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Expert Opinion on Investigational Drugs

ISSN: 1354-3784 (Print) 1744-7658 (Online) Journal homepage: http://www.tandfonline.com/loi/ieid20

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To cite this article: Christoph Stein (2018): New concepts in opioid analgesia, Expert Opinion on Investigational Drugs, DOI: 10.1080/13543784.2018.1516204

To link to this article: https://doi.org/10.1080/13543784.2018.1516204

Accepted author version posted online: 27 Aug 2018.

New concepts in opioid analgesia

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Keywords: acidosis, addiction, analgesia, biased signaling, constipation, G-protein coupled receptors, inflammation, injury, opioid, pain, respiratory depression, sedation

Abstract:

Introduction: Opioids are the oldest and most potent drugs for the treatment of severe pain, but they are burdened by detrimental side effects such as respiratory depression, addiction, sedation, nausea and constipation. Their clinical application is undisputed in acute (e.g. perioperative) and cancer pain, but their long-term use in chronic pain has met increasing scrutiny and has contributed to the current "opioid crisis".

Areas covered: This article reviews pharmacological principles and research strategies aiming at novel opioids with reduced side effects. Basic mechanisms underlying pain, opioid analgesia and other opioid actions are outlined. To illustrate the clinical situation and medical needs, plasticity of opioid receptors, intracellular signaling pathways, endogenous and exogenous opioid receptor ligands, central and peripheral sites of analgesic and side effects are discussed.

Expert opinion: The epidemic of opioid misuse has taught us that there is a lack of fundamental knowledge about the characteristics and management of chronic pain, that conflicts of interest and validity of models must be considered in the context of drug development, and that novel analgesics with less abuse liability are badly needed. Currently, the most promising perspectives appear to be augmenting endogenous opioid actions and selectively targeting pathological conformations of peripheral opioid receptors.

Article Highlights

- Tissue injury leads to excitation of peripheral sensory neurons (nociceptors). These signals are transferred to the spinal cord and brain, where they are integrated to generate the perception of pain. Because central sensitization critically depends on the peripheral drive by nociceptors, therapeutic interventions targeting such neurons are promising. Endogenous mechanisms counteract pain at peripheral and central levels. In injured peripheral tissue, immune cell-derived opioid peptides (endorphin) can silence nociceptors carrying opioid receptors.
- Opioid receptors are expressed by central and peripheral neurons. Agonist binding promotes intracellular coupling of $G_{i/o}$ proteins to the receptor. Downstream signaling pathways lead to blockade of neuronal excitation and analgesia. Subsequent binding of arrestins triggers receptor desensitization and internalization.
- Pathological (e.g. inflammatory) pain can lead to enhanced opioid receptor function. Thereby, significant analgesic effects are mediated by opioid receptors localized on peripheral sensory neurons.
- Opioid agonists inhibit clinical pain after peripheral (topical, intraarticular), neuraxial (intrathecal, epidural, intracerebroventricular), or systemic (intravenous, oral, subcutaneous, sublingual, transdermal) administration. Adverse effects include respiratory depression, sedation, addiction, nausea and constipation. Opioids alone are not appropriate for the treatment of chronic non-cancer pain. Species differences must be considered when comparing preclinical with clinical findings.
- Current research strategies aim at reducing side effects by augmenting endogenous opioid mechanisms, biased ligands and selective activation of peripheral opioid receptors. Both pharmacokinetic and pharmacodynamic concepts are pursued.
- Novel drugs for clinical application have not yet arisen from those strategies, although some compounds have advanced to clinical phase III trials.

1. Introduction

1.1. Pain generation

Pain may be roughly divided into two broad categories: physiological and pathological pain. Physiological (acute, nociceptive) pain is an essential early warning sign that usually elicits reflex withdrawal and thereby promotes survival by protecting the organism from (further) injury. In contrast, pathological (e.g. chronic neuropathic) pain is an expression of the maladaptive operation of the nervous system; it is pain as a disease involving complex biopsychosocial interactions [1]. Physiological pain is mediated by a sensory system consisting of primary afferent neurons, spinal interneurons, ascending tracts, and supraspinal areas. Trigeminal and dorsal root ganglia (DRG) give rise to high-threshold A□□ and C-fibers (nociceptors) innervating peripheral tissues (skin, muscles, joints, viscera). When peripheral tissue is damaged, nociceptors are sensitized and/or activated by thermal, mechanical and/or chemical stimuli. Examples are adenosine triphosphate, neuropeptides, nerve growth factor, prostanoids, bradykinin, proinflammatory cytokines and protons (Table 1) [2]. Many of these agents lead to opening of excitatory cation channels in the nociceptor membrane. This produces inward depolarizing currents and subsequent action potentials that are then conducted along the sensory axon to the dorsal horn of the spinal cord. Thereafter, these impulses are transmitted to ascending spinal neurons, brainstem, thalamus and cortex. Repeated nociceptor stimulation can sensitize both peripheral and central neurons (activity-dependent plasticity, “wind-up”). This can be sustained by changes in the expression of genes coding for neuropeptides, ion channels, receptors and signaling molecules (transcription-dependent plasticity) in peripheral and central neurons [2, 3]. Both induction and maintenance of central sensitization are critically dependent on the peripheral drive by nociceptors, indicating that therapeutic interventions targeting such neurons may be particularly effective [3, 4].

