Pro-algesic versus analgesic actions of immune cells

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Purpose of review

When tissue is destroyed, pain arises. Tissue destruction is associated with an inflammatory reaction. This leads to activation of nociceptors. The following review will concentrate on pro-algesic and analgesic mediators, which arise from immune cells or resident cells in the periphery or the circulation during inflammation.

Recent findings

In early inflammation endogenous hyperalgesic mediators are produced, including cytokines, chemokines, nerve growth factor as well as bradykinin, prostaglandins and ATP. Simultaneously, analgesic mediators are secreted: opioid peptides, somatostatin, endocannabinoids and certain cytokines. Inflammation increases the expression of peripheral opioid receptors on sensory nerve terminals and enhances their signal transduction, as well as the amount of opioid peptides in infiltrating immune cells. Interference with the recruitment of opioid-containing immune cells into inflamed tissue by blockade of adhesion molecules or by intrathecal morphine injection reduces endogenous analgesia.

Summary

Inflammatory pain is the result of the interplay between pro-algesic and analgesic mediators. To avoid central side effects, future analgesic therapy should be targeted at either selectively blocking novel pro-algesic mediators or at enhancing endogenous peripheral analgesic effects.

Keywords

immune cells, opioid peptides, hyperalgesia, analgesia

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Abbreviations

CGRP	calcitonin gene related peptide
CRF	corticotropin releasing factor
DRG	dorsal root ganglia
NGF	nerve growth factor
SRIF	somatotropin release-inhibiting factor
TNF	tumour necrosis factor

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Introduction

When tissue is destroyed or invaded by immune cells in inflammation, numerous mediators are liberated at the site. While these mediators contribute to the body's ability to counteract the destruction of tissue integrity they also elicit pain by activation of specialized receptors localized on primary afferent neurons (nociceptors) [1]. Nociceptors are defined as neurons preferentially sensitive to a noxious stimulus or to a stimulus which would become noxious if prolonged (definition of the International Association for the Study of Pain, website: http://www.iasp-pain.org/terms-p.html) [2]. Noxious stimuli are transduced and propagated by specialized nociceptive fibers, Aδ nerve fibres (myelinated fibre) and C fibres (unmyelinated fibre), to the dorsal horn of the spinal cord. The cell bodies of these nerve fibres are located in the dorsal root ganglia (DRG). At the level of the spinal cord and at supraspinal sites, different neurotransmitters are released which, together with environmental and cognitive factors, contribute to the final perception called pain [3••,4]. While these central mechanisms also play a prominent role, the following review will focus on the peripheral inflamed tissue itself.

Pain in humans is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage [2]. Pain cannot be measured in animals; nociceptive behaviour (e.g. writhing in response to intraperitoneal injection of acid, paw withdrawal to mechanical or thermal stimuli), however, can be quantified. An enhanced sensitivity to stimuli or an increase in nociceptive behaviour is interpreted as pain. More detailed discriminations define 'hyperalgesia' as an increased response to a stimulus which is normally painful (International Association for the Study of Pain). Agents like carrageenin, formalin or Freund's complete adjuvant induce local inflammatory hyperalgesia in animals. Peritoneal inflammation can be elicited by zymosan, glycogen or acetic acid.

During inflammation, hyperalgesic mediators are produced by the immune cells or by resident cells (cytokines, nerve growth factor, prostaglandins, ATP), arise from cell destruction (hydrogen ion) or are formed in the circulation (kinines). It is less well known that analgesic mediators are also produced in inflamed tissue, and counteract pain. These include opioid peptides, somatostatin, endocannabinoids and antiinflammatory cytokines (Table 1). In this review recent advances in our understanding of both pro-algesic and analgesic

Table 1. Mediators of hyperalgesia and analgesia in inflamed tissue

	Pro-algesic	Analgesic
Mediators	Bradykinin Prostaglandins ATP Serotonin Sympathetic amines Protons	Endocannabinoids
Cytokines		
Proinflammatory	IL-1, IL-6 and TNF-α	IL-1 and TNF-α (indirect)
Antiinflammatory		IL-4, IL-10, IL-13, IL-1ra
Chemokines	CCL5, CXCL12 and CCL22	
Growth factors	Nerve growth factor	
Hormones		Corticotropin releasing factor (indirect) Somatostatin
Opioid peptides		Endorphins Enkephalins Dynorphins Endomorphins

IL, interleukin; TNF, tumour necrosis factor; ra, receptor agonist.

mediators will be summarized and potential novel targets to counteract hyperalgesia or to enhance analgesia in peripheral inflammation will be described.

