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PHARMACOLOGICAL ASPECTS OF CALCITONIN GENE RELATED PEPTIDE (CGRP)

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ABSTRACT

This review summarizes the receptor-mediated vascular activities of calcitonin gene-related peptide (CGRP). CGRP is a 37-amino acid neuropeptide, primarily released from sensory nerves. It has a vasodilator activity, though to varying extents depending on species and tissue. CGRP has potent activity in the cerebral circulation, which is possibly relevant to the pathology of migraine. CGRP exhibits potent activity in micro-vascular beds. CGRP acts through G protein-coupled receptors whose presence and changes in function modulate the peptide's effects in various tissues. This peptide acts on its receptor that consist of calcitonin receptor-like receptor (CL) linked to one of three receptor activity-modifying proteins (RAMPs) that are essential for functional activity. The association of CL with RAMP1 produces a CGRP receptor. Several endogenous substances such as glucocorticoids, nitric oxide (NO), nerve growth factors (NGF), and steroid hormones modulate CGRP release and synthesis. Both peptide and non-peptide agonists and antagonists of CGRP receptors are being developed. Also the therapeutic benefits of some antagonists such as BIBN 4096 BS i have been promising. This review provides a preliminary understanding of the diverse biological effects of the CGRP in various systems with a special emphasize on its role in path physiological changes. While the current state of knowledge on CGRP and its receptors in many body systems are not fully explored, yet future pharmacotherapeutic relevant findings are greatly awaited.

KEYWORDS

Calcitonin gene-related peptide CGRP, Calcitonin receptor-like receptor (CL) and Receptor activity-modifying proteins (RAMPs) and Vasodilatation.

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INTRODUCTION

Calcitonin gene related peptide (CGRP) is a 37-amino acid neuropeptide that was identified in 1982 by molecular biology techniques[1]. It was discovered when alternative processing of RNA transcripts from the calcitonin gene were shown to result in the production of distinct mRNA encoding CGRP. The gene encoding the thyroid hormone calcitonin was found to be alternatively spliced in mammalian cells. The pre-mRNA from this gene contains 6 exons; the calcitonin mRNA contains exons 1-4,

and terminates after a polyadenylation site in exon 4. Another mRNA is produced from this pre-mRNA by skipping exon 4, and includes exons 1-3, 5, and 6. It encodes a protein known as CGRP[2]. The calcitonin mRNA predominates in the thyroid while the CGRP- specific mRNA appeared to predominate in the nervous system[3].

A human form of CGRP was isolated from thyroid tissue of patients with medullary thyroid carcinoma. CGRP is highly expressed in certain nerves[4] and is now known to belong to a family that includes the more recently discovered peptides adenomedullin and amylin[5].

The microvasculature appears most sensitive to the physiological effects of CGRP. CGRP is one of the most potent micro vascular vasodilator substances identified to date, with a potency ~10-fold greater than the prostaglandins and 100-1,000 times greater than other classic vasodilators (e.g., acetylcholine, adenosine, 5-hydroxytryptamine, and substance P). A dose of 15 pmol injected into human skin produces an erythema that lasts for 5-6 hr[6]. Further studies of CGRP have shown that its vasodilator activity extends to a wide variety of tissues and organs in other species, with particularly potent activity in the cerebral circulation, suggesting that it plays a role in the vasodilatation associated with the pathology of migraine[7].

Aim of the Work

The aim of the present work is to build up and prepare an up to date integrated and advanced review about the pharmacology of calcitonon gene-related peptide (CGRP) with special reference to its therapeutic potentials.

Objectives

- To know the historical background of the CGRP.
- To understand the basic pharmacology of the CGRP.
- To identify the different reported physiological roles of the CGRP.
- To predict the future perspectives of the CGRP pharmacology.

