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Inhibitory $G_{i/o}$-coupled receptors in somatosensory neurons: Potential therapeutic targets for novel analgesics

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Abstract

Primary sensory neurons in the dorsal root ganglia and trigeminal ganglia are responsible for sensing mechanical and thermal stimuli, as well as detecting tissue damage. These neurons express ion channels that respond to thermal, mechanical, or chemical cues, conduct action potentials, and mediate transmitter release. These neurons also express a large number of G-protein coupled receptors, which are major transducers for extracellular signaling molecules, and their activation usually modulates the primary transduction pathways. Receptors that couple to phospholipase C via heterotrimeric $G_{q/11}$ proteins and those that activate adenylate cyclase via G_s are considered excitatory; they positively regulate somatosensory transduction and they play roles in inflammatory sensitization and pain, and in some cases also in inducing itch. On the other hand, receptors that couple to $G_{i/o}$ proteins, such as opioid or $GABA_B$ receptors, are generally inhibitory. Their activation counteracts the effect of G_s-stimulation by inhibiting adenylate cyclase, as well as exerts effects on ion channels, usually resulting in decreased excitability. This review will summarize knowledge on G_i-coupled receptors in sensory neurons, focusing on their roles in ion channel regulation and discuss their potential as targets for analgesic and antipruritic medications.

Keywords

dorsal root ganglion neuron, GABAB receptor, Gi-coupled, G-protein coupled receptor, opioid receptor, trigeminal ganglion neuron

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Introduction

Chronic pain is an unsolved medical problem,[1] causing immense suffering to millions of people worldwide. The annual costs of chronic pain have been estimated to be hundreds of billions of dollars in the United States alone in medical costs and in lost productivity.[2,3] The mainstream therapy against severe pain is opioids, which activate receptors that couple to inhibitory heterotrimeric G-proteins in the $G_{i/o}$ family. Opioids, while efficient against severe pain, have significant side effects, such as tolerance, sedation, respiratory depression, physical dependence, and addiction. The lack of optimal therapies against chronic pain is thought to be a major contributor to the recent opioid epidemic.[4] Most of the effects of opioids leading to addiction are likely caused by activation of receptors in the central nervous system (CNS). DRG neurons are the primary sensory neurons detecting thermal and mechanical stimuli; their peripheral processes and cell bodies are located outside the CNS. These neurons express opioid receptors, as well as a large number of other GPCRs that activate the $G_{i/o}$ pathway. Selectively activating some of these receptors, in principle, can be utilized to develop novel therapeutic approaches that are devoid of side effects caused by receptor activation in the CNS.

The three major classes of heterotrimeric G-proteins, defined by their alpha subunits, are G_s, $G_{i/o}$, and $G_{q/11}$ (Figure 1); the physiological roles of the forth class

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$G_{12/13}$ are much less understood. The classical view of G-protein activation is that under resting conditions, G_α and $G_{\beta\gamma}$ subunits tightly associate with each other and they are inactive. Upon receptor stimulation, G_α binds GTP, dissociates from $G_{\beta\gamma}$, and the two subunits bind to different effecors, until G_α hydrolyses GTP and re-associates with $G_{\beta\gamma}$, which terminates the biological effect. A more nuanced recent model postulates that G_α and $G_{\beta\gamma}$ are associated with effectors in the resting state, and they activate them via a conformational switch or partial dissociation, see later at G-protein activated Inwardly Rectifying K^+ (GIRK) channels section. G-protein signaling is modulated by many regulatory proteins[5] including regulators of G-protein signaling[6,7] and the G-protein coupled receptor kinase β-arrestin system.[8] Various agonists of the same receptor do not necessarily couple with the same efficiency to downstream targets, a concept called biased agonism.[9] For example, for μ-opioid receptors (μOR), the balanced agonist DAMGO activates both G-protein signaling and recruitment of β-arrestin, whereas other agonists, such as the recently described PZM21, activate G-proteins but induce negligible recruitment of β-arrestin.[10]

Receptors coupling to $G_{\alpha s}$ stimulate adenylate cyclase (AC), and thus the formation of cAMP. Activation of G_s-coupled receptors in DRG neurons, such as prostaglandin D receptors, leads to increased excitability, which contributes to inflammatory sensitization and pain.[11] Downstream effectors of cAMP include protein kinase A, exchange proteins activated by cAMP, and hyperpolarization-activated cyclic nucleotide-gated ion channels, all expressed in DRG neurons.

Activation of G_q-coupled receptors stimulates phospholipase Cβ (PLCβ) enzymes, leading to the hydrolysis of the plasma membrane phospholipid phosphatidylinositol 4,5-bisphosphate.[12] This results in the formation of the two classical second messengers inositol 1,4,5-trisphopshate, which releases Ca^{2+} from intracellular stores, and diacylglycerol, which activates protein kinase C. Activation of G_q-coupled receptors by inflammatory mediators, such as bradykinin, or extracellular ATP in DRG neurons plays an important role in inflammatory hypersensitivity.[11,13] Downstream targets of the G_q pathway include protein kinase C-mediated sensitization of the heat and capsaicin-sensitive Transient Receptor Potential Vanilloid 1 (TRPV1) channels[14] and voltage-gated Na^+ channels.[15] Activation of G_q-coupled receptors may also lead to direct excitation and pain.[16] It was shown, for example, that bradykinin induces acute nociceptive signals by inhibiting M-type K^+ channels as well as activating Ca^{2+}-activated Cl^- channels in DRG neurons.[17] Another set of G_q-coupled receptors highly expressed in DRG neurons is the Mas-related G-protein coupled receptor (Mrgpr) family. While the functions and physiological activators of these receptors are not fully elucidated, some of them serve as itch receptors. The MrgprA3 (human MrgprX1) is responsible for chloroquine-induced itch,[18] while the MrgprD receptor is activated by β-alanine, and it is responsible for the itch evoked by this compound.[19]

The physiological roles of $G_{\alpha 12/13}$ proteins, the fourth class of G_α proteins, are much less understood. They may activate small G-proteins;[20] their expression levels in DRG neurons at the RNA level are lower than that of G_q, G_s, or $G_{i/o}$,[21] and very little if any knowledge is available on their roles in these cells.