1.2. Pain inhibition

Concurrent with such excitatory events, powerful endogenous mechanisms counteracting pain unfold. This was initially proposed in the “gate control theory of pain” of 1965 and has since been corroborated and expanded by experimental data in the central nervous system (CNS) and in the periphery. In 1990, a “peripheral gate” at the source of pain generation was discovered by the demonstration that immune cell-derived opioid peptides can block the excitation of nociceptors carrying opioid receptors within injured tissue [5, 6]. This was confirmed by clinical studies in surgical patients [7], and it represented the first example of many neuro-immune interactions relevant to pain [8-11]. Other antiinflammatory mediators

were also found to be involved [12-14]. In the spinal cord, nociceptive signals are inhibited by the release of endogenous opioid peptides or 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. Descending inhibitory noradrenergic, serotonergic and opioid pathways also become activated. Key regions in the brain are the periaqueductal grey and the rostral ventromedial medulla, which projects to the spinal cord dorsal horn [2, 15]. The central integration of signals from excitatory and inhibitory neurotransmitters, cognitive, emotional and environmental factors eventually results in the perception of "pain". When the intricate balance between biological (neuronal), psychological (e.g. learning, memory, distraction) and social (e.g. attention, reward) factors becomes disturbed, chronic pain can develop [1, 16]. The treatment of acute and chronic pain remains a major challenge in clinical medicine and public health [4, 17, 18]. For example, less than half of patients undergoing surgery report adequate postoperative pain relief [18], and there is a lack of fundamental knowledge about the management of chronic pain which has led to widespread misuse of analgesic drugs [17].

1.3. Opioid receptors, signal transduction, receptor recycling

Opioid receptors are expressed by central and peripheral neurons, by neuroendocrine (pituitary, adrenals), immune, and ectodermal cells [10, 15, 19]. Early binding studies and bioassays defined three main types of opioid receptors in the CNS, the mu-, delta- and kappa-receptors [20, 21]. Additional receptor types were proposed (e.g. sigma, epsilon, orphanin) but are no longer considered "classical" opioid receptors. The identification of complementary DNA and the selective deletion of opioid receptor genes in mice confirmed the existence of only three genes [21, 22]. Opioid receptors belong to the class A gamma-subgroup of seven transmembrane G-protein-coupled receptors (GPCR) and show 50-70% homology between their genes [21, 23]. Additional pharmacological subtypes may result from alternative splicing, posttranslational modifications and/or receptor oligomerization (i.e. physical interaction between two or more receptor monomers) [20-22, 24]. Because many of those studies have relied on antibody-based experimental techniques, it is noteworthy that specificities of currently available antibodies have been questioned, thus raising caveats [25]. High-resolution crystallized tertiary structures of mu-, delta- and kappa-opioid receptors have been resolved [20].

Opioid receptors (and other GPCR) have orthosteric and allosteric binding sites. The former are defined as the sites for endogenous opioid peptides and standard exogenous ligands, the latter are separate sites for endogenous or exogenous modulators (see below) [23, 26]. After orthosteric binding of a ligand, conformational changes (possibly influenced by allosteric modulators) allow intracellular coupling of $G_{i/o}$ proteins to the receptor. At the G_α subunit, GTP replaces GDP, and dissociation of the heterotrimeric 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 membrane ion channels (Fig. 1) [19, 27, 28]. This interaction can modulate pre- and postsynaptic Ca^{++} currents and thereby attenuate the excitability of neurons and/or reduce the release of pronociceptive/proinflammatory neuropeptides [2, 27, 29, 30]. 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 [28, 31]. Opioids also inhibit Na^{+}-, I_h-, transient-receptor-potential (TRP)- and acid sensing ion-channels in DRG neurons, and excitatory postsynaptic currents in the spinal cord [15, 32-35]. In addition, the reduced peripheral release of proinflammatory neuropeptides can result in significant antiinflammatory effects [30]. The combination of these effects synergistically lead to decreased transmission of nociceptive stimuli at all levels of the neuraxis and to profoundly reduced perception of pain.

Various kinases can phosphorylate intracellular regions of opioid receptors and promote binding of arrestin molecules. This leads to receptor desensitization by preventing G-protein coupling, and to receptor internalization *via* clathrin-dependent pathways. Recycling of dephosphorylated opioid receptors and their reintegration into the plasma membrane reinstates signal transduction, whereas the alternative targeting to lysosomes leads to receptor degradation (Fig. 1) [36].

