The search for novel analgesics: targets and mechanisms

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The search for novel analgesics: targets and mechanisms

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Abstract

The management of the pain state is of great therapeutic relevance to virtually every medical specialty. Failure to manage its expression has deleterious consequence to the well-being of the organism. An understanding of the complex biology of the mechanisms underlying the processing of nociceptive information provides an important pathway towards development of novel and robust therapeutics. Importantly, preclinical models have been of considerable use in determining the linkage between mechanism and the associated behaviorally defined pain state. This review seeks to provide an overview of current thinking targeting pain biology, the use of preclinical models and the development of novel pain therapeutics. Issues pertinent to the strengths and weaknesses of current development strategies for analgesics are considered.

Introduction

Failure to manage pain has important deleterious biological consequences including, but not limited to, cardiovascular pathology (hypertension, myocardial ischemia), suppression of gastrointestinal motility, suppression of spontaneous activity leading to joint and muscle deterioration, and the physiological (loss of appetite, failure to thrive) and psychological consequences of stress generated by the aversive state [1]. The negative consequences of pain are readily reflected in quality of life measures and economic indices [2–4].

Currently, there are several families of agents that have recognized clinical utility as pain therapeutics. They have varying degrees of efficacy for the different pain states and attendant adverse event profiles that often limit their utility. Management of inflammatory states typically involve nonsteroidal anti-inflammatory drugs (NSAIDs), for example, inhibitors of cyclooxygenases (COX-1 and/or COX-2) [5,6] and opiates [7]. For the states associated with nerve injury, therapies include antidepressants that block monoamine uptake (amitriptyline, duloxetine, venlafaxine) [8–10], anticonvulsants acting through a block of sodium channels (lidocaine, carbamazepine) [11–13], alteration of calcium channel function (e.g. ziconotide, gabapentin) [14,15] or through increasing extracellular levels of the inhibitory transmitter gamma aminobutyric acid (GABA) (tigabine) [16,17] and, to a lesser extent, opioids [7] and topical medications (lidocaine, capsaicin for patients with cutaneous allodynia and hyperalgesia) [18,19]. Often, combination therapies employing agents with distinct targets and non-overlapping side effect profiles may achieve improved therapeutic benefit in treating neuropathic conditions [20]. A point of interest has been whether any of these agents might be disease-modifying by preventing the transition from an acute reversible injury state to a chronic pain state (see below) [21,22].

While these agents may have demonstrated efficacy, it is frequently the case that, even with aggressive management, the patient's pain remains of such a magnitude that the patient could still be eligible to be admitted into the original study [23–25]. It is often difficult to know if the efficacy is restricted by a limited role for the mechanisms targeted, limitation of the drug target engagement, or that the side effects preclude higher doses [26–28]. Development of novel therapeutics to manage pain has thus been a focus of significant effort and investment [26,29]. In this brief overview, we will

consider current thinking regarding the complex biology of pain processing and how it informs the development of drug targets for analgesic therapeutics, and then offer a commentary on the screening of drugs with analgesic therapeutic potential. It should be emphasized that the above commentary is not limited to human therapies. There is a growing appreciation that the adequate treatment of pain in companion animals also presents important therapeutic issues, and the development of effective analgesics in that clinical population is itself an important target [30].

Pain phenotypes

Pain is the constellation of behavioral and physiological sequelae that can arise from three major classes of initiating conditions: (a) acute high intensity stimuli; (b) tissue injury and inflammation; and (c) injury to a specific peripheral nerve (mononeuropathy, e.g. crush, section) [9,31], or to all peripheral sensory nerves (polyneuropathy, e.g. diabetes, chemotherapy, or an immune-mediated reaction) [32,33]. The behavioral state initiated by these several conditions are intrinsically aversive, in that the state is associated with a negative affect (e.g. vocalization, facial expression) and supports complex escape and avoidance behaviors upon the first (e.g. unconditioned) exposure. These states each have defining properties.

After an acute stimulus, the pain sensation co-varies with stimulus intensity, is limited to the body region to which the stimulus is applied, and is absent with removal of stimuli.

Following tissue injury/inflammation, there is ongoing pain, which persists after removal of the injuring stimulus and the appearance of pain behavior with modest intensity stimuli applied to the injury site (primary hyperalgesia) and to adjacent uninjured regions (secondary hyperalgesia/allodynia).

Nerve injuries (mono- and polyneuropathies) are characterized by ongoing pain states (dysesthesia) and by an exaggerated sensitivity to light touch and cold, typically referred to the peripheral distribution of the injured nerve. Mononeuropathies include direct section or compression of a nerve trunk leading to pain referred to the distribution of the injured nerve. The polyneuropathy is typically bilateral in the distal extremities and may be generated by a variety of chemical insults (cancer chemotherapeutics, retroviral drugs), viral infections such as HIV, metabolic diseases (e.g. diabetes), and autoimmune pathologies. Importantly, while the exaggerated sensitivity to low intensity stimuli may appear similar after tissue and nerve injury, it is appreciated that the mechanical sensitivity observed after nerve injury may be mediated by the sensory afferents that normally encode light touch (e.g. Aß axons) [34].

The pain states arising from local tissue injury or inflammation typically show a time course that parallels the onset and resolution of the injury state [35], whereas nerve injury often leads to a persistent condition and may show little resolution over time. Following nerve injury, long-lasting changes in thresholds and the existence of an aversive state is noted in animal models [36–38].

There is a growing appreciation that the pain state originating from prolonged inflammation may persist even when the inflammatory state (e.g. loss of neutrophils/macrophages and pro-inflammatory cytokines) resolves. Thus, following surgeries such as herniorrhaphies, arthroscopies and thoracotomies, up to 30% of the population may show pain that lasts more than 3 months [39–41]. In the classic clinical example of persistent inflammation, rheumatoid arthritis, the pathology is characterized by joint inflammation and remodeling as well as pain. While the association of pain with inflammation is not unexpected, pain may continue to present as a problem, even in patients in remission or who show minimal inflammatory signs [42,43], suggesting the development of a chronic or enduring pain state. In preclinical models of acute injury and inflammation, a transient change in thresholds is commonly observed with the loss of the pain state observed with resolution of inflammation [44,45]. In contrast, long-lasting inflammatory states may demonstrate a resolution of inflammation with an enduring allodynia and persistent aversive component [46,47]. In animal models, the early phase is characterized by sensitivity to agents such as NSAIDs and agents that block hyperalgesia (gabapentin), while the late phase is characterized by a pharmacology resembling that noted with nerve injury [46,47]. As will be reviewed below, while pain associated with tissue injury and inflammation may be mechanistically and pharmacologically distinguishable from the states initiated by nerve injury, over time, in the persistent/chronic pain patient, there appears to be a convergence in mechanisms underlying chronic inflammation-evoked and nerve injury-evoked pain states.

Preclinical characterization of the "analgesic" actions of agents

In the following sections, we will briefly survey the biological targets that underlie the mechanisms through which the aversive stimulus environment is encoded. It is recognized that the assertion that a particular state represents a pain experience, and that conversely the actions of an agent targeting those mechanisms are "analgesic",

depends on the effects of these manipulations upon the behavior of the intact and unanesthetized animal. Such preclinical models have been widely employed in defining the behavioral correlates of nociceptive processing reflecting acute, post-tissue injury and post-nerve injury (neuropathic) pain states. Conversely, these models may be employed to "screen" the activity of a family of agents in development to define (a) their analgesic efficacy and (b) their relative analgesic versus behavioral side effect profile. Table 1 summarizes examples of the principal classes of models. Several issues may be considered in the implementation of these models.

Model paradigm

The preclinical experimental models outlined in Table 1 may be broadly divided into three major classes. First, those that typically utilize changes in the threshold stimulus intensity (e.g. temperature/mechanical) to initiate a response or the latency to escape (evoked behaviors). Some of these behaviors may employ a response phenotype organized at the spinal level. The simplest example would be the so-called tail flick response [48]. Technically, this is a poly-synaptic reflex movement of the tail, which is evoked by an acute high intensity thermal stimulus applied to the tail that can be observed in a spinalized animal. Other escape models may involve a supraspinal component (even a simple response such as the withdrawal of a hind paw requires complex motor coordination) [49]. A variant is the emission of higher order responses such as vocalization [50] and characteristic facial expressions in rodents [51]. Second, the measurement of ongoing behaviors that are otherwise suppressed/altered in a pain state [52,53], such as ambulation [54–57], weight bearing [58], locomotor patterns, feeding, or social interactions [59–64], rearing [65,66], and burrowing [67,68]. Third, an allied approach has sought to define the intrinsic aversiveness of a stimulus environment by employing a "conditional place preference" (CPP) paradigm to establish that a hypothesized injury state is indeed aversive and that a proposed intervention is able to attenuate that aversive component. In this, the animal with a tissue or nerve injury is exposed to a drug in a distinct environment *versus* a vehicle in a different environment. The animal is then permitted to choose either environment. If the injury induces an aversive state and if the drug treatment prevents/reduces that aversive state and if the treatment is not itself capable of producing a preference in a normal animal, then it is argued that the drug in the presence of the aversive state has a positive reinforcing value and will result in the selection of the paired environment [45,69–74]. A variant on this paradigm employs environments that become intrinsically aversive in the pain state, such as extreme light avoidance in a migraine model [75].

