

# Role of calcitonin gene-related peptide and brain natriuretic peptide to modulate the excitability state of trigeminal neurons: relevance to migraine pathology and treatment

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**Abstract:** Hyperactivity of trigeminal sensory neurons is a major process to generate recurrent headache, typical of migraine attacks. How physiological nociception is converted into strong pathological pain remains, however, poorly understood. In recent years, certain neuropeptides and their receptors have been shown to modulate sensory neuron nociception and to contribute to the persistent hyperalgesia due to the sensory stimulus sensitization that defines the clinical experience of chronic pain syndromes, including migraine. Using calcitonin gene-related peptide (CGRP) and brain natriuretic peptide (BNP) as examples, this review addresses the mechanisms through which neuropeptides might modulate nociceptor activity. One attractive notion is that pain signaling by trigeminal sensory neurons is potently regulated by the ambient levels of these peptides: CGRP is thought to facilitate neuronal firing responsible for trigeminal sensitization necessary to trigger headache, whereas BNP is proposed to act as a negative regulator of trigeminal neuron activity. For either peptide, the key target appears to be the ATP-gated P2X3 receptor that, widely expressed by trigeminal sensory neurons, generates fast, large excitation to release glutamate onto second-order brain neurons. The fine balance between the activities of these peptides is suggested to ultimately determine whether nociception is perceived at higher center as a physiological or pathological response. Hence, the clinical goal of CGRP antagonism using either pharmacological receptor blockers or monoclonal antibodies (to sequester this peptide or to directly inhibit its receptor) is currently considered a novel approach for migraine prophylaxis and to treat acute headache attacks.

**Keywords:** trigeminal ganglion, headache, sensory neurons, P2X3, TRPV1

## Introduction – migraine pain can arise via chronic dysfunction of nociceptive neurons

While the molecular mechanisms of nociceptive neuron signaling have been widely investigated,<sup>1</sup> the pathophysiological basis of chronic pain is less understood. A prototypical case is the recurrent chronic headache of migraine, which is a highly prevalent and disabling neurovascular disorder<sup>2</sup> affecting a significant proportion of the adult population worldwide, and representing an enormous socioeconomic and health care burden for both the individual and society.<sup>3–5</sup>

Migraine is characterized by attacks of headache associated with autonomic nervous system dysfunction, and in about one-third of the cases, with transient neurological symptoms termed “aura”, whereby aberrant visual perceptions are the commonest complaint.<sup>6</sup> Despite the fact that the migraine attack etiology remains largely unknown,

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evidence points to the notion that migraine headache results from strong activation of primary afferent neurons (trigeminal ganglion [TG] nociceptors) that innervate craniofacial tissues (including meninges and their blood vessels) and project to the trigeminal nucleus of the brainstem and spinal cord with further signal processing at higher brain centers.<sup>7–9</sup> In physiological conditions, membrane depolarization (arising from mechanical, thermal, or chemical stimuli) of the peripheral terminals of TG nociceptors widely distributed over the craniofacial territory will, if sufficiently large to reach a certain threshold, evoke action potentials that propagate to the central nervous system. To decode such stimuli and convert them into sensory information, TG neurons express a range of receptors/ion channels that confer stimulus-related excitation. The depolarization induced by sensory stimuli is then modulated by membrane ion channels (e.g. K<sup>+</sup> channels) that constrain the excitation propagating via voltage-gated Na<sup>+</sup> channels responsible for the generation of action potentials. Conveying painful signals to the brain, therefore, demands a delicate balance between excitatory and inhibitory processes at the level of TG neurons. When such a fine equilibrium is pathologically disrupted, sensitization develops, whereby TG neurons become hyperresponsive even to mild stimuli: in the case of migraine, this is an important phenomenon to trigger headache.<sup>10</sup> Furthermore, allodynia, namely pain generation by non-noxious stimuli, can occur in response to peripheral and central inputs.<sup>10–12</sup> Symptoms of sensitization during migraine include throbbing headache and its aggravation during routine physical activities that increase intracranial pressure such as coughing and bending over.<sup>13,14</sup>