Pro-algesic mediators

Different inflammatory mediators can elicit pain, as well as the classical signs of inflammation: bradykinin, serotonin, substance P, prostaglandins, adenosine, ATP and sympathomimetic amines like noradrenalin. These mediators can either directly (e.g. bradykinin, serotonin, excitatory amino acids, hydrogen ions) or indirectly (e.g. prostaglandins, serotonin, noradrenalin, adenosine, nitric oxide, nerve growth factor (NGF), cytokines) stimulate nociceptors. The painful sensation can be mediated by either activation or sensitization of nociceptors. A direct generation of an action potential within the neuronal membrane is called activation. A sensitizing substance does not directly initiate an action potential but it reduces the threshold for depolarization by other substances. This is accomplished by modulation of downstream second messenger cascades or ion channel gating. Some mediators also change gene expression of nociceptors [5].

Proinflammatory cytokines and chemokines

Four cytokines – interleukins 1β and 1α, tumour necrosis factor (TNF)-α and interleukin-6 – have pro-algesic effects if injected subcutaneously into non-inflamed tissue as well as in experimental inflammation [6]. However, their effect is not direct: interleukins 1β and 6 stimulate the production of prostaglandins, whereas TNF-α exerts its effects via prostaglandins as well as sympathomimetic amines [7]. In addition, interleukin-1β can stimulate the secretion of NGF from mast cells and exert hyperalgesic effects through leukotriene B_4. Recently, it was shown that these cytokines induced not only acute but also chronic hyperalgesia lasting up to 30 days after initial repetitive injection over 6 days [8•]. Cytokines (interleukin-1β and TNF-α) might also indirectly induce hyperalgesia to thermal stimuli. Release of calcitonin gene related peptide (CGRP) from primary afferent neurons occurs after nociceptor activation and heat sensitization *in vitro*. Interleukin-1β and TNF-α are able to substantially augment heat-induced release of CGRP indicating an indirect sensitizing effect of cytokines on heat-induced hyperalgesia [9].

Chemokines – CC and CXC – are chemotatic mediators, which are produced in inflamed tissue and lead to the recruitment of immune cells to the site of inflammation. Chemokines bind to a group of chemokine receptors: CCR and CXCR seven transmembrane spanning G-protein coupled receptors. Several observations point towards a role of chemokines in hyperalgesia. Chemokine receptors such as CXCR4 and CCR4 are expressed on subpopulations of DRG neurons and their corresponding ligands can induce calcium influx by receptor activation. Direct local injection of chemokines such as CCL5, CXCL12 and CCL22 induces nociceptive behavior in animals [10]. In addition, chemokines (human CXCL8 or rat CXCL1) can indirectly cause hyperalgesia through release of sympathomimetic amines when applied subcutaneously [6,11].

In summary, proinflammatory cytokines as well as certain chemokines have hyperalgesic effects via prostaglandins and sympathomimetic amines, release of CGRP in the early inflammation or direct excitatory effects.

Nerve growth factor

NGF is a neurotrophic factor important for the development, regeneration and maintenance of the central and peripheral nervous system [12]. NGF is produced and released by T lymphocytes and mast cells and this process is augmented by interleukin-1β and TNF-α [13]. NGF binds to its receptor (trkA) selectively expressed on primary afferent neurons. After ligand binding the receptor complex is internalized and transported to the DRG (retrograde transport), where it stimulates the synthesis of neurotransmitters, receptors and ion channels [14•]. NGF has sensitizing properties on sensory neurons, for example through upregulation of binding sites for bradykinin or heat gated vanilloid receptors [14•] as well as alterations in channel gating of vanilloid receptors through changes in intracellular signalling [15]. In addition, the axonal transport of the heat sensitive vanilloid receptor TRVP1 into the periphery is increased by NGF, enhancing sensitivity to heat independent of transcription [16••]. Furthermore, NGF can upregulate the gene expression of acid-sensing ion channels, and thereby the excitability of nociceptors to protons at low

pH, as typically found in inflammation [17•]. Interestingly, inhibitors of cyclooxygenase can prevent upregulation of acid-sensing ion channels and may limit pain via this mechanism in addition to their spinal and peripheral actions on prostaglandin synthesis [18].

