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Protein kinase C in pain: Involvement of multiple isoforms

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Abstract

Pain is the primary reason that people seek medical care. At present chronic unremitting pain is the third greatest health problem after heart disease and cancer. Chronic pain is an economic burden in lost wages, lost productivity, medical expenses, legal fees and compensation. Chronic pain is defined as a pain of greater than two months duration and can be of an inflammatory or neuropathic origin that can arise following nerve injury or in the absence of any apparent injury. Chronic pain is characterized by an altered pain perception that includes allodynia (a response to a normally non-noxious stimuli), and hyperalgesia (an exaggerated response to a normally noxious stimuli). This type of pain is often insensitive to the traditional pain drugs or surgical intervention and thus the study of the cellular and molecular mechanisms that contribute to chronic pain are of the up-most importance for the development of a new generation of analgesic agents. Protein kinase C isozymes are under investigation as potential therapeutics for the treatment of chronic pain conditions. The anatomical localization of protein kinase C isozymes in both peripheral and central nervous system sites that process pain have made them the topic of basic science research for close to two decades. This review will outline the research to date on protein kinase C involvement in pain and analgesia. In addition, this review will try to synthesize these works to begin to develop a comprehensive mechanistic understanding of how protein kinase C may function as the master regulator of peripheral and central sensitization that underlies many chronic pain conditions.

Keywords

nociception; primary afferent; dorsal horn; spinal cord; opioid analgesia

I. Pain

Pain is defined by the International Association for the Study of Pain as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage.” It is an evolutionarily conserved sensory experience that is physiologically necessary for an organism to detect and avoid injury. The importance of this sensory system is highlighted by conditions in which pain is absent such as congenital insensitivity to pain, leprosy, or diabetic neuropathy. The transmission of nociceptive input from the peripheral site of noxious stimuli to the central nervous system occurs through the activation of primary afferents (Figure 1). There are three main primary afferent fiber types; the large-diameter, heavily myelinated Aβ fibers that transmit non-noxious tactile sensations, the small-diameter, myelinated Aδ fibers that transmit “first pain” - the rapid shooting pain after you hit your thumb with a hammer, and the small-diameter, unmyelinated C fibers that

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transmit “second pain” – the throbbing in the thumb following the blow with the hammer. A noxious stimulus activates primary afferent nociceptors that synapse in the spinal cord on dorsal horn neurons. The dorsal horn of the spinal cord can be viewed as a gate at which millions of peripheral signals arrive and are sorted before being sent to supraspinal processing sites that determine the final response and add an emotional context to nociception (ie: remove your hand from the hot stove and “Ouch, that hurts”). Dorsal horn neurons not only convey pain sensation through ascending spinothalamic and spinoreticular projections, but also participate in segmental polysynaptic spinal reflexes (flexor, or withdrawal reflexes) to activate muscle groups that move a body part away from the stimulus and thus, protect the tissue from damage. These reflexes are commonly assessed as a measure of perceived pain in behavioral nociceptive tests in animal models of pain.

Pain pathways are modulated by both descending inhibitory and facilitatory control. In adult mammals, electrical stimulation of the periaqueductal gray (PAG) or microinjections of morphine into this midbrain site produces analgesia that can be robust enough to permit surgical procedures without the need for anesthetic agents(1). PAG neurons produce analgesia by activating descending inhibitory pathways to the spinal cord. These pathways include PAG axonal projections to rostral ventromedial medulla (RVM) neurons, which in turn project to the spinal cord via the dorsolateral funiculus to inhibit nociceptive primary afferents and spinal dorsal horn neurons(2–4). This effectively blocks pre-synaptic neurotransmitter release from primary afferent nociceptors and hyperpolarizes second order projection neurons in the spinothalamic or spinoreticular ascending pain pathways leading to a decrease in pain.

Pain can be categorized by a number of characteristics including physiological versus pathological, the duration of the painful stimulus (acute, subacute, tonic, chronic), the nature of the noxious insult (chemical/inflammatory, neuropathic, nociceptive), the type of response (spontaneous, evoked), and the location of the insult (cutaneous, subcutaneous, visceral, neuropathic). To this end, a multitude of animal models have been developed to examine nociceptive mechanisms and pharmacological selectivity. The functional role of protein kinase C (PKC) in pain and analgesia has been an area of intense research over the last two decades. In this review article we will summarize the current literature examining the role of PKC family members in pain and analgesia at each level of the neuro-axis involved in pain transmission.

II. Protein kinase C and Pain

PKC is a family of serine/threonine kinases that are divided into three groups based on calcium and diacylgycerol dependence. The α, βI, βII, and γ isozymes are calcium and diacylglycerol-dependent and are termed conventional (c) PKCs. The δ, ε, η, and θ isozymes are calcium-independent but diacylglycerol-dependent and are termed the novel (n) PKCs. Lastly, the ξ and λ/ι isozymes are calcium and diacylglycerol-independent and termed the atypical (a) PKCs. These different PKC isozymes function as key signal transducers in cells allowing them to regulate a number of cellular functions including differentiation, proliferation, cell migration, and apoptosis making them attractive therapeutic targets for a host of human diseases.

Over a decade ago expression of cPKCs (α, βI, βII, γ), nPKCs (ε, δ) and aPKC (ξ) was reported in the brain of rats (5–9). Since then PKC α, βI, βII, δ, ε, and ξ isozymes have been identified in primary afferents that transmit nociceptive signals from the peripheral site of injury to the superficial dorsal horn (10). Within the superficial laminae of the dorsal spinal cord, an area that has been implicated in pain processing, PKC α, βI, βII, and γ isoforms have been identified (11). With the evidence that PKC isozymes were in the anatomical regions that regulate pain the race was on to determine whether these isozymes were therapeutic targets for the treatment of pain. This review will consider the modulation of pain by PKC activity across the neuro-axis beginning in the primary afferent and ending in the brain.