The question then arises as to whether sensitization can originate from functional changes in the receptor proteins transducing the sensory stimulus and/or in the voltage-dependent ion channels controlling neuron excitability. Recent studies have demonstrated that trigeminal sensory neurons express a large number of integral membrane proteins that sense strong external stimuli like pungent odors, chemicals, or temperature changes. The interest in their role related to migraine resides in the possibility that such proteins can be important triggers of migraine pain attacks either via direct activation of trigeminal neuron firing or via release of local mediators, in turn, exciting trigeminal neurons. This complex scenario presumably requires a delicate equilibrium between mechanisms dampening or enhancing neuronal excitability, and its derangement may lead to acute and/or chronic headache.<sup>15</sup> Due to space constraints, the present short review focuses on the mechanisms of trigeminal pain transduction and how two endogenous neuropeptides, namely

calcitonin gene-related peptide (CGRP) and brain natriuretic peptide (BNP), may up- or downregulate it. These (and other) neuropeptides might also be the intermediaries of the action of certain external stimuli on trigeminal sensory neurons as outlined earlier. Updated reviews are currently available for general involvement of CGRP in pain processing,<sup>16,17</sup> while the role of BNP in maladaptive sensory signaling is a more recent finding.<sup>18,19</sup>

## Molecular mechanisms of nociceptors involved in migraine pain signaling: shifting targets can facilitate pain onset

A large body of evidence has identified membrane proteins expressed on dural afferent nerve endings and on the soma of TG neurons and suggested to play an important role in migraine pathophysiology.<sup>20</sup> Immunolabeling and retrograde-labeling experiments in rodents have revealed that most small- and medium-sized TG neurons innervating the dura express voltage-insensitive acid-sensing cationic channels (ASICs) as well as transient receptor potential (TRP) channels and purinergic P2X receptors.<sup>21–23</sup> Although pharmacological block of cortical-spreading depression (the underlying process mediating aura) and TG activation was experimentally elicited by the potassium-sparing diuretic amiloride via an action proposed to suppress ASIC activity,<sup>23</sup> there is little evidence that prior to (or during) a migraine attack, the local extracellular pH values can fall to a level sufficient for strong ASIC activation.

TRP receptor/channels comprise a wide family of membrane proteins with multifarious functions of relevance to pain processing.<sup>15,24</sup> For instance, the potential role of TRPA1 receptors in migraine has been considered because TRPA1 agonists may trigger migraine headache in susceptible individuals.<sup>25–28</sup> Nevertheless, TRPA1 receptors, which are expressed only by a small minority of sensory neurons,<sup>29</sup> are typically stimulated by reactive oxygen species, and it is currently unclear whether such agents are generated in sufficient amount to trigger migraine or are by-products of subsequent neurovascular dysfunction associated with headache and contributing to sustain it. Recent results indicate that chemical irritants found as environmental pollutants may also stimulate TRPA1 channels and evoke headache.<sup>30</sup>

Among the family of TRP receptors that can contribute to triggering migraine attacks, transient receptor potential vanilloid (TRPV)-4 is expressed by meningeal fibers: its activation by cold temperature<sup>31</sup> or airborne irritants<sup>32</sup> produces afferent

nociceptive signaling from the head that may contribute to migraine headache. TRPM8 channels that are activated by cold are also proposed to be involved in triggering trigeminal neuron activity<sup>33</sup> in accordance with the notion that a TRPM8 gene variant confers susceptibility to migraine.<sup>34</sup> While several studies have demonstrated capsaicin (and heat)-sensitive TRPV1 channels to be important for the development of inflammatory, thermal pain conditions,<sup>35–37</sup> their ultimate role in migraine remains unclear, and there are no current antimigraine drugs blocking TRPV1 receptors in clinical use.

Cannabinoids have been commonly associated with analgesic effects. In the case of headache, trigeminal neurons express the G-protein-coupled CB1 receptors that are the principal transducers of the neuronal action of endogenous endocannabinoids like anandamine and 2-acylglycerol.<sup>15</sup> The hypothesis of the role of endocannabinoids in migraine was proposed as part of a syndrome (clinical endocannabinoid deficiency) with facilitated onset of pain.<sup>38</sup> This concept was supported by the clinical observation of low endocannabinoid levels in the cerebrospinal fluid of migraine patients.<sup>39</sup> The role of endocannabinoids in trigeminal pain processing is complex because anandamide can produce contrasting effects on the trigeminovascular unit activity, in which neurons, glia, and local vascular elements interact at meningeal level.<sup>40,41</sup> Furthermore, anandamide exerts several cellular effects distinct from CB1 receptor activation, including activation of TRPV1 receptors.<sup>42</sup> These multifarious actions by endocannabinoids may ultimately converge onto the modulatory effects of neuropeptides.

