Crosstalk between chemokine, opioid, and vanilloid receptors

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Introduction

Increasing evidence indicates that the immune and neural systems interact by a wide variety of mechanisms [1]. The tight blood–brain barrier (BBB) restricts the communication between central nervous system (CNS) and immune system, and protects the brain from the damaging effects of inflammation. Nevertheless, multiple interconnections exist between these two systems. (1) The autonomic nervous system is embedded in many peripheral sites along with the immune system, such as the liver, spleen, bone marrow, thymus, lymph nodes, skin, and gastrointestinal tract. (2) Neurotransmitters produced by stimulation of the sympathetic and parasympathetic system directly influence leukocyte function. For example, acetylcholine, norepinephrine, and Met-encephalin suppress cells engaged in both innate and adaptive immunity [2–4]. In contrast, calcitonin gene-related peptide (CGRP) and substance P released by pain fibers enhance inflammation [5, 6]. (3) The CNS can also suppress immune response by activation of the hippocampal-pituitary-adrenal (HPA) axis. In response to environmental stress, corticotropin-releasing factor (CRF) secreted by the hippocampus activates the pituitary to produce adrenocorticotropin hormones (ACTH) [7]. ACTH in turn activates the adrenals to produce corticosterones; hormones with potent immune suppressive effects. (4) There is also evidence for the existence of highly localized "windows" in the blood-brain barrier, called circumventricular organs. These "windows" allow transmission of soluble mediators released by immune cells to enter the hypothalamus of the brain [8].

We have studied the role of receptor cross-talk in the communication between immune and neural systems. Receptors that are essential for immune system functions have been detected on neuronal cells, and typically neuronal receptors are also

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expressed by peripheral leukocytes. Activation of one receptor often causes an alternation in the function of nearby other receptors expressed on the same cells. For example, opioid receptors, the key neuronal analgesic receptors, have also been detected on leukocytes. Prolonged activation of opioid receptors on leukocytes dampens chemokine receptor responses [4]. In contrast, chemokine receptors are expressed on peripheral sensory neurons and in the CNS. As will be discussed, chemokines are capable of reversing opioid receptor-mediated analgesic effects [9, 10]. The receptors for prostaglandins and bradykinins, two proinflammatory mediators, are also expressed on sensory neurons. Activation of these receptors enhances the perception of pain by increasing the sensitivity of the Vanilloid receptor 1 (TRPV1), a pain receptor, expressed on the same sensory neurons [11–13]. The Vanilloid receptors in the oral cavity have the capacity to respond to capsaicin, spicy components of peppers. In addition, proinflammatory chemokines are also capable of sensitizing TRPV1 by phosphorylation of its Ser/Thr residues. Conversely, it is clearly documented that secretion of CGRP and Substance P from Vanilloid receptor-activated sensory neurons has proinflammatory effects [5, 6]. Further characterization is underway to map the expression of TRPV receptor family members on immune cells and to determine if they have a role in regulating chemokine receptors. In this chapter, we will focus on the bi-directional desensitization between chemokine and opioid receptors that reduces the perception of pain, and sensitization of Vanilloid receptor 1 (TRPV1) by chemokine receptors that promotes pain signals.

Opiates suppress immune responses

Opiates have long been used to suppress "pain" and enhance "pleasure" in human history. However, abusive usage of opiates leads to a greater prevalence of viral hepatitis, HIV infection, bacterial pneumonias, tuberculosis, CNS infection, and endocarditis [14–16]. These pathological conditions can be explained by opioid-induced suppression of a spectrum of immune host defenses. Chronic morphine administration induces lymphoid organ atrophy, loss of natural killer (NK) cell activity, and a diminished ratio of $CD4^+CD8^+$ cells in the thymus [17]. In rats, repetitive morphine treatment impairs the delayed hypersensitivity skin response to tuberculin [18]. Morphine also inhibits transcription of interferon γ in activated T cells, which may contribute to an increase in HIV infection among morphine users [19]. Chemokine receptor-mediated migration of human leukocytes was also compromised by *in vitro* pre-incubation of cells with opioids [20, 21]. In addition to these immunosuppressive effects, it has been reported that opioids also exhibit certain positive effects on immune responses, including enhanced synthesis of tumor necrosis factor-α and interleukin-1β by activated macrophages, and direct induction of leukocyte chemotaxis [4, 22].

