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Molecular Roles of Cdk5 in Pain Signaling

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Abstract

Injury and inflammation trigger activation of several critical cellular pathways in nociceptive signaling in the peripheral nervous system, but their precise molecular mechanisms have not been clearly defined. Cyclin-dependent kinase 5 (Cdk5), a serine/threonine kinase, is mainly expressed in the post-mitotic neurons, and has many important roles in the development, functions and pathophysiology of diseases of the nervous system. Although many functional roles of Cdk5 have been identified in neurons, its precise role in pain signaling has not been well determined. Experimental inflammation in the hind paws of mice resulted in increased mRNA and protein levels of Cdk5 and its activator p35, as well as the Cdk5 activity in nociceptive neurons (Pareek et al., 2006). Furthermore, we also identified that Cdk5 phosphorylates transient receptor potential vanilloid 1 (TRPV1), a key receptor that modulates agonist-induced calcium influx in the neurons (Pareek et al., 2007). We subsequently demonstrated that inflammation triggers increase in Cdk5 activity through activation of early growth response 1 (Egr-1) and p35 expression by tumor necrosis factor alpha (TNF- α) (Utreras et al., 2009). These findings suggest that Cdk5 plays an important role in pain signaling and therefore Cdk5 and its activators are potentially important drug targets for development of novel analgesics to treat neuropathic pain.

Keywords

Pain; nociception; Cdk5; phosphorylation; TNF- α ; analgesic

1. Introduction

The sensation of pain, or nociception, is a critical factor in host defense mechanism during tissue injury and inflammation. It is initiated by activation of primary afferent nerve endings that trigger neuropathic pain. Although the mechanisms underlying neuropathic pain are not well understood, there is sufficient evidence to suggest that protein kinases may contribute to initiation of pain signaling. Repeated or prolonged noxious stimulation following nerve injury activates a number of intracellular second messenger systems, characterized by phosphorylation through the participation of protein kinases. These include mitogen-activated protein kinase (MAPK) (Ji R, 2004; Ma and Quirion, 2005; Ji et al., 2007), protein kinase C (PKC) (Zimmermann M, 2001; Velazquez et al., 2007), calcium/calmodulin kinase II (CaMK-II) (Lou et al., 2008), protein kinase B (PKB), phosphatidylinositol 3-kinase (PI3K) (Xu et al., 2007), protein kinase A (PKA) (Aley and Levine, 1999), and more

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recently cyclin-dependent kinase 5 (Cdk5) (Pareek et al., 2006; Pareek and Kulkarni, 2006; Pareek et al., 2007; Saikkonen et al., 2008; Pareek and Kulkarni, 2008; Utreras et al., 2009).

Cdk5, a proline-directed serine/threonine protein kinase that belongs to the family of cyclin-dependent kinases, is expressed in all tissues. But in order to be a active kinase, it needs to bind to p35 or p39, mainly neuron-specific activators of Cdk5.

This review focuses on: 1) the role of protein kinases in pain signaling; 2) the role of Cdk5 in pain; 3) potential downstream targets modulated by Cdk5; and 4) Cdk5 and its activators as therapeutic targets to treat pain.

2. Role of protein kinases in pain signaling

The human genome encodes over 500 different protein kinases, representing one of the largest protein families (Manning et al., 2002). These kinases regulate almost every signal transduction in eukaryotic cells, and also control many cellular processes such as metabolism, transcription, cell cycle, differentiation and movement, and apoptosis. Although protein kinases are not usually preferred targets, recent studies have demonstrated important roles of these kinases in regulating peripheral and central pain sensitization. They are activated in primary sensory and dorsal horn neurons by nociceptive activity, growth factors, and inflammatory mediators. This contributes to the induction and maintenance of pain sensitization via post-translational, translational, and transcriptional regulation. It is well established that MAPKs participate in both peripheral and central mechanisms of pain sensitization. Other protein kinases such as PKC, CaMK-II, PKA, PKB, PI3K, and Cdk5 are also involved in the regulation of pain.

