripheral drive by nociceptors, indicating that therapeutic interventions targeting such neurons may be particularly effective, even in chronic pain syndromes (1).

Concurrent with tissue injury, endogenous mechanisms counteracting pain unfold in the periphery and in the central nervous system. In peripheral tissue, this leads to upregulation of opioid receptors in DRG neurons as well as enhanced permeability of the perineurium (4, 42, 50, 78). In parallel, opioid peptide–containing immune cells extravasate and accumulate in the inflamed tissue (**Figure 2**). These cells upregulate the gene expression of opioid peptide precursors and the enzymatic machinery for their processing into functionally active peptides (79–81). In response to stress, cytokines, or bacteria, leukocytes secrete opioids, which then activate peripheral opioid receptors and produce analgesia by inhibiting the excitability of nociceptors and/or the release of excitatory neuropeptides (4, 82). The clinical relevance of these mechanisms has been confirmed in studies demonstrating that patients with joint inflammation express opioid peptides in immune cells and opioid receptors on sensory nerve terminals within synovial tissue (65, 83, 84). After knee surgery, the patients' pain and analgesic consumption were enhanced by blocking the interaction

between the endogenous opioids and their receptors (83) and were diminished by stimulating opioid secretion (8, 85).

Opioid receptors are also expressed by immune cells (reviewed in 4, 86). No evidence has suggested that such opioid receptors contribute to analgesia, but they were shown to modulate various immune cell functions. However, these effects were mostly examined in vitro, and they were often contradictory and dependent on specific experimental conditions (e.g., cultured cell types, duration of cultures, doses and timing of opioid exposure). Based on such studies (reviewed in 86), immunosuppressive effects of opioids were frequently proposed but have not been verified in clinical or epidemiologic studies (87, 88). In addition, opioid receptors were shown to modulate cellular growth, inflammation, and wound healing (reviewed in 62, 63). The involvement of opioid receptors in acupuncture effects has been explored extensively in models of physiologic and pathologic pain, but no satisfactory consensus on the underlying mechanisms has been reached, and conclusive evidence from well-controlled clinical trials is lacking (89, 90).

Opioid Receptor Agonists

Consistent with the expression of opioid receptors at all levels of the neuraxis, opioid agonists can effectively inhibit clinical pain after peripheral (topical, intraarticular), neuraxial (intrathecal, epidural, intracerebroventricular), or systemic (intravenous, oral, subcutaneous, sublingual, transdermal) administration (4, 9, 91–94). The commonly available opioid drugs (morphine, codeine, methadone, fentanyl, and their derivatives) are primarily mu agonists. The choice of a particular compound or formulation is based on pharmacokinetic considerations (route of administration, absorption, distribution, desired onset or duration, extended-release formulations, metabolism, excretion) and on side effects (reviewed in 91, 95, 96). Systemically and spinally administered opioids can produce similar side effects (e.g., respiratory depression), depending on dosage and rostral/systemic redistribution. Partial agonists must occupy a greater fraction of the available pool of functional receptors than full agonists to induce a response of equivalent magnitude. Mixed agonist/antagonists (buprenorphine, butorphanol, nalbuphine, pentazocine) may act as agonists at low doses and as antagonists (at the same or a different receptor type) at higher doses. Such compounds typically exhibit ceiling effects for analgesia, and they may elicit an acute withdrawal syndrome when administered together with a pure agonist. All three opioid receptor types mediate analgesia but have differing side effects. This is most likely due to the variable regional expression, plasticity, and functional activity of receptors in different parts of central and peripheral organ systems. For example, mu receptors mediate respiratory depression, sedation, reward/euphoria, nausea, urinary retention, biliary spasm, and constipation; kappa receptors mediate dysphoric, aversive, sedative, and diuretic effects; and delta receptors can mediate reward, respiratory depression, and convulsions (9). Tolerance and physical dependence may occur with prolonged administration of pure agonists, and abrupt discontinuation or antagonist administration can result in a withdrawal syndrome (9, 20).

Both experimental and clinical studies have demonstrated that peripheral opioid receptors mediate a substantial proportion of analgesia produced by systemically applied opioids (reviewed in 4, 63). A recent clinical study showed that the selective blockade of peripheral opioid receptors by methylnaltrexone led to a 55% increase in systemic morphine requirements for sufficient postoperative pain relief during the first four hours after knee replacement surgery (97). Peripheral application of opioids became an area of considerable interest because many pain syndromes depend to a significant degree on the peripheral activation of DRG neurons, and side effects may be reduced (1). The most extensively studied and well-established regimen is intraarticular

morphine administration during surgery (92, 94, 98, 99). Meta-analyses showed that its analgesic efficacy is similar to the local anesthetic bupivacaine (100). In many small clinical studies, locally applied opioids (e.g., dermal formulations, gels) have shown analgesic actions in the treatment of skin ulcers, cystitis, cancer-related oral mucositis, corneal abrasion, neuropathic pain, chronic arthritis, and bone injury (reviewed in 93). No significant adverse effects have been reported so far.