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 [15, 37-39]. The three opioid receptor types all mediate analgesia but differing side effects, mostly due to their variable regional expression and functional activity in different parts of central and peripheral organ systems. For example, mu-agonists produce respiratory depression, sedation, reward/euphoria, nausea and constipation. Kappa-agonists induce dysphoria, sedation and diuresis, and delta-receptors can mediate reward, respiratory depression and convulsions. Most of these effects are elicited in the CNS, while constipation is mainly mediated by opioid receptors in the intestinal myenteric plexus [15, 24, 40-45].

1.4. Endogenous opioids

Endogenous opioid peptides 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 receptors and kappa-opioid receptors, respectively. A fourth group of tetrapeptides

(endomorphins) with yet unknown precursors do not contain the pan-opioid motif but bind to mu-receptors with high selectivity. Opioid peptides are expressed throughout the central and peripheral nervous systems, in neuroendocrine tissues, and in immune cells [10, 11, 13, 15]. Interactions between immune cell-derived opioid peptides and peripheral opioid receptors have been examined extensively, particularly with regard to the generation of analgesia [9-11].

2. Pathophysiology and clinical challenges

2.1. Plasticity of the opioid system under pathological conditions

Pathological pain is associated with multiple adaptations in the nervous, endocrine and immune systems [1, 2, 9, 12]. Numerous investigations have been conducted in models of peripheral tissue or nerve injury associated with inflammation. This is in keeping with the notion that inflammation (and accompanying tissue acidosis) is an essential component of a large group of painful syndromes such as arthritis, neuropathy, cancer, wounds and surgery (Table 1) [2, 46]. Indeed, diseases with an inflammatory component have been termed our greatest health threat [47]. Initial investigations demonstrated upregulation of opioid receptors and peptides in the spinal cord [48]. In addition, evidence emerged that significant antinociceptive effects are mediated by opioid receptors localized on peripheral sensory neurons and that opioid agonists elicit stronger analgesic effects in inflamed than noninflamed tissue of animals and humans [5, 49-51]. These intriguing observations stimulated extensive research into the underlying mechanisms.

We and others found that peripheral tissue inflammation induced upregulation of opioid receptors and their mRNAs in DRG neurons, which was dependent on neuronal electrical activity and on local cytokine production [10, 19]. In addition, the peripherally directed axonal transport of opioid receptors in DRG neurons was increased [52], and the perineural barrier was disrupted, thus facilitating access of opioid agonists to their receptors [53]. These events were ascribed to the influence of various inflammatory mediators such as bradykinin, nerve growth factor and prostaglandins [10, 19]. Furthermore, it was shown that G-protein coupling of opioid receptors was augmented [54], and that low pH (as seen in inflammation; Table 1) increased opioid agonist efficacy *in vitro* [55, 56]. Recordings from sensory nerve fibers supplying injured tissue revealed opioid inhibition of spontaneous and stimulus-evoked action potentials (reviewed in [19]).

Nerve injury resulting in neuropathic pain is another condition influencing opioid receptor expression in peripheral sensory neurons. For example, upregulation of opioid receptors and accumulation of opioid peptide-producing immune cells was detected at the site of nerve injury, accompanied by enhanced antinociceptive activity of opioid agonists [9, 10, 57].

Thus, besides changes in the CNS, the expression, axonal transport, signaling and accessibility of opioid receptors on DRG neurons are augmented, suggesting that tissue or nerve injury are prerequisites to "unmask" peripheral opioid effects [19, 49, 51]. Opioid peptides and receptors expressed by immune cells can also contribute to analgesia [57].

The clinical significance of these observations 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 [7, 58, 59]. After knee surgery, the patients' pain and analgesic consumption was enhanced by blocking the interaction between the endogenous opioids and their receptors [7], and it was reduced by stimulating opioid peptide secretion or by intraarticular application of opioid agonists [1, 39, 50]. Moreover, endogenous opioid peptides within injured tissue were found to produce additive/synergistic analgesic effects rather than cross-tolerance at peripheral opioid receptors, both in animals and humans [57, 58, 60, 61]. After surgical injury, up to half of the analgesic effect produced by intravenous morphine can be mediated by peripheral opioid receptors [62].

2.2. Tolerance, dependence, addiction

Tolerance describes the phenomenon that the magnitude of a given drug effect decreases with repeated administration of the same dose, or that increasing doses are needed to produce the same effect. All opioid-induced effects (e.g. analgesia, nausea, respiratory depression, sedation, constipation) can be subject to tolerance development, albeit to different degrees [36, 44, 63-65]. In contrast to the experimental literature, there is a lack of carefully controlled clinical studies demonstrating the development of pharmacodynamic tolerance in opioid analgesia [66, 67]. Of note, pharmacokinetic (e.g. altered distribution or metabolism) and learned tolerance (e.g. development of compensatory skills), as well as increased nociceptive stimulation by tumor growth, inflammation or neuroma formation are possible reasons for increased dose requirements [63, 68]. Tolerance development was shown to be reduced in inflammatory pain. This was ascribed to the continuous presence of endogenous opioid peptides and enhanced recycling of peripheral opioid receptors [58, 61].