Bradykinin

Bradykinin is a nonapeptide generated from high molecular weight kininogen through cleavage by kallikrein under inflammatory conditions [19]. Bradykinin is a proinflammatory agent and one of the most potent endogenous pro-algesic mediators. It activates bradykinin receptors on primary afferent neurons or sensitizes nociceptors indirectly (through release of prostaglandins, nitric oxide, neurokinins and CGRP, cytokines and histamine). Furthermore, bradykinin sensitizes nociceptors (e.g. via vanilloid receptor channel gating alterations) [15]. Non-peptide orally active B1 and B2 receptor antagonists are currently being developed as peripheral analgesics [20•].

Prostaglandins

Prostaglandins do not directly activate nociceptors but enhance signal transduction within nociceptors by intracellular phosphorylation. Processing of arachidonic acid by cyclooxygenase results in prostaglandin production while alternative metabolism by lipoxygenase yields leukotrienes. Prostaglandin E_2, prostacyclin I_2 and leukotriene B_4 are indirect hyperalgesic mediators. In addition, the leukotriene 12-HPETE has been shown to directly activate the heat-sensitive vanilloid receptor TRVP1 and could thereby directly contribute to thermal hyperalgesia [21].

Consistently, in addition to their well known analgesic effects after systemic administration, the intraarticular injection of cyclooxygenase inhibitors (nonsteroidal antiinflammatory drugs) after arthroscopy significantly inhibited postoperative pain presumably by reducing cyclooxygenase-mediated production of prostaglandins [22]. In a novel approach, prostaglandin-mediated hyperalgesia in a postoperative pain model was inhibited by local injection of an antagonist to the prostaglandin receptor EP-1 [23]. A study in humans demonstrated that oral intake of this antagonist also blocked acid-induced oesophageal pain [24•].

ATP

ATP is a ubiquitously expressed energy source and modulator of intracellular and extracellular functions. It also has a role as a neurotransmitter in the central and peripheral nervous system. One of the receptors for ATP, $P2X_3$, is exclusively expressed by nociceptors [25]. ATP or its more stable analogues can elicit pain in humans [26] and hyperalgesia in animals [27] when applied locally. In the recently cloned $P2X_3$ knockout mouse, nociceptive responses to chronic but not acute inflammation were reduced [28,29].

Studies using an antagonist at the ATP receptor $P2X_7$, oxidized ATP, have provided further insight into the source of ATP in inflammation. Oxidized ATP injected locally can cause sustained analgesia for up to 24 h [30•]. Interestingly, this effect is independent of immune cells, pointing towards nerve terminals or endothelial cells as the source of endogenous algesic ATP [31]. Local application of ATP receptor antagonists could provide a novel approach for treatment of inflammatory pain.

Immune cells

Immune cells and the mediators released by immune cells play a major role in nociception. In different animal models, depletion of immune cells reduces mechanical and thermal hyperalgesia. Granulocyte depletion diminished local hyperalgesia [32] as well as neuropathic pain induced by ligation of the ischiadical nerve [33]. Depletion of tissue macrophages reduced nociceptive behavior (i.e. writhing) due to acid-induced peritonitis whereas enhanced migration of macrophages into the peritoneal cavity augmented hyperalgesia [34]. Immune cells can therefore increase pain by release of hyperalgesic mediators.

Peripheral analgesic mechanisms

Not only are there hyperalgesic mediators in the peripheral inflamed tissue, but endogenous analgesic mediators are also present. The best-characterized and clinically relevant system consists of the endogenous opioid peptides. Other analgesic mediators are somatostatin (somatotropin release-inhibiting factor (SRIF)), the endocannabinoids as well as antiinflammatory cytokines.