Opioids induce immunosuppressive effects by enhancing neurohormone production

Prolonged activation of CNS by morphine leads to a 3- to 4-fold increases in the level of circulating corticosterone, up to 400–450 ng/ml, resulting in splenic and thymic atrophy, a decrease in lymphocyte proliferation, inhibition of IL-2 and IFN-γ synthesis [23]. Conversely, disruption of μ-opioid receptors blocks morphine induced increase in circulating corticosterone. The immunomodulatory effects of chronic morphine treatment are significantly attenuated in *mor*$^{-/-}$ mice. Supplemental infusion of corticosterone partially reproduces the immunodeficiency [24]. Opioids also activate the sympathetic nervous system, resulting in an increase in the level of circulating epinephrine from the adrenal medulla and norepinephrine from sympathetic nerve terminals [25]. Increased catecholamine levels have been linked to suppression of NK cell and lymphocyte function [26].

Opioids downregulate chemokine receptors by heterologous desensitization

Receptor desensitization is a key mechanism for protecting cells from prolonged responses to the agonists. The desensitization process of a GPCR can be initiated with its own ligand, causing homologous desensitization, or by activation of other "nearby" receptors, resulting in heterologous desensitization. Homologous desensitization mainly involves the activation of the feedback inhibitors, GRK and arrestins [27]. Heterologous desensitization is usually mediated by second messenger-activated kinases, such as PKA and PKC [28]. When the cytosolic tail of a GPCR is phosphorylated, the receptor loses its effective coupling to downstream G proteins, and sometimes even undergoes internalization, resulting in the loss of receptor function.

As discussed in previous chapters, chemokine receptors play a critical role in cell trafficking, development, activation of inflammatory and immune cells, and HIV infection. Upon injury, exogeneous microbial products, such as fMLP, and production of endogenous chemokines create an *in vivo* concentration gradient. Chemokine receptors on leukocytes sense the chemical gradient and direct the cells towards the inflammatory site. Chemokine receptors are coupled to Gi/o proteins. Consequently, PI3 kinases are recruited to the leading edge of a cell, which elicits a chain of downstream signaling events, including activation of CDC42/Rac, recruitment of Arp2/3 complex, and assembly of actin filaments. Formation of the actin filaments in the front of a cell is believed to be the driving force of chemotaxis [29]. Chemokine receptors also mediate other signaling pathways, such as G-protein dependent activation of phospholipase C and protein kinase C, and G-protein independent recruitment of G protein coupled receptor kinases (GRK) and arrestins. All three subtypes of opioid receptors, identical to their counterparts in the brain, are co-expressed by leukocytes along with chemokine receptors [30]. Although opioids

Table 1 - Crosstalk between chemokine, opioid, and vanilloid receptors

Effecter receptors	Cell types	Target receptors	Effects
MOR, DOR	Leukocytes	Desensitize CCR1, CCR2, CCR5, CXCR1/2	Immuno-suppression [4, 20–22, 30]
CCR1, CCR2, CCR7, CXCR4, CXCR1/2, CCR5	Neurons and leukocytes	Desensitize MOR, DOR	Hyperalgesia [4, 9, 10]
CCR1, CCR2, CCR5, CXCR1/2	Neurons	Sensitize TRPV1	Hyperalgesia

Abbreviations: MOR, μ-opioid receptors; DOR, δ-opioid receptors. TRPV1, Transient receptor potential vanilloid 1, also called vanilloid receptor

exhibit a moderate capacity to induce opioid receptor-dependent chemotaxis *in vitro*, their principal effect is to suppress inflammation by inhibiting chemokine receptor function [20–21]. Pretreatment with opioids selectively inhibits a number of chemokine receptors, including CCR1, CCR2, CXCR1 and CXCR2 on myeloid cells, such as human monocytes and neutrophils (Tab. 1). Additional studies reveal similar opioid-induced heterologous desensitization of chemokine receptors on T-lymphocytes.

