Current Topics

Lipid Mediators and Pain Signaling

Platelet-Activating Factor and Pain

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Platelet-activating factor (PAF) is a phospholipid mediator that regulates the functions of a variety of cells in the peripheral tissues and in the nervous system. Findings that injection of PAF exogenously at the skin or in the spinal cord induced pain hypersensitivity gave us much attention to its role in pain signaling. Studies using pharmacological and genetic tools to control the functions of the PAF receptor (PAFR) revealed that the PAF/PAFR system plays a role in tissue injury-induced pain, but not in the acute physiological pain evoked by thermal and mechanical stimuli. Recent investigations have focused on the roles of PAFR in pathological chronic pain such as the neuropathic pain that occurs after nerve injury for which there is currently no effective therapy. Nerve injury upregulated PAFRs in dorsal root ganglion (DRG) neurons. Studies using PAFR antagonists and PAFR-deficient mice indicated a crucial role of PAFR in production of tumor necrosis factor α (TNF α) and interleukin-1 β (IL-1 β) in the DRG and in developing and maintaining neuropathic pain. Thus, blocking PAFRs may be a viable therapeutic strategy for treating neuropathic pain.

Key words platelet-activating factor; tissue injury-induced pain; neuropathic pain

1. INTRODUCTION

Platelet-activating factor (PAF, 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine) is a phospholipid mediator that regulates the functions of a variety of cells in the peripheral tissues and the nervous system.^{1,2)} The biological activities of PAF are elicited by activating its cognate receptor, the PAF receptor (PAFR), which belongs to the superfamily of G protein-coupled receptors.^{3,4)} Accumulating evidence indicates the role of the PAF/PAFR system in modulating pain signaling. In this article, we review progress in the current understanding of how the PAF/PAFR system contributes to acute and chronic pain.

2. ROLE OF PAF AND PAFR

Studies showing that local injection of PAF to the periphery such as skin enhances pain sensitivity in animals^{5–7)} and humans⁸⁾ suggest that PAF is an important substrate in the formation of pain. The injection of lyso-PAF, which is a structurally-related, biologically-inactive form of PAF, failed to produce pain responses⁹⁾ and appeared to involve the specific target receptor, PAFR. On the other hand, PAFR antagonists, such as BN52021, produced antinociceptive effects in a model of inflammatory pain evoked by tissue damage.⁹⁾ PAF is produced by various kinds of cells including keratinocytes¹⁰⁾ and inflammatory cells,¹¹⁾ and PAF is increased in inflamed tissue.¹²⁾ Thus, perhaps endogenous PAF is a mediator of pain behavior caused by tissue damage-induced inflammation. Nonetheless, the PAFR antagonists used in these studies were not specific for PAFR^{13,14)} and could not reveal the precise role of the endogenous PAF/PAFR system in pain signaling. However, this issue was solved by studying the behavioral phenotype using mice that lack PAFR by targeted disruption of the gene.³⁾ By analyzing the performances in a

range of acute pain tests, we showed that the PAF/PAFR system is important for tissue injury pain and visceral pain.¹⁵⁾ Indeed, PAFR-deficient mice displayed almost normal responses to thermal and mechanical stimuli but exhibited attenuated persistent pain behaviors resulting from tissue injury by locally injecting formalin to the hindpaw (formalin test).¹⁵⁾ These mice also showed attenuated pain behaviors in models of chemical-induced cutaneous pain evoked by intraplantar injection of capsaicin and of chemical-induced visceral inflammatory pain evoked by intraperitoneal injection of acetic acid. Using intracellular Ca²⁺ imaging techniques, we applied a PAFR agonist to cultured dorsal root ganglion (DRG) neurons which produced a Ca²⁺ response in capsaicin-sensitive DRG neurons. This response was presumably due to stimulating the G-protein/phospholipase C/IP₃ pathway^{1,16)} which suggests the presence of functional PAFR in nociceptors. This notion is supported by previous data that PAFR stimulation induced gene expression in cultured primary sensory neurons.¹⁷⁾ In contrast, no alterations in pain behaviors were seen in response to a direct activation of NMDA and neurokinin 1 receptors in the spinal cord in PAFR-deficient mice.¹⁵⁾ A PAFR agonist did not affect C- or A δ -evoked excitatory postsynaptic currents in substantia gelatinosa neurons in the dorsal horn.¹⁵⁾ Based on these findings, primary sensory neurons might be a target of endogenous PAF in modulating pain signaling. However, the phenotype of PAFR-deficient mice was unlikely due to a developmental deficit in cytoarchitectural and neurochemical properties in DRG neurons.¹⁵⁾ Furthermore, PAFR-deficient mice showed a reduction of extracellular signal-regulated kinase (ERK) activation in DRG neurons following peripheral tissue injury.¹⁵⁾ ERK is a member of the mitogen-activated protein kinase (MAPK) family and ERK sensitizes primary sensory neurons to peripheral inputs.^{18–20)} Thus, PAFR activation may sensitize primary afferent sensory neurons. Tissue-dam-

