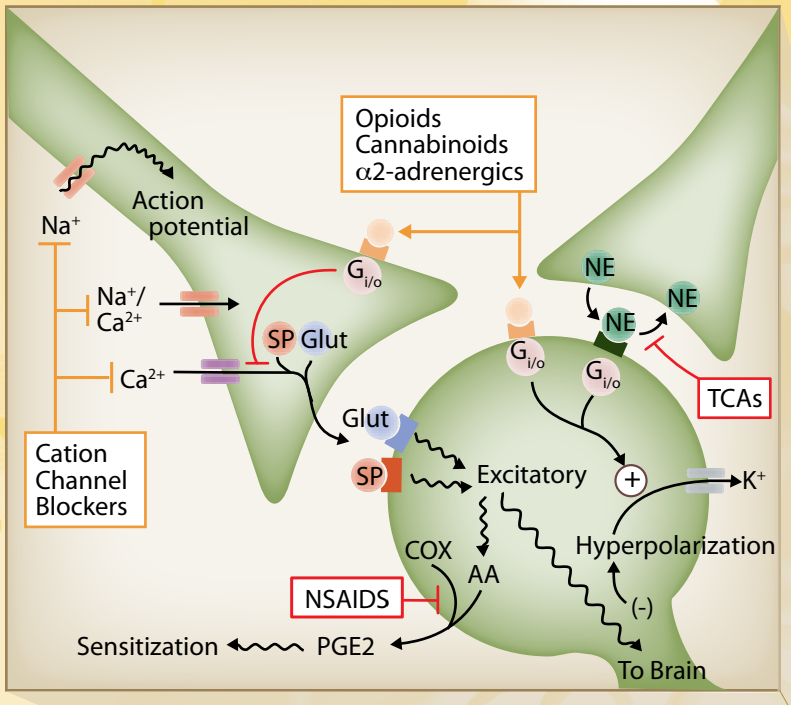
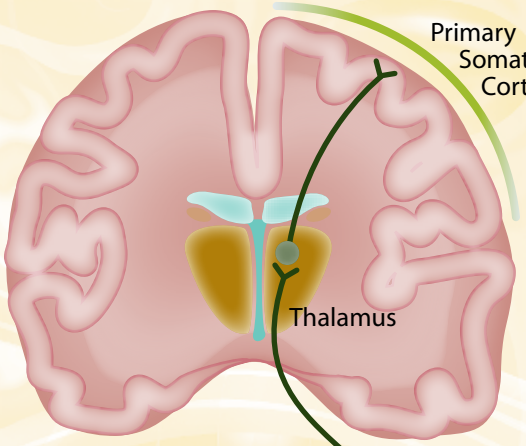
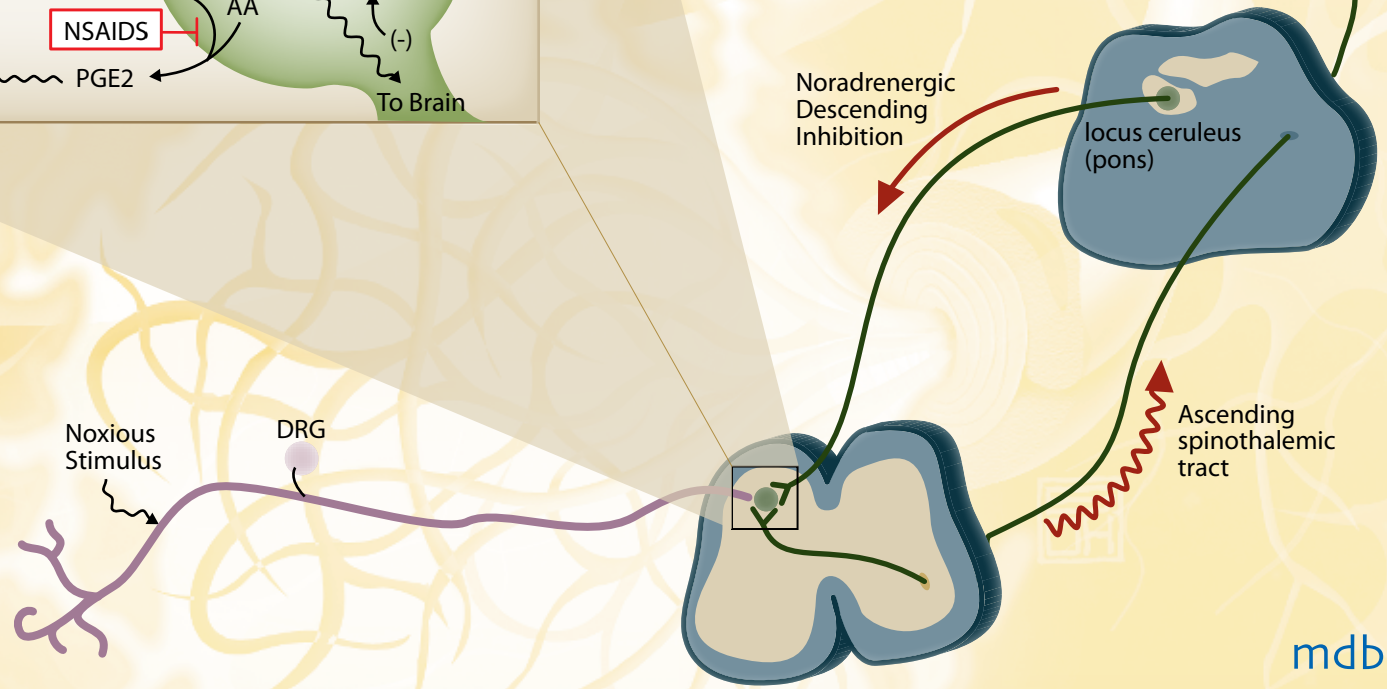
PAIN PROCESSING AND PATHWAYS

