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Role of calcitonin gene-related peptide in cerebral vasospasm, and as a therapeutic approach to subarachnoid hemorrhage

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Calcitonin gene-related peptide (CGRP) is one of the most potent microvascular vasodilators identified to date. Vascular relaxation and vasodilation is mediated via activation of the CGRP receptor. This atypical receptor is made up of a G protein-coupled receptor called calcitonin receptor-like receptor (CGRP), a single transmembrane protein called receptor activity-modifying protein (RAMP), and an additional protein that is required for Ga coupling, known as receptor component protein (RCP). Several mechanisms involved in CGRP-mediated relaxation have been identified. These include nitric oxide (NO)-dependent endothelium-dependent mechanisms or cAMP-mediated endothelium-independent pathways; the latter being more common. Subarachnoid hemorrhage (SAH) is associated with cerebral vasospasm that occurs several days after the hemorrhage and is often fatal. The vasospasm occurs in 30–40% of patients and is the major cause of death from this condition. The vasospasm is associated with a decrease in CGRP levels in nerves and an increase in CGRP levels in draining blood, suggesting that CGRP is released from nerves to oppose the vasospasm. This evidence has led to the concept that exogenous CGRP may be beneficial in a condition that has proven hard to treat. The present article reviews: (a) the pathophysiology of delayed ischemic neurologic deficit after SAH, (b) the basics of the CGRP receptor structure, signal transduction, and vasodilatation mechanisms and (c) the studies that have been conducted so far using CGRP in both animals and humans with SAH.

Keywords: GPCR, CGRP, subarachnoid hemorrhage, cerebral vasospasm, G-proteins

INTRODUCTION

In the US, over 30,000 persons each year experience a subarachnoid hemorrhage (SAH). Whereas intracranial aneurysms are found in 2–5% of all autopsies, the incidence of rupture is only 2–28/100,000 individuals/year (Ingall et al., 2000). SAH is more frequent in women than men (3:2 ratio) over the age of 40, but the reverse is the case in those younger than 40 (The ACROSS Group, 2000; Ohkuma et al., 2002). Peak rupture rates occur between the ages of 50 and 60 years (The ACROSS Group, 2000; Ohkuma et al., 2002). Intracranial aneurysms account for approximately 85% of cases of non-traumatic SAH, whereas 10% have the pattern of non-aneurysmal perimesencephalic hemorrhage (van Gijn et al., 2007). The other causes include bleeding from other vascular malformations, moyamoya syndrome, coagulopathy, and, rarely, extension of an intracerebral hematoma (van Gijn et al., 2007). In up to 15%, no source of bleeding is identified (Kim et al., 2012). Approximately 10–15% of patients die before receiving medical treatment from the initial bleed or its immediate complications (Ingall and van Gelder, 2002) and over 40% of hospitalized patients die within 1 month of the event (Ingall et al., 2000). Those that survive the initial bleed are at risk for a number of secondary insults including rebleeding (Wittem et al., 1977; Ohkuma et al., 2001), hydrocephalus, and cerebral vasospasm (van Gijn et al., 2007).

CGRP has been found to be at least 1,000 times more potent than acetylcholine, substance P, ATP, adenosine, and 5-hydroxytryptamine, and 10–100 times more potent than the β-adrenergic agonist isoprenaline. Consequently, a dose of 15 pmol injected into human skin produces an erythema that lasts for 5–6 h (Brain et al., 1985). As we discuss later, CGRP has a particularly potent vasodilator activity in the cerebral circulation, rendering it a promising agent for the treatment of SAH-triggered cerebral vasospasm.

In the present review, we summarize the etiology and therapy of cerebral vasospasm, the biology of CGRP and its receptors, and review the role of CGRP as a treatment in SAH-associated vasospasm in both animals and humans.
CEREBRAL VASOSPASM AFTER SAH

DEFINITIONS

Throughout the literature, authors have used various means of defining vasospasm including terms like angiographic vasospasm, symptomatic vasospasm, and delayed cerebral ischemia (DCI). Angiographic vasospasm is a narrowing of the lumen of the major cerebral arteries, which is usually focal but may be diffuse. Vasospasm has its onset usually on day 3 after SAH, is maximal at days 6–8, and usually lasts for 2–3 weeks (Wilkins, 1990). Symptomatic vasospasm is characterized by the insidious onset of confusion and decreased level of consciousness, followed by focal motor and/or speech impairments. It is mainly a diagnosis of exclusion, when clinical deterioration occurs and hydrocephalus, rebleeding, hypoxia, and metabolic abnormalities have been ruled out. DCI is defined as symptomatic vasospasm, infarction attributable to vasospasm, or both (Frontera et al., 2009). Although about 70% of patients may develop arterial narrowing, only 30% will manifest neurological deficits. The outcome of DCI itself is death in about one-third and permanent deficit in another third (Dueck, 1995). In the present review the term vasospasm is defined as arterial vessel narrowing.