For systemic administration, novel opioid receptor ligands are being developed. A common approach is the use of hydrophilic compounds with minimal capability to cross the blood–brain barrier. Among the first compounds were the mu-receptor agonist loperamide (known as an antidiarrheal drug) and the kappa-receptor agonist asimadoline. Peripheral restriction was also achieved with glucuronidation, arylacetamide, triazaspiro, morphinan-based and peptidic compounds (reviewed in 4, 101, 102). Although earlier attempts to demonstrate peripheral opioid analgesia in healthy tissue failed, potent antinociception was consistently detected in models of nerve damage and of inflammatory, visceral, cancer, and bone pain (reviewed in 4, 34). In clinical studies, the peripherally restricted opioid morphine-6-glucuronide was shown to reduce visceral and postoperative pain with limited central side effects and efficacy similar to that of conventional opioids (103). Current research pursues the development of systemically applicable opioid agonists and enkephalinase inhibitors that do not permeate the blood–brain, intestinal, or placental barriers (4, 63, 68, 69, 101, 104, 105). In addition, gene-therapeutic approaches enhancing the expression of peripheral opioid receptors and peptides are being investigated (106, 107). Thus, the selective activation of peripheral opioid receptors promises advantages such as antiinflammatory actions and the absence of side effects typical for conventional opioids (sedation, nausea, apnea, fetal/neonatal depression, addiction) or nonsteroidal analgesics (gastrointestinal ulcers, bleeding, stroke, myocardial infarction) (91, 108).

The concept of biased signaling has generated considerable interest in the context of drug development (11, 26), although caveats have been raised. For example, intracellular reaction partners (e.g., arrestins) may be differentially involved in opioid receptor internalization depending on specific cell types, and ligand bias may not be conserved across different neuronal populations (109). Nonetheless, opioid agonists that preferentially activate G protein rather than arrestin binding were sought (110, 111). The underlying hypothesis was that arrestin binding promotes side effects such as sedation and gastrointestinal or respiratory dysfunction, whereas G protein activation primarily underlies analgesic effects. Initial studies in healthy human volunteers yielded mixed results. Compared to morphine, a biased mu agonist produced higher elevations of experimental cold thresholds, equally potent but shorter-lasting respiratory depression, and similar subjective central effects (nausea, dizziness, somnolence) (112).

Other approaches include exploiting selectivity of agonists for delta- or kappa-opioid receptors. However, the adverse side effects (e.g., convulsions, dysphoria) associated with the systemic administration of such agonists have inhibited their clinical development as analgesics (101, 113). Novel extended-release formulations of opioid agonists have become available (reviewed in 95, 114, 115). Besides prolonging the duration of action, many of those formulations aim at reducing the risk of overdose, tampering, and misuse, e.g., by embedding opioid antagonists or by adding features to deter crushing, snorting, or injecting. Unfortunately, such alterations may reduce analgesic efficacy, and even the most sophisticated galenic, pharmaceutic, and pharmacologic strategies have not succeeded in preventing abuse of opioids. This has been demonstrated by some popular and inventive forms of application (e.g., rectal, nasal, inhalant) even of purportedly tamper-resistant or abuse-deterrent formulations (114, 116, 117; **http://www.bluelight.org/vb/archive/index.php/t-419120.html**) and by postmarketing surveys indicating that prescribed extended-release formulations are substituted with other opioids

more amenable to tampering (115). These observations suggest that complete prevention of opioid abuse may never be achieved by pharmaceutical strategies alone but must involve psychosocial, regulatory, and educational approaches (114, 115, 117).

Opioid Receptor Antagonists

Opioid receptor antagonists have been used for treatment of constipation, the most frequent side effect of opioid medication in surgical and cancer patients. Constipation in these settings is mediated by intestinal and (partially) central mu receptors, does not readily exhibit tolerance, and is usually treated by laxatives (9, 118). As therapeutic alternatives, oral naloxone (a conventional nonselective opioid receptor antagonist) and the peripherally restricted antagonists methylnaltrexone and alvimopan were investigated with the aim of avoiding central effects that would reduce analgesia or produce withdrawal. Although some studies demonstrated reversal of constipation, their application in clinical practice is limited by relatively low response rates, adverse effects, increased opioid agonist consumption, and high costs (97, 118, 119).

Opioid Receptor Gene Variants

Personalized pain therapy based on genetics is an attractive concept. The mu-opioid receptor gene *OPRM1* was among the first genes screened for functional relevance with regard to analgesia. The human single-nucleotide polymorphism (SNP) *OPRM1* 118A>G is the most thoroughly investigated candidate to date. In vitro biochemical and molecular assays indicated altered binding affinity, signal transduction, and expression. These differences were assumed to underlie occasionally diminished opioid efficacy in patients. However, meta-analyses revealed that these findings translate into very small clinical effects, such as slightly higher opioid dosing requirements for acute pain; the differences do not affect chronic pain or opioid side effects. Thus, this SNP appears to be without major clinical relevance as a solitary variant (120, 121). Nonetheless, efforts continue to find other genetic variants predicting analgesic efficacy and side effects of opioids (122, 123).

Opioid Tolerance

"Tolerance" describes the phenomenon that the magnitude of a drug effect decreases with repeated administration of the same dose or that increasing doses are needed to produce the same effect. Tolerance is not synonymous with dependence. All opioid effects (e.g., analgesia, nausea, respiratory depression, sedation) can be subject to tolerance development, albeit to different degrees. For example, tolerance to respiratory depression, sedation, and nausea often develops faster than to constipation or miosis (124, 125). Incomplete cross-tolerance between opioids or genetic differences may explain clinical observations that switching drugs ("opioid rotation") is occasionally useful in patients with inadequate pain relief or intolerable side effects (96, 125). Opioid-induced adaptations can occur at multiple levels in the nervous and other organ systems, beginning with direct modulation of opioid receptor signaling and extending to complex neuronal networks including learned behavior. Proposed mechanisms involved in pharmacodynamic tolerance include opioid receptor–G protein uncoupling, decreased receptor internalization/recycling, and increased sensitivity of the NMDA receptor (9, 25, 67) (**Figure 1**). In addition, pharmacokinetic tolerance (e.g., altered distribution or metabolism of the opioid) and learned tolerance (e.g., compensatory skills developed during mild intoxication), as well as increased nociceptive stimulation by tumor growth, inflammation, or neuroma formation are possible reasons for increased dose

requirements (124). There is a lack of carefully controlled studies that unequivocally demonstrate pharmacodynamic tolerance to opioid-induced pain inhibition in patients (72, 126). Tolerance development may be reduced in models of chronic pain (26). In inflammatory pain this has been related to enhanced recycling of peripheral opioid receptors (65, 67).

