Basic/Translational Development of Forthcoming Opioid- and Nonopioid-Targeted Pain Therapeutics

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Opioids represent an efficacious therapeutic modality for some, but not all pain states. Singular reliance on opioid therapy for pain management has limitations, and abuse potential has deleterious consequences for patient and society. Our understanding of pain biology has yielded insights and opportunities for alternatives to conventional opioid agonists. The aim is to have efficacious therapies, with acceptable side effect profiles and minimal abuse potential, which is to say an absence of reinforcing activity in the absence of a pain state. The present work provides a nonexclusive overview of current drug targets and potential future directions of research and development. We discuss channel activators and blockers, including sodium channel blockers, potassium channel activators, and calcium channel blockers; glutamate receptor–targeted agents, including *N*-methyl-D-aspartate, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid, and metabotropic receptors. Furthermore, we discuss therapeutics targeted at γ-aminobutyric acid, α2-adrenergic, and opioid receptors. We also considered antagonists of angiotensin 2 and Toll receptors and agonists/antagonists of adenosine, purine receptors, and cannabinoids. Novel targets considered are those focusing on lipid mediators and anti-inflammatory cytokines. Of interest is development of novel targeting strategies, which produce long-term alterations in pain signaling, including viral transfection and toxins. We consider issues in the development of druggable molecules, including preclinical screening. While there are examples of successful translation, mechanistically promising preclinical candidates may unexpectedly fail during clinical trials because the preclinical models may not recapitulate the particular human pain condition being addressed. Molecular target characterization can diminish the disconnect between preclinical and humans' targets, which should assist in developing nonaddictive analgesics. (Anesth Analg 2017;125:1714–32)

The management of pain is a clinical imperative. Aside from humanistic concerns, failure to adequately control pain has negative consequences in terms of system biology. Opioids, through their potent modulatory effect mediated via canonical receptors on pain processing, have been, and remain, an essential component of pain management. Nevertheless, reliance on this therapeutic approach has limitations and deleterious consequences to the patient and society. Opioid misuse is an expanding crisis with over 36,000 deaths due to opioid overdose in 2015 alone.[1,2] Pharmaceutical companies have pursued abuse-deterrent opioid formulations.[3,4] While these formulations reduce the possibility of the content of the pills being extracted, the underlying properties of the pharmaceutical agent (ie, opioids) remain the same and extraction-deterrent systems are subject to being overcome.[5–8]

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Our understanding of systems that mediate and regulate nociceptive processing has yet to produce a recognized alternative to opioids. Advances in pain biology have, however, yielded remarkable insights and opportunities. We will provide an overview of salient areas of research that focus on current advances in pharmacological targets. Meaningful advances in drug therapy must consider not only (i) analgesic efficacy, but also (ii) therapeutic ratio (separation of pain relief from side effects); (iii) constancy of response over extended use (eg, tolerance); (iv) lack of positive reinforcing properties in the absence of a pain state. Due to space restriction, this review must be considered a nonexclusive overview of advances in terms of analgesic targets.

PAIN PHENOTYPES

Pain is an aversive state that reflects the perceptual covariates of events that arise from stimuli of sufficient intensity to induce tissue damage or which otherwise mimic the activity induced by such stimuli, as in nerve injury. It is heuristically useful to think of mechanisms generating the aversive condition associated with afferent stimulation as having 4 elements.

i. Acute nociception in which an acute, noninjuring, high-intensity stimulus activates small unmyelinated and myelinated afferents, driving intensity-linked excitation of second-order dorsal horn projection neurons, leading to a stimulus-linked pain report/escape.
ii. After tissue injury and inflammation, hyperalgesia occurs at the injury site, causing an enhanced response to moderate stimuli, and an enlarging receptive field, including areas not injured, resulting in a second

hyperalgesia/allodynia. This phenotype reflects a peripheral sensitization (development of ongoing activity and a left shift in the intensity response relationship at the terminal) and a central/spinal sensitization (heightened excitability of the primary afferent terminal and second-order neurons causing an enhanced discharge to a given afferent input).

iii. Injury to the peripheral nerve resulting in ongoing dysesthesias and enhanced sensitivity to light touch and modest changes in temperatures (allodynia), associated with reactive changes in the afferent axon, dorsal root ganglia (DRG) and dorsal horn (typically reflecting a loss of inhibitory regulation).

iv. After persistent inflammation and tissue injury, the evolving pain state displays characteristics, suggesting the development of a nerve injury phenotype, for example, an acute to chronic pain transition.

The biology of these above states has been reviewed in detail elsewhere.[9–11] These comments importantly emphasize that a pain condition may represent multiple mechanistic phenotypes. Accordingly, the regulation of the encoding and trafficking of the nociceptive stimulus to higher centers may reflect a role for engaging multiple targets.

ISSUES IN ANALGESIC DRUG DEVELOPMENT

Demonstration of Target Analgesic Efficacy

Development of analgesic drugs with known targets and mechanisms of action can use models of target engagement, such as in silico and in vitro modeling (eg, opioids and cyclooxygenase [COX] inhibitors), which can move a drug with some predictability into a behavioral assessment. Novel targets often arise based on association of the target with specific systems, but their efficacy in regulating the pain state requires a sense of what role that target plays in mediating the behaviorally defined pain construct. Preclinical behavioral models provide such insights. Detailed reviews of preclinical models that focus on events secondary to inflammation (acute and chronic) and nerve injury (mono- and polyneuropathies) with their strengths and shortcomings have been provided elsewhere.[12,13] While instances of failure of the predictive models have been discussed (as is true for virtually every translational system in biology), mechanistic studies have made a number of valid predictions of clinical efficacy ranging from COX inhibitors to antimigraine drugs.[9] Several issues regarding preclinical models are noted.

i. Each behavioral model has mechanistic components particular to that system. Convergent results from multiple models and comparable dose-effect relationships increase the likelihood of assessing mechanisms relevant to the human state.

ii. Preclinical models have long examined a single sex, for several reasons including economy and the belief there is little difference between the sexes. Numerous instances at the behavioral and mechanistic level can now be cited to dispute this assertion.[14,15]

iii. Many models employ threshold measurements. Alternative models use "spontaneous behaviors," including general activity, rearing, weight bearing, and gait as markers of an aversive condition.[16] There is also an understanding that if there is an aversive condition generated by an injury, a drug that has no intrinsic rewarding property but which serves to diminish that pain state will in fact acquire a positive reinforcing property in the presence of the pain state. Such "conditioned place preference" models have an important place in current drug evaluations.[16,17]

iv. While preclinical analgesic drug evaluation has been largely successful in rodents, characterization of issues of analgesic efficacy and tolerability may also be achieved through naturally occurring pathologies in companion animals, notably dogs. The incidence of canine osteoarthritis and osteosarcoma provides an important way station in defining efficacy in controlled trials using validated inventories and neurological assessments.[18] While safety-toxicology studies in such animals are routinely part of an investigational new drug package during drug development, there may be an advantage to pursuing efficacy studies as well. Such information is pivotal in the development of veterinary analgesic products and their approval by the US Food and Drug Administration (FDA) veterinary division to manage the pain states in this patient population. The predicted spending on analgesics for pets alone was predicted to be ~$335 million in 2011, so there is a secondary market that can incentivize additional testing in the veterinary patient.[19]

v. Human experimental models initiating a local injury (eg, ultraviolet B irradiation, thermode burn) or afferent stimulation (capsaicin) are increasingly used to determine efficacy of both new and existing analgesics. Their apparent ability to demonstrate efficacy with known analgesics provides some validation of their sensitivity[20] and to define a drug effect and corresponding side effects at the effective dose.

vi. The following commentary considers a variety of targets and comments on systemic and neuraxial routes of delivery, reflecting the fact that drug effects upon pain processing frequently reflect an action at the first-order synapse. It must be stressed that these discussions do not raise issues of safety. This commentary is particularly relevant for neuraxial drugs where appropriate assessment of neuraxial safety must be undertaken before such drug implementation.[21]

vii. Finally, it is challenging to find a drug target that can alter a pain state with a favorable therapeutic ratio (eg, little or no effects upon mentation, arousal, or motor function). An important concern we must now consider is that the drug target is not a mediator of positive reinforcement.

