

The role of calcitonin gene-related peptide in peripheral and central pain mechanisms including migraine

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Abstract

Calcitonin gene-related peptide (CGRP) is a 37-amino acid peptide found primarily in the C and A δ sensory fibers arising from the dorsal root and trigeminal ganglia, as well as the central nervous system. Calcitonin gene-related peptide was found to play important roles in cardiovascular, digestive, and sensory functions. Although the vasodilatory properties of CGRP are well documented, its somatosensory function regarding modulation of neuronal sensitization and of enhanced pain has received considerable attention recently. Growing evidence indicates that CGRP plays a key role in the development of peripheral sensitization and the associated enhanced pain. Calcitonin gene-related peptide is implicated in the development of neurogenic inflammation and it is upregulated in conditions of inflammatory and neuropathic pain. It is most likely that CGRP facilitates nociceptive transmission and contributes to the development and maintenance of a sensitized, hyperresponsive state not only of the primary afferent sensory neurons but also of the second-order pain transmission neurons within the central nervous system, thus contributing to central sensitization as well. The maintenance of a sensitized neuronal condition is believed to be an important factor underlying migraine. Recent successful clinical studies have shown that blocking the function of CGRP can alleviate migraine. However, the mechanisms through which CGRP may contribute to migraine are still not fully understood. We reviewed the role of CGRP in primary afferents, the dorsal root ganglion, and in the trigeminal system as well as its role in peripheral and central sensitization and its potential contribution to pain processing and to migraine.

Keywords: Calcitonin gene-related peptide, Peripheral sensitization, Central sensitization, Primary afferent neurons, Dorsal root ganglion, Trigeminal system, Migraine, Pain

1. Introduction

Recent developments in the treatment of migraine, along with advances in the understanding of the structure and function of the calcitonin gene-related peptide (CGRP) receptor have spurred an increased interest in the role of this neuropeptide. Although CGRP was identified over 30 years ago, its precise role in the transmission and modulation of nociceptive signals, especially regarding migraine, is not fully understood. The receptors that bind CGRP have only recently been fully characterized. In contrast to many G-protein-coupled receptors, the CGRP receptor requires the heterodimerization of 2 components, the receptor activity-modifying protein 1

(RAMP1) accessory protein and the calcitonin receptor-like receptor (CLR) protein, to efficiently couple to the G_s subunit.^{152,159,160,190} Moreover, CGRP has affinity to other receptors, including the amylin 1 (AMY1) receptor, which is also a heterodimer containing RAMP1 but with the calcitonin receptor (CT) protein.^{159,160,190} Calcitonin gene-related peptide's affinity for AMY1 is comparable with the CGRP receptor, and has been proposed as a second CGRP receptor site.¹⁸⁵ In addition to its role in the somatosensory system, CGRP is also an extremely potent vasodilator, and vascular beds, including the mesenteric and the meningeal vasculatures, richly express CGRP receptors.^{59,136} In the meninges, the middle meningeal and the cerebral arteries are innervated by primary afferent trigeminal fibers that express CGRP.^{59,136} Evolving evidence of CGRP's role in the trigeminal system and the recent success of clinical trials with monoclonal antibodies directed at CGRP or its receptor, coupled with the clinical efficacy of CGRP receptor antagonists, have provided certain evidence that CGRP is a critical component for the pathogenesis of migraine. However, the mechanism by which CGRP plays a role in migraine is unclear. Moreover, although there is certainly overlap between the function of CGRP at somatic and cephalic sites, there are also some important mechanistic differences. In light of these developments, we have undertaken a review of the literature to better understand the state of the art regarding CGRP in pain-processing pathways, and contrast this with what is understood about migraine pain.

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

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PAIN 158 (2017) 543–559

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<http://dx.doi.org/10.1097/j.pain.0000000000000831>

2. Calcitonin gene-related peptide and the calcitonin gene-related peptide receptor

Calcitonin gene-related peptide and its receptor are widely expressed in somatosensory and autonomic peripheral nerves, the cardiovascular and the enteric systems, and are increasingly described in neuroanatomical tracts of the central nervous system. The prominent localization of CGRP in A δ - and C-fiber peripheral nerves has elicited considerable attention to this peptide, but its functional role in pain transmission is still unclear.

2.1. Pharmacology

This 37-amino acid neuropeptide was discovered serendipitously when Amara et al.⁶ found that alternate splicing of the gene for CT produced a very different peptide, which was eventually designated as α -CGRP. Shortly thereafter, a novel gene coding a homologous peptide was discovered.⁵ This peptide, β -CGRP, differs from α -CGRP by a single amino acid in the rat, and 3 amino acids in humans.^{5,56,138,157} Based on receptor-binding assays and in vitro bioassays, the biological activity of these 2 forms of CGRP is nearly identical, although there are small differences in potency.^{5,56,138,145} α -CGRP and β -CGRP, along with the homologous proteins pro-CT, CT, adrenomedullin (AM), amylin (AMY), and intermedin (AM 2), as well as the C-terminal fragments, CGRP₍₁₈₋₃₇₎ and CGRP₍₁₉₋₃₇₎, form the CT receptor family of peptides.^{11,23,117,152} In addition, these CGRP fragments have been found in the rat spinal cord, and the CGRP₍₁₉₋₃₇₎ fragment is an antagonist of the receptor.⁷

Until fairly recently, the CGRP antagonists available for the examination of CGRP receptors were peptidic fragments and analogs based on the CGRP molecule. These early pharmacologic studies, using isolated tissue preparations, suggested the existence of a CGRP₁ receptor, based on a greater sensitivity to the peptidic antagonist CGRP₈₋₃₇, and a CGRP₂ receptor that was more sensitive to the CGRP analog antagonists [Cys(ACM) 2,7]- and [Cys(Et)2,7]hCGRP.¹⁵⁹ The development of selective, nonpeptidic antagonists has allowed more detailed investigations of the CGRP receptors. The CGRP receptor is considered to be atypical among G-protein-coupled receptors, as the functional receptor is composed from the heterodimerization of 2 different peptides.¹⁵⁹ This construct provided challenges in its discovery and characterization.¹⁵⁹ Consequently, there is overlap among activities of CGRP, AMY, and AM in this family of receptors.^{159,160,190} The proposed CGRP₂ receptor has never been identified, and it is believed that this was an artifact because of the ability of CGRP to bind to the AM and AMY receptors. Expression cloning strategies, which successfully cloned the CT receptor, were applied to the search for the CGRP receptor. Although a CLR (formerly CRLR) was identified, it did not show any binding or activity in response to CGRP and was long considered to be an orphan receptor. However, expression studies performed with isolated cell cultures revealed that the combined expression of CLR with a RAMP was required to express the pharmacologic phenotype of the CGRP receptor. At least 3 RAMPs have been identified (RAMP1, RAMP2, and RAMP3). The dimerization of CLR with RAMP1 shows the highest affinity for CGRP and corresponds to the previously characterized CGRP₁ receptor in pharmacologic activity (Fig. 1). Dimerizations of CLR with RAMP2 or RAMP3 produce the AM1 and AM2 receptors. Moreover, RAMPs can also combine with the CT receptor to form the AMY receptors AMY1–3.^{42,152,159} Importantly, as CGRP, AMY, and AM have considerable homology, they also have differing degrees of binding affinity and function for each of these receptors. For

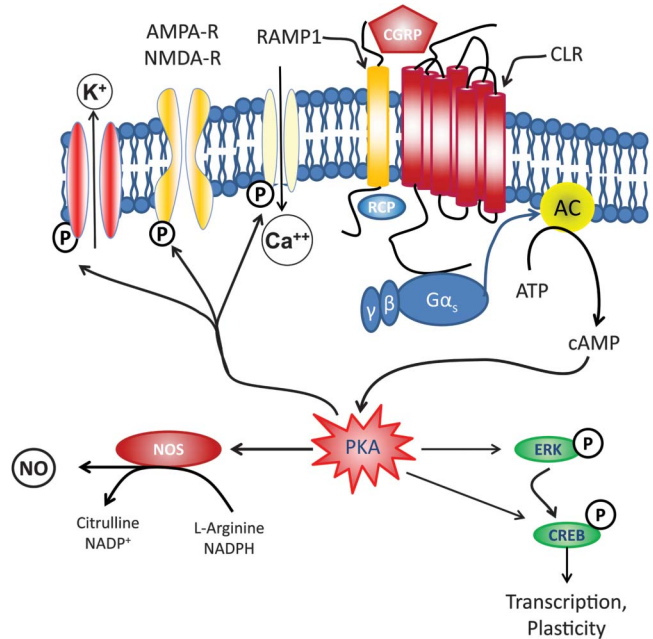


Figure 1. Schematic representation of a CGRP receptor. A functional calcitonin gene-related peptide (CGRP) receptor requires 3 components. The calcitonin receptor-like receptor (CLR) which consists of 7 transmembrane domains, the receptor activity-modifying protein 1 (RAMP1), which confers specificity for binding with CGRP, and the RCP, which seems to be critical for signal transduction by promoting effective coupling to the G_s protein. G_s activates adenylyl cyclase (AC), which catalyzes the conversion of adenosine triphosphate (ATP) to cyclic adenosine monophosphate (cAMP), which in turn activates protein kinase A (PKA). Activated PKA can then regulate the activity of numerous intracellular processes, including the K⁺ channels, L-type Ca²⁺ channels, and cAMP response element binding (CREB) protein. Thus, the downstream effects of activation of the CGRP receptor include neuronal excitability, neurotransmitter release, nitric oxide (NO) production, and vasodilation. AMPA-R, amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor; ERK, extracellular receptor-activated kinase; NMDA-R, N-methyl-D-aspartate receptor; NOS, nitric oxide synthase; P, phosphorylation sites; RCP, receptor component protein.

