IN SEARCH OF ANALGESIA:
Emerging Roles of GPCRs in Pain

Of all clinically marketed drugs, greater than thirty percent are modulators of G protein–coupled receptors (GPCRs). Nearly 400 GPCRs (i.e., excluding odorant and light receptors) are encoded within the human genome, but only a small fraction of these seven-transmembrane proteins have been identified as drug targets. Chronic pain affects more than one-third of the population, representing a substantial societal burden in use of health care resources and lost productivity. Furthermore, currently available treatments are often inadequate, underscoring the significant need for better therapeutic strategies. The expansion of the identified human GPCR repertoire, coupled with recent insights into the function and structure of GPCRs, offers new opportunities for the development of novel analgesic therapeutics.

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Introduction

The G protein–coupled receptors (GPCRs) comprise the largest superfamily of transmembrane receptors. Their function is to transduce extracellular stimuli into intracellular responses. These stimuli can be remarkably diverse, ranging from physical stimuli (e.g., photons or heat) to chemical signals in the form of ions (e.g., Ca^{2+}, H^+), chemical neurotransmitters (e.g., dopamine, noradrenaline, adrenaline, acetylcholine, or nucleotides), peptides and protein hormones (e.g., chemokines or opiates), and lipids and eicosanoids (e.g., sphingolipids or leukotrienes). GPCRs mediate and/or modulate virtually all physiological processes in eukaryotic organisms, including acute and chronic pain (1).

Disorders resulting in persistent pain are among the most common forms of chronic illness in North Americans. In individuals age sixty and under, the prevalence of migraine and chronic back pain is ten and fifteen percent, respectively. Arthritis among people less than sixty years of age occurs at a rate of twelve percent, and the frequency rises to forty-six percent for the population that is older than sixty (2). Medical conditions including diabetes, AIDS, and multiple sclerosis all have a high incidence of chronic neuropathic pain. Because pain impairs one's ability to carry out a productive life, it has serious economic consequences in addition to being a major health problem. In the US alone, an estimated $100 billion is spent each year on health care associated with chronic pain, and an equal amount is further estimated for the related loss of productivity (3, 4). Available therapeutic interventions, such as morphine, are not always able to adequately control pain; not only is drug efficacy at issue, but intolerable side effects, such as sedation, respiratory depression, and gastrointestinal impairment, can also preclude effective pain management. The development of new drugs that target members of the GPCR superfamily holds great promise for the treatment of acute and chronic pain, reaching far beyond the use of traditional opioid receptor agonists.

In this review, we will first provide an overview of GPCR function with regard to the pain signaling system. Second, we will discuss emerging insights into GPCR function that relate to nociceptive transmission. Finally, we will conclude with a brief summary of the role of each GPCR family in nociception.
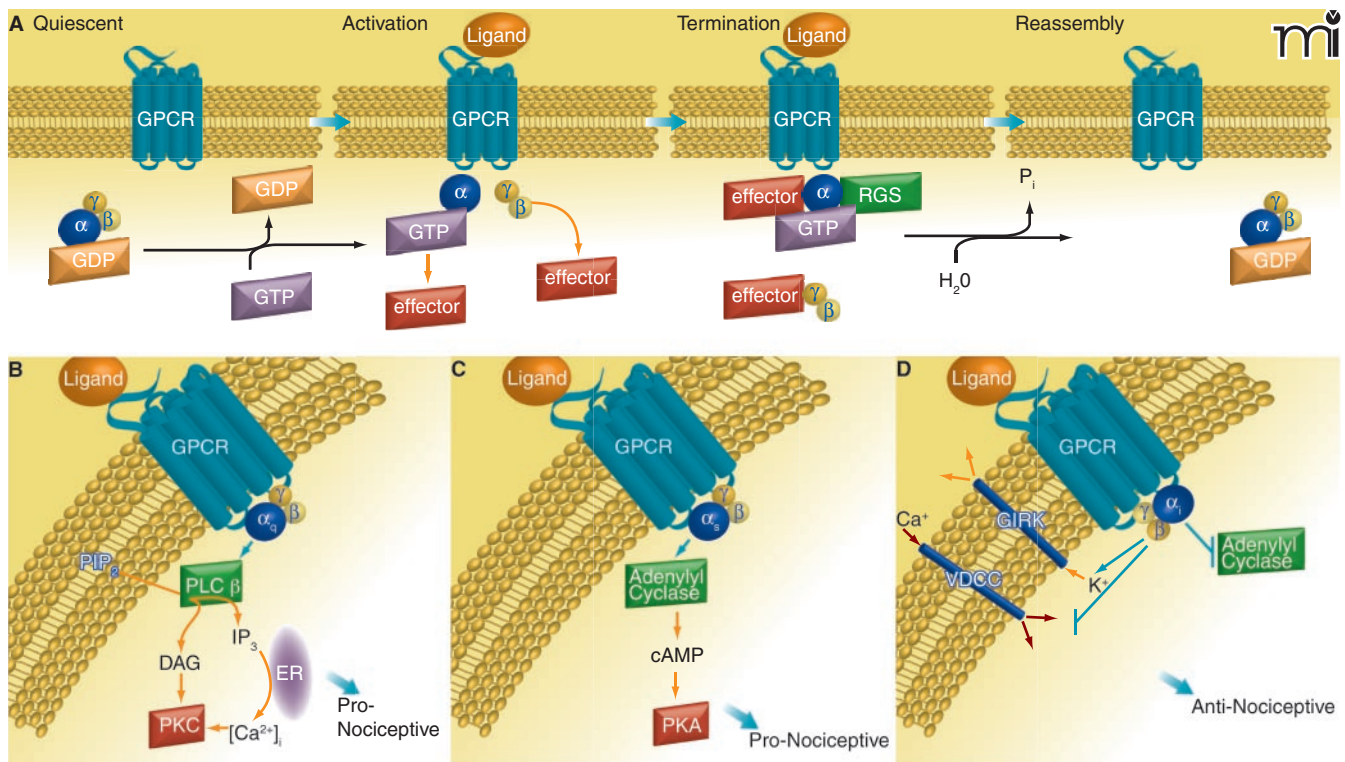

Figure 1. Overview of GPCR signaling. A) Diagram of the cycle of G protein activation and inactivation. In the absence of GPCR signaling (Quiescent), G proteins are present as inactive αβγ trimers; the α subunit is bound to GDP. Binding of ligand to GPCR (Activation) causes a conformational change that promotes binding of the receptor to its preferred trimeric G protein and concomitant displacement of bound GDP by incoming GTP at α subunit. Upon GTP binding, the αβγ trimer dissociates into GTP-bound monomer and βγ dimer, each of which can then interact with respective effectors. Signaling is terminated by the GTPase activity of the α subunit; this GTPase activity can be enhanced by RGS proteins. The α subunit–catalyzed hydrolysis of GTP causes subunits to reassemble into the trimeric G protein. The GPCR is generally desensitized and internalized for recycling or destruction (see text). **B–D)** Diagrams of the canonical signaling pathways for the major G protein families, as described in the text. (ER, endoplasmic reticulum.)

