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Review

Microglial signalling mechanisms: Cathepsin S and Fractalkine

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ABSTRACT

A recent major conceptual advance has been the recognition of the importance of immune system–neuron interactions in the modulation of spinal pain processing. In particular, pro-inflammatory mediators secreted by immune competent cells such as microglia modulate nociceptive function in the injured CNS and following peripheral nerve damage. Chemokines play a pivotal role in mediating neuronal–microglial communication which leads to increased nociception. Here we examine the evidence that one such microglial mediator, the lysosomal cysteine protease Cathepsin S (CatS), is critical for the maintenance of neuropathic pain via cleavage of the transmembrane chemokine Fractalkine (FKN). Both CatS and FKN mediate critical physiological functions necessary for immune regulation. As key mediators of homeostatic functions it is not surprising that imbalance in these immune processes has been implicated in autoimmune disorders including Multiple Sclerosis and Rheumatoid Arthritis, both of which are associated with chronic pain. Thus, impairment of the CatS/FKN signalling pair constitutes a novel therapeutic approach for the treatment of chronic pain.

Contents

Introduction

Chronic pain results from nerve and tissue damage, often accompanying disorders such as diabetes mellitus and arthritis, and the management of this clinical pain remains a difficult task. Mounting preclinical evidence suggests that the generation of inflammatory and neuropathic pain requires neural-immune interactions for chronicity (Austin and Moalem-Taylor, 2010; Romero-Sandoval et al., 2008). Following peripheral nerve/tissue injury, one important contributor to increased nociceptive transmission are microglial cells in the dorsal horn of the spinal cord (McMahon and Malcangio, 2009; Milligan and Watkins, 2009). Under physiological conditions microglial cells perform immune surveillance functions within the Central Nervous System (CNS). However, following a peripheral insult microglia proliferate and contribute to central sensitization and the

Abbreviations: AIA, Adjuvant-Induced Arthritis; ATP, Adenosine Tri-Phosphate; APCs, Antigen Presenting Cells; AD, Alzheimer's Disease; CatS, Cathepsin S; CIA, Collagen-Induced Arthritis; CNS, Central Nervous System; ERK, Extracellular Signal-related Kinase; EAE, Experimental Autoimmune Encephalomyelitis; FKN, Fractalkine; IL-1β, Interleukin-1β; LPS, Lipopolysaccharide; MAPK, Mitogen-Activated Protein Kinase; MS, Multiple Sclerosis; LHVS, morpholinurea-leucine-homophenylalanine-vinyl sulfone-phenyl; PAMP, Pathogen-Associated Molecular Pattern; PI3K, Phosphatidyl Inositol 3-Kinase; PNL, Partial Ligation of the sciatic Nerve; RA, Rheumatoid Arthritis.

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generation of chronic pain states by releasing pro-inflammatory factors (microglial-cell-activation). In experimental animal models withdrawal thresholds to evoked pain e.g. hind paw withdrawal in response to heat are used to measure changes in nociceptive behaviour. In rodents these behavioural changes, including hyperalgesia (increased response to noxious stimuli) and allodynia (response to non-noxious stimuli as noxious), develop within hours and persist for many weeks following tissue-damaging insults. The critical contribution of spinal microglia to these chronic nociceptive behaviours is provided by many observations, including our own, that hyperalgesia and allodynia associated with both peripheral inflammation and nerve injury are reduced by inhibitors of microglial-cell-activation (Clark et al., 2007a; Ledeboer et al., 2005; Sweitzer et al., 2001). However, as most of the tools used are not exclusively microglial inhibitors and may target other immune competent cells and even neurons, there is an obvious need for more selective inhibitors of microglial cells.

Thus, understanding of the sequence and nature of the events that govern neuron-microglia communication is critical for the discovery of new mechanisms and microglial targets for chronic pain treatment. For this reason the signals that mediate cross-talk of nociceptive signalling between neurons and glial cells in the dorsal horn of the spinal cord are being actively investigated. In this context, the chemokine FKN has been proposed to sub-serve this role within the dorsal horn.

FKN is a transmembrane chemokine which is expressed by neurons, whereas its receptor CX3CR1 is expressed by microglia in the spinal cord (Clark et al., 2009; Lindia et al., 2005; Verge et al., 2004). Intrathecal injection of FKN is pro-nociceptive in rats and wild-type mice (Clark et al., 2007b; Milligan et al., 2004, 2005) but devoid of pro-nociceptive activity in CX3CR1 null mice (Clark et al., 2007b). As FKN is a transmembrane protein, in order to activate the CX3CR1 receptor on microglia the extracellular domain of the chemokine needs to be cleaved from the neuronal membrane by a protease. Our own recent work has established a new pathway whereby in the spinal cord soluble FKN is liberated by the cysteine protease Cathepsin S (CatS), which is expressed and released by microglial cells (Clark et al., 2007b, 2009, 2010b).

Altogether these data prompt us to put forward the following mechanisms for neuron-microglia communication (Fig. 1). Following peripheral tissue/nerve damage microglial cells in the spinal cord increase their cellular activity and enter a pain-related enhanced response state (McMahon and Malcangio, 2009). In particular, P2X7 receptor activation by high concentration of extracellular ATP results in activation of the p38 Mitogen-Activated Protein Kinase (MAPK) pathway and the release of CatS (Clark et al., 2010b). Extracellular CatS then liberates soluble FKN from neurons (Clark et al., 2007b, 2009). FKN feeds back onto the microglial cells via the CX3CR1 receptor to further activate p38 MAPK (Clark et al., 2007b) and release inflammatory mediators that activate neurons which signal pain to the higher centres.

