Pain Physician 2009; 12:E309-E318 • ISSN 2150-1149

Back to Basics

Calcineurin as a Nociceptor Modulator

Howard S. Smith, MD

From: Albany Medical College, Albany, NY.

Dr. Smith is Associate Professor and Academic Director of Pain Management for Albany Medical College Department of Anesthesiology, Albany, NY.

Address correspondence:
Howard S. Smith, MD
Albany Medical College
Department of Anesthesiology
47 New Scotland Avenue; MC-131
Albany, New York 12208
E-mail: smithh@mail.amc.edu

Disclaimer: There was no external funding in the preparation of this manuscript.
Conflict of interest: None.

Manuscript received: 12/30/2008
Accepted for publication: 02/13/2009

Free full manuscript:
www.painphysicianjournal.com

Calcineurin may be involved in affecting nociceptive processes in multiple circumstances. It is conceivable that interfering with calcineurin's normal role in contributing to glial resting membrane potential, via its effects on the ion channel (TRESK) [tandem-pore-domain weakly inward rectifying potassium channels (TWIK)-related spinal cord potassium channels] may facilitate nociception. Another aspect of calcineurin function may be its role in the pronociceptive signaling of nuclear factor of activated T-cells (NFAT). NFAT activation via mediators (e.g. Substance P, brain-derived neurotrophic factor, nerve growth factor, bradykinin) appears to be dependent on calcineurin function. This calcineurin-regulated NFAT signaling may subsequently lead to transcription of pronociceptive genes as well as upregulation of pronociceptive chemokine receptors in the dorsal root ganglion. In fact, multiple articles have described the clinical use of calcineurin-inhibitors leading to pain, a phenomenon referred to as calcineurin inhibitor-induced pain syndrome (CIPS). Thus, it appears that calcineurin functions may encompass actions which promote or dampen nociceptive processes. A greater understanding of the physiology of calcineurin, especially as it relates to modulating nociception may lead to the development of novel analgesic targets in attempts to optimally alleviate patient discomfort.

Key words: Pain, neuropathic, calcineurin, NFAT, TRESK-[Tandem-pore-domain weakly inward rectifying potassium channels (TWIK)-related spinal cord potassium channels], CIPS (calcineurin-induced pain syndrome)

Pain Physician 2009; 12:E309-E318

Calcineurin may play a significant role in the modulation of nociception. Calcineurin may contribute to maintaining aspects of "normal" baseline physiologic sensory neural and/or glial function, via its role in maintaining "homeostatic potassium currents of resting membrane potentials." Furthermore, it appears that interruption of this valuable role of calcineurin may spark nociceptive processes and/or hyperexcitability and hence, may be involved in nociceptive signaling pathways, thereby potentially facilitating algesia.

The 2-pore domain K(+) channel, TRESK (TWIK-related spinal cord K(+) channel) is reversibly activated by the calcium/calmodulin-dependent protein phosphatase, calcineurin. Czirják et al (1) reported that 14-3-3 proteins directly bind to the intracellular loop of TRESK and control the kinetics of the calcium-dependent regulation of the channel. Phosphorylation of serine 264 in mouse TRESK was required for the binding of 14-3-3eta. Because 14-3-3 proteins are ubiquitous, they are expected to control the duration of calcineurin-mediated TRESK activation in all the cell types that express the channel, depending on the phosphorylation state of serine 264 (1). This kind of direct control of channel regulation by 14-3-3 is unique within the 2-pore domain K(+) channel family (1).

Czirják et al (2) reported that calcineurin also interacts with TRESK via an NFAT-like docking site, in addition to its enzymatic action. In its intracellular loop, mouse TRESK possesses the amino acid sequence, PQIVID, which is similar to the calcineurin binding consensus motif, PXIXIT (where X denotes any amino acids), necessary for NFAT (nuclear factor of activated T cells) activation and nuclear translocation (2). Mutations of the PQIVID sequence of TRESK to PQIVIA, PQIVAD, or PQAVAD increasingly deteriorated the calcium-dependent activation in the listed order and correspondingly reduced the benzocaine sensitivity (a property discriminating activated channels from resting ones) when it was measured after the calcium signal in Xenopus oocytes. Microinjection of VIVIT peptide, designed to inhibit the NFATcalcineurin interaction specifically, also eliminated TRESK activation. The intracellular loop of TRESK, expressed as a GST fusion protein, bound constitutively active calcineurin in vitro (2). PQAVAD mutation as well as addition of VIVIT peptide to the reaction abrogated this calcineurin binding. Wild type calcineurin was recruited to GST-TRESK-loop in the presence of calcium and calmodulin. Czirják and colleagues (2) suggested that their results indicated that the PQIVID sequence is a docking site for calcineurin, and its occupancy is required for the calcium-dependent regulation of TRESK.

In the K2P channel family, there are 6 subfamilies according to the differences in channel structure and regulatory mechanism: 1) tandem-pore-domain weakly inward rectifying potassium channels (TWIK), 2) tandem-pore domain halothane-inhibited potassium channels (THIK), 3) TWIK-related acid-sensitive potassium channels (TASK), 4) tandem-pore-domain alkaline-activated potassium channels (TALK), 5) TWIK-related arachidonic acid-sensitive and mechano-gated potassium channels (TREK), and 6) TWIK-related spinal cord potassium channels (TRESK).

Tandem-pore-domain weakly inward rectifying potassium channels (TWIK)-related spinal cord potassium channels are a kind of 2-pore domain K+ channel (K2P), first discovered in 2003 (3) which is very different from the other K+ channels including voltage-gated (Kv), calcium-activated (Kca), and inward rectifier (Kir) channels in molecular structure, electrophysiological and pharmacological properties (4). K2P is a collection of ion channel subunits exhibiting 4 transmembrane domains (TMA1-4) and 2 pore-forming domains (P1, P2), while other K+ channel subunits are characterized by having 2, 6, or 8 transmembrane segments and one conserved pore-forming domain (5-7). K2P currents are observed at all membrane potentials and can be activated instantaneously by a voltage step and show no voltage threshold for activation, which also distinguishes them from other K+ channels. So, it can produce leak current that help to set and stabilize the resting potential and thus influences the cell excitability under physiological conditions (8,9).