VASOSPASM PATHOPHYSIOLOGY

Nitric oxide

Loss of the biological effect of nitric oxide (NO) is considered to play a pivotal permissive role in the development of cerebral vasospasm. The principal effect of NO on cerebral vessels is the relaxation of vascular smooth muscle cells, with decreased bioavailability of NO being implicated in the formation of SAH-induced vasospasm. The depletion of NO has been assumed to occur via several mechanisms in the setting of SAH. First, due to its high affinity for hemoglobin (Hb), NO is scavenged by Hb released during the breakdown of subarachnoid blood (Goretski and Hollocher, 1988; Ignarro, 1990). Second, it is possible that the production of NO is decreased in SAH, as a result of the down-regulation of endothelial NO synthase (eNOS) and neuronal NOS (nNOS; Pluta, 2005). This is supported by studies that revealed the down-regulation of eNOS and loss of nNOS in spastic arteries after SAH (Hino et al., 1996; Pluta et al., 1996), as well as the finding that levels of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of eNOS, are elevated in the CSF of patients with SAH and in blood vessel walls exposed to clot (Polin et al., 1998; Dumont et al., 2003). Leukocytes can contribute to vasospasm by promoting free radical formation that may evoke endothelial dysfunction (Gribam et al., 1998; Sullivan et al., 2000), and by producing a variety of vasoactive substances, including ET-1 and cytokines (Fassbender et al., 2000). Several cytokines have been found to be up-regulated in cerebral vasospasm, including TNF-alpha, IL-1, IL-6, and IL-8 (Hirashima et al., 1997; Fassbender et al., 2001; Takizawa et al., 2001).

Oxidative stress

Oxyhemoglobin (OxyHb) may catalyze generation of reactive oxygen species (ROS). Free radicals are considered to play a pivotal role in cerebral vasospasm through various mechanisms. First, they can initiate lipid peroxidation, whose products, lipid peroxides, are capable of producing vasospasm and damaging the structure of arteries (Lin et al., 2006). Second, it has been hypothesized that ROS may act as messengers to activate vasospasm (Duerrschmidt et al., 2000). Lastly, ET-1 antagonists may become vasodilators and reduce the NO bioavailability that is associated with vasospasm (Toda et al., 1991), and intracisternal injections of this agent result in cerebral vasospasm (Macdonald et al., 1991). Indeed, the presence of OxyHb in the CSF of patients after SAH and the extent of hemorrhage are correlated with the distribution, severity, and time course of vasospasm (Mayberg et al., 1990). Ferrous hemoglobin released from subarachnoid clot could lead to delayed arterial narrowing by a number of mechanisms, such as scavenging of decreased production of NO (Pluta, 2005), free radical production, modification of K+ and Ca2+ channels (Ishiguro et al., 2008), differential upregulation of genes (Vikman et al., 2006), and activation of the Rho/Rho kinase and PKC pathways (Wickman et al., 2003).

Intracellular Ca2+

Vasospasm can be regarded as an abnormal and prolonged contraction of vascular smooth muscle. The intracellular free Ca2+
level plays a pivotal role in the regulation of smooth muscle contractility (Horowitz et al., 1996). Following SAH, changes have been reported in the electrical properties of smooth muscle cells of small diameter cerebral arterial tissues leading to enhanced \( \text{Ca}^{2+} \) influx, vasoconstriction, and decreased cerebral blood flow (Koide et al., 2011). Cerebral arteries from healthy animals express only L-type voltage-dependent \( \text{Ca}^{2+} \) channels. Expression of an additional type of voltage-dependent \( \text{Ca}^{2+} \) channels (R-type) occurs after SAH, leading to increased \( \text{Ca}^{2+} \) channel density, increased \( \text{Ca}^{2+} \) influx, and vasoconstriction (Ishiguro et al., 2005).

Cortical spreading depolarization
This is a pathogenetic process that has attracted much attention lately. The term "cortical spreading depolarization" describes the wave of near-complete neuronal depolarization and neuronal swelling in the brain that is ignited when passive cation influx across the cellular membranes exceeds ATP-dependent \( \text{Na}^+ \) and \( \text{Ca}^{2+} \) pump activity. The cation influx is followed by water influx and shrinkage of the extracellular space by \(~70\%\) (Dreier et al., 2009). Although the ignition of cortical spreading depolarization occurs passively, driven by electrical and diffusion forces, energy consumption paradoxically increases since \( \text{Na}^+ \) and \( \text{Ca}^{2+} \) pumps are immediately activated to correct the intracellular \( \text{Na}^+ \) and \( \text{Ca}^{2+} \) surge. As a consequence, regional cerebral blood flow increases during the neuronal depolarization phase. The opposite of this physiological hemodynamic response to cortical spreading depolarization is termed the "inverse hemodynamic response," and occurs when there is local dysfunction of the microvasculature. With the inverse response, severe microvascular spasm instead of vasodilatation is coupled to the neuronal depolarization phase, and the term "cortical spreading ischemia" describes the cortical spreading depolarization-induced perfusion deficit (Dreier et al., 2009).

Neurogenic factors
The cerebral arteries have sympathetic, parasympathetic, and sensory innervation. It has been postulated that SAH causes a derangement of neuronal regulatory mechanisms, which in turn leads to vascular smooth muscle contraction. The vasoconstriction is associated with a decrease in CGRP levels in cerebral perivascular nerves (Edvinsson et al., 1991) and an increase in CGRP levels in blood draining from the external jugular vein (Boul et al., 1990), suggesting that CGRP is released antidromically from trigeminal sensory perivascular nerves to oppose the vasoconstrictor. This evidence has led to the concept that administration of CGRP may be beneficial in SAH-associated vasospasm. The molecular characteristics of CGRP and its use as a treatment option in SAH are reviewed in Sections "Calcitonin Gene-related Peptide Biology" and "Calcitonin Gene-related Peptide and SAH," respectively, of the present article.

TREATMENT OF VA SPASM
The management of vasospasm involves routine "prophylactic" measures as well as more aggressive interventions, reserved for situations where there are signs or symptoms of DCI.