III. Role of protein kinase C in pain and analgesia at different levels of the neuro-axis

III.a. The primary afferent peripheral terminal

Following tissue damage a variety of chemical mediators are released at the site of injury including, ATP, protons, bradykinin, prostaglandins, substance P, calcitonin gene related peptide (CGRP), and proinflammatory cytokines to name just a few. These chemicals can activate and sensitize primary afferent nociceptors leading to pain that can be characterized by hyperalgesia (ie: warm shower water is painful on sunburned skin), and allodynia (ie: the touch of clothing becomes painful). These substances have been used to model hyperalgesia and allodynia in animals and have highlighted the importance of specific PKC isozymes in nociceptive processing in the primary afferent.

The foundation suggesting a role of PKC in nociceptive processing in primary afferents came from work in isolated primary afferent neurons and isolated spinal cord preparations. These early studies showed that 1) PKC activation could depolarize unmyelinated afferent neurons (12,13), 2) PKC activators could sensitize afferent neurons (14), 3) PKC activators could enhance currents activated by noxious thermal stimulus in afferent neurons (15), and 4) PKC inhibitors could block sensitization in afferent neurons (16,17). Moving to *in vivo* models of cutaneous, inflammatory and neuropathic pain a variety of non-specific and isozyme specific PKC inhibitors have shown anti-nociceptive properties as outlined in Table 1.

The development of the PKCε knockout mice (18) and PKC isozyme-selective small peptide inhibitors demonstrate that PKCε in primary afferents has a critical role in nociception (18, 19) (Figure 2). One of the most studied pathways by which PKCε modulates nociception is through activation of the TRPV1 receptor, a member of the transient receptor potential ion channel superfamily. TRPV1 (transient receptor potential vanilloid receptor 1) is a nonspecific cation channel that is activated by capsaicin, the pungent ingredient in hot chili peppers, as well as by noxious heat, extracellular acidification, and potentially the endogenous ligands anandamide (20), leukotriene B, and lipoxygenase products (21). TRPV1 knockout mouse studies have shown this receptor to be essential for thermal and inflammatory pain. TRPV1 receptors are expressed on unmyelinated C fibers that contain substance P and/or CGRP (22). Normally, TRPV1 is activated only at high temperature, but following tissue damage, TRPV1 conducts current at lower temperatures. This response to non-noxious temperatures is thought to underlie thermal hyperalgesia.

Capsaicin applied to the skin produces thermal hyperalgesia and has proven a useful agent to study pain mechanisms in primary afferent neurons. It has been suggested that there are two independent pathways for TRPV1 activation: 1) direct ligand binding (endogenous ligands) which can be modulated by allosteric binding of protons 2) extracellular ligands coupled to PKC by intracellular signaling (23). In rat afferent neurons, PKC activation promotes exocytosis of TRPV1 to the cell surface (24), and increases capsaicin-induced TRPV1 currents while PKC inhibition decreases currents (25). Activation of PKC increases depolarization of TRPV1 allowing the receptor to conduct at lower temperatures (non-noxious temperatures) and in the presence of more physiologically relevant pH (26). As shown in Figure 3, application of capsaicin to the hind paw of a rat increases PKCε immunoreactivity in afferent fibers entering the dorsal horn of the spinal cord. PKCε has been implicated in phosphorylation of the TRPV1 receptor on Ser502 and Ser800 responsible for the potentiation of capsaicin-evoked currents (27,28). Alternatively, it has been reported that PKCα may also activate TRPV1 independent from the binding of TRPV1 ligand. Down-regulation of PKCα does not alter conductance following TRPV1 ligand suggesting that phosphorylation of TRPV1 is not necessary for normal activity only for augmented activity. Further study needs to be done to clarify whether

PKC ε and α may have isozyme specific roles in regard to the necessity of extracellular ligand-binding for activity.

Furthermore, substances released locally following tissue damage such as protons, bradykinin, ATP, and prostaglandins may potentiate the activation of TRPV1 by thermal stimulus. PKC may serve as a master switch by which TRPV1 integrates heat and tissue damage to produce hyperalgesia. There is a large literature that suggests numerous inflammatory mediators may enhance the activity of TRPV1 via PKC-dependent pathways. The integration of these various pathways is briefly outlined below and shown schematically in Figure 4A. Bradykinin is one potential extracellular ligand that has been shown to activate PKCε (29), and induce translocation of PKCε (19) in DRG neurons, thus, enhancing TRPV1 activity in a PKC-dependent manner (30–32). Similarly, galanin (33), ATP via P2Y2 receptors (34, 35), trypsin/tryptase released during inflammation via proteinase-activated receptor (PAR) 2 (36), prostaglandin E2 and I2 acting at EP1 and IP (37), acid sensing ion channels (ASIC) via association with PICK-1 (protein interacting with C-kinase) (38),(39), have been implicated in potentiating TRPV1 currents and shifting TRPV1 activation to a more physiological pH. Further evidence for the role of PKC in primary afferents in pathological pain, have come from studies looking at the role of PKC in hyperalgesic priming. These studies suggest that TNF acting through the TNFR1 during acute carrageenan-induced inflammation induces hyperalgesic priming by activating neuronal PKCε (10, 40, 41).