K2P channels may be involved in certain neuropathic pain states. TRESK is specifically located in the spinal cord in humans whereas it is mainly in the dorsal root ganglion (DRG) in mice, where it functions as an important background potassium channel. The major background K+ channel at 24° C in DRG neurons is TRESK (10). Thus, TREKs and TRESK may represent modulatory targets of nociceptive modulation since they likely play active roles in regulating the excitability of DRG neurons.

TRESK is abundantly expressed in the DRG, as well as in the spinal cord (11). The DRG is important in transmitting nociceptive information and in the onset and development of chronic pain because it is a relay of pain signal transport from periphery to the central nervous system. Previous studies have demonstrated hyperexcitability and ectopic discharges of DRG in several pain models, such as chronic constriction injury (CCI) and partial sciatic nerve ligation model (12-14). TRESK may contribute a significant component of background potassium currents in murine dorsal root ganglion neurons (15). Thus, TRESK might play an important role in the development of pain by regulating the background potassium currents of DRG. Also, the glial cell membrane at rest is exclusively permeable to K+ ions whereas the neuron at rest is permeable to both K+ and Na+, so Hunag et al (15) have speculated that the TRESK may be a key ion channel to modulate the resting potential of glial cell. Glia have emerged as important contributor to mechanisms of persistent pain and studies have suggested that glial cells play a key role in nociceptive processes of various pain states (16-20). On activation, both astrocytes and microglia respond to and release a number of signaling molecules, which have protective and/or pathological functions (21). Ca2+-dependent calcineurin is upregulated in reactive astrocytes in neuroinflammatory models (22). Canellada et al (23) reported that the calcineurin-dependent expression of Cox-2 and Rcan 1-4 is induced (via NFAT activation) by physiological calcium mobilizing agents, such as thrombin, agonists, or purinergic and glutamate receptors, and L-type voltage-

gated calcium channels. Adenovirus-mediated delivery of the NFAT inhibitor, VIVIT, suppressed the IL-1beta-dependent induction of several inflammatory mediators and/or markers of astrocyte activation, including tumor necrosis factor and vimentin (24). Expression of an activated form of calcineurin in one set of astrocyte cultures also triggered the release of factors that, in turn, stimulated NFAT activity in a second set of "naïve" astrocytes (24). This effect was prevented when calcineurin-expressing cultures co-expressed VIVIT, an NFAT inhibitor, suggesting that the calcineurin/NFAT pathway coordinates positive feedback signaling between glia (e.g. astrocytes) [and perhaps between glia and neurons] (24). In quiescent astrocytes, inflammatory mediators such as tumor necrosis factor-alpha (TNF-alpha) recruits calcineurin to stimulate a canonical inflammatory pathway involving the transcription factors nuclear factor kappaB (NFkappaB) and nuclear factor of activated T-cells (NFAT) (25). However, in reactive astrocytes, local anti-inflammatory mediators such as insulin-like growth factor I also recruit calcineurin but, in this case, to inhibit NFkappaB/NFAT (25). In vitro experiments showed that expression of constitutively active calcineurin in astrocytes abrogated the inflammatory response after TNF-alpha or endotoxins and markedly enhanced neuronal survival (25). Regulated expression of constitutively active calcineurin in astrocytes markedly reduced inflammatory injury in transgenic mice, in a calcineurin-dependent manner (25). Thus, TRESK currents might be involved in abnormal electrical activity in DRG and spinal cord signal conduction and thereby promote increasing cell excitability known to be a factor which may contribute to nociception (26,27).

Attenuated TRESK currents induced by accumulation of lactate or arachidonic acid (from tissue injury) might be responsible for some proportion of neuron hyperexcitability in both DRG and spinal cord.

Additionally, inflammatory mediators exerted may bind to G protein-coupled receptor (GPCR) and induce the Gq activation with subsequent elevated cytoplasmic calcium. Calcineurin (calcium/calmodulin dependent phosphatase 2B) is activated with the enhancement of calcium. Then TRESK shows large calcium-dependent increases in current in response to calcineurin activation. Calcineurin has been shown to bind directly to a nuclear factor of activated T-cells (NFAT)-like docking site on TRESK channels and dephosphorylate them (12, 28). So the NFAT-like docking site may be a key for the phosphorylation of TRESK. The phosphorylation of TRESK in NFAT-like docking sites can be inhibited by FK506, an inhibitor of calcineurin. It is a bi-directional influence of Ca2+ and calmodulin on the calcineurin activity. The strength and duration of particular stimulations such as peripheral injury of nerves may cause apparently antagonistic functions of calcineurin to work in concert (28,29) and then downregulate the TRESK. Thus, we suppose the activation of calcineurin can relieve the pain in part because of the increase of TRESK current. Recently, calcineurin-inhibitor induced pain syndrome (CIPS), which is characterized by severe pain in the lower limbs after use of the calcineurin inhibitor, has been recognized in both organ and stem-cell transplantations (30-34). A possible mechanism for CIPS is that an inhibitor of calcineurin blocks the TRESK channel which downregulate the background current and, thus, enhance the excitory signal transduction of neurons by affecting the velocity of action potential propagation.

At present, the pathway(s) phosphorylating TRESK at rest and maintaining their basal activity is unknown and specific activators of TRESK are uncertain.