**Hemodynamic therapy**
The use of triple-H therapy (hypervolemia, hypertension, and hemodilution) stems from numerous clinical observations noting improvement in patients’ clinical symptoms following induced hypertension and volume expansion (Kosnik and Hunt, 1976; Kassell et al., 1982). The relative contribution of each component is debated. However, there are many uncertainties for the use of prophylactic hemodynamic therapy following SAH. Two studies randomly assigned normovolemic or hypervolemic therapy to patients and reported no difference in the incidence of DCI between groups (Tennihan et al., 2008; Egge et al., 2001).

**Nimodipine**
Nimodipine is safe, cost-effective, and reduces the risk of poor outcome and secondary ischemia (Neil-Dwyer et al., 1987; Welty, 1987; Kostron et al., 1988; Mee et al., 1988), but has very modest effects. It is used prophylactically in all patients with SAH. Its precise mechanism of action remains unclear. Despite being shown to reduce the incidence of DCI and cerebral infarction in clinical trials, it has negligible effects on angiographic vasospasm; nimodipine may be neuroprotective by blocking \( \text{Ca}^{2+} \) influx at a neuronal level (Al-Tamimi et al., 2010).

**Intracisternal thrombolysis**
A meta-analysis looking at a total of 652 patients who were treated with intracisternal thrombolitics concluded that thrombolytic therapy had a statistically significant beneficial effect. However, the authors acknowledged the lack of large, randomized prospective trials (Amin-Hanjani et al., 2004).

**Endovascular techniques**
Endovascular techniques frequently play a role in the aggressive treatment of vasospasm. They include transluminal angioplasty and intra-arterial infusion of vasodilators (papaverin, nicardipine, verapamil, etc.; Brisman et al., 2006). Transluminal balloon angioplasty is very effective at reversing angiographic spasm of large proximal vessels and produces a sustained reversal of arterial narrowing (Brisman et al., 2006; Jestaedt et al., 2008). The optimal timing of angioplasty in relation to medical therapy is uncertain. Major complications occur in ~5% of procedures and include vessel rupture, occlusion, dissection, hemorhagic infarction, and hemorrhage from unsecured aneurysms (Zwienenberg-Lee et al., 2006).

**Statins**
Statins have been shown to possess cholesterol-lowering-independent pleiotropic effects in different clinical settings, including a decrease in the incidence and duration of severe vasospasm as well as a reduction in the mortality rate after SAH (Lynch et al., 2005; Tseng et al., 2005, 2007). Statins are thought to be beneficial in the prevention of cerebral vasospasm by down-regulating inflammation and up-regulating the expression of eNOS and therefore NO (Sugawara et al., 2011).

**Other treatments**
Clasosantan, an endothelin receptor antagonist (ET\(_A\) ) decreased the incidence of severe vasospasm, DCI and new infarcts seen on CT scans in a dose-dependent fashion. However, CONSCIOUS 1...
AMY, which is important for maintaining glycemic control, and adrenomedulin (AM), which is a potent vasodilator, amylin from the calcitonin gene. Other members of this family include CGRP, in humans, which were found to contain an alternative peptide product from the calcitonin gene (Amara et al., 1982). CGRP, in the thyroid of aging rats and medullary thyroid carcinomas (Benarroch, 2011).

Involvement of CGRP in migraine headache has led to the hypothesis that CGRP functions as a component of neurogenic inflammation. CGRP may be released in response to noxious stimulation at all these levels, which leads to central sensitization underlying chronic pain states (Benarroch, 2011). CGRP can also be released antidromically in the periphery, eliciting vasodilatation as a component of neurogenic inflammation. CGRP may be involved in the pathophysiology of inflammatory and neuropathic pain. Involvement of CGRP in migraine headache has led to the development of CGRP antagonists for treatment of this disorder (Benarroch, 2011).

Calcitonin gene-related peptide is a 37-amino acid neuropeptide that was identified in 1982 by molecular biological techniques in the thyroid of aging rats and medullary thyroid carcinomas in humans, which were found to contain an alternative peptide product from the calcitonin gene (Amara et al., 1982). CGRP, in common with other members of this peptide family, is derived from the calcitonin gene. Other members of this family include adrenomedullin (AM), which is a potent vasodilator, amylin (AMY), which is important for maintaining glycemic control, and calcitonin, which contributes to calcium metabolism (Hill, 2007). CGRP exists in two forms, named CGRP and β-CGRP. While these two isoforms share the same biological activities, and differ by only three amino acids in the human (Steenbergh et al., 1985, 1986), they are formed from two distinct genes, which share >90% homology, at different sites on chromosome 11. CALC 1 gene forms calcitonin and α-CGRP, whereas CALC II forms β-CGRP (Alevizaki et al., 1986). α-CGRP synthesis is caused by alternative splicing of the calcitonin gene (Amara et al., 1982, Figure 1). β-CGRP is known to be transcribed from its own distinct gene (Steenbergh et al., 1985, 1986). The majority of CGRP within the body is α-CGRP and primarily expressed in the peripheral and central nervous system. β-CGRP is mainly expressed in the gut (Mulder et al., 1988). However, it has also been identified in the central nervous system, pituitary, thyroid, and in medullary thyroid carcinoma as a major CGRP form together with α-CGRP (Petermann et al., 1987).

Data from NMR studies suggest that CGRP consists of a characteristic N-terminal disulfide bridge-linked loop between cysteines Cys1 and Cys7, followed by an alpha-helix in amino acids Val8-Arg14 (Breeze et al., 1991). The next domain at residues 19–27 forms a hinge region (Conner et al., 2002). The C-terminus lies at residues 28–37, and contains two turn regions which form a putative binding epitope (Carpenter et al., 2001). It appears that the N-terminal cyclic portion of the CGRP molecule, containing a ring structure with a disulfide bond, is essential for agonistic activity (Maggi et al., 1990). It is interesting to note that the C-terminal fragment, CGRP1–37, is devoid of any agonist activity at CGRP receptors, although it behaves as a competitive antagonist against the intact peptide (Chiba et al., 1989).