Assessment of Abuse Liability

An important issue in developing novel analgesics is to overcome the potential for abuse. If the drug acts on components of systems associated with positive reinforcement or possessive of stimulant and/or sedative effects, suspicion of its potential for abuse must be elevated. Such examples, at present, include drugs interacting with opioid receptors, central

nervous system (CNS) depressants (γ-aminobutyric acid-A [GABAA] receptors); CNS stimulants (increased dopamine release/block reuptake; nicotine), hallucinogens, glutamate antagonists (ketamine), and cannabinoids.[22,23] It seems reasonable that a molecule lacking CNS bioavailability would show a reduced likelihood of having a reinforcing property (eg, loperamide[24]), but now even large molecules are considered not to be excluded as having potential liability.[25] To this end, locomotor, reinforcing, and dependence-producing effects of the agent must be routinely assessed. A variety of strategies are considered relevant and have been effective in predicting human-drug behavior including self-administration, drug discrimination, and conditioned place preference paradigms.[26–28]

SURVEY OF CURRENT TARGETS OF PAIN THERAPEUTICS

In the following sections, we will consider several current drug targets and potential future directions of research and development. The Figure summarizes these targets as they reflect upon actions at the level of the peripheral terminal and central sites. The Table summarizes those agents that moved into clinical trials.

Sodium Channel Blockers/Potassium Channel Activators

Axon excitability depends directly on voltage-gated sodium channel, while activation of potassium channels produces hyperpolarization reducing membrane excitability. These represent potential peripheral targets for altering afferent transmission.

Sodium Channels. Voltage-gated sodium channels (Na_v) are the target of all clinical "local anesthetics."[29] Nine Na_v isoforms with distinct activation properties and tissue distributions have been identified: $Na_v1.1$ and Na_v1 (large DRG/axons), $Na_v1.4$ and $Na_v1.5$ (skeletal and cardiac

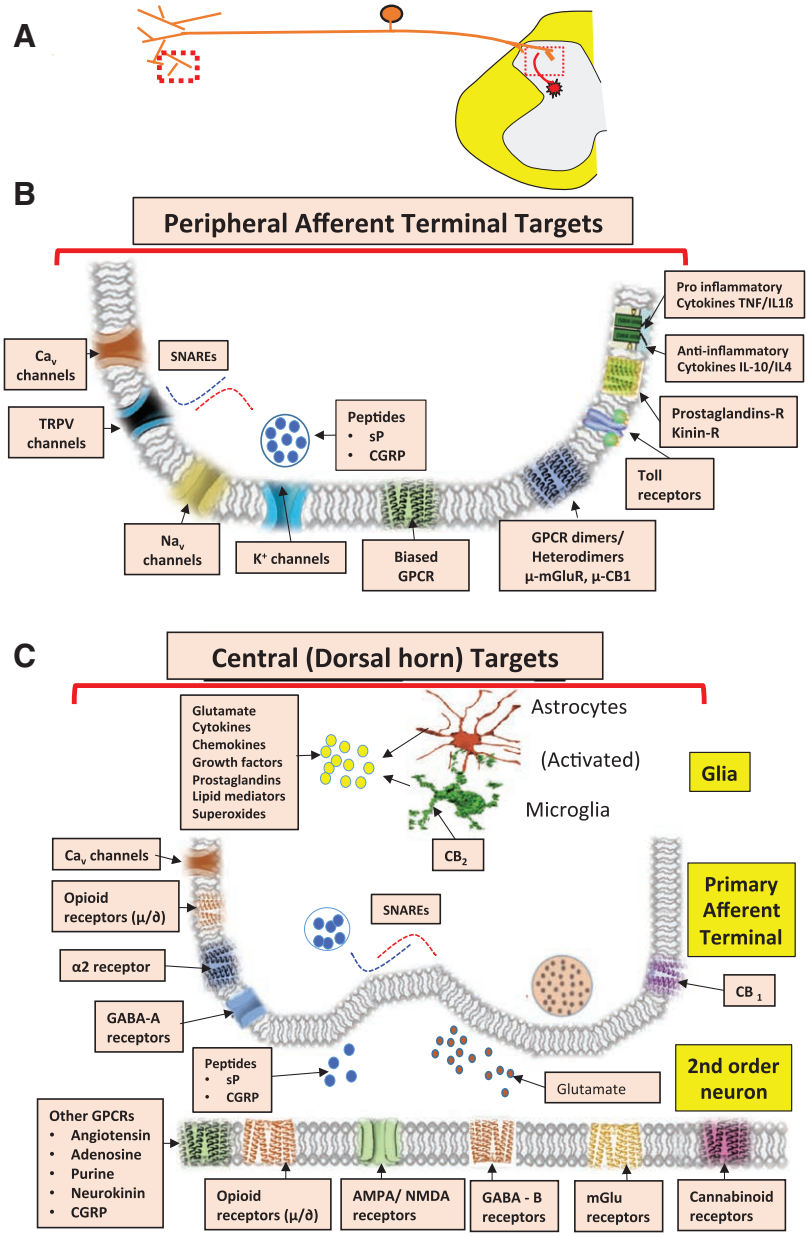

Figure. Potential opioid and nonopioid targeted drugs. Key drug targets at the level of the afferent and dorsal horn (A) that act as peripheral afferent terminal targets (B) and central (dorsal horn) targets (C). See text for discussion. α2 indicates adrenergic alpha 2 receptor; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; 1/2 Cav, voltage-dependent calcium channel; CB1/CB2, cannabinoid type 1/type2; CGRP, calcitonin gene-related peptide; GABA, γ-aminobutyric acid; GPCRs, G-protein–coupled receptors; IL, interleukin; K^+, potassium channel; mGlu, metabotropic glutamate receptor; Na_v, voltage-gated sodium channel; NMDA, *N*-methyl-D-aspartate; SNAREs, Soluble *N*-ethylmaleimide-sensitive fusion protein Attachment protein REceptors; sP, substance P; TNF, tumor necrosis factor; TRPV, transient receptor potential cation channel.

