Calcitonin Gene-Related Peptide (CGRP)

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Abstract: Calcitonin gene-related peptide (CGRP) is a 37 amino acid vasoactive neuropeptide that is widely distributed in central and peripheral nervous systems in mammals. CGRP was discovered in 1982 by molecular cloning of calcitonin (CT) gene. CGRP-specific mRNA appears to predominate in the hypothalamus while CT mRNA predominates in the thyroid. CGRP has an amphiphilic α-helical secondary structure in the amino acid sequence between 8-25. Half-life of CGRP in mammalian plasma is about 10 min. CGRP is immunohistochemically co-localized with substance P in cardiovascular system and it has similar role to that of substance P. CGRP is secreted by primary afferents and causes primary hyperalgesia and its expression increases in dorsal horn, under sensitization conditions. CGRP plays important role in blood pressure system.

Key Words: calcitonin, calcitonin gene-related peptide, cardiovascular, substance P, neuropeptide

1. Introduction

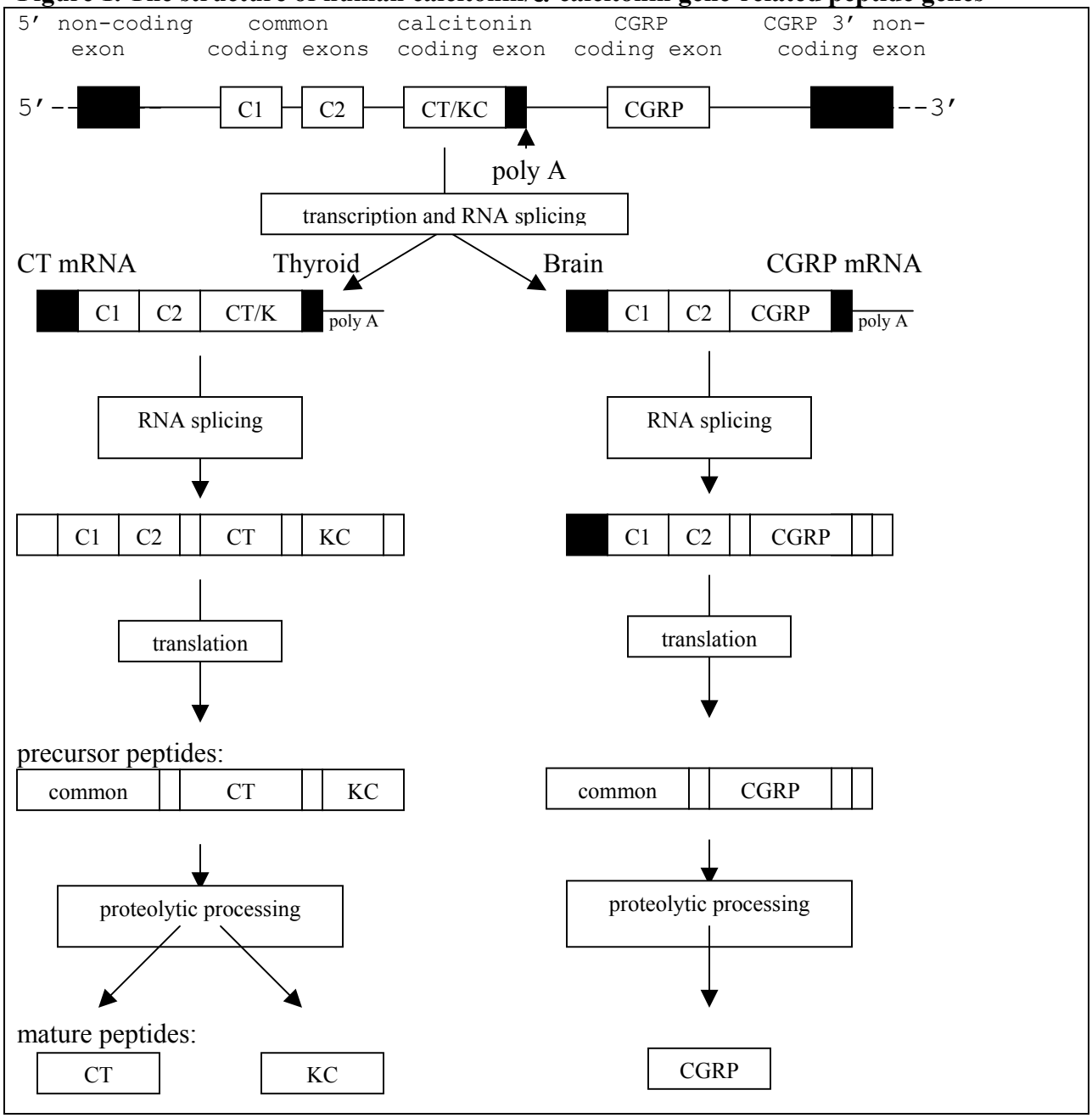
Calcitonin (CT) and calcitonin gene-related peptide (CGRP) are derived from CT/CGRP gene. For human, this gene is localized in chromosome 11. Different splicing of primary RNA transcript arouses the translation of CT and CGRP peptides in a tissue-specific manner (Fig. 1). This alternative tissue-specific processing of primary mRNA from the CT/CGRP gene in rats generates two distinct peptides, CT and CGRP. CT, a calcium-lowering hormone, is a 32 amino acid single chain peptide expressed mainly in the thyroid gland. CGRP is a 37 amino acid vasoactive neuropeptide that is widely distributed in the central and peripheral nervous systems in mammals (DiPette et al. 1995, Wimalawansa et al. 1996). α-CGRP is produced by the tissue-specific alternative splicing of the primary transcript of the CT/α-CGRP gene and is synthesized almost exclusively in neuronal tissues (Breimer et al. 1988, Rosenfeld et al. 1983). There is a second CGRP gene, β-CGRP, which does not produce CT (Amara et al. 1985). Expression of β-CGRP is limited almost exclusively to specific neuronal sites. The two CGRP genes, α and β in rats and I and II in humans, differ in their protein sequences by 1 and 3 amino acids, respectively, and their biological activities are quite similar in most vascular beds (Breimer et al. 1988). CGRP receptors widely exist in animal body. The fact that CGRP gene knockout mouse performs lower blood flow rate shows CGRP plays role in blood pressure (Ma et al. 2002).

