Cellular and molecular mechanisms of calcium/calmodulin-dependent protein kinase II in chronic pain

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Minireview

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Abbreviations:
AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; Aβ-LTMRs: fast conducting low-threshold mechanoreceptors; AIP, autocamtide 2-related inhibitory peptide; AS: antisense; BCP, bone cancer pain; CaMKII,
calcium/calmodulin-dependent protein kinase II; CCI, chronic constriction injury; CeLC, central amygdala; CFA, complete Freund’s adjuvant; CNP, Central neuropathic pain; cPLA2, cytosolic phospholipase A2; CXCL12: C-X-C motif chemokine 12; CXCR4: C-X-C motif chemokine receptor 4; DPN, diabetic peripheral neuropathy; DRG, dorsal root ganglia; HO-2, heme oxygenase type 2; i.c.v., intracerebroventricular injection; i.g., intraganglionic; IL-17A, interleukin 17A; IL-33, interleukin 33; i.p., intraperitoneal; LTP: long-term potentiation; MAP2, microtubule-associated protein 2; NAc, nucleus accumbens; NeuN, neuronal nuclei; NMDA, N-methyl-d-aspartate; NSAID, nonsteroidal anti-inflammatory drugs; ODN: oligodeoxynucleotide; OIH, opioid-induced hyperalgesia; PBN, phenyl-N-tert-butylnitrone; pCaMKII, phosphorylated calcium/calmodulin-dependent protein kinase II; pCREB, phosphorylated cAMP-response element-binding protein; PKA: cAMP-dependent protein kinase; p.o., oral administration; pSNL, partial sciatic nerve ligation; ROS, reactive oxygen species; s.c., subcutaneous injection; SCI, spinal cord injury; SNT, spinal nerve transection; tCaMKII, total calcium/calmodulin-dependent protein kinase II; Y1472F-KI mice, mice lacking phosphorylation of NR2B subunits of NMDA receptors at Tyr1472.
Abstract: Chronic pain, often defined as any pain lasting more than 3 months, is poorly managed due to its multifaceted and complex mechanisms. Calcium/calmodulin-dependent protein kinase II (CaMKII) is a multifunctional serine/threonine kinase, which plays a fundamental role in synaptic plasticity, learning and memory. Recently, emerging evidence demonstrate increased expression and activity of CaMKII in the spinal cord and dorsal root ganglia of various chronic pain models. Moreover, our previous studies also find that inhibiting CaMKII could attenuate inflammatory pain and neuropathic pain. In this review, we provide evidence for the involvement of CaMKII in the initiation and development of chronic pain including neuropathic pain, bone cancer pain and inflammatory pain. Novel CaMKII inhibitors with potent inhibitory effect and high specificity may be alternative therapeutic strategy for the management of chronic pain in the future.
Introduction

Chronic pain represents a major public health concern with a high prevalence ranging from 19% to 50% of the population (van Hecke et al., 2013; Macfarlane, 2016; Zhou et al., 2016b). In addition to significantly affect patients’ quality of life, chronic pain causes a high economic burden (Crown, 2012; Pizzo and Clark, 2012; Zhou et al., 2016a). Currently, nonsteroidal anti-inflammatory drugs (NSAID), opioids and gabapentinoids (pregabalin and gabapentin) remain to be the first-line therapeutics in the treatment of chronic pain (Moulin et al., 2015; Alles and Smith, 2016; Paice et al., 2016). Unfortunately, these conventional drugs often lead to undesirable side effects which eventually limit their use. Despite marked advances in neuroscience research, few new drugs with potent antinociceptive effects and minimal adverse effects have been developed. Therefore, further understanding of the cellular and molecular mechanisms of chronic pain is warranted to discover novel targets for the development of effective analgesic drugs.