False positive versus false negative outcomes in analgesic studies

The incorrect assertion of analgesic activity of an agent may arise for several reasons. In the case of a behavioral paradigm, where the aversive stimulus evokes an escape response, a drug treatment leading to a loss of that

Table 1. Summary of preclinical models

Stimulus environment	Model	Time course	Reference
Acute	Heat/cold/mechanical withdrawal	Acute	[409–411]
Acute Afferent evoked hyperalgesia	Intraplantar formalin Intraplantar capsaicin	Min-hours	[412,413]
Inflammation - Acute	Carrageenan: thermal (heat and cold)/mechanical withdrawal	Min-hours	[320,414]
Inflammation - Chronic	Freunds adjuvant (systemic): allodynia	Days-weeks	[415]
	KBxN serum transfer (joint): allodynia	Days-months	[46]
	CAIA antibody transfer (joint): allodynia	Days-months	[47]
Osteoarthritis	Anterior cruciate ligament section: weight bearing, activity, mechanical thresholds	Days-months	[76,416,417]
	Iodoacetate model: weight bearing, activity, and mechanical thresholds	Days-weeks	[418]
Skin Incision	Plantar incision: tactile allodynia	Hours-days	[419]
Paw Burn	Plantar burn: thermal and mechanical allodynia	Hours-days	[420]
Visceral	Colonic distention/inflammation: pseudoaffective response, tactile sensitivity	Days-weeks	[421,422]
Pancreatitis	Evoked inflammation: affective behavior and hypersensitivity	Days-weeks	[423]
Bladder (Cystitis)	Evoked inflammation: abdominal hypersensitivity	Days-weeks	[424]
Mononeuropathy	Nerve compression: allodynia Nerve ligation: allodynia Needle stick: allodynia	Days-months	[425–427] [428] [433]
Polyneuropathy	Diabetic model (Streptozotocin): allodynia	Days-weeks	[429]
	Chemotherapy: tactile allodynia	Days-months	[430]
Spinal Injury	Compression/section: tactile allodynia	Days-months	[207]
Bone Cancer	Femoral osteosarcoma: pressure/allodynia	Days-weeks	[431,432]

response may be interpreted as a change in the processing of the aversive stimulus suggesting an "analgesic" treatment effect. To minimize the likelihood of a false positive, several controls may be taken. Firstly, at a minimum, this assertion of analgesic action rests upon the absence of a block of the animal's motor capacity to make the required response. Increased thresholds or latencies may reflect changes in motor strength, motor coordination, and competing arousal states (sedation or hyperactivity/stereotypic behaviors), leading to competing behaviors. The presence of ongoing spontaneous activity, normal ambulatory patterns, and simple reflexes may be necessary to corroborate the absence of obtunding behaviors or incapacity [76]. Secondly, the use of these models provides the greatest information when performed in the context of a range of doses, concurrently with a range of doses of standard, well-characterized agents, for example, NSAIDs (such as Ibuprofen or ketorolac), anti-convulsants (such as gabapentin), and/or opiates (such as morphine). Concurrent comparison of the dose response curves for side effect profile (e.g. changes in ambulation) and the anti-nociceptive action allows calculation of therapeutic ratios to permit comparison of the predicted relative safety of different agents or their combinations (see, for example, [77–79]). The use of pharmacologic standards permits comparison between research groups. Thirdly, co-varying results (namely equi-effective dosing and time course) on multiple behavioral paradigms in tests with common underlying mechanisms can also be used. Given comparable hypothesized mechanisms in different models we would not anticipate major differences in potency and time course for a given agent. Such differences should be considered as unanticipated. Fourthly, covariance between pharmacokinetic and pharmacodynamics as regards behavioral effects versus time course and concentrations in plasma and brain provides an indication that the drug as formulated and delivered has a predictable target engagement (see, for example, [80,81]).

Model selection

Model selection in Table 1 would likely be based on one of several factors. Firstly, an assumed target mechanism (an inflammatory model such as carrageenan or KBxN for an anti-inflammatory agent or models of central facilitation, such as intraplantar formalin or capsaicin). Secondly, an apparent comparability of the target model with the intended clinical target (e.g. face validity: an incision model for postoperative pain or a nerve injury model for a mononeuropathy). Thirdly, the specfic clinical pathology (e.g. chemotherapy-induced neuropathy). Fourthly, the specific model choice could depend upon whether the aim was to characterize the role of a novel mechanism in a specific pain state (such as a chemotherapy neuropathy) or whether the drug target is known, and a simple "screening" model is needed to characterize the relative activity of a family of agents. Here, opiates might be examined using acute thermal escape [82,83] or studies with glutamate receptor antagonists might employ the formalin flinching model [84,85]. Neither of those models have face validity for a clinical condition, but both reflect endpoints mediated by specific elements regulated by the respective drug targets. Labor-intensive models, such as the CPP, would be considered more appropriate to comfirm the analgesic properties of a specific target or drug effect, as opposed to the need to screen large numbers of agents.

Species

The majority of such work has involved rodents. Several points should be made. There are strain differences reported amongst mice and rats in various pain models and these must be considered when considering reported results [86]. With regard to the use of non-rodent species, experimental primate models of threshold, tissue and nerve injuries have been described [87–89], but there is no consensus that such models reveal distinctive mechanisms or are associated with increased predictive reliability. There has been an increase in interest in using other models such as companion animals (e.g. dogs and cats) in the context of clinical trials with validated behavioral inventories in animals with an ongoing clinical pathology (e.g. osteosarcoma and osteoarthritis) [90–92].

Sex

The majority of studies considering mechanisms and pharmacology of nociceptive processing have involved males. It has become apparent that, where examined, as with the role of cyclooxygenases [93], ASICs [94], toll-like receptors [95,96], and analgesics such as opiates [97], there can be significant differences between the responses of males and females.

Ethical considerations

While the importance of preclinical evaluations in pain research is apparent [86], an important caveat in the use of the unanesthetized animal to study persistent, inescapable pain states is the need to minimize unnecessary stress and utilize sufficiently powered paradigms to minimize animal use [98,99]. Importantly, the models listed in Table 1 have been subject to approval in the US by the relevant institutional animal care and use committees.