Choosing suitable behavior tests for common drug classes based on the primary mechanism and site of action.







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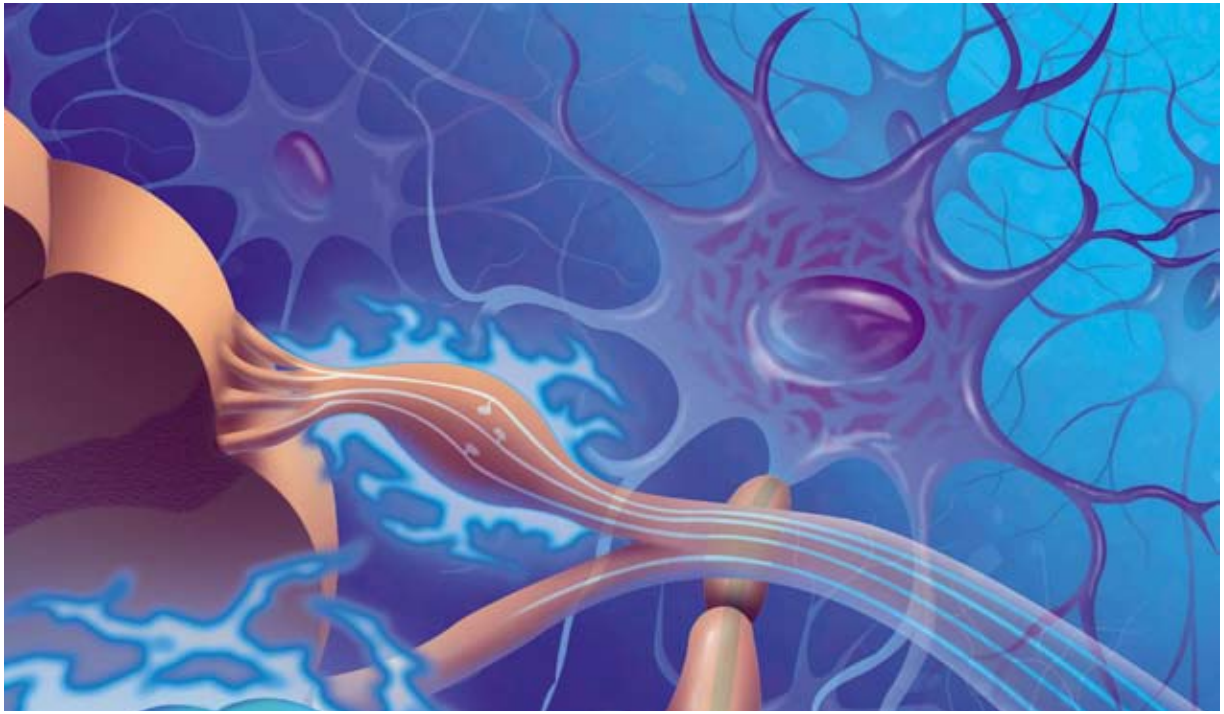
PAIN PROCESSING