Table. Studies Registered on Clinical Trials.gov

Name of the Drug	Target	Registration Number	Testing	Indication	Phase of Clinical Trial	Status of the Study
Eslicarbazepine acetate (ESL)	Voltage-gated sodium channel (VGSC) antagonist	NCT00980746	Assess the efficacy of ESL	Painful diabetic neuropathy	Phase 2	Completed
ESL	VGSC antagonist	NCT00981227	Assess the efficacy of ESL	Postherpetic neuralgia	Phase 2	Completed
ESL	VGSC antagonist	NCT01820585	Three different doses of ESL versus placebo	Fibromyalgia	Phase 2	Completed
ESL	VGSC antagonist	NCT01124097	Assess the efficacy of ESL	Postherpetic neuralgia	Phase 3	Terminated
ESL	VGSC antagonist	NCT01129960	Assess the efficacy of ESL	Painful diabetic neuropathy	Phase 3	Terminated
Neosaxitoxin (NeoSTX)	VGSC antagonist	NCT01786655	NeoSTX alone or in combination with bupivacaine with or without epinephrine	Safety in healthy volunteers	Phase 1	Completed
Tetrodotoxin (TTX)	VGSC antagonist	NCT01655823	Multiple dose levels of TTX versus placebo	Neuropathic pain	Phase 2	Terminated
Z160	Selective N-type calcium channel (Cav2.2) blocker	NCT01757873	Compare Z160 and placebo	Postherpetic neuralgia	Phase 2	Completed
Z160	Selective N-type calcium channel (Cav2.2) blocker	NCT01655849	Compare Z160 and placebo	Lumbosacral radiculopathy	Phase 2	Completed
Ifenprodil Tartrate	NMDA receptor antagonist	NCT01896388	Confirm whether ifenprodil tartrate is effective in the treatment of adolescents PTSD patients	Posttraumatic stress disorder	Phase 1/2	Currently recruiting participants
Etifoxine	$GABA_A$ receptor agonist. Agonist at β2 and β3 subunit of the $GABA_A$ receptor complex	NCT02147548	Confirm the effect of etifoxine and lorazepam on vigilance and cognitive functions in the elderly	Healthy volunteers	Phase 3	Completed
ADL5859	δ-Opioid agonist	NCT00993863	Assess the efficacy and safety of ADL5859 compared with placebo and active control (ibuprofen)	Acute dental pain	Phase 2	Completed
ADL5859, ADL5747	δ-Opioid agonist	NCT00979953	Assess the efficacy of ADL5859 versus placebo and ADL5747 versus placebo	Osteoarthritis of the knee	Phase 2	Completed
TRV130 (oliceridine)	Opioid μ-receptor agonist; β-arrestin inhibitor	NCT02335294; NCT02820324	Analgesic efficacy IV oliceridine compared with placebo	Acute pain after abdominoplasty	Phase 2; phase 3	Completed
TRV130 (oliceridine)	Opioid μ-receptor agonist; β-arrestin inhibitor	NCT02100748; NCT02815709	Analgesic efficacy of IV TRV130 versus placebo	Bunionectomy	Phase 2; phase 3	Completed
TRV130 (oliceridine)	Opioid μ-receptor agonist; β-arrestin inhibitor	NCT02656875	Safety evaluation of TRV130 in patients with acute pain	Moderate to severe pain caused by medical conditions or surgery	Phase 3	Currently recruiting participants
TRV130 (oliceridine)	Opioid μ-receptor agonist; β-arrestin inhibitor	NCT02520297	Analgesic efficacy of TRV130 for moderate to severe acute pain	Fracture pain	Phase 2	Terminated
EMA401	Angiotensin 2 receptor (AT2R) antagonist	NCT02435199	Compare EMA401 300 mg and placebo	Diabetic neuropathies	Phase 2	Withdrawn
EMA401	AT2R antagonist	NCT02426411	Compare 2 different doses of EMA401 and placebo	Postherpetic neuralgia	Phase 2	Withdrawn
EMA401	AT2R antagonist	NCT03094195	3 different doses versus placebo	Postherpetic neuralgia	Phase 2	Not yet open for recruitment
AF219 (gefapixant)	P2X3 receptor antagonist	NCT02349425	Crossover, dose escalation study of gefapixant (AF-219)	Refractory chronic cough	Phase 2	Completed
AF219 (gefapixant)	P2X3 receptor antagonist	NCT01432730	Effectiveness of gefapixant in reducing daytime objective cough frequency	Idiopathic chronic cough	Phase 2	Completed
AF219 (gefapixant)	P2X3 receptor antagonist	NCT02397460	Crossover study in healthy and chronic cough subjects	Chronic cough	Phase 2	Completed
AF219 (gefapixant)	P2X3 receptor antagonist	NCT02476890	Crossover study in healthy and chronic cough subjects	Chronic cough	Phase 2	Completed

(*Continued*)

Table. Continued

Name of the Drug	Target	Registration Number	Testing	Indication	Phase of Clinical Trial	Status of the Study
Resiniferatoxin (RTX)	Transient receptor potential vanilloid 1 (TRPV1) receptor agonist	NCT00804154	Safety of intrathecal RTX	Cancer pain	Phase 1	Suspended participant recruitment
Substance P-Saporin	Neurokinin 1 receptor antagonist	NCT02036281	Eight different doses intrathecally	Terminally ill patients, histologically confirmed advanced cancer, for intractable pain	Phase 1	Currently recruiting participants

Abbreviations: GABA, γ-aminobutyric acid; IV, intravenous; NMDA, *N*-methyl-D-aspartate.

muscle), and $Na_v1.7$, -1. (small sensory DRGs/afferents).[30] After inflammation and nerve injury, increases in small afferent Na_v ($Na_v1.3$, 1.7, 1.8, and 1.9) expression are believed to underlie ectopic afferent traffic and increased responsiveness.[30–32]

Specific confirmation of the role of Nav 1.7 in human pain processing is based on the phenotype of naturally occurring gain and loss of function mutations in Nav1.7 channels, wherein those expressing these mutations, respectively, show pronounced increased and decreased pain states.[33,34]

While local anesthetics given perineurally and neuraxially produce conduction block anesthesia, systemic anesthetics such as intravenous lidocaine have surprisingly selective antihyperpathic effects in a variety of preclinical models and human pain states at concentrations that do not produce a general conduction block, suggesting a differential sensitivity of systems related to facilitated states after tissue and nerve injury.[35,36]

Future development of the sodium channel–blocking drugs focuses on the role of selective blockers of channels expressed on nociceptive linkages. Clinically used local anesthetics (amide and ester) do not selectively block these channels,[37] although several isoforms are sensitive to puffer fish toxin, tetrodotoxin (TTX) ($Na_v1.1$–7), with the remainder resistant to TTX.[37,38] Toxin-based sodium channel blockers, neosaxitoxin and TTX, demonstrated long-lasting nerve blocks after perineural and intrathecal delivery and, surprisingly, after systemic delivery in human and animal models[38–41] (Table). In regard to selective channel antagonists, intrathecally delivered, toxin-based $Na_v1.7$[42] and 1.8[43] inhibitors have shown preclinical efficacy in models of inflammation and nerve injury, with a favorable therapeutic ratio. Development of systemically bioavailable, small-molecule, channel-selective antagonists as analgesics have faced challenges.[44,45] Clinical work with oral-targeted, sodium channel–selective blockade was negative,[46] although promising results from multicenter studies in postherpetic neuralgia and primary erythromelalgia have been reported.[47] Loss of response to local anesthetics (eg, tolerance or tachyphylaxis) has been reported after neural blocks, but the phenomenon does not appear to be robust.[48]

An interesting application of the specific association of transient receptor potential vanilloid 1 (TRPV1) with pain afferents has been the use of protonated local anesthetics such as QX314, which are able to enter the otherwise impermeant axon membrane through TRPV1 channels upon their activation by capsaicin, and result in function block of the sodium channel in the TRPV1 (+) afferent axon.[49] It is now understood that lidocaine by itself is a TRPV1 agonist and can promote passage of the protonated form,[50] allowing quaternary lidocaine (QX314) to enter the TRPV1-bearing axon and selectively block the Na_v channel, resulting in specific block of TRPV1 (+) primary afferents.[50]

Potassium Channels. There are 4 major families of K channels (voltage-gated [Kv], calcium-activated [K Ca], inwardly rectifying [Kir], 2 P domain [K2P] potassium channels), which when activated lead to membrane hyperpolarization through increased potassium conductance. Genetic analyses illustrate that variations in several K^+ channel genes are relevant to the risk for persistent pain after injury