Calcium/calmodulin-dependent protein kinase II (CaMKII), a multifunctional serine/threonine kinase, is comprised of 12 subunits, each encoded by one of four genes (α, β, γ and δ) (Rosenberg et al., 2005). Each of these subunits contains a highly conserved N-terminal catalytic domain responsible for enzymatic activity of the kinase, followed by a core regulatory domain, and a C-terminal association domain responsible for assembly of the dodecameric holoenzyme (Figure 1). The regulatory domain of CaMKII contains a calmodulin binding region and various regulatory sites (Lisman et al., 2012; Erickson et al., 2013; Stratton et al., 2013). Under inactive
conditions, the catalytic domain of CaMKII is restrained by the autoinhibitory sequences within the regulatory domain, thus inhibiting the catalytic activity (Griffith, 2004; Coultrap and Bayer, 2012). CaMKII can be activated by binding of calcium/calmodulin, which releases catalytic domain from the inhibitory effects of the regulatory domain (Hund and Mohler, 2015). The activation leads to autophosphorylation of the kinase at the sites Thr286 or Thr287 depending on the specific isoform (Mattiazzi et al., 2015). CaMKII autophosphorylation markedly enhances the binding affinity of calmodulin and blocks the regulatory domain from inhibiting catalysis, thereby generating autonomous kinase activity (Colbran and Brown, 2004). This autonomous activity persists until dephosphorylated by a regulatory phosphatase (Erickson, 2014) (Figure 2).

It is well established that CaMKII plays a fundamental role in synaptic plasticity (Shen et al., 2000; Bejar et al., 2002; Sanhueza et al., 2007; Fukushima et al., 2008). Long-term potentiation (LTP) is a synaptic substrate for memory and learning (Lynch, 2004). There are emerging evidence suggested that LTP can also be induced in sensory pain-related central synapses (e.g., spinal cord dorsal horn) and cortical areas that are important for pain perception (e.g., cingulate cortex, amygdala) (Luo et al., 2014). Moreover, LTP at spinal C-fiber synapses is considered as a synaptic model of pathological pain since the spinal LTP can only induced by noxious stimulus (Liu and Zhou, 2015). Interestingly, although there are differences between the synaptic plasticity contributing to central sensitization and LTP, there are also striking similarities, indicating that pain and memory may share similar mechanisms (Ji et al.,
CaMKII preferentially localized in the superficial laminae of the spinal cord dorsal horn and in the primary sensory neurons in dorsal root ganglia (DRG), which are vital for transmission and processing of nociceptive signals (Bruggemann et al., 2000; Carlton, 2002). Emerging evidence suggests that CaMKII may be a novel therapeutic target for the management of chronic pain. Our lab has been investigating the mechanisms of chronic pain for decades (Tian et al., 2009; Ye et al., 2014; Guan et al., 2015; Song et al., 2016; Sun et al., 2016; Chen et al., 2017). Previously, we have demonstrated that blocking CaMKII attenuated complete Freund’s adjuvant (CFA)-induced inflammatory pain (Luo et al., 2008) and spinal nerve ligation (SNL)-induced neuropathic pain (Chen et al., 2009). Our recent study also found a critical role of CaMKIIα in the laterocapcular division of the central amygdala (CeLC) in opioid-induced hyperalgesia (OIH) (Li et al., 2016). Moreover, there is accumulating evidence demonstrating increased expression and activity of CaMKII in the spinal cord and DRG of various chronic pain models (Liang et al., 2004a; Crown et al., 2012; Ferhatovic et al., 2013a; Hung et al., 2014; Wang et al., 2014). Additionally, CaMKII inhibitors could alleviate pain-related behaviors in a dose-dependent manner in these models. These studies indicated a pivotal role of CaMKII in chronic pain, suggesting that novel analgesic drugs may be developed targeting CaMKII. Therefore, in this review, the evidence for the involvement of CaMKII in chronic pain is discussed.
**CaMKII and neuropathic pain**

Neuropathic pain is defined as pain caused by a lesion or disease of the somatosensory nervous system (www.iasp-pain.org/Taxonomy#Neuropathicpain). Patients with neuropathic pain may suffer from abnormal sensations (paresthesia, e.g., tingling, tickling, pricking, numbness with no apparent physical cause) and pain from normally non-painful stimuli (allodynia) (Treede et al., 2008; Jensen et al., 2011). Many animal models successfully mimic the clinical symptoms of neuropathic pain patients, exhibiting significant mechanical allodynia. The mechanical allodynia in rodent models are often assessed by measuring the withdrawal threshold of the paw ipsilateral to the site of injury in response to mechanical stimuli delivered by von Frey hairs. A positive response was defined as a brisk withdrawal of the hind paw upon stimulation.

