Lipid Mediators and Pain Signaling

Leukotrienes in Nociceptive Pathway and Neuropathic/Inflammatory Pain

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Received April 11, 2011

The purpose of this review is to summarize the recent studies examining the expression of leukotrienes (LTs) and their receptors in nociceptive pathways, and their crucial roles in pathological pain conditions. LTs belong to a large family of lipid mediators, termed eicosanoids, which are derived from arachidonic acids and released from the cell membrane by phospholipases. LTs are known to be important factors in a variety of local and systemic diseases and allergic/inflammatory diseases. We examined whether LTs were implicated in neuropathic pain following peripheral nerve injury. Using the SN1 model in rats, we investigated the expression of LTs and their receptors mRNAs in the spinal cord and the roles on the pain behaviors. We found the expression of LTB4, or LTC4, LTD4 and LTE4 enzymatically; these are converted to leukotriene A4 (LTA4) and LTA4 is converted to 5-lipoxygenase (5-LO) pathway. Arachidonic acid is converted from arachidonic acid and comprise several products of the 5-lipoxygenase (5-LO), FLAP and the cysteinyl leukotrienes (CysLT1) mRNAs in spinal microglia, LTA4h and LTC4s mRNAs in both spinal neurons and microglia, and BLT1 mRNA in spinal neurons. Administration of the 5-LO inhibitor or the receptor antagonists suppressed mechanical allodynia. Our findings suggest that the increase of LT synthesis in spinal microglia produced via p38 mitogen-activated protein kinase (MAPK) plays a role in the generation of neuropathic pain. We also examined the expression and roles on pain behaviors of LT receptors in the dorsal root ganglion (DRG) using a peripheral inflammation model. The data indicate CysLT2 expressed in DRG neurons may play a role as a modulator of P2X3, and contribute to the potentiation of the neuronal activity following peripheral inflammation. This review summarizes the hypothesis that LTs might work in the spinal cord and primary afferent in pathological pain conditions.

Key words leukotriene; neuropathic pain; inflammatory pain; microglia; spinal cord; dorsal root ganglion neuron

1. INTRODUCTION

Neuropathic pain is often caused by nerve injury or diseases such as diabetes, acquired immunodeficiency syndrome or cancer, which damage peripheral nerves. One common symptom of neuropathic pain is tactile alldynia, which is characterized by painful responses to normally innocuous tactile stimuli. The currently available therapies for this type of chronic pain are relatively ineffective, and the underlying molecular mechanisms are largely unknown. Previous studies suggest that nerve injury induces a number of alterations in gene expression, protein synthesis and intracellular signaling in nociceptive pathways,1–3 and have focused considerable attention on the directly damaged primary afferents and their influence on the activity of spinal dorsal horn neurons. However, recent evidence suggests that glial cells in the spinal cord undergo dynamic changes in their gene expression and produce a number of important mediators, triggering the activation of an excitatory circle among glial cells. And importantly, activated glial cells produce and release pro-inflammatory cytokines, such as interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), and neurotrophins,2,4–7 resulting in enhanced excitability of nociceptive dorsal horn neurons.

Leukotrienes (LTs) are a group of lipid mediators derived from arachidonic acid and comprise several products of the 5-lipoxygenase (5-LO) pathway. Arachidonic acid is converted to leukotriene A4 (LTA4) and LTA4 is converted to LTB4, or LTC4, LTD4 and LTE4 enzymatically; these are known as bioactive LTs. LTC4, LTD4 and LTE4 are collectively termed as the cysteinyl leukotrienes (CysLTs). LTs act by binding to specific receptors that are located on the outer plasma membrane of structural and inflammatory cells.8 Four G-protein coupled LT receptors have been cloned.9–12 Leukotriene B4 receptor 1 (BLT1) is a receptor which recognizes LTB4 with high affinity, and BLT2 binds LTB4 and many other LTs with low affinity. The cysteinyl leukotriene receptor 1 and 2 (CysLT1 and 2) are known to recognize CysLTs with different affinities.13 LTs have a variety of biological actions and have been recognized as important factors in numerous disease processes including allergic diseases such as asthma, atopic dermatitis and allergic encephalomyelitis, local and systemic inflammatory diseases such as viral injection, ischemia/reperfusion injury, traumatic spinal cord injury, rheumatoid arthritis, psoriasis, cancer and cardiovascular diseases, and aging or normal development of central nervous system (CNS).8,13–24 There are lines of evidence suggesting that LTs and their synthesizing enzymes are present in CNS including spinal cord and play important normal and pathological roles.25–27 Recently, it was reported that LTs have a key role in the pain mechanisms of peripheral inflammation and some papers indicated the involvement of spinal lipoxygenase metabolites in hyperalgesic responses.28–30 Here, the detailed expression patterns of enzymes for LTs synthesis and the receptors in the rat spinal cord are summarized and the detailed alteration of expression of these molecules after peripheral nerve injury and their roles in neuropathic pain are also reported.31