Calcitonin gene-related peptide is widely distributed in the central and peripheral nervous systems, primarily in sensory fibers that are closely associated with blood vessels (Uddman et al., 1986). CGRP is often co-localized with other peptides in these fibers, especially the tachykinins substance P (Uddman et al., 1986). In the cerebral circulation, CGRP is released from sensory fibers originating in the trigeminal ganglia and acts to dilate cerebral vessels (McCulloch et al., 1986). In the gut, CGRP is also released from spinal afferents, where it dilates mucosal blood vessels and may protect against the acidic environment (Holzer, 2000). CGRP-containing fibers also innervate coronary arteries of the heart (Gulbikenian et al., 1993).

The regulation of CGRP production is poorly understood. At a cellular level, nerve growth factor (NGF) up-regulates CGRP via the Ras/Raf/mitogen-activated protein kinase-1 (MEK-1)/p42/p44 pathway (Frieland et al., 2000).

In the human circulation, CGRP has a half-life of approximately 7–10 min (Kraenzlin et al., 1985; Struthers et al., 1986). Regarding its metabolism, it seems that there is an obvious mechanism, and it is probably broken down via a number of routes. First, mast cell tryptase has a potent effect in cleaving CGRP into inactive fragments, both in vivo and in vitro. More specifically, if both CGRP and substance P are released simultaneously, then CGRP could be inactivated by enzymes (tryptases), released by mast cells in response to substance P. This mechanism has been demonstrated in skin (Brain and Williams, 1988, 1989).

Second, a matrix metalloproteinase II has the ability to metabolize CGRP and remove its vasodilator activity (Fernandez-Patron et al., 2000). Third, Sams-Nielsen et al. (2001) have provided evidence that CGRP is taken back up into sensory nerve terminals after repolarization in vitro. Finally, in the CSF, α-CGRP is degraded by an endopeptidase that cleaves the peptide at the Leu17–Ser18 bond (Le Groes et al., 1989).
STRUCTURE OF CGRP RECEPTORS

Many peptides, including the CGRP family, mediate their actions via G protein-coupled receptors (GPCRs). The GPCRs form the largest family of cell-surface proteins that are capable of interacting with an extracellular stimulus and transducing that stimulus to produce a reaction inside a cell (Pierce et al., 2002). All GPCRs have seven transmembrane spanning domains, an extracellular N-terminus and an intracellular C-terminus and can be divided into three families based on signature amino acid sequences. Family A is the largest and generally binds small molecules and short peptides. Receptors in this class have been studied extensively, including photoreceptor rhodopsin, as well as adrenergic...
and olfactory receptors. Family B receptors bind larger peptides in the range of 27 to approximately 50 amino acids (secretin, glucagons, VIP, etc.). These receptors mediate the actions of CGRP and related peptides (Poyner et al., 2002; Hoare, 2005).

Family C receptors include glutamate and GABAB receptors (Pierce et al., 2002).

Calcitonin receptor-like receptor (CLR), which belongs to family B of the GPCRs, comprises the main functional unit of the CGRP receptor (Figure 2). It was not until McLatchie’s work (McLatchie et al., 1998) was published that it was recognized that a novel family of single transmembrane domain proteins, called receptor activity-modifying proteins (RAMPs), were required to allow CLR to bind peptide and transduce signal. Three RAMPs have been identified so far (RAMP1, RAMP2, and RAMP3). Each RAMP has a single transmembrane-spanning domain, a short intracellular C-terminal tail (∼9 amino acids) and a long extracellular-terminus (∼100 amino acids; McLatchie et al., 1998).

As a result of CLR and calcitonin receptor (CTR) interactions with RAMP, the International Union of Pharmacology (IUPHAR) nomenclature recognizes that CGRP interacts with CLR/RAMP1 (CGRP1) receptors, whereas AM interacts with CLR/RAMP2 (AM1) or CLR/RAMP3 (AM2) receptors. The CTR without RAMP is sufficient for calcitonin binding, but CTR with RAMP 1, 2, or 3 are AMY1, AMY2, and AMY3 receptors, respectively (Poyner et al., 2002). The discovery of RAMPs has led to evolution of our understanding of how receptor diversity is implemented, providing a novel mechanism for generating receptor subtypes within a subset of family B GPCRs (Sexton et al., 2006).

The primary function of CLR is thought to be related to ligand binding, whereas the RAMP molecule plays a crucial role in receptor trafficking to the membrane and determination of receptor pharmacology. The RAMP family regulate the glycosylation and transport of the CLR. However, they are not CGRP receptors by themselves (McLatchie et al., 1998; Sexton et al., 2009). Terminal glycosylation of the receptor and transit from the endoplasmic reticulum/Golgi apparatus to the cell surface require interaction of CLR with RAMP (Sexton et al., 2009).

Calcitonin gene-related peptide receptor activation is known to involve several crucial elements, in common with other GPCRs, such as the presence of a proline “kink” in transmembrane helix (TM6 (Conn et al., 2005), and a putative DRY motif equivalent (Conn et al., 2007), similar to family A GPCRs. There is also evidence suggesting stabilization of the CLR interaction with G “alpha”s (Gα) by another 17kDa intracellular membrane protein, called RCP (Evans et al., 2000).

