Calcitonin gene-related peptide: Understanding its role

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ABSTRACT

Calcitonin gene-related peptide (CGRP), a 37 amino acid neuropeptide, identified in multiple species, has widespread distribution and expression. CGRP acts through G protein-coupled receptors whose presence and changes in function modulate the peptide's effects in various tissues. Three receptor subtypes have been identified and CGRP's signal transduction through the receptors is dependent on two accessory proteins: Receptor activity modifying protein1 (RAMP1) and Receptor component protein (RCP). 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 in migraine have been promising. This brief review provides a preliminary understanding of the diverse biological effects of the peptide in various systems. The current status of CGRP and its receptors in many pathophysiological states is not fully explored and future findings are greatly awaited.

KEY WORDS: Calcitonin-receptor like receptor, CGRP receptor, cAMP, signal transduction

Introduction

Calcitonin gene-related peptide (CGRP), identified in 1982, belongs to the calcitonin family of neuropeptides which also includes adrenomedullin, amylin, calcitonin, intermedin and calcitonin receptor-stimulating peptide. CGRP results from the tissue-specific alternative splicing of the primary RNA transcripts of the calcitonin gene. It is a 37 amino acid neuropeptide (Figure 1) with widespread expression such as in the heart, blood vessels, pituitary, thyroid, lung, gastrointestinal tract and a wide array of biological effects including neuromodulation, vasodilatation, cardiac contractility, bone growth, and mammalian development. The peptide is released from motor neurons at the neuromuscular junction and sensory neurons of spinal cord. There are two isoforms available: αCGRP and βCGRP[1] which are derived from different genes. βCGRP differs from αCGRP by three amino acids in rats and by one in humans. βCGRP is mainly present in the enteric nervous system whereas αCGRP is present in the sensory neurons. No major differences in the effects between α and βCGRP with respect to adenylyl cyclase stimulation and intracellular cAMP formation are found. Potent subtype selective non-peptide agonists and antagonists for CGRP are being developed for therapeutic use in hypertension, cardiac failure, migraine headaches, Reynaud's syndrome, preeclampsia, and diabetes.[2] The main goal of this review is to summarize recent findings with respect to CGRP, from its physiology and signal transduction to its pharmacological aspects in various systems. The

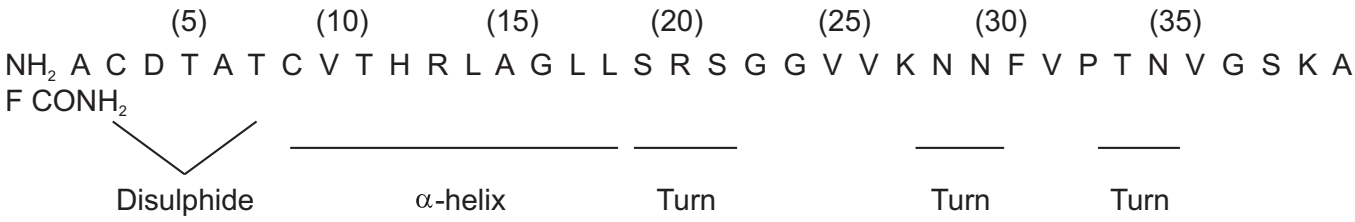

Figure 1: Structure of human αCGRP

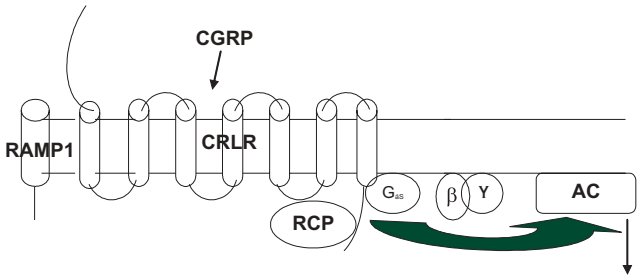

Figure 2: CGRP receptor complex. RAMP: receptor-activity-modifying protein, CRLR: calcitonin-receptor-like receptor, RCP: receptor component protein, AC: Adenylyl cyclase and cAMP: Cyclic AMP.

reader is suggested to look into similar reviews for additional information.[3-5]