Structure of CGRP

The tertiary structure of CGRP has not been conclusively determined. In 1991, Breeze *et al*[8] produced data from NMR and distance geometry studies suggesting that (as shown in figure 1) CGRP consists of a characteristic NH_2-terminal disulfide bridge-linked loop between cysteines Cys^2 and Cys^7, followed by an alpha-helix in amino acids Val^8-Arg^{18} and a poorly defined turn between amino acids Ser^{19}-Gly^{21}. Later, also using NMR and molecular modeling techniques, Boulanger et al[9] produced a suggested structure for CGRP with a disulfide-linked loop between residues Cys^2 and Cys^7, a helix segment between residues Val^8 and Leu^{16} (rather than Arg^{18}), and defined the turn between residues 19 and 21 as a γ-type. The COOH and NH_2 terminals of the peptide can interact independently with its receptors in that the $CGRP_{8–37}$ fragment is an antagonist whilst $CGRP_{1–7}$ is important for efficacy[10]. There are two isoforms of CGRP for most species (α and β) that exhibit similar functional activities and differ by between one and three amino acids.

Basic Pharmacology of CGRP

Distribution and regulation of genes and peptides of CGRP

CGRP is widely distributed in the central and peripheral nervous systems[11,12]. αCGRP, encoded by the calcitonin gene, is the more abundant and found in discrete areas of the central and peripheral nervous system while is primarily located in the gut. Both forms of CGRP possess similarly potent biological activity in terms of vasodilator activity[13], although there are proposed differences in their receptor-mediated effects. In general, it is the effects of αCGRP that are discussed in this review. Amino acid sequences of human alpha and beta CGRP is provided in Figure No.2[10].

CGRP has been identified at many sites complementary to its activity as a vasoactive mediator For example; CGRP-containing nerves innervate smaller arteries, where innervating nerve terminals can pass into the vascular smooth muscle layer. Studies done about cerebral circulation show that CGRP is released from sensory fibers

originating in the trigeminal ganglia and acts to dilate cerebral vessels vessels[14]. The gut has also been intensively studied: here CGRP released from spinal afferents acts to dilate mucosal blood vessels and may protect against the acidic environment. It is possible that CGRP-containing vagal afferents, which originate from nodusa ganglia, have a preferential nociceptive role[15].

The regulation of CGRP production is poorly understood. Plasticity occurs at the level of the ganglia, for example, in models of peripheral axotomy, enteritis, and inflamed arthritic joints. In each case there is an associated increase in CGRP production in the ganglia. One factor of potential importance in influencing plasticity is nerve growth factor (NGF), which has an important role in the growth and maintenance of sensory nerve function. At a cellular level, NGF upregulates CGRP via a cAMP/ras responsive element and via a constitutively active mitogen-activated protein kinase (MAPK)[16]. In some experiments the upregulation of CGRP has been associated with nerve sprouting, an indication of NGF activity. Furthermore, up-regulation of CGRP production in the dorsal root ganglia by NGF has been linked to restoration of the endogenous micro-vascular activity of CGRP in diabetic skin , and in promoting CGRP expression in the spontaneously hypertensive rat. A classical mechanism leading to the release of sensory neuropeptides is that mediated by capsaicin, but the endogenous significance of this release mechanism remains unproven. CGRP immune-reactivity increases in the plasma after the administration of capsaicin, although the elevation is short lived[17]. Capsaicin acts via vanilloid (TRPV1) receptors on sensory C and Aδ fibers to increase permeability to cations. Low pH and heat are also associated with the activation of the capsaicin receptor, leading to a release of CGRP[18-20]. It has been recently suggested that a range of endogenous agents may also act to stimulate this receptor. These include anandamide and leukotriene B_4. Other substances considered to be involved in mediating the release of CGRP include kinins, prostaglandins and NO. It has been suggested that under certain circumstances, such as septic shock, mediators can act in a synergistic manner to potentiate CGRP release. Presynaptic/prejunctional receptors on the sensory nerves themselves also play an important role in modulating CGRP release. Such receptors include those for opioids, 5-hydroxytryptamine ($5\text{-}HT_1$ receptor), γ-aminobutyric acid ($GABA_B$ receptor), histamine (H_3 receptor), neuropeptide Y, somatostatin, vasoactive intestinal polypeptide, purines, and galanin[21,22].

