Comprehensive Review

PAIN

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

Smriti Iyengara,*, Michael H. Ossipovb, Kirk W. Johnsona

Abstract
Calcitonin gene–related peptide (CGRP) is a 37-amino acid peptide found primarily in the C and Aδ 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 Gs subunit. 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. Calcitonin gene–related peptide’s affinity for AMY1 is comparable with the CGRP receptor, and has been proposed as a second CGRP receptor site. 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. In the meninges, the middle meningeal and the cerebral arteries are innervated by primary afferent trigeminal fibers that express CGRP. 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.

a Eli Lilly and Company, Indianapolis, IN, USA. b inVentiv Health Clinical, LLC, Ann Arbor, MI, USA

*Corresponding author. Address: Eli Lilly and Company, Indianapolis, IN 46285, USA. Tel.: 317-276-2479; fax: 317-277-2350. E-mail address: iyengar_smriti@lilly.com (S. Iyengar).

PAIN 158 (2017) 543–559

Copyright © 2017 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the International Association for the Study of Pain. This is an open-access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

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

April 2017 · Volume 158 · Number 4 www.painjournalonline.com 543
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 Aδ- 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.14 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.5,159,160 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.5,159,160,190 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, CGRP18-37 and CGRP19-37, form the CT receptor family of peptides.1,123,117,152 In addition, these CGRP fragments have been found in the rat spinal cord, and the CGRP19-37 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 CGRP receptor, based on a greater sensitivity to the peptidic antagonist CGRP9-37, and a CGRP receptor that was more sensitive to the CGRP analog antagonists [Cys(ACM)2,7]- and [Cys(Et)2,7]-CGRP.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.159,190 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 AMY1–3.15,162,159 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 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 Goα subunit, allowing activation of adenyly cyclase.

![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 confors specificity for binding with CGRP, and the RCP, which seems to be critical for signal transduction by promoting effective coupling to the Go protein. Gα, 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; NO, nitric oxide synthase; P, phosphorylation sites; RCP, receptor component protein.](https://example.com/figure1.png)
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.139 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.146

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.63,64,66,152,158,160 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 was 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, parabrahcial nucleus, Kölner-Fuse nucleus, striatum, colliculi, TNC, medullary cranial motor nuclei, nucleus ambiguous, peripeduncular, posterior, centromedial thalamic nucleus, central gray, and the inferior colliculus.67,70,110,157,159,160,190 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

High densities of binding for [125I]CGRP were found in the molecular layer and Purkinje cell layer of the human cerebellum59 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.62 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.62 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 centrolateral part of the dorsal
motor nucleus of vagus, and the hypoglossal nucleus. 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. 47,48,98,134,150

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. 152,155,160 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–to–diameter Aβ fibers, approximately 70% of which are nociceptors, as well as in some large-diameter Aβ sensory fibers and Aα motor neurons. 8,158

Studies combining electrophysiological and histochemical approaches have shown that most CGRP-expressing neurons are either C-fiber or Aδ-fiber nociceptors and include thermosensitive, high-threshold mechanonociceptors and polymodal nociceptors. 116,158 A small population of high-threshold mechanosensitive Aβ 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 Aβ and Aδ 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
protein extravasation induced by substance P. 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. 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.
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. 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.

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. 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. Intraplantar injection of a CGRP receptor antagonist blocked behavioral signs of hyperalgesia in rats with peripheral nerve injury and attenuated capsaicin-induced mechanical allodynia and hyperalgesia. 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. Importantly, the tetrodotoxin-resistant Na⁺ currents occur mainly in nociceptive DRG neurons. 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. 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. Thus, one mechanism by which NGF increases excitability may be through an induced CGRP-mediated enhancement of Na⁺ currents. Accordingly, the up-regulation of CGRP is believed to contribute to the development of central sensitization and enhanced pain.

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. Primary afferent neurons express the Na⁺/K⁺-2Cl⁻ cotransporter (NKCC1), which maintains high intracellular concentrations of chloride in these fibers. It is proposed that GABA released from GABAergic interneurons activates the GABA_A receptors on primary afferents, opening the Cl⁻ channels. 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. Electrically evoked studies performed in normal, uninjured rats have shown GABA-mediated depolarization of Aβ and C-fiber DRG neurons. 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. Several electrophysiologic and pharmacologic studies performed in rats with capsaicin-induced inflammation showed that Aβ and C-fibers mediate neurogenic inflammation through activation of DRRs and peripheral release of CGRP and substance P. Neurogenic inflammation was blocked by dorsal rhizotomy or intrathecal administration of the GABA_A antagonist bicuculline. Collectively, these studies suggest the possibility that DRRs can preferentially cause release of CGRP from peripheral terminals of Aβ afferent fibers, thus contributing to the sensitization of peripheral nociceptors. 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 nonnoceptive 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). Behaviorally, central sensitization is manifest as enhanced nociception, secondary hyperalgesia, and allodynia. 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. These characteristics are considered to be electrophysiologic correlates that parallel the allodynia, hyperalgesia, and enhanced sensitivity in noninjured regions observed in human studies. 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. 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.

