**SITE OF ACTION OF CGRP AND CGRP RECEPTOR ACTING AGENTS**

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The peptide calcitonin gene-related peptide (CGRP) has a key role in migraine, supported by studies showing that CGRP is released in migraine attacks, infusion of CGRP can trigger migraine-like headache in patients and that studies with 5 different CGRP receptor antagonists (gepants) aborted the migraine pain. The CGRP receptor antagonists have shown clinical efficacy in the treatment of acute migraine attacks. The key question for a CGRP-receptor antagonist, whether a classical antagonist or an antibody, is whether inhibition of CGRP released peripherally from sensory nerves is sufficient for the antimigraine action, or if inhibition of CGRP acting centrally in trigeminal pain-relay nuclei in the brainstem contributes to the clinical effectiveness?

The activation of the trigeminovascular system causes release of CGRP at terminals in cerebral vessels and in the dura mater and its middle meningeal artery branches. Immunohistochemistry has revealed CGRP and CGRP receptors in many places in the brainstem and in cerebellum; however these sites are protected from circulating agents by the blood-brain barrier (BBB). Recent PET studies have shown that telcagepant could only in high doses access these sites, suggesting that the major site of action of this gepants is outside the BBB. Thus, it appears likely that the drugs would act somewhere on the trigeminovascular complex; trigeminal nucleus caudalis, trigeminal ganglion and peripheral on the intracranial vessels.

Our hypothesis suggests that migraine is a CNS disorder caused by an imbalance in the CNS which then activates the trigeminovascular system providing the link to pain and referred pain. Detailed tracing studies have shown that the trigeminal sensory fibres project to the TNC and C1-2 in the brainstem. Recent studies with markers for the BBB do not suggest that this part of the CNS is freely diffusible to circulating agents. Thus, molecules like the gepants and antibodies would not pass to any greater extent.

The intracranial blood vessels differ; the cerebral arteries have a BBB while the dura and the middle meningeal artery do not. A recent clinical MRI study on genuine migraine attacks showed only minor dilatation of cerebral arteries but no change in meningeal artery diameter. A series of systemic compounds have been tested in the “migraine-like model” and there is no common mechanism that can explain the induced headache attacks.

The remaining structure in the trigeminovascular system, the trigeminal ganglion has only to a minor extent been evaluated as a possible “locus minoris” that could serve as a unifying hypothesis in understanding which and how different molecules may have antimigraine efficacy. We have therefore in detail studied; (i) the trigeminal ganglion from rat, monkey and man in relation to binding sites of a CGRP receptor antagonist and protein expression of CGRP and its receptor. (ii) In addition, we have examined whether or not the trigeminal ganglion is protected by the BBB.

**(i)** *In vitro* autoradiography mapping studies were performed on rhesus monkey trigeminal ganglion. Trigeminal ganglion tissue slices were incubated with [$^{3}$H]MK-3207 (a CGRP receptor antagonist) to define the CGRP receptor binding sites. High binding densities of [$^{3}$H]MK-3207 were found in the trigeminal ganglion. The binding sites were mainly located in the ganglion where the cells were located.

Immunofluorescence was used to study the detailed distribution of CGRP and its receptor components, calcitonin receptor-like receptor (CLR) and receptor activity-modifying protein 1 (RAMP1) in rat, monkey and human trigeminal ganglion. Immunofluorescence revealed expression of CGRP, CLR and RAMP1 in the trigeminal cells. CGRP was expressed in the small/medium sized cells whereas the receptor components were mainly expressed in the larger cells. In addition, CLR and RAMP1 expression was found in the satellite glial cells that surround neurons. CGRP and the receptor components were rarely co-expressed. Co-expression of CLR and RAMP1 was found in the large neurons and in the satellite glial cells

**(ii)** To evaluate if the trigeminal ganglion is located within or outside the blood-brain barrier, experiments with Evans blue were performed on rodents. Evans blue administrations revealed that the trigeminal ganglion is not protected by the BBB. Thus, the trigeminal ganglion is located outside the BBB, suggesting that CGRP receptor antagonists do not need to penetrate the BBB to block receptors in the trigeminal ganglion. This study suggests that the trigeminal ganglion may be a key site of action also for CGRP receptor antagonists.