Basic Science of Calcineurin-Inhibitor (CI)-Induced Nociception

Sato et al (35) studied the effects of cyclosporine on pain, using the tail-immersion assay to study the pain response (36,37). Four hours after intraperitoneal injection of cyclosporine (1, 10, and 60 mg/kg), the tails of the mice were immersed 2 cm in water of 48°C and the latency time to a rapid tail flick was measured 5 separate times on each animal over a 15-minute period allowing a 3 – 4 minute recovery period between each trial. To prevent injury, the tail was removed from the water within 10 seconds if the animal did not respond. The times for each animal in each group were then averaged to yield a group mean and standard deviations for comparisons (35).

The Calcineurin-Inhibitor, (Cyclosporine), Enhances Pain Reactions

After administration of cyclosporine (10 and 60 mg/kg), tail-flick latencies in the tail immersion test were significantly shortened in both wild-type [$F(3,63) = 13.18$, $P < 0.001$] and knockout mice [$F(3,68) = 15.00$, $P < 0.001$] by one-way ANOVA (30). Two-way ANOVA showed no significant differences between wild-type and knockout mice [$F(1,130) = 2.59$, $P = 0.11$], indicating that cyclosporine induces hyperalgesia in both

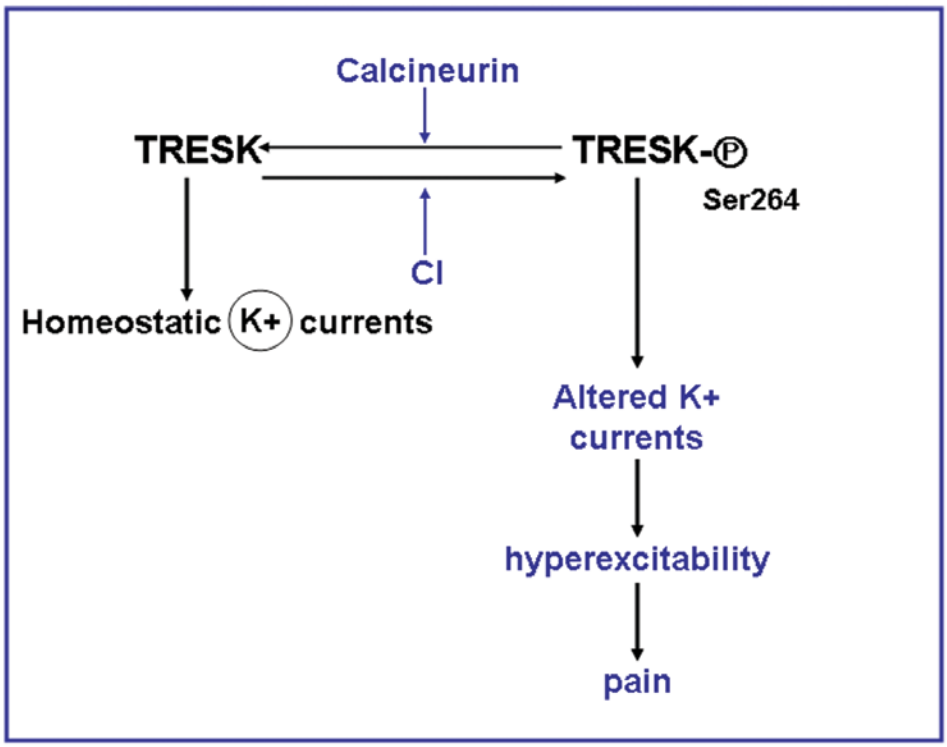

Fig. 1. *Potential mechanism of calcineurin-inhibitor (CI)-induced pain.*

wild-type and multidrug resistant 1 (mdr1) homozygous knockout mice (mdr-1%). (The mdr1 gene encodes for P-glycoprotein, which acts to impede agents like cyclosporine from entering the brain [35].) Knockout mice are genetically engineered mice in which one or more normally existing genes have been inactivated or turned off ("knocked out") in every cell (generally via replacement or disruption).

Sato and colleagues (35) then tested whether cyclosporine would modulate pain responses in wild-type and mdr1a knockout mice. There are several previous studies demonstrating pain sensitivities in mdr1a knockout mice. For example, Thompson et al (38) found that morphine induced greater analgesia in P-glycoprotein knockout mice compared with that in wild-type mice, whereas Scott et al (39) showed that systemic ondansetron increased pain sensitivity in P-glycoprotein knockout mice but had no effect in wild-type mice. Sato et al (35) found that cyclosporine induces hyperalgesia; however, there were no significant differences between wild-type and knockout mice. Therefore, these results suggest that either cyclosporine has a peripheral effect rather than a central effect or that a sufficient quantity reaches the central nervous systems to have hyperalgesic action (35). Thus, Sato and colleagues (35) speculated that the direct action of cyclosporine on peripheral nerves for pain perception might be related to the pathogenesis of calcineurin-inhibitor-induced neuropathic pain in humans.

Potential Mechanisms Which May Contribute to Calcineurin Inhibitor-induced Nociception

Calcineurin-Inhibitors May Affect Nociceptive NO Pathways

Consistent with the findings of Sato et al (36), cyclosporine was shown to attenuate the antinociceptive effects of morphine by activation of a nitric oxide (NO) pathway (40). It has been demonstrated

that cyclosporine reduces catalytic activity of neuronal NO synthase (nNOS) via inhibition of calcineurin-mediated dephosphorylation of nNOS and decreases the production of NO (41,42). nNOS is localized in the peripheral and central nervous systems, although the sites of cyclosporine actions remain unclear. However, desensitization of capsaicin-evoked currents is greatly reduced by cyclosporine in cultured dorsal root ganglion neurons (43).