Development and maintenance of central sensitization is largely dependent on the activation of the glutamate N-methyl-D-aspartate (NMDA) receptors. 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. 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 maintain a sensitized state. Activation of the CGRP receptor by release of the neuropeptide from...
primary afferent terminals activates signaling cascades that also sensitize the NMDA receptor. 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. 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.

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. 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. 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. Animal studies suggest that the development of widespread allodynia with migraine is due to development of central sensitization. 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.

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. Disruption of primary afferent inputs into the spinal cord produced near-complete loss of immunoreactive staining for CGRP in the dorsal horn. 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. These neurons include the nociceptive-specific spinohypothalamic 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. 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. 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. Lenerz et al. 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. 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. 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 α2-adrenergic receptors. It is possible that these CGRP receptors promote the release of glutamate, acting in opposition to the inhibitory effect of the α2-adrenergic receptors, but the fact that some of these neurons also express the enkephalins is a confounding factor.

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. 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 CGRP(9-37) or antiserum to CGRP significantly elevated these thresholds, suggesting an antinociceptive effect. 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. These results suggest that CGRP mediates the enhanced abnormal pain sensitivity but does not mediate normal acute nociceptive signals. Application of CGRP produces a slow, long-lasting depolarization of dorsal horn WDRs and enhances WDR responses to nerve stimulation. For example, noxious electrical stimulation of the rat hind paw evoked firing of WDFs that was enhanced by iontophoresic application of CGRP and blocked by the CGRP antagonist CGRP(8-37), also applied iontophoretically. 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. 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. 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. The CGRP antagonists, BIBN4096BS and CGRP(8-37), blocked the neuronal activity evoked by glutamate microiontophoresis or stimulation of the superior sagittal sinus. It was concluded that release of CGRP in the TNC acts on nonpresynaptic, likely postsynaptic sites to promote transmission of trigeminovascular nociceptive inputs.

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. 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 capsaiscin, 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 response, were abolished by spinal administration of CGRP 8-37.142 Electric currents of dorsal horn units.22 These signs of enhanced synaptic inflammation was associated with synaptic facilitation and trophysiologic studies performed in rats with experimentally (ie, physiologic manifestations of enhanced pain.89 It was also found that peripheral inflammation can promote upregulation of CGRP along with behavioral signs of enhanced abnormal pain.78 Manipulations that abolished CGRP along with behavioral signs of 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 CGRP9-37 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 laminai 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.126 Studies performed with genetically similar rats that expressed either a high (HA) or low (LA) propensity to develop allodynia after a peripheral nerve injury showed that the HA rats showed a 10-fold increase in CGRP in large-diameter DRG neurons.153,144 The increase in CGRP in these neurons corresponded to the development of behavioral signs of tactile allodynia and central
sensitization in the HA rats.\textsuperscript{143,144} The intrathecal administration of the CGRP receptor antagonist BIBN4096BS (olicgepant) blocked behavioral and neurochemical signs of central sensitization without altering baseline nociceptive thresholds.\textsuperscript{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.\textsuperscript{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.\textsuperscript{177} In that study, no CGRP was detected in cell bodies of the PAG or NRM.\textsuperscript{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.\textsuperscript{43} Microinjection of CGRP into the PAG blocked nociception in normal and nerve-injured rats, and this effect was reversed by CGRP\textsuperscript{8-37}.\textsuperscript{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.\textsuperscript{196} Microinjection of CGRP into the CeA produced dose-dependent antinociception in rats blocked by intra-CeA CGRP\textsuperscript{8-37} and systemic naloxone.\textsuperscript{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.\textsuperscript{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.\textsuperscript{198} This increase was blocked by pretreatment with rizatriptan.\textsuperscript{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.\textsuperscript{153} Neuronal responses were enhanced by CGRP microinjected into the ventrolateral PAG and attenuated by microinjection of either oliceranpt or CGRP\textsuperscript{8-37}.\textsuperscript{153} These studies suggest that CGRP may promote enhanced excitability of the trigeminocervical complex in part through activity of the PAG.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{85} These findings were coupled with findings that electrical stimulation of the cat TG also elevated blood CGRP levels.\textsuperscript{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.\textsuperscript{96} Moreover, patients with migraine also showed a normalization of CGRP levels after efficacious sumatriptan treatment.\textsuperscript{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.\textsuperscript{84} Although another study failed to find any change in CGRP levels in either cubital or jugular blood samples during migraine,\textsuperscript{181} this failure may have been due to the low pain levels reported in these patients.\textsuperscript{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.\textsuperscript{2,111,179} Importantly, individuals who do not experience migraine do not develop the delayed migraine-like symptoms.\textsuperscript{2,111,179} However, both migraineurs and normal individuals report developing a mild headache immediately after NO donor administration, lasting 15 to 20 minutes.\textsuperscript{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.\textsuperscript{102,103} The infusion of GTN to normal, healthy volunteers produced an immediate, mild headache but did not elevate plasma CGRP levels.\textsuperscript{111} Sumatriptan attenuated the GTN-induced migraine headache symptoms and reduced the elevated blood levels of CGRP.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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
Importantly, as with NO donors, CGRP infusion to attack with many of the characteristics of migraine without a mild headache during the infusion that was followed by an episodic and chronic migraine, with a favorable safety studies with the CGRP antibody TEV-48125 show efficacy in enhance the activity of the other.12,95,123,135