Similarly, insulin and insulin-like growth factors activate receptor tyrosine kinases that activate PKC leading to both increased TRPV1 receptor translocation to the cell membrane and enhanced receptor sensitivity (42). In a rodent model of painful diabetic neuropathy, PKC inhibitors decrease thermal hyperalgesia and the hyperresponsiveness of C afferent neurons (43). Diabetic neuropathy is accompanied by increased phosphorylation (activation) of PKCα, no change in PKCβII, and a down-regulation of PKCδ and TRPV1 protein (44,45). Functionally, increased capsaicin activated inward currents were observed and PKC activation further potentiated the currents(45). This apparent contradication in expression and activity of TRPV1 can be explained by the observation that although there were fewer TRPV1 receptors, those that remained were more likely to be phosphorylated (via PKC) and present on the plasma membrane. Furthermore, not only does PKC potentiate pain signaling but it may also decrease analgesia. For instance, opioid-mediated activation of inhibitory G protein function is associated with increased PKC-dependent phosphorylation of the Goα subunit (44). In conclusion, PKC appears to be critical to the development of peripheral sensitization in primary afferent fibers resulting in the development of thermal hyperalgesia following injury.

III. b. The primary afferent-spinal cord synapse

In addition to activity in the peripheral nerve terminal, PKC is likely to modulate the neurotransmission at the central terminal in the dorsal horn of the spinal cord. PKC has been shown to increase excitatory neurotransmission and decrease inhibitory tone at the primary afferent-spinal cord synapse (Figure 4B).

Peripheral application of capsaicin releases neuropeptides and excitatory amino acids into the dorsal horn of the spinal cord (46,47). In addition, to the sensitization of the TRPV1 receptor discussed above, PKC activation also enhances tetrodotoxin-resistant Na+ currents (48), thus increasing action potential propagation to the central terminals of afferent neurons. Electrophysiological studies have demonstrated that PKC activation potentiates capsaicin-induced depolarization in afferent neurons (25), a finding that correlates with enhanced capsaicin-induced release of substance P from spinal cord slices (49). While enhanced release of neurotransmitters may be secondary to PKC mediated events at the peripheral terminal there is evidence that local PKC activity at the pre-synaptic terminal also contributes. In afferent neurons, PKC activation alone increases substance P and CGRP release as well as potentiates

potassium- and capsaicin-stimulated release of these neuropeptides (49–51). More specifically, inhibition of PKCε decreases capsaicin-induced release of glutamate and CGRP in isolated spinal cords (52). In addition to topical capsaicin, chemokines stimulate the release of CGRP from afferent neurons in a PKC dependent manner (53). One potential mechanism by which pre-synaptic PKC activity can augment neurotransmitter release is via PKC-dependent sensitization of voltage-dependent L-type Ca2+ channels (54). An alternative mechanism may be a PKC-mediated decrease in pre-synaptic inhibition.

In vitro electrophysiology suggests that PKC is involved in modulating opioid and $GABA_A$ receptor function. PKC activators have been shown to inhibit μ, δ, and κ opioid receptor agonist-stimulated analgesia (55–58). Chronic use of opioid analgesics increases activity and expression of PKC that correlates with a decrease in analgesia. In addition, PKC inhibitors attenuate the development of opioid tolerance (59,60). Whether tolerance is due to a de-sensitization of opioid receptors or to the concomitant development of an opioid-induced hyperalgesia remains un-resolved. PKC may be involved in both the desensitization of opioid receptors as well as in the development of opioid-induced hyperalgesia. De-sensitization can occur in an agonist-dependent (homologous) and agonist-independent (heterologous) manner (For review see (61). While homologous de-sensitization is thought to involve the G protein coupled receptor kinases (GRK) (62–65), heterologous de-sensitization involves PKC mediated phosphorylation of the opioid receptor (62,64,66,67). Both NMDA receptor (68, 69) and insulin-induced tyrosine kinase receptor activity (70) have been reported to activate PKC resulting in heterologous de-sensitization of the μ opioid receptor. PKC mediated phosphorylation of the μ opioid receptor inhibits internalization thus, preventing re-sensitization of the receptor (71). In contrast, in δ opioid receptors, PKC mediated phosphorylation of serine 344 produces internalization (72). This suggests that PKC may differentially modulate the opioid receptor sub-types.

GABA functions as an inhibitory neurotransmitter in the spinal cord and can act pre-synaptically to reduce the release of neurotransmitters from primary afferent terminals. Similar to opioid receptors, the inhibitory $GABA_A$ receptor is modulated by phosphorylation status (73–75). Both cholecystokinin and substance P decrease inhibitory $GABA_A$ currents via PKC-dependent phosphorylation of the receptor (74–76). These findings suggest that PKC acts on numerous receptor types in primary afferents to both enhance excitatory neurotransmission and to attenuate inhibitory tone at the synapse.

III. c. Spinal cord

Increased translocation and activation of PKC in dorsal horn neurons has been shown in a number of pain models(77–79) including following topical administration of capsaicin (Figure 3B). Spinal administration of non-specific inhibitors of PKC has highlighted the importance of spinally located PKC in pain (Table 2). Findings in PKC γ knockout mice suggest that PKCγ is a critical regulator of central sensitization while leaving acute pain processing intact (80).

Sensitization of dorsal horn neurons is characterized by lower thresholds for neuronal activation and increased spontaneous and evoked discharges. As in peripheral nociceptive neurons, in central spinal cord neurons PKC is likely to serve as a point of convergence for the development of sensitization (Figure 4C). In dorsal horn neurons activation of PKC increases excitability, shortens the latency to first spike, increases spike frequency, and increases action potential amplitude (81). Findings in PKCγ knockout mice coupled with the localization of PKCγ in a sub-set of excitatory interneurons in lamina II of the spinal cord (80, 82) have implicated an important role for spinal PKCγ both in inflammatory and neuropathic pain. PKCγ containing glutamatergic interneurons receive input from a subset of TPRV1 positive primary afferents (22), and can modulate lamina V transmission neurons (83). This provides

a bridge from peripheral stimuli to ascending transmission neurons. In addition, to an ideal anatomical localization for the transmission of nociceptive stimuli, PKCγ has been found to potentiate NMDA-induced currents in the spinal cord via alleviation of its voltage-dependent Mg2+ block (84) providing an ideal mechanism for PKC- dependent central sensitization.