INTRODUCTION

Pain is a complex and generally unpleasant experience that serves the important protective function of alerting us to situations that may threaten our well being. As such, pain is typically associated with noxious stimuli, events that are potentially or actually damaging to tissue. Pain processing begins with specialized sensory neurons called nociceptors that are able to distinguish and preferentially respond to noxious stimuli. Nociceptive primary afferent neurons receive information via free nerve endings at their peripheral terminals and pass that information to second order neurons in the dorsal horn of the spinal cord. This first synapse in the pain pathway is one of the most targeted sites for analgesic drugs. From here, several ascending pathways exist to relay messages related to arousal as well as affective and other aspects of pain. The most prominent ascending pain pathway is the



spinothalamic tract, via which axons from second order neurons cross the midline and project rostrally in the ventrolateral part of the spinal cord and medulla to the thalamus. Third order neurons in the thalamus then send projections to the primary somatosensory cortex, as well as other cortical regions, for localization and cognitive processing. One of the ways the body provides some endogenous pain relief is through descending inhibitory connections that originate from several areas of the brain and project back to the spinal cord to decrease the activity of nociceptive neurons there. One example is the noradrenergic pathway, which originates in the locus ceruleus and is the target of several pharmacological agents.

After tissue injury or nerve damage, neurons along the nociceptive pathway may display enhanced sensitivity and responsiveness, referred to as sensitization. A variety of events and agents can contribute to sensitization, including the release of inflammatory mediators such as prostaglandins or release of algesic (painful) substances from damaged cells or even the peripheral terminals of nociceptors themselves. The sensitization of nociceptive neurons can lead to an enhanced response to noxious stimuli, referred to as hyperalgesia, or pain in response to a normally innocuous stimulus, termed allodynia. In addition, peripheral nerve damage may cause nociceptors to fire ectopically, which contributes to spontaneous pain. Pain medications can provide relief either through targeting sensitizing agents or by inhibiting the activity of neurons involved in pain processing directly. This article will review several of the most commonly employed pain drug classes, their primary mechanism and site of action, and which behavioral tests are best suited to test novel compounds from each of these drug classes.

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CANNABINOID RECEPTOR AGONISTS

Appropriate Models of Pain
Tail Flick
Caspaicin
Carrageenan
CFA Inflammatory Pain
Post-operative Pain
Neuropathic Models