Heterologous desensitization of chemokine receptors involves uncoupling of Gi protein by calcium-independent PKC

Met-enkephalin stimulation of opioid receptors activates phospholipase Cβ, resulting in the accumulation of IP3 and diacylglycerol (DAG) from PIP2 (4,5) hydrolysis (Fig. 1) [31]. This opioid-induced production of IP3 is rather modest, as indicated by the lack of transient calcium influx. At the same time, the capacity of Met-enkephalin to induce chemotaxis suggests that PI3 kinase γ is activated as well. Both DAG and PI3 kinase γ activate Protein Kinase C, a family of Ser/Thr kinases. The 12 PKC isozymes can be divided into three subfamilies based on differences in activation: classical PKCs (cPKCs), such as α, βI, βII, and γ, require both Ca^{2+} and DAG for activation; novel PKCs (nPKCs), such as δ, ε, θ, and η, are DAG-dependent but Ca^{2+}-independent; and atypical PKCs, such as ζ and λ, require neither Ca^{2+} nor DAG [32]. Recent studies have suggested that atypical PKCs may be activated by PI3 kinases [33]. Eight PKC isozymes, α, β1, β2, δ, ε, η, μ, and ζ, have been identified in human blood monocytes. Biochemical analysis of human monocytes and HEK cells transfected to express μ-opioid receptors (MOR) and chemokine receptors reveals that opioid induced heterologous desensitization involved calcium-inde-