Calcineurin/Calcineurin-Inhibitors May Regulate Nociceptive Processes Involving TRPV1

The vanilloid receptor TRPV1, a polymodal nonselective cation channel of nociceptive sensory neurons involved in the perception of inflammatory pain, exhibits desensitization in a Ca2+-dependent manner upon repeated activation by capsaicin or protons (44). Mohapatra and Nau (44) generated point mutations at PKA and protein kinase C consensus sites and studied wild type (WT) and mutant channels transiently expressed in HEK293t or HeLa cells under whole cell voltage clamp. Mohapatra and Nau (44) concluded that calcium-dependent desensitization of TRPV1 might be in part regulated through channel dephosphorylation by calcineurin and channel phosphorylation by PKA possibly involving Thr370 as a key amino acid residue.

Calcineurin-inhibitors May Modulate GABA Receptors and the NMDA Receptor Complex

The neurotoxic effects of CIs, such as selective toxicity to glial cells (45) and the induction of apoptosis in oligodendrocytes (46), have been investigated; however, the mechanisms leading to neuropathic pain and causing the allodynia that occurs in the legs remains unclear. The above-mentioned high venous pressure in the lower limbs may have affected not only the bones but also the peripheral nervous system. According to Sander et al (47), CIs have been reported to modulate the activity of both N-methyl-D-aspartate (NMDA) and γ-aminobutyric acid (GABA) receptors. These mechanisms may play a role in contributing to the neuropathic pain-like characteristics of the pain in patients with CIPS, or, alternatively, an unknown neural impairment induced by CIs may have been involved. The itching seen in the area affected by the pain may also have arisen from damage to the peripheral nervous system caused by the CIs. Considering the apparent differences between the features in the patient reported by Noda and colleagues (30) and the others reported, it is tempting to speculate that the pain of CIPS in HSC transplant patients treated with FK might have a pathogenesis and mechanism—which may cause neuropathic pain—that differ from those previously reported for patients with solid organ transplants treated with FK. The use of calcium channel blockers is recommended for the therapy of CIPS because of the ability of these agents to antagonize CSP- or FK-related vasoconstriction (48); however, oral nifedipine had no analgesic effect in the patient of Noda and colleagues (30). Pain relief was obtained for the patient of Noda et al (30) by the administration of intravenous lidocaine combined with oral amitriptyline and clonazepam.

Clinical Science of CI-induced Pain

Aside from the preclinical work which supports the phenomena of CI-induced nociception, it appears that CIs promote pain when used clinically in human subjects. There are multiple case reports of calcineurin-inhibitor induced pain syndrome (CIPS) (34,49-55).

CIPS is an established entity, characterized by severe pain in the lower limbs, frequently with symmetrical involvement, accompanied by radiographic evidence of patchy osteoporosis of bone, bone marrow edema on MRI, and increased uptake on bone scan (48,55). This rare syndrome was first described in organ transplant recipients (56). The pathogenesis remains unclear, but vascular disturbance of bone perfusion and permeability associated with high levels of CSP or FK has been postulated (48,57). Although Kida and colleagues (55) could not perform a thorough radiological examination, they felt that typical clinical symptoms such as debilitating bilateral lower limb pain with preceding intolerable pruritis suggested the diagnosis of CIPS, despite that some of these features might also be seen in patients with complex regional pain syndrome (CRPS) (58,59).

Molina et al (60) reported 9 patients treated with the CI sirolimus who presented with symptoms, clinical findings/features, and scintigraphy changes typical for CRPS. The syndrome was moderate or benign in 8 cases, with total resolution of the symptoms in an average of 3 months without having to modify the SRL dose; the treatment most used was calcitriol at doses of 0.25-0.50 μg per day. Only one patient had intense pain, functional incapacity, erythema, and edema of the affected areas, which remitted immediately after the SRL was replaced by tacrolimus (60).

Fujii et al (33) described a patient with calcineurin

inhibitor-induced irreversible neuropathic pain after allogeneic hematopoietic stem cell transplantation. The patient developed dysesthesia, electric shock-like pain, and severe itching followed by intractable analgesic-resistant pain in the lower extremities). There were no abnormal radiographic findings and there was no improvement with a reduction of CI dosage or with administration of a calcium channel blocker (33).

Calcineurin as a Mediator Signaling in Nociceptive Pathways

Calcineurin may also play a role as a signaling mediator in pronociceptive pathways largely via its role in promoting the activation of nuclear factor of activated T-cells (NFAT).

NFAT activation of gene transcription is regulated by Ca2+ (61). In unstimulated cells, NFAT is phosphorylated and restricted to the cytoplasm. After an increase in [Ca2+]i, cytoplasmic NFAT is dephosphorylated by the Ca2+-dependent serine/threonine phosphatase calcineurin (62). This exposes a nuclear localization signal, allowing NFAT to translocate to the nucleus and initiate transcription through interaction with other transcription factors. Four isoforms of NFAT (NFAT1–4) transcription factors have been identified, several of which have been localized to neuronal tissue (63,64), including DRG neurons (65). Increases in [Ca2+]i and activation of NFAT-mediated transcription have been implicated in the maintenance of pain in response to substance P in spinal neurons (66).

In neurons expressing a green fluorescent protein (GFP)-NFAT4 fusion protein, a 2-minute exposure to bradykinin induced the translocation of GFP-NFAT4 from the cytoplasm to the nucleus (67). Translocation was partially inhibited by the removal of extracellular Ca2+ and was blocked by inhibition of calcineurin (67).