example, CGRP binds to the AMY1 receptor (ie, CT/RAMP1), although at one-tenth the affinity of AMY itself, and this affinity may account for the reported displacement of AMY by CGRP in the nucleus accumbens.^{42,160} Thus, although CGRP acts most prominently via the CGRP receptor, it is nonetheless capable of exerting functional activity through these related receptors as well. Notably, however, a recent study using TaqMan G protein-coupled receptor arrays and immunohistochemistry found that the AMY1 receptor has high affinity for CGRP that is comparable with the CGRP receptor, and proposed that it be considered as a dual CGRP/AMY receptor.¹⁸⁵ It is present in the human trigeminal ganglion (TG), and may represent a second CGRP receptor site pertinent to migraine.¹⁸⁵ In addition to CLR and the RAMPs, a third protein, the receptor component protein, is important for the full expression of the CGRP receptor activity.^{159,160,190} This protein is responsible for effective coupling of the CLR to the G_s subunit, allowing activation of adenylyl cyclase.¹⁵⁹

2.2. Distribution

The distribution of CGRP and the CGRP receptor is largely conserved across mammalian species.¹⁹⁰ The CGRP is almost exclusively found in neurons and is most abundantly expressed in sensory nerves.^{152,159} Immunohistochemical and in situ

hybridization studies revealed that α -CGRP predominates in sensory nerves with cell bodies in the dorsal root ganglion (DRG) and TG, whereas β -CGRP predominates in motor neurons and is almost exclusively expressed in the enteric nervous system.^{138,145} In the TG, labeling for α -CGRP was 10-fold greater than for β -CGRP.⁵ Messages for both α -CGRP and β -CGRP are found in small- and medium-diameter DRG neurons, whereas large-diameter DRG neurons predominantly express mRNA for α -CGRP, suggesting that the expression of the protein is likely regulated at the transcriptional level.¹⁴⁵

The highest concentrations of CGRP have been found in the outer laminae of the spinal cord dorsal horn and in the trigeminal nucleus caudalis (TNC), which correspond to the central terminals of primary afferent neurons with soma in the DRG and TG, respectively.^{63,64,66,152,159,160} Importantly, in these regions, CGRP is localized within the primary afferent terminals, and there is little evidence of CGRP in cell bodies of second-order neurons. This distribution is consistent with the role of CGRP as a primary afferent transmitter.^{64,68,69,119} Calcitonin gene-related peptide is also found in peripheral fibers innervating the heart, coronary arteries, vascular beds, and myenteric system.^{157,159,160,190} The CGRP receptor is highly concentrated in the spinal cord, particularly the dorsal horns of the spinal cord and cerebral gray matter, and overlaps with the distribution of CGRP protein.¹⁴⁵ Of relevance to migraine, in the TG, CGRP is primarily found in the small- and medium-diameter neurons, and the CGRP receptors, as defined by CLR and RAMP1 immunoreactivity, are found on large-diameter TG neurons that do not express CGRP and on satellite glial cells.^{63,66} Other studies also found very little expression of CGRP receptors on neurons that also express the CGRP peptide.¹³⁶ The CGRP receptor is also highly expressed in the meningeal vasculature, which is innervated by primary afferent fibers from the TG that express CGRP.^{63,66} Recent studies using antibodies that specifically recognize the CGRP-binding site, which was discovered by using a fusion protein of the extracellular domains of RAMP1 and CLR, showed binding to human TG neurons and human vascular smooth muscle cells of the meningeal vasculature.¹³⁶ More detailed examination of tissues obtained from cynomolgus monkeys confirmed the presence of the CGRP receptor on dural vascular smooth muscle cell, neurons, and satellite glial cells of the TG and TNC neurons.¹³⁶ In that study, binding to both postsynaptic and presynaptic sites were observed, but the CGRP receptor was predominantly expressed by second-order neurons of the TNC.¹³⁶

There is a growing understanding of the neuroanatomical tracts that include CGRP-expressing neurons in addition to the primary afferent fibers (Fig. 2). Immunohistochemical, autoradiographic, and in situ hybridization studies performed with rat or human brains have provided evidence for the presence of CGRP or for mRNA for CGRP in the cerebellum, hippocampus, hypothalamus, amygdala, basal ganglia, parabrachial nucleus, Kölliker-Fuse nucleus, striatum, colliculi, TNC, medullary cranial motor nuclei, nucleus ambiguus, peripeduncular, posterior, centromedial thalamic nuclei, central gray, and the inferior colliculus.^{67,109,110,157,159,160,180,190} Growing evidence shows that the parabrachial region is an important component of ascending transmission of nociceptive signaling, and there are significant projections from the parabrachial region to the central nucleus of amygdala (CeA) that express CGRP.^{45,88,156} The projections from the parabrachial region to the rostral ventromedial medulla, which are likely pronociceptive and engage descending pain facilitatory systems from the rostral ventromedial medulla, were found recently to not express CGRP.¹⁵⁶

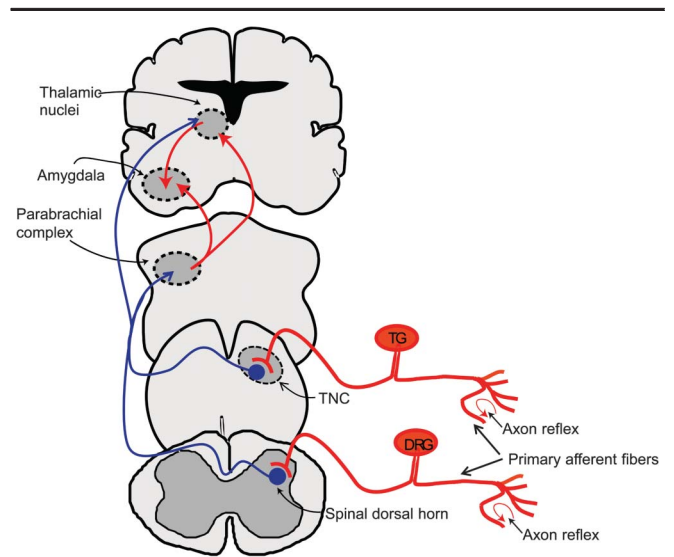


Figure 2. Schematic representation of pain pathways where calcitonin gene-related peptide (CGRP) is expressed. The tracts that contain nerve fibers expressing CGRP are shown in red, and the non-CGRP-expressing tracts are shown in blue. The central terminals of primary afferent fibers, some of which express CGRP, provide nociceptive inputs to second-order neurons of the spinal dorsal horns and the trigeminal nucleus caudalis (TNC). The second-order neurons project to supraspinal sites, particularly the parabrachial and thalamic nuclei, which then can send projections to cortical sites including the insula. Calcitonin gene-related peptide has been found in projections from parabrachial neurons to the posterior intralaminar thalamic complex and to the amygdala/striatal region, including the central nucleus of the amygdala (CeA). This region also receives inputs from the posterior intralaminar thalamic complex that express CGRP. In the periphery, stimulation of sensory nerve endings can elicit an axon reflex, releasing CGRP from adjacent axonal branches, thus propagating a neurogenic inflammation. DRG, dorsal root ganglion; TG, trigeminal ganglion.

High densities of binding for [¹²⁵I]CGRP were found in the molecular layer and Purkinje cell layer of the human cerebellum⁶⁹ and for [³H]MK-3207 in the molecular layer of rhesus cerebellum,⁶⁷ suggesting the possibility of a functional role in primate cerebellar cortex. Using the radiolabeled compound [¹¹C]MK-4232 as a tracer for identification of CGRP-binding sites, positron emission tomography (PET) found the highest concentrations of CGRP receptors in the cerebellum and brainstem of rhesus monkeys and the cerebellum of humans.⁹² In the same study, autoradiography and in vitro binding studies found that the highest densities of CGRP receptors were in the cerebellum and substantia nigra, whereas moderate densities were found in the meninges, brainstem, and hippocampus in both rhesus monkeys and humans.⁹² Although these CGRP receptors are believed to be functional, their role in the cerebellum regarding nociception or migraine remains unclear. Evidence exists showing that the cerebellum may play a role in nociception and migraine, and PET has shown activation of the cerebellum during migraine attacks.^{9,24,137}

Most recently, autoradiography, in situ hybridization, and immunofluorescence were used to localize CGRP receptor expression in rhesus monkey brain.⁶⁵ High densities of the CGRP receptor were found in the pineal gland, medial mammillary nucleus, median eminence, infundibular stem, periaqueductal gray (PAG), dorsal raphe, posterior hypothalamic area, trochlear nucleus, and the medial lemniscus.⁶⁵ Binding to [³H]MK-3207, indicative of CGRP receptors, was also detected in the spinal TNC, nucleus gracilis, dorsal and ventral horns of the spinal cord, pontine raphe nucleus, pontine nuclei, area postrema, inferior olive, the centromedial part of the dorsal

motor nucleus of vagus, and the hypoglossal nucleus.⁶⁵ Many of these regions, such as the PAG, the raphe areas, and the nucleus gracilis, receive somatosensory inputs, are implicated in the transmission or modulation of nociceptive signaling, and have also been implicated in migraine headache.^{47,48,58,134,150}