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G Proteins in Signaling

Upon GPCR activation, intracellular signaling systems are activated that couple to a diverse array of downstream effector systems. By definition, signal transduction through GPCRs involves the heterotrimeric GTP-binding proteins (G proteins) to which these receptors are coupled. Current estimates, based on the sequencing of the human genome, predict that the G proteins in human cells can be assembled from among sixteen α, five β, and fourteen γ subunits, with each heterotrimeric combination corresponding to a distinct complement of effector targets *(5, 6)*. The approximately 400 human GPCRs (exclusive of odorant and light receptors) are differentially expressed by specific tissues, allowing for a diversity of signaling cascades that may further be localized with respect to distinct intracellular domains and associated with specific G proteins. In addition, each GPCR may be sensitive to multiple endogenous agonists, and agonists may act at multiple receptor isoforms. Furthermore, data are emerging that GPCRs also elicit G protein–independent intracellular effects, further increasing the spectrum of possibilities *(7)*.

The basic cycle of G protein activation and inactivation is illustrated in Figure 1. Agonist binding and receptor activation induce a conformational change in the heterotrimeric G protein such that the α subunit binds GTP in exchange for GDP, thereby causing the G protein to dissociate into a GTP-bound α monomer and a $\beta\gamma$ dimer. The α monomer and $\beta\gamma$ dimer are subsequently free to engage target effectors. A mechanism for terminating G –protein signaling to effector systems is built in to the α subunit by means of its intrinsic GTPase activity. Hydrolysis of GTP returns the α subunit to its GDP-bound state, which assembles with the $\beta\gamma$ dimer to reform the inactive, heterotrimeric G protein. A number of regulators of G protein signaling, or RGS proteins, enhance the GTPase function of the α subunit and thereby reduce the duration of GPCR signaling. The G protein families primarily involved in the modulation of neurotransmission utilize α_s, $\alpha_{i/o}$, or $\alpha_{q/11}$ subunits (Figure 1B); members within each family show differences in their patterns of expression *(8)*. Downstream effectors also show isoform-specific intracellular targeting and tissue-specific distribution patterns, providing another level of selectivity in the signaling pathways activated by GPCRs in different cell types.

G_s proteins (i.e., heterotrimeric G proteins that possess an α_s subunit) exert their effects primarily by activating adenylyl cyclase, resulting in increased intracellular cyclic AMP (cAMP), which in turn activates downstream effectors, including protein kinase A (PKA). Activated PKA phosphorylates numerous proteins that determine the physiological properties of nociceptors (see below). There are also reports that in some cell types, including some nociceptors (sensory neurons that detect noxious stimuli), G_s signaling can lead to activation of protein kinase C (PKC) through the cAMP-activated guanine exchange factor Epac *(9, 10)*. These phosphorylation events are regulated in turn by phosphatases and their downstream substrates. PKA may also activate transcription factors, including the cAMP response element binding protein (CREB), leading to long-term changes in the physiological properties of affected neurons. G_s activation typically results in increased neuronal excitability.

$G_{i/o}$ proteins mediate the widespread inhibitory effects of many neurotransmitters. Especially significant for the purposes of our discussion, $G_{i/o}$ proteins also mediate the effects of almost all analgesic GPCR agonists. Several mechanisms account for the inhibitory activity of $G_{i/o}$ proteins. First, the GTP-bound $\alpha_{i/o}$ subunit inhibits adenyly cyclase, counteracting the effects of G_s activation. Second, the dimer acts to inhibit voltage-dependent calcium channels, resulting in reduced neurotransmitter release and negative regulation of calcium-activated transcription. Third, they directly hyperpolarize neurons by activation of the G protein–gated inwardly rectifying potassium channels (GIRKs), which results in reduced excitability. In addition to affecting channel activity, $G_{i/o}$ proteins can also modulate neurotransmitter release by interacting directly with release proteins *(11)*. An important function of $\alpha_{i/o}$ subunits is to activate the ERK/MAPK cascade, resulting in regulation of gene expression. [For a comprehensive review of presynaptic signaling by heterotrimeric G proteins, see *(12)*.]

$G_{q/11}$ proteins function mainly through phospholipase C beta (PLCβ), of which there are four known isoforms. PLCβ hydrolyzes membrane phosphatidylinositol-4,5-bisphosphate (PIP2) to form IP3, which evokes release of intracellular calcium stores (by activation of IP3 receptors), and diacylglycerol (DAG); both products lead to activation of protein kinase C (PKC). DAG may also activate protein kinase D. Increased intracellular calcium can promote neurotransmitter release at the presynaptic terminal, activates calmodulin-dependent mechanisms (e.g., calcium/calmodulin-dependent protein kinase), and may lead to transcription factor activation. PKC is a major effector for the functional modulation of neuronal signaling machinery downstream of GPCRs. PKCϵ appears to be particularly important in the sensitization of primary afferent nociceptors in response to activation of $G_{q/11}$ protein–coupled receptors, but other family members contribute to this process as well *(13)*.

Receptor activation can be terminated by G protein–coupled receptor kinases (GRKs) and arrestins [for review, see *(14, 15)*]. Following prolonged GPCR activation, GRKs phosphorylate the intracellular loops and C terminus of the receptor, which causes arrestins to associate with the GPCR and promotes receptor internalization. Internalized receptors may be recycled or targeted for degradation by ubiquitination. For many receptors, desensitization and internalization appear to be separate processes; the underlying mechanisms are under investigation *(14, 15)*.

In summary, GPCRs alter neuronal functional properties both by covalent modification of the signaling machinery (e.g., phosphorylation) and transcriptional activation of targeted genes. A basic overview of these pathways is provided in Figure 1. Our knowledge of the diverse pathways activated by GPCRs continues to expand [for review see *(16)*].

Box 1. Pain vs Nociception

Pain is defined by the International Association for the Study of Pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage." In contrast, *nociception* refers to the transduction of noxious stimuli, irrespective of cognitive awareness. A *nociceptor* is a sensory neuron preferentially sensitive to a noxious stimulus or to a stimulus which would become noxious if prolonged. Excitatory and inhibitory influences on nociception are referred to as *pro-* or *antinociceptive,* respectively. Increases and decreases in the experience of pain are referred to as *proalgesic* and *analgesic,* respectively.