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The gene expression of the LT synthetic enzymes from the substrate arachidonic acid and the receptors of LTs were examined in the rat spinal dorsal horn using reverse transcription-polymerase chain reaction (RT-PCR) methods. The mRNA level of LT synthases in the L4-5 spinal cord was determined at 0 (naive), 3, 7 and 14 d after peripheral nerve injury (SNI). Three of 4 LT synthases examined, 5-LO, 5-lipoxygenase activating protein (FLAP) and LTC4s, increased their mRNA content after SNI surgery. Significant increases were observed 3 d after nerve injury and continued at least for 14 d except 5-LO. LTA4h mRNA did not change after SNI. The expression of LT receptors was also examined and the mRNA of BLT1 increased gradually and reached significant at 7 d after injury. In contrast, the level of CysLT1 rapidly increased at 3 d after injury and remained statistically significant at least until 14 d. CysLT2 showed no change in mRNA after SNI. We could not detect BLT2 mRNA in the rat spinal cord by the RT-PCR method.

In situ hybridization histochemistry (ISHH) revealed the morphological information about the LT synthesis in the spinal cord after nerve injury. Initially, we examined the mRNA of 5-LO in the spinal cord. In the spinal cord of naïve rats, we detected very few aggregations of silver grains, suggesting very low mRNA levels (Fig. 1A). Three days after SNI surgery, aggregations of signals were observed in the dorsal horn on the ipsilateral side to the nerve injury (Fig. 1B), especially in cells with small nuclei deeply stained by hematoxylin. There was no induction of 5-LO mRNA in the spinal cord was hard to see in darkfield photographs. The brightfield photograph showed the aggregation of silver grains on cells with large nuclei that were lightly stained by hematoxylin. In contrast, the CysLT2 mRNA was expressed in microglia in the spinal cord. These 4 mRNAs were not expressed in cells labeled for GFAP, suggesting no expression in astrocytes.

Next, we performed a detailed morphological analysis of LT receptors in the spinal cord after peripheral nerve injury using ISHH. As predicted from the RT-PCR data, the expression level of BLT1 mRNA was low, even after spinal nerve injury, and therefore the increase in BLT1 mRNA in the spinal cord was hard to see in darkfield photographs. The brightfield photograph showed the aggregation of silver grains on cells with large nuclei that were lightly stained by hematoxylin in the gray matter after SNI. On the contralateral side to the nerve injury, there were fewer aggregations of silver grains. Double labeling study indicated that the BLT1 mRNA was co-labeled with NeuN, but not with GFAP or Iba1. This finding suggests the BLT1 mRNA was expressed in neurons in the spinal cord and increased after SNI.

The expression in CysLT1 mRNA in the spinal cord increased in the dorsal horn after SNI. The labeled cells for CysLT1 mRNA contained small nuclei deeply stained by hematoxylin. In contrast, the CysLT2 mRNA was expressed in the white matter of the spinal cord, and this expression was not changed after SNI compared to the controls. Double labeling study indicated that CysLT2 mRNA was colocalized with Iba1, not with NeuN or GFAP, suggesting this mRNA was expressed in microglia in the spinal cord.
3. THE EFFECTS OF LEUKOTRIENES ON NEUROPATHIC PAIN BEHAVIORS

The presence of the mRNA of synthetic enzymes and the receptors of LTs in the spinal cord led us to behavioral experiments to study whether the LTs have a role in abnormal pain. First, the effect of an intrathecal injection of the 5-LO inhibitor was examined using the SNI model (Figs. 2A, B). The administration of inhibitor started at 2d after SNI surgery using an osmotic pump. The nerve injury induced a decrease in the withdrawal threshold indicating mechanical hypersensitivity and the administration of 5-LO inhibitor (2.4 nmol/d) attenuated the mechanical hypersensitivity significantly compared to the vehicle-treated group. Low dose of the 5-LO inhibitor did not produce a significant effect. Next, the effect of intrathecal injection of a CysLT1 receptor antagonist was also examined (Figs. 2C, D). The intrathecal administration of the CysLT1 antagonist attenuated the decrease in withdrawal threshold after SN1. There was a significant effect of the antagonist from 3 to 7 d after injury, with a similar timecourse to the 5-LO inhibitor. The low dose of the antagonist had no effect.