(KCNS1-Kv9.1, GIRKs-Gir, TRESK-K2P18.1), increased pain sensitivity (KCNS1, GIRKs), and analgesic efficacy of G-protein–coupled receptors (GPCRs; GIRK2).[51] Inwardly rectifying, ATP-sensitive potassium (K-ATP) channels are widely expressed in numerous cell types including neurons and are linked to antiallodynic and antihyperalgesic activity. ATP-sensitive potassium channel agonist–mediated antinociceptive effects are reversed with pretreatment with ATP-sensitive K^+ channel blockers.[52,53]

Interestingly, autoantibodies targeting Kv channels can lead to neuronal hyperexcitability and a pain state.[54] Increasing potassium channel expression and potassium conductance via receptor channel agonists assists in hyperpolarizing (normalizing) otherwise enhanced axon, DRG, and terminal excitability, resulting in antihyperalgesic actions.[55,56]

Calcium Channel Blockers

Movement of calcium into the cell represents a significant source of charge leading to membrane depolarization, while increased intracellular calcium leads to activation of a variety of kinases that phosphorylate: enzymes, channels (lower threshold for activation and increasing ion permeability), and receptors, resulting in hyperalgesic states.[57–60] One source of this intracellular calcium is a variety of high and low voltage-gated calcium channels (VGCCs): high VGCCs include L-(Ca_V1.1–4), P/Q-(Ca_V2.1), N-(Ca_V2.2), and R-(Ca_V2.3) type channels; low VGCCs include T-type (Ca_V3.1–3).[61,62] These are transmembrane channels composed of multiple subunits endowing members of each family with distinguishing properties of voltage gating and antagonist pharmacologies. They are located on primary afferents and postsynaptic membranes in spinal dorsal horn.[63]

N-Type Channel (Cav2.2). The N-type calcium channel is present on presynaptic nerve terminals in the superficial dorsal horn and dorsal root ganglia. Upregulation occurs after peripheral nerve injury.[64] Ziconotide, is an N-type VGCC blocker,[65] possessing potent antihyperpathic properties in rodents and humans when administered intrathecally as a bolus or an infusion[66,67] and is without tachyphylaxis (tolerance).[68] Although ziconotide remains the only approved N-type channel blocker, there are efforts to develop new peptides and small molecules[65,69] and to alter nociceptive properties of N-type VGCC function by hindering its membrane trafficking.[70,71] In humans, a systematically active N-type calcium channel blocker (Z160) failed in phase 2 clinical trials in treatment of postherpetic neuralgia and lumbosacral radiculopathy[72] (Table).

L-Type Channel (Cav1). Channels are largely present postsynaptically and are considered to play a possible role in maintaining facilitated states. Intrathecal delivery preclinically of channel blockers (nifedipine, verapamil, and benzothiazepines) has shown efficiency in altering injury-induced hyperpathia.[73]

T-Type Channel (Cav3.2). T-type calcium channels are present in the dorsal horn and channel blockers, such as ethosuximide and mibefradil, have antihyperalgesic effects in rodents.[74]

Glutamate Receptor–Targeted Agents

Glutamate released from primary afferents, interneurons, and sequestrated stores in astrocytes may interact with a variety of receptor-gated ionophores and receptors with G-protein coupling.

NMDA Receptor. The *N*-methyl-D-aspartate receptor (NMDA-R) is a calcium ionophore composed of 3 subunits (NR1, NR2, and NR3), each with multiple combinations of subunits.[75] This channel is expressed on primary afferents in the dorsal horn, on second-order neurons, and on nonneuronal cells (oligodendroglia and astrocytes). Glutamate is released from afferents and interneurons and binds to the NMDA-R. At the spinal dorsal horn, high-frequency C-fiber stimulation leads to postsynaptic depolarization, removal of an Mg^{2+} ion blocking the pore, and, if the allosterically coupled channel-binding sites for glycine and polyamines are occupied,[76] there is a channel influx of sodium and calcium,[76] leading to a cascade known as windup.[77] NMDA-R blockade inhibits this phenomenon.

Block of NMDA-R function is achieved by competitive glutamate-binding site blockers, noncompetitive channel blockers, and agents blocking associated allosteric binding sites.[78] While NMDA-R function may be prevented by blocking any of these sites, the side effect profile (learning, memory, excitability) for these different agents varies considerably and impacts clinical tolerability.[79]

Preclinical work has demonstrated the antihyperpathic effects in inflammatory and nerve injury models of a variety of intrathecally and/or systemically administered competitive glutamate blockers (2-amino-5-phosphonovalorate), noncompetitive NMDA channel blockers (ketamine, MK-801 and memantine, conantokin-G; agmatine), and glycine site blocker (7-chlorokynurenic acid, ifenprodil).[80–82] Ifenprodil administered into the rostral cingulate cortex alleviated bone cancer pain in rats.[83] While there are surprisingly few high-quality clinical trials, ketamine has a long clinical history of use alone and in combination with opioids in diverse pain states characterized by hyperalgesia and allodynia, including neuropathic pain, surgery, and fibromyalgia.[84–88] Ifenprodil, an inhibitor of the NMDA glycine-binding site, is currently being tested for the treatment of posttraumatic stress disorder in phase 1/2 study (Table).

The abuse potential of NMDA antagonists is controversial and complex.[89] Channel blockers such as ketamine have identified abuse potential. This effect may be mediated by channels associated with specific subunit constituents.[90] The role of other antagonism motifs in contributing to abuse potential is not known.

AMPA Receptor. The α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptor is a glutamate-activated sodium-selective ionophore composed of 4 subunits (GluR1 to GluR4), which plays a pivotal role in acute dorsal horn evoked excitation.[91] In preclinical studies, tezampanel (LY-293558, NGX-424) displayed efficacy in postoperative pain and spasticity.[92,93] In humans, oral

administration showed efficacy upon capsaicin-evoked hyperalgesia[94] and in postoperative pain.[95]

With peripheral injury, the AMPA subunit composition changes, leading to a calcium-permeable channel. Joro spider toxin, selective for calcium-permeable AMPA site, decreases secondary mechanical allodynia development evoked in tissue injury models.[96] The abuse potential of these agents is not known.

Metabotropic Receptors. Eight mGluRs (mGluR1–8) have been identified and are divided into 3 groups[97]: group I (mGluR1 and mGluR5) stimulates phospholipase C (PLC); group II (mGluR2 and mGluR3) and group III (mGluR4 to mGluR8) inhibit adenylate cyclase.[97,98] mGluRs have been localized on the primary afferents, neurons, and glia within the brain and spinal cord.[99,100] Group I resides postsynaptically, and group II and group III are dominantly located on presynaptic terminal.[101]

Activation of group I mGluRs is linked to central sensitization and persistent nociception, while the activation of group II mGluRs suppresses facilitated states.[102] Group I mGluR antagonists have an analgesic action by an effect on peripheral terminal, spinal, and supraspinal sites.[103] Group II mGluR agonists regulate neurotransmitter release and depress pain transmission by acting at different levels of pain neuraxis, including nociceptors, dorsal horn, and supraspinal regions such as the amygdala and periaqueductal gray.[104] Group III mGluR agonists are also involved in the control of hyperalgesia after inflammation. As with the other metabotropic receptors, agonist injections into a peripherally inflamed site or into the spinal dorsal horn regulate glutamatergic transmission in inflammatory and neuropathic pain.[103] Group I mGluRs antagonists and group II and III mGluRs agonists exhibited analgesic properties in neuropathic or inflammatory pain states[105–112] and may serve as a basis to develop future spinally targeted agents.[113,114] Importantly, antagonists affecting group I mGluRs have minimal impact on fast synaptic transmission and minimal cognitive effects as compared to ionotropic glutamate antagonists.[115]