**CaMKII and peripheral neuropathic pain**

Peripheral neuropathic pain is caused by damage to the peripheral nerve fibers (Woolf and Salter, 2000; Xu et al., 2008). Numerous animal models have been developed to examine the cellular and molecular mechanisms of chronic pain due to peripheral nervous system injury. Among these models, surgical intervention of sciatic nerve is used most frequently (Bennett and Xie, 1988; Mosconi and Kruger, 1996; Decosterd and Woolf, 2000; Jaggi and Singh, 2011). A growing body of studies have shown the pivotal role of CaMKII in the generation and maintenance of peripheral neuropathic pain. Garry et al. (Garry et al., 2003) first reported that intrathecal injection (i.t.) of a
very low dose of CaMKII inhibitor KN93 120 pmol reversed chronic constriction injury (CCI)-induced peripheral neuropathic pain in mice. The analgesic effect of autocamtide 2-related inhibitory peptide (AIP) was also examined. AIP is a non-phosphorylatable analog of autocamtide-2, which was identified to be a highly specific and potent inhibitor of CaMKII (Ishida et al., 1995). The results showed that AIP 1 nmol significantly suppressed the thermal hyperalgesia and mechanical allodynia in CCI mice. Using a rat model of mononeuropathy, the CCI model, Dai et al. (Dai et al., 2005) explored the time course of activation of CaMKII and the role of CaMKII in the initiation and development of peripheral neuropathic pain. Their immunohistochemistry data demonstrated that the immunoreactivity of total CaMKII (tCaMKII) was remarkably increased in the superficial laminae of the ipsilateral dorsal horn of CCI rats from 3 to 14 days after surgery, while the immunoreactivity of phosphorylated CaMKII (pCaMKII) showed an increase at 1 day after model establishment, which was 2 days before tCaMKII upregulation. Their western blot data verified that the protein level of tCaMKII was upregulated in CCI rats, starting at 3 days after surgery, but not at 1 day after surgery. To further examine the specific cell type that expressed CaMKII, they performed double immunofluorescence staining with anti-neuronal nuclei (NeuN; neuronal nuclei marker) or anti-microtubule-associated protein 2 (MAP2; neuronal dendrites marker). They found that pCaMKII were colocalized with NeuN and MAP2 in the ipsilateral dorsal horn of CCI rats, while it was mainly located in cell bodies in the contralateral dorsal horn. Moreover, they examined the effect of KN93 on the pain-behavior of CCI rats.
They found that intrathecal injection of KN93 before CCI surgery, but not at 7 days after surgery, significantly delayed the development of mechanical allodynia and thermal hyperalgesia in CCI rats. Furthermore, the upregulation of tCaMKII and pCaMKII were significantly attenuated by intrathecal administration of an N-methyl-d-aspartate (NMDA) receptor antagonist MK801 before CCI surgery. Their findings were corroborated by Hasegawa et al. (Hasegawa et al., 2009), who found that the immunoreactivity of pCaMKII was marked increased in the ipsilateral L5 DRG following L5 SNL, but not contralateral L5 DRG. Additionally, treatment with KN93 (10 nmol, i.t.) before L5 SNL surgery significantly attenuated the development of tactile allodynia in SNL. Interestingly, a single injection of KN-93 near L5 DRG at 7 days after SNL surgery also greatly suppressed the tactile alldynia in SNL rats. Besides, pretreatment with KN93 blocked the phosphorylation and translocation of cytosolic phospholipase A2 (cPLA2) in injured DRG neurons, which contributed to the mechanical alldynia after spinal nerve injury (Tsuda et al., 2007).