3. The presence of calcitonin gene-related peptide in dorsal root ganglion primary afferent neurons

As the distribution of CGRP and its receptor suggests, this neuropeptide is associated with activity in the gastrointestinal, cardiovascular, and nociceptive systems.^{152,159,160} Calcitonin gene-related peptide is expressed in nerve endings of the mesenteric plexus, coronary, and meningeal arteries, and is a very potent vasodilator.^{25,109} In addition, CGRP also plays a prominent role in the transmission and modulation of pain signals.^{14,159} Calcitonin gene-related peptide is expressed in approximately 45% of DRG neurons, predominantly in small-diameter unmyelinated C-fibers.^{8,158} These peptidergic nociceptors, which also coexpress substance P, represent approximately one-half of C-fiber nociceptors.^{8,158} The DRG neurons also express the tyrosine kinase A receptor (TrkA), and are thus responsive to nerve growth factor (NGF); approximately one-half of DRG neurons also express the transient receptor potential vanilloid type 1 (TRPV1) ion channel.¹³³ Calcitonin gene-related peptide is also expressed in small-to-medium-diameter A δ fibers, approximately 70% of which are nociceptors, as well as in some large-diameter A β sensory fibers and A α motor neurons.^{8,158}

Studies combining electrophysiological and histochemical approaches have shown that most CGRP-expressing neurons are either C-fiber or A δ -fiber nociceptors and include thermal-sensitive, high-threshold mechanonociceptors and polymodal nociceptors.^{116,158} A small population of high-threshold mechanosensitive A β fibers has also been identified. Low-threshold mechanosensitive DRG neurons and those responsive to cool stimuli were not found to express CGRP.^{116,158} The central terminations of primary afferent neurons that express CGRP are also consistent with nociceptive processing, as these are found primarily in the outer laminae of the spinal dorsal horn and in lamina V, although there is some CGRP expression around the central canal. A recent study using immunofluorescent histochemistry and anterograde axonal tracing techniques found that the large-diameter CGRP neurons most likely are high-threshold mechanosensitive A β and A δ fibers that synapse with wide-dynamic-range (WDR) neurons of laminae IV and V.¹⁰⁶ Although it is unclear whether these fibers are nociceptive, they are believed to have polymodal mechanoreceptor functions, and their interaction with the WDR neurons would still allow these fibers to influence nociceptive inputs and central sensitization.¹⁰⁶

4. Peripheral effects of calcitonin gene-related peptide

The DRG and TG neurons are pseudounipolar, meaning that the axon bifurcates and sends projections both to the dorsal horn of the spinal cord or brainstem and to the periphery.¹⁰ This unique morphology allows the release of transmitters at central and peripheral sites, and allows bidirectional communication between the 2 terminals.¹⁰ In the periphery, the primary afferent nociceptors terminate as free nerve endings innervating peripheral structures.¹⁰ Tissue injury results in the release of numerous proinflammatory mediators from the injured cells, mast cells, leukocytes, and the nerve terminals. These substances include

the tachykinins, bradykinin, prostaglandins, serotonin, histamine, as well as the release of CGRP and substance P from the nerve endings.¹⁰ These proinflammatory mediators can directly activate the nociceptors, generate action potentials, or facilitate the firing of the nociceptors, resulting in peripheral sensitization and hyperalgesia.¹⁰ Activation of a free nerve ending can produce an axon reflex action, which causes the release of CGRP and substance P from adjacent-free endings of the same axon, resulting in the evoked release of additional proinflammatory mediators. This in turn can further excite adjacent nerve endings and thus propagate an expanding area of neurogenic inflammation, indicated by the erythematous flare response.^{10,93,163,165}

5. Peripheral sensitization and the role of calcitonin gene-related peptide

An increase in the activity of peripheral nerves in response to local tissue injury or inflammatory processes results in sensitization of peripheral nerves. Injured tissue releases proinflammatory substances that can decrease the firing thresholds of peripheral nociceptors (ie, sensitizers) as well as mediators that activate (ie, activators) these neurons. Consequently, the peripheral afferent neurons become sensitive to further inputs, thus amplifying pain signals.^{10,189,192} Over time, the peripheral nerve increases the production of peptidic neurotransmitters and of ion channels, which also lend to an increase in the somatosensory signals.^{10,189,192} Peripheral sensitization included decreased threshold of a peripheral sensory neuron to firing, increased neuronal responses to a stimulus, and enlargement of the receptive field. Peripheral sensitization manifests clinically as hyperalgesia and allodynia.¹⁰

5.1. Peripheral sensitization

Peripheral sensitization and the associated hyperalgesia are initiated and maintained in part by the actions of CGRP (**Fig. 3A**). Studies performed with human volunteers showed that the intradermal injection of up to 0.25 nmol of CGRP into the skin of the forearm or back produced localized erythema and distant flare that lasted over 12 hours, consistent with its potent vasodilatory action.⁷⁵ Others have shown that 15 pmol injected intradermally into the forearm produced vasodilation lasting 5 to 6 hours.²⁵ The long duration of the vasodilatory effect of CGRP contrasts with its short half-life of only 7 minutes, suggesting perhaps prolonged receptor activation or the engagement of long-lasting intracellular mechanisms.⁸⁷ The intradermal application of substance P or CGRP through dialysis tubing in the forearm of human volunteers did not produce any sensations of pain or axon reflex erythema.¹⁸⁸ Substance P provoked vasodilation and plasma protein extravasation, whereas CGRP produced a potent vasodilation only, without plasma extravasation.¹⁸⁸ Importantly, plasma extravasation and histamine release occurred at high concentrations of substance P ($<10^{-8}$ M and 10^{-5} M, respectively) that exceeded normal physiologic concentrations.¹⁸⁸ It was concluded that substance P or CGRP most likely do not directly mediate pain, but that they may contribute to sensitization and pain facilitation.¹⁸⁸ In a different study, intense electrical stimulation of cutaneous nerves of human volunteers, which released substance P and CGRP, did not evoke mast cell degranulation, histamine release, or plasma protein extravasation.¹⁶⁶ Moreover, capsaicin evoked the release of CGRP from human iris slices, whereas substance P was not detectable in these studies.^{80,81} In addition, the failure of substance P antagonists to block acute migraine attacks convincingly demonstrated that migraine is not due to meningeal plasma