It has been shown that stimulation of α_2-adrenoceptors, located presynaptically on sensory neurons, acts to inhibit CGRP release, with CGRP also inhibiting the release of norepinephrine from sympathetic nervous[23]. These results are indicative of an important role for CGRP in the regulation of peripheral blood flow. In the circulation, CGRP has a half-life of ~7–10 min in humans[24]. There is no obvious mechanism for CGRP metabolism, and it is probably broken down via a number of routes. Mast cell tryptase has a potent effect in cleaving CGRP into inactive fragments, both *in vivo* and *in vitro*. This mechanism has been clearly demonstrated in extravascular sites, e.g., skin. A matrix metalloproteinase II has the ability to metabolize CGRP and remove its vasodilator activity[25]. Finally, there has also been a suggestion that CGRP may be taken back up into sensory nerve terminals after repolarization, at least *in vitro*.

Receptor of CGRP

The understanding of these receptors is still at an early stage, and the information available to date is described below. CGRP acts on its own CGRP receptor to mediate its vasorelaxant effects.

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[26]. The 30-amino acid fragment of CGRP, $CGRP_{8–37}$, is an antagonist showing preference for the CGRP1 receptor[27]. In contrast, linearized CGRP analogs such as diacetoamidomethyl cysteine CGRP {[Cys(ACM)2,7]hαCGRP} are considered to show preferential agonist potency for the CGRP2

receptor. [Cys(ACM)2,7]hαCGRP is formed by reduction of the disulfide bond of CGRP. In general, receptors that can be antagonized by $CGRP_{8–37}$ with an approximate pK_b value of 7.0 are designated as CGRP1 receptors, while those that $CGRP_{8–37}$ block with a pK_b of 6 or less are classified as CGRP2 receptors [28, 29]. However, more recent studies have questioned the selectivity of [Cys(ACM)2,7]hαCGRP for the CGRP2 receptor and suggested that it also exhibits potent activity at the CGRP1 receptor.

Components of CGRP Receptor

The calcitonin receptor-like receptor (CL) component is common to all three receptors and is a G protein-coupled 7-transmembrane receptor. The three RAMP components are single transmembrane domain proteins. The active receptor is a functional heterodimer of one CL complexed with a RAMP, at the cell membrane. The interaction of RAMP1 with CL produces a CGRP receptor. The proposed receptor component protein (RCP), is suggested to allow coupling to intracellular signaling pathways (as shown in Figure No.3).

The CGRP receptor that have been cloned and characterized to date consists of a seven-transmembrane G protein-coupled CL in association with one of three single membrane-spanning RAMPs. There is strong evidence from studies in cultured cells that CL, in combination with an appropriate RAMP, acts as a receptor for CGRP[30]. CL is a member of the B family of seven transmembrane G protein-coupled receptors. Members include, in addition to the calcitonin receptor, receptors for vasoactive intestinal polypeptide, pituitary adenylate cyclase activating polypeptide, and parathyroid hormone.

RAMP1, -2, and -3 have been identified in humans, rats, and mice. The same RAMPs in different species show >60% homology, but <30% homology exists between different RAMPs in the same species[31]. The extracellular NH_2 terminus of the RAMP is important for ligand binding. In comparison, the short COOH terminus is not essential to activity. It has been proposed that RAMP1 is dominant, leading to the preferential expression of a functional CGRP receptor when both RAMP1 and RAMP2 are present in a cell[32]. It is now accepted that, while the CL is important for ligand binding, the RAMP proteins have roles in determining receptor phenotype and species selectivity. The trafficking activity of RAMP1 has been studied, and it is now realized that RAMP1, when expressed alone, is located in the endoplasmic reticulum and the Golgi mainly as a disulfide-linked homodimer[33].

The CGRP-receptor component is a 17-kDa intracellular membrane protein that was cloned and shown to provide CGRP receptor activity to *Xenopus* oocytes[34].

Factors Affecting Release of CGRP

Physical Factors

Low pH and heat are associated with the activation of the capsaicin receptor, leading to a release of CGRP. This may be relevant to the release and contribution of CGRP in models of cardiovascular ischemic inflammation, where localized acidity and increased heat are observed.

Chemical Factors

Nitroglycerin Induced Release of CGRP

Nitroglycerin is a nitric oxide (NO) donor that is widely used in the management of ischemic heart disease.