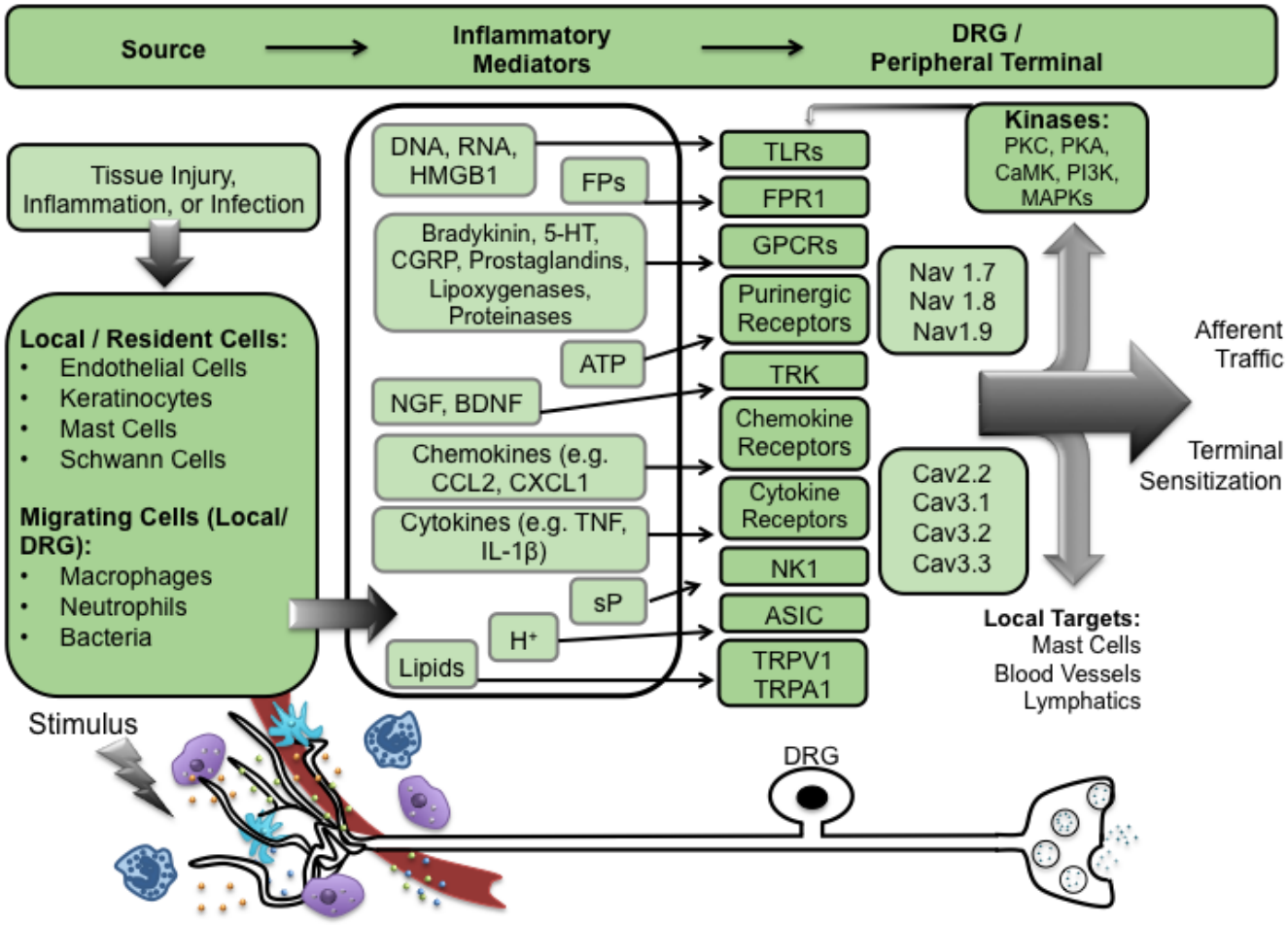
Mechanisms of behaviorally defined pain states

The protocol for defining systems relevant to pain processing involves demonstrating convergence between the underlying physiology of those systems that are activated by stimuli (which evoke the constellation of events defining a pain state) and the pharmacology that modifies the observed behaviorally defined pain state.

Acute high intensity thermal or mechanical stimuli

Acute high intensity thermal or mechanical stimuli result from activation of populations of lightly myelinated (Aδ) and slowly-conducting, unmyelinated (C) fibers. As summarized in Figure 1, the response properties of these afferents are defined by thermal or mechanical transduction channels such as the TRP family of channels (TRPA1/TRPV1) or mechanically sensitive large cation channels [100], which are expressed in dorsal root ganglion (DRG) cells and small afferent terminals. This depolarization of the C nociceptor leads to conducted potentials mediated by several types of voltage-gated sodium channels, some of which are selective for small afferents (e.g. NaV 1.7, 1.8 and 1.9) [101,102]. This afferent traffic invades the central terminal, opens voltage-gated calcium channels of the N type (CaV2.2), which serve to mobilize populations of

Figure 1. Summary of peripheral targets in nociceptive processing



Stimuli, such as tissue injury, inflammation, or infection, lead to the release of pro-inflammatory mediators from local/resident cells (mast cells, Schwann cells), migrating cells (macrophages, neutrophils), damaged cells, and blood vessels, at the peripheral terminal. These mediators act on receptors expressed on dorsal root ganglion cells and, when activated, evoke excitation and activation of intracellular kinases. This results in the phosphorylation of receptors inducing activation at lower thresholds, increased afferent traffic, and terminal sensitization.

DRG, dorsal root ganglion; DNA, deoxyribonucleic acid; RNA, ribonucleic acid; FP, formyl peptide; 5-HT, 5-hydroxytryptamine (serotonin); CGRP, calcitonin gene related peptide; ATP, adenosine triphosphate; NGF, nerve growth factor; BDNF, brain-derived neurotrophic factor; CCL, chemokine (C-C motif) ligand; CX3CL1, chemokine (C-X3-C motif) ligand; TNF, tumor necrosis factor; IL, interleukin; sP, substance P; H+, hydrogen ions; TRK, tyrosine receptor kinase; NK-1, neurokinin 1; ASIC, acid-sensing ion channel; TRPV1, transient receptor potential cation channel subfamily V, member 1; TRPA1, transient receptor potential cation channel, subfamily A, member 1; $Na_v1.7$, voltage-gated sodium channel type 1.7; $Ca_v2.2$, voltage gated calcium channel type 2.2; PKC, protein kinase C; PKA, protein kinase A; CaMK, calcium/calmodulin-dependent protein kinase; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; MAPK, mitogen-activated protein kinase; GPCR, G protein-coupled receptor; TLR, Toll-like receptor.

synaptic proteins (soluble N-ethylmaleimide-sensitive factor activating protein receptor [SNAREs]) [103]. This results in deployment of synaptic vesicles that contain and release a variety of excitatory (glutamatergic and peptidergic) transmitters at the first order synapse; these transmitters act through eponymous ionotropic (AMPA) and metabotropic (metabotropic glutamate receptor [mGluR] 1 and 5; NK1) receptors to acutely depolarize the second order membrane [104].

The first order linkages of the primary afferents are formed with two functionally defined populations of second order neurons: those that receive largely Aδ/C fiber input (and are hence nociceptive specific neurons) and those that receive convergent input from both low threshold (Aß) and the high threshold sensory axons (referred to as wide dynamic range [WDR] neurons). In the nociceptive specific neurons, the frequency of discharge rises as stimulus intensity exceeds the threshold of the nociceptive afferents, while in the WDR neurons, the frequency of discharge progressively increases as the stimulus intensity increases over the thresholds for the low threshold and then high threshold nociceptive afferents, thus encoding as a function of frequency the range of stimulus intensity from non-aversive to aversive [105,106].

The postsynaptic activation resulting from large afferents projecting into the dorsal horn is subject to pre- and postsynaptic inhibitory regulation through GABAergic and glycinergic interneurons. Loss of that inhibition will typically lead to a marked enhancement of the postsynaptic activation otherwise initiated by Aß input [107], leading *in vivo* to a prominent tactile evoked pain behavior (e.g. allodynia) [108].

Tissue/nerve injury

Peripheral terminal/DRG

The ongoing pain represents ongoing small afferent traffic generated at the peripheral terminal by active factors released from blood (vascular injury, plasma extravasation), damaged/disrupted tissue, local-resident cells (keratinocytes, mast cells, endothelial cells, Schwann cells), and migrating inflammatory cells (macrophages, neutrophils) [109,110].

These factors include products derived from injured cells (RNA/DNA; H^+, HMGB1) [111], or DNA released from inflammatory cells (e.g. neutrophil extracellular traps [112], endothelial cells/clotting cascade and blood (bradykinin, serotonin), a variety of local inflammatory cells yielding lipid mediators formed by cyclooxygenase (prostaglandins) and lipoxygenases (hepoxilins, 5,6-epoxyeicosatrienoic acid [EET] [113–115]), proteinases (trypsin), ATP, growth factors (NGF/ brain-derived neurotrophic factor [BDNF]) [115], pro-inflammatory cytokines (tumor necrosis factor [TNF], interleukin [IL]-1β, IL-6) [116,117], chemokines (CCL2, CXCL1 and CXCL5) [118–120], and Gram negative and positive bacterial products acting through toll-like receptor 4 (TLR4) and formyl peptide receptor 1 [121,122]. In addition, the local milieu is altered by products released from sympathetic (epinephrine) and primary afferent terminals (substance P [sP], calcitonin gene related peptide [CGRP]) [123–126].

In each case, these products act upon eponymous receptors that are expressed on peptidergic (C fiber) DRG cells and, when activated, evoke excitation and activation of intracellular signaling through increased intracellular calcium ions (Ca^{2+}) and activation of different cascades, including that signaling through IP3, which leads to activation of a variety of terminal kinases including cyclic adenosine monophosphate (cAMP)-dependent protein kinase (PKA), protein kinase C (PKC), calcium/calmodulin-dependent protein kinase (CaMK) [127], phosphoinositide 3-kinase (PI3K) [128], and mitogen-activated protein kinases (MAPKs), including extracellular signal-regulated kinase (ERK), p38 MAPK and c-Jun N-terminal kinase (JNK). These kinases phosphorylate a variety of terminal membrane channels and protein, leading to their activation at lower thresholds and with increased functionality. The net effect of these events is to initiate and sustain ongoing small afferent traffic and produce a sensitization of the terminals of small afferents, such that they display an enhanced discharge for a given stimulus intensity.