Dependence is not synonymous with tolerance. Physical dependence is defined as a state of adaptation that is manifested by a withdrawal syndrome elicited by abrupt cessation, rapid dose reduction, and/or administration of an antagonist [34, 69]. All opioids produce clinically relevant physical dependence, even when administered only for a relatively short period of time [70].

Addiction is a complex syndrome involving reward/euphoria, the urge to avoid withdrawal, craving, uncontrolled/compulsive drug use despite harmful side effects, and other drug-related aberrant behaviors (e.g. altering prescriptions, manipulating health care providers, drug hoarding or unsanctioned dose

escalation) [69]. Addiction is likely to play a role in opioid misuse by chronic pain patients, and by individuals with opioid use disorder associated with prescription opioids (see below) [71-75].

2.3. Opioid-induced hyperalgesia

There is an ongoing debate whether opioids paradoxically induce hyperalgesia. 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 [34, 76, 77]. At ultra-high doses, occasionally encountered in extreme cancer pain, singular cases of allodynia have been attributed to neuroexcitatory effects of opioid metabolites. To date, there is no conclusive evidence that clinically significant hyperalgesia occurs during the perioperative or chronic administration of regular opioid doses in patients [67, 76, 78, 79].

2.4. 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 non-malignant (e.g. neuropathic, musculoskeletal, abdominal) pain has not proven effective. Meta-analyses show clinically insignificant reduction of pain scores, and epidemiological data suggest that quality of life or functional capacity are not improved [74, 80, 81]. Adverse side effects (e.g. nausea, sedation, constipation, respiratory depression, cognitive deficits) and lack of analgesic efficacy have led to the drop-out of high numbers of subjects in long-term studies [66, 74, 81]. Considering the multifactorial bio-psycho-social etiology of chronic pain, it is indeed not surprising that drugs are not beneficial if affective components, learned pain behavior, dysfunction, psychosocial factors and dependence on the health care system are the main problems [16, 82]. Notwithstanding, opioids are prescribed widely and addiction, overdoses, death rates and misuse have reached epidemic proportions [72-75]. Thus, the use of opioids as a sole treatment modality in chronic non-malignant pain is strongly discouraged. Instead, chronic pain requires a multidisciplinary approach encompassing various pharmacological and non-pharmacological (psychological, physiotherapeutic) treatment strategies [1, 17, 72, 74].

2.5. Genetic variants and species-differences

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* 118 A>G is the most thoroughly investigated candidate to date. *In vitro* biochemical and molecular assays indicated altered

binding affinitiy, signal transduction and expression, and it was assumed that this may underly occasionally diminished opioid efficacy in patients. However, meta-analyses demonstrated that these findings translate only into very small clinical effects without major relevance [83, 84]. Thus, with the possible exception of the metabolic enzyme CYP2D6, current evidence is insufficient to base opioid analgesic prescribing on genetic factors of individual patients [85]. Nonetheless, because personalized pain therapy remains an attractive concept, efforts continue to find new genetic variants predicting analgesic efficacy and side effects of opioids [85-87].

Another important consideration are species differences [21, 24, 88]. Numerous studies on intra- and inter-species differences have demonstrated that even single amino acid variations in opioid receptor proteins can have measurable effects on the structure-activity of the receptor [21, 24, 31, 86, 87, 89]. Extensive alternative splicing of the mu-opioid receptor gene has been described in different species and strains, possibly enabling divergent anatomical distributions, expression levels, oligomers, recycling and intracellular signaling events [24]. In addition, disparate (sometimes opposite) CNS or intestinal phenomena were detected in mice versus rats or humans (e.g. G-protein activation, adenylyl cyclase, locomotion) [45, 90-96]. A recent study reported that tolerance to opioid-induced analgesia was dependent on mu-opioid receptors expressed in DRG neurons in mice [97], contrary to findings in rats and humans [58, 61]. Opioid agonists did not affect K^+ currents [31, 32] or TRPV1 channels [32, 35, 96] in mouse DRG neurons, but did so in rat [31, 33, 34, 96]. Studies on gene expression of K^+ channels suggested a greater similarity between humans and rats than between humans and mice [31]. Thus, mice may not be the most appropriate species to study opioid actions. Similar to other fields (e.g. immunology, oncology), all these findings have to be taken into consideration when preclinical data are analyzed to predict drug effects in humans (see below).

3. Current research strategies

The search continues for novel opioids and formulations to reduce adverse effects. Previously developed selective agonists for delta- or kappa-opioid receptors were troubled by unacceptable side effects (e.g. convulsions, dysphoria) [41, 98]. Thus, alternative strategies are being pursued.