A number of studies have proposed that an acute attack of migraine is associated with a large release of ATP into the extracellular compartment to stimulate nociceptors.<sup>43</sup> While ATP receptors constitute a heterogeneous population, those with ionotropic properties and therefore, producing rapid ion current responses, belong to the P2X subclass. Among ATP-sensitive P2X receptors, the P2X3 receptors are selectively and almost exclusively expressed by sensory ganglion neurons,<sup>44,45</sup> suggesting their key role in processing pain. The subject of P2X3 receptors has been reviewed in detail.<sup>46,47</sup> In the mouse TG, P2X3 receptors are expressed by the vast majority of sensory neurons that may also express receptors for capsaicin (TRPV1)<sup>48</sup> and have been implicated in craniofacial pain,<sup>49,50</sup> including migraine.<sup>47</sup> These data highlight the role of P2X3 receptors in nociception, and suggest that they may be an important target for novel analgesic drugs.<sup>51</sup> For instance, dihydroergotamine (DHE), a nonselective ergot alkaloid derivative extensively used for the acute treatment of migraine,

depresses ATP-mediated sensitization of TG neurons via downregulation of P2X3 receptors:<sup>52</sup> these data suggest that the effectiveness of DHE in treating migraine might be partly due to its inhibitory effect on P2X3 receptors.

Ca<sup>2+</sup>-activated potassium channels may also contribute to controlling the hyperactivity of the trigeminocervical complex. Activation of neuronal large-conductance calcium-activated potassium channels (BK<sub>Ca</sub> or MaxiK) induces hyperpolarization of neurons,<sup>53</sup> while intravenous administration of the MaxiK opener NS1619 dose-dependently inhibits neurogenic dural vasodilation in a model of trigeminovascular nociception.<sup>54</sup> Among the group of voltage-independent K<sup>+</sup> channels, the TRESK K2P channel modulates cellular excitability<sup>55</sup> and is expressed in the TG. Although mutation in the KCNK18 gene that encodes a protein member of the TRESK subfamily of leak K<sup>+</sup> channels was reported to be linked to familial migraine,<sup>56</sup> it was later suggested that KCNK18 might act as a modifier instead of being sufficient to cause typical migraine.<sup>57</sup>

Certain genetic mutations associated with familial migraine and affecting channels directly involved in hyperexcitability have provided further information to understand the primary mechanisms underlying migraine pathophysiology. In particular, the genes known to cause familial hemiplegic migraine (FHM) are all involved in ion homeostasis across the neuronal cell membrane. Missense mutations in CACNA1A gene encoding the pore-forming subunits of the neuronal voltage-gated P/Q-type Ca<sup>2+</sup> channel (Cav2.1) are reported to be the commonest cause of FHM type 1.<sup>58</sup> Such mutation determines a major gain of function of Cav2.1 channels whose activation threshold is shifted to more negative membrane potential.<sup>59</sup> Thus, not only this mutation favors neuronal excitability, but it also facilitates presynaptic release of transmitter, in particular glutamate.<sup>60</sup>

## Migraine pain: sensitization process and its triggers

A number of studies and a large body of clinical as well as preclinical data have provided evidence for the involvement of sterile inflammation as one important mechanism for the activation of the migraine pain pathway.<sup>61</sup> This process is purported to produce abnormal cross talk between neurons and nonneuronal cells in the TG and to reinforce activation and recruitment of immune cells releasing inflammatory agents to perpetuate sensitization.<sup>62–65</sup> A recent study with *in vitro* TG cultures and a cell-line of macrophages has, in fact, shown how co-culturing these cells enhances phagocytic activity of macrophages and augments functional responses

by P2X3 receptors.<sup>64</sup> During neurogenic inflammation, several neuropeptides, neuromodulators, and other signaling molecules are released from neurons and glial and inflammatory cells of the trigeminovascular system. Pro-inflammatory cytokine levels have been found to be elevated in the plasma and in the cerebrospinal fluid of migraineurs.<sup>66–68</sup> Bradykinin, histamine, serotonin (5-HT), and prostaglandin E2 cause mechanical sensitization and increase the excitability of somatic<sup>69</sup> and meningeal nociceptors.<sup>10,11</sup> Other inflammatory mediators like interleukins 1 (IL-1), 6 (IL-6), and 8 (IL-8) and tumor necrosis factor  $\alpha$  exert their effects through the endogenous release of eicosanoids and sympathetic amines.<sup>70,71</sup> Increased nitric oxide (NO) production within the meninges may also contribute to migraine headache in patients.<sup>72</sup> These inflammatory mediators collectively modulate the activity of various ion channels.<sup>73,74</sup>