CGRP promotes that of CGRP, such that both of these substances can promote the synthesis and release of NO and NO loci that receive inputs from the TG. Several studies show that development of sensitization of the TG afferents and/or central strongly suggests that CGRP and NO are likely to act through the episode, but require a substantial delay to exert its effect, that neither CGRP nor NO produce an immediate migraine as well as NO, in the pathogenesis of migraine. However, the fact to together, these studies clearly show a prominent role of CGRP, as well as NO, in the pathogenesis of migraine. Hence, 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.20,21,54,55,173 More recently, RCTs using humanized monoclonal antibodies to CGRP or to the CGRP receptor have shown efficacy in patients with migraine.155 For instance, a 12-week study in patients with episodic migraine demonstrated that the humanized monoclonal antibody galcanezumab reduced the number 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.124 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. More recently, RCTs using humanized monoclonal antibodies to CGRP or to the CGRP receptor have shown efficacy in patients with migraine.155 For instance, a 12-week study in patients with episodic migraine demonstrated that the humanized monoclonal antibody galcanezumab reduced the number 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.124 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.

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.124 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.124 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.77,155 This distribution is consistent with a prominent nociceptive, CGRP-dominated 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 nociceptive 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
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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{17,31,35} 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\textbeta and the inducible form of NO.\textsuperscript{46} Increased IL-1\textbeta levels induced by CGRP resulted in a 3-fold increase in COX\textsubscript{2} levels, which would increase prostaglandin E\textsubscript{2} synthesis, ultimately further sensitizing the TG neurons.\textsuperscript{37,46} This could result in a positive feedback loop, with CGRP promoting neuronal-glial crosstalk within the TG and maintaining a state of peripheral, and ultimately central, sensitization.\textsuperscript{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.\textsuperscript{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 NO 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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{70} Tissue culture studies showed that NO donors increase CGRP release and stimulate CGRP promoter activity in TG neurons.\textsuperscript{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 fibers 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 permeable during a migraine attack.154 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 A6 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. Uprogulation 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

References

[1] Ablin JN, Buskila D, Clauw DJ. Biomarkers in fibromyalgia. Curr Pain Headache Rep 2009;13:343–9.
[2] Afridi SK, Kaube H, Goadsby PJ. Glycerol trinitrate triggers premonitory symptoms in migraineurs. PAIN 2004;110:675–80.
[3] Afridi SK, Matharu MS, Lee L, Kaube H, Friston KJ, Frackowiak RS, Goadsby PJ. A PET study exploring the laterality of brainstorm activation in migraine using glycerol trinitrate. Brain 2005;128:932–9.
[4] Ann AH, Basters AM. Where do triptans act in the treatment of migraine? PAIN 2005;115:1–4.
[5] Amara SG, Anzila JL, Leff SE, Swanson LW, Evans RM, Rosenfeld MG. Expression in brain of a messenger RNA encoding a novel neuropeptide homologous to calcitonin gene-related peptide. Science 1985;220: 1094–7.
[6] Amara SG, Jonas V, Rosenfeld MG, Ong ES, Evans RM. Alternative RNA processing in calcitonin gene expression generates mRNAs encoding different polypeptide products. Nature 1982;298:240–4.
[7] Aghgar MS, Becerra L, Larsson HB, Borsook D, Ashina M. Calcitonin gene–related peptide modulates heat nociception in the human brain—an fMRI study in healthy volunteers. PLoS One 2016;11:e0150334.
[8] Averill S, McMahon SB, Clary DO, Reichardt LF, Priestley JV. Immunocytochemical localization of trkA receptors in chemically identified subgroups of adult rat sensory neurons. Eur J Neurosci 1995;7:1484–94.
[9] Bahra A, Matharu MS, Bucholz C, Frackowiak RS, Goadsby PJ. Brainstem activation specific to migraine headache. Lancet 2001;357:1016–7.
[10] Bastiaan AM, Bautista DM, Scherrer G, Julius D. Cellular and molecular mechanisms of pain. Cell 2009;139:267–84.
[11] Becker KL, Nylen ES, White JC, Müller B, Snider RH. Jr. Clinical review 167: procalcitonin and the calcitonin gene family of peptides in
inflammation, infection, and sepsis: a journey from calcitonin back to its precursors. J Clin Endocrinol Metab 2004;89:1512–25.

[12] Bellamy J, Bowen EJ, Russo AF, Durham PL. Nitric oxide regulation of calcitonin gene-related peptide gene expression in rat trigeminal ganglia neurons. Eur J Neurosci 2006;23:2057–66.

[13] Benaroch EE. CGRP: sensory neuropeptide with multiple neurologic implications. Brain Pathol 2011;21:272–36.

[14] Benemsi S, Nicolletti P, Capone JG, Geppetti P. CGRP receptors in the control of pain and inflammation. Curr Opin Pharmacol 2009;9:9–14.