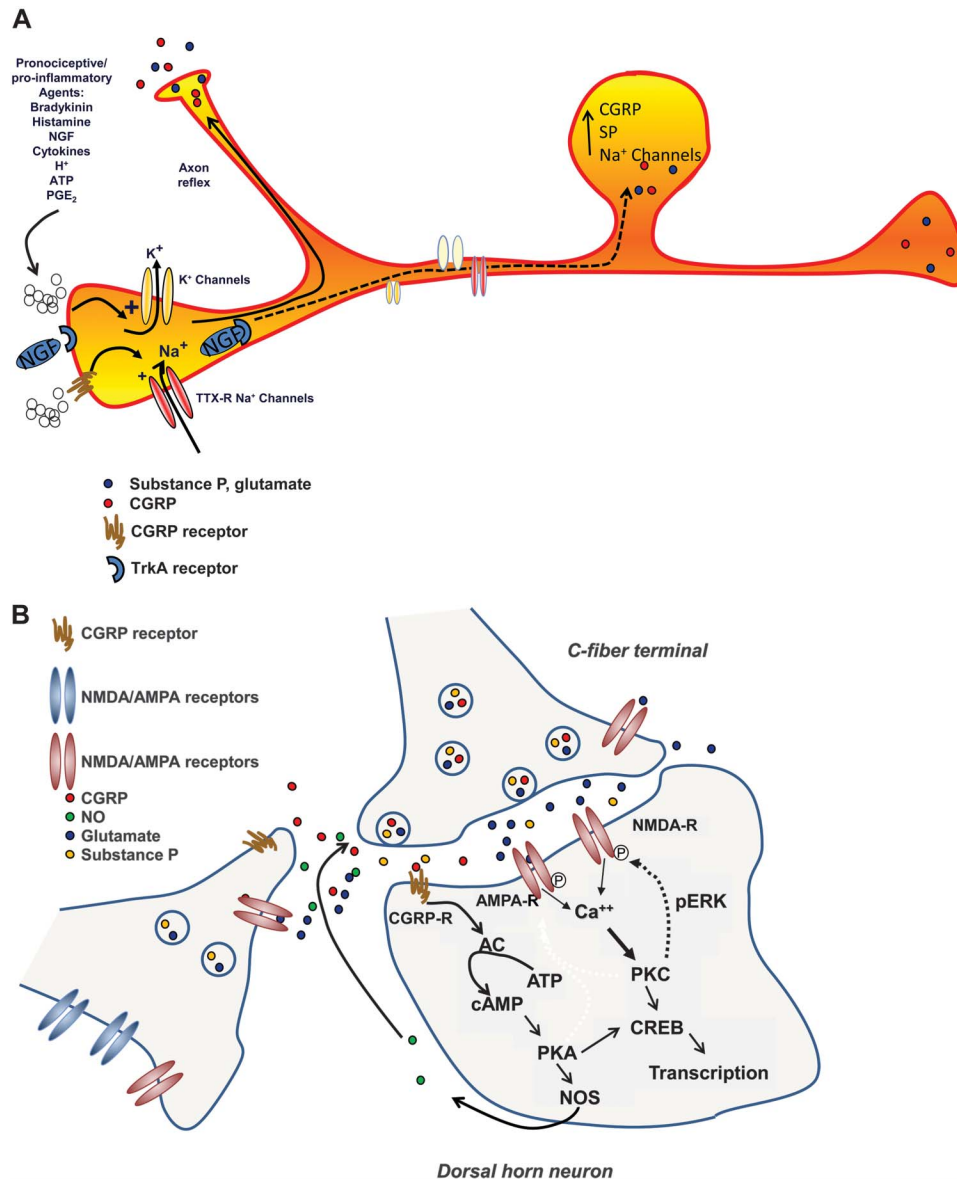


Figure 3. (A) Peripheral sensitization. Damage to tissue or nerves in the periphery results in the localized release of proinflammatory mediators such as bradykinin, histamine, chemokines and cytokines, nerve growth factor (NGF), adenosine triphosphate (ATP), prostaglandin E₂ (PGE₂) and creates acidic conditions (eg, H⁺). These substances can lower the activation threshold of peripheral nociceptors (ie, sensitization) or trigger action potentials, thus activating the nociceptors. Action potentials can cause release of excitatory transmitters from other branching terminals of the same axon (ie, axonal reflex), which elicits the release of inflammatory mediators from adjacent tissue and excites adjacent nerve endings, thus spreading the inflammatory response. Persistent activation of the nociceptors over time, along with the retrograde transport of NGF bound to its receptor, tyrosine kinase A (TrkA-NGF complex) causes transcriptional changes, resulting in the increased production of sodium channels, calcitonin gene-related peptide (CGRP), and substance P (SP). These changes result in enhanced excitability of the peripheral nerve and enhanced release of CGRP and SP, thus maintaining a state of peripheral sensitization. (B) Central sensitization. Increased outputs from primary afferent terminals increases the excitability of postsynaptic neurons, directly and through activation of secondary mechanisms. Activation of the N-methyl-D-aspartate (NMDA) receptor increases calcium inputs and sensitizes the neuron to further inputs, such that inputs that would normally be subthreshold are sufficient to generate an action potential. Calcitonin gene-related peptide receptor activation activates the G_s protein and elicits a number of signaling cascades that serve to enhance neuronal excitability. The downstream activation of protein kinase A (PKA) and protein kinase C (PKC) promotes phosphorylation of NMDA receptors and of calcium ion channels, increasing their activity. Protein kinase A can also increase nitric oxide synthase (NOS) activity, increasing the release of nitric oxide (NO). Nitric oxide along with glutamate and PGE₂ can act as retrograde transmitters increasing the output from primary afferent terminals. Moreover, the enhanced release of CGRP can also activate presynaptic CGRP receptors (CGRP-R) of adjacent terminals, further enhancing transmitter release. Over time, the increased phosphorylation of PKA and PKC can lead to transcriptional changes, such as the upregulation of receptors and ion channels, which increase the excitability of the second-order neuron, thus maintaining a state of central sensitization. AC, adenylyl cyclase; AMPA, amino-3-hydroxy-5-methylisoxazole-4-propionic acid; AMPA-R, amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; CREB, cAMP response element binding; NMDA-R, N-methyl-D-aspartate receptor; pERK, phosphorylated extracellular signal-regulated kinase.

protein extravasation induced by substance P.⁸¹ The dermal application of capsaicin to the forearm of healthy adult volunteers, through the activation of TRPV1 receptors on cutaneous nociceptors, evokes the local release of CGRP, resulting in increased dermal blood flow which presents as the flare

response.¹⁶⁸ Oral administration of the CGRP antagonist telcagepant and injection of the humanized monoclonal CGRP antibody galcanezumab (LY2951742) both blocked capsaicin-induced increases in dermal blood flow, as measured by laser Doppler perfusion imaging in healthy human subjects.^{168,183}

Taken together, these observations all support the contention that CGRP is the major mediator of neurogenic dilator responses arising from the activation of sensory nerves in humans.⁸¹ This situation contrasts with the rat, where these neuropeptides produce not only vasodilation and plasma protein extravasation but also histamine release and mast cell degranulation.¹⁶⁶

Studies performed in several animal models also support the idea that peripheral CGRP release supports nociceptor sensitization associated with hyperalgesia, while not eliciting nociceptive signals alone. For example, repeated subplantar injections of low doses (0.1 fmol) of CGRP into the paws of rats resulted in peripheral sensitization, indicated by a significant reduction in response threshold to a noxious mechanical stimulus.¹³⁹ In that study, a challenge injection with the same low dose of CGRP after response latencies returned to baseline levels resulted in a pronounced and long-lasting reduction in response latency, signaling a restoration of peripheral sensitization.¹³⁹ Intraplantar injection of a CGRP receptor antagonist blocked behavioral signs of hyperalgesia in rats with peripheral nerve injury⁹⁹ and attenuated capsaicin-induced mechanical allodynia and hyperalgesia.¹⁷⁴ Calcitonin gene-related peptide enhances tetrodotoxin-resistant Na⁺ currents in small- and medium-diameter-cultured DRG neurons, and this effect is blocked by the CGRP antagonist CGRP₈₋₃₇.¹⁴¹ Importantly, the tetrodotoxin-resistant Na⁺ currents occur mainly in nociceptive DRG neurons.¹⁴¹ Tissue injury provokes the release of NGF from epithelial cells and fibroblasts, which when bound to the TrkA receptor is retrogradely transported to the cell body in the DRG of the sensory fiber.^{15,121,149,161} Through activation of several intracellular signaling cascades, the NGF-TrkA complex elicits marked upregulation of CGRP protein and mRNA in the DRG and increased CGRP levels in the dorsal horns of the spinal cord.^{15,121,149,161} Thus, one mechanism by which NGF increases excitability may be through an induced CGRP-mediated enhancement of Na⁺ currents. Accordingly, the upregulation of CGRP is believed to contribute to the development of central sensitization and enhanced pain.^{15,149,161}

5.2. Dorsal root reflex, calcitonin gene-related peptide, and peripheral sensitization

Some investigators have suggested that neurogenic inflammation also may be maintained in part through the dorsal root reflex (DRR), which is the generation of action potentials arising from the central terminals and traveling to the periphery.^{124,126,170,189} Primary afferent neurons express the Na⁺K⁺2Cl⁻ cotransporter (NKCC1), which maintains high intracellular concentrations of chloride in these fibers.^{154,182,189} It is proposed that GABA released from GABAergic interneurons activates the GABA_A receptors on primary afferents, opening the Cl⁻ channels.^{154,170,182,189} When the intercellular Cl⁻ concentrations are sufficiently high, opening the channels results in an outflow of Cl⁻, producing primary afferent depolarizations, which can summate to trigger action potentials that are conducted centrifugally to the peripheral terminals.^{154,170,182,189} Electrophysiologic studies performed in normal, uninjured rats have shown GABA-mediated depolarization of Aδ and C-fiber DRG neurons.⁵² Other studies have shown that electrical stimulation of a peripheral nerve generates DRRs in adjacent fibers, which would help spread the neurogenic inflammatory response from the initial site of injury.^{170,189} Several electrophysiologic and pharmacologic studies performed in rats with capsaicin-induced inflammation showed that Aδ and C-fibers mediate neurogenic inflammation through activation of DRRs and peripheral release of CGRP and

substance P.^{122–126} Neurogenic inflammation was blocked by dorsal rhizotomy or intrathecal administration of the GABA_A antagonist bicuculline.¹²⁴ Collectively, these studies suggest the possibility that DRRs can preferentially cause release of CGRP from peripheral terminals of Aδ afferent fibers, thus contributing to the sensitization of peripheral nociceptors.^{122,124–126} Hence, the DRR represents another means through which CGRP may contribute to peripheral sensitization.

6. Central sensitization and the role of calcitonin gene-related peptide

Central sensitization, when considered regarding pain, refers to the enhanced responses of neurons of the central pain transmission pathways in response to stimuli. Central sensitization encompasses increased sensitivity of second-order neurons to afferent inputs, enhancement of receptive fields, and increased excitability, but it also includes long-term effects due to transcriptional changes and an increased net pain facilitatory tone from a reduction of descending inhibition and/or enhancement of descending facilitation. These changes result in enhanced pain sensitivity (hyperalgesia) and pain arising from stimuli that are normally nonnoxious (allodynia).

6.1. Central sensitization

Central sensitization, originally described in the spinal dorsal horn, arises from the concept that excessive nociceptive primary afferent discharges, which increases the release of excitatory transmitters including CGRP, substance P and glutamate, into the spinal dorsal horn, lead to neuroplastic changes that support synaptic efficacy and enhanced nociceptive transmission (**Fig. 3B**).^{115,192} Behaviorally, central sensitization is manifest as enhanced nociception, secondary hyperalgesia, and allodynia.^{115,176,192} Electrophysiologically, it is characterized by increased spontaneous and evoked activity of WDR neurons, lowered response thresholds, responses to novel inputs, enlargement of the receptive field, and neuronal after-discharge.^{115,176,192} These characteristics are considered to be electrophysiologic correlates that parallel the allodynia, hyperalgesia, and enhanced sensitivity in noninjured regions observed in human studies.^{115,176,192} Conditions involving a dysfunction of descending pain modulatory systems such that descending inhibition is deficient or descending pain facilitation is enhanced, so that the net drive is a pronociceptive pain facilitation, can help maintain a sensitized state.^{51,114,150,176,192} Emerging evidence suggests that dysfunctional pain states, such as rheumatoid arthritis, osteoarthritis, visceral pain hypersensitivity syndromes, fibromyalgia, inflammatory bowel syndrome, neuropathic pain, and headaches, including migraine headache, may be caused in part by a deficient endogenous pain inhibitory system that allows the development of central sensitization.^{1,33,96,108,169,192}