## Neuropeptides as modulators of nociceptors in migraine

The seminal observation that a pharmacological antagonist of the endogenous neuropeptide CGRP was a potent anti-migraine drug<sup>75</sup> catalyzed a strong focus on the action of CGRP in migraine. CGRP is a 37-amino acid neuropeptide derived from the gene encoding calcitonin by alternative splicing of mRNA and proteolytic processing of its precursor.<sup>76,77</sup> CGRP exists as two isoforms,  $\alpha$ - and  $\beta$ -CGRP.<sup>78,79</sup>  $\alpha$ -CGRP is predominant in the central and peripheral nervous systems, while  $\beta$ -CGRP is mainly present in the enteric nervous system.<sup>80</sup> Jugular plasma concentrations of CGRP are increased during attacks of migraine.<sup>81</sup> In addition, release of CGRP is produced by electrical stimulation of TG fibers.<sup>82</sup> Chemical stimuli that activate, for example, TRPA1 or TRPV1 receptors, or endogenous mediators liberated by neuroinflammatory cells increase the release of CGRP.<sup>82–84</sup> These data have, therefore, led to consider CGRP as a major “migraine mediator” as recently reviewed.<sup>16,17,85,86</sup>

CGRP is known to mediate neurogenic inflammation in peripheral tissues by increasing blood flow, and by recruiting immune cells, that may, in turn, activate sensory neurons.<sup>87</sup> In accordance with this notion, the peptide evokes strong stimulation of NO synthesis and release from trigeminal ganglia<sup>88</sup> as well as liberation of inflammatory cytokines.<sup>89</sup> Furthermore, CGRP can promote the release of endogenous algogenic mediators like bradykinin from TG satellite cells,<sup>63</sup> thus contributing to the creation of a chemical milieu that facilitates sensory neuron activity. Its receptors are expressed in most peripheral components of the trigeminovascular system, including blood vessels, Schwann cells, dural mast cells,

satellite glial cells, and a subpopulation of TG neurons.<sup>90</sup> The importance of CGRP in the pathophysiology of migraine pain is supported by the fact that its intravenous administration causes delayed migraine-like headache in migraineurs, whereas CGRP receptor antagonists are proving effective in treating migraine,<sup>91</sup> in line with the notion that migraine patients may somehow be hypersensitive to the CGRP-positive modulation of nociception.<sup>75,92</sup> It has been suggested that CGRP may mediate cross talk between TG neurons and satellite glial cells<sup>63</sup> resulting in the increased expression and/or membrane targeting of specific pain receptors, such as ATP-gated P2X3, as well as an increased production of other inflammatory mediators, like IL-1 $\beta$ , thus sensitizing nociceptors and reinforcing neuroinflammation.<sup>93,94</sup>

An interesting issue concerns the origin of endogenous CGRP release with particular relevance to the migraine attack. Recent studies have suggested that low levels of endocannabinoids may decrease their inhibitory control over the trigeminovascular system in migraine patients, a phenomenon that, in turn, may lead to increased CGRP.<sup>95</sup> The action of endocannabinoids can also include a site in the brainstem to regulate trigeminal excitability.<sup>96</sup> The mechanism of action of endocannabinoids in migraine remains, however, completely understood in view of the activation of TRPV1 receptors by anandamide and the implications of this result for the control of the local vasculature at meningeal level.