The results of whether KN93 treatment could reverse peripheral neuropathic pain was controversial, which might be explained by the difference in animal model, route of administration and drug dosage. Considering that these conflicting results might be resolved by observing the degree of CaMKII activity before and after KN93 treatment, we conducted a study to examine the analgesic effect of KN93 (15-45 nmol, i.t.) in SNL mice on day 5, at which time the SNL-induced mechanical allodynia and thermal hyperalgesia were well-established (Chen et al., 2009). Our behavioral results showed that acute intrathecal treatment with KN93 at the dose of 30 and 45 nmol, but not 15
nmol, 2 h before behavioral test was able to reverse the established mechanical allodynia and thermal hyperalgesia. Moreover, KN93 (30, 45 nmol, i.t.) dose-dependently inhibited CaMKII autophosphorylation (pCaMKII), which represents CaMKII activity. To further confirm the role of CaMKII in peripheral neuropathic pain, we tested the analgesic effect of trifluoperazine, a clinically used antipsychotic drug that shows potent inhibitory effect on CaMKII activity. Similar to KN93, intraperitoneal (i.p.) or oral administration (p.o.) of trifluoperazine dose-dependently reversed SNL-induced pain behaviors and the upregulation of pCaMKII. Our results supported a critical role of CaMKII in SNL-induced neuropathic pain and suggested that trifluoperazine may be used for neuropathic pain by targeting CaMKII in clinical setting. In another study, Wang et al. (Wang et al., 2011) examined the antihyperalgesic effect of AIP in a peripheral neuropathic pain model established by partial sciatic nerve ligation (pSNL). Pretreatment with AIP (0.1 nmol, i.t.) considerably delayed the onset of tactile allodynia for 3 days, while postoperative treatment with AIP (0.1 nmol, i.t.) only transiently reversed the developed mechanical allodynia. Moreover, AIP treatment significantly inhibited the protein levels of pCaMKII and phosphorylated cAMP-response element-binding protein (pCREB) in the spinal cord, suggesting that spinal activation of CaMKII participates in CREB phosphorylation during central sensitization processing. The analgesic effect of AIP (3, 6 and 12μg) was also demonstrated via intra-nucleus accumbens (NAc) injection in a periphery neuropathic pain model induced by left common sciatic nerve ligation (Bian and Yu, 2015). Recently, spinal interleukin 33
(IL-33) (Liu et al., 2015) and interleukin 17A (IL-17A) (Yao et al., 2016) were reported to contribute to periphery neuropathic pain via activation of neuronal CaMKII/CREB signaling pathway. The critical role of CaMKII was further proved by Matsumura et al. (Matsumura et al., 2010), who found that knock-in mice lacking phosphorylation of NMDA receptor containing subunit 2B (NR2B) at Tyr1472 (Y1472F-KI mice) failed to exhibit neuropathic pain induced by L5 spinal nerve transection (SNT). Moreover, autophosphorylation of CaMKII at Thr286, but not Thr305, was evidently impaired in Y1472F-KI mice following SNT. This result further demonstrated that autophosphorylation of CaMKII at Thr286 contributed to persistent neuropathic pain state. However, it is worth mentioning that a recent study reported that loss of CaMKII signaling in DRG neurons may contribute to SNL-induced neuropathic pain (Bangaru et al., 2015), which was conflicting with other studies. This inconsistency remains to be elucidated. Nevertheless, increased CaMKII activity was also found in a rat model of oxaliplatin-induced peripheral neuropathic pain, in which KN-93 (50 nmol, i.t.) and trifluoperazine (0.1 and 0.3 mg/kg, p.o.) suppressed both tactile allodynia and increased CaMKII phosphorylation (Shirahama et al., 2012). Similarly, diabetic peripheral neuropathy (DPN) models showed upregulated expression of CaMKII both in the spinal cord and in the DRG (Ferhatovic et al., 2013a; Ferhatovic et al., 2013b; Jelicic Kadic et al., 2013; Jelicic Kadic et al., 2014). Interestingly, Jelicic Kadic et al. (Jelicic Kadic et al., 2013; Jelicic Kadic et al., 2014) found that only intraganglionic (i.g.) injection of CaMKII inhibitors, but not intrathecal injection, could alleviate pain-related behaviors in DPN.
rats.

To sum up, CaMKII, which can be activated by NMDA receptor-mediated Ca\(^{2+}\) influx, is obviously upregulated in animal models of periphery neuropathic pain. Moreover, CaMKII inhibitors considerably alleviated pain-related behaviors in neuropathic pain models. Additionally, IL-33/ST2 signaling and IL-17/IL-17R signaling were demonstrated to contribute to nerve injury-induced neuropathic pain through activating neuronal CaMKII/CREB signaling pathway. These studies provided strong evidence for the essential role of CaMKII in periphery neuropathic pain, suggesting that CaMKII may be a novel therapeutic target for the management of peripheral neuropathic pain.

