
Views and Perspectives

The Journey to Establish CGRP as a Migraine Target: A Retrospective View

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In this retrospective, Dr. Lars Edvinsson recounts early steps and milestones in our understanding of the neuropeptide calcitonin gene-related peptide (CGRP) in the trigeminovascular system and its role in migraine. The discovery of the presence and function of CGRP and other neuropeptides in the cerebral vasculature and its sensory innervation is described. He relates the seminal finding that CGRP is uniquely released during migraine and the journey to develop blockers of CGRP effects. Now, over 30 years since its discovery, CGRP has become the target for a number of promising novel treatments for migraine patients.

Key words: CGRP, migraine, review

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Migraine is the most prevalent of the neurological disorders and adversely affects patients throughout their lifetime. The sensory neurotransmitter CGRP (calcitonin gene-related peptide) is now thought to play a major role in migraine pathophysiology.¹ CGRP and its receptors are currently being targeted for development of promising new migraine therapies.² Yet this has not always been the view in the long history of studying and treating migraine patients. In this retrospective overview, I have been asked to go back to the beginning and recount how the CGRP-neurovascular-migraine story developed. For the most part, this is a personal perspective and is not intended as a comprehensive review.

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DISCOVERY OF NEUROVASCULAR PEPTIDES

Looking back, it is evident that the cerebral vasculature was an ideal model with which to understand the function of CGRP in trigeminal nerves and develop tools to discover the role of CGRP and CGRP-related drugs in migraine. Cerebral arteries are densely innervated by sensory nerves, and the journey to understand this neurovascular system laid the foundation for current insights regarding CGRP-related therapies in migraine.

I first became interested in innervation of cerebral arteries as a doctoral student and presented my thesis on autonomic nerves, receptors and effects on cerebral blood flow.^{3,4} Our research group in Lund continued to investigate perivascular nerves, using the combined approaches of histochemistry, pharmacology, and physiologic measures of vascular contractility and cerebral blood flow. Timing is everything, and this period in our research coincided with an era of unprecedented

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neuropeptide discovery. A breakthrough method for identification of bioactive peptides, spearheaded by Viktor Mutt at the Karolinska Institute, allowed for isolation and characterization of peptides in the gut.⁵ At the same time, new technologies made it possible to synthesize sufficient quantities of peptides for use in functional tests, immunohistochemistry and radioimmunoassay. Our first peptide study focused on vasoactive intestinal polypeptide (VIP), which had been discovered in Mutt's laboratory and shown to have vasodilatory effects in the mesenteric circulation.⁶ In 1976, our group demonstrated the presence of perivascular VIP-containing nerve fibers in the walls of cerebral arteries.⁷ We proceeded to establish that VIP was a parasympathetic transmitter that increased cerebral blood flow and caused cerebral vasodilation via a nonendothelial increase in adenylyl cyclase.^{8,9} With regard to headache pathology, we later found that VIP is released during cluster headache and is involved in the associated facial symptoms of nasal congestion, rhinorrhea, and scleral injection.¹⁰

From the VIP studies we now had the methodologies to investigate other neuropeptides in the cerebral circulation, and for a while, there seemed to be a new peptide discovered every year (eg, neuropeptide Y [NPY], peptide histidine isoleucine [PHI], neurokinin A [NKA], substance P, pituitary adenylyl cyclase activating peptide, and galanin).¹¹ Our interest in mapping peptide localization and vascular function was mainly directed toward understanding the respective role of these neuropeptides in regulation of cerebral blood flow.^{12,13}

CGRP was identified in the early 1980s when it was discovered there could be alternative processing of mRNA transcribed from the calcitonin gene.¹⁴ This produced a novel 37 amino acid peptide, hence named "calcitonin gene-related peptide." CGRP was found to be located in neural tissue including pathways associated with nociception.¹⁵ Our interest in CGRP was immediately stimulated, and we began to investigate this new neuropeptide. Fortunately, we were able to get the peptide synthesized for us so we could perform *in vitro* and *in vivo* experiments. We first described CGRP localization and function in the cerebral cir-

ulation at an INSERM symposium in Paris in 1984.^{12,16} We found early on that CGRP was an impressive vasodilator of the cerebral circulation that acted via adenylyl cyclase in vascular smooth muscle, independent of the endothelium.⁸ CGRP was the strongest vasodilator we had seen so far in cerebral arteries and arterioles *in vivo*, but amazingly there was little effect on veins.^{16,17} This selectivity contrasted with other dilators such as substance P, which affected both the arterial and venous parts of the circulation.