Blocking a glutamate transporter (excitatory amino acid transporter [EAAT-3]) reduces intracellular glutamate, attenuates pain, and decreases cellular activation. In addition to their cytoplasmic location, mGluR5s are nuclear and may mediate these effects of intracellular glutamate. Accordingly, cell-permeable mGluR5 antagonists may show increased efficacy in attenuating neuropathic pain.[116]

Regarding abuse potential, central group I mGluRs appear to be substrates for stimulants.[115] Importantly, antagonists at group I mGluRs reduce self-administration with no alteration in motor function or the reward value of natural rewards, while agonists at group II mGluRs prevent reinstatement of drug seeking after abstinence.[117]

GABA Receptors

GABA, a principal inhibitor transmitter, is expressed in neurons throughout brain and spinal cord. GABAergic spinal interneurons presynaptically regulate large mechanosensitive afferents and postsynaptic excitation input by a potential interaction with 2 GABA receptors: the GABA-A ionophore and the GABA-B metabotropic receptor.[118,119]

γ-Aminobutyric Acid-A. The GABA-A receptor is a GABA-gated chloride ionophore and is composed of 5 subunits, each with 4 transmembrane-spanning domains. The specific subunits define the binding of a number of molecules at the ionophore. Drugs can activate the channel (GABA, Muscimol), while others (benzodiazepines, neurosteroids, alcohol, many anesthetics) act as positive allosteric modulators at channels having specific subunit composition, stabilizing an open conformation in the presence of the agonist and at greater concentrations to directly activate the chloride channel.[120] Studies show dense and variable staining for GABA-A subunits in brain[121] and spinal dorsal horn and on primary afferent terminals,[118,122] regulating their excitability.[123,124] Nonspinal GABA-A ionophore activation leads to sedative, anxiolytic, and amnestic effects, whereas at the spinal level increased GABA-A activity alters motor function.[125] The high degree of GABA-A receptor structure/subtype heterogeneity raises expectations for determining specific structures to target these subtypes.[126–129] Subtype specificity may exhibit different effects upon neuronal inhibition in various systems.[130]

GABA-A agonists, such as muscimol or isoguvacine, display preclinical efficacy in neuropathic pain models.[131–135] Intrathecal benzodiazepines depressed nociceptive reflexes in dogs,[136] while bolus intrathecal midazolam has displayed efficacy in postoperative, low back, and labor pain in humans.[137–140] At allosteric binding sites, neurosteroids, such as allopregnanolone, have shown efficacy in preclinical models of tissue and nerve injury.[141] Of note, etifoxine promotes production of 3alpha-reduced neurosteroids and has efficacy in reducing mechanical and thermal pain symptoms in vincristine-induced neuropathic pain.[142] Further, etifoxine, by binding to GABA-A receptor subunits, has shown to be effective in different pain disorders followed by anxiety.[143] Etifoxine was clinically tested in combination with lorazepam for cognitive improvement in elderly patients (Table).

The abuse potential of GABA-A-targeted drugs is clearly suggested by role of the GABA-A receptor in reward circuitry. It is clear, however, that the potential abuse for any GABA-A-targeted drug must be interpreted in terms of the subunits with which the drug interacts and the systems with which the subunits are associated.[144]

γ-Aminobutyric Acid-B. Two GABA-B receptors have been cloned and are metabotropic receptors serving to block the opening of voltage-gated Ca channel and activate inwardly rectifying K channels. These receptors are expressed peripherally and centrally, including thalamus, brainstem nuclei, and spinal cord. While positive antinociceptive actions have been reported, they tend to be minimal. An important element is the potent effect on motor neuron excitability leading to a clinically useful effect on elevated motor tone underlying spasticity occurring with neuraxial injury.[119] Lioresal, typically used by oral or intrathecal delivery in spasticity, is not a controlled agent, but significant withdrawal can be seen with drug termination.

Opioid Receptor–Targeted Drugs

Mu-opioid receptor–targeted agonists represent the gold standard for modifying acute nociceptive processing. This

action reflects the association of these receptors (i) with small afferent input that encode nociceptive processing at the spinal dorsal horn and, (ii) at supraspinal levels, regulating spinal processing through descending pathways, altering perceptual processing, and initiating reinforcing/reward circuit function.[145] Apart from their analgesic efficacy, the classic opioids display tolerance, physical dependence, respiratory depression, and a high propensity for abuse.

Receptor Targeting. There are 3 identified gene products that yield 3 families of opioid receptors (μ/MOR, δ/DOR, and κ/KOR)[146] that, when activated, alter pain processing in a naloxone-reversible fashion. More recently, identification of a receptor for the neuropeptide nociceptin has led to designation of a fourth receptor family (NOP), which is typically naloxone insensitive. While subtypes have been proposed, it appears likely that differences in pharmacology within a class may reflect on properties endowed by receptor organization and posttranslational processing versus a distinctive receptor protein. These receptors are widely distributed in the brain and spinal cord and are characterized by comparable transmembrane-spanning motifs and intracellular GPCR signaling. At the membrane level, opioid receptors have typically been shown to be coupled, so that there is a presynaptic action reducing terminal release through a block of calcium-mediated exocytosis and membrane hyperpolarization through an increased potassium conductance.[147] At the spinal level, the distribution of opioid receptors on C-fiber terminals and second-order neurons is consistent with the analgesic actions being mediated by a block of excitatory transmitter release from C-fibers and inhibition of second-order neuron excitability.[145] A peripheral opioid action manifested on sensitized afferent nerve terminals is observed reflecting in part the presence of opioid receptors on the peripheral terminals of the afferent.[148] Supraspinal opioid actions have been identified, wherein the classic descending pathways are considered to be activated by the effects of the opioid receptor on GABA interneurons in the mesencephalon removing a tonic modulation of downstream descending projections.[149] Higher order action on forebrain structures have additionally been identified[145] and likely reflect upon the effects of opioids on distress.[150] Recent work has suggested possible efficacy of κ-opioid antagonists as a migraine therapeutic.[151] Preclinical actions of opioids and their effects mediated through the several opioid receptors on pain behavior after systemic and spinal delivery have been reviewed extensively.[145,152–154]

As noted, the common opioid target for the clinically used agents is typically the μ-receptor. The possibility that among these receptors there may be subtypes appears likely to reflect other aspects of signaling, including ligand bias and the role of heteromers (see below). δ-Opioid receptors clearly exert a regulatory role.[153] Intrathecal δ-preferring agonist such as DADL has analgesic efficacy in humans after intrathecal delivery.[155] Two nonpeptide molecules ADL5747 and ADL5859 were 2 orally bioavailable compounds[156] tested for acute (NCT00993863) and chronic (NCT00979953) pain management in phase 2 clinical trials but were not more effective than placebo in osteoarthritic patients.