2. Discovery

The CT gene was unexpectedly found to encode two different mRNAs with identical 5' sequences but with distinct 3' sequence. The mRNAs encode either the 17,500 MW CT precursor protein witch is proteolyzed to yield CT and two other peptides or the 16,000 MW protein which is the predicted translation predict of CGRP mRNA. From the nucleotide sequence of cloned CGRP mRNA it could be predicted that the encoded protein is proteolytically processed to yield three peptides, including the 37 amino acid CGRP (Rosenfeld et al. 1983). A Canadian scientist Copp first postulated the existence of calcium-lowering peptide CT. With the assistance of a number of first-year medical students working during the summer of 1961, Copp and his group developed a precise method for measuring calcium, demonstrated the remarkable constancy of plasma calcium in normal human subjects, and found that normal calcium levels were restored quickly after being artificially raised or lowered. They focused on parathyroid hormone, which plays a key role in controlling hypocalcemia by stimulating osteolysis. While studying the control of its secretion in 1961, they discovered a second calcium-regulating hormone CT that was released by hypercalcemia and lowered plasma calcium by inhibiting osteolysis (Copp 1994). CT gene was first cloned in 1980 (Jacobs et al. 1981), and CGRP was discovered in 1982 by molecular cloning of CT gene (Amara et al. 1982, Rosenfeld et al. 1983). According to Amara and Rosenfeld's report in 1982 and 1983, alternative processing of the RNA transcribed from CT gene appears to result in the production of an mRNA in neural tissue distinct from that in thyroidal C cells. The thyroid mRNA encodes a precursor to the hormone CT whereas that in neural tissues generates a novel neuropeptide, referred to as CGRP. The CT mRNA predominates in the thyroid while the CGRP-specific mRNA appears to predominate in the hypothalamus (brain). The second rat CGRP (β-rCGRP) gene has been discovered in brain and thyroid. This β-rCGRP is different from the α-CGRP by one amino acid (position 1 Ala instead of Ser). The second human CGRP (β-hCGRP) gene has been discovered in

medullary thyroid (Tschopp et al. 1985). This β-hCGRP differs from the α-hCGRP by three amino acids located position 3, 22, and 25 (Table 1).