Recent studies indicate that nitroglycerin causes the release of CGRP in the cardiovascular system[35-38]. Preliminary evidence suggests that once nitrate tolerance is established, CGRP release may be reduced in response to subsequent administration of nitroglycerin. Nitroglycerin is widely recognized as a direct-acting vasodilator. Considerable evidence indicates that nitroglycerin causes smooth muscle relaxation by serving as an NO donor, thereby activating the guanylyl cyclase/cyclic GMP signaling pathway.

CGRP Secretion by Serotonergic Anti Migraine Drug

This belief has been strongly supported by the clinical efficacy of the selective 5-HT_1 receptor drug sumatriptan. Sumatriptan has been shown to decrease the elevated CGRP levels in migraine patients, coincident with relief of headache pain.

Trigeminal nerves play an important role in the regulation of cerebral blood flow during normal and disease states and are the major source of sensory and CGRP innervations to the cerebral vasculature. However, because all of the previous studies have used *in vivo* model systems and 5-HT_1 receptor are expressed by cerebral blood vessels and trigeminal nerves, the site of sumatriptan's action, has remained unclear. In that study, the authors demonstrated that sumatriptan and other 5-HT_1 receptor agonists can directly repress the stimulated, but not basal, release of CGRP from cultured trigeminal neurons[39].

Physiology of CGRP

Effect on cardiovascular system

Vascular mechanisms

There are several mechanisms by which CGRP produces vascular relaxation. It is accepted that vasodilatation is mediated via the $CGRP_1$ receptor and blocked in a competitive manner by $CGRP_{8–37}$. Current evidence points to the existence of an NO- and endothelium-independent pathway, where CGRP administration correlates closely with a rise in intracellular cAMP. This mechanism is observed in the majority of tissues that have been studied to date (e.g., rat perfused mesentery, cat cerebral artery, porcine coronary artery). The ability of CGRP to relax these tissues in the absence of an endothelium implies that, it acts directly on the smooth muscle cells to stimulate adenylate cyclase, and this has been demonstrated in cultured smooth muscle cells [40,41]. Figure No.4, shows cellular mechanism of vasodilatation to CGRP[42-50].

Cardiovascular Regulation

The vasodilator activity of CGRP has been studied extensively in the vasculature *in vitro*. The contribution of the various vasodilator mechanisms of CGRP to the vasodilatation in humans has been examined in a study in the human forearm where the ability of intra-arterial infusion of CGRP to stimulate a decrease in forearm vascular resistance due to vasodilatation is well established. The intravenous administration of CGRP is associated with hypotension and positive inotropic and chronotropic responses in the rat. In comparison, intracerebroventricular injection of CGRP, as well as other members of the calcitonin family of peptides causes an increase in blood pressure in rats, due to sympathetic nerve stimulation and release of the vasoconstrictor norepinephrine[51]. However, the available evidence, as indicated below, suggests that CGRP does not have a role in the regulation of blood flow in the normal rat or mouse.

Studies with intravenously injected $CGRP_{8–37}$ in rodents have shown a lack of effect on basal blood pressure. A recent investigation in the anesthetized rat and conscious dog supports these results and shows that $CGRP_{8–37}$, at doses that antagonized the effects of CGRP, had no effect on either systemic blood pressure or regional vascular beds. This supports the hypothesis that endogenous CGRP acts in a local rather than a systemic manner to modulate blood flow. In addition to studies using CGRP receptor antagonists, elucidation of the role of CGRP in cardiovascular regulation has been aided by the creation of αCGRP knockout mice.

The studies using the αCGRP knockout mice have been important in the absence of a well-characterized, potent, and selective antagonist for the CGRP receptor(s). They have revealed some inconsistencies compared with each other and to the antagonist studies, and these require further investigation. In comparison, it is established that $CGRP_{8–37}$ has little potency as a systemic vasoconstrictor, although effects observed on regional blood flow suggest that CGRP may play a local modulatory/homeostatic role in the control of blood pressure. Furthermore, it has been suggested that the attenuated release of CGRP in spontaneously hypertensive rats may contribute to the observed hypertension[52].