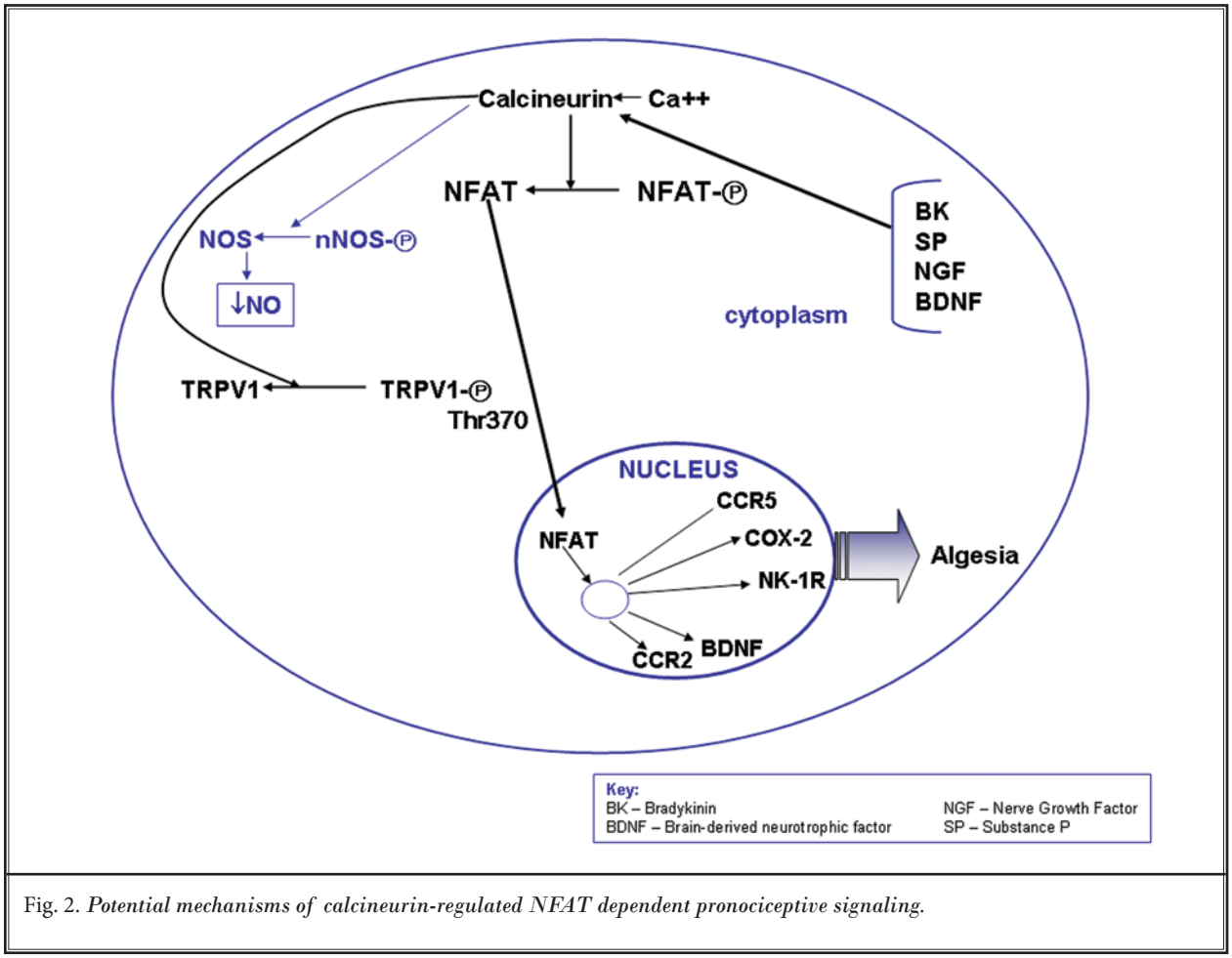

Fig. 2. *Potential mechanisms of calcineurin-regulated NFAT dependent pronociceptive signaling.*

Furthermore, bradykinin triggered a concentration-dependent increase in NFAT-mediated transcription but more of a luciferase-based NFAT gene reporter (ED50 = 24,4 +/- 0.1 nM) which was dependent on the B2 receptor, PLC activation, and inositol triphosphate-mediated Ca2+ release (67). The use of a dual-luciferase (firefly)-based gene reporter assay (known as Promega) (reporter of NFAT activity [pNFAT-luciferase] where the expression of firefly luciferase was normalized to constituitively expressed R. reniformis luciferase activity to correct for differences in transfection efficiency), enabled investigators to reasonably easily assess NFAT activity (67). Quantitative reverse transcription-polymerase chain reaction data indicated that bradykinin elicited an increase in cyclooxygenase mRNA. This increase was sensitive to calcineurin and B2 receptor inhibition. These findings suggest a mechanism by which short-lived bradykinin-mediated stimuli can enact lasting changes in nociceptor function and sensitivity (67).

Neurotrophins have been shown to activate members of the calcineurin (CaN)-regulated, nuclear factor of activated T-cells (NFATc) family of transcription factors within brain (68). Groth et al (68) hypothesized that NFATc transcription factors couple neurotrophin signaling to gene expression within primary afferent and spinal neurons brain. In situ hybridization revealed NFATc4 mRNA within the dorsal root ganglion and spinal cord. In cultured dorsal root ganglion cells, NGF triggered NFAT-dependent and NFAT-independent transcriptional in a CaN-sensitive manner. Further, increased BDNF expression following NGF treatment relied on CaN, thereby suggesting that NGF regulated BDNF transcription via activation of NFATc4. Within cultured spinal cells, BDNF also activated CaN-dependent, NFAT-regulated gene expression. Interestingly, BDNF stimulation increased the expression of the pro-nociceptive genes cyclooxygenase-2, neurokin-1 receptor, inositol trisphosphates receptor type 1, and BDNF itself, through both NFAT-dependent and NFAT-independent transcriptional mechanisms of the brain (68). Groth and colleagues (68) suggested that regulation of pro-nociceptive genes via activation of NFAT-dependent transcription is one mechanism by which NGF and BDNF signaling contributes to the development of persistent pain states in the brain.

Substance *P* activated NFAT-dependent gene transcription in primary cultures of neonatal rat spinal cord transiently transfected with a luciferase DNA report construct (66). The effect of Substance *P* was mediated by neuronal neurokinin-1 receptors that coupled to activation of protein kinase C, L-type voltage-dependent calcium channels and calcineurin (66). Substance *P* had no effect on cyclic AMP response element (CRE)-dependent gene expression, however, calcitonin gene-related peptide, which activated CRE-dependent gene expression, did not activate NFAT signaling (66).