We also examined the effect of intrathecal injection of the BLT1 receptor antagonist on the pain behaviors (Figs. 2E, F). Because constitutive expression of BLT1 expression was observed in the spinal cord, the BLT1 antagonist was administered from the time of SNI surgery for 1 week. Only a high dose of BLT1 antagonist (24 nmol/d) attenuated the mechanical hypersensitivity significantly compared to vehicle control.

In order to determine the effect of antagonists of LT receptors on established pain behaviors, the osmotic pump with antagonist of BLT1 or CysLT1 was implanted at the 6th day after SNI surgery and pain behaviors were measured from 7 to 14 d. Neither antagonist attenuated the decrease of withdrawal threshold after SNI surgery.

These findings suggest a novel idea that LT synthesis increases in the spinal cord after peripheral nerve injury and the inhibitors of these enzymes affect pain behaviors in the rat, especially during the development of neuropathic pain. In the present study, the effects of LT synthesis on pain behaviors were examined just after the upregulation of several LTs mRNAs were confirmed. Previous studies reported that some lipoxigenase metabolites are involved in hyperalgesia in peripheral inflammation. In these cases, LTs were released from infiltrated immune cells, such as neutrophils, and may have an effect on nociceptors in peripheral inflamed tissues. LTs have important roles in a variety of systemic diseases, but few reports have suggested their possible role on neuropathic pain via traspinal mechanisms. Findings that the intrathecal injection of 5-LO inhibitors and CysLT1 receptor antagonists significantly suppressed the development of mechanical allodynia after SNI suggest an intraspinal role of LTs in neuropathic pain. Like other molecules synthesized in activated microglia, LTs have positive effects on pain behaviors for limited periods after nerve injury. Because the delayed application of LTs antagonists showed no effects on pain behaviors, we believe that CysLT1 in microglia and BLT1 in neurons are involved in the early phase of neuropathic pain, not in its maintenance.

4. p38 MITOGEN-ACTIVATED PROTEIN KINASE (MAPK) SIGNALING REGULATES LT SYNTHESIS IN MICROGLIA

5-LO and FLAP are first step enzymes of the LT metabolic pathway (Fig. 3), and the changes in the signal transduction cascade in microglia is known to be important step for microglial activation. MAPK plays a critical role in intracellular signal transduction and consists of ERK1/2, p38 MAPK, and JNK1/2. Emerging evidence indicates that nerve injury results in MAPK activation in spinal glial cells, as well as MAPK inhibitors diminish injury-induced pain hypersensitivity.

We examined whether p38 inhibitor (SB203580) application could suppress or inhibit the upregulation of LT synthesis in microglia after SNI. At first, we confirmed the upregulation of phosphorylated p38 in microglia in the spinal cord after nerve injury, and found that phospho-p38 labeled cells were also labeled for Iba1 extensively after SNI. We tested the effect of several doses of the p38 inhibitor on mechanical allodynia following SNI surgery. Just like previous reports, all doses of the p38 inhibitor significantly suppressed mechanical allodynia. Next, we found that a p38 MAPK inhibitor, SB203580, significantly reduced nerve injury-induced 5-LO mRNA upregulation in spinal microglia, although other enzymes, FLAP, LTC4s, etc., did not change as a result of the treatment with p38 inhibitor. These findings suggest that nerve injury activates the first step of the sequential LTs pathway, 5-LO, in spinal microglia via p38 MAPK activation, and thus increases mechanical hypersensitivity.
5. FUNCTIONAL ROLE OF LEUKOTRIENE SYNTHESIS IN THE SPINAL CORD

Increasing evidence in pain research indicates that glial cells in the spinal cord have substantial roles in the development and maintenance of neuropathic pain.\(^2,7,40,44\) Glial activation after nerve injury is thought to trigger the production and release of pro-inflammatory cytokines and neurotrophins that may augment nociceptive signals in the spinal dorsal horn.\(^37,43\) Moreover, a number of substances released from activated glial cells may have an effect on neighboring glial cells, change gene expression, increase the excitability and enhance the release of pro-inflammatory mediators, resulting in the enhancement of positive feedback in spinal glial networks.\(^2,7,40,44\)