Pain Signaling

Normal nociceptive transmission (see Box 1) begins when nociceptive axons innervating the target organ (e.g., skin, viscera, or joint) are activated by noxious stimuli. Primary sensory neurons transmit this information from the periphery to the spinal cord dorsal horn, where the nerve impulse is subject to local modulatory control. A subset of postsynaptic spinal neurons (i.e., secondary sensory neurons) send ascending axons to the thalamus, where they relay the information to higher cortical centers. The
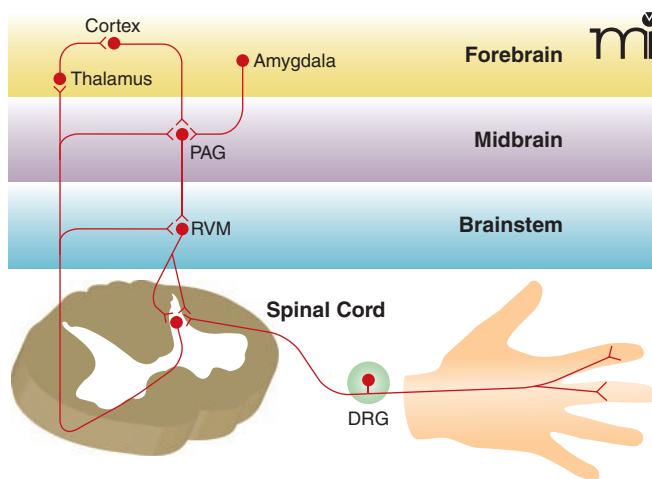

Figure 2. Overview of pain transmission. Nociception begins in the periphery with the activation of nociceptive sensory neurons by noxious stimuli (e.g., heat, acid, or tissue injury). These neurons, which have their cell bodies in the dorsal root ganglia (DRG), synapse on neurons in the spinal cord that send ascending projections to the thalamus, which in turn projects to forebrain regions involved in the subjective experience of pain. Descending inhibitory and excitatory pathways are activated by both ascending input from the spinal cord and descending input from the forebrain and limbic structures, including the amygdala. The major structures modulating descending modulation are found in the brainstem rostral ventral medulla (RVM) and midbrain regions [periaqueductal grey, PAG)].

ascending fibers also send collateral branches into brainstem (i.e., the rostral ventral medulla) and midbrain regions involved in pain modulation (i.e., the periaqueductal grey) and attention and emotion (i.e., the amygdala). These supraspinal centers in turn send descending projections to the spinal cord that can either inhibit or facilitate nociception. Many of the analgesic medications currently available target GPCRs in these descending pathways *(17, 18)*.

In chronic pain conditions, the normal regulation of nociceptive signaling may be altered. For example, inflammatory mediators released by peripheral tissues and immune cells in response to injury act at GPCRs to sensitize peripheral nociceptors (see Box 1), making them more responsive to both noxious and innocuous stimuli. Persistent firing of peripheral nociceptors causes spinal cord neurons to become more responsive to nociceptive input through a process known as central sensitization. Sensitization also occurs at higher-order relays in the brain. Neurons at each step in the pain pathway, both ascending and descending, are subject to modulation by GPCRs that thus represent potential targets for therapeutic intervention into persistent pain. An overview of the pain signaling system is provided in Figure 2.

Emerging Concepts in GPCR Signaling and Pain Modulation

GPCR Signaling and Modulation of Peripheral Nociceptive Channels

GPCRs modulate the function of a wide variety of ion channels and signaling molecules in sensory neurons, allowing neurons to rapidly adjust their sensitivity in response to changes in peripheral target tissues and at the central synapse. In particular, GPCRs modulate ligand-gated and voltage-dependent ion channels that determine key physiological characteristics of nociceptors *(19)*. These channels include members of the transient receptor potential (TRP) family of ligand-gated cation channels, such as TRPV1 and TRPA1, ATP-gated P2X channels, acid-sensing ion channels (ASICs), TTX-resistant sodium channels, voltage-dependent calcium channels, and M-type potassium channels.

TRPV1, a cation channel gated by heat and protons, is selectively expressed in a subset of primary afferent nociceptors and plays a key role in the sensitization of nociceptors in response to inflammation. Numerous GPCRs have been found to regulate TRPV1 *(20, 21)*. Both G_s and G_q protein–coupled receptor signaling enhance TRPV1 function, resulting in peripheral sensitization of nociceptors and reduced pain threshold. Activation of PKCε by G_q signaling plays a major role in the modulation of TRPV1. A related family member, TRPA1, shares many of the same regulatory mechanisms and is largely co-expressed with TRPV1. Several studies suggest that constitutive modulation of TRPV1 by GPCR signaling is required to maintain normal TRPV1 function *(20)*. Additional TRP family members have also been implicated in the transduction of thermal stimuli *(21)*.

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P2X3 is a member of the P2X family of ATP-gated ion channels that is preferentially expressed in the non-peptidergic subset of nociceptive sensory neurons. During inflammation, P2X3 currents are enhanced through phosphorylation by PKC. This occurs through an indirect pathway in which the G_s protein–coupled prostaglandin receptor PGE2 acts through Epac1 to activate PKC (9). ASICs are also positively regulated by GPCRs, including serotonin receptors, through the action of PKC. Phosphorylation appears to be selective for the ASIC2b subunit, although it affects currents through heteromeric channels, including those that contain ASIC3 subunits (22). Whereas ASIC2b is expressed by many sensory neurons, ASIC3 is more restricted to peptidergic nociceptors and a subset of larger-diameter neurons of unknown modality (23).

M-type potassium currents, generated by channels consisting of KCNQ subunits, play a key role in regulating nociceptor sensitivity. M-type potassium currents are negatively modulated through G_q protein–mediated signaling; this negative modulation acts to depolarize the resting membrane potential and thereby enhances nociceptive signaling (24). Voltage-dependent calcium channels regulate action potential kinetics and neurotransmitter release as well as neuronal activity–dependent transcription. In nociceptors, these channels contribute significantly to the duration of the action potential. $G_{i/o}$ protein–coupled receptors such as the opioid receptors inhibit primary afferent signaling in part by inhibiting calcium channels through a direct action of the G protein βγ subunits (25).

Tetrodotoxin-resistant sodium channels Nav1.8 and Nav1.9 are selectively expressed in nociceptors and contribute to injury-evoked changes in neuronal excitability. These channels are modulated through GPCR signaling cascades initiated by inflammatory mediators such as prostaglandins, serotonin, and adenosine (19). Regulation of these sodium channels can occur both through G_s signaling through PKA, which is antagonized by G_i, and by G_q activation of PKC.