G_i-coupled receptors, such as opioid, $GABA_B$, and somatostatin (SST) receptors, are generally considered inhibitory, and their activation reduces hypersensitivity and pain.[22] The $G_{\alpha i}$ family consists of four members in mammals, $G_{\alpha i1}$, $G_{\alpha i2}$, $G_{\alpha i3}$, and $G_{\alpha o}$. $G_{\beta\gamma}$ was originally considered an inactive scaffold molecule, but now it is very well accepted to act as an effector stimulating or inhibiting various signaling enzymes and ion channels. While all G_α proteins associate with $G_{\beta\gamma}$ subunits, documented effects of $G_{\beta\gamma}$ are most pronounced when

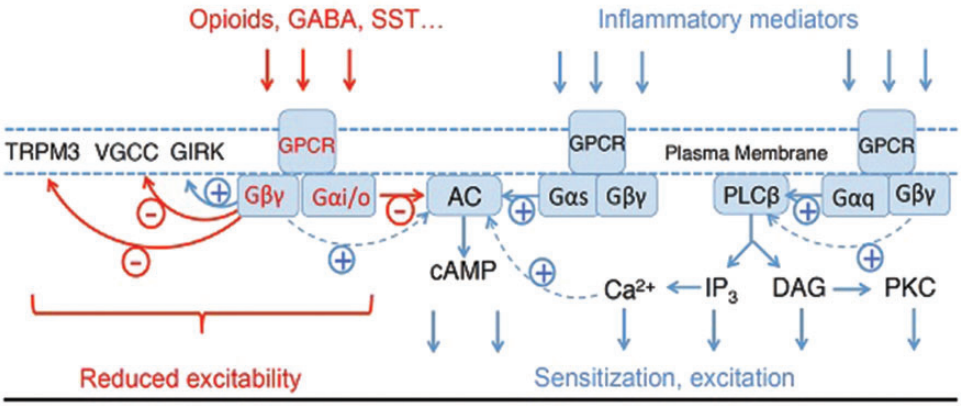

Figure 1. Signaling by GPCRs, abbreviations are explained in the main text.

G_i-coupled receptors are stimulated (see possible explanation under the section on GIRK channels).

DRG neurons are pseudounipolar cells; their cell bodies are located in the intervertebral foramen (opening). These neurons have a long peripheral process reaching from the ganglion to the periphery innervating not only the skin but also internal organs, as well as the bones and muscles. A shorter central process forms a synapse with secondary neurons in the dorsal horn of the spinal cord, thus transmitting the stimulus to the CNS. The equivalent primary sensory neurons innervating the orofacial region are located in the trigeminal ganglia (TG). G_i-coupled receptors are often presynaptic, and some of them are located in the central processes of DRG or TG neurons, and their activation reduces transmitter (glutamate) release. Many of the G_i-coupled receptors, however, are also found on the cell bodies and on the peripheral sensory processes, where they can inhibit the generation of receptor potential. Much of the electrophysiological characterization of native sensory ion channels is based on measurements performed on isolated and cultured cell bodies of DRG neurons. Due to this fact, it is often difficult to tell if a regulatory effect described in isolated DRG neurons takes place physiologically on the central, the peripheral, or both processes. When drugs are administered locally, such as in the hind paw in rodent models, the assumption is that they mainly exert their effects at the peripheral termini, unless they are injected at concentrations high enough to reach distant targets via the bloodstream. When drugs are injected intrathecaly, they exert effects both at the central termini of DRG neurons and on secondary neurons in the spinal cord. Systemically injected drugs reach both central and peripheral targets, unless they do not cross the blood brain and blood spinal-cord barrier,[23] in that case, they reach the peripheral processes and potentially the cell bodies in the DRG,[24] but not the central termini.

DRG neurons are also notoriously heterogeneous, a detailed description of the different cell types can be found in a recent review.[25] Briefly, larger cells generate myelinated fibers mediating light discriminatory touch (Aβ) and proprioception (Aα). Medium-sized and small neurons give rise to lightly myelinated Aδ fibers and non-myelinated C-fibers, which mediate thermosensation, pain, and itch. These latter neurons have been divided into peptidergic and non-peptidergic neurons, depending on the expression of various markers, such as CGRP, Substance P, and IB4. A recent article divided mouse DRG neurons into 11 groups based on single cell RNA sequencing and principle component analysis of ~900 cells.[26] Further resources based on their data are available at http://linnarssonlab.org/drg/. See also Table 1 for expression of selected G_i-coupled receptors and sensory ion channels in the different cell populations. Additional single cell RNA sequencing articles have been also published for DRG neurons[27,28] and for TG neurons.[29] As this technology advances, it is likely that we will have higher coverage data available in the near future as it has happened for other organs such as the brain[30] and the kidney.[31]

Targets of G_i-coupled receptors

Here, we will briefly discuss three classical targets of G_i signaling and a recently discovered new one. Other targets will be discussed at the parts dedicated to individual receptors. For example, the heat and capsaicin-sensitive TRPV1 is affected by $GABA_B$ receptors via a G-protein independent manner;[32] this effect is not shared by other G_i-coupled receptors, thus we will discuss it at the $GABA_B$ receptors section.

Adenylate cyclase

The letter "i" in $G_{\alpha i}$ stands for "inhibitory" because receptors coupled to $G_{\alpha i}$ proteins inhibit AC, as opposed to "stimulatory" $G_{\alpha s}$ proteins. G_s-coupled receptors, such as prostaglandin D receptors, generally increase excitability of DRG neurons; thus contribute to inflammatory hypersensitivity. Concurrent activation of G_i-coupled receptors, in principle, counteracts this effect by decreasing cAMP levels. There are nine mammalian membrane bound AC isoforms. AC5 and AC6 are inhibited by all $G_{\alpha i}$ isoforms via protein–protein interactions, while AC1 is inhibited by $G_{\alpha o}$, and $G_{\beta\gamma}$ may also contribute to inhibition of these AC isoforms. AC2, AC4, and AC7 on the other hand are potentiated by $G_{\beta\gamma}$ subunits in the presence of G_s stimulation (Figure 1).[33] Further complicating this picture is the finding that sustained stimulation of G_i-coupled receptors paradoxically potentiates cAMP production, especially after cessation of the stimulus.[34] This may underlie hypersensitivity upon repeated application of G_i-coupled agonists such as morphine and adenosine.[35,36] Also, increased cytoplasmic Ca^{2+} stimulates several isoforms of AC (Figure 1), while inhibits others, providing a cross talk from G_q-coupled receptors.[33]

GIRK (Kir3.x) channels

GIRK channels are stimulated by activation of G_i-coupled cell surface receptors, leading to hyperpolarization and thus decreased excitability. GIRKs are members of the inwardly rectifying K^+ (Kir) family of ion channels[37]; four subunits, Kir3.1, Kir3.2, Kir3.3, and Kir3.4, form homo- or hetero-tetramers to produce functional GIRK channels. The GIRK1/GIRK4 (Kir3.1/Kir3/4) combination forms the classical cardiac K^+ channel activated by acetylcholine and contributes to slowing the heart rate, while GIRK2 is generally expressed in the nervous system. Activation of various

Table 1. Expression of various G_i-coupled receptors, $G_{\alpha i}$ subunits, and some sensory TRP channels in mouse DRG neurons.