Development and maintenance of central sensitization is largely dependent on the activation of the glutamate N-methyl-D-aspartate (NMDA) receptors.^{114,171,191,192} Spinal administration of NMDA antagonists or selective knock-down of the NR1 subunit of the NMDA receptor blocks development of experimentally induced central sensitization in rodents.^{114,171} The activation of the NMDA receptors of postsynaptic neurons elicits the release of glutamate, prostaglandin E₂, and nitric oxide (NO), which act as retrograde transmitters to promote further release of excitatory transmitters from primary afferent terminals and thus maintaining a sensitized state.^{114,127,171,191–194} Activation of the CGRP receptor by release of the neuropeptide from

primary afferent terminals activates signaling cascades that also sensitize the NMDA receptor.¹³ Sustained enhancement of dorsal horn neuronal activity triggers the activation of the mitogen-activated protein kinase signaling cascade, promoting the phosphorylation of the NR1 subunit of the NMDA receptor and the activation of transcription factors that increase expression of pronociceptive transmitters, thus facilitating pain transmission.^{100,114,171,192} The disruption of this cascade with inhibitory agents or receptor antagonists has abolished behavioral signs of hyperalgesia in several animal models of inflammation or nerve injury.^{100,114,150,171,192}

Although most studies on central sensitization regarding pain enhancement have focused on the spinal or trigeminal medullary dorsal horns, several studies have shown that central sensitization at supraspinal sites also can support enhanced abnormal pain. Sensitization of neurons of the thalamus or of the amygdala has been associated with enhanced nociceptive responses which were blocked by NMDA receptor antagonists.^{88,104} Development of central sensitization has also been described in the anterior cingulate cortex, insula, and the ventrolateral orbitofrontal area, and has implications for enhanced abnormal pain.⁹⁷ The spread of central sensitization is believed to contribute to mirror-image pain, where hypersensitivity or pain may be expressed on the uninjured side, and to growing allodynia and enhanced pain that occur as chronic pain conditions progress.^{30,34,71–73,97,192} Animal studies suggest that the development of widespread allodynia with migraine is due to development of central sensitization.^{30,32–34} Imaging studies with migraine patients using functional magnetic resonance imaging scans with recent blood-oxygenation-level-dependent (BOLD) signals indicated a sensitization of the thalamus associated with extracephalic allodynia.³³

6.2. Calcitonin gene-related peptide physiology and central sensitization

The distribution of CGRP within the spinal cord is almost exclusively within the outer laminae of the dorsal horn, with some distribution in lamina V and very little expression in the intermediate laminae of the spinal cord. Importantly, CGRP, like substance P, is found exclusively within the terminals of primary afferent neurons innervating these laminae.^{98,159,200} Disruption of primary afferent inputs into the spinal cord produced near-complete loss of immunoreactive staining for CGRP in the dorsal horn.^{98,159,200} This distribution is consistent with a role for CGRP as a primary afferent neurotransmitter acting on second-order transmission neurons in the spinal dorsal horns.^{98,159,200} These neurons include the nociceptive-specific spinothalamic projections of the outer laminae and the WDR neurons of lamina V. However, the exact nature of the interactions between CGRP and second-order spinal dorsal horn neurons is still not well understood. Early studies of nerve fibers immunoreactive for CGRP in the spinal dorsal horn, including human, described them as a mesh, network, or plexus.⁹⁸ A fine-scale light microscopic analyses of human spinal cord tissue revealed that CGRP fibers in lamina I and lamina V can form multiple intimate axodendritic and axosomatic synaptic connections with targeted neurons, whereas those in lamina II form a homogenous plexus.⁹⁸ Thus, it seems that CGRP can act as a released neurotransmitter and also as a secreted neuromodulator.

Evidence also exists to suggest a presynaptic role for CGRP as well as a postsynaptic one on second-order neurons. Lennerz et al.¹¹⁹ found immunoreactivity for CGRP and RAMP1 on “glomerular structures,” but not on cell bodies, in the TNC, and

suggested that the CGRP receptor probably resides on neuronal processes, including primary afferent endings. Immunohistochemical studies performed in the human TG with novel antibodies found CGRP expressed in small- and medium-sized neurons and CLR/RAMP1 in larger neurons including on nerve fibers.⁶⁶ No cell bodies expressing CGRP or components of the CGRP receptor were found in the TNC, but are likely present on nerve terminals, suggesting that CGRP may act presynaptically.⁶³ In the spinal dorsal horn, CGRP receptors were identified presynaptically, on nerve terminals of glutaminergic neurons, some of which also express enkephalins that also express presynaptic α_2 -adrenergic receptors.¹³¹ It is possible that these CGRP receptors promote the release of glutamate, acting in opposition to the inhibitory effect of the α_2 -adrenergic receptors, but the fact that some of these neurons also express the enkephalins is a confounding factor.¹³¹

Considerable evidence supports a pronociceptive role for CGRP in the spinal cord. However, it is not clear if CGRP itself is nociceptive or whether it facilitates nociceptive signals in the spinal cord. Early studies suggested that spinal CGRP might have a nociceptive role.^{76,105,112,128} Stimulation of peripheral nociceptors evokes release of CGRP from their central terminals in the spinal cord.^{128,129,159,190} The intrathecal administration of CGRP did not alter response thresholds of normal rats to noxious mechanical or thermal stimuli, but intrathecal injection of the CGRP antagonist CGRP₈₋₃₇ or antiserum to CGRP significantly elevated these thresholds, suggesting an antinociceptive effect.^{112,199} However, many studies suggest that spinal release of CGRP may support central sensitization, rather than produce nociception. For example, spinal administration of CGRP antisera blocked behavioral signs of hyperalgesia in rats with carrageenan-induced inflammation or experimental arthritis, but did not alter nociceptive behavioral responses in the noninflamed hind paws of these animals or in normal rats.¹⁰⁵ These results suggest that CGRP mediates the enhanced abnormal pain sensitivity but does not mediate normal acute nociceptive signals.¹⁰⁵ Application of CGRP produces a slow, long-lasting depolarization of dorsal horn WDRs and enhances WDR responses to nerve stimulation.^{197,202} For example, noxious electrical stimulation of the rat hind paw evoked firing of WDRs that was enhanced by iontophoretic application of CGRP and blocked by the CGRP antagonist CGRP₈₋₃₇, also applied iontophoretically.²⁰² The convergence of sensory afferents from the bladder and the gastrointestinal organs with somatic afferents in the spinal dorsal horn can account for referral of visceral pain.^{26,27} Cross-organ sensitization, where inflammation of the gut can manifest also as referred hypersensitivity to cutaneous sites, is mediated in part by increased levels of CGRP in the spinal dorsal horns.^{26,27}

The microiontophoresis of glutamate or of CGRP onto WDR or nociceptive-specific neurons in the TNC of the cat enhanced neuronal responses to stimulation of the superior sagittal sinus.¹⁷² The CGRP antagonists, BIBN4096BS and CGRP₍₈₋₃₇₎, blocked the neuronal activity evoked by glutamate microiontophoresis or stimulation of the superior sagittal sinus.¹⁷² It was concluded that release of CGRP in the TNC acts at non-presynaptic, likely postsynaptic sites to promote transmission of trigeminovascular nociceptive inputs.¹⁷²

Other studies showed that CGRP can promote central sensitization, thus enhancing synaptic function, at supraspinal sites. For example, the laterocapsular division of the central nucleus of the amygdala (CeLC) receives CGRP immunoreactive fibers from the parabrachial region and expresses the CGRP receptor.⁸⁸ Patch-clamp recordings performed on coronal slices

of the rat brain showed that CGRP increases the amplitude of excitatory postsynaptic currents and neuronal excitability in postsynaptic neurons of the CeLC.⁸⁸

7. Calcitonin gene-related peptide and pain models

Numerous behavioral and electrophysiologic studies provide strong evidence that CGRP contributes to central sensitization in animal models of inflammation or nerve injury.^{13,14,159,175,200} Studies performed with α -CGRP-null mutant mice showed that these mice had markedly attenuated behavioral responses in the capsaicin, formalin, and carrageenan models of peripheral inflammation but did not change nociceptive responses to noxious radiant heat.¹⁶² Moreover, visceral responses to intraperitoneal acetic acid, which produces a delayed inflammatory effect, but not to MgSO₄, which produces a noninflammatory visceral nociceptive response were significantly decreased in α -CGRP-null mutant mice.¹⁶² These results are consistent with a pronociceptive sensitizing role of CGRP. The selective ablation of DRG neurons that express α -CGRP through the use of a LoxP-stopped cell ablation construct (human diphtheria toxin receptor) resulted in a >90% loss of all α -CGRP DRG neurons and spinal afferents as well as a loss of approximately one-half of TRPV1⁺ DRG neurons.¹³² These mice had reduced behavioral and electrophysiologic nociceptive responses in models of inflammation and neuropathic pain.¹³²