One of most important findings in our study was that 5-LO and FLAP mRNAs increased in spinal microglia and after SNI surgery. 5-LO is the key enzyme in the process of synthesis of LTs and FLAP enhances the ability of 5-LO to interact with its substrate. We found 5-LO and FLAP mRNAs in spinal microglia, suggesting that LTs are synthesized and playing a role in the microglia in the spinal cord. The following enzymes, LTA4h and LTC4s mRNAs, were also increased in the spinal cord by ISHH. However, RT-PCR confirmed the significant increase in mRNA content only of the LTC4s, but no increase of LTA4h after nerve injury. This discrepancy may be derived from the ample constitutive expression of mRNA in numerous cells, including ventral horn neurons, which results in the saturation of RT-PCR signals in all conditions. In any case, we believe that LTB4 and CysLTs are synthesized in spinal microglia a few days after peripheral nerve injury (Fig. 3).

Another important finding in the present paper is that the receptors of LTs, BLT1 and CysLT1, are expressed in the gray matter in the spinal cord. ISHH and double-labeling studies revealed that BLT1 is localized in neurons and CysLT1 in microglia. We also found that these receptors were up-regulated by peripheral nerve injury in each cell type. These findings are interesting, because LTs synthesized in the spinal cord after SNI may have an effect on different cells, neurons and microglia (Fig. 3). LTs induced and released from activated microglia may have pro-nociceptive effects onto spinal neurons as well as within glial networks. LTs in the spinal cord may be important mediators in the neuropathic pain mechanisms, in addition to other lipid mediators, prostaglandins or lysophosphatidic acid.\(^45,46\)

We propose a hypothesis based on the findings described in this present study (Fig. 3), which indicates a novel lipid mediator working in the spinal cord during peripheral nerve injury. The LTs synthetic enzymes, 5-LO, FLAP, LTA4h and LTC4s, increased in microglia after SNI injury via the p38 MAPK pathway. At the same time, the LTs receptors, BLT1 and CysLT1, increased in spinal neurons and microglia, respectively. The signaling via LTs and the receptors between microglia and neurons or among microglia may be involved in the development of mechanical allodynia after peripheral nerve injury. Of course, how neurons in the dorsal horn with BLT1 contribute to increased pain behaviors should be examined, and how the signaling in microglial network via LTs affects nociception is also a next important research question.

6. THE EXPRESSION OF LT RECEPTORS IN THE DORSAL ROOT GANGLION (DRG)

So far, we have examined the role of leukotrienes in the spinal cord after peripheral nerve injury and suggested their novel role in neuro-microglial networks. Next, we investigated whether the leukotriene system in primary sensory neurons has roles in pathological conditions, such peripheral inflammation.\(^47\) To examine whether sensory neurons express the LT receptor mRNAs, we performed RT-PCR and ISHH using adult rat DRG. The mRNAs for BLT1 and CysLT2 mRNAs were expressed in the DRG, but not the BLT2 and CysLT1 mRNAs. The ISHH revealed that the BLT1 mRNA was expressed in an extremely limited population of non-neuronal cells. In contrast to the BLT1 mRNA, a subpopulation of DRG neurons expressed CysLT2 mRNA (Fig. 4). The darkfield photograph displayed distinguishable clusters of silver grains over the tissue with minimal background signals. Highly magnified brightfield images confirmed the presence of CysLT2 on neuronal cell bodies (Fig. 4). We evaluated objectively the expression of the CysLT2 mRNA in DRG neurons, and we plotted the signal-to-noise (S/N) ratio and cross-sectional area of each neuron. In this scattergram, neuronal profiles with a grain density of 20-fold the background level or higher (S/N ratio > 20) were considered positively labeled for this mRNA. With this criterion, 35.8±3.3% of profiles were positively labeled for CysLT2 mRNA of the total DRG neurons. The scattergram revealed that CysLT2 mRNA was expressed more intensely by the neurons with cell profiles less than 600 μm² compared with the medium or large-size neurons. The CysLT2 mRNA was
expressed in a limited population of small (<600 μm²) and medium-size (600—1200 μm²) neurons, whereas large-size (>1200 μm²) neurons were not labeled for this mRNA.