Microvascular Mechanisms

The intravenous infusion of subvasodepressor doses into the conscious rat led to specific relaxant effects in a range of tissues, for example, a reduction in hindquarters vascular resistance[53]. The concept of CGRP as a highly targeted vasodilator is enhanced by the observation of increased microvascular blood flow induced in the ipsilateral, but not contralateral, skin of the hindleg of the anesthetized rat after

stimulation of CGRP-containing nerves, demonstrating that its activity is primarily at the site of release. It is also in keeping with the observation of selective facial flushing observed after intravenous CGRP administration in humans[54].

The beneficial role of sensory nerves is considered to be due to the vasodilator activity of CGRP. Peripheral vascular conditions associated with a deficit of CGRP-containing nerves, vascular dysfunction, and slow wound healing includes diabetes and Raymond's disease, where a lack of reflex vasodilatation is observed. In comparison, excess release of CGRP is associated with blushing syndromes[55].

In many species, the coronary arteries and left anterior descending artery receive innervation from a high density of CGRP-containing nerve fibers[56]. CGRP is released from the heart in laboratory species in response to ischemia and low pH; with evidence that endothelium-derived prostacyclin (PGI_2) may also play a role in CGRP release. There is also evidence for a protective role of endogenous CGRP in a myocardial infarction model in the pig; however, exogenously administered CGRP caused hypotension but no cardioprotection, as observed by a lack of reduction in the size of infarct size. Li et al, Suggest that CGRP is involved in ischemic preconditioning; possibly via protection of microvascular endothelial cells, and that the protective role of nitroglycerin may be related to stimulation of CGRP release[57].

Furthermore, it has been suggested that the delayed cardioprotection observed after intestinal ischemic preconditioning is mediated by endogenous CGRP in an NO-dependent manner as a Nitric oxide synthase (NOS) inhibitor abrogated the response[58].

Action of CGRP on Heart

Evidence indicates that CGRP can have a protective influence in dilating coronary arteries at locations of atheromatous stenosis, and delaying the onset of myocardial ischemia in patients with chronic angina undergoing treadmill exercise[59].

Effect of CGRP on Cerebral Vessels

The most compelling evidence of a role for CGRP in a pain syndrome comes from sufferers of migraine and cluster headache. Studies have shown that CGRP levels are raised during the painful phases of both conditions and are restored to basal levels by successful migraine treatment with triptan 5-HT_1 agonists, providing evidence that the trigeminal sensory nervous system is activated. It is now known that exogenous CGRP induces a delayed migraine-like headache in migraineurs. However there is little evidence for a role of CGRP in tension-type headache[60]. The release of CGRP and the role of the blood - brain barrier, in migraine are still poorly understood. CGRP can be released from either the trigeminal ganglia or perivascular nerves. It has recently been suggested that triptans act by causing a prolonged elevation of intracellular calcium in trigeminal neurons, which blocks the MAPK activation of CGRP synthesis and release. 5-HT_1 agonist treatment of migraine is associated with a rebound effect; the pain is removed, but then returns several hours after the initial attack[61]. This has spurred on the search for a new class of antimigraine drugs. The non-peptide CGRP antagonist, BIBN4096BS, has been tested in phase II clinical trials as a potential novel treatment and found to be effective against the symptoms of migraine, without significant acute side effects.

It has been shown that gene transfer of recombinant adenoviral prepro-CGRP can prevent fatal cerebral vasoconstriction after subarachnoid hemorrhage in a rabbit model[62]. It is therefore possible that gene therapy may be appropriate in this condition and of greater benefit than current therapies or intravenous infusion of CGRP, although mechanisms for the administration of such treatments will have to be developed.

Effect of CGRP on Reproductive System

CGRP influences many stages of mammalian development by affecting the function of female and male reproductive organs. It regulates blood flow to the female reproductive organs, and has a role in the innervations of the uterus and aids in fetal growth and survival. It is involved in uterine relaxation during pregnancy[63]. It is suggested that the peptide is involved in maintaining the human myometrium in quiescence during pregnancy by

antagonizing the actions of uterine stimulants like oxytocin, and a decrease in the CGRP receptors towards the end of pregnancy aids in the initiation of labor. It has been reported that CGRP plays an important role in sperm function in mice[64,65]. CGRP's status in the human male reproductive system is still being studied though it is reported to be present in the semen, prostate, and seminal vesicles.