CGRP receptors

Therapeutic targeting of CGRP has always been a hindrance due to the presence of multi components of its receptor. Cell surface receptor for CGRP has been cloned recently and it is found to contain two components. A seven transmembrane protein, calcitonin receptor-like receptor (CRLR) belonging to the B family of G protein-coupled receptors (GPCRs) has been identified as the receptor for CGRP (Figure 2). It has a long extracellular N-terminus and a short intracellular C-terminus. However, when transfected into COS-7 cells it was found out that CRLR alone could not act as the functional receptor. An accessory protein, receptor activity modifying protein 1 (RAMP1), acts as a chaperone for CRLR, thus specifying the RAMP1+CRLR complete receptor complex.[6]

RAMPs are a three-member family of integral membrane proteins. They are 14-17 kDa single transmembrane domain polypeptides with a 100 amino acid N-terminal extracellular domain and a short intracellular region. RAMP-1, -2 and -3, have been identified in man, rat and mouse sharing a common topology and ~60% similarity.

CGRP receptor component protein (RCP), an intracellular peripheral membrane protein, is reported to couple the receptor complex to the cellular signal transduction pathway.[7] It is also known that heterodimeric RAMP2+CRLR is the defined adrenomedullin receptor complex.[7] Recently, a different CGRP receptor without any CRLR+RAMP1 has been expressed in rat cerebellum and human embryonic kidney (HEK-293) cell lines. This seven transmembrane receptor may be associated with RCP and it is speculated that this receptor may be present in RCP-rich tissues where CRLR is not expressed.[8]

Receptors for CGRP have been characterized in a variety of tissues including pituitary, adrenal gland, heart and blood vessels. The comparative potencies of CGRP and its analogs in various *in vitro* and *in vivo* functional assays have led to the suggestion of the existence of multiple classes of CGRP receptors. Three subtypes of CGRP receptors have been reported. Those are $CGRP_1$, $CGRP_2$ and $CGRP_3$[9] and the first two subtypes share pharmacological similarities. CGRP receptors are classified based on their affinity for the truncated CGRP peptide, $CGRP_{8-37}$. pA_2/pK_B values of various tissues in different species are mentioned in Table1. Tissues having pA_2 values 7.5-8.5 (high-affinity) are considered as $CGRP_1$ and 5.5-6.5 (low-affinity) as $CGRP_2$. But it is difficult to categorize the tissues whose pA_2 values are between 6.5 and 7.5.[10] Apparent pK_B values of BIBN 4096 BS for hαCGRP and hβCGRP in the left anterior descending coronary artery are, respectively, 8.0 and 6.6[4]. The effects of CGRP on the heart as well as on the blood vessels are mediated through $CGRP_1$. The relaxation induced by CGRP on the smooth muscle such as the urinary bladder and vas deferens appears to be largely mediated through the $CGRP_2$ receptor subtype. Various novel non-peptide $CGRP_1$ receptor antagonists such as BIBN 4096 BS (a Lys–Tyr dipeptide derivative), WO 98/11128 (Compound 1) and SB-273779[4] are used as pharmacological tools to gain insights into CGRP receptor heterogeneity in tissues. BIBN 4096 BS is a competitive reversible antagonist with pK_B 11. Reduction of the disulfide bond in CGRP, which destroys the N-terminal ring structure of the peptide, yields a linear analog,

Table 1

Antagonistic affinities for $h\alpha CGRP_{8-37}$ in terms of pA_2/pK_B values in various species

Animal/Tissue/Cells	*pA_2/pK_B*	*Reference*
Rat		
Tissue		
Thoracic aorta	<5.0 and 7.0	12
Vas deferens	5.9-6.6	13
Pulmonary artery	6.9	14
Intramural coronary artery	6.9	15
Mesenteric resistance artery	7.2	16
Basilar artery	7.5	17
Perfused tissues		
Mesenteric Vasculature	7.4	18
Kidney	8.0	19
Heart	8.5	20
Cells		
Adipocytes	6.9	21
Ventricular cardiomyocytes	7.9	22
Aortic smooth muscle cells	8.0	23
Liver plasma membranes	8.1	24
Glomerular mesangial cells	8.2	25
L6 myocytes	8.3	26
Pig		
Left anterior descending coronary artery (large)	5.7, 6.3 and 7.2	27 and 28
Left anterior descending coronary artery (small)	7.0	27
Coronary artery (not specified the actual part)	6.7	29
Guinea-pig		
Ileum	7.2	30
Isolated left atrium	6.6	31
Human		
Cerebral artery (Intact endothelium)	9.7	32
Cerebral artery (endothelium denuded)	9.1	32
Middle meningeal artery (Intact endothelium)	8.8	32
Middle meningeal artery (endothelium denuded)	8.5	32