As in the peripheral nervous system in which PKC activation was a point of convergence for the numerous peripheral effectors to ultimately alter current flow through the TPRV1 receptor resulting in thermal hyperalgesia, PKCγ activation in lamina II interneurons and the resulting change in NMDA currents serves as a point of convergence for multiple excitatory neurotransmitter and neuromodulatory peptides released at the primary afferent-spinal cord synapse. For instance, group I metabotropic glutamate receptors and neurokinin 1 receptors (binds Substance P) are G protein coupled receptors that couple to $G_{\alpha q}$ activating PLCβ leading to the production of IP_3, DAG, and liberation of intracellular Ca^{2+} facilitating the activation of PKC. Group I metabotropic glutamate receptor activation has been shown to enhance NMDA receptor activity via PKC (84–88) and inhibition of these metabotropic receptors or PKC alleviate pain (78,89). Phosphorylation of the NMDA receptor appears to occur indirectly via the formation of a signalling complex that includes metabotropic glutamate receptor 5, Shank (postsynaptic density protein associated with metabotropic glutamate receptors), postsynaptic density-95, and the tyrosine kinase Src (which is activated up-stream by PKC). Phosphorylation of the NMDA receptor NR2B subunit by Src increases channel kinetics and time spent open (90–93). Similarly, substance P can bind spinal neurokinin 1 receptors and activate PKC (94,95). This evidence supports a role of PKC in directly mediating neuronal excitability within the spinal cord.

As in afferent neurons, PKC in dorsal horn neurons also plays a significant role in opioid tolerance. Daily intrathecal administration of morphine increases membrane bound PKC in lamina I and II (96). In addition, PKC α and γ are up-regulated in the spinal cord of morphine tolerant rats (97,98). The NMDA NR1 sub-unit is also up-regulated in morphine tolerant animals (99) and blockade of the NMDA receptor can diminish the development of tolerance (100,101). These findings suggest overlap in nociception and opioid tolerance highlighting the potential dual utility of PKC isozyme specific inhibitors in the treatment of chronic pain and the prevention of opioid tolerance.

III. d. Descending modulation from the brain

Much less is known about PKC in the brain as it relates to pain and analgesia. Descending pathways from the PAG through the RVM to the dorsal horn of the spinal cord is an important mechanism for modulating ascending spinal nociceptive transmission and another potential level for modulation of pain and analgesia by PKC (Table 3). Electrical stimulation of the PAG results in a potent analgesia at the spinal level. PKC activation in the spinal cord can antagonize this descending inhibition (102). In addition, PKC activation in a model of inflammatory pain can enhance descending facilitation from the RVM via phosphorylation of the AMPA receptor (103), and the NR2a subunit of the NMDA receptor (104). So, similar to at the level of the spinal cord, activation of PKC in the brain can enhance nociception. In addition, as at the other levels of the neuro-axis supraspinal morphine exposure activates PKC and enhances excitatory glutamate signaling (105). While much work remains in understanding how PKC in the brain modulates pain and analgesia the data to date suggests similarity to mechanisms described in afferent and spinal cord neurons.

IV. Conclusions

In summary, this review has highlighted the importance of different PKC isozymes at different levels of the neuro-axis in both pain and analgesia such that PKC α and ε appear to be involved in peripheral nociception while PKCγ is important to central nociception. This does not exclude

a role for other PKC isozymes in pain and analgesia but highlights the limited research on these other isozymes. This review also highlights the importance of PKC isozymes in mediating a switch from a protective and evolutionarily conserved physiological pain (those necessary to avoid tissue damage) to a more prolonged and pathological pain (pain of a higher intensity or longer duration than required to avoid or resolve tissue damage) as highlighted by the intact acute pain but absent inflammatory or neuropathic pain in PKC ε and γ knockout mice. This bodes well for the pursuit of isozyme specific inhibitors for the treatment of pain since the end goal in the development of novel analgesic agents is to develop therapeutics that leave intact normal physiological pain systems while preventing or attenuating pathological pain. New therepeutics that target specific isozymes of PKC may be on the horizon. In fact, preclinical development of small peptide PKC ε and γ inhibitors to treat inflammatory and neuropathic pain is underway (Kai Pharmaceuticals, South San Francisco, CA).

In conclusion, this review highlights the role of PKCε in peripheral sensitization and PKCγ in central sensitization. In both cases of sensitization it appears that PKC functions to integrate numerous receptor pathways into final effectors that increase excitatory signaling and decrease inhibitory signaling, thus promoting pain. These results highlight the idea that adequate analgesia will require either a cocktail approach at the level of the individual receptor or alternatively inhibition of second messenger pathways in an isozyme specific manner.