Effect of CGRP on Gastrointestinal System

CGRP receptors are present in the D-cells of the gastric mucosa demonstrating control of secretion and production of somatostatin. The secreted somatostatin inhibits gastric acid secretion both directly and indirectly[66]. The inhibitory influences of CGRP on the gastrointestinal tract result in decreased motility and contraction. CGRP antagonists could be used as spasmolytics, antidiarrheal and antinociceptive drugs in gastrointestinal diseases. The increase in gastric blood flow by CGRP resulting in gastric protection may provide new drug targets in the treatment of gastric ulcer. CGRP stimulated release by capsaicin was shown to result in hyperemia in the gastric lining[67], which explained the earlier finding that capsaicin protects against peptic ulcer[68], which was later confirmed by others on human stomach.

Effect of CGRP on Pulmonary System

In situ hybridization and immunohistochemistry revealed the existence of CGRP in the pulmonary system[6]. In the airways, the peptide acts on bronchial smooth muscle and submucosal glands to promote airflow obstruction and hyperemia, hence it's implication in bronchoconstriction. CGRP causes a concentration-dependent relaxation of the pulmonary artery and also effectively dilates precontracted pulmonary arteries. It is further reported that in isolated rat lungs CGRP's mitigating effect on hypoxic pulmonary vasoconstriction could involve the suppression of pressor response to angiotensin II[69]. Studies on the extent of CGRP accumulation in allergic conditions such as asthma are under way and its potential role in regulating pulmonary vasculature is being investigated.

Pathophysiologic Roles of CGRP

CGRP has many important pathologic functions as in inflammation, different Cellular Effects, Pulmonary Hypertension, Raynaud's Disease, Sepsis, Blushing Syndromes, Migraine, and much other abnormalities[70-76].

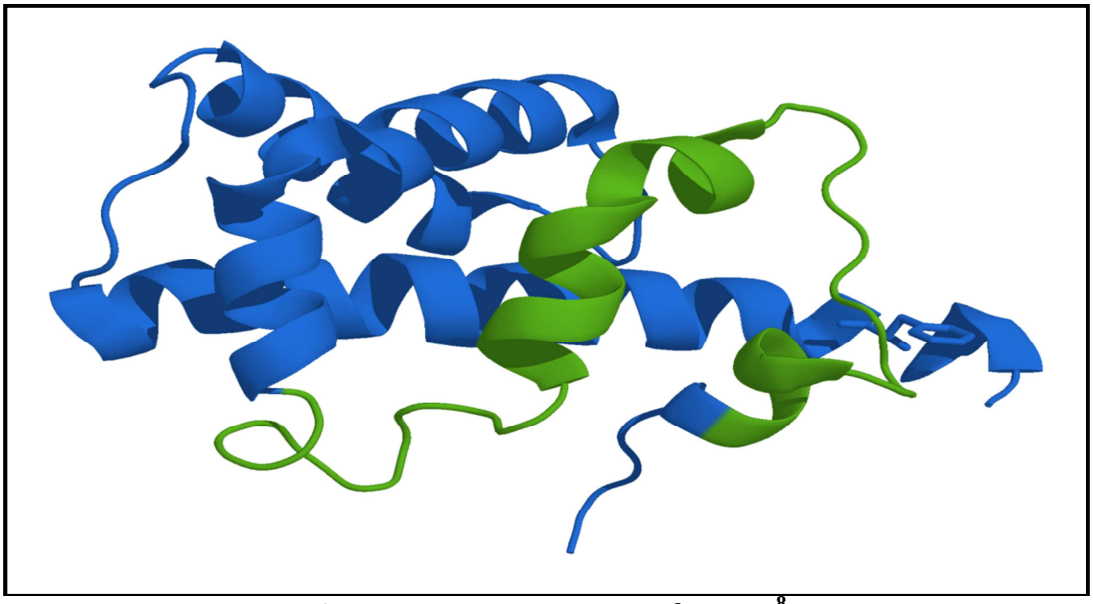

Figure No.1: 3D structure of CGRP[8]