Somatostatin

SRIF is widely distributed in the brain and periphery including small diameter DRG neurons. Three subtypes (SST_{2a} SST_{2b} and SST_3) of the SRIF receptors are localized on DRG neurons and on peripheral non-myelinated nociceptive nerve terminals in the skin (SST_{2a}) [35]. Experimental studies in animals [36] as well as a few clinical case reports [37] have shown that local intraarticular injection of SRIF agonists can inhibit pain. Furthermore, local application of SRIF antagonists or SRIF antiserum enhances nociceptive behaviour in normal and formalin-injected animals [38]. This indicates that SRIF may play a role in a tonic control of peripheral nociception independent of endogenous opioid peptides. SRIF or its longer acting analogues like octreotide (available for symptomatic treatment of endocrine tumours) might be suitable for local treatment of inflammatory pain [37,39].

Interestingly, SRIF is not only expressed in nerve terminals but also in immune cells (mostly macrophages). Expression in macrophages requires stimulation and is inhibited by substance P [40]. It is possible that macrophages secrete SRIF and, thereby, counteract inflammatory pain. However, this concept of endogenous SRIF mediated analgesia from immune cells in inflammatory pain needs to be further evaluated in experimental and clinical studies.

Endocannabinoids

Endocannabinoids (anandamide, 2-arachidonylglycerol and palmitoylethanolamide) are produced by immune cells (e.g. macrophages) and platelets from plasma membrane phospholipids and have analgesic properties [14•]. Stimulation of leukocytes induces release of endocannabinoids [41,42]. Two cannabinoid receptors, CB_1 and CB_2, have been cloned. CB_1 is exclusively expressed in the brain, spinal cord and DRG. CB_2 expression is restricted to immune cells. Endocannabinoids mediate analgesia via spinal and peripheral mechanisms. When injected locally anandamide and palmitoylethanolamide as well as synthetic cannabinoids reduce nociceptive behaviour in many animal models of inflammatory pain [14•]. Furthermore, cannabinoids are antiinflammatory and may counteract the NGF-induced hyperalgesic effects on immune cells and nerve terminals.

Although the hypothesis that endogenous endocannabinoids contribute to a physiological analgesic tone is attractive the data provided so far remain inconclusive [43]. Furthermore, CB1 as well as CB2 knockout mice do not exhibit more nociceptive behaviour, arguing against an endogenous analgesic tone [44,45]. Peripheral analgesic effects of synthetic cannabinoids are currently being investigated [20•].

Antiinflammatory cytokines

In later stages of inflammation antiinflammatory cytokines are produced, which limit inflammation and also counteract hyperalgesia. Cytokines including interleukins 4, 10, 13 and the interleukin-1 receptor antagonist are analgesic through inhibition of proinflammatory cytokines like TNF-α, interleukins 1β and 6 and the chemokine CXCL8 [46–49]. Analgesic properties of interleukins 4, 10 and 13 are independent of endogenous production of opioid peptides [50]. The analgesic effects have been demonstrated in different models of inflammatory pain induced by intraplantar injection of carrageenin, TNF-α or bradykinin as well as experimental peritonitis. In summary, during inflammation analgesic cytokines are produced which counteract the effects of the proinflammatory hyperalgesic cytokines generated in the early stages of inflammation.

Opioid peptides and opioid receptors

Opioid peptides are produced in peripheral inflamed tissue by immune cells and can be released upon certain stimulation. They bind to peripheral opioid receptors and thereby elicit potent endogenous analgesia.

Changes of peripheral opioid receptors in inflammation

The opioid receptors, μ, δ and κ, are synthesized in DRG neurons and transported to a peripheral site of inflammation via axonal transport in sensory neurons [51,52]. Peripherally active opioid analgesics are more effective under inflammatory conditions [53] and gain functional relevance with the chronicity of inflammation [54] due to four factors: (1) Inflammation increases μ opioid receptor binding sites in DRG neurons and μ opioid receptor axonal transport leading to an increased density of opioid receptors on the peripheral nerve endings [55,56]. (2) Inflammation enhances G-protein coupling to the intracellular signaling cascade [56]. (3) Inflammation destroys the perineural sheath, the barrier to diffusion of hydrophilic and high molecular weight substances, facilitating the access to the neuronal membrane of agonists like morphine or opioid peptides [57]. (4) Inflammation increases the surface of accessible nerve endings by sprouting of nerve terminals [55]. All these factors contribute to enhanced analgesic effects of opioids under inflammatory conditions.