After local injury to the nerve, there is a dying back of the injured axon and reactive changes in the DRG neuron (including an increase in protein expression and the expression of various transcription factors, such as activation transcription factor 3: ATF3) and sprouting of the peripheral afferent terminal. Of interest, after persistent peripheral inflammation, peripheral terminals also show evidence of extensive sprouting and changes in the DRG, resembling those observed after nerve injury [46,129]. Physiologically, the injured afferent axon shows prominent ongoing activity that is believed to provide a source for the ongoing pain that often characterizes the nerve and tissue injury states [130,131]. The ectopic afferent activity in the injured axon has been shown to arise from the neuroma and from the dorsal root ganglion cell body [132]. The origin of this ongoing activity reflects changes in channel expression where local nerve injury and persistent inflammation can lead to the following: (a) an upregulation of sodium channels in the C-fiber afferent axon [133–135] and a downregulation of potassium channels

[136]; (b) sympathetic ingrowth [137]; (c) cytokines and chemokines released from migration of inflammatory cells [138–140]; (d) activation of non-neuronal (satellite) cells [141]; and (e) cross-talk between ganglion neurons [142]. These effects jointly enhance excitability of the DRG membrane, leading to ectopic activity that projects into the dorsal horn. Further, as noted above, nerve injury pain states are often characterized by an exaggerated response to light touch (tactile allodynia), which is mediated, in part, by activity in large, low threshold mechanoreceptive afferents [31]. The ability of the large Aß axons to drive a pain state may reflect an exaggerated activation of the WDR neurons with which they make synaptic contact, secondary to loss of intrinsic inhibition otherwise regulating large afferent input (see below).

Central (spinal) sensitization

Ongoing small afferent input leads to increased transmitter release at the first order synapse, and a robust increase in the frequency of the response evoked by large and small afferent input in dorsal horn projection neurons. The increased activity secondary to C fiber input leads to increased neuronal excitability, such that subsequent afferent input will evoke a greater response in these second order projection neurons, a phenomenon characterized by the classic description of the enhanced response initiated by repetitive small afferent input ("wind-up") [143]. Convergence between the pharmacology of spinal facilitation and hyperpathic states emphasizes that this enhancement of spinal afferent processing contributes mechanistically to the enhanced pain states observed after tissue and nerve injury. Components of the biology of the facilitated state are summarized in Figure 2.

Enhanced excitability of the second order neuron

Enhanced excitability of the second order neuron results from engagement of the N-methyl-D-aspartate (NMDA) receptor (a calcium ionophore) secondary to removal of a magnesium channel block [144], increased trafficking of AMPA (2-amino-3-(5-methyl-3-oxo- 1,2-oxazol-4-yl) propanoic acid) receptor subunits, leading to an increase in the expression of calcium-permeable AMPA sites [145–147] and activation of mGluRs. For glutamate, activation of group I ($mGluR_1$ and $mGluR_5$), present on neurons and glia [148], leads to stimulation of phospholipase C, increased intracellular Ca^{2+} levels, activation of adenylate cyclase and induction of the ERKs/MAPKs [149]. Agents blocking these ionotropic [150–154] and group 1 metabotropic receptors (mGluR1 and mGluR5) [155,156] have potent effects upon the associated hyperalgesic states.

Increased intracellular calcium and activation of cAMP and IP3-DAG

Increased intracellular calcium and activation of cAMP and IP3-DAG pathways by the metabotropic receptors lead to increases in the activity of dorsal horn kinases, including CaMK [127], PI3K [128], PKA, PKC, and MAPKs serving to phosphorylate local membrane receptors and channels enhancing their functionality, such as the NR1 and NR2A or NR2B subunits of the NMDA receptor [157–159].

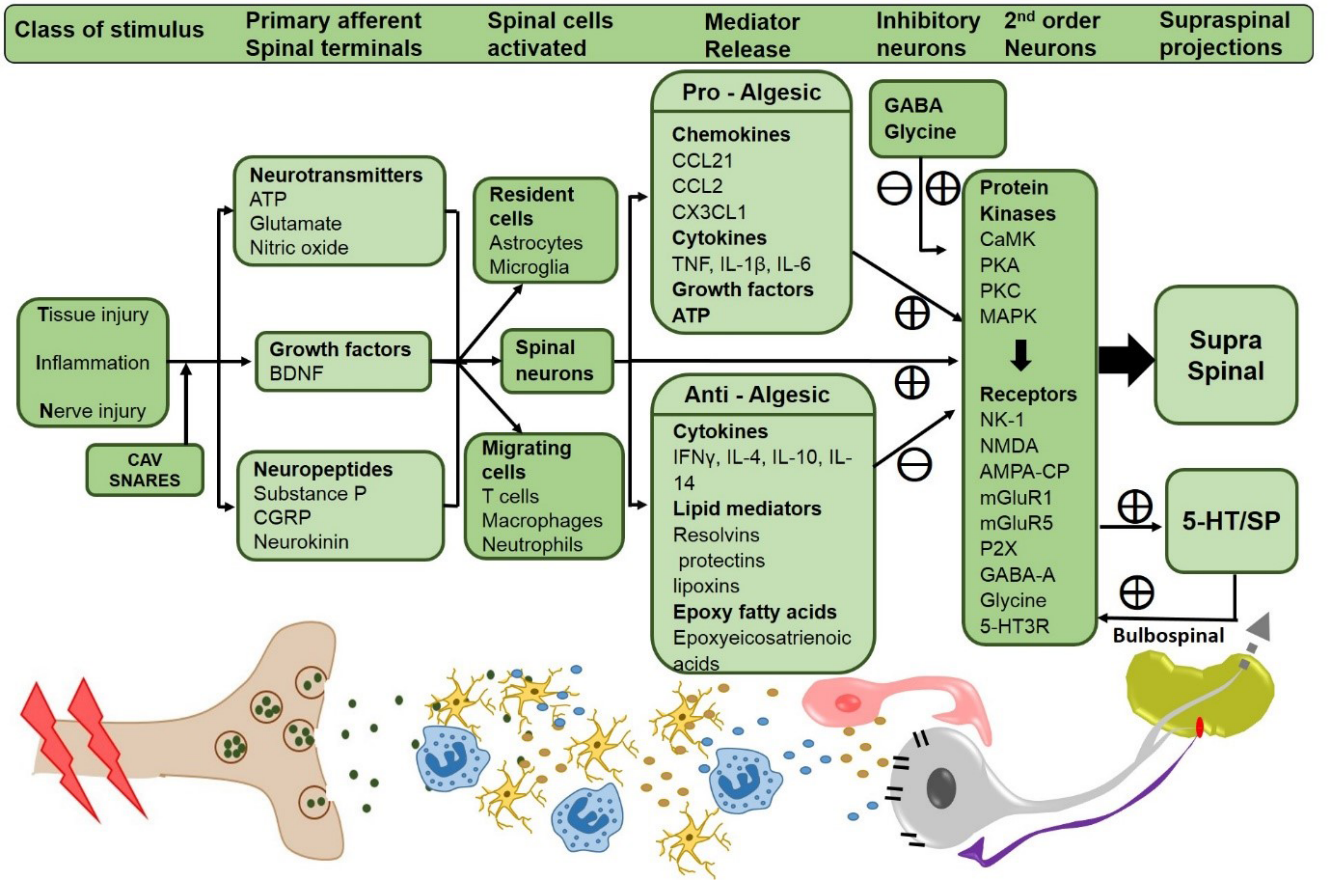
Other calcium-dependent pathways

Aside from being a charge carrier, increased intracellular calcium further serves to activate a variety of intracellular enzymes that are part of multiple facilitatory cascades. Several examples can be noted. Isoforms of phospholipase A2 lead to activation of signaling by a wide variety of constitutively expressed cyclooxygenases (COX-1 and COX-2) and lipoxygenase metabolites [110,160,161]. Prostaglandin E2, which through several eponymous receptors serves by activation of protein kinases (e.g. A and C) [162] to enhance pre-synaptic voltage-gated sodium and calcium channel function (in part through enhanced channel trafficking to the membrane) [163], also acts post-synaptically by attenuating glycine receptor function, thus, reducing constitutive dorsal horn glycine-mediated inhibition [164]. Nitric oxide synthases (NOSs) are constitutively expressed in neurons and glia and are activated by afferent traffic (through calcium ionophores such as NMDA and voltage gated calcium channels), leading to increased intracellular calcium [165,166]. Nitric oxide initiates downstream signaling through cyclic guanosine monophosphate (cGMP) and protein kinase G (PKG) to modulate neurotransmission through various ion channels [167] leading to the release of a variety of proalgesic factors [168]. Drugs targeted at several isoforms, notably neuronal NOSs, have been shown to possess efficacy in a variety of hyperpathic pain states [166,169–171].