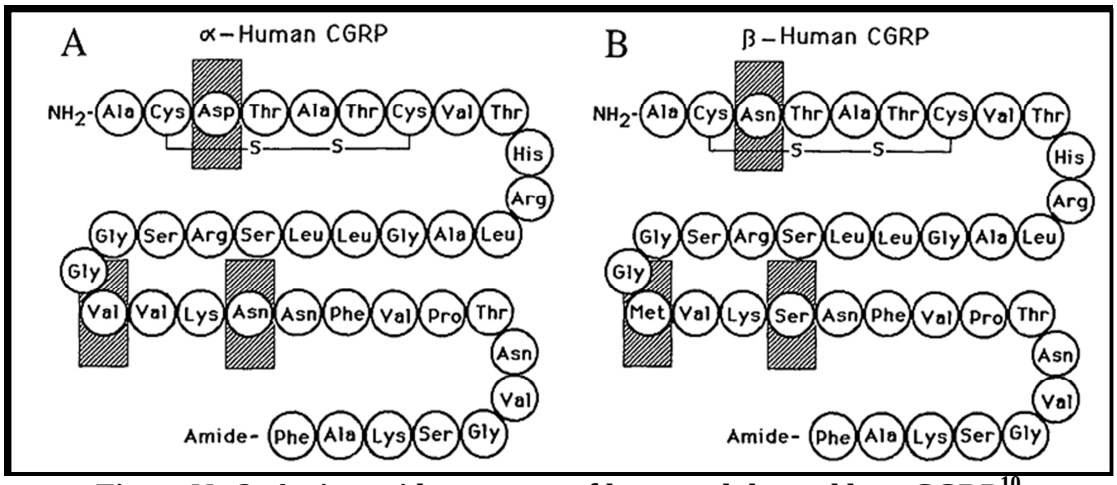

Figure No.2: Amino acid sequences of human alpha and beta CGRP[10]