PROTEIN SCAFFOLDING AND THE ORGANIZATION OF SIGNALING MOLECULES

The great diversity of GPCR signaling entails a large number of receptors that must evoke selective, often tissue-specific, cellular responses, despite a relatively small complement of G proteins. There is increasing evidence that the functional selectivity of GPCRs is tightly regulated through targeting of signaling components to macromolecular signaling complexes (so-called transducisomes) at specialized membrane compartments known as lipid rafts. Lipid rafts are membrane domains of reduced fluidity, enriched in cholesterol and glycosphingolipids, that promote the assembly of signaling protein complexes (26). These complexes are organized through protein–protein interaction domains (e.g., PDZ, SH2, and SH3 domains) and specialized scaffold proteins that physically coordinate the signaling effector molecules in the transducisome (27). This structural organization allows for highly-
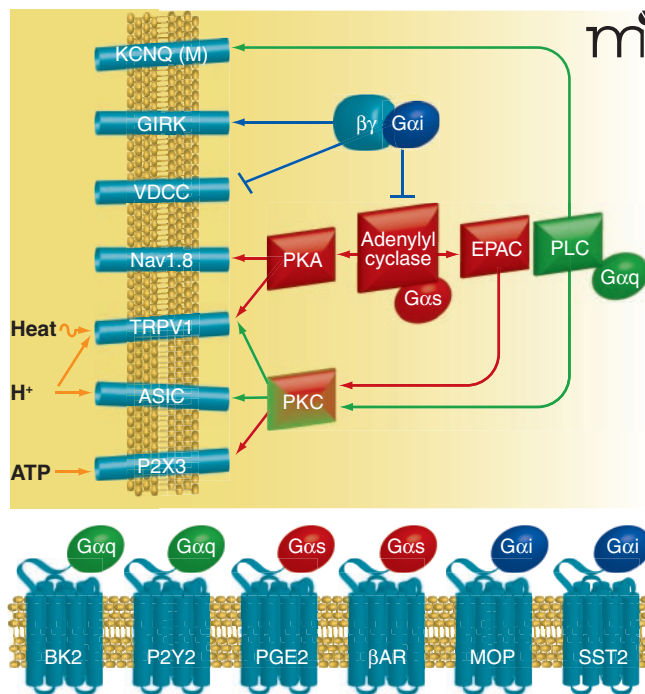

Figure 3. GPCR modulation of nociceptor excitability. GPCR activation, in response to tissue damage or inflammatory mediators, can often result in the covalent modification (e.g., phosphorylation) of ion channels; such channel modification can modulate important physiological properties of nociceptors. A few examples of GPCRs and ion channels that are involved in the modulation of nociceptor activity are shown (see text for details); many other GPCRs have also been implicated in this process. GPCRs also regulate functional properties of neurons at the level of transcription (not shown here). Channels that are regulated by GPCR activation include the ligand-gated ion channels TRPV1, ASICs, and P2X3; voltage-dependent channels including tetrodotoxin-in-resistant sodium channels Nav1.8 and Nav1.9; voltage-dependent calcium channels; KCNQ channels mediating the M-type potassium current; and G protein–activated potassium channels (GIRK). GPCR-mediated pathways regulating channel function represent an active area of investigation.

efficient regulation of effector function. For example, in some cells, G_q protein–coupled glutamate receptors in the plasma membrane are physically associated with the IP3 receptors on endoplasmic reticulum that regulate calcium stores. This process occurs through binding of the scaffolding protein Homer (28). In nociceptors, some ion channels modulated by G protein signaling are also associated with the transducisome. Examples of channels that are rapidly modulated upon nociceptive GPCR activation and are likely to be associated with scaffolding proteins include the heat- and acid-gated channel TRPV1 and the M-type potassium channel (discussed above) (29).

Available evidence suggests that GPCRs and G proteins are modified by fatty acid acylation, particularly palmitoylation and myristylation, and that these modifications, along with specific polypeptide sequences within the GPCR transmembrane domains, are responsible for directing these proteins to lipid rafts. However, other mechanisms, as yet unidentified, are likely to be important in determining whether a GPCR is targeted to lipid rafts (30). The
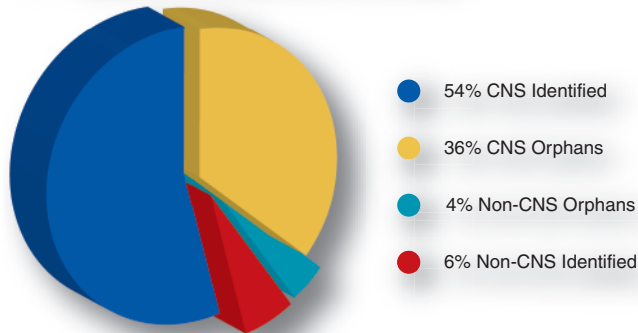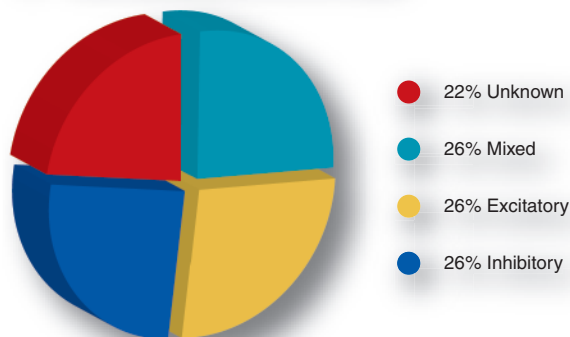
A   Orphan GPCRs in the Nervous System

- 54% CNS Identified
- 36% CNS Orphans
- 4% Non-CNS Orphans
- 6% Non-CNS Identified

B   Pain Modulation by GPCR Family

- 22% Unknown
- 26% Mixed
- 26% Excitatory
- 26% Inhibitory

Figure 4. Current status and opportunities in GPCR targeting. A) Predicted proportions of human GPCRs by nervous system expression (90%) and orphan status (40%). Approximately one-third of all GPCR genes encode orphan receptors that are expressed in the CNS. The endogenous ligand(s) and physiological function(s) of these receptors remain to be discovered, representing enormous opportunities for drug development. **B)** Modulatory effects of GPCRs organized by family. The equal distribution of inhibitory, excitatory, and mixed functional families suggests an equal balance between pro- and anti-nociception.

formation of signaling complexes in lipid rafts provides a mechanism for specialized and highly efficient signal transduction, with pathway selectivity determined by the association of specific effector molecules and receptors through scaffolding proteins.

Integrins are transmembrane proteins associated with lipid rafts and mediate focal adhesions at which the intracellular cytoskeleton connects to extracellular matrix. Integrins contribute to the formation of signaling complexes that are activated in response to binding of extracellular matrix proteins. Studies by Levine and colleagues indicate that integrin binding to the extracellular matrix, along with intact lipid rafts, is essential for signaling through a number of GPCRs in inflammatory hyperalgesia *(31)*. The extent to which aberrant nociceptive GPCR signaling or malformation of signaling complexes might underlie persistent pain states is a largely untapped area of investigation.

Ligand- and G Protein–Independent GPCR Signaling

There have been several reports that GPCRs can engage with components of the intracellular signaling complex and activate G protein signaling in the absence of extracellular ligands. Signaling complex components that have been implicated in such interactions include adhesion molecules such as integrins, scaffolding molecules such as Homer, and growth factor receptor tyrosine kinases such as the nerve growth factor receptor TrkA and the epidermal growth factor receptor. This kind of receptor transactivation has been described in both directions; GPCRs may also activate signaling through receptor tyrosine kinases or integrins *(16)*.