**CaMKII and central neuropathic pain**

Central neuropathic pain (CNP) refers to pain initiated or caused by a primary lesion or dysfunction in the central nervous system (1986; Hulsebosch et al., 2009; Han et al., 2015). Multiple diseases may lead to CNP including spinal cord injury (SCI), multiple sclerosis and stroke (Siddall and Loeser, 2001; Osterberg et al., 2005; Frese et al., 2006). Currently, various rodent models are established to investigate the initiation and maintenance of CNP after SCI, such as spinal contusion injury (Basso et al., 1996; Hulsebosch et al., 2000), spinal hemisection injury (Gwak et al., 2009; Martini et al., 2016) and intrathecal injection of quisqualic acid (Yezierski et al., 1993; Yezierski et al., 1998). One of the mechanism of CNP is neuronal hyperexcitability which may in part caused by imbalanced neurotransmitter release (e.g. glutamate) (Gray, 2007). It is
well-established that enhanced glutamate release contributes to neuropathic pain (Osikowicz et al., 2013). Interruption of the calcium influx may lead to reduced glutamate release, thus alleviating CNP.

Using a rat model of CNP established by a contusion injury at spinal level T10, Crown et al. (Crown et al., 2012) provided the first converging evidence that chronically activation of CaMKII contributed to CNP after SCI. They found that the expression of pCaMKII was markedly increased in the T7/8 spinal dorsal horn of SCI rats in neurons, but not glial cells. Compared with sham rats, SCI rats showed considerably greater neuronal activity without stimulation and to brush, press, pinch and mechanical stimuli. Moreover, intrathecal administration of KN-93 dose-dependently reversed the mechanical allodynia in SCI rats. Most importantly, KN-93 not only significantly decreased the background rate of neuronal firing in SCI rats, but also decreased the neuronal responses to brush, press, pinch and mechanical stimuli. These data suggested that CaMKII phosphorylation plays a pivotal role in neuronal membrane hyperexcitability under SCI conditions. In another study, Gwak et al. (Gwak et al., 2013) reported that SCI-induced overproduction of reactive oxygen species (ROS) may contribute to the activation of CaMKII, which leads to CNP following T10 spinal contusion injury. Their results showed that treatment with phenyl-N-tert-butyl nitrate (PBN, a ROS scavenger) significantly attenuated mechanical allodynia and dorsal horn hyperexcitability in SCI rats. The upregulated expression of pCaMKII was also suppressed by PBN treatment. Furthermore, naïve rats treated with t-BOOH (a ROS donor) showed significantly decreased paw
withdrawal threshold (a sign of mechanical allodynia) and increased expression of pCaMKII, indicating that ROS may contribute to CPN via activation of CaMKII.

Taken together, increased activity of CaMKII in the spinal cord was detected under CPN situation. The enhanced expression of pCaMKII may be the result of spinal cord injury-induced increased production of ROS, as ROS scavengers suppress the upregulation of pCaMKII in CNP rats and ROS donors lead to increased expression of pCaMKII in naive rats. Therefore, targeting CaMKII may alleviate CNP.

**CaMKII and bone cancer pain**

75% of advanced cancer patients suffer from severe pain due to bone metastasis, which significantly affect their quality of life (Costantini et al., 2009; Zhou et al., 2015; Fu et al., 2016). Currently, the role of CaMKII in bone cancer pain (BCP) remains largely unknown. KIF17 is a member of the kinesin superfamily motor protein, which plays a critical role in the dendritic transport of NR2B (Hirokawa and Takemura, 2004). Using a mice model of BCP established by intramedullary injection of osteosarcoma cells, Liu et al. (Liu et al., 2014) reported that the protein levels of pCaMKII, NR2B and KIF17 were significantly upregulated in BCP mice. Moreover, intrathecal injection of KN93 obviously alleviated BCP in a time- and dose-dependent manner and suppressed the upregulation of pCaMKII, NR2B and KIF17, indicating an important role of CaMKII-mediated KIF17/NR2B trafficking in the development of BCP. A very recent study provided various lines of evidence demonstrated that chemokine receptor CXCR4 contributed to the development of BCP via activating
neuroal CaMKII/CREB signaling pathway (Hu et al., 2017). Their western blot and immunochemistry results showed upregulated expression of pCaMKII and pCREB in the spinal cord neurons in BCP rats. Moreover, intrathecal injection of CaMKII specific inhibitor AIP suppressed mechanical allodynia and thermal hyperalgesia and upregulation of p-CREB in BCP rats. Interestingly, intrathecal injection of CXCR4 siRNA inhibited the upregulated expression of both p-CaMKII and p-CREB in BCP rats, thus exhibiting analgesic effect. To further understanding the role of CaMKII/CREB signaling pathway in CXCR4-mediated BCP, they intrathecal injection of stromal-derived factor-1 (SDF-1), a principal ligand for CXCR4 into naive rats. They found that both p-CaMKII and p-CREB expression levels were upregulated after SDF-1 injection, which was prevented by post-treatment with CXCR4 inhibitor Plerixafor. Taken together, these results provided strong evidence that CaMKII/CREB signaling pathway may be a critical downstream pathway of CXCR4 under BCP situation, indicating that suppressing the activation of CaMKII/CREB signaling pathway may be an alternative therapeutic strategy for the management of BCP. In addition, our recent study also confirmed the role of CREB under BCP condition (Zhou et al., 2017).