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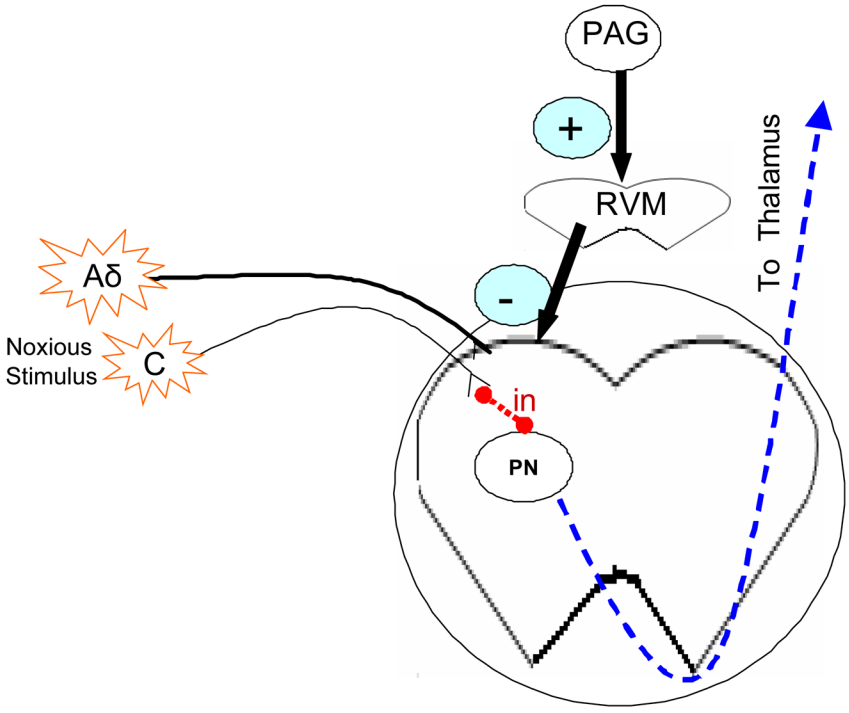

Figure 1. The pain pathway and its descending modulation

A noxious stimulus will excite peripheral nociceptors (Aδ and C fibers). These fibers synapse on second order dorsal horn neurons. Some of these dorsal horn neurons are excitatory or inhibitory interneurons (in). Others are ascending spinothalamic projection neurons (PN) that ascend via the contralateral ventrolateral funiculus to convey pain sensation to the brain. Dorsal horn neurons are also subject to descending modulation from the midbrain periaquaductal gray (PAG) through polysynaptic circuits through the medulla including through the rostral ventromedial medulla (RVM).

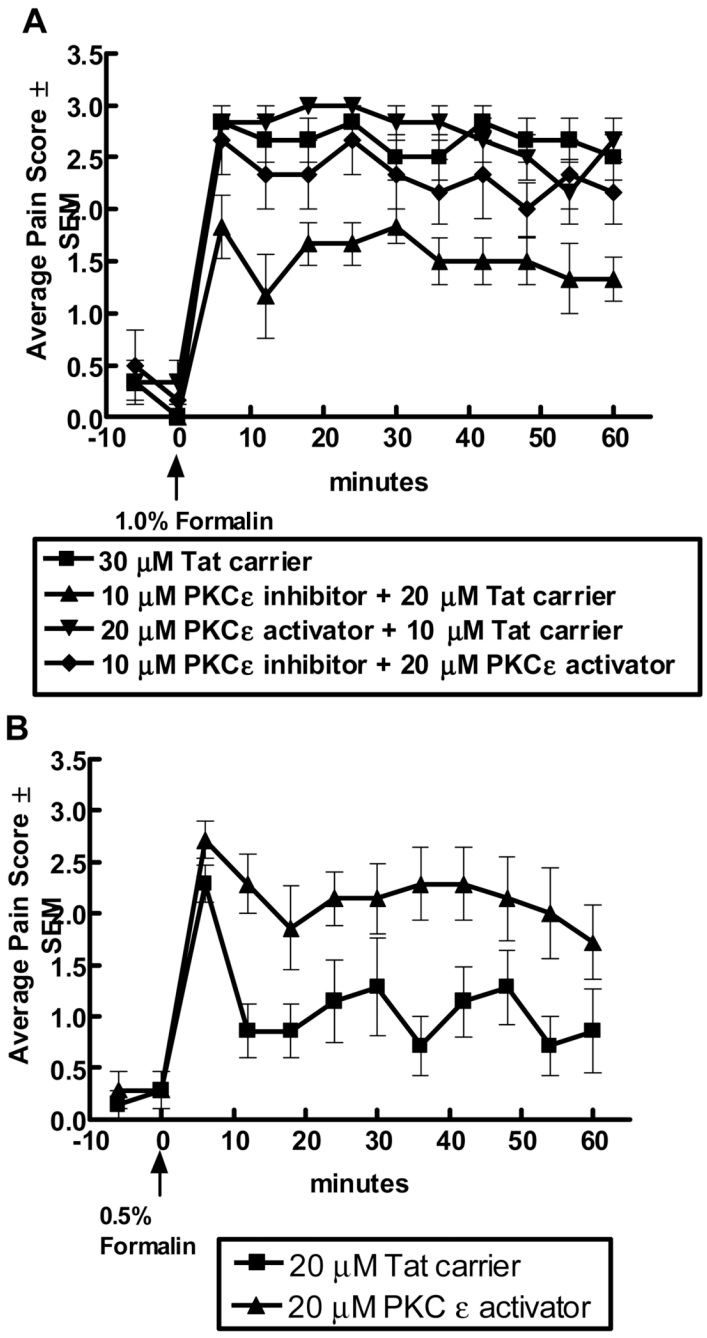

Figure 2. Protein kinase C ε modulates formalin-induced nociception

Subcutaneous administration of dilute 1% formalin in the plantar hindpaw of a one week old rat produces spontaneous flinching and guarding of the hind paw that can be scored in 6 minute bins to provide a Pain Score. A score of 0 represents the absence of paw flinching and a score of 3 represents the presence of flinching or guarding of the hind paw. In panel A, intrathecal administration of an isozyme specific PKCε peptide inhibitor linked to a Tat carrier peptide reduced the average pain score. Attenuation by the PKCε peptide inhibitor could be reversed by co-adminstration with a PKCε peptide activator. The Tat carrier peptide did not alter behaviors as compared to saline or no treatment (148). PKCε peptide activator alone did not increase behaviors with the recognition that behaviors were already maximally stimulated with

1% formalin. In panel B, intrathecal administration of the PKCε peptide activator potentiated pain-associated behaviors stimulated with a sub-maximal concentration of formalin (0.5%). These findings indicate a role for PKCε in nociception.