In addition, increasing evidence supports the idea that signaling by GPCRs may occur independently of G proteins. The molecules most clearly involved in this process are the β-arrestins, which are widely understood to be involved in the desensitization and recycling of GPCRs. β-arrestins are also able to function as scaffolding molecules for GPCRs and downstream effectors, such as Src tyrosine kinase family members and the MAP kinases, and may actually allow transactivation of these molecules independently of G protein actions *(32)*. However, pathways also exist for the activation of MAP kinases by G protein subunits, suggesting that the pathway used for activation of a specific effector molecule in a given cell type is highly context-dependent *(16)*. These data raise the possibility that there are GPCR-mediated effects in nociceptors that are ligand- and/or G protein–independent *(33)*.

GPCR Oligomerization

It is now recognized that GPCRs, traditionally envisaged monomeric, can form oligomeric complexes. These associations can result in novel pharmacological properties distinct from either component receptor, including alterations in ligand binding affinity, changes in signal transduction, and altered receptor trafficking [for review see *(34–37)*]. The recognition of oligomeric GCPRs has led to significant re-evaluation of the in vivo mechanisms thought to be involved in GPCR function. Homo- and hetero-oligomerization has been documented within GPCR families, (e.g., the opioid receptor family) and across GPCR families. For example, the functional implications of GPCR–GPCR interactions includes the "unmasking" of opioid binding sites when both the μ- and δ-opioid receptors are co-expressed *(38)*. In addition, the formation of functional GABA_B receptors is predicated on a requirement for co-expression of both GABA_BR1/GABA_BR2 receptor species *(39–41)*. Although the potential in vivo relevance of these data for neuronal function remains an open question, the existence of oligomers has significant implications for drug development. For example, if the functional receptor is heteromeric, strategies to identify GPCR ligands that rely on cell systems expressing only a single receptor type may not be successful. It is therefore possible that the large number of currently orphaned GPCRs reflects the

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use of screening paradigms that rely on monomeric rather than heteromeric systems.

Regulation of Cell Surface Expression

The regulation of GPCR internalization and recycling to the cell surface following agonist activation is an area of intense research and has been reviewed extensively elsewhere *(42, 43)*. An interesting new development, with relevance to analgesic drug discovery, is the observation that receptor signaling, internalization, desensitization, and recycling can differ, depending on the specific ligand used *(44)*. This ligand-specific regulation has enormous implications for the development of clinically useful agents with reduced risk of tolerance.

To become functionally competent, GPCRs must be properly synthesized and trafficked to the cell membrane, processes that are under tight cellular control [for review, see *(7)*]. The cell surface expression of the δ-opioid receptor (DOP) subtype is a case in point. In axon terminals, DOP is associated with large, dense-core vesicles (LDCVs) and in sensory and spinal cord neuron cell bodies, expression is primarily intracellular. In both axons and cell bodies, DOP appears to be inserted into the plasma membrane in a stimulus-dependent manner [see *(37, 45, 46)*]. DOP may also be translocated in response to chronic morphine exposure, peripheral inflammation, inflammatory mediators, and chronic nociceptive stimuli. As a consequence, sensitivity to DOP agonists is increased. For example, chronic morphine treatment results in an increase both in intrathecal DOP agonist–induced analgesia and in the number of plasma membrane–associated DOP-immunoreactive particles *(47)*.

GPCR Families in Pain Modulation

Early attempts to study, classify, and target GPCRs relied on measurable functional endpoints and on the availability of compounds to selectively stimulate or antagonize those responses. Historically, the modification of these compounds provided the primary approach to the development of new drugs with improved properties. For example, there are at least two dozen different chemical entities in clinical use that target opioid receptors (e.g., morphine and methadone), and most exist in multiple formulations optimized with regard for route of administration or half-life in the plasma. As a result, currently available drugs target only a small fraction of GPCRs.

The human genome project has identified more than 800 different GPCRs, approximately half of which are predicted to respond to endogenous (non-light and non-odorant) ligands *(48, 49)*. Of the 379 GPCRs (i.e., exclusive of odorant and light receptors) by the International Union of Basic and Clinical Pharmacology Committee on Receptor Nomenclature and Drug

Classification (NC-IUPHAR), an estimated ninety percent are expressed in the central nervous system *(50)*, and nearly forty percent of all GPCRs remain orphans with no identified ligand *(49)*. These numbers predict that over 100 new GPCRs of currently unknown function remain to be identified in the central nervous system, and indeed, new potential targets for drug development have been identified along with previously unknown neurotransmitters. Further insights into GPCR identification and characterization will undoubtedly advance our understanding of pain transmission.

We used the NC-IUPHAR classification system (www.iuphar. org/nciuphar.html) to survey the role(s) of each of the currently proposed GPCR families in pain processing. We were astounded to discover that nearly eighty percent (47/61) of the currently identified families have a known role in the modulation of pain. These survey results speak to the enormous physiological importance of pain modulation by GPCRs. A brief summary of our current understanding of the role of each GPCR family in nociception is provided in Table 1.

Conclusions

Regulation of pain transmission by GPCRs occurs throughout the central nervous system, providing a dominant focus for clinical analgesic therapy. However, modulation of nociceptive transduction and processing also occurs in the primary afferent neurons and in peripheral tissues, and all of these sites represent potential targets for novel analgesics. Indeed, it is becoming increasingly clear that GPCRs provide a fundamental mechanism of regulation in an integrated network of communication among sensory axon terminals, their peripheral target tissues, and immune cells *(20)*. The identification of receptors and mechanisms of regulation of GPCRs in pain transmission remains a fertile and largely unexplored field for the development of novel therapeutics for acute and chronic pain, particularly given the paucity of currently available drugs. ♥ doi:10.1124/mi.9.5.7

Table 1. GPCR Families and Their G Protein–Dependent Roles in Pro- and Antinociceptive Processing