**CaMKII and inflammatory pain**

Inflammatory pain is associated with tissue injury-induced hyperexcitability of peripheral nociceptive sensory neurons (Ji, 2004). Numerous animal models are established to investigate the mechanisms of inflammatory pain including intraplantar
injection of formalin, capsaicin, carrageenan and CFA (Jeske, 2015). Fang et al. (Fang et al., 2002) provided the first evidence that CaMKII contributes to spinal cord central sensitization of nociceptive dorsal horn neurons after intradermal capsaicin injection. Their western blot results showed that the protein level of CaMKII significantly increased by 15 min and pCaMKII significantly increased by 5 min following the intradermal injection of capsaicin. Moreover, the increased expression of CaMKII and pCaMKII were only detected in the ipsilateral part of the spinal cord, but not in the contralateral side. The immunochemistry data confirmed that the expression of CaMKII and pCaMKII were increased in the superficial laminae of the spinal cord dorsal horn ipsilateral to the capsaicin injection site. Additionally, treatment with KN-93 (100 μM) considerably blocked the capsaicin injection-induced increases in background activity and in the responses of nociceptive dorsal horn neurons, indicating that these electrophysiological responses are CaMKII-dependent. Intrathecal injection of KN-93 also prevented capsaicin injection-induced reduction of the number of entries and traveled distance and increase in the resting time. Moreover, carrageenan injection-induced upregulation of phosphorylation of AMPA receptors was significantly blocked by intrathecal injection of KN-93, indicating that CaMKII directly regulates the phosphorylation state of AMPA receptors during central sensitization. These results provided several lines of evidence demonstrating that CaMKII plays a vital role in the intracellular signal transduction pathways that cause central sensitization after intradermal capsaicin injection. As mentioned above, CaMKII has four isoforms, α, β, γ and δ. Although KN-93 is a selective CaMKII
inhibitor, it cannot differentiate the contribution of calcium-dependent CaMKII activity from its autonomous activity. Therefore, Zeitz et al. (Zeitz et al., 2004) conducted a study to determine whether CaMKIIα contributed injury-induced inflammation and pain using autophosphorylation (T286A) mutant mice which are unable to autophosphorylate. No difference was found between wild-type and CaMKIIα T286A mutant mice regarding acute nociception, including thermal nociception, mechanical nociceptive thresholds, and chemical nociception. Similarly, first phase formalin behavior between wild-type and mutant mice showed no difference. It is worth mentioning that the first phase behavior provides a measure of acute chemical pain due to direct activation of primary afferent nociceptors, while the second phase behavior represents ongoing and spontaneous pain owing to central sensitization (Tjolsen et al., 1992). However, pain behaviors-induced by intraplantar injection of formalin during the second phase were considerably suppressed in the mutant mice. These results suggested that phosphorylation of CaMKIIα at position 286 (threonine) plays a fundamental role in generating the ongoing/spontaneous pain behaviors during the second phase following intraplantar formalin injection, but that this phosphorylation do not affect the acute pain behaviors. In another study, Liang et al. (Liang et al., 2004b) demonstrated that the expression of spinal CaMKIIα was almost unchanged after hindpaw formalin injection in heme oxygenase type 2 (HO-2) null mutant mice, indicating that CaMKII activity is modulated by HO-2. Increased CaMKII activity was also found in the mouse hippocampus after subcutaneous injection (s.c.) of formalin and intracerebroventricular injection (i.c.v.) of KN-93
alleviated formalin-induced pain behaviors (Seo et al., 2008). Moreover, our previous study also examined the analgesic effect of CaMKII inhibitors on CFA-induced inflammatory pain (Luo et al., 2008). We found that pretreatment with KN-93 (30 nmol, i.t.) prevented CFA-induced mechanical allodynia and thermal hyperalgesia and acute inhibition of CaMKII (KN-93 45 nmol, i.t.) reversed established CFA-induced pain behaviors in mice. Additionally, trifluoperazine also alleviated CFA-induced inflammatory pain by suppressing the activity of CaMKII. Recently, clonidine, a α2 noradrenergic receptor agonists, was demonstrated to attenuate inflammatory pain induced by intraplantar injection of CFA via suppressing the autophosphorylation of CaMKII at Threonine 286 in a cAMP-dependent protein kinase (PKA) -dependent manner. Taken together, these studies proved the critical role of CaMKII activation in the initiation and development of inflammatory pain.