Gene	whole DRG	purified DRG neuron	TRPV1 lineage	TRPV1 depleted	NF1	NF2	NF3	NF4	NF5	NP1	NP2	NP3	PEP1	PEP2	TH
Gabbr1	148.86	175.317	133.64	79.23	0.323	0.354	0.417	0.273	0.385	0.440	0.375	0.250	0.281	0.588	0.391
Gabbr2	44.778	53.985	48.63	35.09	0.129	0.167	0	0.045	0.154	0.136	0.063	0.083	0.031	0.059	0.172
Oprm1	4.6222	5.31885	7.75	2.98	0	0	0	0.045	0	0.056	0.125	0.250	0.047	0.118	0.004
Oprd1	4.8355	2.22282	1.74	5.89	0	0.063	0.250	0	0	0	0	0	0	0	0
Oprk1	0.9234	0.833171	1.29	0.96	0	0.104	0.083	0	0	0	0	0	0	0	0
Oprl1	8.7985	3.96903	3.15	7.61	0.129	0.208	0.083	0.136	0.154	0.008	0	0	0.063	0	0.052
Sstr1	1.358	1.1629	1.81	0.39	0.065	0	0	0.136	0.115	0	0.031	0	0.031	0	0.021
Sstr2	2.8183	3.29239	11.17	0.30	0	0	0	0	0	0	0	0.083	0.156	0	0
Sstr4	1.5131	0.859453	1.86	0.47	0	0	0	0	0	0.008	0	0	0	0.059	0.004
Grm2	0.0389	0.0933552	0.05	0.03	0	0	0	0	0.077	0.008	0	0	0	0	0
Grm3	0.2625	0.182401	0.78	0.18	0	0.021	0	0	0	0	0	0	0.031	0	0
Grm4	10.154	1.40604	1.32	14.79	0.355	0.167	0.333	0.136	0.154	0.008	0	0	0	0.235	0.004
Grm7	25.656	32.1534	24.73	8.02	0.032	0	0	0.045	0.038	0.120	0.125	0	0.141	0.118	0.013
Grm8	9.3652	2.30104	1.40	6.52	0	0.063	0.583	0.318	0.192	0.016	0.031	0	0	0.294	0.039
Adora1	34.493	43.8114	26.44	15.89	0.452	0.354	0.500	0.227	0.192	0.456	0.125	0.167	0.094	0.294	0.506
Npy1r	8.3534	6.94382	17.33	2.40	0	0.042	0	0.091	0	0	0	0	0.328	0	0.021
Npy2r	8.2259	16.8471	14.64	1.37	0	0.021	0	0	0	0	0.094	0.833	0.031	0.294	0
Htr1a	2.8533	4.61	6.05	0.55	0	0.021	0	0.045	0	0	0.063	0.250	0.078	0.059	0
Htr1b	6.6005	2.62054	6.43	9.09	0	0	0	0	0.038	0	0	0	0	0.059	0.004
Htr1d	18.537	6.73259	3.88	24.09	0.677	0.688	0.917	0.273	0.500	0.016	0.031	0.083	0.047	0.059	0.258
Htr1f	2.4569	4.09502	5.62	2.35	0	0.250	0	0.182	0.154	0	0.094	0.833	0	0	0
Cnr1	12.014	5.04595	4.65	7.31	0.000	0.271	0	0	0.154	0.032	0	0	0	0.118	0.000
Cnr2	0.0557	0.0823272	0.02	0.11	0.000	0.000	0	0	0.000	0.000	0	0	0	0.000	0.004
Gnai1	59.773	25.0302	20.43	46.27	0.484	0.500	0.750	0.636	0.808	0.088	0.188	0.083	0.109	0.529	0.223
Gnai2	117.32	158.576	133.48	56.09	0.355	0.188	0.167	0.136	0.077	0.584	0.469	0.333	0.422	0.412	0.472
Gnai3	30.677	34.3675	24.53	16.11	0.097	0.063	0	0.045	0.038	0.216	0.250	0.167	0.188	0.059	0.197
Gnao1	305.81	558.236	395.80	99.89	0.194	0.021	0.083	0	0	0.760	0.813	0.417	0.313	0.353	0.245
TRPV1	44.431	65.284	151.22	1.34	0	0	0	0.045	0	0.032	0.281	0.583	0.313	0.059	0
TRPA1	18.674	34.2468	23.57	0.95	0	0	0	0	0	0.512	0.219	0.167	0.063	0	0.176
TRPM8	10.388	7.30955	15.20	0.73	0	0	0	0	0	0	0	0	0.063	0	0
TRPM3	9.8029	9.43739	6.31	3.86	0	0	0	0	0	0.104	0.031	0	0.078	0	0

Gene names for G_i-coupled receptors and G_α subunits: Gabbr1&2: $GABA_B$ receptor 1&2; Oprm1: μOR; Oprd1: δOR; Oprk1: κOR; Sstr1,2 & 3: SST receptor 1,2,3; Grm2,3,4,7,8: metabotropic glutamate receptors 2,3,4,7,8; Adora1: adenosine receptor 1, Npy1r: NPY receptor 1; Npy2r: NPY receptor 2, Htr1a,b,d,f: 5HT receptors 1a, 1b, 1d, 1f; Cnr1,2 Cannabinoid receptors 1,2; Gnai1,2,3: $G_{\alpha i1,2,3}$; Gnao1: $G_{\alpha o}$. Columns *Whole DRG* and *purified DRG* neurons enriched in small neurons are from Thakur et al.[41] expression levels are expressed as FPKM. *TRPV1 lineage* and *TRPV1 depleted* are from Goswami et al.,[21] from FACS isolated TRPV1 lineage DRG neurons, and from DRGs where this lineage was ablated, values are expressed as RPKM. *NF1-NF5* are five different populations of neurofilament positive neurons, *NP1-NP3* are three populations of non-peptidergic neurons, *PEP1–2* are peptidergic neurons, and *TH* is tyrosine hydroxylase positive neurons from Usoskin et al.,[26] based on single cell RNA sequencing of mouse DRG neurons, from external resource table available at http://linnarssonlab.org/drg/. Numbers note the fraction of cells that had detectable RNA for the given gene.