[15] Bennett DL. Neurotrophic factors: important regulators of nociceptive function. Neuroscientist 2001;7:13–7.

[16] Bensouf JP, Collins EC, Darling RJ, Allan BW, Leung D, Conner EM, Nelson J, Geppetti P, Xu J, Wang XF, Lynch RA, Li B, McCurdy D, Nisenbaum ES, Oskins JL, Lin C, Johnson KW, Chambers MG. Development of a novel antibody to calcitonin gene-related peptide for the treatment of osteoarthritis-related pain. Osteoarthritis Cartilage 2014;22:578–85.

[17] Bernstein C, Burstein R. Sensitization of the trigeminovascular pathway: perspective and implications to migraine pathophysiology. J Clin Neurol 2012;8:89–99.

[18] Bigal ME, Dodick DW, Rapoport AM, Silberstein SD, Ma Y, Yang R, Levy D, Jakubowski M. Effects of sensitization of trigeminal ganglion neurons innervating pelvic organs in the mouse express trophic neuropeptide y. Neuroreport 2014;25:1453–6.

[19] Bigal ME, Escandon R, Bronson M, Walter S, Sudworth M, Huggins JP, Garzone P. Safety and tolerability of LBR-101, a humanized monoclonal antibody that blocks the binding of CGRP to its receptor: results of the phase 1 program. Cephalalgia 2014;34:483–92.

[20] Bigal ME, Escandon R, Bronson M, Albicy A, Escandon R. Cardiovascular and hemodynamic parameters in women following prolonged CGRP inhibition using LBR-101, a monoclonal antibody against CGRP. Cephalalgia 2014;34:889–96.

[21] Bird GC, Han JS, Fu Y, Adwaniyet H, Willis WD, Neugebauer V. Pain-related synaptic plasticity in spinal dorsal horn neurons: role of CGRP. Mol Pain 2006;2:31.

[22] Born W, Fischer JA. The calcitonin peptide family: what can we learn from receptor knock out and transgenic mice. In: Hay LD, Dickerson MI, editors. The calcitonin gene-related peptide family: form, function and future perspectives. Dordrecht: Springer, 2010. p. 75–86.

[23] Bosco D, Moulton EA, Tully S, Schmahmann JD, Becerra L. Human CGRP gene-related peptide is a potent vasodilator. Nature 1985;313:54–6.

[24] Burstein R. Sensitization of the trigeminal pathway: relevance for migraine pathology. Headache 2009:56;281–9.

[25] Burstein R, Noseda R, Burstein R. Migraine: multiple processes, complex pathophysiology. J Neurosci 2015;35:6619–29.

[26] Burstein R, Noseda R, Burstein R. Migraine associated with chronic trigeminal pain: a race against the development of central sensitization. J Clin Neurol 2011;7:27–36.

[27] Burstein R, Jakubowski M. Analgesic triptan action in an animal model of intracranial pain: a race against the development of central sensitization. J Neurol Sci 2011;306:237–41.

[28] Burstein R, Jakubowski M. Managing migraine associated with sensitization. Handb Clin Neurol 2010;97:207–15.

[29] Burstein R, Jakubowski M, Garcia-Nicas E, Kainz V, Bajwa Z, Hargreaves R, Becerra L, Bosco D. Thalamic sensitization transforms localized pain into widespread allodynia. Ann Neurol 2010;68:81–91.

[30] Burstein R, Levy D, Jakubowski M. Effects of sensitization of trigeminovascular neurons to triptan therapy during migraine. Rev Neurol (Paris) 2005;161:658–60.

[31] Burstein R, Noseda R, Bosco D. Migraine: multiple processes, complex pathophysiology. J Neurosci 2015;35:6619–29.
[57] Dussor G, Yan J, Xie JYS, Ossipov MH, Dichkew DW, Porreca F. Targeting TRP channels for novel migraine therapeutics. ACS Chem Neurosci 2014;5:1085–96.

[58] Edelmayer RM, Vanderah TW, Majuta L, Zhang ET, Fioravanti B, De Felice M, Chichorro JG, Ossipov MH, King T, Lai J, Kori SH, Nielsen AC, Cannon KE, Heinricher MM, Porreca F. Medullary pain facilitating neurons calculate alloglubia in headache-related pain. Ann Neurol 2009; 65:184–93.

[59] Edvinsson L. CGRP receptor antagonists and antibodies against CGRP and its receptor in migraine treatment. Br J Clin Pharmacol 2015;80: 195–9.

[60] Edvinsson L, Ho TW. CGRP receptor antagonism and migraine. Neurotherapeutics 2010;7:164–75.

[61] Edvinsson L, Nilsson E, Jansen-Olesen I. Inhibitory effect of BIBN4096BS, CGRP(8–37), a CGRP antibody and an RNA-Spiegeler on CGRP induced vasodilatation in the perfused and non-perfused rat middle cerebral artery. Br J Pharmacol 2007;150:633–40.

[62] Edvinsson L, Tfelt-Hansen P. The blood-brain barrier in migraine treatment. Cephalalgia 2009;28:1245–58.