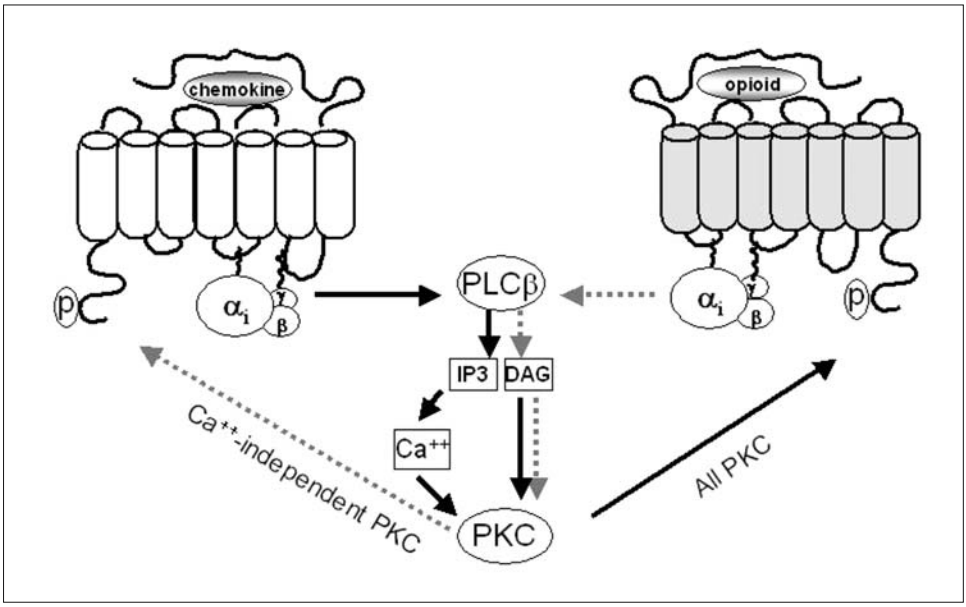

Figure 1
Molecular mechanism of bi-directional heterologous desensitization between chemokine and opioid receptors. In leukocytes, opioids induce heterologous desensitization of chemokine receptors through Gi proteins, phospholipase Cβ (PLCβ), and Ca^{2+}-independent protein kinase C (PKC), resulting in an immunosuppressive effect. In sensory neurons, treatment with proinflammatory chemokines downregulates opioid receptor function through both Ca^{2+}-dependent and -independent protein kinase C, resulting in hyperalgesia. Phosphorylation of the cytoplasmic tail and intracellular loops of a seven-transmembrane receptor by PKC decouples the receptor from downstream Gi-proteins, resulting in a decrease in receptor function. (IP3, inositol 1, 4, 5-triphosphate; DAG, diacylglycerol)

pendent PKC [21]. Activation of PKC is associated with the enhanced phosphorylation of chemokine receptors, resulting in a decrease in their affinity and in reduced coupling to G-proteins. Consequently, chemokine receptor mediated chemotaxis, calcium influx, and HIV infection are impaired.

Opioid-induced heterologous desensitization exhibits selectivity

In human monocytes, only μ and δ opioid receptors were detected to inhibit chemokine receptors [20]. Furthermore, opioid treatment inhibits the chemotactic response of human monocytes and neutrophil to a limited selection of chemokines,

including IL-8, MIP-1α, RANTES, and MCP-1, but not NAP-1, MIP-1β, SDF-1α, or fMLP [4, 22]. The availability of chemokine receptors to be desensitized may be based on their intrinsic properties: the accessibility of their C-terminal tails to phosphorylation, the impact of phosphorylation on their capacity to activate G-protein, and/or the activation threshold of each chemokine receptor. Chemokine receptors are arranged in a hierarchy in their capacity to induce heterologous desensitization [28]. For example, certain receptors, such as the fMLP receptor, have a higher capacity to desensitize other GPCRs than to be desensitized. Treatment with fMLP causes a greater phosphorylation and internalization of C5a and IL8 receptors, resulting in over 50% inhibition of their function. In contrast, IL8 has lower inhibitory effects on fMLP receptors. The capacity of a receptor to cross-desensitize GPCRs seems to correlate with its ability to induce greater phosphoinositide hydrolysis and sustained calcium mobilization [21, 28]. Opioid induced heterologous desensitization has only modest inhibitory effects on leukocyte chemotactic responses. The lower inhibitory effects are probably due to a lower expression of opioid receptors on leucocytes than on certain neuronal cells, resulting in a limited activation of downstream PKC [21].

Chemokines inhibit opioid receptors on leukocytes and sensory neurons

Pretreatment of monocytes with chemokines inhibits δ- and μ-opioid receptor mediated chemotaxis [10]. The inhibitory effects are elicited by ligand activation of selective chemokine receptors, including CCR2, CCR5, CCR7, and CXCR4, but not by CXCR1 or CXCR2. The heterologous desensitization of opioid receptors by chemokine receptors is also mediated by Gi protein mediated protein kinase C activation. Prolonged treatment with chemokines induces the phosphorylation of MOR, resulting in loss of surface MOR via receptor internalization and uncoupling of MOR from downstream effector G proteins [9]. The pathophysiological relevance of chemokine-induced desensitization of opioid receptors on leukocytes is unclear. We therefore decided to consider whether chemokine receptors expressed on neuronal cells desensitized nearby opioid receptors.

Expression of chemokine receptors in the central and peripheral nervous system

Many chemokine receptors, with the exception of CCR6 and 7, have been reported to be normally expressed by cells of the CNS, including astrocytes, microglial cells, oligodendrocytes, and neurons [34]. Chemokines and their receptors in the CNS participate in pathological, inflammatory, and neurodegenerative conditions, such as multiple sclerosis, experimental autoimmune encephalitis, Alzheimer's disease,