G_i-coupled receptors including $GABA_B$ and SST receptors have been shown to activate GIRK currents in rat DRG neurons,[38,39] and mRNA has been detected for all four Kir subunits in those cells.[38] Another study reported that GIRK channels were present in rat and human DRG neurons, but they were absent in mouse DRG neurons. In vivo nociceptor-specific transgenic expression GIRK2 in mouse DRG neurons using $Na_v1.8$ promoter restored peripheral analgesia induced by the μOR agonist DAMGO.[40] Unbiased RNA sequencing in mouse DRG neurons detected low levels of GIRK channel expression, with the exception of GIRK1 (KCNJ3),[41] and single cell RNA sequencing of mouse DRG neurons showed significant enrichment of GIRK2 in the tyrosine-hydroxylase positive subpopulation.[26]

GIRK channel activation is mediated by direct interactions between $G_{\beta\gamma}$ and the channel.[42,43] Interestingly, the channels are activated by G_i-coupled receptors, but not by G_q- or G_s-coupled receptors, even in heterologous expression systems. The mechanism of this selectivity has been a subject of intensive research; it cannot be explained by different subunit composition, because all G_β-s ($G_{\beta 1-4}$) with the exception of $G_{\beta 5}$ activate GIRK channels,[44] and no clear differences were identified in subunit composition of G_β and G_γ associating with different G_α-s. The most likely explanation is that the $G_{\alpha i}$-$G_{\beta\gamma}$ complex associates with high affinity with GIRK channels in resting cells, and upon receptor activation, a local conformation switch, similar to a clamshell opening, rather than full dissociation of $G_{\beta\gamma}$ from $G_{\alpha i}$, activates the channel. This model is based largely on fluorescent resonance energy transfer measurements between the channel, receptor, and the G-proteins.[45,46] The key findings supporting this model are that upon receptor stimulation, fluorescent resonance energy transfer may increase or decrease between Gβ and the channel,[46] and between Gβ and $G_{\alpha i}$,[45] depending on the location of the CFP and YFP tags on the individual proteins. It remains to be seen if other effectors of $G_{\beta\gamma}$ show similar mechanism.

Voltage-gated Ca^{2+} channels

N-type ($Ca_v2.2$, CACNA1B) and P/Q-type ($Ca_v2.1$, CACNA1A) voltage-gated Ca^{2+} channels (VGCC) are also classical targets of $G_{\beta\gamma}$ released from $G_{\alpha i}$.[47] N-type channels are usually found presynaptically, where they play an important role in initiating neurotransmitter release. Inhibition of N-type channels by G_i-coupled receptors reduces transmitter release, an effect expected to take place in the central process in the context of DRG neurons. Indeed, inhibition of N-type Ca^{2+} channels by several G_i-coupled receptors including $GABA_B$ receptors and opioid receptors have been reported in DRG neurons.[48]

DRG neurons also express low-voltage activated Ca^{2+} channel (T-type), and the $GABA_B$ receptor agonist baclofen has been shown to inhibit both low- and high-voltage activated Ca^{2+} channels in DRG neurons.[49] T-type channels ($Ca_v3.2$) are expressed both in the soma and the in the peripheral nerve termini and play important roles in initiating the receptor potential in response to mechanical stimuli.[50,51]

Transient receptor potential melastatin 3

Transient receptor potential melastatin 3 (TRPM3) channels are activated by heat,[52] and chemical agonists such as pregnenolone sulphate (PregS)[53] and the synthetic agonist CIM0216.[54] These channels are expressed in small nociceptive DRG neurons, and their genetic deletion in mice reduces sensitivity to noxious heat.[52] Recent reports from three different laboratories identified TRPM3 as a novel target of $G_{\beta\gamma}$ upon activation of G_i-coupled receptors in DRG neurons using a wide range of overlapping techniques.[55–57] Activation of μ-opioid,[56,57] $GABA_B$,[55–57] SST,[55,57] or Neuropeptide Y (NPY) receptors[57] inhibited Ca^{2+} signals evoked by PregS in DRG neurons. Activation of recombinant $GABA_B$, M2 muscarinic, and Dopamine 2 receptors also inhibited TRPM3 expressed in HEK cells, and the effect on M2 receptor activation was inhibited by co-expressing the $G_{\beta\gamma}$ binding C-terminal fragment of the β-adrenergic receptor kinase.[55] Co-expressing $G_{\beta 1\gamma 2}$ in HEK cells inhibited PregS-induced Ca^{2+} signals and currents, but various $G_{\alpha i/o}$ isoforms including the constitutively active $G_{\alpha 1}$-Q204L had no effect.[56] Similarly, co-expressing $G_{\beta 1\gamma 2}$ in Xenopus oocytes inhibited PregS-induced currents, but none of the tested $G_{\alpha i/o}$ isoforms had a significant effect.[55] The effect of $G_{\beta\gamma}$ likely proceeds via direct protein–protein interaction as application of purified $G_{\beta\gamma}$, but not $G_{\alpha i2}$ inhibited TRPM3 currents in excised inside out patches[55,57] and TRPM3 co-immunoprecipitated with G_β.[55,57] Nocifensive responses evoked by hind paw injection of either CIM0216 or PregS were inhibited by co-injection of baclofen,[55,57] DAMGO,[56] morphine,[57] or NPY.[57] On the other hand, Ca^{2+} responses in DRG neurons evoked by agonists of other sensory TRP channels TRPV1,[56] TRPA1,[55,56] and TRPM8[55] were not affected by G_i-coupled receptor activation, and accordingly, nocifensive responses to the TRPV1 agonist capsaicin were not inhibited by co-injection of DAMGO,[56] and baclofen did not inhibit nocifensive responses to the TRPA1 agonist mustard oil.[55] Overall, the three articles described here convincingly demonstrate that TRPM3 is a *bona fide* novel ion channel target of $G_{\beta\gamma}$ in DRG neurons, see also discussion by Csanady.[58]

Other targets

Downstream targets of $G_{\beta\gamma}$ also include phosphoinositide 3-kinase-γ (PI3Kγ) and mitogen-activated protein kinases.[59,60] While there is an extensive literature on mitogen-activated protein kinases in DRG neurons, most studies focused on its role in inflammatory hypersensitivity, and little is known if they play any roles in signaling by $G_{\alpha i/o}$-coupled receptors.[61] Similarly, PI3K enzymes have been studied largely in the context of hypersensitivity, NGF-signaling[62] and inflammation,[63] and little is known about their role in $G_{\alpha i/o}$-coupled receptor signaling.