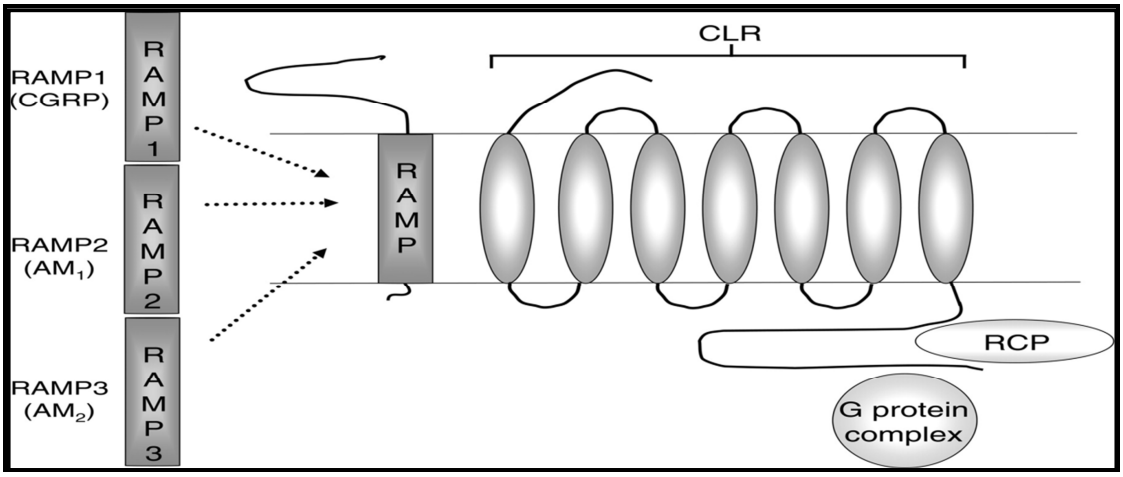

Figure No.3: Components of CGRP receptor

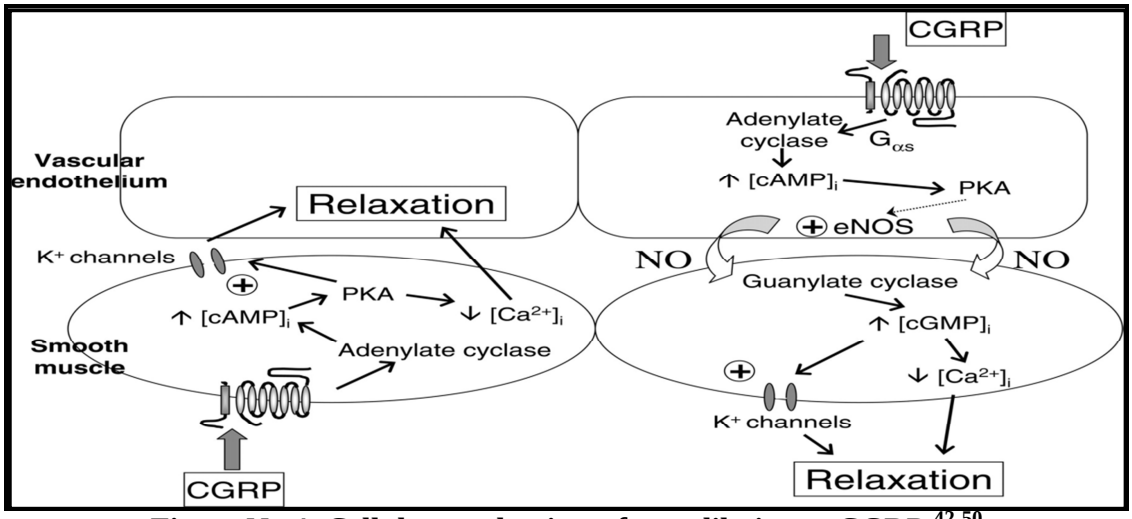

Figure No.4: Cellular mechanism of vasodilation to CGRP [42-50]

The cellular mechanisms of vasodilatation to CGRP. ***Left*:** endothelium-independent vasodilatation to CGRP. Activation of CGRP receptors on smooth muscle cells is coupled to production of cAMP by adenylate cyclase. The increase in intracellular cAMP concentration ($[cAMP]_i$) then stimulates protein kinase A (PKA), which opens K^+ channels and activates Ca^{2+} sequestration mechanisms to cause smooth muscle relaxation. ***Right*:** endothelium-dependent vasodilatation to CGRP. CGRP interacts with receptors on endothelial cells and stimulates production of nitric oxide (NO). This is mediated via cAMP accumulation, although a direct effect of Protine Kinase A (PKA) on endothelial NO synthase (eNOS) is yet to be fully characterized. Diffusion of NO into adjacent smooth muscle cells, activating guanylate cyclase, then leads to relaxation.

CONCLUSION

In conclusion, this review acts to integrate evidence from recent developments in molecular and cardiovascular research which predicts a pivotal role of CGRP. Research in this field is at an exciting stage where selective ligands (agonist and antagonists) are in the processes of experimental investigating as well as clinical trials for use as selective therapies for several disorders (such as migraine, Reynaud's disease, pulmonary hypertension ….etc).

CGRP is one of the most important and widely distributed neuropeptides with a very potent vasodilatory effect. It is involved in most of human tissues. Hence, with the advanced studies on CGRP and the possibly reacting ligands with its receptors, indispensable benefits to the pharmaco-therapeutics of many diseases will take place. For example, it is expected that new drugs, acting on the CGRP receptors, are on their way to be utilized for alleviating disease conditions such as migraine, hypertension, flushing syndrome, diarrhea, spasms, ischemia and many other pathological conditions. In addition, research on ligands to those receptors will help in the propping for improving the understanding of the different body functions. It will be interesting to follow the success of such compounds especially when considering the pleiotropic nature of the CGRP family of peptides. Thus, it may be worth noting to emphasize that, within few years the medical importance of CGRP and its interactions will be of more or less similar weight as that of the earlier discovered neurotransmitters ,such as dopamine and nor adrenaline.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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