## Gain of function of P2X3 receptors is an important mechanism of CGRP action

CGRP has been demonstrated to upregulate the membrane expression and function of P2X3 receptors<sup>86,94,97</sup> through enhancing gene transcription and receptor membrane targeting.<sup>98</sup> The mechanisms of P2X3 facilitation mediated by CGRP involve the activation of complex intracellular pathways and depend on protein kinase A (PKA) and protein kinase C (PKC) activity.<sup>94,98</sup> It is noteworthy that only a minority of TG neurons can bind CGRP<sup>94</sup> suggesting that the action of this peptide is discrete and can perhaps be amplified via further signaling processes within and between TG cells. In vivo studies confirm that CGRP injection into the temporomandibular joint capsule leads to early, PKA-dependent increase in P2X3 receptors of the spinal trigeminal nucleus neurons.<sup>97</sup>

The potentiating action by CGRP on P2X3 receptors is typically slow as it requires approximately 1 hour to develop fully, and it persists long after the peptide is eliminated.<sup>94</sup> Importantly, this effect of CGRP is selective

for P2X3 receptors because, in TG cultures, it does not affect TRPV1 receptors.<sup>94</sup> Furthermore, it has been proposed that neuronal P2X3 receptor activation and ensuing depolarization can also release CGRP, thereby perpetuating a self-supporting process for TG neuron sensitization.<sup>20</sup>

A significant component of the action by CGRP occurs indirectly, through the release of the algogenic neurotrophin brain-derived neurotrophic factor (BDNF).<sup>98</sup> In accordance with this view, P2X3 receptor expression is promoted by CGRP/BDNF-dependent activation of calcium/calmodulin-dependent kinase II and of the transcription factor CREB.<sup>98</sup> Patients show significantly higher BDNF serum levels during migraine attacks.<sup>68,99</sup> BDNF is a key mediator of plasticity in trigeminal nociceptive pathways,<sup>100</sup> and it has been shown to facilitate P2X3 receptor function.<sup>98</sup> Thus, CGRP and BDNF may be viewed as a combinatorial stimulus to facilitate TG sensitization.

The constitutive upregulation of P2X3 receptors in TG of the mouse model of FHM-1 is due to the higher level of CGRP release probably caused by increased Ca<sup>2+</sup> inflow via gain of function of Cav2.1 channels.<sup>63,101,102</sup> Furthermore, the perturbed intracellular Ca<sup>2+</sup> homeostasis of such neurons enhances calcineurin activity with consequent change in the delicate intracellular balance between phosphorylation and dephosphorylation processes of P2X3 receptors with a positive impact on their function.<sup>101</sup> Thus, preclinical and clinical data concur to show a pivotal role of CGRP in the migraine pain attack and suggest upregulated P2X3 receptors as a major contributor to TG sensitization.

## Clinical pharmacology of CGRP antagonists to treat headache

The growing evidence linking migraine to neuropeptide signaling emphasizes the view that neuropeptide receptors represent an important area for pharmacological treatment.

Despite recent advances in the structure of CGRP receptors obtained with crystallography, the design of effective CGRP antagonist remains a significant challenge in view of the heteromeric nature of the receptor complex.<sup>103</sup> Table 1 summarizes a few recent data for CGRP inhibitors based on receptor antagonists (the “gepants”) or monoclonal antibodies used for current clinical investigations. Detailed reviews on this subject are also available.<sup>17,91,103</sup>

CGRP antagonists appear to be attractive new drugs because they lack vasoconstrictor effects that limit the use of other migraine therapies by patients with cerebral or coronary vascular disease.<sup>114–116</sup> It is noteworthy that, since antagonists can possess distinct species specificity for the CGRP receptor,<sup>103</sup> the present discussion is primarily centered on human studies. In a Phase II double-blind randomized clinical trial of 126 patients with migraine, the olcegepant response rate to block a migraine attack was comparable to that of triptans.<sup>75</sup> Its tolerability was good, but this drug could only be administered by intravenous injection,<sup>75</sup> thus representing a pitfall for treatment accessibility and repetition. More recently developed CGRP antagonists are available as oral formulation. Some of them (namely telcagepant/MK-0974 and MK-3207) were shown to be effective against migraine, but, due to liver toxicity concerns, their development was discontinued.<sup>117,118</sup> Three new CGRP receptor antagonists (BI 44370 TA, BMS-927711, and MK-1602) have completed Phase II trials, although only two have been reported (BI 44370 TA and BMS-927711) (Table 1).