2.1. MAPKs and pain signaling

The role of MAPKs in pain signaling has been reviewed in detail (Obata and Noguchi, 2004; Ji, 2004; Ma and Quirion, 2005; Ji et al., 2006). The MAPK is a family of serine/threonine protein kinases that is activated by several extracellular stimuli, including cellular stress (ultraviolet rays, osmotic and heat shock), lipopolysaccharide, and proinflammatory cytokines such as tumor necrosis factor alpha (TNF- α) and interleukin 1- β (IL-1 β) (Ji R, 2004). The MAPK family includes extracellular signal-regulated kinase 1/2 (ERK1/2), p38, c-Jun N-terminal kinase (JNK), and ERK5. Each are activated by inflammatory mediators in sensory neurons, and participate in the generation and maintenance of inflammatory pain (Ji, 2004; Ma and Quirion, 2005; Ji et al., 2006). The activation of ERK1/2 contributes to nociceptive responses in the dorsal horn and DRG following inflammation or nerve injury (Obata and Noguchi, 2004). Studies on animal models of neuropathic pain have revealed a rapid phosphorylation of ERK after nerve injury (Ma and Quirion, 2005). Other MAPK members, such as p38 and JNK, have also been implicated in pain (Ji and Suter, 2007; Gao and Ji, 2008). P38 MAPK is activated in spinal microglia after nerve injury, and contributes significantly to development and maintenance of neuropathic pain (Ji and Sutter, 2007). In addition, JNK activation has been clearly implicated in inflammatory responses and is activated in astrocytes of the spinal cord after nerve injury. This activation can maintain central sensitization and mechanical allodynia (Gao and Ji, 2008).

2.2. Other protein kinases and pain signaling

The molecular role of PKC in pain signaling has been recently reviewed in detail (Velazquez et al., 2007). PKC is a family of serine/threonine kinases composed of different isozymes expressed in brain and primary afferent neurons, that transmit nociceptive signals from the peripheral site of injury to the superficial layers of the dorsal horn of the spinal cord. Inflammatory mediators produced by tissue damage can activate and sensitize primary afferents through activation of PKC isozymes. For example, it has been proven that PKC ϵ ,

which modulates nociception through activation of TRPV1 is important in peripheral sensitization (Cesare et al., 1999). In contrast, another isozyme, PKC γ , is involved in central sensitization (Malmberg et al., 1997). In conclusion, different PKC isozymes are important at different levels in sensitization processes (Velazquez et al., 2007).

CaMK-II plays a diverse role in virtually all cell types. It is regulated by local changes in calcium ion concentration that induce calmodulin binding and subsequent activation (Colbran, 2004). CaMK-II regulates synaptic transmission by phosphorylating various proteins, including neuronal membrane receptors and intracellular transcription factors. The expression and activity of CaMK-II were enhanced in rat spinal cord neurons after intradermal injection of capsaicin, an agonist of TRPV1 (Fang et al., 2002). In addition, intraplantar injection of Complete Freund's Adjuvant produced an increase in spinal activity of CaMK-II, which was blocked by a CaMK-II inhibitor, indicating a critical role of CaMK-II in inflammatory pain (Luo et al., 2008).

PKA is typically activated by cyclic AMP (cAMP), and has been strongly implicated in peripheral and central sensitization. PKA mediates sensitization of the peripheral nociceptor terminals induced by prostaglandin E₂ (PGE₂), a major inflammatory mediator (Aley and Levine, 1999). In addition, PKA is also required in central sensitization (Sluka and Willis, 1997).

Recent evidence also suggests that PKB and PI3K are involved in pain hypersensitivity (Zhuang et al., 2004; Xu et al., 2007). Intradermal injection of capsaicin and NGF produces heat hypersensitivity by activating TRPV1, and this phenomenon is mediated in part by PI3K activation and subsequent PKB phosphorylation (Zhuang et al., 2004). In addition, spinal nerve ligation in rats induced activation of PI3K and PKB in dorsal root ganglion (DRG) and spinal cord (SC), contributing to neuropathic pain (Xu et al., 2008).