[63] Eftekhari S, Edvinsson L. Possible sites of action of the new calcitonin gene-related peptide receptor antagonists. Ther Adv Neurol Disord 2010;3:369–78.

[64] Eftekhari S, Edvinsson L. Calcitonin gene-related peptide (CGRP) and its receptor components in human and rat spinal trigeminal nucleus and spinal cord at C1-level. BMC Neurosci 2011;12:112.

[65] Eftekhari S, Gaspar RC, Roberts R, Chen TB, Zeng Z, Villarreal S, Edvinsson L, Salvatore CA. Localization of CGRP receptor components and receptor binding sites in rhesus monkey brainstem: a detailed study using in situ hybridization, immunofluorescence, and autoradiography. J Comp Neurol 2016;524:90–118.

[66] Eftekhari S, Salvatore CA, Calamari A, Kane SA, Tajti J, Edvinsson L. Differential distribution of calcitonin gene-related peptide and its receptor components in the human trigeminal ganglion. Neuroscience 2010;169:683–96.

[67] Eftekhari S, Salvatore CA, Gaspar RC, Roberts R, O’Malley S, Zeng Z, Edvinsson L. Localization of CGRP receptor components, CGRP, and receptor binding sites in human and rhesus cerebellar cortex. J Neurosci 2015;35:807–40.

[68] Eftekhari S, Salvatore CA, Johansson S, Chen TB, Zeng Z, Edvinsson L. Localization of CGRP, CGRP receptor, PACAP and glutamate in trigeminal ganglion. Relation to the blood-brain barrier. Brain Res 2015;1600:93–109.

[69] Eftekhari S, Warvinge K, Blixt FW, Edvinsson L. Differentiation of nerve fibers storing CGRP and CGRP receptors in the peripheral trigeminovascular system. J Pain 2013;14:1289–303.

[70] Feistel S, Albrecht S, Messlinger K. The calcitonin gene-related peptide receptor antagonist MK-8825 decreases spinal trigeminal activity during nitroglycerin infusion. J Headache Pain 2013;14:93.

[71] Fernández-Camero J, Fernández-de-las-Peñas C, de la Llave-Rincón Al, Gállego SN, Zambrenau L, Wise RG, Buchanan TJ, Huggins JP, Smart TG, Vennart W, Tracey I. Pharmacological modulation of pain-related brain and behavior responses using in situ hybridization, immunofluorescence, and autoradiography. J Comp Neurol 2004;150:633–40.

[72] Fernández-Camero J, Fernández-de-las-Peñas C, Sterling M, Sourliás T, Arendt-Nielsen L, Vicenzino B. Exploration of the extent of somato-sensory impairment in patients with unilateral lateral epicondylopathy. J Pain 2009;10:1179–85.

[73] Fernández-de-las-Peñas C, de la Llave-Rincón Al, Gállego SN, Zambrenau L, Pareja JA, Arendt-Nielsen L. Widespread mechanical pain hypersensitivity as sign of central sensitization in unilateral epicondylogia: a blinded, controlled study. Clin J Pain 2009;25:555–61.

[74] Fernández-Camero J, Fernández-de-las-Peñas C, Sterling M, Sourliás T, Arendt-Nielsen L, Vicenzino B. Exploration of the extent of somato-sensory impairment in patients with unilateral lateral epicondylopathy. J Pain 2009;10:1179–85.

[75] Fernández-de-las-Peñas C, de la Llave-Rincón Al, Gállego SN, Zambrenau L, Pareja JA. Widespread mechanical pain hypersensitivity in carpal tunnel syndrome: evidence of central sensitization in unilateral mechanical pain hypersensitivity. J Neurosurg 2009;1:33–40.

[76] Fiazza TS, Vennart W, Tracey I. Pharmacological modulation of pain-related brain and behavior responses using in situ hybridization, immunofluorescence, and autoradiography. J Comp Neurol 2004;150:633–40.

[77] Hotta H, Sato A, Sato Y, Uchida S. Stimulation of saphenous afferent nerve produces vasodilatation of the vasa nervorum via an axon reflex-like mechanism in the sciatic nerve of anesthetized rats. Neurosci Res 1998;64:305–8.

[78] Hou M, Kaneji M, Longmore J, Tajti J, Uddman R, Edvinsson L. 5-HT(1B) and 5-HT(1D) receptors in the human trigeminal ganglion: co-localization with calcitonin gene-related peptide, substance P and nitric oxide synthase. Brain Res 2001;909:112–20.

[79] Hughes SR, Brain SD. Nitric oxide-dependent release of vasodilator quantities of calcitonin gene-related peptide from capsaicin-sensitive nerves in rabbit skin. Br J Pharmacol 1994;111:425–30.

[80] Iannetti GD, Zambreanu L, Wise RG, Buchanan TJ, Huggins JP, Smart TG, Vennart W, Tracey I. Pharmacological modulation of pain-related brain activity during normal and central sensitization states in humans. Proc Natl Acad Sci U S A 2005;102:18195–200.

[81] Jaggi AS, Singh N. Role of different brain areas in peripheral nerve injury-induced neuropathic pain. Brain Res 2011;1381:187–201.