κ-Opioid agonists that are peripherally restricted have shown minimum abuse potential and efficacy in inflammatory and visceral pain. This along with the potential of a reduced side effect profile and lower abuse potential suggests such agonists as promising candidates for treating pain.[157,158]

Interestingly, while there is a typical aim to seek selective agonists, some have argued that effective improvements in efficacy side effect profiles may be achieved through ligands targeting multiple opioid receptors.[159,160]

Biased Ligands. One of the major strategies that is gaining interest is that GPCRs can associate with multiple second messengers (such as G α proteins, β-arrestin) and ligands can modulate GPCR response via one of those functional pathways, thereby exhibiting "biased agonism."[161,162] Such biased agonists at the μ-opioid receptors produce analgesia with limited side effects.[163] Currently, a biased ligand (TRV130) shows analgesia with reduced respiratory depression in phase 2 clinical trials[164] (Table). Recently, an in silico screening approach has identified PZM21, as a μ-opioid-biased agonist that shows promising analgesic data with reduced side effects.[165]

Heteromeric Receptors. Many GPCRs couple to yield homo- and heteromers.[166–168] Such oligomerized receptors serve as targets for developing novel analgesics. For instance, a bivalent ligand containing μ-agonist and δ-antagonist pharmacophores linked via a spacer (MDAN-21) effectively bridges μ-δ opioid receptor heteromers and exhibits enhanced efficacy and a reduced tendency for tolerance.[169,170] Better understanding of μ- and δ-opioid receptor heteromers will help in understanding peripheral pain, as well as development of tolerance, as it has been shown that several clinically used opioids are also selective for these heteromers.[171–173] A combination of μ-receptor agonists and cannabinoid receptor agonists in rhesus monkey models showed significant antinociception.[174] μ-Opioid receptor and CB1 (cannabinoid) receptor heterodimers[175] and μ-mGluR5[176,177] heteromers with opioid and nonopioid binding sites expressed strong antinociceptive effects in a range of models. In addition, a small-molecule agonist for the μ-κ opioid receptor heteromer, *N*-naphthoyl-β-naltrexamine, is a potent antinociceptive agent with no propensity to display physical dependence or drug-seeking behavior.[178]

Tissue Target-Selective Opioids. Inflamed tissues display an acidic environment as compared to a healthy tissue. NFEPP ([{±}-N-{3-fluoro-1-phenethylpiperidin-4-yl}-N-phenylpropionamide]) is a μ-opioid agonist that displays pH-sensitive binding and is thus limited in its activity to a peripheral action at injured/inflamed tissues inflammatory. It is reported to be absent CNS effects or display addiction potential.[179]

Abuse liability of the classical analgesic opioid agonists reflecting an effect on higher order neuraxial function is clear. To the degree that a pain state reflects on activity generated by a peripheral stimulus (eg, tissue injury, inflammation, neuroma), opioids with a peripherally restricted action acting upon systems outside the blood–brain barrier offer a potential way forward. As reviewed above, there is anticipation that NOP agonists or opioid agonists restricted to a peripheral action do not have intrinsic reinforcing effects.[154] Additional work on the biased ligands and heterodimer systems is required.

α2 Adrenergic Receptor-Targeted Drugs

α2 Adrenergic agonists have a potent analgesic action that is accompanied by sedation. The analgesic effects are mediated in large part by spinal α2 receptors of which there are 3 subtypes (α2A, B, C).[180] These are GPCRs that regulate dorsal horn excitation produced by small primary afferent input. Studies with mutations, antisense, and antagonists suggest an important role for the α2A subtype.[181–183] α2 Agonists delivered systemically or intrathecally have significant effects upon acute, inflammatory, and nerve injury hyperpathias.[184,185] In humans, neuraxial α2 agonist (clonidine) and systemic (clonidine, tizanidine, dexmedetomidine) have analgesic properties with sedation being a common sequelae of the actions of these agents. Dexmedetomidine is not a controlled substance. While the dependence potential of dexmedetomidine has not been studied in human, preclinical studies have shown, as with clonidine, withdrawal upon discontinuation.[186]

Cannabinoids

Cannabinoids can produce strong antinociceptive results in various animal models of acute, tissue injury, and nerve injury-induced nociception.[187] Cannabinoid receptors (CB1 and CB2) are G-protein–bound receptors that negatively bind via Gi/o proteins.[188] CB1 receptors are found in spinal neurons, particularly in the dorsal root ganglia,[189] and its agonists decrease excitatory transmitter release, whereas CB2 receptors reside in spinal microglia and attenuate microglial activation.[190,191] Cannabinoids mediate their psychotropic effects through CB1, not CB2.[192,193] Ligands that interact with CB1 and CB2 demonstrated the ability to regulate nociceptive processing.[194,195] Agents that block the metabolism of CB1 endogenous agonists consequentially increase its concentration and may be used to activate cannabinoid receptor function.[196] CB1- and CB2-selective agents, when intrathecally delivered, decreased facilitated states such formalin model, hyperpathia in neuropathy models and in tumor bone pain in rodents.[197–199] Cannabinoid role in pain processing is based on spinal and nociceptive neuron inhibition, although peripheral sites of action have also been identified. The use of spinal cord stimulation in a rodent neuropathic pain model revealed long-lasting and incremental reduction of hyperalgesia mediated by endocannabinoids. The effect was amplified by coadministration of LY2183240, an endocannabinoid reuptake/breakdown inhibitor, and inhibited by a CB1 receptor antagonist, AM251, but not by a CB2 receptor antagonist, AM630.[200] CB2 receptor antagonists may be a potential target in treating chronic pain of several etiologies by modifying cytokine profile when blocking peripheral immune tissue receptors and by blocking receptors in neurons and glial cells.[201]

The antinociceptive effect of eslicarbazepine acetate (ESL), an antiepileptic drug derived from carbamazepine/oxcarbazepine, has been shown to be mediated by serotonergic 5-HT1B/1D and cannabinoid CB1/CB2 receptors. ESL showed beneficial effect in different neuropathic and visceral pain models.[202] ESL has been tested clinically in different pain conditions (diabetic neuropathy, postherpetic neuralgia [PHN], fibromyalgia, etc; Table).

Abuse potential associated with CB1 receptor agonists has been well documented.[203] The CB2 receptors has been shown to modulate ventral tegmental dopamine neuron activity, circuitry considered pivotal in the addictive process.[204]

Angiotensin 2 Receptor Antagonist

Angiotensin may reside in primary afferents and can activate facilitatory cascades mediated through AT1 and AT2 receptors.[205,206] An AT2 antagonist, EMA401, has been tested in phase 2 clinical trials for the treatment of PHN, and preliminary data showed that it is well tolerated and it exhibited a primary analgesic efficacy end point.[207] Two phase 2b studies with EMA401 for PHN and painful diabetic neuropathy were put on hold.[208] However, recently, the new phase 2 study for PHN was registered at clinicaltrials.gov (Table).

Adenosine Agonists/Antagonists

In models of acute nociceptive processing,[209] neuropathy[209–213] and inflammatory pain[214] administration of adenosine and related ligands yielded significant antihyperalgesic effects. Intrathecally administered adenosine lowered allodynia in experimental pain models[215,216] and in patients experiencing neuropathic pain,[217,218] although negative results have also been reported.[219] Adenosine activates 4 G-protein–bound receptors: A_1, A_{2A}, A_{2B}, A_3.[220] A_1 receptors, which presynaptically inhibit neurotransmitter release and postsynaptically inhibit excitatory transmission,[221,222] are found on dorsal horn neurons and on small- to medium-sized neurons of the DRG.[221,223–226] A_{2A} receptor agonists were reportedly able to cause long-term reversal of allodynia in mononeuropathies,[227] while the possible explanation was the role of A_{2A} agonists as potential glial inhibitors. In addition, A_{2A} receptors may enhance glutamate release and A_{2A} antagonists may behave protectively by reducing such excitatory effect.[228] A_{2A} receptor knockout mice showed a significant reduction of the mechanical allodynia and a suppression of thermal hyperalgesia and allodynia, as well as attenuated expression of microglia and astrocytes, confirming potential beneficial role of A_{2A} receptor antagonists in the treatment of neuropathic pain.[229] Activation of the A3 adenosine receptor (A3AR) blocked hyperalgesia in mono- and polyneuropathies.[230] The abuse potential of agonists at these A1-3 receptors is not known.