Opioid-Induced Hyperalgesia

There is an ongoing debate on whether opioids paradoxically induce hyperalgesia. However, upon closer scrutiny of the available data it appears that most studies have in fact shown withdrawal-induced hyperalgesia, a well-known phenomenon following the abrupt cessation of opioids (20, 127). At ultrahigh doses, occasionally encountered in extreme cancer pain, singular cases of allodynia have been observed and attributed to neuroexcitatory effects of opioid metabolites. There is no conclusive evidence that hyperalgesia occurs during the perioperative or chronic administration of regular opioid doses in patients (126, 127).

Long-Term Opioid Use in Chronic Pain

Conventional opioid agonists are undisputed in the treatment of severe acute and cancer pain, but their long-term use in chronic nonmalignant (e.g., neuropathic, musculoskeletal) pain has not proven effective (128). Instead, addiction, overdoses, death rates, and abuse of prescription opioids have reached epidemic proportions and have become a public health problem (129–131). Meta-analyses show clinically insignificant reduction of pain scores, and epidemiologic data suggest that quality of life and functional capacity are not improved (128, 132). Adverse side effects (nausea, sedation, constipation, dizziness, respiratory depression, cardiac arrhythmia, cognitive deficit, endocrinopathy) and lack of analgesic efficacy have led to the dropout of high numbers of subjects in long-term studies (72, 128, 131, 133–136). Indeed, considering the multifactorial biopsychosocial etiology of chronic pain, it is not surprising that opioids alone do not produce analgesia if, for example, there is a major affective component or if learned pain behavior is the main problem (137, 138). The target of intervention is not only the source of nociception (if at all identifiable) but suffering, dysfunction, psychosocial factors, and dependence on the healthcare system. Thus, the use of opioids as a sole treatment modality in chronic nonmalignant pain is not recommended. Instead, chronic pain requires a multidisciplinary approach encompassing various pharmacologic, psychological, and physiotherapeutic treatment strategies (8).

CONCLUSIONS AND OUTLOOK

Opioids are the oldest and most potent drugs for the treatment of severe acute and cancer pain. However, their long-term use in chronic nonmalignant pain has not proven effective and carries the risks of addiction, overdosage, and abuse (128, 129). Thus, it is crucial to select suitable patients and to consider alternative (e.g., nonpharmacologic) therapeutic avenues in multifaceted syndromes such as chronic pain. Several avenues of research are being pursued to find new treatment approaches that reduce current risks.

A field of intense investigation is the endocytic trafficking and processing of opioid receptors in sensory neurons, particularly the influence of injury and the implications for tolerance development and for the efficacy and potency of opioid agonists. Furthermore, the recent flurry of studies on GPCR structures enables novel approaches to elucidate ligand-biased signaling, as well as allosteric, oligomeric, and heteromeric modulation of opioid receptor function. Whether

genetic or epigenetic variations in opioid receptor expression play a significant role in the clinical efficacy of opioid analgesics needs further investigation (10). For example, species differences in the expression of GIRK channels and opioid effects in DRG neurons have been demonstrated, raising questions about the most suitable animal species to model humans (18).

Despite convincing data on the attenuation of neuropathy-induced hypersensitivity in vivo, the expression, axonal transport, and signaling of opioid receptors after nerve injury have not been thoroughly examined. In addition, the role of leukocytic opioid receptors in pain modulation needs to be explored. An area that has not received much attention is the influence of opioids on inflammation and wound healing. Although there is ample evidence for beneficial effects from basic research, clinical studies are lacking to date (63). Similarly, clinical studies on the augmentation of effects of endogenously released opioid peptides by inhibiting their degrading enzymes are needed (68, 69).

The epidemic of opioid misuse in chronic pain illustrates the persistent (and worsening) problems that result from nonselective activation of ubiquitous opioid receptors throughout central and peripheral compartments. The potential of peripheral actions is increasingly recognized by researchers and clinicians (1, 4, 68, 92, 94, 99, 104). Peripheral opioid receptor activation can reduce pain and inflammation while avoiding sedation, respiratory depression, addiction, and adverse effects typical of nonsteroidal analgesics. However, beyond the described ongoing efforts in drug development, technology-oriented research (e.g., development of nanocarriers) is needed to find novel approaches to peripheral restriction of opioids (**Figure 3**). In oral application, such compounds should be able to penetrate the gastrointestinal barrier but not the blood–brain or placental barrier (139). In topical application, they should sustain slow release of active drug. Manipulating the perineurium of peripheral sensory neurons is another interesting approach (42, 43). These endeavors may eventually lead to novel pain medication with fewer side effects.