Cannabinoid receptor agonists and FAAH inhibition

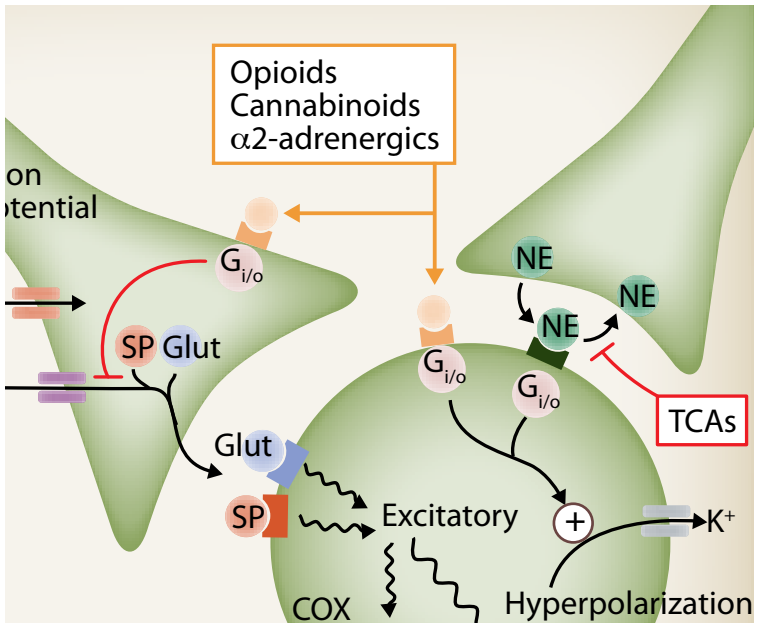
The body's cannabinoid system consists of two cannabinoid receptors, CB1 and CB2, their endogenous ligands, which include 2-arachidonoyl glycerol (2-AG) and anandamide (AEA), and the enzymes that regulate the synthesis and degradation of these ligands. While the endogenous cannabinoid system serves naturally to modulate pain transmission, it can be exploited to provide more robust relief, either through administration of agonists at CB1 or CB2 receptors or through inhibition of degrading enzymes to increase endogenous cannabinoid levels.

Although activation of either receptor can promote pain relief, CB1 receptors are responsible for the centrally-mediated psychomimetic side effects that sometimes accompany administration of cannabinoid receptor agonists such as tetrahydrocannabinol (THC).

Both CB1 and CB2 are GPCRs that signal predominantly through Gi/o to decrease VGCC conductance and activate GIRKs to hyperpolarize cells. Therefore, ligand binding to cannabinoid receptors results in decreased release of excitatory neurotransmitters from nociceptive neurons

Cannabinoid receptor agonists

CB1 receptors are expressed in neurons throughout the central and peripheral nervous system, including in the DRG, where noiciceptor cell bodies reside, the dorsal horn of the spinal cord, and the PAG, all of which are important sites for modulation of pain transmission. CB2 receptors, on the other hand, are not found in the CNS under normal conditions (although they may be upregulated in nociceptive neurons after injury) and are instead expressed in a variety immune cells and microglia [1].



and post-synaptic cells exhibiting decreased excitability for signals they do receive. Activation of cannabinoid receptors on immune cells can similarly inhibit their function and thereby indirectly modulate pain processing. Since CB2 receptors are found primarily on immune cells and microglia, this indirect, anti-inflammatory effect is the primary mechanism by which CB2-selective agonists modulate pain responses.

FAAH inhibition

Enzymes that degrade endogenous cannabinoids are another pharmacological target for pain therapy. One of the most commonly studied is fatty acid amide hydrolase (FAAH), the major degrading enzyme of the endogenous cannabinoid AEA. Normally, AEA is rapidly degraded, limiting its analgesic efficacy. Inhibition of FAAH results in more robust, longer lasting AEA action and does so without producing the psychomimetic effects elicited by some cannabinoid receptor agonists, making it a particularly attractive target [2].

Since pharmacological FAAH inhibition increases endogenous cannabinoid levels, it should not be surprising that activation of CB1 and/or CB2 receptors is the primary mechanism by which this drug class produces its antinociceptive effects. However, FAAH catabolizes fatty acid amides other than AEA, and cannabinoid receptor agonists can act at other receptor types. As such, non-cannabinoid mechanisms of action can contribute to the analgesic effects produced by FAAH blockade. For example, both TRPV1 and opioid receptor activation have been shown to play a role in FAAH inhibitor-mediated antinociception in some models [2].