Neuronal chloride homeostasis

As reviewed above, current work emphasizes the importance of constitutively active glycine and GABA-A receptors (chloride ionophores) in down regulating dorsal horn afferent-evoked activation, particularly that initiated by large afferent input [172]. Following nerve injury and in the face of high levels of spinal activation, there is an increased release of BDNF from the primary afferent and from microglia [173]. BDNF, acting through tyrosine receptor kinase (TRK)B, evokes down regulation of the K^+-Cl^- co-transporter (KCC2) in dorsal horn neurons [174]. In addition, activation of WNK-SPAK kinases inhibits KCC2 via phosphorylation [175]. This decrease in KCC2 activity leads to an increase in intracellular Cl^- [176–180]. Alternately, increasing intracellular Cl^- can occur secondary to increasing Na+-K+-2Cl- cotransporter-1 (NKCC1) [176,181]. In either case, these changes alter the result of chloride ionophore

Figure 2. Summary of central targets in nociceptive processing



Stimuli, such as tissue injury, inflammation, or nerve injury activates the primary afferents and induces CaV and SNARE-dependent release of neurotransmitters, growth factors and neuropeptides from the spinal primary afferents. Release of these substances activates the resident glial cells and migrating cells (T cells, macrophages and neutrophils) in the spinal cord along with the second order neurons. These cells release a constellation of pro-inflammatory and anti-inflammatory molecules which further act on the second order neurons activating several protein kinases responsible for the phosphorylation of several membrane bound receptors leading to the activation of second order neurons, thus initiating and maintaining the hyperexcitable state of these neurons, and further sending the nociceptive signals to higher brain centers. The second order neurons also project onto raphe-spinal serotonergic neurons which, through the bulbospinal pathway, terminate in dorsal horn neurons and serve to facilitate the excitability of dorsal horn projection neurons.

5-HT3, 5 hydroxytryptamine 3 receptor; AMPA-CP, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor-calcium permeable; ATP, adenosine triphosphate; BDNF, brain-derived neurotrophic factor; CaMK, calmodulin-dependent protein kinase; CaV, voltage-gated calcium channels; CCL, chemokine (C-C motif) ligand; CGRP, calcitonin gene related peptide; CX3CL1, chemokine (C-X3-C motif) ligand; GABA-A, gamma aminobutyric acid; IFNγ, interferon gamma; IL, interleukin; MAPK, mitogen-activated protein kinase; mGluR, metabotropic glutamate receptor; NK-1, neurokinin-1; NMDA, N-methyl D-aspartate; PKA, protein kinase A; PKC, protein kinase C; SNARE soluble N-ethylmaleimide-sensitive factor activating protein receptor; sP, substance P; TNF, tumor necrosis factor.

(GABA-A and glycine receptors) activation to yield membrane depolarization and hyperexcitability, rather than inhibition. Inhibition of NKCC1 with bumetanide or the recently discovered KCC2 activators lowered intracellular chloride concentrations and attenuated injury-evoked neuronal hyperactivity and hyperpathia in rodent models [181,182], emphasizing the potential utility of this approach in managing a variety of hyperpathic states influenced by this aberration of inhibitory regulation [175].

GTP Cyclohydrolase 1 (GCH1) and tetrahydrobiopterin (BH4)

GCH1 is present in spinal cord neurons and glia. Inflammation and nerve injury have been shown to result in its upregulation in glia and in neurons [183–185]. GCH1 is a pivotal regulator of BH4, an essential co-factor for nitric oxide synthase and aromatic hydroxylases that synthesize tyrosine, and several monoamines [186]. Excess BH4 results in a pain state and, conversely, inhibiting its synthesis reduces hyperpathic states [187]. Importantly, a GCH1 haplotype resulting in reduced BH4 synthesis has

been associated with a pain-protective effect in experimental and clinical pain phenotypes [188,189].

Activation of resident non-neuronal cells

After persistent inflammation and several forms of nerve injury, morphological changes are observed in a variety of glial cells in the peripheral nerve: Schwann cells [190], the dorsal root ganglion (satellite cells) [141], and spinal dorsal horn (microglia, astrocytes, and oligodendrocytes) [191]. Aside from morphology, persistent afferent traffic and nerve injury rapidly leads to phosphorylation of p38 MAPK isoforms and mediates the upregulation of pro-inflammatory pathways in microglia [168,192]. While glial activation is evident in many inflammatory and neuropathic states, it is not uniform. Thus, in some chemotherapeutic models, microglial responses are not evident [73]. This glial activation can be initiated by synaptic overflow through release from primary afferents and intrinsic neurons of a variety of molecules, including glutamate and peptides, such as sP, CGRP, angiotensin and purines (ATP). These products (delivered spinally or in cell culture) will lead to increased expression of gliotypic markers in DRG and spinal cord, and phosphorylation of intracellular transcription factors, such as ERK and p38 MAPK. Based on the anti-nociceptive suppression of glial activation of various antagonists, the action of purine in a variety of tissues and nerve injury pain states is considered to be mediated through P2X4/P2X7 [193,194] and angiotensin through the AT receptor 1 [195–197], chemokines (such as fractalkine via the CX3CR1 receptor) [198], and monocyte chemoattractant protein-1 ([MCP1] through the CCR2 receptor) [199], growth factors (e.g. neuregulin-1, NRG1) through tyrosine kinase receptors erbB3 and erbB4) [200], cytokines such as TNF through TNF-r 1 and 2 [201], and a variety of products such as HMGB1 and HSP90 via the TLR4/RAGE and TLR4 receptors, respectively. Many of these products are increased in spinal cord neurons and glia with persistent inflammation and nerve injury and act upon glial TLR4 [202–204], which has been shown to be expressed on astrocytes and microglia [96]. Glial activation by the above extracellular hormones, acting through eponymous receptors on microglia and astrocytes, serves to directly increase the excitability of the primary afferent terminal and second order neuron through the increased synthesis and glial release of purines (ATP), pro-inflammatory cytokines (TNF, IL-1β, IL-6, and interferon [IFN]γ) [71, 113, 205], lipid mediators (e.g. prostaglandin E2, hepoxylins, chemokines, such as CCL2) [206], and reactive-free radicals [207]. It has long been appreciated that tissue injury and inflammation increases spinal dynorphin message and expression [208,209] and intrathecal dynorphin delivery initiates proalgesic factor release and hyperalgesia [210,211]. Recent work has shown that spinal astrocytes produce and secrete dynorphin [212].

Conversely, injury and inflammation can reduce astrocyte expression of excitatory amino acid transporter 1 (EAAT1) and EAAT2, leading to increased extracellular concentrations of glutamate and excitation [213,214]. The emphasis upon glial activation has led to the effort to characterize the ability of agents reducing glial activation to attenuate nerve and post-tissue injury pain states. Agents such as cannabinoid (CB2) agonists, minocycline and propentofylline suppress glial activation and attenuate injury-evoked hyperpathia in animal models, though systematic trials have not shown promise [215,216]. Other approaches suppressing glial activation and injury-evoked hyperpathia include adenosine agonists [217,218], inhibitors of purinergic receptors including P2X7 [219–221] and P2Y12 [222,223], inhibitors of P38 MAPK [224,225], inhibitors of NF-kß [226], and inhibitors of TLR4 signaling [227–230].

Non-resident inflammatory cells in spinal cord and DRG

Following persistent inflammation and nerve injury, the presence of macrophages and T cells in the DRG and spinal cord has been identified [138–140]. The origin of this transmigration arises from several mechanisms. Endothelial cells respond to chemokines such as CC-chemokine ligand 2 (CCL2/MCP1) and CX_3C-chemokine ligand 1 (CX_3CL1: fractalkine) with increased expression/release of chemo-attractants such as CCL2 acting through CCR-2 to increase the presence of receptors and tethering proteins that facilitate trans-endothelial migration of monocytes and T cells across the blood–CNS barrier. CCL2 and CX_3CL1 also attract monocytes and T cells to the CNS [199,231], and produce an increase in neurovascular permeability secondary to the local release of transmitters from the peptidergic afferents, such as sP and CGRP [232,233].