[82] Janetty CP, Salamon I, Petrusz P, Réthelyi M. Termination patterns of calcitonin gene-related peptide-immunoreactive nerve fibers in the dorsal horn of the human spinal cord. Exp Brain Res 1990;80: 609–17.

[83] Jang JH, Nam TS, Paik KS, Leem JW. Involvement of peripherally released substance P and calcitonin gene-related peptide in mediating mechanical hypersalgesia in a traumatic neuropathy model of the rat. Neurosci Lett 2004;375:320–2.

[84] Jil RR, Kohno T, Moore KA, Woolf CJ. Central sensitization and LTP: do pain and memory share similar mechanisms? Trends Neurosci 2003;26: 696–705.
Li D, Smith C, Monteith D, Brown R, Camporeale A, McNeary T, Deeg M, Raddad E, de la Pena A, Kivity S, Schnitzer T. 2006;28:425–59.

Lee KJ, Rolle A, Mathieu A, Desmette P, Descombes P, Benolken A, et al. 2015;11:219–32.

LeGrice SJ, Bennet MH, Calverley PM, Cooper JA. 2011;10:105–11.

Lewin GR, Mendell LM. Nerve growth factor and nociception. Trends Neurosci 2016;39:644–58.

Lei Y, Smith C, Monteith D, Brown R, Camporeale A, McNeary T, Deeg M, Raddad E, de la Pena A, Kivity S, Schnitzer T. 2017;58:688–705.

Lewin GR, Mendell LM. Neuronal growth and nocicreception. Trends Neurosci 1993;16:353–9.

Li D, Ren Y, Xu X, Zou X, Fang L. Sensitization of primary afferent nociceptors induced by intradermal capsaicin involves the peripheral release of calcitonin-gene-related Peptide driven by dorsal root reflexes. J Pain 2009;9:1155–68.

Liu LF, Vause CV, Durham PL. Calcitonin gene-related peptide stimulation of nitric oxide synthesis and release from trigeminal ganglion glial cells. Brain Res 2008;1190:22–32.

Lin Q, Li D, Xu X, Zou X, Fang L. Roles of TRPV1 and neuropeptidic receptors in dorsal root reflex-mediated neurogenic inflammation induced by intradermal injection of capsaicin. Mol Pain 2007;3:30.

Lin Q, Zou X, Williams D. Adelia and C primary afferent convey dorsal root reflexes after intradermal injection of capsaicin in rats. J Neurophysiol 2000;84:2696–8.

Lobanov OY, Peng YB. Differential contribution of electrically evoked dorsal root reflexes and peripheral vasodilatation and plasma extravasation, J Neuroinflammation 2011;8:20.

Ma QP, Woolf CJ. Noxious stimuli induce an N-methyl-D-aspartate receptor-dependent hypersensitivity of the flexion withdrawal reflex to touch: implications for the treatment of mechanical allodynia. PAIN 1996;61:383–90.

Ma W, Chabot JG, Schorcher-Petru A, Hong Y, Wang Z, Quiron R, CGRP and adrenomedullin as pain-related peptides. In: Hay LD, Dickerson MI, editors. The calcitonin gene-related peptide family: form, function and future perspectives. Dordrecht: Springer, 2010. p. 151–71.

Maggi CA, Meli A. The sensory-efferent function of capsaicin-sensitive primary sensory neurons. Glia 1998;19:1–19.

Marcus R, Goadsby PJ, Dodick D, Stock D, Manos G, Fischer T. BM 2011;158:2281–92.

Marcus R, Goadsby PJ, Dodick D, Stock D, Manos G, Fischer T, BM 2011;158:2281–92.

Marzio JC, Perez OA, Song B, Chen W, Sunakawa M, Okiji T, Suda H. The role of N-methyl-D-aspartate receptor subunits in the rat thalamic mediodorsal nucleus during central sensitization. Brain Res Bull 2011;1371:16–22.

Mc Coy ES, Taylor-Blake B, Street SE, Pribisko AL, Zheng J, Zylka MJ. Peptidogic CGRP primary sensory neurons encode heat and itch and tonically suppress sensitivity to cold. Neuroreport 2013;24:138–41.

Mc Coy ES, Taylor-Blake B, Zylka MJ. CGRP-expressing sensory neurons respond to stimuli that evoke sensations of pain and itch. PLoS One 2012;7:e36355.

Meng ID, Dodick D, Ossipov MH, Porreca F. Pharmacology of medication overuse headache: insights and hypotheses from preclinical studies. Cephalalgia 2011;31:851–60.

Messler K, Lennerz JK, Eberhardt M, Fischer MJ. CGRP and NO in the trigeminal system: mechanisms and role in headache generation. Headache 2012;52:1411–27.

Miller S, Liu H, Warfvinge K, Shi L, Dovlatyan M, Xu C, Edvinsson L. Immunohistochemical localization of the calcitonin gene-related peptide binding site in the primate trigeminal vasculomotor system using functional and immunohistochemical techniques. Neurosci 2011;43:2288–93.

Moulton EA, Schmahlmann JD, Becerra L, Borsook D. The cerebellum and pain: passive integrator or active participant? Brain Res Rev 2010;64:15–27.