7.1. Calcitonin gene-related peptide in inflammatory pain models

Animal models of adjuvant arthritis and of chronic inflammatory pain show a marked upregulation of CGRP and mRNA in DRG neurons, as well as elevated CGRP levels in primary afferent terminals of the spinal dorsal horn.^{13,14,200} For example, carrageenan-induced inflammation of the hind paw of the rat increased CGRP levels in exudate and cerebrospinal fluid, but not plasma, 24 hours after the inflammation.²⁰⁰ In another study, inflammation induced by injection of complete Freund adjuvant into the L₅-L₆ facet joint increased CGRP expression in DRG from L₁ through L₅.¹⁴⁷ Moreover, blocking the effects of CGRP, either with the CGRP antagonists, BIBN4096BS or CGRP₈₋₃₇, or with galcanezumab, blocked behavioral and electrophysiologic signs of enhanced pain in animals with inflammation.^{13,14,16,89,200} Rats with inflammation induced by complete Freund adjuvant or with monoiodoacetate injected into the knee joint, which serves as a model for osteoarthritis, reduced mechanical thresholds to elicit a nocifensive response and lowered the thresholds required to evoke electrophysiologic responses of WDR neurons.⁸⁹ The intravenous administration of the small-molecule CGRP antagonist BIBN4096BS abolished both the behavioral and electrophysiologic manifestations of enhanced pain.⁸⁹ It was also found that peripheral inflammation can promote upregulation of CGRP in the DRG through exposure of TRPV1 receptors of primary afferent fibers to an acidic environment.¹⁴⁰ Collagen-induced arthritis was associated with increased CGRP levels in DRG, enhanced abnormal pain, and significant microgliosis.¹⁴² These were abolished by spinal administration of CGRP₈₋₃₇.¹⁴² Electrophysiologic studies performed in rats with experimentally (ie, kaolin/carrageenan) induced arthritis of the knee showed that the inflammation was associated with synaptic facilitation and enhancement of the monosynaptic excitatory postsynaptic currents of dorsal horn units.²² These signs of enhanced synaptic function were blocked by the addition of CGRP₈₋₃₇ to the artificial cerebrospinal fluid superfusate.²² Taken together, these studies

show that central sensitization associated with the enhanced abnormal pain responses in the inflammatory pain state is mediated in large part by elevated CGRP expression in DRG neurons. Thus, animal models of osteoarthritis or of rheumatoid arthritis showed enhanced CGRP in peripheral nerves, and the small-molecule CGRP antagonist BIBN4096BS abolished behavioral and electrophysiologic signs of enhanced pain.^{28,29,186} Benschop et al.¹⁶ demonstrated that systemic administration of galcanezumab blocked behavioral signs of hyperalgesia in 2 rat models of osteoarthritis (ie, monoiodoacetate and meniscal tear) and suggested that clinical studies with galcanezumab were warranted in patients with osteoarthritis. However, a preliminary abstract on a phase II randomized controlled trial (RCT) study of galcanezumab in patients with osteoarthritis found that the antibody did not show efficacy against moderate-to-severe knee pain.¹⁰¹ No other clinical studies have been reported to have examined the utility of CGRP receptor antagonists or CGRP antibodies in inflammatory pain.

7.2. Calcitonin gene-related peptide in neuropathic pain models

Ligation of the L₅ and L₆ spinal nerves produces behavioral signs of hyperalgesia and allodynia, suggestive of central sensitization associated with enhanced abnormal neuropathic pain, along with an increase in capsaicin-evoked release of CGRP from dorsal spinal cord sections.^{77,79,151} Ablation of descending modulation from the nucleus raphe magnus (NRM) by neurochemical lesioning of neurons expressing the μ -opiate receptor, which is believed to correlate with descending pain facilitatory neurons, or by surgical lesioning of the dorsolateral funiculus, which contains the spinopetal projections from the NRM, abolished behavioral signs of enhanced abnormal pain (ie, hind paw hyperalgesia and allodynia) and reduced the enhanced capsaicin-evoked release of CGRP from sections of the dorsal lumbar spinal cord.^{77,79,151} Systemic administration of the neurotrophic artemin, which restored functionality of the injured spinal nerves, produced similar results.⁷⁹ Intrathecal administration of CGRP₈₋₃₇ attenuated behavioral signs of mechanical hyperalgesia in rats with a peripheral nerve injury.¹¹⁸ Importantly, CGRP expression is reduced in the injured DRG, but there seems to be a compensatory upregulation in adjacent DRG. Spinal nerve ligation of L₅ spinal nerve of rats produced a marked reduction of CGRP in the L₅ DRG, but marked increases in L₄ and L₆.¹⁸⁷ Repeated injections of ketanserin attenuated thermal hyperalgesia and tactile allodynia in a manner that corresponded to the reduction in upregulation of CGRP in the DRG.¹⁸⁷ A recent study in an animal model of nerve compression and disk herniation showed mechanical hyperalgesia and upregulation of CGRP in primary afferent nerve terminals in laminae I and II, as well as in the deeper laminae.¹⁰⁷ In other studies, the persistent exposure of rats to morphine resulted in the gradual development of enhanced abnormal pain along with upregulation of CGRP in the spinal dorsal horn and enhanced capsaicin-evoked release of CGRP from dorsal spinal cord sections.⁷⁸ Manipulations that abolished endogenous pain facilitatory systems attenuated the upregulation of the neuropeptides and reduced the enhanced release of CGRP along with behavioral signs of enhanced abnormal pain.⁷⁸ Studies performed with genetically similar rats that expressed either a high (HA) or low (LA) propensity to develop allodynia after peripheral nerve injury showed that the HA rats showed a 10-fold increase in CGRP in large-diameter DRG neurons.^{143,144} The increase in CGRP in these neurons corresponded to the development of behavioral signs of tactile allodynia and central

sensitization in the HA rats.^{143,144} The intrathecal administration of the CGRP receptor antagonist BIBN4096BS (olcegepant) blocked behavioral and neurochemical signs of central sensitization without altering baseline nociceptive thresholds.^{143,144} No clinical studies on neuropathic pain have been reported to date on the effects of CGRP antagonists or antibodies. Thus, to date there is limited information on clarifying the role of CGRP in this condition.

Taken together, studies overall suggest that CGRP may contribute to the development and maintenance of central sensitization, which underlies enhanced chronic pain conditions including idiopathic pain states, as well as inflammatory and neuropathic pain. However, there is a paucity of information on CGRP's direct effects on pain and the utility of CGRP receptor antagonists or CGRP antibodies in non-migraine chronic pain conditions.

8. Supraspinal effects of calcitonin gene-related peptide

The CGRP peptide and the CGRP receptors are present in different regions of the brain that modulate nociception and that have been implicated in the pathophysiology of migraine.^{3,65,67,177} In a 2001 study, it was estimated that up to 80% of cell bodies in the locus coeruleus, which is implicated in cardiovascular, autonomic, and nociceptive functions, expressed CGRP.¹⁷⁷ In that study, no CGRP was detected in cell bodies of the PAG or NRM.¹⁷⁷ An earlier study had found CGRP expression in nerve fibers that terminated in the PAG of the cat as well as CGRP in cell bodies in the caudal aspect of the ventrolateral PAG.⁴³ Microinjection of CGRP into the PAG blocked nociception in normal and nerve-injured rats, and this effect was reversed by CGRP₍₈₋₃₇₎.^{195,201} The CeA, which also contributes to the regulation of pain, was found to contain CGRP receptors as well as nerve fibers containing CGRP.¹⁹⁶ Microinjection of CGRP into the CeA produced dose-dependent antinociception in rats blocked by intra-CeA CGRP₍₈₋₃₇₎ and systemic naloxone.¹⁹⁶ Retrograde fluorescent tracing studies along with immunohistochemistry indicated that CGRP released within the CeA activated enkephalinergic projections to the PAG, thus producing an antinociceptive effect.^{195,196} In a study where the subcutaneous injection of 10 mg/kg of nitroglycerin to rats was proposed to act as an experimental migraine model, this treatment resulted in an increase in mRNA and protein levels of CGRP in the PAG.¹⁹⁸ This increase was blocked by pretreatment with rizatriptan.¹⁹⁸ However, no behavioral signs of pain or headache were measured in that study. In a more recent study, the responses of neurons in the trigeminocervical complex with cutaneous or meningeal receptive fields were recorded.¹⁵³ Neuronal responses were enhanced by CGRP microinjected into the ventrolateral PAG and attenuated by microinjection of either olcegepant or CGRP₍₈₋₃₇₎.¹⁵³ These studies suggest that CGRP may promote enhanced excitability of the trigeminocervical complex in part through activity of the PAG.¹⁵³ Most recently, it was found that functional CGRP receptors are present in many sites in the brainstem of the rhesus monkey, including sites important to nociceptive processing such as the PAG, area postrema, pontine raphe nucleus, gracile nucleus, and the TNC.⁶⁵ Moreover, CGRP receptors were detected in areas that lack protection of the blood-brain barrier (BBB), such as the area postrema, median eminence, and the pineal gland.⁶⁵ These studies point to the involvement of CGRP in areas of the brain involved in central sensitization and descending modulation, implicated in both migraine and nociceptive processing. However, the role of CGRP in these different brain regions

remains to be elucidated, as both pro- and antinociceptive roles have been suggested, depending on the region involved. In addition, the roles of the CGRP receptor as well as potential roles for the AMY1 receptor in migraine headache, and nociceptive processing in general, also require further clarification. A better understanding of the regional effects of CGRP, and of receptors stimulated by CGRP, in the brain will lead to a better understanding of the role of this peptide in pain and its modulation.