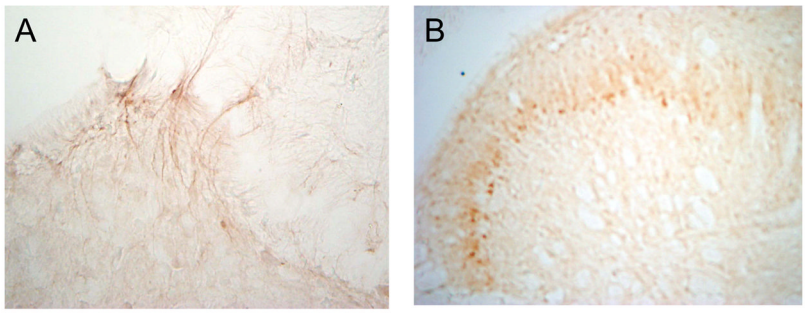

Figure 3. Protein kinase C ε and γ immunoreactivity in the spinal cord after topical capsaicin

Topical capsaicin was applied to the rat hind paw resulting in thermal hyperalgesia that lasted at least one hour. At the end of the hour rats underwent transcardiac perfusion and lumbar spinal cords were collected for immunohistochemical analysis of protein kinase C ε and γ. In panel A, PKC ε immunoreactivity is seen in the dorsal horn of the spinal cord. The PKC ε appears to be on primary afferent fibers entering into the superficial lamina of the dorsal horn. Using serial spinal cord sections in panel B, PKC γ immunoreactivity is seen in lamina II of the dorsal horn of the spinal cord. In contrast with PKCε, PKCγ is located in dorsal horn neurons.