NFAT-mediated transcription plays a role in neuronal development and synaptic plasticity (69). Jackson and colleagues (67) reported that bradykinin, a pronociceptive peptide secreted from the site of injury, activates NFAT signaling in DRG neurons resulting in NFAT-dependent transcription of COX2. Seybold and colleagues (66) reported that NGF and BDNF, pronociceptive neurotrophins, also activate NFAT-dependent transcription. It is likely that the NFAT pathway might not only be activated in the development phase of neuropathic pain as a result of the action of multiple proinflammatory molecules, but also in the maintenance phase where activity and Ca2+-dependent transcription takes place (69). Activation of diverse transcriptional pathways, including NFAT might turn on the transcription of a cohort of genes responsible for sustained hyperexcitability of the DRG in neuropathic pain (69).

Summary

Calcineurin may play various roles in modulating nociception depending on clinical/environmental circumstances. Calcineurin may contribute to normal glial resting membrane potential and interruption of this function may facilitate pronociceptive processes. Furthermore, calcineurin may be involved in the pronociceptive signaling of nuclear factor of activated T-cells (NFAT). NFAT may promote the transcription of pronociceptive genes as well as upregulation of pronociceptive chemokine receptors in the dorsal root ganglion. Thus, it appears that calcineurin functions may promote or dampen nociceptive processes. An appreciation of the various aspects of calcineurin function may be important in addressing suboptimal pain control in certain patients under certain circumstances.

References

1. Czirják G, Vuity D, Enyedi P. Phosphorylation-dependent binding of 14-3-3 proteins controls TRESK regulation. *J Biol Chem* 2008; 283:15672-15680.
2. Czirják G, Enyedi P. Targeting of calcineurin to an NFAT-like docking site is required for the calcium-dependent activation of the background K+ channel, TRESK. *J Biol Chem* 2006; 281:14677-14682.
3. Sano Y, Inamura K, Miyake A, Mochizuki S, Kitada C, Yokoi H, Nozawa K, Okada H, Marsushime H, Furuichi K. A novel two-pore domain K+ channel, TRESK, is localized in the spinal cord. *J Biol Chem* 2003; 278:27406-27412.
4. Ocaña M, Cendán CM, Cobos EJ, Entrena JM. Potassium channels and pain: Present realities and future opportunities. *Eur J Pharmacol* 2004; 500:203-219.
5. Ketchum KA, Joiner WJ, Sellers AJ, Kacxmarek LK, Goldstein SA. A new family of outwardly rectifying potassium channel proteins with two pore domains in tandem. *Nature* 1995; 376:690-695.
6. Yost CS. Update on tandem pore (2P) domain K+ channels. *Curr Drug Targets* 2003; 4:347-351.
7. Lesage F, Lazdunski M, Molecular and functional properties of two-pore-domain potassium channels. *Am J Physiol Renal Phsyiol* 2000; 279:F793-F801.
8. Kim D. Physiology and pharmacology of two-pore domains potassium channels. *Curr Pharm Des* 2005; 11:2717-2736.
9. Goldstein SA, Bockenhauer D, O'Kelly I, Zilberberg N. Potassium leak channels and the KCNK family of two-P-domain subunits. *Nat Rev Neurosco* 2001; 2:175-184.
10. Kang D, Kim D. TREK-2 (K2P 10.1) and TRESK (K2P 18.1) are major background K+ channels in dorsal root ganglion neurons. *Am J Physiol Cell Physiol* 2006; 291:C138-C146.
11. Keshavaprasad B, Liu C, Au JD, Kindler CH, Cotton JF, Yost CS. Species-specific differences in response to anesthetics and other modulators by the K2P channel TRESK. *Anesth Analg* 2005; 101:1042-1049.
12. Czirják G, Toth ZE, Enyedi P. The two-pore domain K+ channel, TRESK, is activated by the cytoplasmic calcium signal through calcineurin. *J Biol Chem* 2004; 279:18550-18558.
13. Chung JM, Chung K. Importance of hyperexcitability of DRG neurons in neuropathic pain. *Pain Pract* 2002; 2:87-97.
14. Song XJ, Vizcarra C, Xu DS, Rupert RL, Wong ZN. Hyperalgesia and neural excitability following injuries to central and peripheral branches of axons and somata of dorsal root ganglion neurons. *J Neurophysiol* 2003; 89:2185-2193.