GPCR family[a]	Role[c]	Subtype(s)	G protein(s) [a, b]	Description	Source
5-HT (Serotonin)	+/−[d]	$5\text{-HT}_{1A,B,D,E,F}$	$G_{i/o}$	Descending facilitation and inhibition; peripheral sensitization. 5-HT_1 agonists are used to treat migraine.	17, 51
		$5\text{-HT}_{2A,B,C}$	$G_{q/11}$		
		$5\text{-HT}_{4,5A,6,7}$	G_s		
Acetylcholine (muscarinic)	−[e]	M_2, M_5	$G_{i/o}$	Antinociceptive by supraspinal and spinal mechanisms; support analgesic effects of opioid and α_2AR agonists. Cholinesterase inhibition (neostigmine) produces analgesia in acute and chronic pain in humans.	52, 53
		M_1, M_3, M_5	$G_{q/11}$		
Adenosine	−	A_1	$G_{i/o}$	Antinociceptive and antihyperalgesic actions in spinal cord. Systemic agonists elicit severe motor effects, but enzymatic conversion of extracellular AMP to adenosine in the spinal cord produces analgesia without motor effects, suggesting novel approach for analgesic therapeutics.	54–56
		$A_{2A,B}$	G_s		
		A_3	$G_{i/o}$		
Adrenergic	+/−	$\alpha_{1A,B,D}$	$G_{q/11}$	α_1ARs are pronociceptive, particularly in sympathetic nervous system dysfunction. α_2AR agonists have analgesic actions throughout the CNS in acute, inflammatory, and neuropathic pain. βARs are involved in the negative affective component of pain and endogenous pain sensitivity in humans.	57–60
		$\alpha_{2A,B,C}$	$G_{i/o}$		
		$\beta_{1,2,3}$	G_s		
Angiotensin	+[f]	AT_1	$G_{q/11}, G_{i/o}$	Supraspinal angiotensin is pronociceptive. Spinal administration appears ineffective.	61, 62
		AT_2	$G_{i/o}$		
Anaphylatoxin	+	C3a	$G_{i/o}$	C3a and C5a, components of the activated complement system, mediate neuroimmune function. Blockade of the complement system reduces pain in animal models of acute, inflammatory, and neuropathic pain.	63–66
		C5a	$G_{i/o}, G_{16}$		
		C5L2	NA		
Apelin	−	APJ	$G_{i/o}$	One study, linking the apelin receptor to nociception, reports that supraspinal administration of apelin-13 produced antinociception and potentiated morphine.	67
Bile aid	NA[g]	GPBA	G_s	Bile acids induce visceral pain, but GPBA is not nociceptive per se; levels of GPBA are low in CNS.	68, 69
Bombesin	+/−	BB_{1-3}	$G_{q/11}$	Bombesin receptors exist throughout CNS. Supraspinal bombesin receptors produce antinociception, but spinal activation results in hypersensitivity.	70, 71
Bradykinin	+	B_1	$G_{i/o}, G_{q/11}$	Peripheral injection of bradykinin causes pain behavior and heat hyperalgesia. B_2 is constitutively expressed in sensory neurons. B_1 expression is upregulated in response to inflammation, nerve injury, and trophic factor application.	72–74
		B_2	$G_s, G_{i/0}, G_{q/11}$		
Calcitonin	+/−	CT, AMY_{1-3}, CGRP, $AM_{1,2w}$	G_s	CGRP is expressed by a subset of predominantly nociceptive sensory neurons and mediates central and peripheral sensitization and neurogenic inflammation. CGRP antagonists are under exploration as antimigraine agents. Calcitonin is reportedly analgesic in humans. Amylin is expressed by small sensory neurons, implying nociception. Activation of AM receptors in the spinal cord produces heat hyperalgesia.	75–77

GPCR family[a]	Role[c]	Subtype(s)	G protein(s) [a, b]	Description	Source
Cannabinoid	–	$CB_{1,2}$	$G_{i/0}$	CB_1 receptors are expressed in CNS and produce both analgesic and psychoactive effects. CB_2 receptors, putatively absent in CNS, inhibit acute, inflammatory, and neuropathic pain. Clinical utility of cannabinoid agonists is under study.	18, 78, 79
Calcium-sensing	NA	CaS	$G_{i/o}$, $G_{q/11}$	Calcium-sensing GPCRs are widespread among neurons, oligodendrocytes, and keratinocytes and respond to extracellular calcium, magnesium, polyamines, L-amino acids, ionic strength, and pH. GPRC6A was identified by genomic screening and is a promiscuous amino acid receptor.	49
		GPRC6	$G_{q/11}$		
Chemokine	+	CCR_{1-10}, $CXCR_{1-6}$, CX3CR1, XCR1	$G_{i/0}$	Chemokine receptors modulate calcium channel activity through $G_{i/o}$. Chemokines have emerged as key modulators of neuropathic and inflammatory chronic pain. Chemokines and their receptors form a link between the immune and nervous systems.	80–83
Cholecystokinin	+	$CCK_{1,2}$	$G_{q/11}$	Activation of CCK receptors has pronociceptive and/or antiopioid activity at several levels along the neuroaxis.	17, 84
Corticotropin-releasing factor	+/−	$CRF_{1,2}$	G_s	Brain CRF receptors are critical to hypothalamic-pituitary axis responses to pain and stress. Acute activation of this system results in analgesia; chronic activation may enhance pain. Many chronic pain states are associated with HPA axis dysfunction.	85–87
Dopamine	+/−	$D_{1,5}$	$G_{q/11}$	Dopamine receptors are widely expressed throughout the brain and contribute to descending pain modulation. Evidence exists for both antinociceptive D_2 and pronociceptive D_1 actions in spinal cord.	17, 88
		D_{2-4}	$G_{i/0}$		
Endothelin	+/−	ET_A	$G_{q/11}$	Endothelins are involved in the maintenance of vascular tone. Peripheral ET_A activation promotes pain-like behavior; ET_B receptor activation is analgesic (possibly by endogenous peripheral opioid activation analgesia.	89, 90
		ET_B	$G_s, G_{i/0}, G_{q/11}$		
Estrogen	+	GPER	G_s	Previously designated as orphan receptor GPR30, the G protein–coupled estrogen receptor functions autonomously from the steroid receptors ERα and ERβ. GPER is expressed in small sensory neurons; its activation increases CFA-induced allodynia in the masseter muscle.	91
Formylpeptide	NA	FPR_1, FPR_2	$G_{i/o}$	Formylpeptide receptors are expressed in brain, spinal cord and peripheral nervous system. A role for the formylpeptide receptors in nociception has not been investigated.	92
		FPR_3	NA		
Frizzled	NA	$FZD_{1,7}$	$G_{q/11}$, $G_{i/o}$	Frizzled receptors are important in embryonic development and adult tissue homeostasis; their modulation of bone remodelling may have relevance to arthritis.	93, 94
		$FZD_{2,4,6,9,10}$	$G_{i/o}$		
		FZD_3	$G_{q/11}$, $G_{i/o}$, G_s		
		$FZD_{5,8}$	$G_{q/11}$		
		SMO	NA		