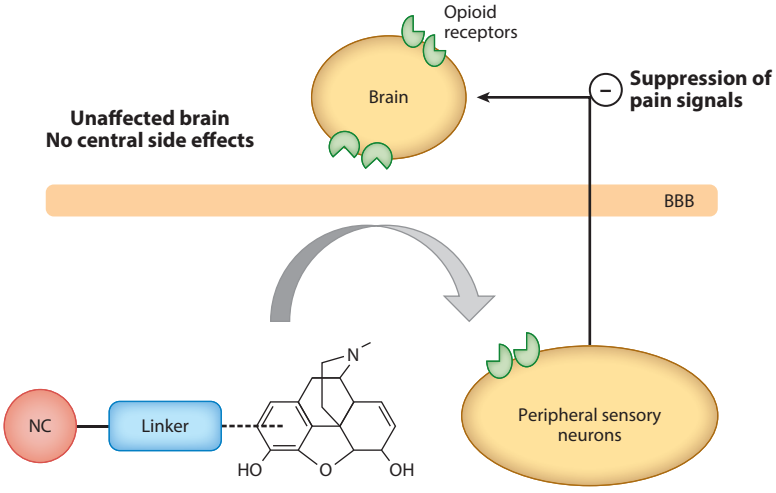

Figure 3

Example of a strategy to reduce central side effects by linking morphine to a nanocarrier (NC) that does not cross the blood–brain barrier (BBB) but selectively releases morphine in the vicinity of peripheral sensory neurons.

DISCLOSURE STATEMENT

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LITERATURE CITED

1. Baron R, Hans G, Dickenson AH. 2013. Peripheral input and its importance for central sensitization. *Ann. Neurol.* 74:630–36
2. Basbaum AI, Bautista DM, Scherrer G, Julius D. 2009. Cellular and molecular mechanisms of pain. *Cell* 139:267–84
3. Stein C, Hassan AH, Przewlocki R, et al. 1990. Opioids from immunocytes interact with receptors on sensory nerves to inhibit nociception in inflammation. *PNAS* 87:5935–39
4. Stein C, Machelska H. 2011. Modulation of peripheral sensory neurons by the immune system: implications for pain therapy. *Pharmacol. Rev.* 63:860–81
5. Rittner HL, Brack A, Stein C. 2008. Pain and the immune system. *Br. J. Anaesthesia* 101:40–44
6. Herz A, Millan MJ, Stein C. 1989. Arthritic inflammation in rats as a model of chronic pain: role of opioid systems. *NIDA Res. Monogr.* 95:110–15
7. Cheng HY, Pitcher GM, Laviolette SR, et al. 2002. DREAM is a critical transcriptional repressor for pain modulation. *Cell* 108:31–43
8. Stein C. 2013. Opioids, sensory systems and chronic pain. *Eur. J. Pharmacol.* 716:179–87
9. Zöllner C, Stein C. 2007. Opioids. *Handb. Exp. Pharmacol.* 177:31–63
10. Cox BM. 2013. Recent developments in the study of opioid receptors. *Mol. Pharmacol.* 83:723–28
11. Law PY, Reggio PH, Loh HH. 2013. Opioid receptors: toward separation of analgesic from undesirable effects. *Trends Biochem. Sci.* 38:275–82
12. Katritch V, Cherezov V, Stevens RC. 2013. Structure-function of the G protein-coupled receptor superfamily. *Annu. Rev. Pharmacol. Toxicol.* 53:531–56
13. Schmidt Y, Gaveriaux-Ruff C, Machelska H. 2013. mu-Opioid receptor antibody reveals tissue-dependent specific staining and increased neuronal mu-receptor immunoreactivity at the injured nerve trunk in mice. *PLoS ONE* 8:e79099
14. Wang HB, Zhao B, Zhong YQ, et al. 2010. Coexpression of delta- and mu-opioid receptors in nociceptive sensory neurons. *PNAS* 107:13117–22
15. Bradbury A, Pluckthun A. 2015. Reproducibility: standardize antibodies used in research. *Nature* 518:27–29
16. Tedford HW, Zamponi GW. 2006. Direct G protein modulation of Cav2 calcium channels. *Pharmacol. Rev.* 58:837–62
17. Luscher C, Slesinger PA. 2010. Emerging roles for G protein-gated inwardly rectifying potassium (GIRK) channels in health and disease. *Nat. Rev. Neurosci.* 11:301–15
18. Nockemann D, Rouault M, Labuz D, et al. 2013. The K channel GIRK2 is both necessary and sufficient for peripheral opioid-mediated analgesia. *EMBO Mol. Med.* 5:1263–77
19. Endres-Becker J, Heppenstall PA, Mousa SA, et al. 2007. Mu-opioid receptor activation modulates transient receptor potential vanilloid 1 (TRPV1) currents in sensory neurons in a model of inflammatory pain. *Mol. Pharmacol.* 71:12–18
20. Spahn V, Fischer O, Endres-Becker J, et al. 2013. Opioid withdrawal increases transient receptor potential vanilloid 1 activity in a protein kinase A-dependent manner. *Pain* 154:598–608
21. Cai Q, Qiu CY, Qiu F, et al. 2014. Morphine inhibits acid-sensing ion channel currents in rat dorsal root ganglion neurons. *Brain Res.* 1554:12–20