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aging stimuli generate a complex cascade of transmitters and modulators at the periphery, which in turn develop tissue injury-induced persistent pain.²¹⁾ Findings obtained from mice lacking PAFR have now provided evidence that PAFR signaling activated by endogenous PAF may be an important event in producing persistent pain in primary sensory neurons after peripheral tissue injury.

Neuropathic pain, a debilitating chronic pain condition, is a common consequence of damage to the nervous system. Optimal treatment of neuropathic pain is a major clinical challenge. By using PAFR-deficient mice, we demonstrated the crucial role of PAFR in neuropathic pain.²²⁾ After peripheral nerve injury, wild-type mice showed tactile allodynia which is pain hypersensitivity to innocuous stimuli, but PAFR-deficient mice show reduced allodynia.²²⁾ The role of PAFR in the DRG was suggested by an anti-allodynic effect of a PAFR antagonist that had been injected near the L5 DRG. The expression of PAFR mRNA was increased in the DRG ipsilateral to the nerve injury and, interestingly, the macrophages in the DRG were markedly increased. Thus, peripheral nerve injury may cause the transcriptional upregulation of PAFR in macrophages in the DRG.²²⁾ Alternatively, the upregulation of PAFR mRNA may be due to infiltration of macrophages expressing PAFR in the DRG after nerve injury, because PAFR was expressed in macrophages,¹⁾ was involved in macrophage chemotaxis²³⁾ and the number of macrophages in the DRG was increased following peripheral nerve injury.²⁴⁾ More interestingly, PAFR-deficient mice showed a marked suppression of the upregulation of two major proinflammatory cytokines, tumor necrosis factor α (TNF α) and interleukin-1 β (IL-1 β), in the injured DRG.²²⁾ Increased synthesis and release of TNF α and IL-1 β can directly modulate neuronal activity and elicit spontaneous action potential discharges in the DRG.²⁴⁾ TNF α enhances tetrodotoxin-resistant Na⁺ currents in DRG neurons through TNF receptor 1.²⁵⁾ Similarly, IL-1 β application increases the excitability of sensory neurons by potentiating voltage-dependent Na⁺ currents.²⁶⁾ Therefore, these cytokines in the injured DRG might be involved in sensitization of primary sensory neurons. As macrophages are one of the major sources of these proinflammatory cytokines,²⁷⁾ the PAF/PAFR system may contribute to tactile allodynia following peripheral nerve injury through a regulation of TNF α and IL-1 β production in macrophages in the injured DRG.

In addition to the role of the PAF/PAFR system at primary afferent sensory neurons, spinal PAF contributes to tactile allodynia. Intrathecal injection of PAF, but not lyso-PAF, to normal mice induced tactile allodynia.²⁸⁾ The effect of PAF was suppressed by several PAFR antagonists. The PAFR transcript was detected in the spinal cord and in the DRG. The PAF-induced tactile allodynia was suppressed by inhibitors of purinergic P2X receptors (P2XRs), *N*-methyl-D-aspartate (NMDA) receptors, and nitric oxide (NO) synthetases.^{28,29)} Furthermore, cGMP-dependent protein kinase (PKG) inhibitors blocked tactile allodynia induced by intrathecal PAF injection. The allodynic behaviors evoked by intrathecal administered PAF or a cGMP analog were not observed in mice with the knockdown of GlyR $\alpha 3$ expression in the spinal cord. Thus, it was suggested that the glutamate-NO-cGMP-PKG pathway in the spinal cord, which is presumably activated by P2XRs, may be a mechanism under-

lying spinal PAF-induced tactile allodynia.

3. ROLE OF CYTOSOLIC PHOSPHOLIPASE A₂ (cPLA₂)

Cytosolic phospholipase A₂ (cPLA₂), a Ca²⁺-dependent subclass of the PLA₂ family,³⁰⁾ is a crucial enzyme that catalyzes the hydrolysis of phospholipids to release arachidonic acid and lysophospholipid, and subsequently generates lipid mediators including PAF by lyso-PAF acetyltransferase. Intrathecal administration of an inhibitor of cPLA₂ suppresses nerve injury-induced tactile allodynia.³¹⁾ Furthermore, nerve injury increased phosphorylation of serine at amino acid position 505 of cPLA₂ in injured DRG neurons and translocation of phosphorylated cPLA₂ at the plasma membrane of injured DRG neurons. Phosphorylation and translocation are required for full activation of cPLA₂.³²⁾ The time-dependent activation of cPLA₂ in damaged DRG neurons paralleled the emergence of tactile allodynia.³¹⁾ Contrastingly, neither the level of the phosphorylated cPLA₂ nor its localization was altered in DRG neurons after intraplantar injection of complete Freund's adjuvant in an animal model of chronic inflammatory pain. Thus, it is likely that cPLA₂ activation in DRG neurons is specifically associated with nerve injury.