9. Calcitonin gene-related peptide, migraine, and the trigeminal system

There is considerable, growing evidence that implicates CGRP in the genesis of migraine (**Fig. 4**). This role is ultimately emphasized by the recent success of early phase clinical trials with CGRP receptor antagonists and humanized monoclonal antibodies.^{20,21,54,55,82}

9.1. Calcitonin gene-related peptide and migraine

The possibility that CGRP might be germane to migraine was first raised by Goadsby and Edvinsson who in 1988 found that patients who were undergoing thermocoagulation of the TG for trigeminal neuralgia and who showed facial flushing had significantly elevated CGRP levels in blood taken from the external jugular vein.⁸⁵ These findings were coupled with findings that electrical stimulation of the cat TG also elevated blood CGRP levels.⁸⁵ It was then discovered that blood collected from the external jugular vein, but not from the cubital fossa, from patients during a migraine attack showed elevated levels of CGRP.⁸⁶ Moreover, patients with migraine also showed a normalization of CGRP levels after efficacious sumatriptan treatment.⁸⁴ Studies performed in parallel showed that elevated plasma CGRP levels following electrical stimulation of the TG of cats were reduced by subsequent administration of sumatriptan.⁸⁴ Although another study failed to find any change in CGRP levels in either cubital or jugular blood samples during migraine,¹⁸¹ this failure may have been due to the low pain levels reported in these patients.¹¹¹

The administration of NO, by means of an NO donor such as glyceryl trinitrate (GTN), to some migraineurs precipitates a typical migraine attack, but after a delayed onset of several hours.^{2,111,179} Importantly, individuals who do not experience migraine do not develop the delayed migraine-like symptoms.^{2,111,179} However, both migraineurs and normal individuals report developing a mild headache immediately after NO donor administration, lasting 15 to 20 minutes.^{2,111,179} The intensity of GTN-induced migraine was associated with significant elevations in levels of CGRP in blood obtained from the external jugular vein.^{102,103} The infusion of GTN to normal, healthy volunteers produced an immediate, mild headache but did not elevate plasma CGRP levels.¹¹¹ Sumatriptan attenuated the GTN-induced migraine headache symptoms and reduced the elevated blood levels of CGRP.¹⁰² Sumatriptan elicited a moderate increase in CGRP levels in blood samples obtained from cubital fossa, but not external jugular, venipuncture. However, it did not alter CGRP levels after GTN infusion.¹¹¹ It was also found that patients with chronic migraine had significantly elevated blood CGRP levels during the interictal (ie, headache-free) periods when compared with healthy normal volunteers and patients with episodic migraine.³⁸ Plasma CGRP levels were predictive of the efficacy of onabotulinumtoxinA in preventing migraine episodes in patients with chronic migraine. The probability of a patient responding to onabotulinumtoxinA was 28-fold greater for patients with interictal plasma CGRP levels of

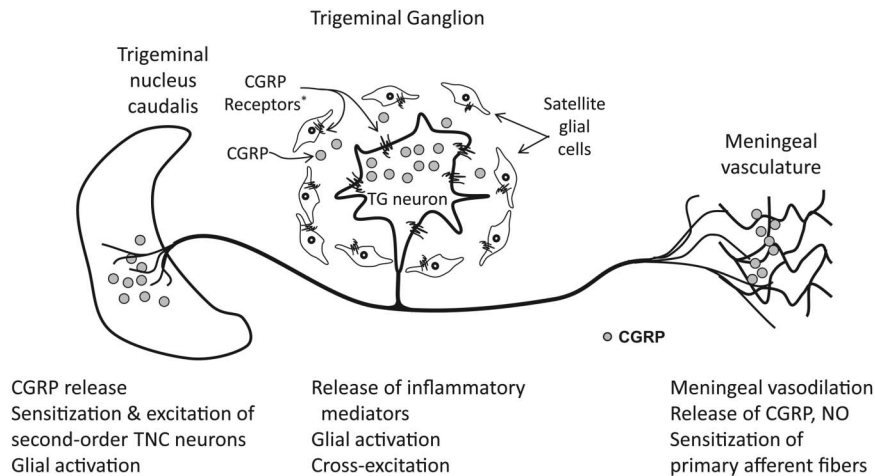


Figure 4. Activation of trigeminal ganglion (TG) neurons, perhaps through the release of inflammatory mediators or in response to ischemia or a localized insult, results in the release of calcitonin gene-related peptide (CGRP) into the meningeal vasculature, which results in vasodilation and release of nitric oxide (NO), producing a neurogenic inflammation. Calcitonin gene-related peptide can also be secreted from the cell body within the TG, where it excites satellite glial cells, which in turn can release inflammatory mediators, thus resulting in cross-excitation and facilitation of an inflammatory loop. The CGRP receptors are also present on TG neurons that do not express CGRP. Thus, CGRP released in the meninges and TG can initiate and maintain peripheral sensitization. The release of CGRP and other excitatory neurotransmitters from the central terminals of the TG neurons in the trigeminal nucleus caudalis (TNC) can excite second-order neurons in the TNC, leading to central sensitization and the manifestation of hyperalgesia and allodynia. Calcitonin gene-related peptide can also sensitize primary afferent nerves by acting on the presynaptic CGRP receptors that are present on presynaptic terminals of primary afferents that do not express CGRP. *CGRP receptors are found on large-diameter TG neurons that do not express CGRP.

>72 pg/mL than for those with lower CGRP levels.³⁹ Moreover, the interictal CGRP levels were significantly reduced by onabotulinumtoxinA in responsive patients, whereas levels did not change in nonresponders.⁴⁰ Similar to GTN, the intravenous infusion of human α -CGRP to patients with migraine provokes a mild headache during the infusion that was followed by an attack with many of the characteristics of migraine without aura.¹¹³ Importantly, as with NO donors, CGRP infusion to nonmigraineurs does not provoke migraine-like attack.¹¹³ Taken together, these studies clearly show a prominent role of CGRP, as well as NO, in the pathogenesis of migraine. However, the fact that neither CGRP nor NO produce an immediate migraine episode, but require a substantial delay to exert its effect, strongly suggests that CGRP and NO are likely to act through the development of sensitization of the TG afferents and/or central loci that receive inputs from the TG. Several studies show that CGRP promotes the synthesis and release of NO and NO promotes that of CGRP, such that both of these substances can enhance the activity of the other.^{12,95,123,135}

The important role of CGRP in the pathogenesis of migraine is further supported by recent phase II RCTs that show that the small-molecule CGRP antagonists, olcegepant, telcagepant, MK-3207, BI 44370 TA, rimegepant, and ubrogepant, all were efficacious for the acute treatment of migraine.^{53,90,91,130,148,167,184} More recently, RCTs using humanized monoclonal antibodies to CGRP or to the CGRP receptor have shown efficacy in patients with migraine.^{20,21,54,55,173} For instance, a 12-week study in patients with episodic migraine demonstrated that the humanized monoclonal antibody galcanezumab reduced the frequency of migraine headache days with low incidence of adverse events.⁵⁵ Another recent study demonstrated that the monoclonal CGRP antibody ALD403 reduced the number of migraine headache days in patients with episodic migraine.⁵⁴ Phase II studies with the CGRP antibody TEV-48125 show efficacy in episodic and chronic migraine, with a favorable safety profile.^{18,19} AMG 334 is a CGRP receptor antibody that was demonstrated to be effective in a phase II study for migraine

prevention in patients with episodic migraine.¹⁷³ Taken together, these trials present strong evidence that disruption of the effects of endogenous CGRP is an efficacious approach to attenuate migraine headaches.⁵⁹ However, the means through which CGRP may initiate and/or sustain migraine, as well as the site(s) of action of the anti-CGRP therapeutics are still not well understood.

9.2. Calcitonin gene-related peptide, the trigeminal system, and sensitization

The TG and its central terminal field, the TNC, are often likened to the DRG and spinal dorsal horn. Although there are many similarities, there are also some differences that become important in the context of migraine. Like in the DRG, the TG sensory neurons are pseudounipolar, with one end of the axon terminating in the TNC and the antipodal end projecting to peripheral sites. In addition to providing cutaneous innervation of the face and scalp, the TG also supplies a dense innervation of the meningeal vasculature with nociceptive primary afferents. Moreover, CGRP is the most abundant peptidic neurotransmitter in the TG, expressed in 70% of TG neurons in rats, compared with 49% of DRG neurons.¹⁵⁵ Also, there is a greater proportion of CGRP neurons that coexpress TRPV1 in the TG in comparison with DRG neurons.¹⁵⁵ These differences highlight the importance of CGRP in the trigeminovascular innervation.^{57,155} This distribution is consistent with a prominent nociceptive, CGRP-dominant innervation of the trigeminovascular system. It was recently reported that elevated levels of CGRP in the rostral spinal cord are associated with sensitization of primary nociceptive trigeminal neurons.⁴⁴ The intracisternal injection of CGRP enhanced nocifensive withdrawal of the head to sensory stimuli, and the enhancement of this response was inhibited by the CGRP antagonist CGRP₍₈₋₃₇₎.⁴⁴

As reviewed above, there is considerable evidence to indicate that CGRP is instrumental in the development and maintenance of peripheral and central sensitization. Based on the results of

a series of animal and clinical studies, Burstein et al. have advanced the idea that migraine is a product of peripheral and central sensitization.^{17,30,34,35,146} In support of this hypothesis, some patients develop cutaneous allodynia, and the allodynia spreads eventually to include extracephalic areas.^{17,30,34,35,146} In these patients, triptans would effectively block migraine headache only if given before the onset of cutaneous allodynia.^{17,30,35} Functional magnetic resonance imaging performed in patients during a migraine attack showed enhanced activation of the TG and TNC in response to innocuous mechanical stimuli applied to the periorbital skin during migraine and enhanced activity of the thalamus to innocuous mechanical stimulation applied to the dorsum of the hand.^{17,30,35} Thus, it was hypothesized that peripheral sensitization mediates the painful throbbing felt at the beginning phases of a migraine episode. Left unchecked, the sensitized TG neurons provoke central sensitization of the second-order TNC neurons, which mediates the development of cutaneous allodynia.^{17,30,35} In a recent BOLD functional magnetic resonance imaging study with normal, healthy volunteers, the intravenous infusion of CGRP increased BOLD-signal in the brainstem and insula and decreased the BOLD-signal in the caudate nuclei, thalamus, and cingulate cortex in response to the application of noxious heat stimuli to the V1 trigeminal area.⁷ These changes in BOLD-signal were reversed by sumatriptan. These data support the notion that CGRP modulates nociceptive transmission in the trigeminal pain pathways.⁷