22. Ingram SL, Williams JT. 1994. Opioid inhibition of Ih via adenylyl cyclase. *Neuron* 13:179–86
23. Gold MS, Levine JD. 1996. DAMGO inhibits prostaglandin E2-induced potentiation of a TTX-resistant Na^+current in rat sensory neurons in vitro. *Neurosci. Lett.* 212:83–86
24. Waldhoer M, Bartlett SE, Whistler JL. 2004. Opioid receptors. *Annu. Rev. Biochem.* 73:953–90
25. Williams JT, Ingram SL, Henderson G, et al. 2013. Regulation of mu-opioid receptors: desensitization, phosphorylation, internalization, and tolerance. *Pharmacol. Rev.* 65:223–54
26. Pradhan AA, Smith ML, Kieffer BL, Evans CJ. 2012. Ligand-directed signalling within the opioid receptor family. *Br. J. Pharmacol.* 167:960–69
27. McPherson J, Rivero G, Baptist M, et al. 2010. Mu-opioid receptors: correlation of agonist efficacy for signalling with ability to activate internalization. *Mol. Pharmacol.* 78:756–66
28. Walwyn W, Evans CJ, Hales TG. 2007. Beta-arrestin2 and c-Src regulate the constitutive activity and recycling of mu opioid receptors in dorsal root ganglion neurons. *J. Neurosci.* 27:5092–104
29. Mohr K, Schmitz J, Schrage R, et al. 2013. Molecular alliance—from orthosteric and allosteric ligands to dualsteric/bitopic agonists at G protein coupled receptors. *Angew. Chem. Int. Ed. Engl.* 52:508–16
30. Walwyn W, John S, Maga M, et al. 2009. Delta receptors are required for full inhibitory coupling of mu-receptors to voltage-dependent Ca^{2+} channels in dorsal root ganglion neurons. *Mol. Pharmacol.* 76:134–43
31. Berg KA, Rowan MP, Gupta A, et al. 2012. Allosteric interactions between delta and kappa opioid receptors in peripheral sensory neurons. *Mol. Pharmacol.* 81:264–72
32. Stein C, Clark JD, Oh U, et al. 2009. Peripheral mechanisms of pain and analgesia. *Brain Res. Rev.* 60:90–113
33. Tabas I, Glass CK. 2013. Anti-inflammatory therapy in chronic disease: challenges and opportunities. *Science* 339:166–72
34. Stein C. 1993. Peripheral mechanisms of opioid analgesia. *Anesth. Analg.* 76:182–91
35. Stein C. 1995. The control of pain in peripheral tissue by opioids. *N. Engl. J. Med.* 332:1685–90
36. Busch-Dienstfertig M, Stein C. 2010. Opioid receptors and opioid peptide-producing leukocytes in inflammatory pain—basic and therapeutic aspects. *Brain Behav. Immun.* 24:683–94
37. Hassan AHS, Ableitner A, Stein C, Herz A. 1993. Inflammation of the rat paw enhances axonal transport of opioid receptors in the sciatic nerve and increases their density in the inflamed tissue. *Neuroscience* 55:185–95
38. Zöllner C, Shaqura MA, Bopaiah CP, et al. 2003. Painful inflammation-induced increase in mu-opioid receptor binding and G-protein coupling in primary afferent neurons. *Mol. Pharmacol.* 64:202–10
39. Mousa SA, Cheppudira BP, Shaqura M, et al. 2007. Nerve growth factor governs the enhanced ability of opioids to suppress inflammatory pain. *Brain* 130:502–13
40. Jeanjean AP, Moussaoui SM, Maloteaux JM, Laduron PM. 1995. Interleukin-1 beta induces long-term increase of axonally transported opiate receptors and substance P. *Neuroscience* 68:151–57
41. Zhou L, Zhang Q, Stein C, Schäfer M. 1998. Contribution of opioid receptors on primary afferent versus sympathetic neurons to peripheral opioid analgesia. *J. Pharmacol. Exp. Ther.* 286:1000–6
42. Antonijevic I, Mousa SA, Schäfer M, Stein C. 1995. Perineurial defect and peripheral opioid analgesia in inflammation. *J. Neurosci.* 15:165–72
43. Rittner HL, Amasheh S, Moshourab R, et al. 2012. Modulation of tight junction proteins in the perineurium to facilitate peripheral opioid analgesia. *Anesthesiology* 116:1323–34
44. Patwardhan AM, Berg KA, Akopain AN, et al. 2005. Bradykinin-induced functional competence and trafficking of the delta-opioid receptor in trigeminal nociceptors. *J. Neurosci.* 25:8825–32
45. Berg KA, Patwardhan AM, Sanchez TA, et al. 2007. Rapid modulation of mu-opioid receptor signaling in primary sensory neurons. *J. Pharmacol. Exp. Ther.* 321:839–47
46. Bao L, Jin SX, Zhang C, et al. 2003. Activation of delta opioid receptors induces receptor insertion and neuropeptide secretion. *Neuron* 37:121–33
47. Gendron L, Lucido AL, Mennicken F, et al. 2006. Morphine and pain-related stimuli enhance cell surface availability of somatic delta-opioid receptors in rat dorsal root ganglia. *J. Neurosci.* 26:953–62
48. Zhang N, Rogers TJ, Caterina M, Oppenheim JJ. 2004. Proinflammatory chemokines, such as C-C chemokine ligand 3, desensitize mu-opioid receptors on dorsal root ganglia neurons. *J. Immunol.* 173:594–99