MAPKs,^{33–35)} Ca²⁺/calmodulin-dependent protein kinase II (CaMKII)^{36,37)} and MAPK-interacting kinase 1 (MNK1) or a closely related isoform³⁸⁾ phosphorylate serine residues in the catalytic domain of cPLA₂. Pharmacological inhibition of either p38 or ERK in the injured DRG did not affect cPLA₂ phosphorylation and translocation in injured DRG neurons, but inhibiting CaMKII activity abolished the cPLA₂ activation as well as the development and expression of nerve injury-induced tactile allodynia.³⁹⁾ Phosphorylated CaMKII, an active form, co-localized with activated cPLA₂ in injured DRG neurons. Also, similar to cPLA₂, phosphorylated CaMKII translocated to the plasma membrane in injured DRG neurons. Thus, CaMKII might be an intracellular regulator of cPLA₂ activation following peripheral nerve injury.

What extracellular signal(s) activates the CaMKII-cPLA₂ pathway in DRG neurons after nerve injury? We demonstrated an involvement of purinergic P2XRs.³¹⁾ P2XRs are a family of ligand-gated cation channels activated by extracellular ATP.^{40,41)} Molecular cloning has so far identified seven genes encoding P2XRs (P2X1R—P2X7R). P2XRs are permeable to Ca²⁺ as well as to Na⁺ and K⁺.^{42,43)} Among the seven subtypes, pharmacological blockade of P2X3R and P2X2/3R by the selective antagonist A-317491 inhibits cPLA₂ activation in DRG neurons after nerve injury.³¹⁾ Applying ATP to cultured DRG neurons phosphorylates CaMKII and cPLA₂ and translocates these two proteins to the plasma membrane. Phosphorylation and translocation of CaMKII and cPLA₂ was abolished by A-317491 and by EGTA or a nonselective voltage-dependent Ca²⁺ channel blocker. Furthermore, after P2XR stimulation, a physical association of phosphorylated cPLA₂ with phosphorylated CaMKII was detected in cultured DRG neurons. Because activated CaMKII is implicated in the trafficking of receptor proteins to the plasma membrane,^{44,45)} it is possible that CaMKII activation leads to an physical interaction with cPLA₂ and subsequently phosphorylates and translocates cPLA₂ to the plasma membrane of DRG neurons.

Fig. 1. Schematic Representation of the Proposed Mechanism Underlying the PAF/PAFR System-Mediated Neuropathic Pain after Peripheral Nerve Injury

4. SUMMARY

Based on the results obtained from these studies, we propose the following mechanism (Fig. 1). After peripheral nerve injury, cPLA₂ is activated by Ca²⁺ signaling evoked by P2X3 or P2X2/3 receptors.³¹⁾ These receptors are activated by extracellular ATP, which is released from neighboring cells such as Schwann cells,⁴⁶⁾ satellite glia,⁴⁷⁾ or injured nerves.⁴⁸⁾ The activity of voltage-gated Ca²⁺ channels is also involved in cPLA₂ activation.³⁹⁾ The Ca²⁺-dependent cPLA₂ activation is mediated by CaMKII.³⁹⁾ cPLA₂ supplies lyso-PAF, a precursor to PAF, which is converted into PAF by lysophosphatidylcholine acyltransferase 2 (LPCAT2).²²⁾ PAF is released from injured DRG neurons and then activates the PAFR expressed in macrophages. PAFR activation produces and releases proinflammatory cytokines such as TNFα and IL-1β. These cytokines increase the excitability of DRG neurons that are linked to nerve injury-induced tactile allodynia. Based on these findings, preventing the upregulation of PAFR expression in the DRG or pharmacologically inhibiting these receptors represent novel therapeutic approaches for treating pain caused by nerve damage, for which there is currently no effective therapy. Importantly, as basal pain sensitivity was not altered in PAFR-deficient mice and animals treated with PAFR antagonists,^{15,22)} a predicted therapeutic benefit of interfering with PAFR might be that normal pain sensitivity would be unaffected.

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