diacetoamidomethyl cysteine CGRP ([Cys $(ACM)_{2,7}$] CGRP), a selective agonist for $CGRP_2$ receptors. $CGRP_{(12-37)}$, $CGRP_{(19-37)}$, $CGRP_{(28-37)}$, [Tyr^0] $CGRP_{(27-37)}$ are the other novel antagonists of the $CGRP_1$ receptor.[11]

Signal transduction

GPCRs interact with G proteins to initiate signal transduction. Activation of sensory cells by calcium influx releases CGRP and coupling of receptor complex to $G_{\alpha S}$ results in increased cellular cAMP levels. Although not a receptor itself, RCP is reported to channel CRLR to the cell surface and couple the receptor to the cell-signaling pathway.[7] The function of RCP is not universal to all GPCR signaling but is limited to CGRP receptors only. Protein Kinase A (PKA) is activated by cAMP formed by adenylyl cyclase. PKA in turn causes activation of many transcription factors like c-JUN NH_2-terminal protein kinase (JNK) and phosphorylation of other proteins such as cAMP responsive element binding protein (CREB).[33] PKA-mediated activation is limited not only to JNK but also to other kinases like extracellular signal-regulated kinase-1 (ERK-1) and p38 Mitogen-activated protein kinase (p38 MAPK).[34]

CGRP and its biological significance

CGRP receptors are present in a wide variety of tissues and are implicated in several pathophysiological conditions. The activation of these receptors mainly produces potent vasodilatation and smooth muscle relaxation. CGRP receptors not only increase cAMP levels but also downregulate the expression of acetylcholinesterase at transcription level.[35] In vascular smooth muscle cells, elevated cAMP levels are observed during the opening of potassium channels resulting in decreased vascular tone.[36] Recent studies have shown that CGRP increases mRNA levels of α subunit of acetylcholine receptor (AChR), which is mediated through PKA. Local administration of CGRP activates AChR in the brain, which modulates sympathetic nervous system actions.[37] The main biological effects of CGRP on various systems are summarized in the following sections.

Nervous system

CGRP is widely distributed in the brain suggesting its involvement in sensory and motor systems. With the exception of the dorsal motor nucleus of the vagus nerve, CGRP is reported to be present in all cranial nuclei. CGRP binding sites are also observed in the olfactory system. The presence of a cholinergic/CGRP vestibular system suggests the role of CGRP in the processing of auditive information.[1] Immunohistochemical studies showed the presence of RCP of CGRP receptors in the human trigemino-vascular system where CGRP receptors are co-localized with $5\text{-}HT_{1B/1D}$ receptors.[38] Elevated CGRP levels in jugular venous blood correlate with the timing and severity of migraine and cluster headaches.[39] This might be due to the increased gene expression of CGRP by activated MAPK pathways.[40] Sumatriptan, a $5\text{-}HT_{1B/1D}$ receptor agonist, is used to treat increased CGRP levels in migraine.[32] It owes its therapeutic activity solely to a presynaptic action inhibiting CGRP release and therefore neurogenic inflammation. Recent clinical trials in migraine patients have demonstrated higher rate of response to BIBN 4096 BS, a CGRP receptor antagonist. These trials identified BIBN 4096 BS as an effective agent in the acute treatment for migraine.[41]