7. CHARACTERIZATION OF CysLT2-LABELED DRG NEURONS

To characterize the expression of CysLT2 mRNA in DRG neurons, we used double labeling ISHH with immunohistochemistry (IHC) for NF-200, a maker of myelinated A-fiber neurons. We found NF-200-immunoreactive neurons in 36.3 ± 1.5% of the total neurons. The results of the double labeling analysis of CysLT2 mRNA with NF-200 showed that 9.6 ± 3.4% of the CysLT2 mRNA-positive profiles expressed NF-200; conversely, 8.0 ± 2.3% of NF-200-profiles expressed CysLT2 mRNA (Fig. 5A). The CysLT2 mRNA was expressed in 44.0% of NF-200 negative profiles, which were considered unmyelinated neurons (C-fiber). We tested the co-expression of CysLT2 mRNA with CGRP and IB4 in order to identify the peptide-dependent neuronal subpopulations, using double labeling of ISHH with IHC (Figs. 5B, C). We observed CGRP-immunoreactive and IB4-binding neurons in 39.0 ± 3.1% and 37.5 ± 2.9% of the total neuronal profiles, respectively. The results of the double labeling analysis of CysLT2 mRNA with CGRP and IB4 showed that 27.5% of the CysLT2 mRNA-positive profiles expressed CGRP; conversely, 25.6% of CGRP-profiles expressed CysLT2 mRNA (Fig. 5B), and 85.6% of the CysLT2 mRNA-positive profiles expressed IB4, while 82.0% of IB4-profiles expressed CysLT2 mRNA (Fig. 5C). These results indicated that CysLT2 mRNA was expressed in non-peptidergic neurons rather than peptidergic neurons.

Next, to examine whether CysLT2 mRNA was co-expressed with TRPV1 and P2X3 that are considered as pivotal nociceceptors in primary afferent fibers, we tested the percentage of colocalization of CysLT2 mRNA with TRPV1 and P2X3. We observed TRPV1 and P2X3 immunoreactive neurons in 36.7 ± 1.5% and 34.0 ± 1.9% of the total neuronal profiles, respectively. Further, 71.2% of the CysLT2 mRNA-positive profiles expressed TRPV1; conversely, 69.6% TRPV1-positive profiles expressed CysLT2 mRNA (Fig. 5D) and 80.7% of the CysLT2 mRNA-positive profiles expressed P2X3; conversely, 88.8% P2X3-positive profiles expressed CysLT2 mRNA (Fig. 5E). This high percentage of co-localization with CysLT2 and P2X3 indicated a possible relationship the leukotrienes might have a role in the modulation through CysLT2 receptor in the P2X3 responses, because the role of P2X3 in inflammatory pain mechanisms has been well established.

8. EFFECT OF LTC4, A CysLT2 RECEPTOR AGONIST, ON PAIN-RELATED BEHAVIORS

Leukotrienes are known as proinflammatory lipid mediators, and CysLT2 was co-localized with TRPV1, a heat sensor, in DRG neurons shown in Fig. 5D. We examined whether LTC4, a CysLT2 receptor agonist, leads to thermal hyperalgesia. We tested heat sensitivity of the hind paw after intraplantar injection of LTC4 (8 fmol, 0.8, 80 pmol). None of the doses affected heat sensitivity at 10, 30 and 60 min after LTC4 injection. LTC4 alone (0.8 pmol) did not contribute to the nocifensive behaviors (pain-like behaviors) and swelling of the hind paw.

Next, because CysLT2-positive cells heavily co-localized with P2X3, we examined whether intraplantar injection of LTC4 can enhance the nocifensive behaviors induced by alpha, beta-methylene adenosine 5’-triphosphate (αβ-me-ATP), a P2X3 receptor agonist. In normal rats, αβ-me-ATP (100 μmol) consistently induced periods of intermittent hind paw-lifting behavior, which mostly began within 30—40 s after the injection and continued for the first 4 min. Intraplantar injection of LTC4 at 0.8 pmol before the αβ-me-ATP injection induced a remarkable increase of paw-lifting behavior. The increased duration of paw lifting was significantly longer than that after the injection of PBS plus αβ-me-ATP. Lower and higher doses of LTC4 (<80 pmol, 8 pmol>) did not show the alteration of nocifensive behaviors by αβ-me-ATP injection. Potentiation of nocifensive behaviors induced by LTC4 showed a bell-shaped concentration—effect curve, with no significant effect at lower and higher amounts.