49. Rowan MP, Ruparel NB, Patwardhan AM, et al. 2009. Peripheral delta opioid receptors require priming for functional competence in vivo. *Eur. J. Pharmacol.* 602:283–87
50. Cayla C, Labuz D, Machelska H, et al. 2012. Impaired nociception and peripheral opioid antinociception in mice lacking both kinin B1 and B2 receptors. *Anesthesiology* 116:448–57
51. Rasenick MM, Childers SR. 1989. Modification of G_s-stimulated adenylate cyclase in brain membranes by low pH pretreatment: correlation with altered guanine nucleotide exchange. *J. Neurochem.* 53:219–25
52. Selley DE, Breivogel CS, Childers SR. 1993. Modification of G protein-coupled functions by low-pH pretreatment of membranes from NG108-15 cells: increase in opioid agonist efficacy by decreased inactivation of G proteins. *Mol. Pharmacol.* 44:731–41
53. Vetter I, Cheng W, Peiris M, et al. 2008. Rapid, opioid-sensitive mechanisms involved in transient receptor potential vanilloid 1 sensitization. *J. Biol. Chem.* 283:19540–50
54. Andreev N, Urban L, Dray A. 1994. Opioids suppress spontaneous activity of polymodal nociceptors in rat paw skin induced by ultraviolet irradiation. *Neuroscience* 58:793–98
55. Wenk HN, Brederson JD, Honda CN. 2006. Morphine directly inhibits nociceptors in inflamed skin. *J. Neurophysiol.* 95:2083–97
56. Moshourab R, Stein C. 2012. Fentanyl decreases discharges of C and A nociceptors to suprathreshold mechanical stimulation in chronic inflammation. *J. Neurophysiol.* 108:2827–36
57. Schmidt Y, Labuz D, Heppenstall PA, Machelska H. 2012. Cutaneous nociceptors lack sensitisation, but reveal mu-opioid receptor-mediated reduction in excitability to mechanical stimulation in neuropathy. *Mol. Pain* 8:81
58. Machelska H. 2011. Dual peripheral actions of immune cells in neuropathic pain. *Arch. Immunol. Ther. Exp.* 59:11–24
59. Hall KE, Liu J, Sima AA, Wiley JW. 2001. Impaired inhibitory G-protein function contributes to increased calcium currents in rats with diabetic neuropathy. *J. Neurophysiol.* 86:760–70
60. Mousa SA, Shaqura M, Khalefa BI, et al. 2013. Rab7 silencing prevents mu-opioid receptor lysosomal targeting and rescues opioid responsiveness to strengthen diabetic neuropathic pain therapy. *Diabetes* 62:1308–19
61. Labuz D, Machelska H. 2013. Stronger antinociceptive efficacy of opioids at the injured nerve trunk than at its peripheral terminals in neuropathic pain. *J. Pharmacol. Exp. Ther.* 346:535–44
62. Wei LN. 2011. The RNA superhighway: axonal RNA trafficking of kappa opioid receptor mRNA for neurite growth. *Integr. Biol.* 3:10–16
63. Stein C, Küchler S. 2012. Non-analgesic effects of opioids: peripheral opioid effects on inflammation and wound healing. *Curr. Pharm. Des.* 18:6053–69
64. Picard PR, Tramer MR, McQuay HJ, Moore RA. 1997. Analgesic efficacy of peripheral opioids (all except intra-articular): a qualitative systematic review of randomised controlled trials. *Pain* 72:309–18
65. Stein C, Pflüger M, Yassouridis A, et al. 1996. No tolerance to peripheral morphine analgesia in presence of opioid expression in inflamed synovia. *J. Clin. Invest.* 98:793–99
66. Likar R, Mousa SA, Philippitsch G, et al. 2004. Increased numbers of opioid expressing inflammatory cells do not affect intra-articular morphine analgesia. *Br. J. Anaesth.* 93:375–80
67. Zöllner C, Mousa SA, Fischer O, et al. 2008. Chronic morphine use does not induce peripheral tolerance in a rat model of inflammatory pain. *J. Clin. Invest.* 118:1065–73
68. Roques BP, Fournie-Zaluski MC, Wurm M. 2012. Inhibiting the breakdown of endogenous opioids and cannabinoids to alleviate pain. *Nat. Rev. Drug Discov.* 11:292–310
69. Schreiter A, Gore C, Labuz D, et al. 2012. Pain inhibition by blocking leukocytic and neuronal opioid peptidases in peripheral inflamed tissue. *FASEB J.* 26:5161–71
70. Whiteside GT, Adedoyin A, Leventhal L. 2008. Predictive validity of animal pain models? A comparison of the pharmacokinetic-pharmacodynamic relationship for pain drugs in rats and humans. *Neuropharmacology* 54:767–75
71. Woolf CJ. 2010. Overcoming obstacles to developing new analgesics. *Nat. Med.* 16:1241–47
72. Galer BS, Lee D, Ma T, et al. 2005. MorphiDex (morphine sulfate/dextromethorphan hydrobromide combination) in the treatment of chronic pain: three multicenter, randomized, double-blind, controlled clinical trials fail to demonstrate enhanced opioid analgesia or reduction in tolerance. *Pain* 115:284–95