3.1. Abuse-deterrent formulations

One approach to the problem of addiction was the development of “abuse-deterrent” formulations, e.g. by increasing resistance to crushing, chewing or dissolving, or by adding antagonists or other aversive ingredients. However, the active agents still retain euphoric or respiratory depressant properties. Indeed, the dissemination of such formulations has spawned sophisticated ways of defeating them, and has led to

increasing heroin use and death rates. This has reinforced the notion that complete prevention of abuse will not be achieved by pharmaceutical strategies alone, but must include psycho-social and other (e.g. regulatory, educational) approaches [99-101].

3.2. Augmenting endogenous opioid mechanisms

Endogenous opioid peptides are susceptible to rapid enzymatic degradation by aminopeptidase N and neutral endopeptidase ("enkephalinases"). Preventing this degradation by inhibitors (in the CNS or in peripheral tissues) has been shown to produce analgesic effects in many animal models and in some human trials [8, 13, 102]. This strategy avoids unphysiologically high concentrations of exogenous agonists at (ubiquitously distributed) receptors and, thus, diminishes the risk for development of receptor downregulation, tolerance, desensitization, off-site or paradoxical excitatory effects [13]. Another interesting strategy is based on vaccination-induced recruitment of opioid peptide-producing lymphocytes to inflamed tissue [103].

3.3. Allosteric modulators

Allosteric modulators of opioid receptors were shown to influence the affinity and/or efficacy of orthosteric ligands *in vitro*, but evidence for *in vivo* efficacy is lacking so far [26]. Nonetheless, this concept is intriguing because positive allosteric modulators may enhance the activity of endogenous opioid peptides which are elevated during stress and pain. This activity would be confined to opioid receptors that are exposed to released endogenous opioids and would thereby avoid side effects (similar to the concept of enkephalinase inhibitors) [13, 26].

3.4. Bivalent ligands

Opioid receptors (and other GPCR) can form dimers or oligomers (i.e. two or more monomers physically interacting with each other). A number of bivalent ligands incorporating distinct pharmacophores for two receptors are being investigated [23, 100]. The underlying idea is that the combination of agonist and antagonist properties at different opioid or nonopioid receptors may reduce side effects. Indeed, some of these compounds have shown promising pharmacological profiles in preclinical investigations [100]. However, previous clinical studies have demonstrated that such compounds typically exhibit ceiling effects for analgesia and may elicit a withdrawal syndrome when administered together with a pure agonist [15].

3.5. Biased signaling

The concept of biased signaling (i.e. preferential activation of distinct intracellular pathways) has generated considerable excitement [22, 23, 36, 88, 100, 104]. Opioid agonists that primarily activate G-proteins rather than arrestins were sought for, based on the hypothesis that arrestin binding promotes side effects, while G-protein activation underlies analgesic effects [22, 88, 104]. However, upon systemic administration of opioid agonists, G-protein activation occurs not only in nociceptive neurons (which promote pain), but also in neurons driving respiration, arousal and intestinal peristalsis [42, 43, 45, 105]. Thus, an opioid agonist that (*via* G-proteins) effectively reduces electrical excitation of sensory neurons (an essential prerequisite for analgesia) will likewise (*via* G-proteins) inhibit the above mentioned neurons and thereby produce respiratory depression, sedation and constipation [42, 43, 45, 105]. Recent studies directly demonstrated opioid receptor activation in the brain [104, 106], nausea, vomiting [106] and respiratory depression [65] induced by purported biased agonists. Besides, intracellular reaction partners (e.g. arrestins) may be differentially involved in opioid receptor internalization depending on species and cell types, and ligand bias may not be conserved across different neuronal populations [36, 107]. Indeed, both animal [65, 108] and human [109] studies have demonstrated that prototype biased agonists produced similar side effects as conventional opioids.

3.6. Peripherally restricted opioid agonists

Targeting peripheral opioid receptors became an area of renewed interest. While earlier attempts to demonstrate peripheral opioid analgesia in healthy tissue failed, potent antinociception was consistently detected in models of nerve damage, inflammatory, visceral, cancer and bone pain [10, 49, 51, 98], in keeping with the notion that injury and inflammation unmask peripheral opioid effects (see above). In addition, awareness increased that many acute and chronic pain syndromes depend mainly on the stimulation of DRG neurons [3, 4], and that adverse effects of conventional opioids or of nonsteroidal analgesics (gastrointestinal ulcers, bleeding, stroke, myocardial infarction) [100, 110] may be avoided. Moreover, in contrast to other analgesic drug targets (e.g. the selective blockade of individual excitatory ion channels or receptors on neurons [4, 100]), a significant advantage of opioid receptor activation is the simultaneous and synergistic modulation of multiple molecules, e.g. Ca^{2+}-, K^{+}- and TRP-channels [10, 31-34], thus implying a wider range of efficacy.