Spinobulbospinal facilitatory pathways

Afferent input activates lamina I neuron excitatory projections onto raphe-spinal serotonergic neurons, which, in turn, terminate in dorsal horn neurons and serve to facilitate the excitability of the dorsal horn projection neurons [234]. Lesions of this system at the level of the ascending neuron or at the descending (bulbospinal) link [235–237] abolish allodynia. These effects are thought to be mediated through a spinal 5HT3 [235] (but see [238]), 5-HT_4, 5-HT_6 and 5-HT_7 receptors [239].

Intrinsic regulation of neuraxial processing

Spinal nociceptive encoding of afferent input is subject to a robust up-regulation of the input-output function.

Conversely, this excitation is subject to an extensive inhibitory control. Several examples of this regulation can be noted.

GABAergic and glycinergic interneuronal systems

These systems, through GABAA and strychnine-sensitive glycine receptors, constitutively regulate the excitation initiated by large afferent input. Thus, low-threshold primary afferent drive superficial GABAergic interneurons [240]. As reviewed above, loss of this constitutive inhibitory regulation, as occurs after peripheral inflammation and nerve injury, leads to a prominent allodynia [164,241].

Glutamate initiates excitation through ionotropic and metabotropic group I (mGLu1) receptors

The metabotropic group III receptors are primarily located on presynaptic terminals, where they act through $G_{i/o}$-coupled receptors to inhibit transmitter release through inhibition of voltage-gated calcium channels and activation of potassium channels [242]. Their activation can attenuate hyperpathic states after inflammation and nerve injury [243,244].

Bulbospinal regulatory systems

Descending catecholamine-containing projections act presynaptically on dorsal horn α(2A)-adrenoceptors on nociceptive primary afferent terminals and on second order projection neurons to regulate small afferent throughput [245]. Small afferent traffic has been shown to increase activity in these descending projections [246], and activation leads to regulation of nociceptive processing [245,247].

Anti-inflammatory cytokines

As noted, inflammation and nerve injury can lead to the appearance of macrophages and lymphocytes. When activated, these cells can release significant quantities of cytokines. While widely considered in terms of their pro-inflammatory phenotype, populations of these monocytic cells can express an anti-inflammatory phenotype [248,249], and reduce hyperpathic states [250,251]. The presence of such cells is consistent with the increased spinal expression of a variety of anti-inflammatory cytokines, including IFNγ, IL-4 and IL-10 and IL-17 following nerve injury and inflammation [159,252,253]. Agents activating increasing expression of anti-inflammatory agents reduce allodynia following neuropathies [254]. Increasing the presence of these anti-inflammatory cytokines in the spinal space, such as after transfection with viruses [255,256] or plasmids [257], has significant anti-hyperalgesic actions [258].

Anti-inflammatory lipid mediators

Considerable attention is paid to proalgesic actions of the lipid mediators released by neuronal and non-neuronal cells. However, a variety of anti-algesic lipid mediators are released in the periphery and spinal cord after peripheral injury and inflammation and can serve to suppress generation of the proalgesic and proinflammatory milieu. Several examples can be noted, including those agents synthesized by a cascade of 5- and 12-lipoxygenase, and cyclooxygenase 2 enzymes, the resolvins, protectins and lipoxins. Their actions are mediated by families of G protein-coupled receptors (GPCRs) present on macrophages and glia [259]. Local peripheral delivery of several of these lipids reduced the expression of proinflammatory cytokines and chemokines, including TNF, IL-1β, IL-6 and CCL2 [260]. In mice, their intrathecal delivery reduced inflammation and nerve injury-induced hyperpathia [260–263]. Another example of anti-inflammatory lipids is the metabolites of cytochrome P450 enzymes, specifically the EETs. These epoxy-fatty acids, along with epoxides of parent docosahexaenoic acid and eicosapentaenoic acid, have been shown to be anti-hyperalgesic in several pain models. The EETs are subject to rapid enzymatic degradation via the soluble epoxide hydrolase (sEH). sEH inhibition increases the concentrations of these EETs and exerts an anti-hyperalgesic action in inflammatory and neuropathic states [264,265].

Consideration of selected drug targets regulating nociceptive processing

A variety of targets have been identified that can potently regulate nociceptive processing at the level of the brain and spinal cord. Several exciting areas of work are briefly noted.

Opiates

Opiates exert their potent analgesic effects through one of three identified G protein-coupled opioid receptors (mu, delta and kappa) [266,267]. Mechanistically, these agents exert potent effects through spinal and supraspinal interactions [268,269]. Although well-studied, opiates have been shown to have mechanisms beyond simple receptor affiliation for future target development. Mu opiates have been shown to have a number of splice variants. The development of novel mu opiates has been targeted at selectivity for these splice variants [270,271]. Mu opiates are normally considered to couple through Gi/o protein in a ß arrestin-dependent fashion that serves to promote receptor internalization and desensitize G protein signaling [272]. Recent work has suggested activation can be achieved independently of ß arrestin signaling and these "G protein biased" ligands yield

increased analgesic efficacy and persistence [273–275]. Recognizing that opiate receptors can form heterodimers with a variety of G protein-coupled receptors has suggested the interesting development of a bivalent ligand, for example, interacting with opioid heterodimers, such as with a mu and delta receptor. Here, a combined mu agonist and delta antagonist ligand displays enhanced efficacy and a reduced propensity for tolerance [276,277]. Similar results have been observed with opioid and nonopioid heterodimers such as bivalent ligands for Mu - CB1 [278] and Mu-mGluR5 [279,280]. Importantly, the enhanced properties are not mimicked when unlinked ligands focusing on the two targets are given concurrently, or if short or longer linkers are employed [281].

Alpha-adrenergic agonists

Three major alpha-2 receptor subtypes (A-C) have been identified [282]. Dexmedetomidine and xylazine are potent alpha 2 adrenergic agonists approved as analgesics and induction (sedative) agents for human and veterinary use, respectively [283,284]. The specific roles of the several subtypes, namely pain processing versus the principal side effect (sedation), have been suggested [285], but are controversial.

Oxytocin

Oxytocin receptors are distributed in the superficial dorsal horn of the human spinal cord [286] and their activation has been shown to depress spinal excitability and reduce depression [287,288]. This spinal analgesic action may be mediated through an enhancement of GABAergic inhibition [289,290].

Cannabinoids

Cannabinoids interact with two receptors (CB1/CB2), both coupling through $G_{i/o}$ proteins [291]. Behavioral studies have shown that cannabinoids can yield prominent antinociceptive effects in a variety of animal models of acute, tissue injury-, and nerve injury-induced nociception [291]. The psychotropic effects of cannabinoids appear mediated through CB1 and not CB2 [292,293]. CB1 receptors are located on CNS neurons, notably the dorsal root ganglia [294] (while CB2 has been identified on monocytes and microglia), and their activation is believed to regulate inflammatory processes [291] in glia and to suppress glial activation [295]. Ligands interacting with the G protein-coupled CB1 and CB2 receptors have been shown to regulate nociceptive processing [296,297]. Agents increasing the presence of endogenous CB1 agonists by blocking its metabolism (through fatty acid hydrolase) have been implicated in activating cannabinoid receptor function [298]. Cannabinoid-initiated change in pain processing is based on the suppression of spinal and thalamic nociceptive neurons, but peripheral sites of action have also been identified.

Novel targeting strategies for analgesics

An exciting component of pain modulation has been the implementation of several novel approaches to modify the function of systems that process nociceptive information. A brief note on these approaches will be given below.

Targeted toxins

This approach takes advantage of toxin delivery to specific cell systems. Several examples include use of toxins coupled to agonists for G-protein-coupled receptors, which are internalized along with the receptor ligand into cells expressing the particular receptors upon activation. Such receptors include those for mu opiates and neurokinin 1 for sP, present on cells within the spinal pain pathway. A toxin, such as saporin, coupled to these ligands is taken up by cells expressing the relevant receptor. The internalized saporin blocks ribosylation and the targeted cells die, leading to a robust analgesia in a variety of models in several species, including the rodent [299] and dog [300,301], and is currently in clinical trials (NCT02036281). The irreversibility of toxins such as saporin and resiniferatoxin will likely preclude their use in non-terminal states. Other toxins that can be delivered in this manner are the light chain of Botulinum toxins, which, when attached to sP (or other G protein coupled receptor ligands that undergo internalization when activated by an agonist), will lead to cleavage of SNARE protein and block transmitter release [302]. Such an approach is reversible and may find utility in long-lasting, but non-terminal pathophysiological states. TRPV1 receptors are present on the terminals of small high threshold primary afferents and, when activated by ligands such as resiniferatoxin or capsaicin, will yield desensitization of the central (after spinal delivery) or peripheral (after peripheral delivery) afferent terminals expressing the channel, leading to a robust antinociception in rodents and dogs [303,304]. Topicals targeted at TRPV1 receptors have been approved [305], and intrathecal resiniferatoxin is in clinical trials (NCT00804154). A further modality for targeting TRPV1 channels takes advantage of the fact that, when activated, TRPV1 forms a pore that is able to pass large charged molecules. Protonated local anesthetics (e.g. QX314), which cannot normally enter the axon, can pass through TRPV1 channels that have been opened by TRPV1 agonists such as capsaicin and produce a selective block of sodium channel function in the TRPV1 (+) afferent axons [306].