3. The role of Cdk5 in pain signaling

Since the discovery of Cdk5 more than 15 years ago, several important functions have been assigned to this unique kinase, establishing it as a key regulator of neuronal functions. These functions include cell differentiation, neuronal migration, neurotransmitter release, neuronal plasticity, addiction to drugs, apoptosis, learning, memory and pain (Dhavan and Tsai, 2001; Dhariwala and Rajadhyaksha, 2008; Pareek and Kulkarni, 2008). Although the expression and activity of Cdk5 in the peripheral nervous system (PNS) had been reported earlier (Ino et al. 1994; Terada et al., 1998), the precise molecular functions of Cdk5 in pain signaling have not yet been fully characterized. Wang et al (2004) were the first to demonstrate that roscovitine, a inhibitor of Cdk5, injected intrathecally in rats produced an antinociceptive effect. Additionally, intrathecal administration of roscovitine inhibited Cdk5 activity and attenuated formalin-induced nociceptive responses in rats (Wang et al., 2005).

3.1. Direct evidence of Cdk5 in pain signaling

We have recently identified Cdk5 as an important kinase in sensory pathways (Pareek et al., 2006). We first demonstrated that Cdk5 and p35 are expressed in DRG, SC, and trigeminal ganglion (TG) and that Cdk5 activity is increased during a peripheral inflammatory response (Pareek et al., 2006). Cdk5 and p35 protein levels were increased after inflammation induced by injection of carrageenan in the plantar region of the hind paws of rats, with subsequent activation of calpain, a calcium-dependent protease that catalyzes the hydrolysis of p35 to p25 and p10. This activation resulted in much higher levels of p25 which triggered increased Cdk5 activity in DRG, TG and SC (Pareek et al., 2006). We also showed that p35 knockout mice (p35^{-/-}) with significantly decreased Cdk5 activity had delayed responses to painful thermal stimulation, as compared with control mice. In contrast, mice

overexpressing p35 (Tgp35), which exhibit elevated levels of Cdk5 activity, were more sensitive to painful thermal stimuli than the corresponding controls (Pareek et al., 2006). In agreement with our findings, Yang et al (2007) also found that Cdk5 and p35 are expressed in primary sensory and dorsal horn neurons, that Cdk5 activity was increased after CFA injection, and that Cdk5 activity was associated with hyperalgesia to heat but not with mechanical allodynia. Recently, Peng et al (2009) found that Cdk5 was also involved in cross-organ reflex sensitization, and that colon irritation caused an increase in Cdk5 and p25 protein expression in spinal cord and DRG. These findings indicate important molecular roles for Cdk5 in pain signaling, that make it a potential target for the development of a new class of analgesics.

Transient receptor potential vanilloid 1 (TRPV1), a ligand-gated cation channel highly expressed in small diameter sensory neurons, is activated by heat, protons, and capsaicin. The phosphorylation of TRPV1 is a versatile regulation of intracellular calcium levels and is critical for TRPV1 function in responding to a pain stimulus. Our findings suggest that Cdk5-mediated phosphorylation of TRPV1 at threonine 407 can modulate agonist-induced calcium influx. Inhibition of Cdk5 activity in cultured DRG neurons resulted in a significant reduction of TRPV1-mediated calcium influx. This effect could be reversed by restoring Cdk5 activity. Primary nociceptor-specific Cdk5 conditional knockout mice showed reduced TRPV1 phosphorylation, resulting in significant hypoalgesia. Therefore, these findings confirm that Cdk5-mediated TRPV1 phosphorylation is important in the regulation of pain signaling.

3.2. Implication of Cdk5 in morphine tolerance

The involvement of Cdk5 in tolerance to opioids was first suggested by a report of decreased Cdk5 and p35 levels in the prefrontal cortex of postmortem human brains of opioid addicts (Ferrel-Alcon et al., 2003). These studies also revealed that chronic morphine treatment of rats induced a decrease in Cdk5 and p35 levels associated with abnormal phosphorylation of neurofilament-H. This suggests that opiate addiction may have significant consequences in the development of neural plasticity in humans. Moreover, Wang et al (2004) found that intrathecal co-administration of roscovitine with morphine enhanced the morphine's antinociceptive effect in tolerant rats. Intrathecal administration of roscovitine inhibited Cdk5 activity and attenuated formalin-induced nociceptive responses (Wang et al., 2005). Furthermore, Bhat et al (2004) found that chronic administration of morphine to pregnant rats decreased Cdk5 activity in the brain, suggesting that alterations in Cdk5 may play a role in some of the neural and behavioral effects produced by this treatment (Bhat et al., 2006). Inhibitors of Cdk5 and glycogen synthase kinase 3 beta (GSK3 β) are reported to abolish tolerance to morphine analgesia, suggesting the involvement of Cdk5 and GSK3 β in opioid antinociception and tolerance (Parkitna et al., 2006).