Figure 4a

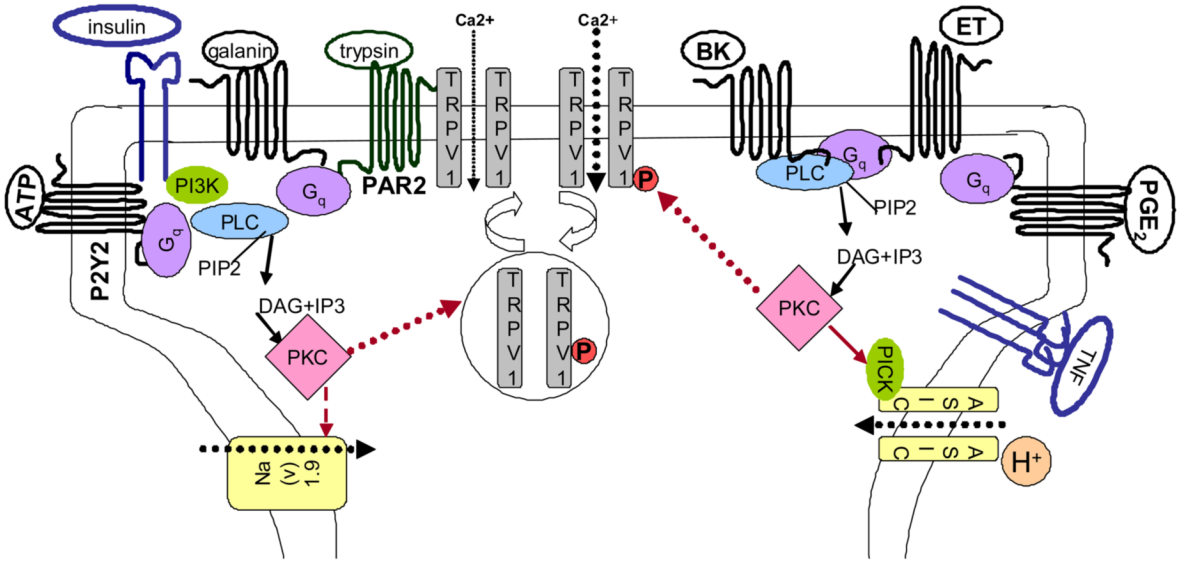

Figure 4b

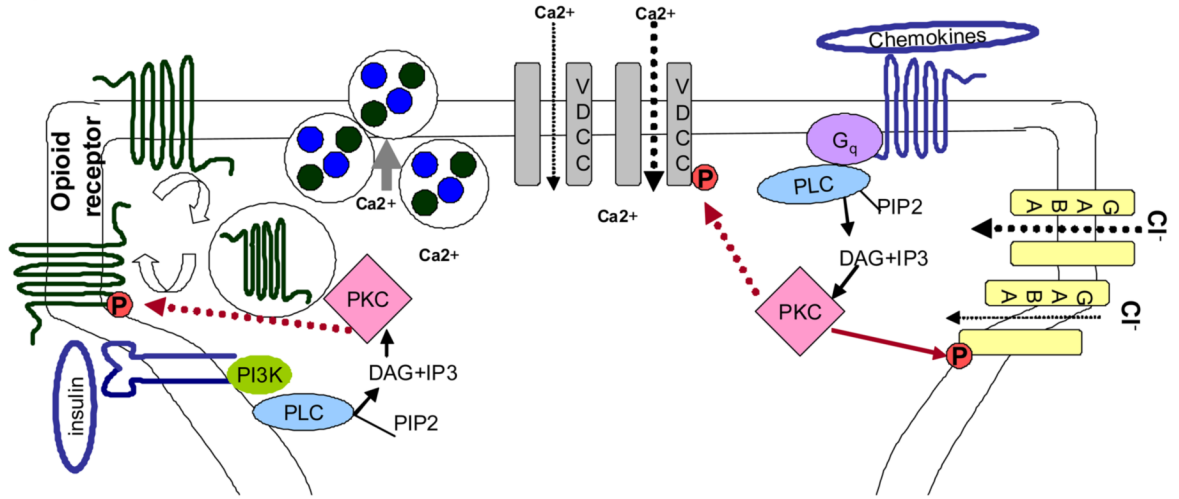

Figure 4c

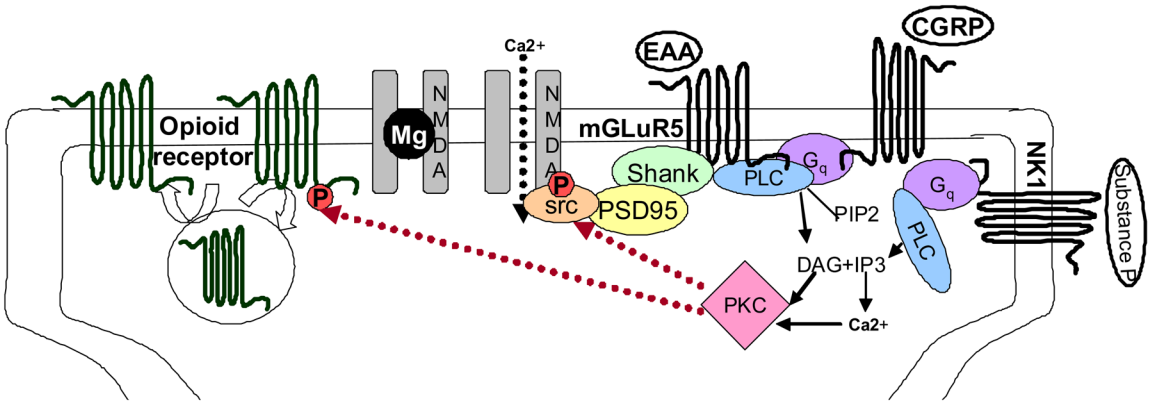

Figure 4. PKC is positioned to play a central role in peripheral and central sensitization

Panel A, PKC in peripheral sensitization in the primary afferent peripheral terminal. PKC is activated in the primary afferent by a large number of substances that are released in response to injury. These include bradykinin (BK), endothelin-1 (ET), prostaglandins (PGE2), cytokines (tumor necrosis factor, TNF), ATP, trypsin, galanin, and insulin. The receptors for these proteins activate phospholipase C (PLC) and stimulate the production of diacylgycerol (DAG) which activates PKCs. Activated PKC subsequently can increase cation flow into the peripheral terminal via actions on the capsaicin receptor (TRPV1), the acid sensing ion channel (ASIC), and tetrodotoxin-insensitive sodium channels (Nav 1.9). **Panel B, PKC is positioned to influence neurotransmitter release from the central primary afferent terminals in the spinal cord.** Binding of G-protein coupled pre-synaptic receptors and receptor tyrosine kinases signal through phospholipase C (PLC) to activate PKC. This activated PKC can then enhance calcium influx into the pre-synaptic terminal by phosphorylation of voltage gated calcium channels (VDCC). Simultaneously, PKC can decrease inhibitory tone in the pre-synaptic terminal by phosphorylation of opioid receptors and inhibitory $GABA_A$ receptors effectively decreasing pre-synaptic inhibition. This increase in excitatory tone and decrease in inhibitory tone can potentiate the release of neurotransmitter from the pre-synaptic terminal. **Panel C, PKC in the post-synaptic dorsal horn neuron regulates neuronal activity.** PKC is activated in dorsal horn neurons subsequent to G protein coupled signaling through phospholipase C (PLC). A variety of G proteins are involved including, but not limited to, neurokinin receptor (NK1), metabotrophic glutamate receptors (mGluR) that bind excitatory amino acids (EAA), and receptors for calcitonin gene-related peptide (CGRP). Activted PKC then phosphorylates Src thus increasing NMDA receptor activity. As in pre-synaptic primary afferent terminals, activated PKC can also modulate post-synaptic opioid receptor activity.

Table 1

Inhibition of nociception by peripheral administration of PKC inhibitors

A summary of the studies that implicate PKC in primary afferents in cutaneous, inflammatory, and neuropathic pain models.

Pain Model	PKC treatment	Change elicited	Refs
	CUTANEOUS PAIN		
PKC activation	Phorbol ester PKCε constituitively active	Induces spontaneous nociception, TH, MA. Enhanced heat activated membrane currents	(19,106, 107)
Bradykinin	PKCε inhibitor bisindolylmaleimide I	Decreased membrane currents MA decreased.	(19,106)
Thermal stimulus	Chelerythrine	Decreased allodynia	(108)
Epinephrine	PKCε knock-out mice εV1-2 inhibitory peptide	Decreased TH and MH Decreased MH	(18)
Nerve Growth Factor	εV1-2 inhibitory peptide	Decreased MH	(18)
Capsaicin	PKC activator Bisindolylmaleimide	Increased capsaicin-induced currents. Decreased capsaicin-induced currents. Contributes to C-fiber induced ERK activation	(25) (93)
PAR2 agonist & capsaicin	PKCε antagonist	Decreased TH	(109)
Endothelin-1	GF109203X	Decreased MH	(32,110)
	INFLAMMATORY PAIN		
Formalin	chelerythrine	Decreased second phase behaviors.	(106)
Carregeenan	εV1-2 inhibitory peptide	MH is attenuated	(18)
	NEUROPATHIC PAIN		
Sciatic nerve transection	No-treatment	Up-regulation of PKCα, β1, δ	(111)
Diabetic Neuropathy	PKC(19–36) Staurosporine	MH and C-fiber hyperexcitability blocked	(43)
Alcohol Neuropathy	non-specific inhibitors & εV1-2 inhibitory peptide	MH and C-fiber hyperexcitability blocked.	(112)

MA – mechanical allodynia, MH – mechanical hyperalgesia, TH – thermal hyperalgesia

Table 2

Inhibition of nociception by spinal (intrathecal) administration of PKC inhibitors

A summary of the studies that implicate PKC in spinal cord in cutaneous, inflammatory, and neuropathic pain models.