Pharmacological targeting of the cannabinoid system, either through receptor activation or FAAH blockade, is a useful analgesic strategy for a wide variety of pain models. Cannabinoid agonists and FAAH inhibitors have shown efficacy in acute models such as tail flick and capsaicin injection, as well as carrageenan and CFA inflammatory pain models. Translation from animal models to the human condition has been documented for a variety of neuropathic conditions as well as for post-operative pain relief; therefore, both neuropathic and post-operative pain models would be appropriate for testing novel compounds designed to target the cannabinoid system as well [1]. ■

FAAH INHIBITION

Appropriate Models of Pain

Tail Flick

Caspaicin

Carrageenan

CFA Inflammatory Pain

Post-operative Pain

Neuropathic Models

Appropriate Models of Pain
Tail Flick (ARs only)
Caspaicin (ARs only)
Carrageenan (ARs only)
CFA Inflammatory Pain (ARs only)
Post-operative Pain
SNL Neuropathic Pain
STZ Diabetic Neuropathy

α_2-adrenergic agonists and tricyclic antidepressants

α_2-adrenergic receptors (α_2ARs) are found in many areas in throughout the nervous system, but the α_2ARs on pre- and post-synaptic neurons in the dorsal horn of the spinal cord are the main target for both endogenous and exogenous analgesia. One of the major descending inhibitory pain pathways involves the projection of noradrenergic neurons in the locus ceruleus back down to the spinal cord to activate α_2ARs at this site. These receptors can also be targeted pharmacologically through administration of selective agonists or through the inhibition of noradrenaline (also known as norepinephrine) reuptake by drugs such as tricyclic antidepressants.

α_2ARs are divided into three subtypes, the α_2A-, α_2B-, and α_2C-ARs. All three are Gi/o coupled GPCRs. α_2AARs are expressed mostly on the central, pre-synaptic terminals of nociceptors and inhibit VGCC on these terminals to reduce the release of excitatory neurotransmitters such as glutamate and substance P. At the same time, α_2CARs, expressed primarily on the second order neurons in the dorsal horn, reduce excitability of these neurons by increasing conductance through GIRK channels [1].

Tricyclic antidepressants (TCAs) are used clinically for the treatment of various neuropathic pain conditions, including nerve injury and diabetic neuropathy. Importantly, their analgesic efficacy is independent of the co-existence of depression in patients. Most TCAs have some action on both serotonin and norepinephrine reuptake, but their analgesic actions are largely mediated by increasing spinal noradrenergic tone coming from descending pathways, which then increases activation of α_2ARs to produce pain relief as described.

In accordance with their clinical usage, animal models of neuropathic pain are widely used to test novel TCAs. In fact, TCAs show little efficacy in animal models of acute or inflammatory pain. Although neuropathic and other forms of chronic pain are common indications for the clinical use of α_2AR agonists as well, they show robust antinociception in a much wider variety of animal models, including acute and inflammatory ones. Also, they are used both clinically for and in animal models of postoperative pain [1]. ■

Opioids

Opioid receptor agonists such as morphine have been used for pain relief for thousands of years and remain the standard treatment for many moderate to severe pain conditions. Opioid receptors are expressed in a variety of pain-modulating regions of the nervous system, including the peripheral terminals of nociceptors (where they are functionally upregulated after injury), the dorsal horn of the spinal cord, and several brain areas involved in descending inhibition of pain signaling.

Four distinct opioid receptors have been identified: mu, delta, kappa, and ORL-1. Mu opioid receptor agonists are the most widely used for the treatment of pain, although agonists at other opioid receptors have also been shown to be antinociceptive. All are GPCRs that couple primarily to $G_{i/o}$. The dorsal horn of the spinal cord is main site of action for opioids, where inhibition of VGCCs resulting from $G_{i/o}$ signaling causes a decrease in firing rate and release of excitatory neurotransmitters from DRG nociceptors onto second order neurons, which are also hyperpolarized due to GIRK activation. The same intracellular mechanisms are responsible for opioid-induced disinhibition of descending inhibitory pathways to promote pain relief [1].

Although opioid receptor agonists are of somewhat limited utility in the treatment of chronic pain due to the development of tolerance and other side effects, they are used clinically to treat a wide array of painful conditions. Accordingly, these agonists have been studied and shown to be effective in many animal models, especially acute nociceptive and inflammatory ones. Opioids may also be studied in post-operative models, and peripherally-acting kappa opioid receptor agonists in particular are among the few drug types that been have shown efficacy in models of visceral pain. In addition, although opioid receptor agonists are not always reliably effective in the treatment of neuropathic pain, they are sometimes used clinically for that purpose; therefore, neuropathic pain models may be used to test novel opioid compounds as well.