We recently found a differential nociceptive response after chronic morphine exposure in p35^{-/-} and Tgp35 mice, suggesting that Cdk5 activity is important for opioid tolerance (Pareek and Kulkarni, 2006). Perhaps Cdk5 regulates MEK1 activity through a negative loop during the peripheral inflammatory response (Pareek and Kulkarni, 2006). In contrast, Contet et al (2008) found that the expression of Cdk5 does not change in the caudate-putamen, nucleus accumbens, or the prefrontal cortex after chronic treatment with morphine in mice. This suggests there may be an alternate molecular mechanism that underlies analgesic tolerance induced by chronic morphine administration.

3.3. Regulation of Cdk5 during inflammation

A range of inflammatory mediators such as neurotrophins, prostaglandins, bradykinin, and proinflammatory cytokines are released during tissue injury and consequent inflammatory

response. These mediators increase the sensitivity of sensory neurons to noxious thermal or mechanical stimuli, enhancing the activity of nociceptive receptor and ion channels (Huang et al. 2006, Cheng and Ji, 2008). Some of these inflammatory mediators have been reported as regulators of Cdk5 activity (Tokuoka et al. 2000; Harada et al., 2001; Quintanilla et al., 2004; Song 2005; Bogen et al. 2008; Ojala et al. 2008; Utreras et al., 2009) (see Figure 1).

Neurotrophins are a family of proteins that play critical role in the development of the nervous system, and there is increasing evidence of their involvement in pain signaling (Mendell et al. 1999). Members of the neurotrophin family, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), neurotrophin-4/5 (NT-4/5), and glial cell-line derived neurotrophic factor (GDNF), have been implicated in peripheral and central sensitization of nociceptors (Mendell et al., 1999). NGF, BDNF, and GDNF can also regulate Cdk5 activity (Tokuoka et al., 2000; Harada et al., 2001; Bogen et al. 2008). NGF, through activation of the ERK pathway, induces sustained and strong expression of p35, as well as the subsequent increase of Cdk5 activity in PC12 cells (Harada et al., 2001). Moreover, BDNF also induces p35 expression and Cdk5 activity in PC12 cells (Harada et al., 2001), and increases Cdk5 activity in primary cultures of rat embryo cortical neurons (Tokuoka et al., 2000). GDNF has been shown to induce Cdk5 activity in motor neurons (Ledda et al., 2002). GDNF-induced hyperalgesia was reported to be mediated in part by Cdk5 (Bogen et al., 2008).

The role of bradykinin in pain signaling is generally well accepted (Wang et al., 2006), and may be linked with Cdk5. Bradykinin enhances NMDA receptor activity in the spinal cord by activating ERK to produce pain hypersensitivity (Kohno et al., 2008). We found that Cdk5 can phosphorylate NMDA receptors in hippocampal neurons (Li et al., 2001). This suggests that bradykinin activates ERK to regulate Cdk5 activity, inducing phosphorylation of NMDA receptors and subsequent pain hypersensitivity. Prostaglandins, including PGE₂, are known to exert a critical role in the generation and maintenance of the nociceptive response (Samad et al., 2002) however their interactions with Cdk5 are not known. Intraplantar injection of PGE₂ into the mouse paw causes nociceptive behavior and mechanical allodynia mediated through activation of prostaglandin receptor and MAPK (Kassuya et al., 2007), but the possible relationship with Cdk5 still needs to be investigated further.