β-arrestin 1 (arrestin 2) and β-arrestin 2 (arrestin 3) were originally identified to bind to phosphorylated GPCRs and induce their desensitization and internalization[8]; their roles, however, are emerging as independent signaling mediators.[8,64] Arrestins have been extensively studied in the context of opioid receptor signaling, and new biased opioid receptor agonists with minimal arrestin recruitment are being developed with the hope of minimizing the side effects of these drugs.[10] Relatively little is known about the roles of arrestins in DRG neurons. It has been shown that δ-opioid receptor (δOR) signaling to VGCC was enhanced in β-arrestin1 knockout mice, and the behavioral effects of δOR agonists were enhanced in the absence of β-arrestin1.[65] The authors concluded that these effects are due to δOR activation of cofilin through Rho-associated coiled-coil containing protein kinase, LIM domain kinase, and β-arrestin1 to regulate actin polymerization.[65] Another article found that the high-internalizing δOR agonist (SNC80) preferentially recruited β-arrestin 1, and genetic deletion of β-arrestin 1 induced a significant increase in the potency of SNC80 to inhibit mechanical pain and decreased acute tolerance. In contrast, the low-internalizing δOR agonists (ARM390) preferentially recruited β-arrestin 2 with unaltered behavioral effects in β-arrestin 2 knockout animals.[66]

There are several less common targets of G_i signaling; some of them with relevance to DRG neurons are discussed below. Substance P released from nociceptive nerve endings is generally thought to be pronociceptive, but acute antinociceptive effects of this peptide have also been described.[67] Substance P activates Neurokinin receptors (NK1–3), which are generally thought to couple to G_q and activate PLC, but they may also couple to G_i. Substance P has been shown to inhibit T-type VGCC[68] and potentiate M-type K^+ channels[69] in DRG neurons, both of which reduce excitability. These effects were mediated by production of reactive oxygen species, and they were eliminated by overnight pertussis toxin (PTX) treatment showing the involvement of G_i signaling.

While G_i-coupled receptors are generally inhibitory, there are examples where pro-nocicepitive mediators increase excitability with the involvement of G_i-coupled receptors. Three examples are listed below on tetrodotoxin-resistant voltage-gated Na^+ channels $Na_v1.8$ and $Na_v1.9$. The pro-inflammatory prostaglandin PGE2 has been reported to potentiate $Na_v1.9$ currents in mouse DRG neurons, and PTX inhibited the effect, pointing to the role of G_i signaling.[70] The chemokine CCL2 potentiated $Na_v1.8$ channels in rat DRG neurons; the effect was blocked by PTX and gallein, suggesting the involvement of G_i signaling and $G_{\beta\gamma}$.[71] The chemokine CXCL12 increased the activity of $Na_v1.8$ and $Na_v1.9$ currents in rat DRG neurons; PTX and the PI3K inhibitor LY294002 eliminated the effect on $Na_v1.9$, but not on $Na_v1.8$.[72]

G_i-coupled receptors in DRG neurons

DRG neurons express a number of different G_i-coupled receptors. We compiled RNA expression levels for G_i-coupled receptors, $G_{\alpha i}$ subunits, and some selected sensory ion channels from three different publications based on RNA sequencing of mouse DRG neurons (Table 1). The first two columns show data from Thakur et al.,[41] who performed RNA sequencing on whole mouse DRG, as well as purified DRG neurons enriched in small nociceptive neurons. As can be seen in Table 1, RNA levels for many neuron-specific receptors and ion channels show some enrichment in purified neurons (e.g., TRPV1, TRPA1, and NPY2-receptors), while some transcript levels drop significantly (e.g., Grm4), indicating that they are mainly expressed in non-neuronal cells. The "TRPV1 lineage" and "TRPV1 depleted" data are from Goswami et al.,[21] who used FACS sorted DRG neurons from a TRPV1 cre-based reporter mouse, which labels all TRPV1-expressing neurons and neurons that expressed the channel developmentally. The column "TRPV1 depleted" denotes DRG tissue depleted of the TRPV1-lineage by Cre-mediated excision of a floxed transcriptional stop-codon preceding the DTA coding sequence.[21] We also included data from a single cell RNA sequencing article;[26] the numbers for each subset of cells (NF1–5, NP1–3, PEP1–2, and TH) show the fraction of cells where transcripts were detected for a given gene.

We chose to present these data as they were obtained in an unbiased fashion, and the results for the two cell population-based RNA sequencing papers were expressed in comparable units, RPKM (Reads Per Kilobase Million) or FPKM (Fragments Per Kilobase Million). The single cell RNA sequencing data provides some estimate on the expression levels in different cell populations. The limitations of these data also need to be acknowledged. RNA levels do not necessarily

correlate well with protein expression levels, and single cell RNA sequencing with relatively low cell number can result substantial false negative rate. Also note that all data in Table 1 are from mice, and other species may show different expression levels of some of these proteins.

Opioid receptors

Morphine and other opioid receptor agonists are mainstream therapy against severe pain. Most clinically relevant effects and many side effects of opioids are mediated by G_i-coupled μOR. The two other opioid receptor subtypes δOR and κ-opioid receptors (κOR) also couple to $G_{i/o}$, and have been studied as alternative targets for analgesics.[73] Specific activation of both δOR and κOR has also been reported to induce analgesic effects, but κOR activation has been associated with dysphoria, while δOR activation has been reported to have anxiolytic and antidepressant effects.[74] The nociceptin receptor or opioid receptor like 1 shares homology with opioid receptors; it is activated by its endogenous ligand nociceptin, but not by most opioid drugs.[75]

Opioid receptors are expressed both centrally, in the brain and spinal cord, as well as peripherally in cell bodies and peripheral processes of DRG neurons. DRG neurons express all three opioid receptors and opioid receptor like 1 at different levels and cellular distribution[41] (Table 1). Both locally administered morphine and opioid receptor agonists such as the μOR agonist DAMGO, which do not cross the blood brain barrier, have been shown to have analgesic effects.[76,77] The idea of peripherally acting opioids targeting DRG neurons, potentially devoid of central side effects, such euphoria and tolerance, have been raised, but so far, there are no clinically useful antinociceptive drugs available.[76,78] Loperamide or Imodium is a peripherally acting μOR agonist, used as an over the counter antidiarrheal medication.[79] Loperamide has no antinociceptive effect when taken orally, but it was reported to alleviate painful symptoms of oral or skin ulcers when applied topically.[79] The main reason for the lack of the analgesic effect of oral loperamide is that it does not reach the systemic circulation, due to its almost complete degradation by the liver.[79] Loperamide has been shown to have analgesic effect when injected subcutaneously[80,81] or applied topically.[82]

A recent review on δOR in primary sensory neurons provides a thorough description of the roles of δOR as well as μOR in DRG neurons.[83] Briefly, most research in DRG neurons focused on μOR and δOR, and experiments based on immunocytochemistry suggested that μOR and δOR are expressed in an overlapping set of cells.[84] A more recent study by Scherrer et al.[85] using a δOR-GFP reporter mouse line showed that μOR and δOR are expressed in different cell populations; δOR were restricted to medium-to-large myelinated NF200 expressing cells and non-peptidergic IB4 positive smaller neurons. μOR on the other hand was mainly expressed in small, peptidergic TRPV1- and substance P-positive neurons.[85] These data are also consistent with the distribution of RNA expression of these receptors in a recent single cell RNA sequencing article[26] (see also Table 1). Consistent with μOR and δOR being expressed in different cell populations, selective activation of μOR or δOR also had functionally distinct effects. Intrathecal administration of the μOR selective agonist DAMGO decreased sensitivity to noxious heat, without significant effect on mechanical pain; the δOR-specific SNC80 on the other hand significantly attenuated mechanical pain, without having an effect on heat sensitivity.[85]