9. FUNCTIONAL CONSIDERATION OF CysLT2 IN DRG NEURONS

Here we demonstrated the expression of LT receptors, BLT1, BLT2, CysLT1, and CysLT2, in the adult rat DRG. We could not detect BLT2 and CysLT1 mRNAs in the DRG. We found the BLT1 mRNA expression in non-neuronal cells, but Andoh and Kuraishi reported expression of BLT1 in
mRNA, CysLT2 mRNA was expressed in DRG neurons. This discrepancy may be due to the difference of the species (rat versus mouse) or the methods (ISHH versus IHC). In contrast to the expression of BLT1 mRNA, CysLT2 mRNA was expressed in DRG neurons. CysLT2 was cloned in 2000, but there has been limited information of its tissue distribution in nervous system, such as in the astrocytes in brain. CysLT2 is involved in apoptosis induced by oxygen-glucose deprivation in vitro, but its functional role remains largely unknown. We found that about 40% of DRG neurons expressed CysLT2 mRNA and small sized DRG neurons preferentially expressed CysLT2. Double-labeling analysis with NF-200 and CysLT2 showed that most CysLT2-labeled cells did not express NF-200. Moreover, a lot of CysLT2-positive profiles exclusively co-localized with IB4-binding, a quarter of CGRP-positive neurons expressed CysLT2 mRNA. These results indicate that CysLT2 was mainly expressed in unmyelinated and non-peptidergic neurons.

Interestingly, we found that CysLT2 mRNA expressing neurons were heavily co-localized with TRPV1- or P2X3-positive neurons. TRPV1, one of the TRPV family, has been cloned and is a thermosensitive channel and is modulated by various G-protein coupled receptors, such as PGE2 receptor, protease-activated receptor 2 and neurokinin-1 receptor via the protein kinase C (PKC) and/or PKA pathways. 12-(S)-HPETE, a product of 12-lipoxygenase, also potentiates the TRPV1 current in HEK cells. Thompson et al. have reported that the signaling pathway for CysLT2 is involved in the activation of PKC pathway via Gq-proteins.

We expected that CysLT2 can sensitize TRPV1 in primary sensory neurons, however one of the doses of LTC4 (8 fmol, 0.8, 80 pmol) affected on heat sensitivity at 10, 30 and 60 min after the injection in normal rats. The data indicate LTC4 does not have a role on thermal hyperalgesia in a normal condition.

A ligand-gated ion channel for ATP, P2X3 is of particular interest in the context of pain pathways, because it is selectively expressed at high levels by nociceptors, and electrophysiological studies suggest that the P2X receptors in sensory neurons may play an important role in the generation and/or modulation of the pain signaling from the periphery to the spinal cord. Furthermore, we previously reported that P2X3 in peripheralafferents plays a role in the induction of the hyperalgesia to mechanical stimulation observed during peripheral inflammation and many P2X3s are co-expressed with protease-activated receptor 2 in the rat dorsal root ganglion neurons. Nocifensive behaviors induced by αβ-me-ATP injection to the hind paw were significantly augmented after the application of protease-activated receptor 2 agonists. These previous studies led us to behavioral experiments to study whether the LTC4 have a role in potentiation of pain sensation induced by αβ-me-ATP. Intraplantar injection of LTC4 before the αβ-me-ATP injection induced a significant increase of paw-lifting behaviors and Fos expression in the spinal dorsal horn. Based on the finding described in the present study, we concluded that CysLT2, the receptor of LTC4, located in the primary afferent, might modulate the activation of P2X3 by the injection of αβ-me-ATP.

10. CONCLUSION

It has been well known that leukotrienes have a key role in the abnormal pain mechanisms, especially peripheral inflammation, however, few papers have expressed interest in the involvement of spinal lipoxygenase metabolites in hyperalgesic responses. Here, we had a hypothesis that spinal lipoxygenase metabolites might have a role in pathological pain, such as neuropathic pain through glial activation. In the latter half of this review, we reported the novel role of leukotriene, CysLT2 in the primary afferent neurons. We found that the CysLT2 is preferentially expressed by small-sized, non-peptidergic and nociceptive neurons expressing TRPV1 or P2X3 in the DRG. Intraplantar injection of LTC4, a CysLT2 receptor agonist, itself did not induce the thermal hyperalgesia, spontaneous pain behaviors or swelling of hind paw. However, pretreatment of LTC4 remarkably enhanced the painful behaviors produced by αβ-me-ATP, a P2X3 receptor agonist. These data suggest that CysLT2 expressed in DRG neurons may play a role as a modulator of P2X3 and contribute to a potentiation of the neuronal activity following peripheral inflammation.

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