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CGRP receptor antagonists and antibodies against CGRP and its receptor in migraine treatment

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Recently developed calcitonin gene-related peptide (CGRP) receptor antagonistic molecules have shown promising results in clinical trials for acute treatment of migraine attacks. Drugs from the gepant class of CGRP receptor antagonists are effective and do not cause vasoconstriction, one of the major limitations in the use of triptans. However their use had to be discontinued because of risk of liver toxicity after continuous exposure. As an alternative approach to block CGRP transmission, fully humanized monoclonal antibodies towards CGRP and the CGRP receptor have been developed for treatment of chronic migraine (attacks >15 days/month). Initial results from phase I and II clinical trials have revealed promising results with minimal side effects and significant relief from chronic migraine as compared with placebo.

The effectiveness of these various molecules raises the question of where is the target site(s) for antimigraine action. The gepants are small molecules that can partially pass the blood–brain barrier (BBB) and therefore, might have effects in the CNS. However, antibodies are large molecules and have limited possibility to pass the BBB, thus effectively excluding them from having a major site of action within the CNS. It is suggested that the antimigraine site should reside in areas not limited by the BBB such as intra- and extracranial vessels, dural mast cells and the trigeminal system. In order to clarify this topic and surrounding questions, it is important to understand the localization of CGRP and the CGRP receptor components in these possible sites of migraine-related regions and their relation to the BBB.

Introduction

Migraine is a common neurological disorder that affects up to 16 % of the adult population in Western countries [1]. It is characterized by episodic, often disabling headache, associated with sensory (aura), autonomic (nausea, vomiting), phonophobia and photophobia, and cognitive symptoms. Although still debated, the general view is that migraine is a disorder in which central nervous system (CNS) dysfunction plays a pivotal role while various parts of the trigeminal system are necessary for the expression of peripheral symptoms and aspects of pain [2]. In support, a recent study reported brain activation already during the premonitory phase of glycerol trinitrate-induced migraine attacks [3].

Although the triptan group of drugs provides effective relief from acute migraine attacks for many patients, a substantial number (up to 40% in the case of oral triptans) of affected individuals are unresponsive [4]. Subcutaneous sumatriptan provides about 81% headache relief at 2 h [5] while the efficacy of oral triptans is lower. Ferrari *et al.* reported sumatriptan 100 mg oral had a response rate of 58% improvement at 2 h (therapeutic gain was 33%) while the pain-free response was 35% (therapeutic gain was 26%) [4]. In addition, such therapy can lead to cardiovascular symptoms in 10% of the subjects [6]. The gepants represent a new class of antimigraine drugs that act as calcitonin gene-related peptide (CGRP) receptor blockers. They have proven efficacy in clinical trials [7] and act at several sites in the trigeminal system and in the CNS resulting in pain relief [8]. The gepants do not cause vasoconstriction *per se*, either in cranial or in coronary arteries [9–11], which avoids one of the major limitations of using triptans [6]. In comparisons with triptans in head-to-head clinical trials on acute treatment of migraine attacks, it has been revealed that the clinical efficiency of gepants is comparable with that of triptans and superior to placebo [7]. Recently, telcagepant was reported to have a prophylactic effect [12]. However, this group of molecules was terminated for further development because of liver toxicity during

repeated exposures. This effect was attributed to the molecular structure of the compound.

In a subgroup of migraine patients (1–2%) the frequency of migraine may increase over time to multiple monthly attacks. These patients are extremely difficult to treat. Furthermore, their attacks may become chronic (attacks $>$ 15 days per month) which is often associated with medication overuse [13]. The development of monoclonal antibodies to CGRP or to its receptor has reopened the development of therapeutics for this group of patients. The first published reports indicate that this novel antibody strategy is effective in such patients [14, 15]. It is suggested that these molecules act by binding to CGRP that is released from the trigeminovascular system or attached to CGRP receptors during the migraine attack. The antibodies, however, act in various parts of the body and are not limited to cranial structures only [16].

However, the site action of CGRP and CGRP receptor interacting agents in migraine therapy is still debated. The gepants pass poorly through the BBB [17]. For telcagepant the CSF : plasma ratio in primates was found to be about 1.4% which suggests the potential for a small amount of brain penetration [18]. On the other hand, the antibodies represent a different class of molecules that are considerably larger in size with even less possibility to cross the BBB. It is often argued that triptans, gepants or antibodies may pass the BBB to some degree and that this is enough to deliver antimigraine efficiency. However, it should be kept in mind that the agonist–antagonist behaviour at a receptor site follows operational criteria for pharmacological interactions as outlined by Black & Leff [19]. It seems unlikely, given their poor penetration of the BBB, that these molecules will achieve the CNS concentrations necessary to provide reasonable antagonism at the receptor sites. The neurotransmitter is released at a synapse at a very high concentration (the site of interaction where the antagonist competes). In addition to poor penetration into the brain, telcagepant is also a substrate for P-glycoprotein transport out of the brain. Based on the above considerations, there is a need to examine the localization of CGRP receptors in relation to the brain, the trigeminal ganglion and the meninges in order to understand where these molecules may act in migraine therapy.