GPCR family[a]	Role[c]	Subtype(s)	G protein(s) [a, b]	Description	Source
Free fatty acid	NA	FFA_1, FFA_2	$G_{q/11}$	Free fatty acids as ligands for a family of orphan GPCRs is a new concept in cell surface receptor signaling. Fatty acids are involved in the immune response and may be important in neuroimmune interactions; a role in pain remains to be investigated.	92
		FFA_3	$G_{i/o}$		
		GPR42	NA		
Galanin	–	$GAL_{1,3}$	$G_{i/o}$	Endogenous galanin modulates spinal nociception primarily in an inhibitory manner and is highly upregulated in sensory neurons following peripheral nerve injury. Galanin is also expressed by neurons that send descending projections to spinal cord.	17, 95, 96
		GAL_2	$G_{q/11}$		
$GABA_B$	–	$GABA_{B1}$, $GABA_{B2}$	$G_{i/o}$	The $GABA_B$ agonist baclofen is used clinically to treat pain in patients with spasticity, but also has efficacy in neuropathic, stroke, spinal cord injury, and musculoskeletal pain.	6, 18
Ghrelin	–	Ghrelin	$G_{q/11}$	Ghrelin has antinociceptive activity following central, systemic, and local peripheral administration. The ghrelin receptor is expressed in the dorsal horn, directly inhibiting spinal neurotransmission.	97, 98
Glucagon	–	GHRH, GIP, GLP-1, glucagon, secretin	G_s	Neither glucagon or the glucagon-like peptide have been reported to directly modulate nociception; however, activation of the growth hormone–releasing hormone receptor attenuates inflammatory hypersensitivity. Reduced GHRH levels have been associated with fibromyalgia.	99–101
		GLP-2	NA		
Glycoprotein hormone	+/–	FSH, LH, TSH	G_s	Acute activation of the hypothalamic-pituitary-adrenal axis results in analgesia; chronic activation may enhance pain. Disturbances in the HPA axis are observed in chronic pain.	86, 87, 102
Gonadotrophin-releasing hormone	+/–	GnRH	$G_{q/11}$	The GnRH receptor, regulating the biosynthesis and secretion of the gonadotropins, may affect pain thresholds by regulating estrogen and testosterone levels. Altered gonadotropin levels have been detected in spinal fluid from chronic headache patients.	103, 104
		GnRH2	NA		
Histamine	+	$H_{1,2}$	$G_{q/11}$	Histamine activates nociceptors, releases pronociceptive neuropeptides, and is painful when injected into skin. It contributes to neurogenic inflammaton and peripheral sensitization. H_1 receptor antagonists and other antihistaminics are analgesic. Histamine receptors in the CNS may be involved in nociception.	105
		$H_{3,4}$	$G_{i/o}$		
KiSS1-derived peptide	NA	$KISS_1$	$G_{q/11}$	Formerly known as the orphan receptor GPR54, $KISS_1$ is expressed in brain and spinal cord.	106
Leukotriene	+	$BLT_{1,2}$, $CysLT_{1,2}$	$G_{q/11}$, $G_{i/o}$	Leukotrienes are pro-inflammatory lipid mediators synthesized by leukocytes, macrophages, and mast cells, and can sensitize nociceptors.	81, 107–109
		OXE	$G_{i/o}$		
Lysophospholipid	+	$LPA_{1,2}$, $S1P_{1-3}$	$G_{i/o}$	Lysophospholipids, produced by phospholipase C activity, agonize a newly recognized GPCR family. Lysophospholipids contribute to peripheral sensitization via direct action on LPA_1 on sensory neurons, produce hyperalgesia if injected intrathecally, and are required for nerve injury–induced hypersensitivity. Sphingolipid S1P receptor activation is implicated in peripheral sensitization.	110, 111
		LPA_3	$G_{q/11}$		
		$S1P_{4,5}$	G_{12}		

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GPCR family[a]	Role[c]	Subtype(s)	G protein(s) [a, b]	Description	Source
Melatonin	–	$MT_{1,2}$	$G_{i/o}$	Melatonin receptors are localized in brain and spinal cord; spinal and supraspinal levels of melatonin are antinociceptive in acute, inflammatory, and neuropathic pain. Melatonin inhibits the production of pro-inflammatory cytokines.	112
Melanocortin	+/–	MC_{1-5}	G_s	Peptide agonists encoded by the pro-opiomelanocortin POMC gene activate CNS receptors and are reportedly analgesic, hyperalgesic, and anti-opioid. Variants of the MC_1 gene are associated with altered pain sensitivity in humans.	113, 114
Glutamate metabotropic	+/–	$mGlu_{1,5}$	$G_{q/11}$	All three groups are expressed throughout CNS. Group I (listed first) is pro-nociceptive and contributes to central sensitization. Group II and III receptors produce analgesia both centrally and in the periphery.	18, 115
		$mGlu_{2,3}$	$G_{i/o}$		
		$mGlu_{4,6-8}$	$G_{i/o}$		
Melanin-concentrating hormone	NA	MCH_1	$G_s, G_{i/o}, G_{q/11}$	Categorized as orphan receptors prior to 1999, the MCH receptor family is most highly expressed in brain and is implicated in regulating food intake, emotion, stress, and motivation.	116
		MCH_2	$G_{q/11}$		
Motilin	NA	Motilin		Injection of motilin induces visceral pain behavior reversed by a selective receptor antagonist. Drugs targeting this receptor may be useful for gastrointestinal disorders such as irritable bowel syndrome.	117, 118
Neurotensin	+/–	$NTS_{1,2}$	$G_{q/11}$	Activation of neurotensin receptors in the brainstem is pronociceptive. In contrast, intrathecal neurotensin produces antinociception via actions on both spinal cord and dorsal root ganglia neurons. Neurotensin knockout mice display defects in both basal nociceptive responses and stress-induced analgesia.	17, 119
Neuromedin U	+	NMU_1	$G_{q/11}$	Intrathecal administration of neuromedin U causes hyperalgesia. Studies in knockout mice suggest that NMU_2 mediates these pronociceptive effects.	120, 121
		NMU_2	NA		
Neuropeptide S	NA	NPS	NA	The NPS receptor, isolated as an orphan GPCR ligand in 2002, is expressed nearly exclusively in the brain. Central administration of NPS results in increased arousal and an altered anxiolytic profile.	122, 123
Neuropeptide Y	+/–	$Y_{1,2,5}$	$G_{i,o}$	After peripheral nerve injury, there is a dramatic increase of NPY in DRG neurons, especially in larger cells. NPY apparently has both pro- and antinociceptive actions, but the antinociceptive actions appear to dominate.	124, 125
		Y_4	$G_{q/11}$		
Neuropeptide W/ Neuropeptide B	–	$NPBW_{1,2}$	$G_{i,o}$	Neuropeptides B and W were recently identified as endogenous ligands of the GPR7 NPBW1 and GPR8 NPBW2 receptors. Intrathecal injection of either peptide inhibits inflammation-induced mechanical but not thermal hyperalgesia.	126–128
Neuropeptide FF/ neuropeptide AF	+/–	$NPFF_{1,2}$	$G_{i/o}$	NPFF receptors are present in superficial layers of spinal cord and in brain. Supraspinal injection of NPFF analogs results in pronociceptive and anti-opioid activity; intrathecal injection induces analgesia and enhances opioid effects.	129, 130