Mulder PK, Ghatei MA, Spokes RA, Johns PH, Person AM, Hamid QA, Karse S, Ousavi S, Burren JM, Polak JM, Bloom SR. Differential expression of α-CGRP and β-CGRP by primary sensory neurons and enteric autonomic neurons of the rat. Neurosci 2012;195:205–20.

Naikama-Craig M, Gill IK. Effect of neurokinin A, substance P and calcitonin gene-related peptide in peripheral hyperalgesia in the rat paw. Neurosci Lett 1991;124:49–51.

Nakanishi M, Hata K, Nagayama T, Sakurai T, Nishisho T, Wakabayashi H, Hiraga T, Ebisu S, Yoneda T. Acid activation of Trpv1 leads to an hyperalgesia in the rat spinal dorsal horn after sciatic nerve compression and application of nucleus pulposus in rats. Spine (Phila Pa 1976) 2014;39:455–62.

Nieto FR, Clark AK, Grist J, Chapman V, Malcangio M. Calcitonin gene-related peptide on thermal and mechanical noxious stimuli in experimental hyperalgesic rats. Brain Res 1989;497:199–203.

Kestell GR, Anderson RL, Clarke JD, Haberberger RV, Gibbins IL. Afferent neurons containing calcitonin gene-related peptide but not substance P in forepaw skin, dorsal root ganglia, and spinal cord of mice. J Comp Neurol 2015;59:2555–69.

Kimura S, Sugawa Y, Suzuki M, Orta S, Yamauchi K, Inoue G, Asahi K, Ishikawa T, Miyagi M, Kanda H, Kubota G, Okawa Y, Image K, Sainoh T, Sato J, Nakamura J, Toyone T, Takahashi K, Ohtori S. Evaluation of pain behavior and calcitonin gene-related peptide immunoreactive sensory nerve fibers in the spinal dorsal horn after acute nerve compression and application of nucleus pulposus in rats. Spine (Phila Pa 1976) 2014;39:455–62.

Kranzler JD, Gendreau RM. Role and rationale for the use of milunilac in the management of fibromyalgia. Neuropsychiatr Dis Treat 2010;6:197–208.

Kresse A, Jacobowitzy DM, Skofitsch G. Distribution of calcitonin gene-related peptide in the central nervous system of the rat by immunocytochemistry and in situ hybridization histochemistry. Ann N Y Acad Sci 1992;657:455–7.

Kruger L, Sterpin C, Brecha NC, Mantyh PW. Distribution of calcitonin gene-related peptide immunoreactivity in relation to the rat central somatosensory projection. J Comp Neurol 1988;273:149–62.

Kruus C, Iversen HK, Jansen-Olesen I, Edvinsson L, Olesen J. Calcitonin gene-related peptide (CGRP) levels during cyclo-oxygen (GTX)-induced headache in healthy volunteers. Cephalalgia 2010;30:467–74.

Kuraishi Y, Nanayama T, Onno H, Minami M, Satoh M. Antinociceptive effect of intrathecal administration of antiserum against calcitonin gene-related peptide. Neurosci Lett 1988;92:255–9.

Lassen LH, Hadar-Strick PA, Jacobsen VB, Iversen HK, Sperling B, Olesen J. CGRP may play a causeative role in migraine. Cephalalgia 2002;22:54–61.

Latreilouiere A, Woolf CJ. Central sensitization: a generator of pain hypersensitivity by central neural plasticity. J Pain 2009;10:905–926.

Latreilouiere A, Woolf CJ. Synaptic plasticity and central sensitization: an overview. J Pain 2010;11:801–3.

Lawson SN, Crepps B, Perl ER. Calcitonin gene-related peptide immunoauctivity and afferent receptive properties of dorsal root ganglion neurons in guinea-pigs. J Physiol 2002;540:989–1002.

Le Greves P, Andersson K, Silberring J. Isolation and identification of CGRP C-terminal fragments in the rat spinal cord. Neuropeptides 1997;31:19–29.

Lee SE, Kim JH. Involvement of substance P and calcitonin gene-related peptide in development and maintenance of neuropathic pain from spinal nerve injury model of rat. Neurosci Res 2007;58:425–9.

Lennerz JK, Rolle A, Mathieu A, Desmette P, Descombes P, Benolken A, et al. Calcitonin gene-related peptide (CGRP) immunoreactivity and afferent receptive properties in the peripheral nervous system: differences between peripheral and central CGRP receptor distribution. J Comp Neurol 2008;507:1277–99.

Lewin GR, Mendell LM. Calcitonin gene-related peptide does not excite or sensitize meningeal nociceptors: implications for the pathophysiology of migraine. Ann Neurol 2005;58:698–705.

Litman GR, Mitchell LM. Nerve growth factor and nociception. Trends Neurosci 1993;16:353–9.

Li D, Ren Y, Xu X, Zou X, Fang L. Sensitization of primary afferent nociceptors induced by intradermal capsaicin involves the peripheral release of calcitonin gene-related Peptide driven by dorsal root reflexes. J Pain 2008;9:1155–68.
differential neuropathic pain phenotype. Exp Neurol 2013;250:
194–204.