73. Berge OG. 2011. Predictive validity of behavioural animal models for chronic pain. *Br. J. Pharmacol.* 164:1195–206
74. Mogil JS, Davis KD, Derbyshire SW. 2010. The necessity of animal models in pain research. *Pain* 151:12–17
75. Lee MC, Wanigasekera V, Tracey I. 2012. Imaging opioid analgesia in the human brain. *Trends Anaesth. Crit. Care* 2:244–48
76. Davis KD, Racine E, Collett B. 2012. Neuroethical issues related to the use of brain imaging: Can we and should we use brain imaging as a biomarker to diagnose chronic pain? *Pain* 153:1555–59
77. Roberts NJ, Vogelstein JT, Parmigiani G, et al. 2012. The predictive capacity of personal genome sequencing. *Sci. Transl. Med.* 4:133ra58
78. Stein C. 2013. Towards safer and more effective analgesia. *Vet. J.* 196:6–7
79. Mousa SA, Shakibaei M, Sitte N, et al. 2004. Subcellular pathways of beta-endorphin synthesis, processing, and release from immunocytes in inflammatory pain. *Endocrinology* 145:1331–41
80. Sitte N, Busch M, Mousa SA, et al. 2007. Lymphocytes upregulate signal sequence-encoding proopiomelanocortin mRNA and beta-endorphin during painful inflammation in vivo. *J. Neuroimmunol.* 183:133–45
81. Busch-Dienstfertig M, Labuz D, Wolfram T, et al. 2012. JAK-STAT1/3-induced expression of signal sequence-encoding proopiomelanocortin mRNA in lymphocytes reduces inflammatory pain in rats. *Mol. Pain* 8:83
82. Rittner HL, Hackel D, Voigt P, et al. 2009. Mycobacteria attenuate nociceptive responses by formyl peptide receptor triggered opioid peptide release from neutrophils. *PLoS Pathog.* 5:e1000362
83. Stein C, Hassan AHS, Lehrberger K, et al. 1993. Local analgesic effect of endogenous opioid peptides. *Lancet* 342:321–24
84. Mousa SA, Straub RH, Schäfer M, Stein C. 2007. Beta-endorphin, Met-enkephalin and corresponding opioid receptors within synovium of patients with joint trauma, osteoarthritis and rheumatoid arthritis. *Ann. Rheum. Dis.* 66:871–79
85. Likar R, Mousa SA, Steinkellner H, et al. 2007. Involvement of intraarticular corticotropin-releasing hormone in postoperative pain modulation. *Clin. J. Pain* 23:136–42
86. Sharp BM. 2006. Multiple opioid receptors on immune cells modulate intracellular signaling. *Brain Behavior Immun.* 20:9–14
87. Brack A, Rittner HL, Stein C. 2011. Immunosuppressive effects of opioids—clinical relevance. *J. Neuroimmune Pharmacol.* 6:490–502
88. Ekholm O, Kurita GP, Hojsted J, et al. 2014. Chronic pain, opioid prescriptions, and mortality in Denmark: a population-based cohort study. *Pain* 155:2486–90
89. Lin JG, Chen WL. 2008. Acupuncture analgesia: a review of its mechanisms of actions. *Am. J. Chin. Med.* 36:635–45
90. Kim W, Kim SK, Min BI. 2013. Mechanisms of electroacupuncture-induced analgesia on neuropathic pain in animal model. *Evid. Based Complement. Alternat. Med.* 2013:436913
91. Schumacher MA, Basbaum AI, Way WL. 2009. Opioid analgesics and antagonists. In *Basic and Clinical Pharmacology*, ed. BG Katzung, SB Masters, AJ Trevor, pp. 531–52. New York: McGraw-Hill Med.
92. Kalso E, Smith L, McQuay HJ, Moore RA. 2002. No pain, no gain: clinical excellence and scientific rigour—lessons learned from IA morphine. *Pain* 98:269–75
93. Graham T, Grocott P, Probst S, et al. 2013. How are topical opioids used to manage painful cutaneous lesions in palliative care? A critical review. *Pain* 154:1920–28
94. Zeng C, Gao SG, Cheng L, et al. 2013. Single-dose intra-articular morphine after arthroscopic knee surgery: a meta-analysis of randomized placebo-controlled studies. *Arthroscopy* 29:1450–58
95. Mesgarpour B, Griebler U, Glechner A, et al. 2014. Extended-release opioids in the management of cancer pain: a systematic review of efficacy and safety. *Eur. J. Pain* 18:605–16
96. Drewes AM, Jensen RD, Nielsen LM, et al. 2013. Differences between opioids: pharmacological, experimental, clinical and economical perspectives. *Br. J. Clin. Pharmacol.* 75:60–78
97. Jagla CA, Martus P, Stein C. 2014. Peripheral opioid receptor blockade increases postoperative morphine demands—a randomized, double-blind, placebo-controlled trial. *Pain* 155:2056–62

98. Stein C, Comisel K, Haimerl E, et al. 1991. Analgesic effect of intraarticular morphine after arthroscopic knee surgery. *N. Engl. J. Med.* 325:1123–26
99. Valverde A, Gunkel CI. 2005. Pain management in horses and farm animals. *J. Vet. Emerg. Crit. Care* 15:295–307
100. Wei J, Lei GH, Gao SG, et al. 2014. Single-dose intra-articular bupivacaine versus morphine after arthroscopic knee surgery: a meta-analysis of randomized-controlled studies. *Clin. J. Pain* 30:630–38
101. Kivell B, Prisinzano TE. 2010. Kappa opioids and the modulation of pain. *Psychopharmacology* 210:109–19
102. Stein C, Küchler S. 2013. Targeting inflammation and wound healing by opioids. *Trends Pharmacol. Sci.* 34:303–12
103. Dahan A, van Dorp E, Smith T, Yassen A. 2008. Morphine-6-glucuronide (M6G) for postoperative pain relief. *Eur. J. Pain* 12:403–11
104. Vadivelu N, Mitra S, Hines RL. 2011. Peripheral opioid receptor agonists for analgesia: a comprehensive review. *J. Opioid Manag.* 7:55–68
105. US Securities and Exchange Commission. 2014. Cara Therapeutics, Inc., 2013 Annual Report. **http://secfilings.nasdaq.com/edgar_conv_html%2f2014%2f03%2f28%2f0001193125-14-119005.html#FIS_BUSINESS**
106. Machelska H, Schroff M, Oswald D, et al. 2009. Peripheral non-viral MIDGE vector-driven delivery of beta-endorphin in inflammatory pain. *Mol. Pain* 5:72
107. Raja SN. 2012. Modulating pain in the periphery: gene-based therapies to enhance peripheral opioid analgesia: Bonica lecture, ASRA 2010. *Reg. Anesth. Pain Med.* 37:210–14
108. Coxib and Traditional NSAID Trialists' (CNT) Collaboration, Bhala N, Emberson J, et al. 2013. Vascular and upper gastrointestinal effects of non-steroidal anti-inflammatory drugs: meta-analyses of individual participant data from randomised trials. *Lancet* 382:769–79
109. Charfi I, Audet N, Bagheri Tudashki H, Pineyro G. 2015. Identifying ligand-specific signalling within biased responses: focus on delta opioid receptor ligands. *Br. J. Pharmacol.* 172:435–48
110. White KL, Scopton AP, Rives ML, et al. 2014. Identification of novel functionally selective kappa-opioid receptor scaffolds. *Mol. Pharmacol.* 85:83–90
111. DeWire SM, Yamashita DS, Rominger DH, et al. 2013. A G protein-biased ligand at the mu-opioid receptor is potently analgesic with reduced gastrointestinal and respiratory dysfunction compared with morphine. *J. Pharmacol. Exp. Ther.* 344:708–17
112. Soergel DG, Subach RA, Burnham N, et al. 2014. Biased agonism of the mu-opioid receptor by TRV130 increases analgesia and reduces on-target adverse effects versus morphine: a randomized, double-blind, placebo-controlled, crossover study in healthy volunteers. *Pain* 155:1829–35
113. van Rijn RM, Defriel JN, Whistler JL. 2013. Pharmacological traits of delta opioid receptors: pitfalls or opportunities? *Psychopharmacology* 228:1–18
114. Raffa RB, Taylor R Jr, Pergolizzi JV Jr. 2014. Sequestered naltrexone in sustained release morphine or oxycodone—a way to inhibit illicit use? *Expert Opin. Drug Saf.* 13:181–90
115. Alexander L, Mannion RO, Weingarten B, et al. 2014. Development and impact of prescription opioid abuse deterrent formulation technologies. *Drug Alcohol. Depend.* 138:1–6
116. Kruger R, Meissner W, Zimmer A. 2014. [Misuse of opioid analgesics. An internet analysis]. *Schmerz* 28:473–82. In German
117. Passik SD. 2014. Tamper-resistant opioid formulations in the treatment of acute pain. *Adv. Ther.* 31:264–75
118. Holzer P. 2009. Opioid receptors in the gastrointestinal tract. *Regul. Pept.* 155:11–17
119. Diego L, Atayee R, Helmons P, et al. 2011. Novel opioid antagonists for opioid-induced bowel dysfunction. *Expert Opin. Invest. Drugs* 20:1047–56
120. Mura E, Govoni S, Racchi M, et al. 2013. Consequences of the 118A>G polymorphism in the OPRM1 gene: translation from bench to bedside? *J. Pain Res.* 6:331–53
121. Walter C, Doehring A, Oertel BG, Lötsch J. 2013. Mu-opioid receptor gene variant OPRM1 118 A>G: a summary of its molecular and clinical consequences for pain. *Pharmacogenomics* 14:1915–25
122. Busch-Dienstfertig M, Roth CA, Stein C. 2013. Functional characteristics of the naked mole rat mu-opioid receptor. *PLoS ONE* 8:e79121