OPIOIDS

Appropriate Models of Pain

Tail Flick

Caspaicin

Visceral

CFA Inflammatory Pain

Carrageenan

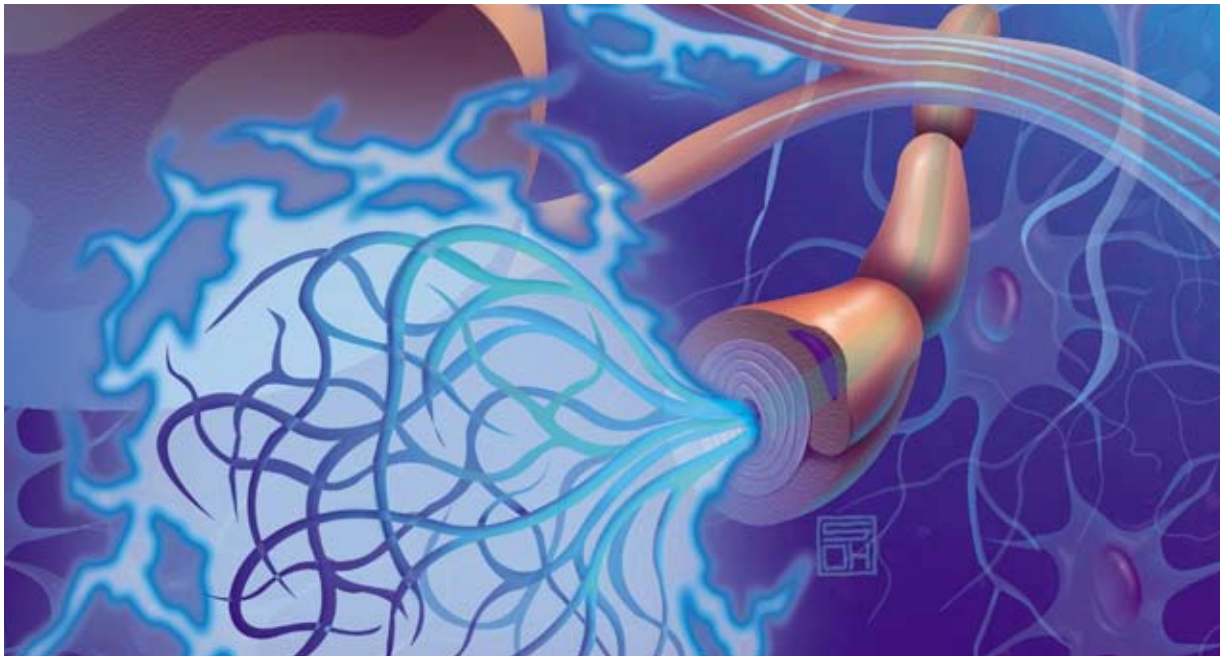
Post-operative Pain

Neuropathic Models

NSAIDs

Non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin and ibuprofen work indirectly to reduce pain by targeting prostaglandins, sensitizing agents produced following tissue damage or injury. Prostaglandins are released at the site of injury in the periphery and bind to their receptors on nociceptive sensory neurons, initiating a signaling cascade that leads to nociceptor sensitization. The repetitive firing of sensitized nociceptors, in turn, causes the release of prostaglandins in the spinal cord, augmenting sensitization and creating hyperalgesia.

NSAIDs reduce prostaglandin production by inhibiting cyclooxygenase (COX), the enzyme that converts arachidonic acid released by damaged cells to prostaglandins such as PGE2 and PGI2. Because of their indirect mechanism of action, neither acute nor neuropathic pain is sensitive to NSAID treatment. They are, however, effective in reducing carrageenan or CFA-induced inflammatory hyperalgesia and in models of postoperative pain [3]. NSAIDs are most commonly studied in models of arthritic pain and are among the standard treatment options for arthritis in humans.