The existence of two receptors, CGRP1 and CGRP2, was originally proposed in the late 1980s, with the CGRP1 receptor being the predominant mediator of cardiovascular effects. This receptor classification was developed as a consequence of pharmacological studies carried out with different agonists and antagonists in a range of tissue preparations, especially the positive inotropic effect in the guinea pig or rat atrium for determination of CGRP1 receptor activity, and the inhibition of electrically evoked twitch responses in the rat vas deferens for determination of CGRP2 receptor activity (Dennis et al., 1989, 1990; Dumont et al., 1997). In general, receptors that can be antagonized by the 30-amino acid fragment of CGRP, CGRP8−37, with an approximate pA2 value of 7.0 are designated as CGRP1 receptors, while those that CGRP8−37 blocks with a pA2 of 6.0 or less are classified as CGRP2 receptors (Quirion et al., 1992; Poyner, 1995). However, it is questionable whether the CGRP2 receptor is a single receptor type or whether it is, in fact, explained by multiple molecular entities (Hay, 2007).
In contrast, CGRP1 is a well-defined receptor type consisting of CLR and RAMP1.

**SIGNAL TRANSDUCTION OF CGRP RECEPTOR**

Several mechanisms involved in CGRP-mediated vasorelaxation have been identified. These mechanisms include either NO-dependent endothelium-dependent mechanisms or cAMP-mediated endothelium-independent pathways. The most common pathway is NO- and endothelium-independent. Activation of the CGRP receptor is generally accepted to result in Gaq-mediated activation of adenylate cyclase, with a subsequent increase in cAMP and activation of protein kinase A (PKA). In the absence of endothelium, CGRP is able to cause relaxation, suggesting it must directly act on the smooth muscle cells to stimulate adenylate cyclase (Edvinsson et al., 1985, 1998; Crossman et al., 1990). The resulting rise in cAMP then activates PKA, which phosphorylates and opens up ATP-sensitive K⁺ channels, thus leading to relaxation (Figure 3A; Nelson et al., 1990).

Endothelium-independent relaxation to CGRP occurs in the majority of tissues examined to date. Exceptions include the rat aorta, where the relaxation to CGRP occurs only in the presence of an intact endothelium and is attenuated by inhibitors of NO synthase, implying an NO-dependent mechanism (Brain et al., 1985; Gray and Marshall, 1992a,b). A significant increase in both cAMP and cGMP occurs and is also dependent on the presence of endothelium (Gray and Marshall, 1992a). This implicates the release of NO from the endothelium, which then relaxes the smooth muscle cells through activation of guanylate cyclase and accumulation of cGMP. Moreover, it has been shown that cAMP is able to stimulate eNOS activity, leading to increased synthesis and release of NO (Ferro et al., 1999; Queen et al., 2000). The activation of eNOS via cAMP is probably mediated via PKA, as a study demonstrated that various protein kinases can phosphorylate and activate eNOS (Burt et al., 2000). It is a possibility that CGRP causes an increase in cAMP in endothelial cells, which leads to PKA activation. PKA, in turn, activates eNOS, which results in NO release, and thus relaxation of the smooth muscle (Figure 3A).

There is some evidence for Gaq, signaling by the CGRP receptor, which is traditionally identified by sensitivity to pertussis toxin (PTX, Figure 3B). The CGRP-mediated stimulation of Ca²⁺ transients in rat nodose neurons and the activation of c-Jun N-terminal kinase (INK) in SK-N-MC cells (which express endogenous CGRP receptors) both displayed PTX sensitivity (Wiley et al., 1992; Dias et al., 2000).

![Figure 3](image-url)
The CGRP receptor may also be able to stimulate intracellular activity through a different G protein. Ayar et al. (1999) reported that CGRP was able to activate phospholipase C (PLC) in HEK293 cells, leading to an increase in intracellular Ca\(^{2+}\) via inositol triphosphate (IP3) activity. This increase in Ca\(^{2+}\) occurred concomitantly with the stimulation of adenylyl cyclase and accumulation of cAMP. Activation of PLC is considered to occur through G\(_{i}\), rather than through G\(_{q}\), suggesting that the activated CGRP receptor is able to interact with both types of G protein. If this mechanism is present in endothelial cells, it provides an alternative explanation for CGRP activation of eNOS (which is traditionally considered to be dependent on Ca\(^{2+}\)/calmodulin for activation), independently of cAMP accumulation. The possibility that CGRP receptors may be coupled to phosphatidylinositol turnover is supported by another study that found this secondary messenger pathway in skeletal muscle (Laufer and Changeux, 1989; Figure 3C).

Recently, Meens et al. (2012) reported that activated CGRP receptors induce cyclic nucleotide-independent relaxation of vascular smooth muscle cells in mesenteric resistance arteries and terminate arterial effects of ET-1 via G\(_{q}\). More specifically, CGRP receptor activation causes cAMP production but the relaxation of rat mesenteric resistance arteries induced by activation of this receptor involves G\(_{q}\), and is not dependent on cAMP (Figure 3D).

Another study by Meens et al. (2010) discovered that CGRP released from peri-arterial sensory motor nerves terminates long-lasting vasoconstrictor effects of ET-1 by promoting dissociation of ET-1/ETA-receptor complexes.

The CGRP receptor can also potentially activate other downstream signaling molecules, such as PKC and mitogen-activated protein kinase (MAPK) cascades, such as p38, JNK, and extracellular receptor-activated kinase 1/2 (ERK 1/2; Walker et al., 2010). CGRP receptor signaling is regulated by desensitization, internalization, and trafficking, which, as with otherGPCRs, involves GPCR kinases (GRK), β-arrestin, and clathrin- and dynamin-dependent endocytosis (Walker et al., 2010). Padilla et al. (2007) proposed a mechanism by which endosomal endothelin converting enzyme-1 (ECE-1) degrades CGRP in endosomes to disrupt the peptide/receptor/β-arrestin complex, freeing internalized receptors from β-arrestins and promoting recycling and resensitization, resulting in long-lasting vascular relaxing response to CGRP.