A recent positron emission tomography study in man has shown that telcagepant achieved only low receptor occupancy at an efficacious dose, suggesting that large-scale antagonism of brain CGRP receptors is unlikely to be required for migraine efficacy.<sup>119</sup> It is, however, feasible that central CGRP receptor antagonism may provide additional therapeutic benefits.<sup>119</sup>

**Table 1** Current CGRP antagonists/antibodies in clinical trial for migraine therapy

Code name	Type	Indication	Administration route	Clinical phase	References
BIBN4096BS (olcegepant)	CGRP receptor antagonist	Acute treatment	Intravenous	Phase II	75,104–106
BI 44370 TA	CGRP receptor antagonist	Acute treatment	Oral	Phase II	107
BMS-927711	CGRP receptor antagonist	Acute treatment	Oral	Phase II	108,109
MK-1602	CGRP receptor antagonist	Acute treatment	Oral	Phase II	In progress (NCT01613248)
LY2951742	Humanized antibody against CGRP	Prophylactic treatment	Subcutaneous	Phase II	110,111
ALD403	Humanized antibody against CGRP	Prophylactic treatment	Intravenous	Phase II	112
LBR-101	Humanized antibody against CGRP	Prophylactic treatment	Subcutaneous	Phase II	113
AMG 334	Humanized antibody against CGRP receptor	Prophylactic treatment	Subcutaneous	Phase II	In progress (NCT01952574)

**Abbreviation:** CGRP, calcitonin gene-related peptide.

An alternative approach to pharmacological antagonists is the use of antibodies to target CGRP or its receptor. The sustained inhibition of CGRP signaling produced by monoclonal antibodies pharmacokinetically differentiates them from the CGRP antagonists, and renders the use of antibodies more suitable as prophylactic treatment rather than acute migraine medication.

One advantage with the antibody therapies is that they are structurally very different from the CGRP antagonists; thus, it is less likely that similar liver toxicity problems will be encountered. However, they are of high cost, and at least, some of them require an injectable route of administration. Moreover, the potential onset of immunological reactions should be borne in mind, and chronic depletion of CGRP may have adverse side effects actually induced by the lack of this peptide. While monoclonal antibodies are not expected to cross the blood–brain barrier in physiological conditions, the integrity of this barrier during an attack of migraine with associated release of circulating inflammatory factors remains a subject for future studies. Hence, it is not clear whether these antibodies exert their pharmacological effects simply through a peripheral site of action. There are, in fact, suggestions that CGRP antagonists may work partly through a central mode of action,<sup>120</sup> even if this hypothesis is not without controversies<sup>121,122</sup> and not fully consistent with recent functional imaging data.<sup>119</sup> It should, however, be recalled that the TG is outside the blood–brain barrier even in physiological conditions, and it remains an accessible target for these antibodies (or, indeed, pharmacological blockers). Thus, it will be of great interest to examine if the CGRP antibodies display comparable long-term efficacy as the CGRP antagonists.

There are no clinical data based on multicenter trials, in which the efficacy of antibodies against the CGRP receptor (or the peptide itself) has been compared versus administration of CGRP pharmacological antagonists. Furthermore, it is not known if there is any advantage in terms of migraine control in using an antibody therapy directed against the CGRP receptor rather than the circulating peptides. These issues will also need extensive future studies in view of the possible long-term changes in CGRP receptor function, once the natural ligand activity has been inhibited in a persistent manner.

LY2951742 and ALD403 are fully humanized monoclonal antibodies that potently and selectively bind to CGRP.<sup>111,123</sup> ALD403, in particular, binds to both  $\alpha$  and  $\beta$  forms of human CGRP (affinity  $K_d < 20$  pM<sup>123</sup>), and LBR-101 binds to the peptide receptor-binding site of CGRP.<sup>113</sup> After antibody-

dependent sequestration of circulating CGRP, the peptide receptors might remain available to other endogenous ligands supporting ongoing receptor signal transduction in neurons and nonneuronal cells.

Advances in antibody design technology have paved the way for an improved generation of therapeutic antibodies.<sup>124</sup> Thus, therapeutic antibodies can be modified to increase their specificity<sup>125</sup> and decrease their risk of adverse reactions, for example, by modulating their immune effector functions, extending their half-life, and optimizing their antigen-binding domains. The antibody stability is an important issue since, in order to neutralize CGRP, whose plasma levels are fluctuating during the course of the illness, high antibody concentrations should be attained. This goal is, however, not without problems as mechanism-related and nonspecific adverse reactions have been reported, including immunogenicity.<sup>126</sup> Guidelines for the therapeutic use of antibodies have recently been published (<http://www.ema.europa.eu/ema/>). It should be noted that certain pharmacological antagonists of CGRP can also induce adverse reactions as shown, for example, by interruption of the trials with the CGRP receptor antagonist telcagepant.<sup>127</sup>