GPCR family[a]	Role[c]	Subtype(s)	G protein(s) [a, b]	Description	Source
Nicotinic acid	NA	GPR81, GPR109A, GPR109B temporary names	$G_{i/o}$	Nicotinic acid was the first orally available drug to treat high cholesterol, but only recently have three GPCRs been identified to recognize this ligand.	131
Opioid	−	DOP, MOP, KOP, NOP	$G_{i/o}$	Activation of DOP, MOP or KOP reduces nociceptive transmission throughout the CNS. Synthetic agonists are common in pain management, but side effects include constipation and respiratory depression. The long-term use of opioids for non-terminal chronic pain is a topic of intense debate.	18, 132
Orexin	−	OX_1	$G_{i/o}$, $G_{q/11}$	In the last decade, orexins were identified as endogenous ligands for two orphan GPCRs. Spinal and supraspinal administration of orexins inhibits nociceptive transmission in acute, inflammatory, and neuropathic pain.	18
		OX_2	$G_{q/11}$		
P2Y	+	$P2Y_{1,2,4,6,11}$	$G_{q/11}$	$G_{q/11}$- and $G_{i/o}$-coupled P2Y receptors are expressed in sensory neurons, including nociceptors, and glia. $P2Y_2$ mediates thermal sensitivity and inflammatory pain. $P2Y_{12}$ mediates tactile allodynia after nerve injury. Stimulated keratinocytes release ATP, suggesting a role in sensory transduction.	20, 133, 134
		$P2Y_{12-14}$	$G_{i/o}$		
Parathyroid hormone	NA	$PTH_{1,2}$	G_s	Parathyroid hormone regulates calcium metabolism and bone growth and remodeling. PTH function in nociception is unclear; its protective effects on bone destruction may reduce arthritic pain.	135
Platelet-activating factor	+	PAF	$G_{q/11}$	PAF is a lipid mediator of peripheral inflammation; intrathecal injection induces thermal and mechanical hypersensitivity; inhibition of PAF attenuates inflammatory pain.	136, 137
Prostanoid	+	DP_1, $EP_{2,4}$, IP_1	G_s	The COX enzymes participate in prostaglandin and prostanoid synthesis. Prostanoids commonly act as localized pro-inflammatory/pro-nociceptive mediators. Prostanoid receptors on sensory neurons may cause direct excitation. COX inhibitors mediate analgesia mainly by reducing prostaglandin synthesis.	81
		DP_2, EP_3	$G_{i/o}$		
		EP_1, FP, TP	$G_{q/11}$		
Prokineticin	+	PKR_1	$G_{q/11}$	The two newly recognized PKRs are expressed by DRG neurons; prokineticin peptides produce hyperalgesia. PKRs on nociceptors mediate heat hyperalgesia by sensitizing TRPV1. PKR_1 mediates pain behavior in mice as well as TRPV1 function.	138–140
		PKR_2	G_s		
Protease-activated	+/−	$PAR_{1,2,4}$	$G_{q/11}$	The proteolytic generation of an N-terminal "tethered" receptor-stimulating ligand is unique to PARs. The release of proteinases in arthritis orchestrates joint tissue remodeling and degeneration. Activation of PAR subtypes on sensory neurons can be either pronociceptive (i.e., PAR_2) or antinociceptive. PAR_2 triggers neuropeptide release, neurogenic inflammation, and TRPV1 sensitization. Sub-inflammatory doses of PAR_1 and PAR_4 activators reduce inflammatory pain.	141–143
		PAR_3	NA		
Prolactin-releasing peptide	−	PRRP	$G_{i/o}$	PRRP was formerly designated as orphan receptor GPR10. Intracerebral injection of PrRP is normally antinociceptive and reduces allodynia in neuropathic rats.	144

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GPCR family[a]	Role[c]	Subtype(s)	G protein(s) α, [b]	Description	Source
Relaxin	NA	RXFP$_1$, RXFP$_2$	G$_s$, G$_{i/o}$	Relaxins modulate the formation of connective tissue. Serum relaxin levels correlate to pelvic pain during pregnancy but more likely reflect the remodeling of pelvic connective tissue rather than a direct role in nociception.	145
		RXFP$_3$, RXFP$_4$	G$_{i/o}$		
Somatostatin	–	SST$_{1-5}$	G$_{i/o}$	Somatostatin is expressed in non-peptidergic C-fibers; most SST$_2$ receptors are expressed by TRPV1-containing neurons. Peripheral somatostatin, released upon injury, inhibits neuropeptide release and prevents nociceptor sensitization. Peripheral SST receptors may tonically inhibit TRPV1. Intrathecal administration of agonists inhibits inflammatory and neuropathic pain.	18, 146, 147
Tachykinin	+	NK$_{1-3}$	G$_{q/11}$	Tachykinins (i.e., substance P and neurokinins A and B) are positively implicated in pain modulation. SP and NKA are expressed by small and medium-sized primary sensory neurons. Tachykinins contribute to peripheral and central sensitization. NK$_1$ receptors are expressed by ascending spinal cord neurons; the analgesic inefficacy of NK$_1$ antagonists in humans was unexpected.	148, 149
Thyrotropin-releasing hormone	–	TRH$_1$	G$_{q/11}$	TRH receptors can activate descending inhibitory pathways. A systemically administered TRH analog mediates antinociception at the supraspinal level.	150
Trace amine[h]	NA	TA$_1$	G$_s$	Trace amines are metabolites of biogenic amine neurotransmitters. TA modulation of the CNS was only recently linked GCPR signaling. Of nine family members, eight receptors remain orphans with unidentified ligands; the potential for relevant drug targets is unexplored. Ingested TA can precipitate headaches.	151, 152
Urotensin	NA	UT	G$_{q/11}$	Urotensin, expressed in brainstem and spinal cord, was recently identified as the ligand for an orphan GPCR. Supraspinal injection reportedly causes anxiogenic and depressant-like effects in mice without altering nociceptive.	153
Vasopressin/ oxytocin	+/–	V$_{1A,1B}$	G$_{q/11}$	Vasopressin and oxytocin are synthesized in the hypothalamus and stored in vesicles at the posterior pituitary. Although released predominately into the bloodstream, a fraction acts directly in the brain. V$_{1B}$ receptors appear to have pronociceptive and anti-opioid actions, whereas V$_{1A}$ receptors in the spinal cord are analgesic.	154–156
		V$_2$	G$_s$		
		OT	G$_{q/11}$		
VIP/PACAP	+	PAC$_1$, VPAC$_{1,2}$	G$_s$	Vasoactive intestinal peptide and pituitary adenylate cyclise–activating polypeptide are expressed in nociceptive sensory neurons and are upregulated following nerve injury. PAC$_1$ mediates hyperalgesia in inflammatory and neuropathic pain; VIP and PACAP receptor antagonists are analgesic.	157–159

[a] *Family name and nomenclature are based on the recommendations of the International Union of Basic and Clinical Pharmacology Committee on Receptor Nomenclature and Drug Classification NC-IUPHAR as of July 2009 (49).*

[b] *Refers only to each receptors primary or preferred α-subunit as provided by IUPHAR or the selected references. In several cases more than one is listed as equally preferred. Note that most GPCRs will couple to more than one Gα with varying affinity.*

[c] *A family was considered to be positive if at least one study showing a direct action on nociception was identified. In some of the negative cases, appropriate experiments have not yet been performed.*

[d] *Mixed effects.*

[e] *Inhibitory.*

[f] *Excitatory.*

[g] *None ascertained.*

[h] *Trace amine associated receptors, TAAR$_{2-9}$, are currently classified as orphans.*

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