HIV infection, demential complex, brain injury, and tumors. Furthermore, chemokines are also involved in brain development [35]. Knockout of the mouse gene for CXCR4 or its ligand CXCL12 causes the disruption of the laminar architecture, probably due to a premature and disorganized inward migration of external granular layer cells [36]. Chemokines also indirectly regulate neuronal signaling. For example, high levels of KC, the murine homolog of CXCL1, cause a progressive neurological dysfunction, characterized by ataxia, postural instability, and rigidity [37]. Furthermore, CXCL8 and CXCL12 enhance synaptic activity by increasing neurotransmitter release and suppressing the induction of long-term depression [38]. On the other hand, soluble CX3CL1/fractalkine was able to reduce calcium oscillations in synoptically coupled hippocampal neurons by decreasing glutamate secretion and blocked gp120-induced apoptosis [39]. Thus, there is considerable evidence for the expression of various functional chemokine receptors by neuronal cells.

Molecular mechanism of opioid receptors-mediated analgesic effects

Opioid receptors consist of a family of seven-transmembrane receptors, with three subtypes, μ, δ, and κ [31]. They exert analgesic effects by blocking either the sensing or the propagation of pain signals. Endogenous peptides, such as endorphins and Met-enkephalin, have been shown to bind to opioid receptors and to exert an analgesic effect similar to that of morphine, heroin, and other plant extracts, indicating that opioids and their receptors provide an intrinsic mechanism to enable a host to perceive "pain" and "pleasure". Binding of opioids induces a conformational change in the receptors and causes the dissociation of heterotrimeric Gi/o proteins immediately downstream of the opioid receptors. Consequently, both Gα and Gβγ orchestrate a spectrum of downstream responses, including activation of G-protein coupled inward rectify potassium channel (GIRK), inhibition of adenylyl cyclase and various calcium channels. Activation of GIRK hyperpolarizes neuronal membranes, thereby preventing the excitation and transmission of pain signals. Inhibition of calcium channels impairs the release of neurotransmitters, which is also critical for the perception of pain. Furthermore, opioids also induce a transient calcium influx in both primary neurons and opioid-receptor-transfected cell lines, probably due to the activation of phospholipase C.

Pro-inflammatory chemokines suppress the function of opioid receptors

Chemokine receptors are detected on the same neuronal cells expressing opioid receptors [9]. Immunohistochemical staining shows the co-expression of CCR1 and MOR on sensory neurons in rat dorsal root ganglion. Several proinflammatory

chemokines, such as CCL2, CCL3, CCL5 and CXCL8, are able to induce a transient but robust calcium influx in a subpopulation of sensory neurons, indicating that these neuronal chemokine receptors are functional [40]. Pretreatment of sensory neurons from rat dorsal root ganglion by these chemokines downregulates the function of MOR. The molecular mechanism of chemokine-induced heterologous desensitization of MOR was further investigated in a HEK293 cell line transfected to stably express both MOR and CCR1. CCL3 treatment causes marked inhibitory effects by phosphorylating the receptors, decoupling MOR from G protein, followed by internalization of MOR. Thus, chemokine induced heterologous desensitization of MOR on sensory neurons is also dependent on Gi-mediated activation of PKC (Fig. 1). The *in vitro* observation on desensitization of MOR on primary sensory neurons was confirmed by a cold-water tail flick assay [10]. Introduction of a specific ligand for MOR, DAMGO, into the rat periaqueductal gray center (PAG) significantly enhances the tail-flick latency, indicative of MOR-mediated analgesic effects. Pre-administration of chemokines impaired the DAMGO-induced analgesic effects, suggesting chemokine-induced heterologous desensitization of opioid receptor restores the sensing of pain [9, 10].