Pulmonary system

In situ hybridization and immunohistochemistry revealed the existence of CGRP in the pulmonary system.[42] Released CGRP has the ability to degranulate mast cells and release various chemical mediators triggering inflammatory cycle.[43] Nerve fibers projecting into the airways and pulmonary neuroendocrine cells release CGRP into lungs. In the airways, the peptide acts on bronchial smooth muscle and submucosal glands to promote airflow obstruction and hyperemia,[44] hence it's implication in bronchoconstriction. CGRP has a potent vasodilatory effect in human pulmonary arteries and veins as evidenced by the presence of its receptors in pulmonary artery endothelium.[45] It has already been reported that these receptors play an important role in pulmonary hypertension.[46] αCGRP causes a concentration-dependent relaxation of the pulmonary artery[45] and also effectively dilates precontracted pulmonary arteries.[47] Certain N-terminal CGRP components are useful in the treatment of hypoxic pulmonary hypertension (HPH). Artery pressures elevated in HPH are lowered by the CGRP interventions and this action has been shown to be mediated through CGRP putative receptor and RAMP1. 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.[47] 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.

Gastrointestinal system

CGRP receptors are present in the D-cells of the gastric mucosa demonstrating control of secretion and production of somatostatin.[48] The secreted somatostatin inhibits gastric acid secretion both directly and indirectly. The gastrointestinal actions of CGRP are mainly mediated by $CGRP_1$ receptors. The inhibitory influences of CGRP on the gastrointestinal tract result in decreased motility and contraction.[49] In rats, CGRP regulates food intake through receptors present in CNS.[50] Following central administration of βCGRP in humans, inhibition of gastric motor functions and suppression of acid secretion were noted. The peptidergic innervations are altered in various gastrointestinal disease conditions. In short the centrally induced gastro-protective effect of CGRP helps in the maintenance of gastric mucosal homeostasis. CGRP receptors are also present in the D-cells of the pancreas[52] and the exogenous CGRP gene therapy has been shown to mitigate autoimmune diabetes by suppressing reactive oxygen species.[53] It is reported that in conscious rats, central administration of CGRP inhibited basal pancreatic secretion, acting through an α-adrenergic mechanism.[54] Also, CGRP acts on the gut mucosal immune system as an immunomodulator. This was supported by the presence of CGRP receptors on T and B lymphocytes.[51] 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 ulcers.

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, has a role in the innervations of the uterus and aids in fetal growth and survival. CGRP receptors are reported in human myometrium, uterus, and placenta.[55] It is involved in uterine relaxation during pregnancy.[56] 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. Downregulation of the receptors at the end of term and in postpartum is evident in rats also.[57] Increased receptor number in pregnancy signifies its importance in the maintenance of normal systemic hemodynamics in that condition.[58] It is postulated that hormonal cycle has an influence in CGRP release and its adaptor functions in pregnancy.[58] Progesterone stimulates and estrogen inhibits the CGRP receptor expression in the placenta.[57] These hormones also modulate the effects of CGRP on blood pressure in pregnancy.[58] Postmenopausal women have less plasma CGRP levels than normal due to vasomotor changes and hormone replacement therapy (HRT) causes CGRP levels to return back to basal values.[59] It has been reported that CGRP plays an important role in sperm function in mice.[60] 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.[60] New ligands of the $CGRP_1$ receptor have therapeutic relevance in conditions such as hot flushes and premature labor.

Cardiovascular system

CGRP has various actions on the human cardiovascular system such as control of peripheral vascular tone, potent vasorelaxation, increase in rate and force of contraction of heart. Sensory nerve terminals of capsaicin-sensitive C- and A- delta fibers release CGRP by chemical, thermal and mechanical stimuli. Various factors such as glucocorticoids, nerve growth factor, and vascular wall tension and sympathetic nervous system at the local level modulate CGRP release. CGRP is present in a network of nerve fibers that surrounds the arteries as well as on the smooth muscle membrane.[61] Because of the dense perivascular network of the CGRP nerves, CGRP dilates various vascular beds such as mesenteric, renal and hindquarter skeletal muscles, acting through an endothelium-dependent and independent mechanism.[62-63] Autoradiography studies reveal that CGRP receptors are present in specific sites in the intima and media of the aorta, coronary arteries and heart valves.[64] It is reported that endogenous αCGRP has no role in regulating basal vascular tone under normal, resting conditions but exogenous administration causes a marked relaxation of different regional vascular beds.[65] The aortic endothelium of the rat seems to express the $CGRP_1$ subtype, which is sensitive to αCGRP, βCGRP and $CGRP_{8-37}$.[28] $CGRP_1$ is predominant in small intramural coronary arteries of the rat.[15] Systemic administration of CGRP decreases blood pressure in humans and animals along with increased cardiac performance and blood supply to vital organs.[66]