The debate on whether or not μOR and δOR are co-expressed in the same DRG neurons however is not yet settled. Recent studies demonstrated the coexistence of μORs and δORs in small DRG neurons using single-cell PCR, in situ hybridization, immunostaining, and electrophysiology.[86] Heteromers of μOR and δOR were shown in DRG neurons using antibodies that recognize those heteromers.[87] Finally, facilitation of the degradation of μOR-δOR heteromers by δOR agonists have been shown to be alleviated by disrupting heteromer formation.[88]

A recent paper showed that nociceptor-specific deletion of μOR had no effect on morphine-induced analgesia, but eliminated both tolerance and opioid-induced hyperalgesia.[89] The same study also showed that methylnaltrexone bromide, a peripherally restricted μOR antagonist, was sufficient to abrogate tolerance and hyperalgesia induced by morphine, without diminishing its antinociceptive effect. These data raise doubt about the usefulness of peripherally acting μOR agonists as analgesics. As mentioned earlier, it was suggested that in mice, analgesic effect of the peripherally acting μOR agonist DAMGO required transgenic expression of GIRK2 in DRG neurons.[40]

Significant recent efforts used innovative approaches to target peripheral opioid receptors for pain relief.[90] A recent article reported a peripherally acting μOR agonist, which acts selectively at the site of injury. Spahn et al.[91] synthesized a fentanyl analog that only activates μOR at low pH, which is characteristic of inflamed and injured tissues, and they showed that the compound reduced inflammatory hyperalgesia to both thermal and mechanical stimuli in rats. Another recent article reported covalently attaching morphine to hyperbranched polyglycerol by a cleavable linker, which prevents blood-brain barrier permeation and selectively releases morphine in injured tissue. This conjugated morphine produced analgesia in inflamed rat paws without major side effects.[92]

While RNA levels for κOR are lower than those of other opioid receptors (Table 1) both κOR expression[84] and inhibitory effects of κOR agonists on VGCC[93] have been reported in DRG neurons. As mentioned earlier, DRG neurons play important roles not only in pain, but also in itch, which in chronic forms is a significant medical problem. Pruritus, or itch, is one of the side effects of activation of μOR,[73] but activation of κOR has the opposite effect. The κOR agonist nalfurafine,[73] as well as two different peripherally acting κOR agonists, asimadoline and CR845, were shown to be effective against itch.[94] CR845 showed promising results in phase II clinical trials against pruritus associated with chronic kidney disease in hemodialysis patients.[95]

DRG neurons also express ORL-1 nociceptin receptors (Table 1), and a recent study using a mouse line in which the ORL-1 protein was tagged with GFP found that 43% of DRG neurons were GFP-positive. GFP was expressed both in small and large neurons, with a slight dominance (58%) of neurofilament positive myelinated neurons.[96] Nociceptin receptors were reported to inhibit N-type VGCC in DRG neurons in a tonic, agonist independent manner.[97] Nociceptin receptors were also reported to be expressed in human DRG neurons, and the same study showed that their activation reduced capsaicin-induced Ca^{2+} signals in rat DRG neurons.[98]

Overall, there are conflicting data on the efficiency of stimulating peripheral opioid receptors in alleviating pain in mice, and there are clear receptor subtype specific effects. Peripheral κOR-s on the other hand are promising targets against itch in humans.

$GABA_B$ receptors

$GABA_B$ receptors are obligate heteromers of $GABA_{B1}$ and $GABA_{B2}$ subunits; the presence of both subunits is required for functional G-protein signaling for the following two reasons. First, the GABA binding site is on $GABA_{B1}$ receptors and the G_i-activating domain is on the $GABA_{B2}$ subunit. Second, $GABA_{B1}$ subunits have an ER retention signal, which prevents trafficking of the subunit in the absence of $GABA_{B2}$ receptors, which masks this signal when they form a dimer with $GABA_{B1}$.[99]

$GABA_B$ receptors are the highest expressing GPCRs in DRG neurons on the RNA level[41] (Table 1). The only widely available $GABA_B$ agonist baclofen is used clinically as a central muscle relaxant; its effect is attributed to inhibiting neurotransmitter release onto motoneurons in the ventral horn of the spinal cord.[100] The use of systemic baclofen is limited by its severe side effects at higher doses such as drowsiness, mental confusion, and even coma,[101] which is not surprising, given the abundance of these receptors in the CNS.[99] Systemic side effects can be limited by administering baclofen intrathecally, which is often done to reduce spasticity in various conditions. Baclofen is also used to treat pain conditions, as an adjuvant therapy, but its effect is mainly attributed to acting as a central muscle relaxant.

As discussed earlier, $GABA_B$ receptor activation by baclofen was shown to activate GIRK channels,[38] inhibit VGCC,[49] and inhibit the heat-activated TRPM3 channels[55–57] in DRG neurons; these effects are mediated by the $G_{\beta\gamma}$ arm of classical heterotrimeric G-protein signaling. All of these mechanisms, in principle, may mediate antinociceptive effects.

$GABA_B$ receptors can also be activated by α-conotoxins. These toxins are generally considered to be inhibitors of nicotinic acetylcholine receptors, but some of them such as Vc1.1 and RgIA also inhibit N-type VGCC via activation of $GABA_B$ receptors[102] reviewed in Adams et al.[103] Accordingly, intramuscular injection of Vc1.1 was shown to induce a long-lasting reversal of mechanical allodynia, which was prevented by the $GABA_B$ receptor antagonist, SCH50911.[104]

Activation of $GABA_B$ receptors in DRG neurons by baclofen was recently shown to inhibit the sensitized state of TRPV1, but not the basal heat or capsaicin activation of TRPV1. The effect was independent of $G_{\beta\gamma}$ signaling; it was mediated by direct protein–protein interaction between $GABA_{B1}$ receptors and TRPV1.[32] While $GABA_{B2}$ receptors were not detected in the protein complexes of TRPV1 and $GABA_{B1}$ receptors in DRG neurons, $GABA_{B2}$ receptors were required for the effect of baclofen both in a heterologous expression system and in DRG neurons. Baclofen was effective when injected locally, showing the presence of the receptors in the peripheral processes, and GABA was shown to be released from nociceptive nerve terminals, suggesting an autocrine feedback mechanism.[32] The growing evidence that these receptors have important antinociceptive effects in the periphery, raise the possibility that peripherally acting $GABA_B$ receptor agonists can be developed as novel analgesics with less side effects.