Cross-talk between "pain" and chemokine receptors

Since the crosstalk between chemokine and opioid receptors resulted in increased pain perception, we wondered whether there also would be any crosstalk between chemokine and pro-pain receptors. Painful signals are detected by a group of specialized sensory neurons called nociceptors [41]. Recently, the first "pain" receptor, TRPV1 (vanilloid receptor 1, VR1), was identified to be a ligand-gated six-transmembrane calcium channel, highly expressed in nociceptors [42]. Noxious stimuli, such as capsaicin, heat, cold, pressure, acid, and inflammatory mediators, induce the opening of this calcium channel. As a consequence, the membrane is depolarized and the action potential is propagated to the CNS as a pain signal. It has been well documented since ancient Greece that inflammation enhances pain and that pain represents another host defense mechanism. A variety of cellular mediators, such as bradykinin, nerve growth factor, and prostaglandins (PGE2), have been shown to contribute to hyperalgesia by regulating the expression, sensitivity, and desensitization of TRPV1 [41]. Bradykinin, a potent inflammatory mediator, does so by inducing the production of endogenous "pain" ligand, 12-HPETE [43]. Inflammation elicits the accumulation of nerve growth factor (NGF) and activation of p38 MAPK, resulting in the enhancement of the translation of TRPV1 in primary neurons [44]. Nerve growth factor (NGF), a member of the interleukin 1 family, can also sensitize TRPV1 by inducing hydrolysis of PtdIns(4,5)P2, an inhibitor of TRPV1 [45]. PGE2, by coupling to Gs, induces the phosphorylation of TRPV1 by PKA, resulting in a significant decrease in desensitization, i.e., TRPV1 maintains sensitivity despite

repetitive stimulation [46]. Thus TRPV1 is an appropriate target for chemokine receptor signals.

Chemokine receptors sensitize TRPV1 on sensory neurons

The expression pattern of CCR1 partially overlaps that of TRPV1 on the sensory neurons of dorsal root ganglion and about 39±3% of DRG neurons express both receptors. Chemokine receptors have been proposed to directly contribute to the inflammation-induced hyperalgesia by inducing a transient calcium influx in neuronal cells [40]. Such a chemokine-induced calcium influx is capable of eliciting an action potential. However, we consider it unlikely that any neuronal calcium influx will result in the perception of pain, since opioids which also induce neuronal calcium influx are far from painful [9]. Pretreatment with CCL3 enhanced the sensitivity of TRPV1 to capsaicin by three- to five-fold as measured by calcium flux responses *in vitro*. The sensitization effects are likely due to the removal of PIP2, a TRPV1 endogenous inhibitor, and phosphorylation of the calcium channel by PKC (Fig. 2). Intrathecal injection of CCL3 to the spinal cord enhanced the rate of mouse hind paw withdrawal from the painful stimulation by heat, indicating the relevance of the *in vitro* observation. The fact that a proinflammatory chemokine, by interacting with its receptor on small diameter neurons, indirectly sensitizes TRPV1 suggests that the process of receptor cross-sensitization may contribute to hyperalgesia during inflammation.

Effects of activation of TRPV receptors on inflammatory responses

Opening of TRPV1 calcium channel induces the production and secretion of calcitonin gene-related peptide (CGRP) and Substance P, two potent neuropeptides regulating leukocyte function [5, 6]. CGRP in the airways causes vasodilatation, and in a few instances, bronchoconstriction. It also induces eosinophil migration, stimulates secretion of cytokines from antigen-specific T cells, and enhances of beta-integrin-mediated T cell adhesion to fibronectin at the site of inflammation. On the other hand, CGRP also impairs the capacity of macrophages to activate T-cells, a potent anti-inflammatory effect. Substance P acts through NK1 receptor expressed on T cells, macrophages, dendritic cells and probably other cell types, resulting in an increase in IFN-γ production and amplification of the Th1 response. TRPV1 may also directly modulate leukocyte function. Treatment of T cells with capsaicin inhibits IkappaB kinase activation, resulting in impaired T cell activation [47]. Whether the cross-talk between chemokine and TRPV receptors on leukocytes is bidirectional remains to be determined. Although painful stimuli may promote inflammatory host defenses, the net effects of TRPV receptor on the immune system