## BNP is a potential endogenous negative regulator of nociceptive signaling

While CGRP is the prototypical neuropeptide for the sensitization of TG neurons, it seems likely that there are also endogenous neuropeptides to downregulate the activity of P2X3 receptors and they would, thus, represent an intrinsic negative feedback system to control pain sensitivity. Recent evidence has been gathered in support of a potential involvement of the natriuretic peptide system in the modulation of peripheral sensory neuron nociceptive signaling.<sup>128–131</sup> The natriuretic peptides are a family of structurally related hormones widely known for their important effects on the cardiovascular system and the regulation of water–electrolyte homeostasis.<sup>132</sup> These include atrial natriuretic peptide (ANP), BNP, and the C-type natriuretic peptide (CNP).<sup>133</sup> All three peptides showed distinct amino acid sequence that hints to their separate genetic origin.

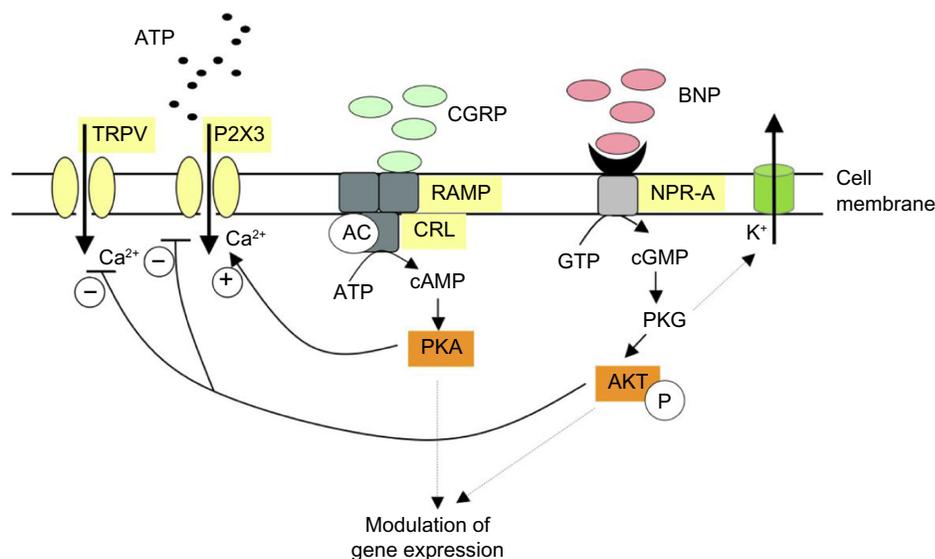
Natriuretic peptides exert their physiologic effects through binding, with different affinities, to three membrane receptor subtypes: the natriuretic peptide receptors A (NPR-A), B (NPR-B), and C (NPR-C), which are also known as guanylate cyclase A (GC-A), B (GC-B), and the clearance receptor, respectively. NPR-A is activated mainly by ANP and BNP.<sup>133,134</sup>

Unlike CGRP, the functional consequences of BNP action on neurons are less understood. Nevertheless, while BNP and its receptor NPR-A are both expressed in the rat dorsal root ganglia (DRG), their signaling may attenuate inflammatory pain through a mechanism involving the opening of large-conductance  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  ( $\text{BK}_{\text{Ca}}$ ) channels.<sup>129</sup> Microarray gene profiling has indicated that chronic pain models enhance BNP and its NPR-A in rat DRG. NPR-C is also present in DRG, where it colocalizes with TRPV1 channels. Here, however, NPR-C activation by CNP displays diametrically opposite effects compared to NPR-A, as it enhances thermal hyperalgesia in a TRPV1- and PKC-dependent fashion.<sup>128</sup> These results indicate the coexistence of functional NPRs in DRG that exhibit contrasting effects on pain transduction, which raises question on how the two functions are inter-related and whether the natriuretic peptide modulatory role can also be generalized to cranial ganglia and other pain conditions.

In relation to migraine mechanisms, ANP gene expression has been shown to increase following experimental cortical-spreading depression.<sup>135</sup> Moreover, a recent clinical report documents that the BNP precursor (proBNP) levels are raised in the jugular vein blood during a migraine attack,<sup>136</sup> indicating a potential involvement of BNP in migraine pathophysiology.