Role of CGRP

A significant role of CGRP in migraine pathophysiology was established by the demonstration that CGRP concentrations are increased in the cranial circulation during genuine migraine attacks [20]. CGRP in saliva has been shown to be elevated in acute migraine [21], and its concentrations correlate with pain and abortion of the attack by triptan administration [22]. Technical issues related to peptide analysis, such as specificity of the detection antibodies and concerns regarding breakdown of CGRP and other peptides in the circulation, are important to keep in mind when evaluating positive as well as negative results [23, 24]. Further support for the role of CGRP is established by the antimigraine effect of CGRP receptor blockade. This was initially demonstrated using intravenous olcegepant [25] and subsequently with several other gepants given orally [7].

The trigeminal origin of perivascular CGRP-containing nerve fibres was first revealed using immunohistochemistry and quantitative radioimmunoassay combined with specific denervation [26]. Subsequent neuronal tracing studies in rat using True Blue in temporal [27], middle meningeal [28] and cerebral arteries [29] localized the tracer in a subpopulation of CGRP-containing trigeminal neurons. Later studies using the sensory transganglionic tracers wheat germ agglutinin-conjugated horseradish peroxidase (WGA-HRP) and cholera toxin subunit b (CTb) revealed a specific somatotopic localization in brainstem projection areas, the trigeminal nucleus caudalis, and the spinal C1–2 levels of projections of both the thin nociceptive (unmyelinated C-fibres and small myelinated Aδ - fibers) and the thick low threshold mechanoreceptive myelinated A- fibres. These projections are connected to the middle meningeal [30], superior sagittal [31], temporal [32] and cerebral arteries [33].

Over many years, our research group has carefully mapped out available messenger molecules in the trigeminovascular system and revealed the central role of the trigeminal sensory system in migraine. Of the various neuronal messenger molecules found in this system, CGRP stands out as the most prevalent molecule with a particular relation to primary headache disorders [34]. CGRP is expressed throughout the central and peripheral nervous systems, consistent with modulation of vasodilatation, nociception, motor function, secretion and olfaction [8]. Two forms of CGRP are expressed, αCGRP is prominently localized in primary spinal afferent C and Aδ fibres of sensory ganglia, while ßCGRP is the main isoform in the enteric nervous system [7, 31]. There is a rich plexus of CGRP containing perivascular nerves in intracranial blood vessels, both in cerebral and meningeal arteries and in the dura mater [7, 34]. Tracing and denervation studies have revealed their origin in the first division of the trigeminal ganglion [34–36]. The peripheral projections of the trigeminal system are involved in neurogenic vasodilatation, sensitization and inflammation, while central release may induce hyperalgesia. Trigeminal nerve activation results in antidromic release of CGRP [36] which acts via a CGRP receptor complex that consists of calcitonin receptor-like receptor (CLR) and receptor activity modifying protein 1 (RAMP1) [37]. This receptor complex is linked to a G-protein that is coupled to the 'receptor component protein (RCP) - adenylate cyclase' [38]. This pathway underlies non-endothelium

mediated and cAMP-associated vasodilatation in cerebral and meningeal arteries [39, 40]. At synapses in the trigeminal nucleus caudalis/C1–2 levels of the brain stem, CGRP, with or without a co-transmitter, acts on other neurons to transmit nociceptive signals centrally via the brainstem and midbrain to the thalamus and higher cortical regions [35]. CGRP binding sites are widely expressed throughout the brain [41]. CLR and RAMP1 have been localized to the cytoplasm of trigeminal neurons, at peripheral sites on the intracranial vasculature (in the smooth muscle cells), in the dura mater and in the brainstem [42–44]. Most CGRP containing neurons in the trigeminal ganglion, however, do not have the receptor elements. Instead CLR and RAMP1 are co-expressed on non-CGRP neurons and on glial cells, suggestive of intraganglionic interaction [45].

Localization of CGRP receptors

Clinical studies with imaging techniques such as positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) have demonstrated that certain brainstem areas are activated during migraine attacks [46]. These include the midbrain, pons, substantia nigra, red nucleus, periaqueductal grey (PAG), nucleus raphe magnus (NRM) and the locus coeruleus (LC) [47]. Recently, investigation of the premonitory phase of migraine (without headache) using functional neuroimaging revealed that the right ventral midbrain and the right PAG in the dorsal pons were activated by systemic administration of glyceryl trinitrate in episodic migraine subjects [3]. However, it is not known what drives this early activation of the brainstem, but these data support the involvement of diencephalic and brainstem mechanisms in the pathophysiology of migraine [2].