Both animal and human studies have demonstrated that peripheral opioid receptors mediate a substantial proportion of analgesia produced by conventional opioids [10, 19]. In clinical trials the selective blockade of peripheral opioid receptors led to about 50% increase in intravenous morphine requirements for pain relief during the first four hours after knee replacement surgery, suggesting that about half of the

analgesic effect produced by systemic opioids is mediated outside the CNS [62]. The most extensively studied regimen is the intraarticular administration of low doses of morphine during surgery [39, 50, 111]. Meta-analyses showed that it produces postoperative pain relief of similar efficacy to local anesthetics [112]. In many small clinical trials, locally applied opioids (e.g. dermal formulations, gels) produced analgesic actions in skin ulcers, cystitis, oral mucositis, corneal abrasion, neuropathic pain, chronic arthritis and bone injury. No significant adverse effects have been reported so far [38, 39]. In addition, gene-therapeutic approaches enhancing the expression of peripheral opioid receptors and peptides have been investigated [113, 114].

Different strategies to obtain peripherally restricted opioids were pursued. A common approach is the development of hydrophilic substances with minimal capability to cross the blood-brain-barrier. Among the first compounds were the mu-agonist loperamide and the kappa-agonist asimadoline. Peripheral restriction was also aimed for with glucuronidation, arylacetamide, morphinan-based, triazaspiro and peptidic compounds [10, 30, 37, 98, 100, 115-117]. Several preclinical studies have described enkephalinase inhibitors with reduced barrier permeability, but clinical studies are lacking to date [8, 13]. In collaboration with a group of chemists, we used a strategy applying a cleavable linker to attach morphine to a polyglycerol-based nanocarrier [118]. This conjugate (PG-M) was devised to selectively release morphine in inflamed tissue and to preclude blood-brain barrier permeation due to its high molecular weight and hydrophilicity. Preclinical experiments showed that this construct exclusively activated peripheral opioid receptors to produce analgesia in injured tissue without evoking sedation or constipation [118].

In a recent cooperative project with mathematicians, we pursued a pharmacodynamic concept that is independent of pharmacokinetic issues such as barrier permeability, but relies on acidosis in damaged tissue (Table 1). We hypothesized that opioid receptors and ligands exhibit different conformation dynamics in inflamed compared to noninflamed tissue (brain, intestinal wall) [49, 119]. Novel methods of computer simulations indicated that opioid ligands assume a much more stable binding position at low pH than at physiological pH, suggesting that agonists have an enhanced potential to activate the receptor under acidic (inflamed) conditions. Based on these *in silico* studies, a prototype (NFEPP) was designed that selectively activated peripheral opioid receptors and induced analgesia in injured tissue (at low pH), while typical adverse effects (respiratory depression, reward, sedation, motor disturbance, constipation) elicited in noninjured environments (at normal pH in brain or intestinal wall) were absent [55, 120]. These results were attributed both to acidosis-induced conformational alterations of peripheral opioid receptors, and to the low acid dissociation constant of NFEPP (pK_a = 6.8). The latter property precludes protonation of a tertiary amine in the ligand (an essential prerequisite for activation of opioid receptors) in noninflamed tissues [55, 56, 121, 122]. This is unique because conventional opioid agonists have pK_a values above 7.5 and are therefore protonated and capable of activating opioid receptors at both low and normal pH values

[55, 56]. Importantly, both NFEPP and PG-M produced analgesic effects of similar magnitude to conventional opioids [55, 118, 120]. These findings await further toxicological evaluation and confirmation in clinical trials.

4. Conclusion

The most serious problems of currently available opioid analgesics arise from the non-selective activation of ubiquitous opioid receptors throughout central and peripheral compartments. To preclude adverse effects, promising perspectives are augmenting endogenous opioid actions and selectively targeting peripheral opioid receptors. Species differences and validity of animal models are important considerations when comparing preclinical and clinical data. Although some biased and peripherally selective agonists have advanced to phase III trials, novel drugs have not become available for routine clinical application. Besides pharmacological treatments, non-pharmacological approaches must be recognized, particularly in chronic non-cancer pain.

5. Expert opinion

The treatment of acute and chronic pain remains a major challenge in clinical medicine and public health. For example, less than half of patients undergoing surgery report adequate postoperative pain relief [18], and a lack of fundamental knowledge about the management of chronic pain has contributed to the widespread misuse of analgesic drugs [17]. This epidemic has not only taught us that novel analgesics with less abuse liability are badly needed, but that conflicts of interest and validity of models must be considered in the context of drug development. Although basic research on pain and analgesia continues at a rapid pace, translation into clinical applications has been difficult [4, 100]. Both diagnostic and therapeutic approaches (e.g. brain imaging, genetics) are being investigated, but have only rarely reached practical applicability in patients [84, 85, 123, 124]. Many obstacles have been discussed, including overinterpretation of data, reporting bias towards neglecting negative results, flawed study design, inadequate animal models, genetic and species differences [4, 66, 75, 100, 123, 125, 126].