Appropriate Models of Pain

Carrageenan

CFA Inflammatory Pain

Arthritic Pain

Post-operative Pain

Cation Channel Blockers

Sodium and calcium channel blockers

There are several types of drugs that have been developed to decrease the firing rate of nociceptive neurons by blocking cation channels. Among the most commonly known are lidocaine and bupivacaine, typically used as local anesthetics, which form an intracellular block of the voltage gated sodium channels (VGSCs) that are necessary for action potential generation. Without action potential firing, nociceptors are unable to propagate their message, and pain is thereby blocked. The main disadvantage of this class of drugs is that without selectivity for nociceptive sensory neurons, tactile input is also lost, leading to the numbness that accompanies local anesthetic administration.

Sodium channel blockers are most commonly used to treat neuropathic and other types of chronic pain; as such, models of neuropathic pain, particularly peripheral neuropathy models, are an excellent option for testing novel compounds of this drug class. Their analgesic efficacy may be more widespread, however, as they have shown to be useful against inflammatory pain and in some post-operative pain models. Notably, they are also among the few drug types shown to be effective in models of visceral pain.

Voltage gated calcium channels (VGCC) are another pharmacological target for pain relief. Gabapentin and pregabalin fall under the classification of gabapentinoids, which, while structurally similar to the endogenous neurotransmitter GABA, do not function as such. Instead, they bind to the α2–δ subunit of VGCC to reduce calcium influx into nerve terminals and thereby decrease neurotransmitter release. The α2–δ subunit of VGCC is highly expressed in the dorsal horn of the spinal cord, and decreasing the release of glutamate and substance P from nociceptive primary afferent neurons here is likely the main mechanism of action for drugs of this type. However, disinhibition of endogenous descending inhibitory pathways at supraspinal sites may also contribute to their analgesic effects [4]. Gabapentinoids are tested primarily in models of neuropathic pain, including both nerve injury and neuropathy models, which reflects their clinical utility.

TRPV1 ligands

The development of more selective cation channel blockers as a solution to avoiding the side effects that accompany a general neuronal blockade has been the subject of much investigation recently. Transient receptor potential

CATION CHANNEL BLOCKERS

Appropriate Models of Pain

CCI Neuropathic

SNL Neuropathic

Inflammatory Pain (Na channels)

Post-operative Pain (Na Channels)

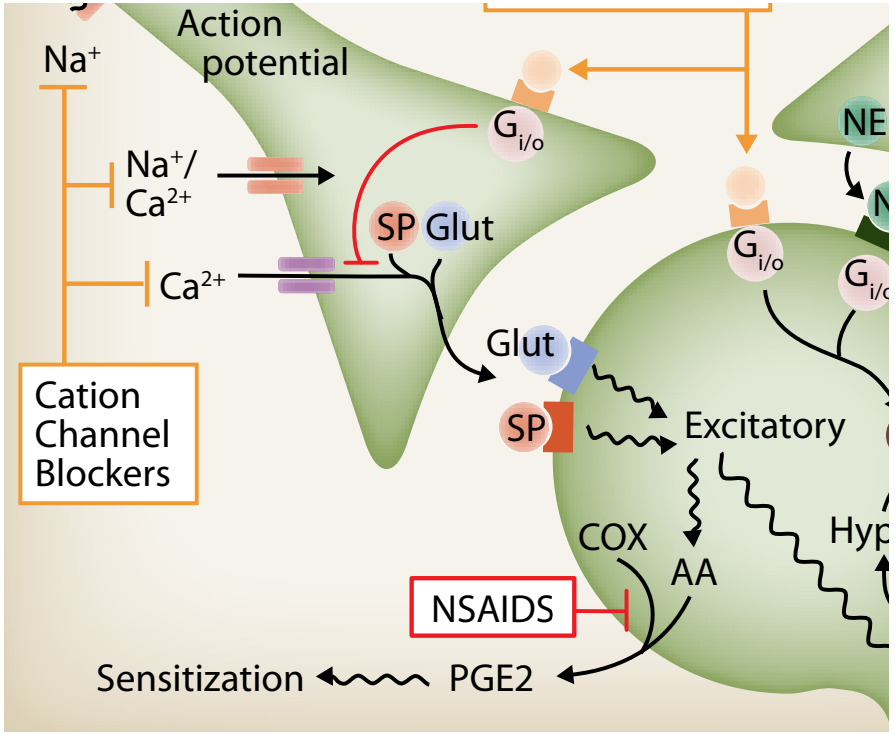
Visceral Pain (Na Channels)