[145] Noguchi K, Senba E, Morita Y, Sato M, Tofyama M. Co-expression of alpha-CGRP and beta-CGRP mRNAs in the rat dorsal root ganglion cells. Neurosci Lett 1990;108:1–5.

[146] Noseeda R, Burstein R. Migraine pathophysiology: anatomy of the trigeminovascular pathway and associated neuronal systems, cortical spreading depression, sensitization, and modulation of pain. Pain 2013;154(suppl 1):S44–53.

[147] Ohtori S, Takahashii K, Chiba T, Yamagata M, Samedha H, Moriya H. Phenotypic inflammation switch in rats shown by calcitonin gene-related peptide immunoreactive dorsal root ganglion neurons inactivating the lumbar facet joints. Spine (Phila Pa 1976) 2001;26:1009–13.

[148] Olesen J, Diener HC, Hutscheidt WW, Goodyard PJ, Hall D, Meier U, Pollenier S, Lesko LM; IBiN4096 BS Clinical Proof of Concept Study Group. Calcitonin gene-related peptide receptor antagonist IBiN4096 BS for the acute treatment of migraine. N Engl J Med 2004;350:1104–10.

[149] Ossipov MH. Growth factors and neuropathic pain. Curr Pain Headache Rep 2011;15:185–92.

[150] Ossipov MH, Dusser GO, Porecica F. Central modulation of pain. Br J Pharmacol 2003;142:1171–81.

[151] Ossipov MH. Growth factors and neuropathic pain. Curr Pain Headache Rep 2011;15:185–92.

[152] Ossipov MH, Dusser GO, Porecica F. Central modulation of pain. Br J Pharmacol 2003;142:1171–81.

[153] Ossipov MH. Growth factors and neuropathic pain. Curr Pain Headache Rep 2011;15:185–92.

[154] Ossipov MH, Dusser GO, Porecica F. Central modulation of pain. Br J Pharmacol 2003;142:1171–81.
gene-related peptide in human skin—a microdialysis study. J Invest Dermatol 2000;115:1015–20.

[189] Willis WD Jr. Dorsal root potentials and dorsal root reflexes: a double-edged sword. Exp Brain Res 1999;124:395–421.

[190] Wimalawansa SJ. Calcitonin gene-related peptide and its receptors: molecular genetics, physiology, pathophysiology, and therapeutic potentials. Endocr Rev 1996;17:533–89.

[191] Woolf CJ. Windup and central sensitization are not equivalent. PAIN 1996;66:105–8.

[192] Woolf CJ. Central sensitization: implications for the diagnosis and treatment of pain. PAIN 2011;152(suppl):S2–15.

[193] Woolf CJ, Chong MS. Preemptive analgesia—treating postoperative pain by preventing the establishment of central sensitization. Anesth Analg 1993;77:362–79.

[194] Woolf CJ, Thompson SW. The induction and maintenance of central sensitization is dependent on N-methyl-D-aspartic acid receptor activation; implications for the treatment of post-injury pain hypersensitivity states. PAIN 1991;44:293–9.

[195] Xu S, Lundeberg T, Wang YT, Li Y, Yu LC. Antinociceptive effect of calcitonin gene-related peptide injected into periaqueductal grey of rats with mononeuropathy. Brain Res 2000;859:358–60.

[196] Xu W, Lundeberg T, Wang YT, Li Y, Yu LC. Antinociceptive effect of calcitonin gene-related peptide in the central nucleus of amygdala: activating opioid receptors through amygdala-periaqueductal gray pathway. Neuroscience 2003;118:1015–22.

[197] Xu XJ, Wiesenfeld-Hallin Z. Neuropeptides: pain A2. In: Squire LR, editor. Encyclopedia of neuroscience. Oxford: Academic Press, 2009. p. 931–4.

[198] Yao G, Han X, Hao T, Huang Q, Yu T. Effects of rizatriptan on the expression of calcitonin gene-related peptide and cholecystokinin in the periaqueductal gray of a rat migraine model. Neurosci Lett 2015;587:29–34.

[199] Yu LC, Hansson P, Lundeberg T. The calcitonin gene-related peptide antagonist CGRP8-37 increases the latency to withdrawal responses in rats. Brain Res 1994;653:223–30.

[200] Yu LC, Hou JF, Fu FH, Zhang YX. Roles of calcitonin gene-related peptide and its receptors in pain-related behavioral responses in the central nervous system. Neurosci Biobehav Rev 2009;33:1185–91.

[201] Yu LC, Weng XH, Wang JW, Lundeberg T. Involvement of calcitonin gene-related peptide and its receptor in antinociception in the periaqueductal grey of rats. Neurosci Lett 2003;349:1–4.

[202] Yu Y, Lundeberg T, Yu LC. Role of calcitonin gene-related peptide and its antagonist on the evoked discharge frequency of wide dynamic range neurons in the dorsal horn of the spinal cord in rats. Regul Pept 2002;103:23–7.

[203] Yu YJ, Watts RJ. Developing therapeutic antibodies for neurodegenerative disease. Neurotherapeutics 2013;10:459–72.