Purine Agonist/Antagonists

Adenosine triphosphate, widely present in the CNS,[231] reacts with P2 receptor family with several subtypes: the P2X ligand-gated ionotropic receptors (consisting of 7 subtypes) and P2Y\GPCRs (divided into 8 subtypes). P2Y receptors may signal either via G_q/G_{11} to initiate the phospholipase C/inositol triphosphate ($InsP_3$) endoplasmic reticulum Ca^{2+}-release pathway (the $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, and $P2Y_{11}$ receptors) or via Gi/o, blocking adenylate cyclase and modulating ion channel function.[232] Both P2X and P2Y receptors reside in dorsal root ganglia, spinal neurons, and glia.[233] These receptors serve to activate glia, leading to the spinal release of proinflammatory proteins and cytokines underlying a facilitated pain state.[234–238] Transient reversal of hyperpathia after nerve injury was achieved via intrathecal

administration of P2X and P2Y inhibitors.[239–242] The P2X3 subtype is predominantly on C- and Aδ-fiber primary afferent neurons. P2X3 antagonists have shown efficacy in inflammatory and in mono- and polyneuropathic pain states.[243] P2X4 subtype is important in spinal facilitation that originated from tissue and nerve injury.[244] P2X4R antisense oligodeoxynucleotide intrathecal delivery prevented P2X4R protein expression and restrained mechanical allodynia development.[239] P2X4R, by modulating neuroimmune interactions in the spinal cord and DRG, could have an important role in development of neuropathic pain, signifying potential therapeutic effects of P2X4 receptor antagonists.[245] Electroacupuncture showed beneficial effect in neuropathic pain models by attenuating interferon-γ release and reduced expression of P2X4R in microglia.[246] Furthermore, duloxetine, a serotonin and noradrenaline reuptake inhibitor, showed results in neuropathic pain models by inhibition of P2X4 receptors.[247] AF-219, a P2X3 antagonist, is in clinical development as an antitussive. The abuse potential of purine receptor agonists and antagonists is unknown[248] (Table).

Innate Immune Signaling

Toll-like receptors (TLRs), a key sensory component in innate immune function, are found on neuronal and nonneuronal cells in the spinal cord and function by recognizing injury-associated molecular structures, while being strongly associated with proalgesic/inflammatory cytokines (DRG).[249,250] Intrathecal TLR4 antagonist administration resulted in improved effects within inflammatory and neuropathic pain states[251] and was associated with opioid-induced hyperalgesia phenomenon.[252] Another perspective on the role of TLR4 signaling was noted when it was found that the spinal delivery of a TLR4 antagonist (lipopolysaccharide-RS [LPS-RS]) would prevent the transition from an acute inflammatory state to chronic postinflammatory state with neuropathic pain phenotype,[253] while a small-molecule TLR4 antagonist (TAK242) would prevent the onset of late-phase allodynia after intraplantar formalin.[254] Repeated intrathecal administration of LPS-RS (TLR2 and TLR4 antagonist) and LPS-RS Ultrapure (TLR4 antagonist) attenuated allodynia and hyperalgesia and potentiated the effect of buprenorphine but not morphine.[255] Effort has been put into developing new structures to block TLR activation by interacting with the TLR4 ligand or downstream signaling[256–258] as shown by the antihyperpathic effects achieved by inhibition of MyD88 signaling.[259]

Lipid Mediators

Prostaglandins. The role of lipid mediators, such as the omega-6–derived prostaglandins, which produce a sensitized primary afferent and is centrally facilitated and mediated by eponymous receptors, has been long appreciated. Discovery of cyclooxygenase isoforms led to the rational development of prostanoid receptor antagonists and isoform-specific inhibitors, which were shown to have both a peripheral anti-inflammatory and a central action on spinal facilitatory processing.[260] Nonselective and COX-2 inhibitors have been shown to have significant antihyperpathic effects in a variety of tissue injury pain states in animal models[260] and in humans.[261] Unfortunately, typical limiting issues involve target-related actions on cyclooxygenase (gastrointestinal, platelet function, and cardiovascular) side effects.[262] An interesting parallel to the nonsteroidal anti-inflammatory drugs is the actions of acetaminophen.[263] This molecule has been shown to be efficacious in a variety of preclinical models and in clinical pain states associated with inflammation and tissue injury and in mono- and polyneuropathies, with dose-dependent effects on hyperalgesia and allodynia.[264–266] Available as an over the counter, this agent, in the United States for more than 100 years, has revealed no abuse liability. In spite of its utility, its mechanism of action is at best controversial.[265]

Soluble Epoxide Hydrolases. The epoxidized metabolites obtained from omega-3 long-chain fatty acids show anti-inflammatory and an antihyperpathic effect in a variety of preclinical models. However, they are being rapidly metabolized by enzymatic hydrolysis by soluble epoxide hydrolases. Of interest, inhibitors of soluble epoxide hydrolase have been shown to have significant antihyperalgesic actions in a variety of preclinical models.[267]

Proresolvins. Inflammatory cascades are typically self-limited leading to the healing phase of an injury. One of the mechanisms of this resolution has been a variety of lipid mediators referred to as proresolvins. These endogenous mediators include omega 3- (resolvins, protectins, and maresins) and omega 6-derived lipoxins. It is increasingly recognized that anti-inflammation and proresolution cascades represent distinct mechanisms for controlling the inflammatory response.[268] Delivery of a variety of these proresolvin molecules has shown to have significant antihyperpathic actions in a variety of inflammatory, mono-, and polyneuropathic models.[269,270] The abuse potential of these lipid mediators is not known.

Anti-inflammatory Cytokines

Upon activation of various glial signaling cascades, numerous cytokines (including activation of nuclear factor-κB) influence the proinflammatory mediators' production (eg, tumor necrosis factor, interleukin [IL]-6, and IL-1β), which, in turn, activate proalgesic cascades.[271] Additionally, such cascades can aid in the release of anti-inflammatory products (eg, IL-4, IL-6, IL-10, IL-11, IL-13, TGF-β) and soluble cytokine receptors,[272] which control the inflammatory cascade.[273]

Interleukin-10. It has been shown that IL-10 is one of the most powerful endogenous anti-inflammatory cytokines in the nervous system.[274] In animal models, IL-10 intrathecal delivery demonstrated therapeutic efficacy in various chronic pain models, primarily in treating different types of neuropathic pain.[274] In different animal models, viral vector-mediated expression of IL-10 in DRGs prevented development of painful diabetic neuropathy[275] and helped in treatment of HIV-induced neuropathy.[276]

Interleukin-4. Another anti-inflammatory cytokine IL-4 showed beneficial role in treating different types of neuropathic pain in different animal models.[277–279] An interesting variation is the intrathecal transfection of an

IL4/IL10 fusion protein leading to a potent and persistent antihyperalgesia.[280]

Toxins

There is an increasing interest in the potential of producing long-term changes in neuraxial pain processing by the peripheral or spinal delivery of agents that target the functionality of systems processing pain information. Here the consideration is for the treatment of persisting pain states. The role of these therapeutic approaches is not clear at the present time. For those approaches leading to permanent loss of cells, such as the saporin conjugates or the TRPV1 agonists, it appears less likely that they would be used outside the terminal patient (as in cancer). Toxins that result in long-lasting but irreversible effects such as the botulinum toxins (BoNTs) might be a therapeutic approach for persistent pain states in a nonterminal patient.