15. Huang DY, Yu BW, Fan QW. Roles of TRESK, a novel two-pore domain K+ channel, in pain pathway and general anesthesia. *Neurosci Bull* 2008; 24:166-172.
16. Wen YR, Suter MR, Kawasaki Y, Huang J, Pertin M, Kohmo T, Berde CB, Decosterd I, Ji RR. Nerve conduction blockade in the sciatic nerve precents but does not reverse the activation of p38 mitogen-activated protein kinase in spinal microglia in the rat spared nerve injury model. *Anesthesiology* 2007; 107:312-321.
17. Guo W, Wang H, Wantanbe M, Shimizu K, Zou S, LaGraize SC, Wei F, Dubner R, Ren K. Glial-cytokine-neuronal interactions underlying the mechanisms of persistent pain. *J Neurosci* 2007; 27:6006-6018.
18. Tsuda M, Mizokoshi A, Shigmoto-Mogami Y, Koizumi S, Inoue K. Acitvation of p38 mitogen-activated protein kinase in spinal hyperactive microglia contributes to pain hypersensitivity following peripheral nerve injury. *Glia* 2004; 45:89-95.
19. Ji RR, Suter MR. p38 MAPK, microglial signaling, and neuropathic pain. *Mol Pain* 2007; 3:33.
20. Zhuang ZY, Kawasaki Y, Tan PH, Wen YR, Huang J, Ji RR. Role of the CX3CRI/p38 MAPK pathway in spinal microglia for the development of neuropathic pain following nerve injury-induced cleavage of fractalkine. *Brain Behav Immun* 2007; 21:642-651.
21. Milligan ED, Watkins LR. Pathological and protective roles of glia in chronic pain. *Nat Rev Neurosci* 2009: 10:23-36.
22. Pérez-Ortiz JM, Serrano- Pérez MC, Pastor MD, Martin ED, Calvo S, Rincón M, Tranque P. Mechanical lesion activates newly identified NFATc1 in primary astrocytes: Implication of ATP and purinergic receptors. *Eur J Neurosci* 2008; 27:2453-2465.
23. Canellada A, Ramirez BG, Minami T, Redondo JM, Cano E. Calcium/calcineurin signaling in primary cortical astrocyte cultures: Rcan 1-4 and cyclooxygenase-2 as NFAT target genes. *Glia* 2008; 56:709-722.
24. Sama MA, Mathis DM, Furman JL, Abdul HM, Artiushin IA, Kraner SD, Norris CM. Interleukin-1beta-dependent signaling between astrocytes and neurons depends critically on astrocytic calcineurin/NFAT activity. *J Biol Chem* 2008; 283:21953-21964.
25. Fernandez AM, Fernandez S, Carrero P, Garcia-Garcia M, Torres-Aleman I. Calcineurin in reactive astrocytes plays a key role in the interplay between proinflammatory and anti-inflammatory signals. *J Neurosci* 2007; 27:8745-8756.
26. Ji RR, Woolf C. Neuronal plasticity and signal transduction in nociceptive neurons implications for the initiation and maintenance of pathological pain. *Neurobiol Dis* 2001; 8:1-10.
27. Vadivelu N, Sinatra R. Recent advances in elucidating pain mechanisms. *Curr Opin Anaesthesiol* 2005; 18:540-547.
28. Groth RD, Dunbar RL, Mermelstein PG. Calcineurin regulation of neuronal plasticity. *Biochem Ciophys Res Common* 2003; 311:1159-1171.
29. Mansuy IM. Calcineurin in memory and bidirectional plasticity. *Biochem Biophys Res Commun* 2003; 311:1195-1208.
30. Noda Y, Kodama K, Yasuda T, Takajashi S. Calcineurin-inhibitor-induced pain syndrome after bone marrow transplantation. *J Anesth* 2008; 22:61-63.
31. Goffin EJ. Calcineurin-inhibitors and bone pain after organ transplantation. *Kidney Int* 2007; 71:468.
32. Takashima S, Numata A, Miyamoto T, Shirakawa T, Kimoshita R, Kato K, Takenanka K, Harada N, Nagafuji K, Taniguchi S, Harada M. Acute lymphoblastic leukemia presenting with calcineurin-inhibitor induced pain syndrome after a second allogreneic bone marrow transplantation. *Rinsho Ketsueki* 2006; 47:1372-1376.
33. Fujii N, Ikeda K, Koyama M, Aoyama K, Masunari T, Kondo E, Matsuzaki T, Mizobuchi S, Hiraki A, Teshima T, Shinagawa K, Ishimaru F, Tanimoto M. Calcineurin inhibitor-induced irreversible neuropathic pain after allogeneic hematopoietic stem cell transplantation. *Int J Hematol* 2006; 83:459-461.
34. Kida A, Ohashi K, Tanaka C, Kamata N, Akiyama H, Sakamaki H. Calcineruin-