At that time, we also injected animals with CGRP to produce antibodies for use in immunohistochemistry and radioimmunoassay (RIA). The antibodies allowed us to localize CGRP to thin nonmyelinated nerve fibers (C-fibers) in the cerebral vasculature, middle meningeal artery and dura mater, and we also found CGRP in cells within the trigeminal ganglion (TG).^{18,19} These findings were confirmed using RIA to measure CGRP in the cerebral vessels. To verify the unique localization of CGRP to trigeminal ganglion (TG) neurons, we transected the trigeminal nerve. These experiments were quite difficult, but we were able to show the total disappearance of perivascular CGRP from cerebral arteries without any effect on sympathetic and parasympathetic innervation, which allowed for subsequent functional studies.¹⁸

FINDING A ROLE FOR CGRP IN THE CEREBRAL CIRCULATION

We were intrigued that CGRP had such a potent and long-lasting effect on arterial dilation; and with my collaborator James McCullough, we set out to understand its role during my frequent visits to the Wellcome Research Institute in Glasgow. We tried many experiments and tested if lesioning the trigeminal nerve would modify flow-metabolism coupling, the hypercapnic response, or autoregulation to changes in arterial blood pressure.^{17,20} However, we saw no effect on any of these basic regulatory mechanisms of the brain circulation. We also tried manipulations such as altering body temperature or lifting the animal's body to increase cranial pressure, but there was no specific response to be found that was modulated by

CGRP and the TG when comparing lesioned and nonlesioned animals. The experiments used available methods like iodoantipyrine for regional cerebral blood flow (rCBF), 2-deoxyglucose for glucose metabolism in rodents, and cranial window methodology in the cat for studies of cortical vessel vasomotion. With the latter method we also tested and compared the responses of CGRP to those of a large number of other vasoactive molecules.

On one unforgettable Monday in 1985, with typical Scottish weather outside (rain), we were following the response time of vasoconstriction induced by perivascular injection of norepinephrine (NE) in cortical pial arterioles in the cat cranial window method. In these experiments, the arterioles normally respond with a rapid constriction to NE and then reestablish baseline tone in about one minute. However, when the same experiment was done in cats with trigeminal denervation, the constrictor response to NE was markedly prolonged. Finally, We had cracked the riddle: the trigeminal CGRP nerves trigger a protective, reflex vasodilation that counteracts prolonged vasoconstriction of cerebral arteries supplying blood to the brain.¹⁷ At the height of our excitement, the door to the laboratory opened and in came Professors Harper (head of institute), Gillespie (chairman of pharmacology), and Teasdale (now Sir Graham and responsible for the Glasgow Coma Scale); they were having an institutional meeting and had taken a break. We explained the findings to them, and they added their congratulations. Of course many more experiments with different agonists and antagonists were performed to validate our concept of the trigeminovascular reflex.^{17,20-22} It is possible that this reflex plays a role in migraine. We speculated that spreading depression may cause vasoconstriction that elicits activation of the trigeminovascular reflex with release of CGRP to maintain vascular tone.²³ Experiments to test this possibility, however, have not been performed as of yet.

To further our understanding of trigeminal-vascular transmission, we needed to consider that CGRP is often co-localized with neuropeptides substance P and neurokinin A in perivascular nerves and TG cells.^{18,24} All of these peptides, including

the other members of the CGRP family of peptides (amylin, adrenomedullin, calcitonin), have vasodilatory effects^{8,12,25} and could play a role in trigeminal regulation; however, CGRP was by far the most potent and induced the strongest relaxation. We decided to see if we could measure neuropeptide release from the trigeminal nerves using RIA methodology. At that time, I had met Peter Goadsby, a young neurologist from Jim Lance's group in Sydney, who was interested in working with me on the project. We knew the peptides had short half-lives in blood (11 minutes for CGRP), so it was necessary to be quick and careful in handling the samples.²⁶ We showed that we could measure the release of CGRP and substance P in the jugular vein of cats and humans following stimulation of the trigeminal nerve.²⁷ With this validation, we were excited to extend our study to patients in the clinic. We were able to monitor blood levels of CGRP and substance P in samples from the jugular vein of patients with trigeminal neuralgia and found a marked increase in the release of both peptides during thermocoagulation of the TG.²⁷ We now had a method to look at trigeminal neuropeptides in headache patients.

EVIDENCE FOR A ROLE OF CGRP IN MIGRAINE

In a landmark study in 1990, we collected a series of clinical samples that provided the first clear evidence that CGRP is uniquely released into the extracerebral circulation (external jugular vein) during the headache phase of migraine.²⁸ In patients with migraine, either with aura or without aura, there was a marked increase in craniovascular levels of CGRP, but no change in levels of substance P, VIP (parasympathetic), or NPY (sympathetic nerve activity marker). These findings prompted us for the first time to speculate that CGRP blockade could be a possible strategy for treatment of migraine.²³

The next year (1991), sumatriptan was approved for use in the United States; and with the success of triptan therapy, most of the focus in the migraine field shifted to serotonin mechanisms. However, triptan action is consistent with the

CGRP-trigeminal theory of migraine. We showed that triptans and other 5-HT₁ receptor agonists block trigeminal activation and CGRP release in animals^{29,30} as well as in humans with migraine or cluster headache.^{10,29} We later showed that 5-HT_{1B} and 5-HT_{1D} receptors are co-localized with CGRP in human TG neurons and trigeminal sensory fibers,^{31,32} consistent with the view that these serotonin receptor subtypes operate presynaptically to suppress CGRP release.