Several cytokines are known to mediate chronic pain caused by inflammation or sensory nerve damage (Myers et al., 2006). In animal models of inflammatory or neuropathic pain, the mRNA and protein levels of interleukin-6 (IL-6), IL-1 β , TNF- α are elevated in the spinal cord, as well as at the site of injury (Safieh-Garabedian et al. 1995; Arruda et al., 1998; Schafers et al., 2003; Loram et al., 2007; Yang et al., 2007). Accumulating evidence suggests that cytokines can regulate Cdk5 activity (Quintanilla et al., 2004; Song et al., 2005; Ojala et al., 2008; Utreras et al., 2008). IL-6 induced an increase in Cdk5 activity in the hippocampal neurons of mice, and this effect was mediated by activation of ERK. Increase in the expression of early growth response gene 1 (Egr-1), a transcription factor that induces p35 protein expression, has been also reported (Quintanilla et al., 2004). Interferon gamma (IFN- γ) has been shown to increase Cdk5 activity in Paju cells through the same mechanism (ERK-Egr-1-p35) (Song et al., 2005). Apparently, the activation of the ERK and increased Egr-1 is a component of a canonical pathway of the regulation of Cdk5 activity (Harada et al., 2001; Quintanilla et al., 2004; Song et al., 2005; Utreras et al., 2009). IL-18 also has been reported to increase Cdk5 and p35 protein levels in SH-SY5Y neuroblastoma cells (Ojala et al., 2008). In addition, we recently demonstrated the role of TNF- α in the regulation of Cdk5/p35 expression (Utreras et al., 2009). We discovered that TNF- α induces sustained and robust expression of p35 in PC12 cells through activation of the ERK1/2 pathway, thereby increasing Cdk5 kinase activity. The activation of ERK1/2 by TNF- α leads

to an increase of Egr-1 expression and subsequent elevation of p35 expression (Utreras et al., 2009). Moreover, we found in a mouse model of inflammation, that the injection of carrageenan in a mouse paw induced an increase in TNF- α , Egr-1 and p35 mRNA. These studies and our previous report, in which carrageenan-induced inflammation in rats increased Cdk5 activity in DRGs (Pareek et al., 2006), suggest that TNF- α increases Cdk5 activity during inflammation-induced pain through induction of Egr-1 and p35 expression (Utreras et al., 2009).

4. Possible downstream target genes and proteins modulated by Cdk5

Several known substrates and proteins that interact with Cdk5 have been linked to nociceptive pathways, including MAPK (ERK, p38 and JNK), CaMK-II, and ion channels (NMDA receptors, P/Q-type voltage-dependent calcium channel [VDCC], and TRPV1), suggesting that Cdk5 may be involved directly and indirectly in nociception (Figure 1).

Activation of MAPK has been involved in pain hypersensitivity (Ji R, 2004; Malik-Hall et al., 2005; Ma and Quirion, 2005; Ji et al., 2006), and Cdk5 has been involved in regulation of MAPK pathways (Sharma et al., 2002; Li et al., 2003; Otth et al., 2003; Zheng et al., 2007). Phosphorylation of MEK1 by Cdk5 down-regulates the MAPK pathway (Sharma et al., 2002), and this modulation may also be implicated in the regulation of neuronal survival (Zheng et al., 2007). Moreover, Cdk5 prevents neuronal apoptosis by negative regulation of JNK3 (Li et al., 2003). In addition, Cdk5 can modulate JNK and p38 pathways in a transgenic mouse model of Alzheimer's disease (Otth et al., 2003). On the other hand, CaMK-II plays a key role in nociceptive transmission (Fang et al., 2002; Luo et al. 2008), and Cdk5 activators p35 and p39 interact with CaMK-II in a calcium-dependent manner (Dhavan et al., 2002). This interaction may possibly inhibit the activation of CaMK-II by auto-phosphorylation (Hosokawa et al., 2006).