Many migraine mediators have, in common, the ability to alter both neuronal and vascular function (eg, CGRP, substance P, bradykinin). This property is also shared by natriuretic peptides that activate cGMP-dependent pathways via GC in vascular cells to induce vasodilation<sup>137,138</sup> and to stimulate nitric oxide synthase and NO production probably via activation of the NPR-C receptor.<sup>138,139</sup> Due to the vasoactive properties of natriuretic peptides, it has been recently postulated that these substances can induce headache.<sup>140</sup> Future studies are, however, needed to determine the vasoactive properties of the natriuretic peptides on cerebral vessels and their potential role in migraine. Several lines of evidence show that vasodilation of cranial (meningeal) and extracranial arteries is not necessary to directly provoke migraine pain as reviewed.<sup>61</sup>

In rodent TG and DRG, the NPR-A receptor has been reported to be expressed only by sensory neurons. In mouse TG, NPR-A is present at membrane level in almost all (>95%) neurons, and application of exogenous BNP rapidly evokes a large increase in cGMP level, a canonical effector of BNP,<sup>133</sup> and phosphorylation of AKT, a kinase downstream of NPR-A activation.<sup>141</sup> Such a strong and widespread activity of NPR-A receptors outlines a major role of this system in controlling neuronal excitability. In fact, although application of exogenous BNP does not change



**Figure 1** Idealized scheme depicting modulation by CGRP or BNP of P2X3 and TRPV1 receptors of trigeminal sensory neurons.

**Notes:** Extracellular CGRP binds to its receptor complex comprising, in addition to the CGRP-binding site, the accessory proteins RAMP and CRL. Once this receptor is activated, it catalyzes the synthesis of cAMP that, in turn, activates PKA to phosphorylate the intracellular domain of the ATP-sensitive P2X3 receptor with subsequent gain of function and facilitation of trigeminal pain signaling. In addition, CGRP receptor activity stimulates P2X3 gene expression to promote synthesis and trafficking of these receptors. No apparent effect by CGRP on TRPV1 receptors is reported. Extracellular BNP binds to its receptor NPR-A, which catalyzes the synthesis of cGMP that, in turn, stimulates phosphorylation of AKT, thereby activating this kinase. Even though the multiple intracellular targets for AKT are not fully known, it is proposed to downregulate (probably via a complex intracellular cascade) the activity of TRPV1 as well as P2X3 receptors with an ultimately inhibitory role of the receptor activity.  $\text{K}^+$  channels crucial for stabilizing membrane potential and controlling firing of action potentials are also shown. Positive and negative signs indicate activation and inhibition, respectively.

**Abbreviations:** AC, adenylate cyclase; BNP, brain natriuretic peptide;  $\text{Ca}^{2+}$ , calcium channels; CGRP, calcitonin gene-related peptide; CRL, calcitonin receptor-like receptor;  $\text{K}^+$ , potassium channels; NPR-A, natriuretic peptide receptor A; PKA, protein kinase A; PKG, protein kinase G; RAMP, receptor activity-modifying protein; TRPV, transient receptor potential vanilloid.

P2X3 receptor activity, the same protocol depresses TRPV1-mediated currents in TG.<sup>142</sup> Furthermore, anantin, a selective NPR-A antagonist, slowly and greatly enhances P2X3 receptor function, implying that ambient BNP produced a constitutive downregulation of P2X3 receptors, which could not be further intensified by exogenous BNP.<sup>142</sup> These data indicate the role of this endogenous peptide as a negative regulator of trigeminal sensory neuron excitability to nociceptive stimuli and should prompt future clinical studies to explore the role of NPR-A receptors in migraine. Further studies are required to clarify how BNP and CGRP may interact in migraine pain: an idealized scheme of the potential regulation by CGRP and BNP of TG neuronal function is illustrated in Figure 1.

## Conclusion

Somatosensory cortical maps show that the trigeminal territory is large and very sensitive to nociceptive stimuli. Thus, to prevent unwanted pain generation with associated allodynia and hyperalgesia as observed in chronic pain syndromes, potent intrinsic regulators should exist to counteract the sensitization elicited by neuropeptides like CGRP. While current interest in novel treatments for migraine is centered on the goal of blocking either CGRP or its receptors, future studies are necessary to find out whether boosting intrinsic negative regulators that may comprise K<sup>+</sup> channels, endocannabinoids, or neuromodulatory peptides might be a viable approach to treat migraine.

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## Disclosure

The authors declare no conflict of interest.

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