TRPV1 Receptors. TRPV1 channels are with few exceptions located on the central and peripheral terminals of high-threshold primary afferents. Topical[281] and spinal delivery[282] of TRPV1 agonists such as capsaicin or analogues such as resiniferatoxin desensitize the TRPV1 (+) afferent and destroy the DRG terminal[283] by calcium cytotoxicity[284] and analgesia. The effects after topical delivery have led to the approval of transdermal capsaicin.[285] Neuraxial delivery of TRPV1 agonists has been shown to result in robust antinociception in dogs.[286,287] Intrathecal resiniferatoxin showed potent and persistent antihyperalgesic effects refractory bone cancer pain in canines, without evidence of deafferentation sequelae.[288] One clinical trial testing the use of intrathecal resiniferatoxin for intractable cancer pain was begun and is currently on hold (Table).

Saporin Conjugates. GPCRs undergo internalization when occupied by their respective agonists.[289] Appropriate linking of a G-protein–targeted agonist such as substance P (SP) and a toxin such as saporin (plant product from *Saponaria officinalis* which is not otherwise taken up by the cell) will result after agonist binding to the neurokinin 1 (NK1) receptor, internalization of the agonist, and toxin complex into the cell expressing that receptor.[290] Saporin blocks ribosylation and protein synthesis, resulting in cell death. The NK1 receptor, a GPCR, found on postsynaptic second-order dorsal horn nociceptive neurons[291] is taken up into the neuron and the neuron dies. Intrathecally administered sP-saporin, but not saporin, robustly destroys NK1(+) dorsal horn neurons and attenuates pain states in rodents and bone cancer pain in dogs.[292–294] Intrathecal administration of sP-saporin is currently in phase 1 clinical trial for the treatment of intractable cancer pain (Table). Importantly, this functional coupling of a ligand to saporin is effective for any ligand for any GPCR that displays internalization.[290]

Botulinum Toxin. These toxins are composed of a heavy chain and a light chain (LC). The heavy-chain portion enables the toxin to be taken into the cell. Once inside, the complex is cleaved, freeing LC, which serves as enzyme cleaving Soluble *N*-ethylmaleimide-sensitive fusion protein Attachment protein REceptors (SNARES).[295,296] SNAREs mobilize vesicles for transmitter release and aid in the transport of GLUA1 AMPA receptor subunits to the membrane.[297] In case of SNARE cleavage, transmitter release is blocked. Preclinically, intrathecally administered BoNTs produced antihyperalgesic effects in various inflammatory and neuropathic hyperpathia.[298–301] The BoNT uptake is ubiquitous, and the potent effects on transmitter release may include inhibitory interneurons and motor neurons.[151,302] Several BoNT serotypes have been shown after topical application to be taken up and to block both local (peripheral) release from a nociceptor and to be transported centrally to inhibit downstream nociceptive processing, with indications of a possible pre- and postsynaptic effect.[303] Intravesical injections of onabotulinumtoxin-A (BoNT-A) showed significant pain reduction in patients with interstitial cystitis/bladder pain syndrome refractory to other treatments, suggesting a local effect upon the urothelium.[304] Coupling of the LC of BoNT-A with substance P showed a beneficial role in treating chronic pain after intrathecal delivery.[305]

Transfection Targets

The use of viral transection at the spinal level represents an exciting approach to modify spinal function. Intrathecal delivery of various transfection systems has been used to increase the expression of cytokines,[151,303] knock down of pivotal targets with shRNAs,[304,305] expression of transcription factor decoy proteins,[306] overexpression of micro-RNAs are among many spinal targets that have been successfully manipulated through transfection approaches. Technically, it is clear that, while intrathecal delivery of AAV may transfect ganglion neurons, parenchymal transfection may be limited, in part, by the diffusion barrier presented by the pia and transfection enhanced by subpial delivery.[307] Intraganglionic injections have also been suggested as an efficient tool to alter afferent function.[308] The ability to produce long-term, regulated changes in processing offers a potential to modify the pathological expression of pain by modifying system function.

CONCLUSIONS

The FDA has been mandated to address the national epidemic of opioid abuse with policies aimed at reversing the epidemic. One element of this plan, recognizing the pivotal role opioid receptors play in pain management, is to stimulate development of more effective pain medications with abuse-deterrent properties and abuse-deterrent formulations of opioids. The rational way forward is to develop analgesics that minimize abuse potential. To improve the translations, the FDA launched the Analgesic, Anesthetic, and Addiction Clinical Trial Translations, Innovations, Opportunities, and Networks (ACTTION) that issued recommendations to improve the reproducibility of research pertaining to pain studies.[306]

In the past 3 decades, by virtue of funding from the national funding agencies and by pharma, we have obtained an increased understanding of pain mechanisms and, accordingly, an abundance of relevant targets for which we must develop druggable molecules. The development of novel targets and the implementation of approaches that

can alter processing for extended periods (transfection and toxins) represent exciting advances in managing the chronic condition.

An important issue in analgesic drug discovery is that apparently promising preclinical candidates can fail during clinical trials. Several reasons for this may be entertained. It is straightforward to model human conditions for which the initiating mechanisms are likely known, as for example in chemotherapy-induced neuropathy. Conversely, it is difficult, if not impossible, to rationally define surrogate models for a pain state such as fibromyalgia, where the mechanisms of hyperpathia observed in the human conditions are not known.[307] Thus, preclinical models may fail to recapitulate the human pain condition being studied. Research into mechanisms and the appreciation of the role played by innate and adaptive immunity are likely to shed light on these complex problems, revealing novel, mechanistically defined targets that lack congruence of the clinical and preclinical target, where minor species differences in a receptor sequence may yield a drug that does not engage the human target. Modern molecular techniques and target sequencing make this disconnect less likely. Further research into biological biomarkers (exosomes) and genetic characterization will provide an important link to define human and animal covariates.[308,309]

It is interesting to note, as outlined in the Figure, that a preponderance of the targets producing therapeutic efficacy as analgesics (versus anesthetics) displays a robust effect on primary afferent and dorsal horn processing that leads to surprisingly specific changes in pain behavior, denoting the role played by the content of the ascending message in characterizing components of the aversive nature of the stimulus event. This emphasis does not exclude the likelihood that many agents, notably opioids, can exert a potent effect on pain behavior after supraspinal action with such actions accounting for changes in the affective-motivational component of the pain state. While it appears likely that specific supraspinal systems may be found that possess a pharmacology specifically targeting the pain state, current research has provided little evidence that what affects pain processing/behavior at supraspinal sites does not also have pronounced effects upon behavior and perception, aspects of which are associated with positive reward and the addictive potential. These results suggesting the interdigitation of these affective components are in parallel with the early work involving surgical resection of limbic and forebrain structures. While such interventions were reported to lead to a loss of the affective components of the pain state, they also led to profound changes in personality and judgment.[310]

Finally, the translational development of analgesics for the clinic must increasingly consider the issues of drug abuse. The aim is to address the specific management of pain and suffering. Clearly, agents minimizing the psychological underpinnings of suffering may well display a positive reinforcing component in the absence of pain. This conflation emphasizes the complexity of the problem and the challenges to selectively modify one of the most basic cognitive elements, the pain experience.

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Name: Nebojsa Nick Knezevic, MD, PhD.
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Conflicts of Interest: Ajay Yekkirala holds a patent on an analgesic agent and is co-founder, Chief Scientific Officer, and shareholder of Blue Therapeutics, Inc, a biotechnology company developing novel analgesics.
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