**CALCITONIN GENE-RELATED PEPTIDE AND SAH**

**PRELIMINARY OBSERVATIONAL STUDIES**

An animal study of experimental SAH in rats revealed that the sensory innervation of the cerebral circulation by CGRP-containing fibers appeared to be reduced after SAH (estimated by the number of fibers present), and there was also a larger vasodilating response to CGRP in basilar arteries after SAH as compared to vessels from control animals. The reduction in CGRP could be due to the release of the transmitter from the perivascular nerve terminals caused by blood in the subarachnoid space (Edvinsson et al., 1990).

In another study (Edvinsson et al., 1991), the proximal parts of the middle cerebral artery (MCA) were collected within 24 h after death from five humans suffering SAH (5–10 days beforehand) and from six subjects dying from myocardial infarction. In humans who had died from SAH the level of CGRP was nearly not detectable, being in contrast to that seen in age and sex matched subjects who had died of myocardial infarction. The trigemino-cerebrovascular system was suggested by the authors to act as an anti-vasoconstrictor system by releasing stored peptides, CGRP being the most likely candidate.

Juul et al. (1995) measured CGRP levels with specific radiomunoassays (RIA) in patients with SAH, after operation with aneurysm clipping and nimodipine treatment. They used samples taken either from the external jugular vein (n = 20) or from the CSF (n = 14) during the postoperative course. They also used samples from healthy volunteers. The degree of vasoconstriction in the patients was monitored with Doppler ultrasound recordings. CGRP concentrations from the external jugular vein were significantly higher than from controls. Also, the CGRP level was measurable in SAH CSF but not in CSF of controls.

Others (Tran Dinh et al., 1994) showed that the basal level of endogenous CGRP in CSF was 0.77 nmol/L in rabbits. The CGRP concentration peaked at 14 nmol/L within 30 min, and at 8 nmol/L within 24 h, after SAH. They further showed that 3 days after SAH the CGRP concentration in CSF declined to 3.5 nmol/L.

Nozaki et al. (1989a) produced a model of SAH by a single injection of fresh autologous arterial blood into the cisterna magna of dogs. Then, they examined changes of CGRP immunoreactivity immunohistochemically in perivascular nerve fibers of the large pial arteries. CGRP in cerebrovascular nerve fibers was suppressed after SAH. The suppression was first detected on the third day after SAH, and was most marked during the 7th to 14th day. CGRP , after SAH. The suppression was first detected on the third day after SAH, and was most marked during the 7th to 14th day. CGRP, however, recovered to a normal level by the 42nd day after SAH.

Arimura et al. (1991) isolated the basilar artery from five rabbits subjected to SAH and five control animals. A mild or severe vasospasm was observed in the basilar artery about 15 min after injection of blood in the cisterna magna, while fluorescence immunohistochemistry revealed a marked decrease of the perivascular nerves containing CGRP in the animals of the experimental group, as compared to the control group.

**EFFECTS OF CGRP ADMINISTRATION ON CEREBRAL VASOSPASM AFTER EXPERIMENTAL SAH IN ANIMALS (Table 1)**

Nozaki et al. (1989b) produced experimental SAH in 30 dogs by injecting autologous arterial blood into the cisterna magna. They used two models of injection: in the first, single-injection model, 1 ml/kg of blood was injected on day 0, while 0.5 ml/kg of blood was injected successively 48 h apart in the second, double-injection model, on day 0 and day 2. The diameter of the basilar artery was measured by angiography. The most marked constriction of the basilar artery was seen on day 3 after SAH in the single-injection model and on day 7 in the double-injection model. When 10\(^{-11}\) to 2 × 10\(^{-10}\) mol/kg of CGRP was administered intracisternally (i.c.) on day 3 in the single-injection model, cerebral vasospasm reversed completely. The effect began to appear 5 min after CGRP administration, continued for 4 h, and disappeared by 24 h after the administration. When CGRP was administered at doses of 10\(^{-11}\) to 2 × 10\(^{-10}\) mol/kg on day 7 after SAH in the double-injection model, the cerebral vasospasm was reversed in a dose-dependent manner: 2 × 10\(^{-11}\) mol/kg of CGRP reversed the vasospasm completely. The effect began to appear 5 min after the CGRP
### Table 1 | Studies of CGRP administration after experimental SAH in animals.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Animal model</th>
<th>Total sample size (intervention/control)</th>
<th>Way of CGRP administration</th>
<th>CGRP dose</th>
<th>Results</th>
<th>Adverse events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nozaki et al. (1998b)</td>
<td>Dog</td>
<td>30 (23/8)</td>
<td>i.c.</td>
<td>2 × 10⁻¹⁰ mol/kg</td>
<td>Vasospasm was reversed completely</td>
<td>AP and HR slightly increased</td>
</tr>
<tr>
<td>Imaiizumi et al. (1996)</td>
<td>Rabbit</td>
<td>16 (8/8)</td>
<td>i.c.</td>
<td>10⁻¹⁰ mol/kg</td>
<td>Basilar artery dilated from 73 to 117%, significantly larger than 67% in control (p &lt; 0.001)</td>
<td>None</td>
</tr>
<tr>
<td>Toshima et al. (1992)</td>
<td>Rabbit</td>
<td>41 (17/24)</td>
<td>i.c., i.v.</td>
<td>100 ng/kg/min i.c./ 100 ng/kg/min i.v.</td>
<td>Vasospasm was significantly greater than that of the respective control group</td>
<td>AP drop-in i.v. CGRP administration</td>
</tr>
<tr>
<td>Ahmad et al. (1996)</td>
<td>Rabbit</td>
<td>45 (22/23)</td>
<td>i.c. slow-release tablet</td>
<td>24 or 153 μg</td>
<td>Vasospasm was completely reversed</td>
<td>None</td>
</tr>
<tr>
<td>Inoue et al. (1996)</td>
<td>Monkey</td>
<td>10 (5/5)</td>
<td>i.c. slow-release tablet</td>
<td>1,200 μg</td>
<td>Cerebral vasospasm was significantly ameliorated</td>
<td>None</td>
</tr>
<tr>
<td>Toyoda et al. (2000)</td>
<td>Rabbit</td>
<td>16 (8/8)</td>
<td>i.c. adenovirus-mediated CGRP gene transfer</td>
<td>NA</td>
<td>Arterial diameter was similar before and after SAH in CGRP group</td>
<td>None</td>
</tr>
<tr>
<td>Satoh et al. (2002)</td>
<td>Dog</td>
<td>20 (8/12)</td>
<td>i.c. adenovirus-mediated CGRP gene transfer</td>
<td>NA</td>
<td>Vasospasm was significantly reduced compared with the control group</td>
<td>None</td>
</tr>
</tbody>
</table>