The recent flurry of publications on GPCR structures enables novel approaches to elucidate biased signaling, allosteric and oligomeric modulation of opioid receptor function. Recent studies indicate that the dynamics of ligand-receptor interactions are different under normal versus pathological conditions [55, 56]. The most serious unresolved problems arise from the non-selective activation of ubiquitous opioid receptors throughout central and peripheral compartments. To avoid these, promising perspectives appear to be augmenting endogenous opioid actions and selectively targeting peripheral opioid receptors. Indeed, the potential of peripheral actions is increasingly recognized by researchers and clinicians [3, 4, 13, 14, 19,

37, 39, 74, 100, 111]. The selective activation of peripheral opioid receptors may be achieved by pharmacokinetic (e.g. PG-M) or pharmacodynamic (e.g. NFEPP) approaches. This strategy not only eliminates the source of pain generation in injured tissues, but also provides synergistic modulation of multiple excitatory molecules in nociceptive neurons, in contrast to other methods such as the blockade of individual ion channels or biased ligands [100].

Funding

This paper was funded by Bundesministerium für Bildung und Forschung

0316177B / C1, 01EC1403E, 01EC1403F, European Commission

EU FP7-HEALTH-2013-INNOVATION-1; No. 602891-2 and Helmholtz-Gemeinschaft

Helmholtz Virtual Institute "Multifunctional Biomaterials for Medicine"

Declaration of interest

C. Stein is listed as inventor on US Patent 9133120 B2 and European Patent 2 801 046.
The author has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

Reviewer disclosures

Peer reviewers on this manuscript have no relevant financial or other relationships to disclose

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Figure legends:

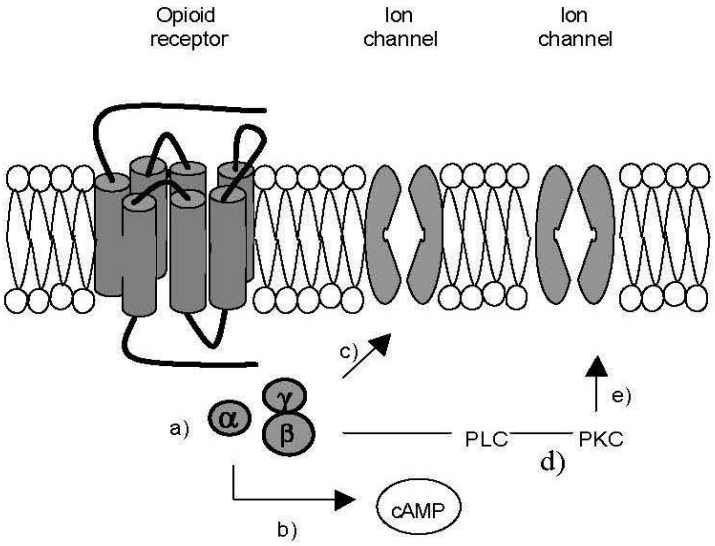
Fig. 1: Opioid receptor signaling and recycling (adapted from [127])

Upper panel: Opioid receptor ligands induce a conformational change at the receptor which allows coupling of G-proteins to the receptor. The heterotrimeric G-protein dissociates into active G_{α} and $G_{\beta\gamma}$ subunits (a) which can inhibit adenylyl cyclase and reduce cAMP production (b), decrease the conductance of voltage-gated Ca^{++} channels or open rectifying K^{+} channels (c). In addition, the phospholipase C / phosphokinase C pathways can be activated (d) to modulate Ca^{++} channel activity in the plasma membrane (e). Lower panel: Opioid receptor desensitization and endocytosis is activated by G-protein-coupled receptor kinases (GRK). After arrestin binding, the receptor is in a desensitized state at the plasma membrane (a). Arrestin-bound receptors can then be internalized via a clathrin-dependent pathway, and either be recycled to the cell surface (b) or degraded in lysosomes (c).

Adapted from *Handb Exp Pharmacol*. 2006/11/08 ed 2007:31-63. Published with permission of Springer.

Table 1: pH values in inflamed tissues measured *in vivo/ex vivo* (adapted from [46])

Figure 1


Opioid receptor
Ion channel
Ion channel
c)
e)
a)
α
γ
β
PLC
PKC
d)
cAMP
b)

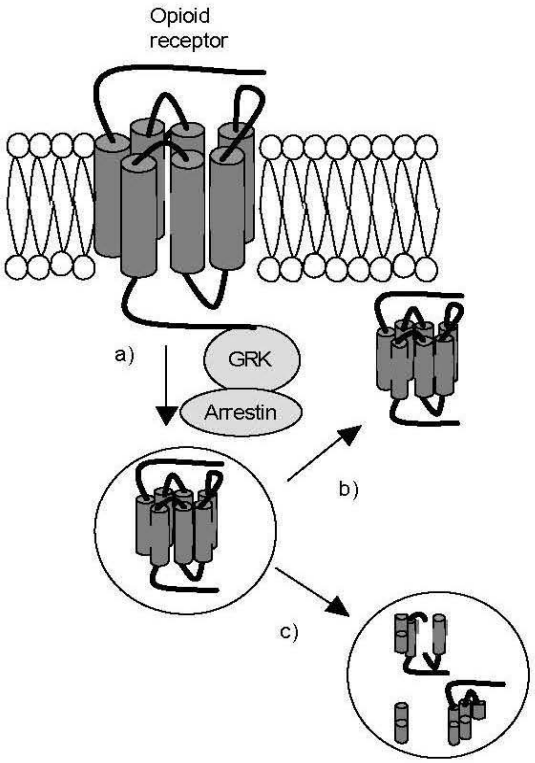

Opioid receptor
a)
GRK
Arrestin
b)
c)