Conclusions

In this review, we discussed the cellular and molecular mechanisms of CaMKII in the initiation and development of chronic pain including neuropathic pain, BCP and inflammatory pain. The role of CaMKII under neuropathic pain situation has been extensively studied (Figure 3). IL-33/ST2 signaling and IL-17/IL-17R signaling were demonstrated to contribute to nerve injury–induced neuropathic pain through activating neuronal CaMKII/CREB signaling pathway. Increased activity of CaMKII in the spinal cord was detected under CPN situation. The enhanced expression of pCaMKII may be the result of spinal cord injury–induced increased production of
ROS, as ROS scavengers suppress the upregulation of pCaMKII in CNP rats and ROS donors lead to increased expression of pCaMKII in naive rats. Under BCP situation, CaMKII/CREB signaling pathway may be a critical downstream pathway of CXCR4, as CXCR4 inhibitor could attenuate BCP-related pain behaviors by suppressing the phosphorylation of CaMKII and CREB (Figure 4). Moreover, selective CaMKII inhibitor AIP also attenuated BCP, indicating that suppressing the activation of CaMKII/CREB signaling pathway may be an alternative therapeutic strategy for the management of BCP. Currently, there are only a few studies investigating the role of CaMKII in inflammatory pain (Figure 5). It was reported that CaMKII phosphorylation was enhanced after intra-plantar injection of CFA, which was abolished by α2 noradrenergic receptor agonists and PKA inhibitior. Moreover, HO-2 null mutant mice show no significant change in CaMKIIα mRNA expression after formalin injection. However, further studies are warranted to investigate the detailed mechanisms.

It is worth mentioning that CaMKIIα may also plays a fundamental role in hyperalgesic priming, a phenomenon implicated in the transition from acute to chronic pain (Ferrari et al., 2013). Intradermal injection of PKCε agonist ψεRACK could induce hyperalgesic priming, which was prevented by intrathecal administration of αCaMKII oligodeoxynucleotide (ODN) antisense (AS) combined with local inhibition of CaMKII inhibitor CaM2INtide. Additionally, intradermal injection of activated αCaMKII on the dorsum of the hindpaw produced hyperalgesia, which was not prevented by pretreatment with PKCε AS, indicating that PKCε is upstream of
αCaMKII in the induction of priming. Moreover, intradermal injection of ryanodine-induced hyperalgesic priming was also prevented by intrathecal administration of αCaMKII ODN AS combined with CaM2INtide, suggesting that the priming induced by ryanodine is dependent on αCaMKII activation. These results demonstrated an indispensable role αCaMKII in the induction of hyperalgesic priming. Interestingly, a recent study reported that CaMKII may control whether touch is painful (Yu et al., 2015). The sensation of touch is initiated when impulses at the terminals in the skin was generated by specialized sensory neurons termed fast conducting low-threshold mechanoreceptors (Aβ-LTMRs) (Abraira and Ginty, 2013).

It was shown that the flow of sensory information in Aβ-LTMR sensory neurons (e.g., impulse generation, AP propagation, and dorsal horn synaptic transmission) was regulated by CaMKII (Yu et al., 2015). Moreover, loss of CaMKII signaling in sensory neurons may contribute to neuronal dysfunction and pain, indicating a vital role of CaMKII in the transition of touch pathway to pain system (Bangaru et al., 2015; Yu et al., 2015).