The interpretations of these clinical results were supported by correlative studies in rats. The application of a solution of inflammatory mediators to the dura of rats increased the spontaneous activity of second-order TNC neurons. Moreover, the TNC neurons also show reduced firing thresholds, increased firing frequencies, and enlarged receptive fields in response to light mechanical stimulation of the periorbital cutaneous region or to the dura.^{17,31,35} These electrophysiological properties are hallmarks indicative of central sensitization. The intravenous administration of sumatriptan 2 hours after application of inflammatory mediators (ie, late application) reduced the expanded receptive fields and the neuronal responses to light tactile stimuli applied to the dura. However, the increased spontaneous firing rates and the enhanced responses to light cutaneous tactile stimuli and reduced response thresholds to heat stimuli remained.^{17,31,35} In contrast, administration of sumatriptan at the same time as the inflammatory mediators blocked the development of all electrophysiologic signs of central sensitization. Because the 5-HT_{1B/D/1F} receptors are coexpressed with nitric oxide synthase (NOS) and CGRP in trigeminal neurons, it is very possible that sumatriptan acts peripherally, by inhibiting the release of CGRP from the primary afferent TG nociceptors.^{4,12,17,31,35,94}

Although there is strong evidence that CGRP can mediate neuronal sensitization, the mechanism of action remains unclear. One study showed that CGRP neither excites nor sensitizes meningeal nociceptors directly.¹²⁰ In that study, the spontaneous and noxious-evoked discharge rates of TG nociceptors with receptive fields in the dura were measured along with dural blood flow. Neither intravenous infusion nor direct application to the dura of CGRP increased ongoing neuronal activity or responses to mechanical stimulation of the dura, but both increased dural blood flow.¹²⁰ These investigators suggested that the results indicate a central effect of CGRP, perhaps facilitation of the second-order neurons of the TNC. The absence of an activation of nociceptors by CGRP is consistent with studies showing that intradermal CGRP does not evoke nociceptive responses in normal individuals.¹⁸⁸ This observation is not necessarily

surprising, as intravenous infusions of CGRP, like NO donors, produce migraine-like headaches after a considerably long latency period of several hours in migraineurs but not in healthy controls.^{2,111,179} It should be noted that in the electrophysiologic study, neuronal recordings were performed for no more than 30 minutes after CGRP administration.¹²⁰ Moreover, these studies were performed in rats that have not been sensitized, and may not mimic the sensitized state believed to exist in migraineurs.^{17,31,35,47} This suggests the activation of processes secondary to CGRP rather than a direct excitatory effect of CGRP. One such possibility is that CGRP acts on nonneural cells to promote sensitization. The TG neurons can secrete CGRP from the soma within the TG, and satellite glial cells in the TG express CGRP receptors. The satellite glial cells of the TG are stimulated by CGRP to upregulate proinflammatory substances, such as IL-1 β and the inducible form of NOS.⁴⁶ Increased IL-1 β levels induced by CGRP resulted in a 3-fold increase in COX₂ levels, which would increase prostaglandin E₂ synthesis, ultimately further sensitizing the TG neurons.^{37,46} This could result in a positive feedback loop, with CGRP promoting neuronal-glia crosstalk within the TG and maintaining a state of peripheral, and ultimately central, sensitization.^{36,37,46,56,119,123}

The interaction among the trigeminal afferents, meningeal vasculature, and the meningeal environment may also provide a means through which CGRP may sustain central sensitization. The release of CGRP provokes an intense vasodilation of the meningeal vasculature. Vasodilation itself is no longer held to be a mechanism driving migraine, and the vascular theory of migraine has been largely abandoned.^{41,83} However, the effects of CGRP could still be linked to a neurogenic mechanism promoting sensitization. Calcitonin gene-related peptide released from peripheral trigeminal nerve terminals can activate CGRP receptors on the endothelial cells of the meningeal vasculature, which results in cyclic adenosine monophosphate (cAMP) formation, which in turn stimulates endothelial NOS to enhance production of NO. Nitric oxide can sensitize the peripheral trigeminal afferent fibers, causing enhanced release of CGRP at the peripheral and central terminals.^{14,35,135,159} Because both NO and CGRP facilitate nociceptive transmission, this mechanism provides a means through which a state of enhanced excitability might be maintained.^{14,35,135,159} Electrophysiologic studies in rats showed that blockade of CGRP receptors by systemically administered CGRP antagonist (MK-8825) blocked the increased neuronal activity of trigeminal neurons with meningeal afferent inputs during sustained infusion of nitroglycerin.⁷⁰ Tissue culture studies showed that NO donors increase CGRP release and stimulate CGRP promoter activity in TG neurons.¹² Taken together, these studies provide a potential mechanism where CGRP can act in concert with NO to facilitate trigeminal nociceptive transmission and potentially promote migraine headache. The complex interaction among CGRP, meningeal vasculature, afferent fibers, and NO suggest that perhaps CGRP-induced vasodilation may not be directly responsible for migraine headache. The consequences of the action of CGRP on meningeal vasculature, involving nociceptive afferent neurons and the release of NO, could mean that meningeal vasodilation is in some way germane to migraine. Clearly, further investigations into the complex interactions among these components are warranted.

9.3. Calcitonin gene-related peptide, latent sensitization, and relevance to migraine

The concept of latent sensitization is that an initiating event can produce a sensitized condition that lies dormant, to be

“awakened” after exposure to a triggering stimulus.^{48–50} In an animal model of latent sensitization, sustained sumatriptan infusion to rats produced the development of periorbital cutaneous allodynia along with a marked upregulation of neuronal NOS (nNOS) and CGRP, but not substance P, in trigeminal neurons with meningeal afferent inputs.^{48–50} After 2 weeks, behavioral signs of allodynia resolved, but the trigeminal levels of CGRP and nNOS remained elevated.^{48–50} The administration of an NO donor or exposure to environmental stress, which are known migraine triggers in migraineurs, precipitated periorbital allodynia that was blocked by nNOS inhibitors.^{48–50} Moreover, the administration of the NO donor to rats with sumatriptan-induced latent sensitization also elevated blood CGRP levels, but did not change them in the nonsensitized (ie, saline-infused) rats.⁵⁰ These studies show that a state of latent sensitization can be produced in rats that, when challenged with putative migraine triggers, can provoke periorbital cutaneous allodynia and elevated blood levels of CGRP.

10. Central vs peripheral site of action of calcitonin gene-related peptide in migraine

Despite the advances in the study of migraine neuropathology and of the role of CGRP in migraine, the site of action of CGRP in alleviating migraine remains unsettled. However, there is a growing body of evidence that points to the peripheral site as being more likely in mediating the observed antimigraine activity. The triptans, which likely act by inhibition of release of CGRP and other neurotransmitters from primary afferents to abort migraines, in general have poor penetration of the BBB.^{4,74} Moreover, their efficacious clinical doses do not correlate well with BBB penetration.^{4,74} The small-molecule CGRP receptor blockers, which also show clinical efficacy in aborting migraine, show little (telcagepant) to no (olcegepant) ability to cross the BBB.^{59–61} Likewise, the recently developed humanized antibodies to CGRP or the receptor are unlikely to act via a central site in preventing migraine, as these substances have very little to no ability to penetrate the BBB.^{55,62,178,203} It has been suggested that migraine episodes are accompanied by changes in BBB permeability, which would allow penetration of these substances.⁴ However, increased BBB permeability during migraine has not been consistently demonstrated and would not explain how these therapeutics may access the brain to prevent migraine attacks.⁴ A recent study using PET with the novel radioligand ¹¹C-dihydroergotamine showed that the radioligand was unable to penetrate into the brain of patients with migraine when migraine was provoked by exposure to GTN suggesting that the BBB is not compromised during a migraine attack.¹⁶⁴ A recent PET study using CGRP PET tracer [¹¹C]MK-4232 as a validated ligand for the CGRP receptor showed very low (<10%) receptor occupancy of the CGRP receptor by telcagepant at therapeutic doses.⁹² It was concluded that occupancy of central CGRP receptors is not required for the therapeutic effect of telcagepant, but the central occupancy that could occur with higher doses may confer some additional therapeutic benefit.⁹²

11. Summary

Collectively, these studies provide strong evidence that an important physiologic function of CGRP seems to be its association with pain transmission and modulation. It is found in high concentrations primarily in peptidergic nociceptive A δ and C-fiber DRG and TG neurons and in their terminals in the outer laminae of the spinal cord dorsal horn and in the TNC. The

terminations of the CGRP-expressing primary afferents correlate well with the distribution of second-order neurons with ascending projections to supraspinal sites involved in pain perception and modulation. Considerable evidence indicates that peripheral sensitization and the associated hyperalgesia are initiated and maintained in part by the actions of CGRP, and enhanced release of CGRP at peripheral and central terminals of primary afferent fibers is associated with nociceptor sensitization and hyperalgesia. Upregulation of CGRP is believed to contribute to the development of central sensitization and enhanced pain in neuropathic and inflammatory pain states. Calcitonin gene-related peptide is a pronociceptive neuromediator, both at somatic and cephalic sites. Moreover, CGRP plays a critical role in the trigeminovascular innervation, where it can activate positive feed-forward circuitries that can initiate and/or sustain a painful event. Interactions between the TG neurons and satellite cells and with the meningeal vasculature can help maintain the state of enhanced excitability by promoting the synthesis and release of both NO and CGRP, which act to promote each other's activities. Disruption of this feed-forward loop, by blocking the activity of CGRP, appears to be therapeutically relevant in migraine.

Conflict of interest statement

S. Iyengar and K. W. Johnson are employed by and are stockholders of Eli Lilly and Company. M. H. Ossipov is employed by inVentiv Health Clinical, LLC.

Eli Lilly and Company contracted inVentiv Health Clinical, LLC, for writing and editorial services.

Teri Tucker of inVentiv Health Clinical, LLC provided editorial assistance. This work was sponsored and funded by Eli Lilly and Company, Indianapolis, IN, USA.

Article history:

Received 14 September 2016

Received in revised form 9 December 2016

Accepted 22 December 2016

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