SST receptors

SST receptors are expressed not only in DRG neurons but also centrally, as well as in inflammatory cells, and can affect nociception and inflammation; the topic is reviewed in literature.[105,106] Briefly, it has been shown that SST is released from activated capsaicin-sensitive nerve endings, and it can exert both local and systemic anti-nociceptive and anti-inflammatory effects.[105,107] Intraplantar injection of SST reduced mechanical allodynia in a rat inflammatory pain model.[108] The SST receptor agonist octreotide inhibited formalin-induced nociceptive behaviors when injected locally, and it also reduced the responses of C-fibers to bradykinin-induced

excitation and sensitization to heat.[109] It was also shown that intraplantar injection of octreotide inhibited capsaicin-induced nocifensive responses in rats, and it also inhibited capsaicin-induced nerve activity in the skin-nerve preparation.[110] Furthermore, intra-articular injection of SST was shown to inhibit knee pain in humans.[111] The SST4 receptor agonist J-2156 was shown to inhibit capsaicin-induced Ca^{2+} signals in rat DRG neurons,[112] as well as activate GIRK channels and inhibit VGCC.[39] SST4 receptor deficient mice showed increased mechanical hyperalgesia after carrageenan-induced inflammation, and the antinociceptive effect of the SSTR4 agonist J-2156 was absent in these animals.[113] Lipopolysaccharide-induced airway inflammation and bronchoconstriction were also markedly enhanced in SSTR4 knockout animals, pointing to the important role of these receptors in inflammatory cells.[113] SST was also shown recently to inhibit Ca^{2+} signals induced by the TRPM3 agonist PregS in a subset of mouse DRG neurons.[55,56] Targeting SST receptors for pain control is complicated by the fact that activation of these receptors have significant other effects, including inhibition of insulin release and inhibition of exocrine secretion and motor activity of the gastrointestinal tract, which may be overcome by developing subtype specific agonists.[105]

Metabotropic glutamate receptors

Metabotropic glutamate receptors (mGlur-s) can function either as homodimers, or as heterodimers.[114] They are divided into group I receptors (mGluR1 and 5) which signal via $G_{\alpha q}$ and group II (mGluR2 and 3), and group III (4,6,7, and 8), which signal via $G_{\alpha i}$.[115] Group I mGluRs, similar to other PLC-coupled receptors, have been shown to be present on peripheral terminals of DRG neurons and play roles in inflammatory hyperalgesia,[116] reviewed in study by Neugebauer.[117]

There are several articles showing antinociceptive effects of the activation of peripheral group II $G_{i/o}$-coupled mGluR-s. Subcutaneous injection of a selective group II mGluR agonist (APDC) into the plantar surface of the hind paw inhibited prostaglandin E2 (PGE2)-induced thermal hyperalgesia in mice.[118] The same study also showed that in cultured DRG neurons, APDC blocked PGE2-induced potentiation of capsaicin-induced Ca^{2+} responses, which was abolished when neurons were pretreated with PTX. Another article from the same group showed that subcutaneous injection of group II mGluR agonists into the plantar surface of the mouse hind paw did not alter basal mechanical thresholds, but inhibited PGE2- or carrageenan-induced mechanical allodynia.[119] Group II metabotropic glutamate receptor agonists also inhibited forskolin-induced potentiation of tetrodotoxin-resistant sodium currents in mouse DRG neurons.[120] Finally, it was shown that membrane hyperexcitability in mouse and human DRG neurons exposed to PGE2 was prevented by the group II mGluR agonist APDC.[121]

While several studies focused on group II mGluR-s, on the RNA level, group III mGluR-s show substantially higher expression in mouse DRG neurons (Table 1). Recent studies also demonstrated potential antinociceptive roles of this group; mGluR8 was found to be present in peripheral nociceptive terminals, and ipsilateral, but not contralateral hind paw injection of the group III mGluR agonist L-AP-4 inhibited nociceptive behavioral responses to capsaicin in rats.[122] Local L-AP-4 injection also attenuated forskolin-induced thermal hyperalgesia.[122] It was also shown that mGluR7 was expressed in small peptidergic and large rat DRG neurons.[123] Nerve ligation experiments in the same study also showed that mGluR7 was anterogradely transported from the cell body to the peripheral site, and after peripheral nerve injury, mGluR7 expression was downregulated. It was also shown that inhibiting peripheral group II/III mGluR-s by intraplantar injection of various antagonists increased capsaicin-induced nociceptive behaviors and nociceptor activity,[124] indicating peripheral glutamate release. On the other hand, the mGluR group III agonist L-AP4 did not have a significant effect on TRPM3 activity, as assessed by PregS-induced Ca^{2+} signals,[57] while agonists of many other $G_{\alpha i/o}$-coupled receptors showed robust inhibition.[55–57]

Overall, both excitatory group I and inhibitory groupII/III mGluR-s are expresed at peripheral nerve terminals, but the opposing effects of the two different receptor groups makes the effects of a potential peripheral glutamate release complex. Neverthelesss, in principle, both group I antagonsist and group II/III agonists may induce beneficial antinociceptive effects.[117]

Adenosine receptors

ATP is released from many cell types and acts as a paracrine signal; it activates both metabotropic (P2X) and ionotropic (P2Y) receptors. Activation of both P2X and P2Y receptors in DRG neurons is generally excitatory. Secreted ATP becomes dephosphorylated rapidly to adenosine by ectoenzymes.[125] Adenosine receptors are distinct from purinergic receptors and couple to different G-proteins.[126] Adenosine 1 receptors (A1R, adora1) couple to $G_{i/o}$-proteins, and they are the most abundant adenosine receptors in DRG neurons; however, G_s-coupled Adenosine 2A receptors (A2AR) are also expressed in DRG neurons at lower levels[21,41] (see also Table 1). Adenosine release has been detected in response to capsaicin and formalin from nociceptive nerve fibers.[127] Due to the presence of receptors with different signal transduction pathways, as well as to the fact that A1R

may also couple to G_q, the local effects of adenosine can be quite complex, both pro- and antinociceptive effects have been observed, reviewed in study by Sawynok and Liu.[126] The presence of various adenosine receptors on many other cell types including immune and vascular cells makes the overall effects of pharmacological modulation of this pathway quite complex.[128]

NPY receptors

NPY is a 36 amino acid peptide; it has five receptors Y1R–Y5R, all couple to $G_{\alpha i/o}$ proteins. DRG neurons express Y1R and Y2R (Table 1). NPY was shown to inhibit VGCC in rat DRG neurons[129,130] and it also inhibited depolarization-induced Ca^{2+} signals and release of substance P from DRG neurons.[130] While both nociceptive and antinociceptive effects of NPY have been described, in general, it is believed that this peptide is mainly antinociceptive.[131] Two independent mouse lines with genetic deletion of Y1R have been generated, and the two studies largely agree that the knockout mice display hyperalgesia to mechanical and thermal stimuli.[132,133] NPY receptors are also expressed in the dorsal horn, and the analgesic effects of NPY may be due to activation of spinal receptors.[134] Consistent with the main role of central NPY receptors, it was shown that intrathecal, but not local administration of NPY reduced guarding behavior in a rat model of plantar incision pain.[135] As mentioned earlier, application of NPY or peptide YY inhibited PregS-induced activation of TRPM3 in mouse DRG neurons,[55,57] and local injection of peptide YY inhibited nocifensive responses evoked by the TRPM3 agonist PregS.[57]