Gene based approaches: specific block of protein synthesis

Targeting specific proteins that play a role in nociceptive processing has been accomplished with several methodologies, including intrathecally-delivered antisense and viral and nonviral transfection [307–309]. Intrathecal antisense has been employed to reduce expression of a variety of transmitters, including sP cellular matrix proteins (thrombospondin) [120] and receptors relevant to nociceptive processing, including the NK1 receptors [310], NMDA receptor subunits [311], P2X3 [118,312], TrpV1 receptors [313], and a variety of kinases, such as p38 MAPK [192,314], phospholipases [315], sodium channels – such as Nav 1.8: [316], TRKB [317], and TRPV1 [313] and transcription factors, such as transcription factor EGR1. This knock-down has been shown to yield analgesia. Transfection of small interfering RNAs has been shown to reduce $Nav_{1.7}$ miRNA and protein in DRG and to reduce hyperpathia in diabetic rats [318].

Gene based approaches: transfection

Intrathecal delivery of viral vectors encoding a given protein or the use of nonviral methods, such as nanoparticles or permeabilizing systems, can enhance targeted protein expression *in vivo*. Several examples are noted. DRG neurons virally transduced with a vector coding for glutamic acid decarboxylase (QHGAD67) release GABA to produce an analgesic effect. Increased expression of endomorphin-2, an opioid peptide, or the preprohormone for enkephalin or ß endorphin after intrathecal viral vector transfection was found to be analgesic [319–321]. Increased spinal expression of the anti-inflammatory cytokine IL-10 after intrathecal viral vector or plasmid delivery was anti-hyperalgesic [257,319,322]. Increasing expression of MAPK phosphatase-1 reduces the phosphorylation of p38 MAPKs and reduces nerve injury-evoked increases in inflammatory cytokines and chemokines and the associated hyperalgesia [323]. The Ca(2+) channel-binding domain 3 (CBD3) peptide was found to block the function of CaV2.2 channels and block nerve injury-induced hyperpathia [324]. Increased expression of the potassium channel Kv1.2 reduced nerve injury-induced hyperpathia [325].

Routes of analgesic delivery

Aside from the normal routes of oral and parenteral drug delivery, several routes have particular promise in delivering analgesic therapies.

Spinal delivery

Delivery of a compound to a target site within the central nervous system may be achieved by systemic administration if the agent passes the blood-brain barrier and can be delivered in doses that achieve an analgesic effect, but do not have impairing side effects (e.g. sedation). Agents with poor CNS penetration that are believed to act at spinal targets (as many analgesics do) may be delivered intrathecally or epidurally. While not routine, where the mechanism of action results in a long duration effect (as with many of the targeted toxins and gene therapeutics discussed above) there are increasing examples of neuraxial delivery of therapeutics [326,327]. An important issue characteristic for neuraxial delivery is the formulation requirement. Typically neuraxial formulations are water-based products with a pH between 5 and 7 and osmolarity in the range of 300 mOsM with minimal adjuvants (e.g. surfactants, antioxidants, or antimicrobial constituents) (see [326,328]). An additional issue to consider in developing a spinal agent is the need for specific preclinical safety evaluations employing this route of delivery (see [326]).

Cutaneous delivery

Application to the skin may be employed to achieve a circulating analgesic dosage (as with agents such as lipophilic opiates) [329], or to achieve a local effect as with topical capsaicin [330,331] or lidocaine [332] in the treatment of post-herpetic neuralgia and diabetic peripheral neuropathic pain. Topical NSAIDs provide significant pain relief in arthritis patients when applied as patches, gels, or in solutions such as dimethyl sulfoxide (DMSO), and have a lower incidence of gastrointestinal effects (see [333]). There is a growing appreciation that locally applied products, such as Botulinum toxin, may undergo uptake in the sensory afferents and fast axon transport to the DRG and central terminals [334,335] and may, in fact, undergo a transcytotic movement into adjacent neurons [336] and glia [337,338], leading to a potent homotopic analgesia [334]. These mechanisms may account for the reported efficacy of peripheral Botulinum toxin in migraine (a pain state believed to originate from intracranial meningeal afferents) [103]. The development of delivery enhancers for transdermal movement of such large molecules may yield increased therapeutic utility [339]. Transfer of drugs through the skin faces diffusion barriers (stratum corneum), methods to enhance such transfer have included microneedles to enhance skin permeability [340]. Enhanced cutaneous penetration has also been achieved through the use of nanoparticle formulations constructed from a variety of materials ranging from silica to titanium dioxide [341,342], coated microneedles [343,344], as well as electrically driven molecule movements [345–347].

Transmucosal delivery

Agents delivered orally undergo significant hepatic first pass metabolism and degradation within the gastrointestinal tract. Delivery systems can involve sprays, wafers and

sublingual tablets. A growing focus is on transmucosal drug administration employing the nasal, rectal, and oral (buccal or sublingual) cavities. To enhance local absorption, agents may be formulated in mucoadhesive systems to enhance mucosal contact [348–350]. Buccal delivery of fentanyl has been shown to be effective in producing acute onset pain relief [351]. Inhalation and nasal absorption is another route for drug delivery. The absorption is effected through the nasal mucosa and lung [352]. This approach represents a favorable route for delivery of a variety of low and high molecular weight compounds, including peptides [353]. Of particular note, the nanoparticle formulation noted above lends itself to transmucosal absorption [354]. Of particular interest, there is evidence that delivering the agent by nasal mucosa may allow access to the central nervous system along the olfactory pathway [355,356]. Oxytocin has been shown to have a pain modulatory component. Delivery by the nasal route has been shown to have efficacy in migraine [357] and on pain sensitivity [358].

Predictive successes/failures of preclinical models of nociception

Preclinical work with analgesic targets has resulted in several notable successes.

COX-2 inhibitors

COX-2 inhibitors were developed to target the inducible form of the cyclooxygenases. They were found to be active in a variety of preclinical models that defined both a central and peripheral action [160] and led to trials showing clinical efficacy and subsequent approval [359,360]. While well tolerated in the short-term, long-term use of these agents was unexpectedly found to have deleterious cardiovascular side effects that had not been appropriately assessed in the relevant preclinical models [361].

Anti-NGF therapeutics

Anti-NGF therapeutics arose from the appreciation that NGF acting through a Trk receptor could initiate pain states, and that NGF levels rose in a variety of models of hyperpathia [362]. Development of humanized antibodies revealed important clinical efficacy in joint disease [363,364]. A caninised anti-NGF monoclonal antibody has been developed for veterinary therapeutics [365]. Osteonecrosis secondary to anti-NGF activity is an important limiting concern [366].

N-type calcium channel antagonist

Components of marine snail venom were found to block voltage-gated calcium flux and were the ligand(s) by which the N-type calcium channel was characterized [367]. Binding sites for ziconotide were found to be present in high concentrations in the spinal dorsal horn [368]. Initial preclinical work showed its efficacy in models of hyperpathic states [369,370]. Subsequent work led to the development of and clinical approval for Prialt for spinal drug delivery [371]. Recent efforts to develop a systemically active N-type calcium channel blocker failed to meet efficacy standards in Phase II clinical studies for activity in lumbosacral radiculopathy (NCT01655849), and in post-herpetic neuralgia (NCT017578) (see [372]).

Gabapentinoids

The anti-convulsant gabapentin was identified as an analgesic by off-label clinical use, prior to work showing its anti-hyperpathic activity in animal models. However, the preclinical work was essential in identifying the $\alpha 2\partial$ auxiliary subunit of the CaV2.2 as its target [21,373]. Subsequent work with it and the drug pregabalin has led to its approval for use in post-herpetic neuralgia and painful diabetic neuropathy [15], as well as fibromyalgia.

T-type calcium channels

T-type calcium channels are thought to regulate the excitability of nociceptive afferents [374] and alter nociceptive processing. When used in humans, an orally active compound was found to reduce nociceptive-evoked potentials (see [372]).