Figure 1

Table 1: pH values in inflamed tissues measured *in vivo/ex vivo* (adapted from [46])

Reference	Species, tissue	lowest pH
Häbler C: Klin. Wochenschrift 1929;34:1569-71	Human, abscess	5.4
Koldajew B, Altschuler M: Z. Immunitätsforschung 1930;69:18-24	Guinea pig, intraperitoneal bacterial inoculation Mouse, s.c., i.p. bacterial inoculation	5.6 5.8
Menkin V: A 1934;10:193-210	Dog, turpentine-induced pleural exudate	6.6
Voegtlin C et al: Natl. Inst. Health Bulletin 1935;164:1-14	Rat, implanted tumors	6.82
Menkin V, Warner CR: Am. J. Pathol. 1937;13:25-43	Dog, turpentine-induced pleural exudate	6.5
Meyer et al: Cancer Res. 1948;8(11):513-8	Human, malignant tumors, inflamed tissues	5.44
Revici E et al: Bull. Inst. Appl. Biol. 1949;1:21-38	Human, malignant tumor	5.7
Menkin V. In: Biochemical Mechanisms in Inflammation. Ed. CC. Thomas. pp. 66-103, 1956	Dog, turpentine-induced pleural exudate	6.0

Jebens E, Monk-Jones Me: J Bone Joint Surg Br. 1959;41-B(2):388-400	Human, osteoarthritis, joint injury, synovial fluid	6.5
Pampus F: Acta Neurochir. 1963;11:305-18	Human, astrocytoma	5.85
Ashby BS, Cantab MB: Lancet 1966;Aug 6:312-5	Human, melanoma	6.4
Cummings NA, Nordby GL: Arthritis & Rheumatism 1966;9(1)47-56	Human, rheumatoid arthritis synovial fluid	7.08
Goldie I, Nachemson A: Acta orthop. Scandinav. 1969;40:634-41	Human, rheumatoid arthritis synovial fluid	6.0
Goldie I, Nachemson A: Acta orthop. Scandinav. 1970;41:354-62	Human, rheumatoid arthritis synovial fluid	6.4
Falchuk et al: Am J Med. 1970;49(2):223-31	Human, rheumatoid arthritis	6.84
Treuhaft & McCarty: Arthritis & Rheumatism 1971; 14(4): 475-84	Human, arthritis	6.60
Hutchins & Sheldon: Proc. Soc. Exp. Biol. Med. 1972;140(2):623-7	Rabbit, diabetic skin wounds	6.9
Silver: Philos. Trans. R. Soc.	Rabbit, brain, wounds, ischemia	5.0

Lond. B Biol. Sci. 1975;271(912):261-72		
Jacobus et al: Nature 1977;265:756-8	Rat, ischemic heart, intracellular	5.7
Levine & Kelly: J. Reprod. Fert. 1978;52:333-5	Rat, seminiferous tubules and epididymis	6.57±0.08
Vaupel et al: Cancer Res. 1981;41:2008-13	Mouse mammary carcinoma	5.8
Farr et al: Clin. Exp. Rheumatol. 1985;3:99-104	Human, rheumatoid and osteoarthritis synovial fluid aspirated	6.85
Punnia-Moorthy: J. Oral Pathol. 1987;16:36-44	Rat, air pouch granuloma induced by carrageenan, dextran, staph. aureus	6.87
Pan et al: PNAS 1988;85:7836-9	Human, exercised muscle, intracellular pH	6.1
Hood et al: Am. J. Physiol. 1988;255:F479-85	Human, exercised muscle, calculated intracellular pH	6.31±0.09
Geborek et al: J. Rheumatol. 1989;16:468-72	Human, rheumatoid arthritis synovial fluid	7.03
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Woo et al. Anesthesiology 2004;101:468-75	Rat, plantar/gastrocnemius incision	6.54±0.12
Gallagher et al. Nature 2008; 453(7197):940-3	Mouse, implanted subcutaneous lymphoma	6.0
Spahn, Del Vecchio et al. Science 2017;355:966-9	Rat, Freund's adjuvant paw inflammation; paw incision	6.8 7.02
González-Rodríguez et al. eLife 2017;6:e27081	Rat, Freund's adjuvant paw inflammation	6.82
Rodriguez-Gaztelumendi et al. Pain 2018, in press	Rat, chronic sciatic nerve constriction; intraperitoneal acetic acid injection	6.91 4.52 (5 min) 6.97 (15 min)