Opioid-containing immune cells in inflammation

Several naturally occurring endogenous ligands for opioid receptors have been described: β-endorphin, Leu- and Met- enkephalin, dynorphin, and the more recently discovered endomorphins-1 and -2. All immune cell subpopulations including lymphocytes, monocytes and granulocytes contain opioid peptides in the peripheral blood as well as in inflamed and non-inflamed lymph nodes [58–62]. Inflammation increases the expression of opioid peptides both *in vitro* and *in vivo* [63] and all opioid peptides can be detected at the site of an experimentally induced inflammation [61,64]. Quantitative analysis revealed that in early inflammation granulocytes are the major source of opioid peptide production [65]. Later in the inflammatory course, monocytes and macrophages are the predominant supply of opioid peptides. The number of infiltrating immune cells as well as total β-endorphin content at the site of inflammation increase steadily with the duration of inflammation [65]. In parallel, stress-induced analgesia is stronger in late than in early stages of inflammation.

Immigration of opioid-containing immune cells

Migration of immune cells into inflamed tissue is a multi-step process governed by adhesion molecules. Blockade of the adhesion cascade by anti-selectin treatment [66] and by an antibody against intercellular adhesion molecule-1 (or CD54) [67••] inhibits the

migration of opioid-containing immune cells and markedly reduces peripheral opioid analgesia. An intact adhesion molecule cascade is, therefore, a prerequisite for peripheral endogenous opioid mediated analgesia and inhibition of only one of the steps is already leading to impaired peripheral analgesia. Immigration of opioid containing immune cells is also dependent on neuroimmune interactions [68•] since the central inhibition of pain by intrathecal application of morphine reduces the number of opioid-containing immune cells at the site of inflammation and also impairs endogenous peripheral opioid analgesia. Efficient central analgesia apparently signals a reduced need for recruitment of opioid-containing immune cells to injured sites.

Secretion of opioid peptides in vitro *and* in vivo*: analgesic effects*

In vitro, secretion of β-endorphin from immune cells is mediated by corticotropin releasing factor (CRF) and interleukin-1. Inflammation increases CRF expression in human inflamed synovial tissue and rat subcutaneous paw tissue [69]. Likewise, the CRF and interleukin-1 receptors are located on immune cells and are upregulated in inflamed tissue [70]. Both interleukin-1 and CRF can induce opioid peptide release from lymphocytes and macrophages [58,59]. Opioid peptide release from granulocytes has only been demonstrated after non-specific activation [62].

In vivo, release of opioid peptides from immune cells by CRF, interleukins 1 and 6 and TNF-α can elicit analgesia [71–73]. Opioid peptides can also be released endogenously after exposure to stress (e.g. cold water swim) [74,75]. An endogenous mediator for stress-induced opioid peptide release is CRF because a CRF antagonist can inhibit peripheral analgesia [69].

Efficacy of exogenous and endogenous opioids in clinical practice

The local injection of opioids into injured tissue (e.g. knee joint) has been shown to produce significant postoperative analgesia in humans [76••]. In addition, endogenous opioid peptides secreted by local immune cells have been demonstrated to enhance postoperative analgesia [77]. Postoperative stress as well as certain cytokines could be the stimulus for endogenous peptide release from immune cells in the inflamed knee joint. Data from animal models also point towards clinical consequences of immunosuppression for pain sensation. Treatment with immunosuppressive drugs as well as diseases with involvement of the immune system may impair endogenous pain control, although sufficient clinical data are lacking. Future strategies should be directed at improving endogenous analgesic mechanisms by augmenting opioid-mediated peripheral analgesia.

Conclusion

Inflammation induces pain by the local production and the release of numerous pro-algesic mediators (e.g. cytokines, chemokines, prostaglandins, bradykinin, ATP and NGF). Concurrently, pain caused by these pro-algesic mediators is partially counteracted by analgesic mediators (endocannabinoids, somatostatin, antiinflammatory cytokines and opioid peptides). While our understanding of these mechanisms has expanded considerably in recent years, highly effective strategies to exclusively combat peripheral nociception are still lacking. This is highly desirable to avoid central unwanted side effects. Strategies could be developed that improve analgesia through selective enhancement of naturally occurring analgesic mediators within peripheral injured tissue, for example by recruitment of opioid producing cells.

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