*Note: i.c., intracisternal; i.v., intravenous; AP, arterial pressure; HR, heart rate; SAH, subarachnoid hemorrhage; NA, non-applicable.*

administration, continued for 4 h, and disappeared by 24 h. Of note, when the amounts of CGRP mentioned above were administered i.c., both mean arterial blood pressure and heart rate were only slightly increased and returned to the previous levels within several minutes.

In a similar study by Imaiizumi et al. (1996), experimental SAH was produced by i.c. injection of arterial blood in rabbits. The animals were treated with intrathecal administration of CGRP 3 days after SAH. The degree of vasospasm and the effect of CGRP were evaluated angiographically by measuring the basilar artery diameter. The basilar artery constricted to 73% of the pre-SAHT diameter. The basilar artery constricted to 73% of the pre-SAH value was produced by i.c. injection of arterial blood in rabbits. The vasospasm was significantly larger than 67% in control (p < 0.001). Basilar artery diameter in either i.v. or i.c. CGRP groups was significantly greater than that of the respective control group (p < 0.001). Similarly, the diameter of basilar artery in group 5 (i.v. CGRP) was significantly greater than that in group 4 (i.v. vehicle, p < 0.01). Although no significant difference was observed in mean arterial blood pressure between groups 2 and 3 (i.c. groups), there was a significant difference between i.c. groups 4 and 5 (lower in group 5, p < 0.01).

Ahmad et al. (1996) implanted a CGRP slow-release tablet i.c., containing either 24 or 153 μg of human αCGRP, 24 h after experimental SAH was induced in rabbits. Following implantation of the tablet in the CSF remained elevated for 5 days. The implantation of the tablet almost completely ameliorated angiographic vasospasm. Moreover, no significant systemic hypotension or neurological adverse event was associated with the treatment.

In a similar approach, Inoue et al. (1996) investigated the efficacy of a CGRP slow-release tablet for the prevention of cerebral vasospasm after SAH in monkeys. Experimental SAH was produced by the method of Espinosa et al. (1984). The animal underwent a right frontotemporal craniectomy under sterile conditions. The dura mater was opened, and the arachnoid membrane was microsurgically incised until the ipsilateral internal carotid...
Table 2: Studies of CGRP administration after aneurysmal SAH in humans.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Sample size/patient type</th>
<th>Study design</th>
<th>Way of CGRP administration</th>
<th>Dose</th>
<th>Primary outcome</th>
<th>Results</th>
<th>Adverse events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juul et al. (1994)</td>
<td>5</td>
<td>Not randomized, not controlled study</td>
<td>i.v. infusion</td>
<td>0.6 μg/min</td>
<td>H.I.</td>
<td>Significant reduction in H.I. during CGRP infusion compared to that before infusion</td>
<td>HR increase during CGRP infusion</td>
</tr>
<tr>
<td>Johnston et al. (1990)</td>
<td>15</td>
<td>Multicenter randomized placebo-controlled study</td>
<td>i.v. infusion</td>
<td>0.035 μg/min, doubled every 10 min, max. 1.15 μg/min</td>
<td>GCS</td>
<td>Of the nine patients who showed a treatment preference, eight favored CGRP (p &lt; 0.05)</td>
<td>None</td>
</tr>
<tr>
<td>European CGRP in SAH study (1992)</td>
<td>117</td>
<td>Multicenter randomized placebo-controlled study</td>
<td>i.v. infusion</td>
<td>0.6 μg/min</td>
<td>Glasgow outcome</td>
<td>Relative risk of a bad outcome in CGRP-treated compared with controls 0.88 (95% CI: 0.6−1.26)</td>
<td>2/3 of the patients included in the CGRP group had medical air and direct compression treatment</td>
</tr>
</tbody>
</table>
artery (ICA) and proximal portions of the MCA and anterior cerebral artery (ACA) were exposed. An autologous blood clot (1 ml/kg) was then placed around the exposed arteries to produce experimental SAH. For animals in the CGRP (n = 5) and placebo (n = 5) groups, a total of three tablets (total drug 1200 μg) were ipsilaterally placed under the frontal and temporal lobes at the time of SAH production. In the control group, cerebral vasospasm developed on day 7 (56% as an average of the ICA, MCA, and ACA). In the CGRP group, vasospasm was significantly ameliorated on average (75%, p = 0.02). The CGRP concentration in CSF was measurable only on day 7 for the CGRP group (6.5 nmol/L).