NaV 1.7

As noted, small afferents express NaV 1.7 channels. In the human population, gain and loss of function mutations in the NaV 1.7 sodium channel lead, respectively, to enhanced and diminished pain states [135], providing a promising support for developing blockers for such targets. Original work with novel targeted sodium channel blockers was negative [375], but more recent work has born positive fruit in models of postherpetic neuralgia and primary erythromelalgia [376] (https://clinicaltrials.gov/ct2/results?term=Xen+402&Search=Search).

Toxin based local anesthetics

Toxin-based sodium channel blockers, such as neosaxitoxin and tetrodotoxin, have been shown to produce long-lasting nerve blocks (in a variety of preclinical models (see [377,378]). Human clinical trials have shown a corresponding long duration of action and efficacy [378,379] (NCT01786655, NCT01655823).

Angiotensin 2 receptor antagonist

As noted, evidence suggests that angiotensin may be present in primary afferents and can activate a variety of facilitatory cascades mediated through AT1 and AT2 receptors [380,381]. In Phase II clinical trials, an AT2 antagonist was found to be tolerated and it met its

primary analgesic efficacy endpoint in post-herpetic neuralgia patients [382].

Monoamine uptake inhibitors

As reviewed, preclinical work has shown that a noradrenergic bulbospinal projection into the spinal cord regulates dorsal horn nociceptive processing through alpha2 adrenergic receptors with efficacy shown in modulating hyperpathia in a variety of tissue and nerve injury models [383]. In addition, forebrain projections of serotonin and norepinephrine play an important role in the regulation of affect. Such preclinical work has led to approval of agents such as duloxetine to treat chronic musculoskeletal pain (fibromyalgia), neuropathic pain associated with diabetes, including discomfort from osteoarthritis, and chronic lower back pain [384–386], and may contribute to preventing a transition from an acute to a chronic pain state [387].

Clinical trial target failures

Conversely, a number of targets have not been confirmed in clinical trials. These include a lack of apparent clinical activity in trials with neurokinin-1 receptor antagonists ([388], but see [389]), NMDA-glycine-site antagonists [390], glial inhibitors (minocycline) [391] and CB2 agonists [392].

The apparent false-positive results of the preclinical models have been discussed elsewhere (see, for example, [26,393]). A number of variables may alone or jointly contribute to the lack of predictive validity: (a) lack of adequate target site engagement (e.g. pharmacokinetics, lack of blood barrier penetration); (b) difference between target properties in the model and in humans (e.g. species differences in binding sites or enzyme active sites); (c) dose-limiting side effects (or off-target site actions) that were not addressed in the preclinical model; and (d) preclinical results that could not be reproduced; or (e) marginal (but statistically significant) evidence of efficacy in the preclinical model. In the case of demonstrated efficacy, robust assessments must present results based on appropriate and achievable drug target levels in models where active standards can be shown to have robust actions. In the last case, there is increasing use of multiple laboratories to confirm in-house results along with the use of an adequate blinding protocol (see a provocative discussion of these issues in [394]). Finally, absence of an adequate therapeutic effect in the human may equally reflect upon the complexity of the human state and that, for some pain phenotypes, a particular target may engage only a component of that phenotype. It is important to note that clinical pain therapies often employ agents with multiple targets and combination therapies often display an improved therapeutic benefit *versus* higher doses of either agent alone [20,395].

Selection of targets for development

The above overview of the biology underlying nociceptive processing emphasizes the complexity and diversity of drug targets. Rational selection of a target for development in humans is aided by a number of convergent observations. Consider these points in the context of the development of specific NaV channels blockers. Firstly, the selection of a drug target that has an identified action in humans. NaV 1.7 gain-of-function and loss-of-function mutations are associated with hyperpathia and analgesia in humans [132]. There is an increasing appreciation that genetic screening of pain patient populations will likely reveal not only differences between patient populations with pain phenotypes, but also confirm or reveal the role of novel targets [396,397]. Secondly, confirmation of a class action with an existing drug. Systemic lidocaine at concentrations that do not block sensory axon conduction will reduce neuropathic pain states in humans [398]. Thirdly, mechanistic convergence. NaV 1.7 protein shows increased expression after nerve injury in animal models and the increased expression correlates with the development of ectopic activity [399]. Fourthly, covariance of pharmacodynamic and pharmacokinetics of drug action. Parallels between pharmacokinetics and pharmacodynamics as regards behavioral effects versus time course and concentrations in plasma and brain provides an indication that the drug as formulated and delivered has a predictable target engagement (see, for example [80,81]. A similar analysis may be considered as having been useful in the development of other novel targets such as GTP cyclohydrolase 1 (GCH1) [187], and opioid receptor splice variants [400].

Development of veterinary analgesics

The above commentary has focused on the development of human pharmaceutics through preclinical models. Though not often considered, numbers obtained from the veterinary community emphasize a similar impact in companion animal populations with the treatment costs borne by owners approaching those in the human arthritic patient [30,401,402]. Not surprisingly, the increasing demand for efficacious veterinary analgesics other than NSAIDs and opiates has spurred a growth in interest in veterinary products, with many large pharma having veterinary divisions or spinoffs. Noted above, a caninised anti-NGF product for osteoarthritis in dogs has been developed [365]. Spinal actions of IL-10 have been shown to display efficacy in regulating hyperpathic states [403]. Increasing IL-10 release by delivery of intrathecal transfection

methodologies is reported to have salutary effects in canine models of persistent pain and development of a canine therapeutic would be important (http://xaludthera.com/products/). The intrathecal toxins sP-saporin and resiniferotoxin were shown to have efficacy in canine models of osteoarthritis and osteosarcoma (see [301]). Such work has enabled their development for human use for pain in advanced cancer and they are now in clinical trials (NCT02036281; NCT00804154). These studies also provide a corresponding link to furthering their development for approval in the veterinary patient. Novel delivery of formulations, such as transmucosal delivery of buprenorphine and dexmedetommidine, have been developed for the feline patients [404]. Pathways for drug development for veterinary use resemble those required for human approval and are in the tract of development that would be employed for human application (see: http://www.fda.gov/AnimalVeterinary). It is important to note that the veterinary patient suffering from osteosarcoma and osteoarthritis displays complex behavioral patterns consistent with the pain states. Considerable effort has been expended by members of the veterinary community to develop validated behavioral inventories to define the gravity of the pain state in these patients [405–407]. It has not been lost on those interested in analgesic drug development that these clinical patients with these inventories can provide an important clinical validation in the larger animal models of analgesic efficacy and side-effect profiles [408].

Closing comments

While there are evident limitations and pitfalls in the translation of mechanisms defined in one species to another, it is clear that the insights provided by the merging of molecular and physiological studies with the role of the characterized systems in pain behavior provides a rational tool for developing analgesic targets and drugs to engage these targets. This brief review emphasizes the complexity of a system that has evolved over the millennia to encode the events that started with the task of recognizing fundamental threats to viability and, as such, is intimately embedded in every aspect of our biological system. It is thus not surprising that modifying the function of these systems will be fraught with the likelihood that to alter one component is to have far-reaching effects on many aspects of system function. It is important to note that many of the agents with defined efficacy have a prominent effect upon afferent processing at the spinal level where alterations in the message initiated by tissue and nerve injury are encoded.

Abbreviations

BDNF, brain-derived neurotrophic factor; CaMK, calmodulin-dependent protein kinase; CaV, voltage-gated calcium channels of the N type; CCL, chemokine (C-C motif) ligand; CGRP, calcitonin gene related peptide; CPP, conditional place preference; CX3CL1, chemokine (C-X3-C motif) ligand; DRG, dorsal root ganglion; EAAT, excitatory amino acid transporter; EET, epoxyeicosatrienoic acid; ERK, extracellular signal-regulated kinase; GABA, gamma aminobutyric acid; GPCR, G protein-coupled receptor; IFNγ, interferon gamma; IL, interleukin; JNK, c-Jun N-terminal kinase; MAPK, mitogen activated protein kinase; MCP, monocyte chemoattractant protein; mGluR, metabotropic glutamate receptor; NMDA, N-methyl D-aspartate; NOS, nitric oxide synthase; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; PKA, protein kinase A; PKC, protein kinase C; PKG, protein kinase G; sEH, soluble epoxide hydrolase; SNAREs, soluble N-ethylmaleimide-sensitive factor activating protein receptor; sP, substance P; TLR, toll-like receptor; TNF, tumor necrosis factor; TRK, tyrosine receptor kinase; WDR, wide dynamic range.

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