123. Bruehl S, Apkarian AV, Ballantyne JC, et al. 2013. Personalized medicine and opioid analgesic prescribing for chronic pain: opportunities and challenges. *J. Pain* 14:103–13
124. Collett BJ. 1998. Opioid tolerance: the clinical perspective. *Br. J. Anaesth.* 81:58–68
125. McNicol E. 2008. Opioid side effects and their treatment in patients with chronic cancer and noncancer pain. *J. Pain Pall. Care Pharmacother.* 22:270–81
126. Schneider JP, Kirsh KL. 2010. Defining clinical issues around tolerance, hyperalgesia, and addiction: a quantitative and qualitative outcome study of long-term opioid dosing in a chronic pain practice. *J. Opioid Manag.* 6:385–95
127. Fishbain DA, Cole B, Lewis JE, et al. 2009. Do opioids induce hyperalgesia in humans? An evidence-based structured review. *Pain Med.* 10:829–39
128. Reinecke H, Weber C, Lange K, et al. 2015. Analgesic efficacy of opioids in chronic pain: recent meta-analyses. *Br. J. Pharmacol.* 172:324–33
129. Paulozzi LJ. 2012. Prescription drug overdoses: a review. *J. Saf. Res.* 43:283–89
130. Vowles KE, McEntee ML, Julnes PS, et al. 2015. Rates of opioid misuse, abuse, and addiction in chronic pain: a systematic review and data synthesis. *Pain* 156:569–76
131. Chou R, Cruciani RA, Fiellin DA, et al. 2014. Methadone safety: a clinical practice guideline from the American Pain Society and College on Problems of Drug Dependence, in collaboration with the Heart Rhythm Society. *J. Pain* 15:321–37
132. Eriksen J, Sjogren P, Bruera E, et al. 2006. Critical issues on opioids in chronic non-cancer pain: an epidemiological study. *Pain* 125:172–79
133. Noble M, Treadwell JR, Tregear SJ, et al. 2010. Long-term opioid management for chronic noncancer pain. *Cochrane Database Syst. Rev.* 1:CD006605
134. Gustavsson A, Bjorkman J, Ljungcrantz C, et al. 2012. Pharmaceutical treatment patterns for patients with a diagnosis related to chronic pain initiating a slow-release strong opioid treatment in Sweden. *Pain* 153:2325–31
135. Schiltenwolf M, Akbar M, Hug A, et al. 2014. Evidence of specific cognitive deficits in patients with chronic low back pain under long-term substitution treatment of opioids. *Pain Phys.* 17:9–20
136. Rhodin A, Stridsberg M, Gordh T. 2010. Opioid endocrinopathy: a clinical problem in patients with chronic pain and long-term oral opioid treatment. *Clin. J. Pain* 26:374–80
137. Stein C. 1997. Opioid treatment of chronic nonmalignant pain. *Anesth. Analg.* 84:912–14
138. Fordyce WE. 1991. Opioids and treatment targets. *Am. Pain Soc. Bull.* 1:1–4
139. Rubelt MS, Amasheh S, Grobosch T, Stein C. 2012. Liquid chromatography-tandem mass spectrometry for analysis of intestinal permeability of loperamide in physiological buffer. *PLoS ONE* 7:e48502