Serotonin receptors

The neurotransmitter serotonin (5-hydroxytryptamine, 5-HT) is part of the inflammatory soup that sensitizes nociceptors. It binds to a variety of receptors including ionotropic 5-HT3 receptors, and a variety of GPCRs coupling to G_q (5HT2), G_s (5HT4,6,7), and $G_{i/o}$ (5HT1,5).[136] Many of these receptors are expressed in DRG and TG neurons, and the overall effect of serotonin is complex, but pro-algesic effects likely dominate. Ionotropic HT3a receptors, for example, are expressed in the central termini and play roles in central sensitization to painful stimuli.[137] Injecting serotonin or a 5HT2 receptor agonist in the hind paw of mice evoked hyperalgesia to mechanical stimuli indicating the presence of stimulatory 5HT2 receptors in the peripheral nerve termini.[138] Serotonin also induced action potentials and potentiated TRPV1 currents in isolated DRG neurons through 5HT2C receptors.[139] Sumatriptam, a drug, which is used to treat migraine headaches,[140] selectively activates $G_{i/o}$-coupled 5HT1B and 5HT1D receptors, which are expressed in DRG neurons (Table 1). It is not clear to what extent direct effects of sumatriptam on TG neurons contribute to its beneficial effects,[140] but the drug was shown to inhibit TRPV1 activity in TG neurons.[141] Serotonin application was shown to potentiate calcium signals and CGRP release induced by capsaicin in TG neurons, but sumatriptam had an inhibitory effect, showing opposing effects of activating different 5HT receptors expressed in those neurons.[142] Sumatriptam was also shown to induce hyperalgesic priming in rats, which may explain the clinical finding that the drug may contribute to migraine chronification.[36]

Designer receptors exclusively activated by designer drugs

Designer receptors exclusively activated by designer drugs (DREADDs) are mutated GPCRs that do not respond to endogenous ligands, but can be activated by synthetic compounds. Most of them are based on muscarinic acetylcholine receptors; they are activated by the inert clozapine derivative clozapine-N-oxide (CNO).[143] DREADDs based on other receptors are also available, and are being developed.[144] By expressing various forms of these receptors in specific cell types, the effect of activating G_i-, G_q-, or G_s-coupled receptors can be studied by applying their chemical activator. Together with optogenetic approaches,[145] DREADDs, in principle, are promising selective tools to study the effects of activation or inhibition of specific neuronal populations in various pain conditions. DREADDs can be expressed in vivo either by crossing mice expressing cre-dependent DREADDs with cell-type specific cre-mice[146] or by injecting DREADD-expressing viral particles.

Expressing inhibitory DREADDs in DRG neurons is a compelling strategy to achieve pain relief. Currently, there are two published articles using this strategy.

Iyer et al.[147] showed that viral expression of the hM4-based G_i-coupled DREADD in small-diameter nociceptors enabled chemogenetic increase of mechanical and thermal nociception thresholds. In the same article, the authors found that transdermal illumination in mice expressing an inhibitory channelrhodopsin inhibited pain.

Another article however raised doubts about the peripheral G_i-coupled DREADD-based approach to inhibit pain. Saloman et al.[148] expressed the G_i-coupled hM4Di receptor in nociceptive DRG neurons expressing the heat- and capsaicin-sensitive TRPV1 ion channel, by crossing TRPV1-cre mice with floxed hM4Di expressing mice. As expected, injection of CNO produced a significant increase in the heat threshold in these animals. Consistent with TRPV1 positive cells being largely insensitive to mechanical stimuli, mechanical sensitivity was not affected by CNO. Surprisingly, however,

expression of these receptors induced significant changes in the absence of CNO, including changes in voltage-gated Na^+ and Ca^{2+} currents, as well as an increase in the expression of $Na_v1.7$ channels. Expression of the G_i-coupled DREADD also reduced the effectiveness of stimulating endogenous μOR by DAMGO on PGE2-induced inflammatory thermal hyperalgesia. The authors concluded that while DREADDs are useful tools, they need additional refinement, especially for potential clinical use. Recognizing the imperfections in currently available DREADDs, novel receptors and compounds are being developed.[149,150]

Additional caution on using these designer receptors have been raised by a recent paper showing that CNO is converted to clozapine in vivo, and the latter is responsible for activating them.[151] Clozapine is an atypical antipsychotic medication; its mechanism of action is not fully understood, but it inhibits certain dopamine and serotonin receptors. The doses required in vivo activation of DREADDs were below that required to exert effects in animals not expressing DREADDs, suggesting that this compound can be more useful than CNO for *in vivo* use.[151]

Optogenetic approaches

Optogenetic approaches classically use light-activated ion channels to study the effects of activating or inhibiting specific neurons and have been used in pain research, see Copits et al.[145] for review. In addition to light-activated ion channels, various GPCRs have also been engineered to become light sensitive. Among G_i-coupled receptors, a photoactivatable μOR was created by splicing together the transmembrane and extracellular parts of the light-activated GPCR rhodopsin, and the intracellular loops and C-terminus of μ-opioid receptor.[152] This opto-μOR was virally expressed in isolated DRG neurons, where they were shown to increase the phosphorylation of extracellular signaling-regulated kinase. Opto-μOR and other light inducible G_i-coupled receptor constructs are promising tools to study the effects of G_i-coupled receptor activation in DRG neurons.

Conclusions

Activation of cell surface receptors coupling to $G_{\alpha i}$ proteins in DRG neurons generally inhibits various processes involved in initiation of painful signals, and therefore, in principle, they can be targets for novel antinociceptive drugs. Several factors complicate this seemingly simple idea. First, DRG neurons are highly heterogeneous, and the expression patterns of the various receptors are different; therefore, the activation of distinct $G_{\alpha i/o}$-coupled receptors is likely to affect different cell types. Second, repeated application of G_i-coupled receptor agonists may induce hyperalgesia.[153] Third, the signaling mechanisms induced by different receptor agonists may not be identical, leading to diversity of the effects. Fourth, expression at the central versus peripheral terminal may induce distinct effects. Clearly, further research is needed to understand the effects of the activation of individual receptors and to explore the potential of targeting these receptors for pain relief. In additions to pain, activation of some of these receptors may also relieve itch, and peripherally acting κOR agonists are currently in clinical trials against uremic pruritus.

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