In this article we will focus on the role of FKN and CX3CR1 in neuropathic pain mechanisms as the role played by this chemokine in pain and inflammation has been recently reviewed (Clark et al., 2011). We will also review the proteolytic activity of CatS in antigen presenting cells and consider both intracellular and extracellular roles of this lysosomal protease. We will discuss CatS' biological relevance in autoimmune diseases such as Multiple Sclerosis (MS) and Rheumatoid Arthritis (RA), as well as in chronic pain which is tightly linked to the FKN/CX3CR1 system.

Fractalkine and CX3CR1

FKN (CX3CL1) is a member of the chemokine family of cytokines which is comprised of over 50 individual chemokines and nearly 20 different receptors have been identified. Chemokines are subdivided into four groups based on their structure. FKN was identified

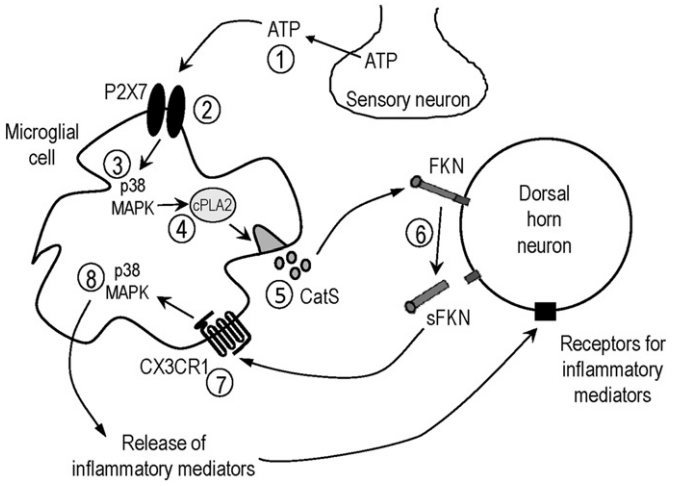

Fig. 1. Schematic of proposed pro-nociceptive mechanism of CatS. In the dorsal horn area innervated by damaged fibres, high concentrations of extracellular ATP (1), possibly released by primary afferent terminals, leads to P2X7 receptor activation on microglia (2). Following P2X7 activation intracellular signalling mechanisms including p38 MAPK (3) and phospholipase A2 (4) result in release of CatS (5) which then liberates soluble FKN from neurons (6). FKN feeds back onto the microglial cells via the CX3CR1 receptor (7) to further activate the p38 MAPK pathway (8) and release inflammatory mediators that activate neurons and result in chronic pain.

relatively recently (Bazan et al., 1997; Pan et al., 1997) and is at present the only member of the CX3C class of chemokines. Generally chemokines are secreted proteins, however, FKN is one of only two chemokines that exists as a membrane bound form (Bazan et al., 1997; Pan et al., 1997). FKN is also found in soluble forms (Bazan et al., 1997) following enzymatic cleavage from the membrane by metalloproteases ADAM-10 and ADAM-17 and the cysteine protease CatS. The binding of chemokines to their receptors is highly promiscuous, although this does not imply that several chemokines and chemokine receptors mediate the same effect in the *in vivo* situation (Schall and Proudfoot, 2011). Until recently FKN was the only described ligand of the CX3CR1 receptor. However, CCL26 (Eotaxin-3) has been recently identified as functional CX3CR1 ligand at high concentrations (Nakayama et al., 2010).

Many cell types, including immune and non-immune cells, are known to produce chemokines, and their action covers a wide range of biological functions. The primary role of chemokines is control of immune cell trafficking, a process important in host immune surveillance, as well as acute and chronic inflammation. In addition, they regulate numerous other cellular responses such as cytokine secretion, adhesion, apoptosis, proliferation and phagocytosis. The role of FKN in inflammation has been recently reviewed (Clark et al., 2011; D'Haese et al., 2010). However, in recent years a large number of chemokines and their receptors have been identified in the CNS under both normal and pathological conditions (Bajetto et al., 2002), and evidence suggests that chemokines play a role in the regulation of neuronal activity and synaptic plasticity.

In particular, FKN has been proposed to mediate neuron-microglial communication in the CNS. FKN and its receptor CX3CR1 are qualified to function as neuron-microglia signals due to their expression profile within the CNS. FKN is expressed by neurons (but not endothelial cells), both in the spinal cord (Clark et al., 2009; Lindia et al., 2005; Verge et al., 2004) and in the brain (Harrison et al., 1998; Hughes et al., 2002; Nishiyori et al., 1998; Schwaeble et al., 1998;), whereas its receptor CX3CR1 is expressed by microglia (Harrison et al., 1998; Hughes et al., 2002; Lindia et al., 2005; Nishiyori et al., 1998; Verge et al., 2004).

In the peripheral vascular structure (endothelial or smooth muscle cells), membrane bound and soluble FKN forms exert differing biological effects. Soluble forms of FKN mediate chemotaxis of immune cells