inhibitor pain syndrome following haematopoietic stem cell transplantation. *Br J Haematol* 2004; 126:288.

35. Sato Y, Onaka T, Kobayashi E, Seo N. The differential effect of cyclosporine on hypnotic response and pain reaction in mice. *Anesth Analg* 2007; 105:1489-1493.
36. Dougherty PM, Aronowski J, Samorajski T, Dafny N. Opiate antinociception is altered by immune modification: The effect of interferon, cyclosporine and radiation-induced immune suppression upon acute and long-term morphine activity. *Brain Res* 1986; 385:401-404.
37. Sarton E, Teppema LJ, Olievier C, Nieuwenhuijs D, Matthes HW, Kieffer BL, Dahan A. The involvement of the mu-opioid receptor in ketamine-induced respiratory depression and antinociception. *Anesth Analg* 2001; 93:1495-1500.
38. Thompson SJ, Koszdin K, Bernards CM. Opiate-induced analgesia is increased and prolonged in mice lacking P-glycoprotein. *Anesthesiology* 2000; 92:1392-1399.
39. Scott JA, Wood M, Flood P. The pronociceptive effect of ondansetron in the setting of P-glycoprotein inhibition. *Anesth Analg* 2006; 103:742-746.
40. Homayoun H, Babaie A, Gharib B, Etminani A, Khavandgar S, Mani A, Dehpour AR. The involvement of nitric oxide in the antinociception induced by cyclosporine A in mice. *Pharmacol Biochem Behav* 2002; 72:267-272.
41. Tsuji A. P-glycoprotein-mediated efflux transport of anticancer drugs at the blood-brain barrier. *Ther Drug Monit* 1998; 20:588-590.
42. Golden PL, Pollack GM. Blood-brain barrier efflux transport. *J Pharm Sci* 2003; 92:1739-1753.
43. Docherty RJ, Yeats JC, Bevan S, Boddeke HW. Inhibition of calcineurin inhibits the desensitization of capsaicin-evoked currents in cultured dorsal root ganglion neurones from adult rats. *Pflugers Arch* 1996; 431:828-837.
44. Mohapatra DP, Nau C. Regulation of Ca2+-dependent desensitization in the vanilloid receptor TRPV1 by calcineurin and cAMP-dependent protein kinase. *J Biol Chem* 2005; 280:13424-13432.
45. Stoltenburg-Didinger G, Boegner F. Glia toxicity in dissociated cell cultures induced by cyclosporine. *Neurotoxicology* 1992; 13:179-184.
46. McDonald JW, Goldberg MP, Gwag BJ, Chi SI, Choi DW. Cyclosporine induces neuronal apoptosis and selective oligodendrocyte death in cortical cultures. *Ann Neurol* 1996; 40:750-758.
47. Sander M, Lyson T, Thomas GD, Victor RG. Sympathetic neural mechanism of cyclosporine-induced hypertension. *Am J Hypertens* 1996; 9:121S-138S.
48. Grotz WH, Breitenfeldt MK, Braune SW, Allmann KH, Krause TM, Rump JA, Schollmeyer PJ. Calcineurin-inhibitor induced pain syndrome (CIPS): A severe disabling complication after organ transplantation. *Transplant Int* 2001; 14:16-23.
49. Ferrari U, Empl M, Kim KS, Sostak P, Förderreuther S, Straube A. Calcineurin inhibitor-induced headache: Clinical characteristics and possible mechanisms. *Headache* 2005; 45:211-214.
50. Malat GE, Dupuis RE, Kassman B, Rhoads JM, Freeman K, Lichtman S, Johnson MW, Gerber D, Andreoni K, Fair J. Tacrolimus-induced pain syndrome in a pediatric orthotopic liver transplant patient. *Pediatr Transplant* 2002; 6:435-438.
51. Franco M, Blaimont A, Albano L, Bendini C, Cassuto E, Jaeger P. Tacrolimus pain syndrome in renal transplant patients: Report of two cases. *Joint Bone Spine* 2004; 71:157-159.
52. Collini A, De Bartolomeis C, Barni R, Ruggueri G, Bernini M, Carmellini M. Calcineurin-inhibitor induced pain syndrome after organ transplantation. *Kidney Int* 2006; 70:1367-1370.
53. Rahman AH, O'Brien C, Patchett SE. Leg bone syndrome in a patient with ulcerative colitis treated with cyclosporin. *Ir J Med Sci* 2007; 176: 129-131.
54. Nishikawa T, Okamoto Y, Tanabe T, Shinkoda Y, Kodama Y, Tsuru Y, Kawano Y. Calcineurin-inhibitor-induced pain syndrome after a second allogenic bone marrow transplantation for a child with aplastic anemia. *Pediatr Transplant* 2008, ahead of print.
55. Kida A, Ohashi K, Kobayashii T, Sakai M, Yamashita T, Akiyama H, Kishida S, Sakamaki H. Incapacitating lower limb pan syndrome in cord blood stem cell transplant recipients with calcineurin inhibitor. *Pathol Oncol Res* 2004; 10:204-206.
56. Lucas VP, Ponge TD, Plougastel-Lucas ML, Glemain P, Hourmant M, Soulillou JP. Musculoskeletal pain in renal-transplant recipients. *N Engl J Med* 1991; 325:1449-1459.
57. Villaverde V, Cantalejo M, Balsa A, Mola EM. Leg bone pain syndrome in a kidney transplant patients treated with tacrolimus (FK506). *Ann Rheum Dis* 1999; 58:653-654.
58. Blombery PA. A review of reflex sympathetic dystrophy. *Aust Fam Phsycian* 1995; 24:1651-1655.
59. Munoz-Gomez J, Collado A, Gratacós J, Campistol JM, Lomeña F, Liena J, Andreu J. Reflex sympathetic dystrophy syndrome of the lower limbs in renal tranplant patients treated with cyclosporin A. *Arthritis Rhuem* 1991; 34:625-630.
60. Molina MG, Diekmann F, Burgos D, Cabello M, Lopez V, Oppenheimer F, Navarro A, Campistol J. Sympathetic dystrophy associated with sirolimus therapy. *Transplantation* 2008; 85:290-292.
61. Dolmetsch RE, Xu K, Lewis RS. Calcium oscillations increase the efficiency and specificity of gene expression. *Nature* 1998; 392:933-936.
62. Beals CR, Sheridan CM, Turck CW, Gardner P, Crabtree GR. Nuclear export of NF-ATc enhanced by glycogen synthase kinase-3. *Science* 1997; 275:1930-1934.
63. Graef IA, Wang F, Charron F, Chen L, Neilson J, Tessier-Lavigne M, Crabtree GR. Neurotrophins and netrins require calcineurin/NFAT signaling to stimulate outgrowth of embryonic axons. *Cell* 2003; 113:657-670.
64. Groth RD, Mermelstein PG. Brain-derived neurotrophic factor activation of NFAT (nuclear factor of activated T-cells)-dependent transcription: A role for the transcription factor NFATc4 in neurotrophin-mediated gene expression. *J Neurosci* 2003; 23:8125-8134.
65. Kim M, Thayer SA, Usachev YM. Mitochondria regulates activation of the transcription factor NFAT in DRG neurons. *Soc Neurosci Abstr* 2006; 32:321-329.
66. Seybold VS, Coicou LG, Groth RD, Mermelstein PG. Substance P initiates NFAT-dependent gene expression in spinal neurons. *J Neurochem* 2006; 97:397-407.
67. Jackson JG, Usachev YM, Thayer SA. Bradykinin-Induced nuclear factor of activated t-cells-dependent transcription in rat dorsal root ganglion *Neurons Mol Pharmacol* 2007; 72:303-310.
68. Groth RD, Coicou LG, Mermelstein PG, Seybold VS. Neurotrophin activation

of NFAT-dependent transcription contributes to the regulation of pro-nociceptive genes. *J Neurochem* 2007; 102:1162-1174.

69. Bhangoo SK, Ren D, Miller RJ, Chan DM, Ripsch MS, Weiss C, McGinnis C, White FA. CXCR4 chemokine receptor signaling mediates pain hypersensitivity in association with antiretroviral toxic neuropathy. *Brain Behav Immun* 2007; 1:581-591.