TRPV1

(TRP) channels are attractive targets, as they are predominantly expressed in nociceptive DRG neurons. Activation of TRP channels, therefore, has little or no effect on normal mechanical sensation, and drugs that target these channels could potentially avoid centrally-mediated side effects as well.

TRPV1 channels, in particular, are widely studied as a potential therapeutic target. TRPV1 is a non-selective cation channel is activated by capsaicin, the active ingredient in chili peppers, as well as heat. The function of TRPV1 is also modulated by a variety of sensitizing agents released after injury, including protons. Inflammation resulting from injury can reduce tissue pH, thereby activating TRPV1, causing an increase in sodium and calcium influx into the cell, and thereby contributing to the sensitization of nociceptors under these conditions [5]. TRPV1 can be targeted through either antagonists to block activation directly or with agonists, which work by causing desensitization of the receptor following robust activation.

Given the effects of inflammation on TRPV1 function, it is not surprising that ligands for this receptor have shown efficacy in a variety of inflammatory pain models, including post-surgical and arthritic pain as well as standard inflammatory pain models [5]. Similar to other cation channel blockers, they are also effective in models of neuropathic pain, particularly peripheral neuropathy models, and in some models of visceral pain. ■



Appropriate Models of Pain

Inflammatory Pain

Arthritic Pain

Post-operative Pain

Nerve-Injury Neuropathic Pain

Visceral Pain

ANIMAL MODELS OF PAIN:

NOCICEPTIVE PAIN		
Inducer	Species	Mediating Factors
Capsaicin	Rats, mice	VR1
Tail Flick	Rats, mice	external stimuli
Visceral	Mice	Acid

INFLAMMATORY & ARTHRITIC PAIN		
Inducer	Species	Mediating Factors
Carrageenan	Rats	PGE2 and Mast cells
CFA	Rats	Cyokines, PG, Macrophages, Neutrophils
CFA (mono-RA)	Rats, mice	Cyokines, PG, Macrophages, Neutrophils
Collagen (RA)	Rats	Cytokine, Macrophages, Tcells
Adjuvant (RA)	Rats	Cyokines, PG, Macrophages, Neutrophils

NEUROPATHIC PAIN		
Inducer	Species	Mediating Factors
Surgery of sciatic nerve (CCI)	Rats	Inflammation of the nerve
Surgery of sciatic nerve, cutting (SNL)	Rats, mice	Damage to the nerve
STZ (diabetic)	Rats	Damage to nerve ending due to hyperlgycemia and hypoxia
Taxol	Rats	Taxol mediated neurotoxicity by interfering with sensory neuron skeleton structure

POST-OPERATIVE PAIN		
Possible Inducer	Species	Mediating Factors
Incisional Pain (Brennan)	Rats	Inflammation, wound
Incisional Pain	Pigs	Inflammation, wound

Please contact MD Biosciences to discuss evaluating compounds in preclinical animal models.

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Abbreviations:

DRG	Dorsal root ganglion
GPCR	G-protein coupled receptor
VGCC	Voltage gated calcium channel
VGSC	Voltage gated sodium channel
GIRK	G-protein coupled inwardly rectifying K+ channel
PAG	periaqueductal grey
SP	Substance P
Glut	Glutamate
$G_{i/o}$	G-protein coupled to receptor
NE	norephinephrine
AA	arachidonic acid
TCAs	Tricyclic antidepressants