Besides vasodilatory activity at the vascular level, CGRP produces positive inotropic and chronotropic actions of the heart in various mammals. CGRP receptor gene is highly expressed in the rat heart.[67] Some β-adrenoceptor blockers have the ability to release CGRP from cardiac sensory neurons, which activates cardiac CGRP receptors.[68] $CGRP_1$ receptors mediate increase in cell surface area through enhanced skeletal α-actin expression in hypertrophy of cardiac myocytes.[69] The highest density of receptors is found in the bundle of His of the guinea-pig.[70] the atrial myocardium has dense CGRP fibers, which upon stimulation releases CGRP. This in turn increases L-type calcium channel current through adenylyl cyclase-cAMP pathway resulting in increased atrial contraction. The peptide has also been shown to produce a positive contractile response in the ventricular myocytes.[22] CGRP produces tachycardia by reflex activation of the sympathetic nervous system.[62] Both $CGRP_1$ and $CGRP_2$ receptors are implicated in the inotropic effect of atria and ventricles[71-72] in all mammals except in dogs where the hemodynamic changes are not mediated by $CGRP_1$ receptors.[73] In metabolic acidosis, CGRP receptors facilitate the deleterious effects of acidic pH by decreasing contractility and relaxation of isolated rat atria.[74] It has a protective action on myocytes and endothelial cells.[75]

CGRP plays a role in the modulation of platelet function. CGRP inhibits platelet aggregation by increasing the platelet cAMP concentration. This antiplatelet activity is mediated by the activation of nitric oxide synthase.[76] Elevated levels of circulating CGRP levels have been noted in experimental and clinical models of sepsis while that of increased mRNA for CGRP were observed in all tissues in a hamster peritonitis model.[77] In humans the upregulation of CGRP occurs as the sepsis progresses. It is suggested that the small intestine is a major source of these elevated CGRP levels.[78]

Nitroglycerin activates sensory nerve fibers to release CGRP and the cardiovascular effects of nitroglycerin (vasorelaxation and cardioprotection) are partly mediated by endogenous CGRP.[79] The reductive species of NO, nitroxyl anion, increases CGRP but not cGMP.[80] In rats, CGRP levels are decreased during nitrate tolerance but are restored after NTG withdrawal. Endogenous CGRP plays an important role in the development of nitrate tolerance in rat thoracic aorta.[81] Nitrate tolerance is reversed by using N-Acetyl cysteine and captopril, which is thought to be due to an increase in CGRP release.[82] CGRP released by nitroglycerin plays a role in cardiac preconditioning which also activates K_{ATP} channels. Exogenous CGRP prevents myocardial and endothelial injury caused by ischemia.[83] Vascular CGRP is cleaved at Gly-Leu peptide bond by vascular matrix metalloproteinase, which abolishes its vasodilatory effect.[84] Acute myocardial infarction is a condition where there is a demand for increased cardiac output and vasodilatation. This suggests the potential benefits of CGRP agonists in ischemia and congestive heart failure.

Other actions

CGRP receptors are present in synovial joints and mediate

various neural regulations in inflammatory responses.[85] In kidneys, infusion of CGRP at low doses causes vasodilatation and an increase in glomerular filtration rate, whereas high doses cause diuresis.[86] CGRP elevates body temperature in male rats and probably is involved in hot flashes in men.[87] Increase in body weight has been shown to be associated with decreases in B_{max} and affinity of CGRP for the receptor.[88] CGRP is involved in skin and immune cell functions such as cell proliferation and cytokinin production.[89]

Conclusions

CGRP receptor antagonists may prove beneficial in many prevalent diseases such as migraine, arthritis, temporomandibular-joint disorders, in which CGRP levels are elevated. Future research with respect to complete cloning of CGRP and its peptide receptors is greatly awaited in order to fully elucidate its role in various systems and pathophysiological conditions. It is necessary to develop highly potent and selective analogues that will permit further characterization and the functional role(s) of each of the CGRP receptor subtypes.

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