DEVELOPMENT OF CGRP RECEPTOR ANTAGONISTS FOR MIGRAINE

As it became apparent that suppression of CGRP signaling could be an effective therapeutic strategy, there was also progress in characterizing CGRP receptors and receptor antagonists. Our cerebral artery model proved to be useful for functional pharmacological characterization of CGRP receptors.^{19,33} Moreover, throughout our research, we have had the good fortune to have access to human, as well as animal, cerebral arteries;^{25,34} and this turned out to be especially important for pre-clinical testing of available CGRP receptor antagonists. Initially, it was not easy to find a blocker. After several decades of basic research, Boehringer Ingelheim was the first company to produce a small molecule (a dipeptide; BIBN4096BS) that selectively blocked CGRP receptors.³⁵ We were the first to study this molecule and related analogs in human cerebral, coronary and omental arteries.³⁶ We then tested novel nonpeptide blockers as they were developed.^{33,37,38} These antagonists were unique in the sense they have much stronger affinity for primate CGRP receptors than for CGRP receptors in other species. This species difference has been explained by a specific mutation in the RAMP1 component of the receptor.³⁹

In 2004, the first test of a CGRP receptor antagonist (olcegepant) in migraine patients⁴⁰ provided “proof of concept” that targeting CGRP was an effective migraine strategy.^{23,37,41} Oral CGRP blockers, such as telcagepant, were then developed that also effectively treated migraine.⁴¹⁻⁴³ Most recently, monoclonal antibodies directed against either CGRP or the CGRP receptor are in develop-

ment as specific prophylactic therapies for frequent episodic and chronic migraine.^{2,44,45}

DETERMINING WHERE CGRP-RELATED THERAPEUTICS ACT IN MIGRAINE

The pathophysiology of migraine is complex, and the location of the antimigraine effect of different drugs has been debated for decades. The prevailing theory for many years was that migraine headache was primarily a vascular disorder in which pain was caused by distention of cerebral and/or meningeal arteries.⁴⁶ Our initial studies showing that CGRP was a powerful dilator of cerebral arteries were consistent with the vascular theory.^{8,17,19} Moreover, we found that human cerebral and dural arteries express relatively high levels of CGRP and of CGRP receptors.^{25,47-49} Thus, intracranial vascular effects of CGRP blockers may contribute to their anti-migraine action.³⁷

However, in recent years, a number of observations have shifted the focus away from the vasculature and highlight the importance of the sensory trigeminal pathway and related neuronal circuits in migraine origin and pain.^{1,50-52} We have identified the components of CGRP receptors [calcitonin-like receptor (CLR) and receptor activity-modifying protein-1 (RAMP1)] in both sensory ganglia and central neural pathways.^{47,53-56} In the human trigeminal ganglion, CGRP receptors are expressed in cells that do not contain CGRP, indicating a postsynaptic regulation by CGRP-containing nerves.^{57,58} Within the trigeminal nerve, CGRP is expressed in thin unmyelinated C-fibers, whereas its receptors (CLR and RAMP1) are expressed in thicker myelinated A-fibers, suggesting cross-talk between the nerves.⁴⁹ Interestingly, a number of migraine therapies (eg, sumatriptan, CGRP and CGRP receptor antibodies) do not readily cross the blood-brain barrier, suggesting a peripheral site of action.² We recently showed that the trigeminal ganglion lacks a blood-brain barrier, suggesting this could be an important site for therapeutic action in migraine.⁵⁴

Central CGRP pathways likely also contribute to migraine pathology; however, we are just beginning to define the localization of CGRP receptors

within the brain. Areas rich in receptors, such as the brainstem and cerebellum,^{55,59} may be additional targets for CGRP blockers in migraine. In the journey to establish CGRP as a migraine target, we have learned much over the last 3 decades. However, in order to best optimize treatment options, it will be important going forward to establish the critical site(s) where CGRP signaling contributes to migraine pathology.

It is an important fact that all CGRP blockers tested in clinical trials so far have shown a positive outcome when compared to placebo. Moreover, CGRP-related antibodies tested in initial Phase II studies have shown significant effects both in frequent episodic migraine and chronic migraine prophylaxis. These findings underscore the unique role that CGRP plays in migraine headache. An often asked question is why is CGRP blockade effective and not substance P antagonists? Perhaps it is due to the relative abundance of CGRP and its highly potent effects in intracranial structures. We are just in the beginning of understanding the molecular mechanisms involved in primary headaches, and hopefully the next stage of the journey will allow us to shift focus from symptomatic relief to a deeper mechanistic viewpoint and treatment of migraine as well as other pain syndromes.

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