Pain Model	PKC treatment	Change Elicited	Refs
CUTANEOUS PAIN			
Acute pain	PKC γ KO mice	No change	(80)
PKC activator	Phorbol esters	Induced pain-like behaviors (mice) Increased activity in spinothalamic tract neurons (primate)	(113,114) (115)
Tail flick	calphostin C	Enhanced [D-Ala2]deltorphin II-induced antinociception	(57)
Capsaicin	NPC15437	Reversed MA	(116)
Thermal injury	GF109203X chelerythrine	decreased MH in the contralateral paw	(117)
INFLAMMATORY PAIN			
Formalin	GF109203X chelerythrine εV1-2 inhibitor γV3-5 inhibitor	decreased nociception – 2nd phase decreased c-fos in lumbar dorsal horn Decreased nociception – 1st & 2nd Decreased nociception – 2nd phase	(118–120) (121)
Bee Venom	Chelerythrine	Decreased primary TH No effect on MH Decreased spontaneous nociception Decreased mirror image TH	(122,123)
Complete Freud's Adjuvant	RO-320432	No effect	(124)
Mustard Oil	PKC inhibitors	attenuation of neuronal activity mustard oil-induced	(125)
NEUROPATHIC PAIN			
sciatic nerve ligation	PKC γ KO mice Calphostin C RO-320432	Decreased MA & TA Decreased TH Decreased TH Increased cPKC activity in dorsal horn	(80) (126) (124)
Chung Model	chelerythrine	Anti-allodynic	(127)
Chronic Constriction Injury	PKCγ knock-out mice Chelerythrine MK-801	Decreased neuropathic pain Decreased TH Decreased PKCγ immunoreactivity	(78,97,128)
Pertussis toxin	Chelerythrine	Decreased TH	(129)
Dihydroxyphenylglycine	GF109203X	Decreased spontaneous nociceptive behaviors & TH	(130)
Diabetic Neuropathy	calphostin	Increased tail flick latencies	(131)
Alcohol Neuropathy	ODN to PKCε	inhibits hyperalgesia	(132)
OTHER PAIN			
Substance P	GF109203X H7	Inhibits TH	(119,133)
Brain Derived Neurotrophic Factor	RO-320432	Inhibits TH, MA	(134)
Muscle Pain	GF109203X NPC15437 Chelerythrine	No change in mechanical thresholds	(135)
Acute Ethanol withdrawal	εV1-2 inhibitor γV3-5 inhibitor	Prevents MA & TH	(136)
MORPHINE HYPERALGESIA & TOLERANCE			
Opioid analgesia	PKC activator	Suppression of analgesia Decreased opioid reward	(55,57) (114)
Opioid analgesia	PKCγ knock-out mice	Enhanced μ opioid receptor analgesia & G protein signaling	(137)
Acute morphine withdrawal	εV1-2 inhibitor γV3-5 inhibitor	Decreases MA & TH	(138)

MA – mechanical allodynia, MH – mechanical hyperalgesia, TH – thermal hyperalgesia, ODN - antisense oligodeoxynucleotide

Table 3

Inhibition of nociception by brain (intracerebrobentricular) administration of PKC inhibitors

A summary of the studies that implicate PKC in the brain in cutaneous, inflammatory, and neuropathic pain models.

Pain Model	PKC treatment	Change Elicited	Refs
CUTANEOUS PAIN			
Patch clamp PAG neurons	PMA GF109203X	modulated Cl- current	(139)
Abdominal constriction	calphostin C chelerythrine	Acute tolerance to N_2O-induced antinociception	(140)
Acetyl-L-carnitine antinociception	Calphostin C and chelerytrine	Potentiated the antinociceptive effect of ALCARe	(141)
INFLAMMATORY PAIN			
Brain Derived Neurotrophic Factor	chelerythrine	Decreased NR2A receptor phosphorylation in RVM	(104)
Complete Freud's Adjuvant	chelerytrine	Attenuated upregulation of phosphoserine 831 GluR1 RVM	(103)
Histamine agonist	Calphostin C chelerytrine	Reverse hyperalgesia	(142)
MORPHINE HYPERALGESIA & TOLERANCE			
Acute Morphine	NPC 15437 (PKC antagonist)	Decrease c-Fos expression in striatum and cingulate cortex Translocation of PKC βII to plasma membrane in cortical and striatum neurons	(143)
Low dose morphine hyperalgesia	Calphostin C ODN to PKCγ	Prevent morphine hyperalgesia Mild analgesic Downregulated PKCγ in PAG	(105)
Opioid analgesia	ODN to PKCγ PKC α, γ, ε inhibitors	Enhanced analgesia	(105,144)
Chronic morphine tolerance	GF109203X Bisindolylmaleimide I, Go-7874	Decreased glutamatergic synaptic transmission in NRM Reversed tolerance	(145–147)

MA – mechanical allodynia, MH – mechanical hyperalgesia, TH – thermal hyperalgesia, ODN - antisense oligodeoxynucleotide, PAG – periaqueductal gray, RVM – rostral ventromedial medulla, NRM – nucleus raphe magnus