No significant untoward reactions were recorded.

Soyoda et al. (2000) sought to determine whether adenovirus-mediated gene transfer in vivo of CGRP ameliorates cerebral vasocostriction after experimental SAH. Arterial blood was injected into the cisterna magna of rabbits to mimic SAH 5 days after injection of adenovirus or vehicle. After injection of adenovirus (n = 8), there was a 400-fold increase in CGRP in CSF. In rabbits treated with vehicle (controls, n = 8), basilar artery diameter after SAH was 25% smaller than before SAH (p = 0.0005). In rabbits treated with adenovirus, arterial diameter was similar before and after SAH. Furthermore, treatment of rabbits with adenovirus after experimental SAH prevented spasm of the basilar artery 2 days after SAH.

Likewise, Satoh et al. (2002) investigated whether a delayed treatment with adenovirus encoding CGRP gene, 2 days after experimental SAH, reduces cerebral vasospasm in a double-hemorrhage model (on days 0 and 2) of severe vasospasm in dogs. Severe vasospasm was observed in control SAH dogs (n = 12) on day 7, and the mean basilar artery diameter was 53% of baseline. In the group treated with adenovirus (n = 8), vasospasm was significantly reduced (the basilar artery diameter was 78% of baseline, p = 0.05 compared with the control SAH group). High levels of CGRP were measured in CSF from dogs that received adenovirus (115-fold greater than baseline levels).

Intracisternal gene transfer of CGRP was initially thought to be more useful than i.v. infusion, because the local gene transfer might avoid systemic effects of CGRP and achieve its sustained release into the central nervous system. However, there are several concerns, such as the inflammatory process induced by adenovirus, the difficulty in approaching the target cells in the presence of a large subarachnoid blood clot, and its potential ability for cancerous transformation of the affected cells.

**EFFECTS OF CGRP ADMINISTRATION ON CEREBRAL VASOSPASM AFTER SAH IN HUMANS (TABLE 2)**

Juul et al. (1994) investigated the effect of i.v. CGRP infusion at a rate of 0.6 μg/min in five patients with vasocostriction in the postoperative course after SAH, where the hemodynamic index (ratio between middle cerebral and ICA mean velocities) was used as an indicator of vasocostriction. A significant reduction was found in the hemodynamic index during the CGRP infusion as compared to that before infusion (4.3 vs. 6.2, p < 0.05). However, no significant change was observed in pulsatility index (another indicator of vasospasm, equal to the difference between the systolic and diastolic flow velocities divided by the mean flow velocity), blood pressure, or consciousness during CGRP infusion. A significant increase in heart rate was observed during the infusion, while blood pressure remained unaltered.

Johnston et al. (1990) undertook a multicenter, randomized, placebo-controlled trial to study the safety and efficacy of i.v. CGRP treatment to reverse neurological deficits after surgical clipping of a ruptured intracranial aneurysm. Patients were enrolled if they had postoperative neurological deficit. Patients received CGRP or placebo in random order, 24 h apart. Fifteen patients were eventually included in the study. Infusion started at a rate sufficient to deliver 0.035 μg/min CGRP, and was doubled every 10 min until either a clinical response was obtained or a maximum dose of 1.15 μg/min was reached at 1 h. If the neurological deficit had not deteriorated and the patient had no side-effects by that time, the maximum infusion rate was continued for another 20 min. Regarding neurological changes according to the modified Glasgow Coma Scale, five patients did not improve on either treatment, one improved on both, eight improved on CGRP but not on placebo, and one improved on placebo but not on CGRP. Of the nine patients who showed a treatment preference, eight (88.9%) favored CGRP (p < 0.05). The mean duration of neurological improvement was 25 min, after which patients returned to their previous neurological status. There was a significant decrease in both systolic and diastolic blood pressures during the infusion of CGRP.

A larger, multicenter, randomized controlled trial (European CGRP in SAH study, 1992) investigated the effect of a postoperative infusion of CGRP on outcome at 3 months. Patients with aneurysmal SAH who underwent surgery entered the trial if an ischemic neurological deficit developed after the operation. A total of 117 patients entered the study (62 patients received CGRP and 55 standard management). The CGRP-treated patients received the drug by i.v. infusion at a rate of 0.6 μg/min. If systemic hypotension developed, the infusion rate was reduced to 0.45 μg/min, then to 0.3 μg/min, if the hypotension was still apparent. CGRP treatment was given for at least 4 h; patients who showed a satisfactory neurological response continued to receive treatment for up to 10 days (minimum of 4 days). The percentage of patients with a good outcome was slightly but not significantly higher in the CGRP than in the control group. The relative risk of a bad outcome in CGRP-treated compared with control patients was 0.88 (95% CI: 0.60–1.28). Interestingly, only a third of patients randomized to receive CGRP completed treatment, so two-thirds included in the treatment group for the analyses had limited exposure to CGRP, mainly due to arterial hypotension.

**CONCLUSION**

The pathogenesis of vasospasm after SAH is complex, multifactorial, and incompletely understood. CGRP has shown promising results both in vitro and in vivo, mainly in animal models of experimental SAH. However, there is a lack of studies in humans. Systemic hypotension induced by the i.v. administration of the drug seems to be a serious problem. The encouraging results from the i.c. application of CGRP in animals could warrant large studies in humans with CGRP instillation into the subarachnoid space, in order to avoid hypotension and achieve even more efficient dilatation of the cerebral arteries.


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Calcitonin gene-related peptide is metabolized by an endopeptidase brekoyting substance C' Regal Pro 25, 277–286.


Human calcitonin gene-related peptide: a potent endogenous vasodila-

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