In summary, treatment with CaMKII inhibitors could attenuate chronic pain-induced mechanical allodynia and thermal hyperalgesia in rodent models. Currently, the most commonly used CaMKII inhibitors in animal experiments are KN-93, KN-62 and AIP. Our previous study also demonstrated a potent inhibitory effect on CaMKII activity of trifluoperazine, a clinically used antipsychotic drug. However, no CaMKII inhibitors have been tested in clinical trials yet due to their absence of highly specific inhibition. Therefore, novel CaMKII inhibitors with potent inhibitory effect and high specificity
should be developed in the future researches. Additionally, further studies are warranted to investigate the intensive mechanisms of how activation of CaMKII contributed to chronic pain.
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Footnote

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Figure legends

Figure 1. Schematic representation of calcium/calmodulin-dependent protein kinase II (CaMKII) structure. (A) CaMKII monomer contains a highly conserved N-terminal catalytic domain responsible for enzymatic activity of the kinase, followed by a core regulatory domain, and a C-terminal association domain responsible for assembly of the dodecameric holoenzyme. (B) CaMKII holoenzyme is comprised of 12 subunits.

Figure 2. Schematic representation of the regulation of calmodulin-dependent protein kinase II (CaMKII) activity. Under inactive conditions, the catalytic domain of CaMKII is restrained by the autoinhibitory sequences within the regulatory domain, thus inhibiting the catalytic activity. CaMKII can be activated by binding of calcium/calmodulin, which releases catalytic domain from the inhibitory effects of the regulatory domain. The activation leads to autophosphorylation of the kinase at the sites Thr286 or Thr287 depending on the specific isoform. CaMKII autophosphorylation markedly enhances the binding affinity of calmodulin and blocks the regulatory domain from inhibiting catalysis, thereby generating autonomous kinase activity. This autonomous activity persists until dephosphorylated by a regulatory phosphatase.

Figure 3. Schematic representation of possible mechanisms of calmodulin-dependent protein kinase II (CaMKII) in the processing of
neuropathic pain. IL-33/ST2 signaling and IL-17/IL-17R signaling were demonstrated to contribute to nerve injury–induced neuropathic pain through activating neuronal CaMKII/CREB signaling pathway. Increased activity of CaMKII in the spinal cord was detected under CPN situation. The enhanced expression of pCaMKII may be the result of spinal cord injury–induced increased production of ROS, as ROS scavengers suppress the upregulation of pCaMKII in CNP rats and ROS donors lead to increased expression of pCaMKII in naive rats. CREB: cAMP-response element-binding protein; IL-17: interleukin 17; IL-17R: interleukin 17 receptor; IL-33: interleukin 33; NMDA: N-methyl-D-aspartic acid; ROS, reactive oxygen species; ST2: interleukin 33 receptor.

Figure 4. Schematic representation of possible mechanisms of calmodulin-dependent protein kinase II (CaMKII) in the processing of bone cancer pain. Under BCP situation, CaMKII/CREB signaling pathway may be a critical downstream pathway of CXCR4, as CXCR4 inhibitor could attenuate BCP-related pain behaviors by suppressing the phosphorylation of CaMKII and CREB. Moreover, selective CaMKII inhibitor AIP also attenuated BCP, indicating that suppressing the activation of CaMKII/CREB signaling pathway may be an alternative therapeutic strategy for the management of BCP. CREB: cAMP-response element-binding protein; CXCL12: C-X-C motif chemokine 12; CXCR4: C-X-C motif chemokine receptor 4.
Figure 5. Schematic representation of possible mechanisms of calmodulin-dependent protein kinase II (CaMKII) in the processing of inflammatory pain. It was reported that CaMKII phosphorylation was enhanced intra-plantar injection of CFA, which was abolished by α2 noradrenergic receptor agonists and PKA inhibitor. Moreover, HO-2 null mutant mice show no significant change in CaMKIIα mRNA expression after formalin injection. AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; α2 NAR: α2 noradrenergic receptor; HO-2, heme oxygenase type 2; PKA: cAMP-dependent protein kinase.
Figure 1
Figure 3

Neuropathic pain
Figure 4
Figure 5

- α2 NAR → PKA
- AMPA Receptor → CaMKII
- HO-2

CaMKII → Nucleus → Inflammatory pain