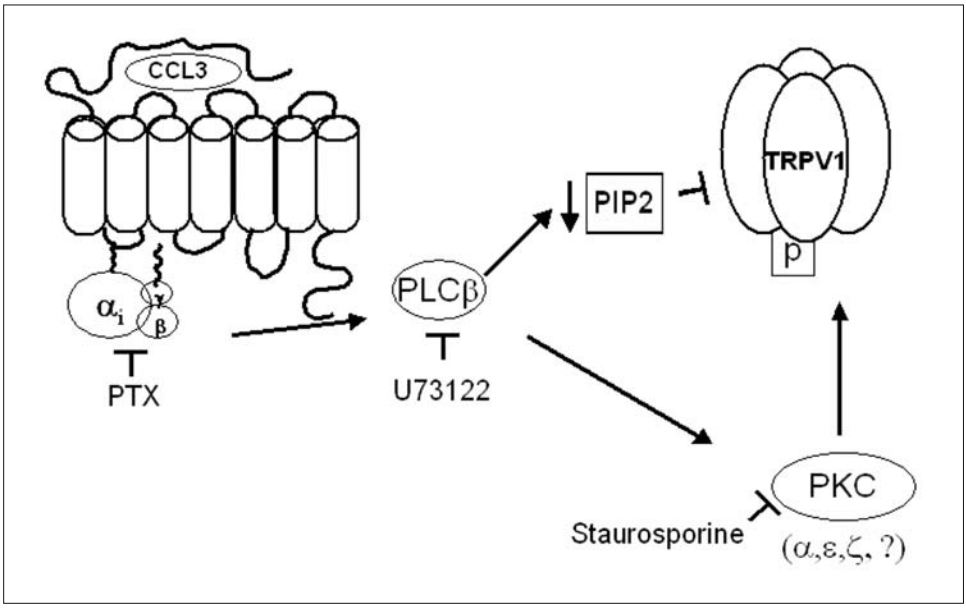

Figure 2
Molecular mechanism of chemokine-induced sensitization of Vanilloid receptor 1 (TRPV1). Activation of CCR1 by CCL3/MIP-1a enhances the sensitivity of TRPV1, a "pain" receptor, through a signal transduction cascade involving Gi protein, phospholipase Cβ (PLCβ), and protein kinase C (PKC). PLCβ hydrolyzes phosphoinositol 4,5-biphosphate (PIP2), an endogenous inhibitor of TRPV1, thereby sensitizing the TRPV1 pain receptor. Phosphorylation of TRPV1 by PKC enhances the sensitivity of TRPV1. CCL3/MIP-1α-induced sensitization of TRPV1 can be blocked at various steps of the signaling cascade by pertussis toxin (PTX), U73122, or staurosporine.

are still not clear. Further *in vivo* and *in vitro* investigations are needed to establish the pathophysiological relevance of the cross-talk between TRPV and chemokine receptors.

Conclusions

Cross-talk between chemokine and neuronal receptors provides a mechanism for integrating neuronal and immune responses. Chemokine receptors play a pivotal role during this communication. Pretreatment with opioids induces heterologous desensitization of chemokine receptors on leukocytes by activating Gi proteins and calcium-independent PKC. Conversely, chemokines also desensitize neuronal recep-

tors for opioids, which enhance pain perception. Furthermore, exposure to chemokines sensitizes TRPV1 "pain" receptors which generate a "painful" signal from sensory neurons to the host CNS. Both of the opioid and Vanilloid pathways warn the host of the existence of a pathological condition. In the future, it will be interesting to investigate the communication between chemokines and neuronal responses in several disease settings. For example, herpes zoster and rheumatoid arthritis are extremely painful inflammatory diseases. Blocking chemokine receptors may significantly reduce the painful symptom. Furthermore, a decrease in nociceptive neuron activity will in turn reduce the secretion of proinflammatory neurotransmitters, such as CGRP and Substance P. Therefore, blocking proinflammatory chemokines may serve as an effective approach to block the positive feedback loops between inflammation and hyperalgesia.

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