Ion channels such as the NMDA receptor (NMDAR), P/Q-type VDCC and TRPV1 play a vital role in peripheral and central sensitization control in pain pathways (Petrenko et al., 2003; Gribkoff VK, 2006; Szallasi et al., 2007; Wang et al., 2008), and Cdk5 can regulate its functions (Li et al., 2001; Tomizawa et al., 2002; Hawasli et al., 2007; Pareek et al., 2007; Zhang et al., 2008; Peng et al., 2009). Cdk5 associates with, and phosphorylates, NR2A subunits at Ser-1232 of NMDAR. This phosphorylation is inhibited by roscovitine, which prevents induction of long-term potentiation (LTP) in CA1 pyramidal neurons (Li et al., 2001). Cdk5 facilitates the degradation of NR2B, another subunit of NMDAR, by directly interacting with both the receptor and its protease (calpain), thus controlling learning and synaptic plasticity (Hawasli et al., 2007). In addition, Cdk5 regulates the phosphorylation of NR2B subunits (Peng et al., 2009), and the surface expression of NMDAR (Zhang et al., 2008). Cdk5 also regulates neurotransmitter release through phosphorylation of P/Q-type VDCC, and downregulation of the channel activity (Tomizawa et al., 2002).

5. Inhibition of Cdk5 as a therapeutic target to treat pain

Cdk5 is an attractive drug target for the development of novel therapies to treat a number of diseases and disorders. Several classes of chemicals inhibitors for Cdk5 have been identified (Sausville EA, 2002; Sondhi et al., 2005; Oumata et al., 2008). Most of these inhibitors target the ATP binding site, resulting in a lack of specificity for cyclin-dependent kinases. It is therefore critical to develop more specific inhibitors directed specifically to Cdk5/p35 (Glicksman et al., 2008). A promising approach is a 125 amino acid peptide of p35, called Cdk5 inhibitor peptide (CIP), which has a higher affinity for Cdk5/p25. CIP was found to inhibit aberrant tau phosphorylation in cortical neurons, and protect against amyloid beta peptide toxicity (Zheng et al., 2005; Kesavapany et al., 2007). This suggests that CIP and smaller molecules derived from this endogenous inhibitor will allow more selective

targeting of Cdk5 hyperactivation (Kesavapany et al., 2007). Conversely, regulation of the Cdk5 activator, p35 and p39, could be an alternative approach for inhibiting Cdk5 activity. Several inflammatory mediators have been reported to regulate p35 expression and subsequently Cdk5 activity (Tokuoka et al. 2000; Harada et al., 2001; Quintanilla et al., 2004; Song 2005; Ojala et al., 2008; Bogen et al. 2008; Utreras et al., 2009). Therefore, if it is possible to regulate the release, activity, receptor-binding, or signaling pathways of these modulators, it may be possible to selectively regulate Cdk5 activity to treat chronic pain.

6. Conclusions

It is clear now that Cdk5 plays an important role in pain signaling. Our findings provide a key molecular mechanism for functional regulation of TRPV1 by Cdk5 and suggest a new paradigm for developing analgesics that target Cdk5-mediated phosphorylation. In addition to current use of opioids and anti-inflammatory drugs, Cdk5-targeted analgesics will prove to be novel therapeutic options for effectively treating many painful conditions and disorders.

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Figure 1. Schematic representation of Cdk5 signal transduction in dorsal root ganglia after nerve injury

Nerve injury induces a release of several inflammatory mediators such as cytokines (IL-6, TNF- α , IFN- γ etc), neurotrophins (NGF, BDNF, and GDNF), leukotrienes, prostaglandins (PGE₂), histamine, bradykinins and ions from adjacent cells (black arrows). These molecules bind to receptors and activate MAP kinase signaling pathways (MEK, p38 MAPK, JNK) among other pathways. TNF- α can regulate Cdk5 activity, and this effect is mediated by MEK through subsequent activation of ERK1/2, Egr-1, and p35 expression (red arrows). Besides, Cdk5 may regulate the function of other proteins (dashed black arrows) involved in pain signaling (JNK, p38, prostaglandins and/or bradykinin receptors, etc.). The increased Cdk5 activity due to inflammation potentially may cause hyperphosphorylation of TRPV1 and other known substrates involved in pain (NMDAR; P/Q VDCC). Also, Cdk5 could phosphorylate unknown substrates (e.g., opioid receptors), leading to pain sensitization and pain transmission towards spinal cord.