In the CNS there is a wide distribution of CGRP with the highest concentrations in the striatum, amygdalae, colliculi and cerebellum [8, 41]. The first CGRP ligand binding study was carried out by Sexton and revealed a number of binding sites in the CNS of rat [48]. Recent studies have verified many of these findings in the rhesus monkey brainstem with the use of novel methodologies and specific antibodies towards CLR and RAMP1 [49]. These studies in primate brain also show CLR and RAMP1 mRNA and protein expression in the pineal gland (related to light and darkness registration and circadian rhythm control), the medial mammillary nucleus, PAG, area postrema, the raphe nucleus, the gracile nucleus and the spinal trigeminal nucleus [49].These data indicate multiple localizations of CGRP receptor components and suggests several putative functions of CGRP within the CNS.

The cerebellum is important in modulating many cortical motor and sensory inputs. The cerebellum exerts an inhibitory control in the cerebral cortex and, thus, may play an important role in filtering of sensory inputs [50]. Purkinje cell bodies store large amounts of vesicular CGRP and the CLR/RAMP1 complex is expressed both on the cell body and in axons and dendrites [51, 52]. Early studies of acute migraine attacks by PET revealed activation of cerebellar regions [46, 53]. However, the studies did not provide any deeper explanation for the activation that was observed.

Based on the view that migraine starts in the CNS [2, 3] and the fact that triptans and gepants can modify acute attacks, it is logical to search for sites in the CNS that are targeted by these drugs. Hence, Merck (West Point, USA) developed a CGRP receptor related PET ligand that passed the BBB and could be used to test the hypothesis in a small group of patients. They first administered a brain penetrant PET tracer that binds to the CNS. Amazingly, the main binding was observed in the cerebellum of the patients [54]. Then the group administered telcagepant in two doses, one in a clinically effective dose and one nearly 10 times higher [54]. Surprisingly, the brain PET signal was not modified by telcagepant when it was given in the clinically effective dose that aborts the migraine headache [7]. However, when telcagepant was given in the higher dose, they observed a competition with the PET signal, demonstrating interaction at CNS binding sites with this dose. However, there was no further clinical efficiency reported in this study. These findings are compatible with the view that the principle site of antimigraine action of telcagepant lies outside the CNS.

Is the trigeminal ganglion located outside the BBB?

Migraine is considered a neurovascular disorder that originates in the brain, involving the hypothalamus and thalamus, as well as certain brainstem regions [2]. The attack is often preceded by prodromal symptoms, which again suggests the CNS as a likely point of origin [3]. The pain during a migraine attack is associated with the release of CGRP which appears to have a key role in migraine pathophysiology [8, 20–22, 25]. Basic studies have revealed that CGRP does not pass the cerebral vessel walls [55]. Hence it is suggested that the elevated concentrations of CGRP found in the jugular venous blood during migraine attacks derives from various parts of the trigeminovascular system during its activation [56]. Systemic intravenous infusion of CGRP has been found to trigger migraine-like headache in migraine patients [57]. With magnetic resonance angiography imaging, the authors observed that CGRP-induced migraine was associated with dilatation of extracranial branches of the middle meningeal artery. The headache was located in association

with the dilatation and hence it was proposed that vasodilatation was causative for the attack because there was at the same time no dilatation of the middle cerebral artery [57]. However, a more recent MRI angiography study showed that pain in spontaneous migraine attacks was not accompanied by extracranial meningeal artery dilatation but with a small intracranial artery dilatation [58]. This shows a difference in intra- and extracranial arterial diameter alterations in drug induced migraine-like attacks *vs*. genuine migraine attacks [58]. The interpretation of these results remains to be related to other neuroimaging findings [47].

Antibodies towards CGRP and the CGRP receptor, as well as 'Spiegelmer' molecules with CGRP-binding RNA [59, 60] provide new tools that may assist in explaining the site of action of antimigraine drugs [16]. These molecules are relatively large and therefore appear to have little chance to pass the BBB in effective doses. However, some studies have shown that IgG molecules can pass the BBB via transcytosis [61], via transfer mechanisms [62] or via facilitated transport [63, 64]. In addition, some regions of the CNS are more penetrable than others which also offers possibilities for systemic drugs to interact with the CNS. Since gepants as well as antibodies to CGRP and CGRP receptors are therapeutically effective in migraine, it could be suggested that they share a site of action. However, it is my view that it is unlikely that these drugs act in the CNS in clinically effective doses.

It has been well known for decades that the dura mater and the middle meningeal artery are devoid of a BBB [65]. In order to shed some light on this issue, research has recently been focused on the trigeminal ganglion to determine if CGRP receptor antagonists may act there [66]. In addition, it is known that trigeminal CGRP-containing nerve fibres innervate cerebral [26] and dural blood vessels [67] and that release of CGRP mainly causes vasodilatation [36, 39]. CGRP also was found to degranulate rodent mast cells, which contain CGRP receptor components [42].

The trigeminal ganglion contains CGRP where the peptide is found in small to medium-sized neurons (about 50%) and in nerve fibres that are dispersed within the ganglion and in perivascular CGRP positive nerve fibres. In addition large neurons (37%), satellite glial cells and vascular smooth muscle cells have CGRP receptor elements CLR/RAMP1 [45, 66] (Figure 1). Centrally the trigeminal system projects to parts of the CNS where nociceptive information is processed to higher cortical regions [35, 43, 44]. This pattern of expression is similar in rat, monkey and human trigeminal ganglia.

In order to determine if the trigeminal ganglion could be one possible site of action for CGRP receptor antagonists, it has recently been shown that Evans blue (which couples with plasma albumin in the circulation to form a large complex) is found in the trigeminal ganglion, among the different cell types and in blood vessel walls within the ganglion (Figure 1) [66]. The experiments showed that the trigeminal ganglion, like the dura mater [65], lacks a BBB and is freely accessible to circulating compounds [66]. Furthermore, a recent study using labelled EDTA, another tracer that allows for quantitative calculation of the transfer constant, showed that the trigeminal ganglion is >100 times more accessible to the passage of this tracer than the cerebral cortex, cerebellum, brainstem and trigeminal nucleus caudalis [68]. Thus, it is possible that current antimigraine drugs may reach sites in the dura mater and the trigeminal ganglion, but not in the CNS apart from regions lacking a BBB. However, the suggestion needs more confirmation with functional data and with imaging techniques because it is still often suggested that the BBB is disrupted during migraine attacks [69] and following cortical spreading

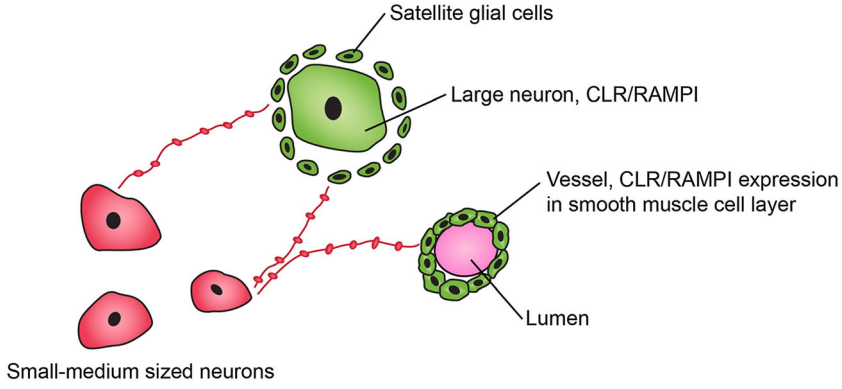

Figure 1

A schematic illustration of the immunofluorescence results [62]. CGRP is expressed in small/medium sized cells and in thin 'pearl'-like fibres among the trigeminal cells (red). The receptor components, CLR and RAMP1, are co-expressed in larger neurons, satellite glial cells and in the vascpular smooth muscle cells located within the trigeminal ganglion (green). ■, co-expression CLR/RAMPI; ■, CGRP expression.

depression [70]. This question has been addressed in a few clinical studies which collectively provide no support for alteration in the BBB during the attacks [71–73].

Conclusion

Clinical trials have demonstrated good efficacy of gepants and triptans in migraine therapy, but they only pass the BBB to a minor degree. The newly developed CGRP and CGRP receptor antibodies are effective prophylactics but they are large molecules that do not readily penetrate the BBB in reasonable doses, suggestive of an action outside the CNS. Therefore, it is suggested that the meninges and, in particular, the trigeminal ganglion are the most logical sites of action for the observed clinical activity. There are neurons and fibres that store CGRP, neurons and satellite glial cells that have CGRP receptors, and the smooth muscle cells of blood vessels have CGRP receptors. All these sites are reachable by molecules present in the circulation.

Competing Interests

The author has completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declares no support from any organization for the submitted work, no financial relationships with any organizations that might have an interest in the submitted work in the previous 3 years and no other relationships or activities that could appear to have influenced the submitted work.

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