Figure 1. The structure of human calcitonin/α-calcitonin gene-related peptide genes



3. Gene

The CT mRNA predominates in the thyroid while the CGRP-specific mRNA appears to predominate in the hypothalamus. The schematic representation of rat CT/CGRP gene structure is illustrated in Fig. 1. CT/CGRP gene consists of 6 exons. The first 3 exons are constitutively spliced in both mRNAs. Exon 1 is untranslated, exon 2 codes for the signal peptide, and exon 3 codes for the N-terminal propeptide. CT sequence is localized in exon 4 and CGRP sequence is localized in exon 5. Exon 6 is part of CGRP mRNA but is untranslated. The primary transcript includes all the 6 exons, and CT and CGRP mRNAs are formed subsequently. The second human CT/CGRP

(hCT/CGRP) gene was identified in 1985 (Hoppener et al. 1985). It contains sequences highly homologous to exons 3, 5 (CGRP-encoding), and 6 of the first hCT/CGRP gene, but sequences closely related to exon 4 (CT-encoding) could not be demonstrated. Southern blot hybridization analysis of DNA from human-rodent somatic cell hybrids showed that the second hCT/CGRP gene is located in the q12-pter region of chromosome 11. The first hCT/ CGRP gene has previously been assigned to the p13-p15 region of chromosome 11.

Table 1. Primary sequences of nine calcitonin gene-related peptides (CGRP)

Human α-CGRP	ACDTATCVTH RLAGLLSRSG GVVKNNFVPT NVGSKAF
Human β-CGRP	ACNTATCVTH RLAGLLSRSG GMVKSNFVPT NVGSKAF
Rat α-CGRP	SCNTATCVTH RLAGLLSRSG GVVKDNFVPT NVGSEAF
Rat β-CGRP	ACNTATCVTH RLAGLLSRSG GVVKDNFVPT NVGSKAF
Porcine CGRP	ACNTATCVTH RLAGLLSRSG GVVKSNFVPT DVGSEAF
Chicken CGRP	ACNTATCVTH RLADFLSRSG GVGKNNFVPT NVGSKAF
Frog CGRP	ACNTATCVTH RLADFLSRSG GMAKNNFVPT NVGSKAF
Rabbit CGRP	GCNTATCVTH RLAGLLSRSG GMVKSNFVPT NVGSEAF
Bovine CGRP	ACNTATCVTH RLAGLLSRSG GVVKSNFVPT NVGSEAF

According to the analysis of the human CT/α-CGRP gene locus, about 39 kb of DNA containing the gene has been mapped and a common Pvu II RFLP identified downstream of the gene. DNA sequence analysis revealed an extensive CpG island containing several rare restriction enzyme sites at the 5' end of the gene. The structure of this island is unusual in that it contains two distinct CpG-rich regions, one located around exon 1 and the other about 1.5 kb further upstream. Msp I sites within both CpG-rich regions were found to be unmethylated, regardless of whether the CT/α-CGRP gene was being expressed. However, a correlation was found between demethylation of Msp I sites in intron 2, downstream of the CpG island, and CT/α-CGRP gene expression. DNA sequence analysis also revealed the presence of several binding sites for constitutive and regulatory transcription factors in the promoter of the gene. These results suggest that both unmethylated CpG islands and specific demethylation of internal sequences may play a role in the activation of CT/a-CGRP gene transcription (Broad et al. 1989).

4. Structure

Primary sequences of nine CGRP peptides are shown in Table 1. From Table 1 it can be seen that rabbit CGRP is identical to human β-CGRP in 35 of 37 amino acid residues. Two amino acid differences were detected at position 1, with Gly in rabbit CGRP instead of Ala in human β-CGRP, and at position 35, with Glu instead of Lys, respectively. Rabbit CGRP differs from human α-CGRP by three additional amino acids at positions 3 (Asn instead of Asp), 22 (Met instead of Val), and 25 (Ser instead of Asn). Rabbit CGRP is more closed to human β-CGRP than human α-CGRP. In the nine CGRP peptides, rabbit CGRP is the only CGRP that has Gly as the amino terminal amino acid and rat α-CGRP is the only CGRP that has Ser as the amino terminal amino acid. Since the amino terminus of CGRP seems to be important for expression of bioactivity, the biological activity of rabbit CGRP and rat α-CGRP may differ from other forms of CGRP. Human α-CGRP is the only CGRP that has the amino acid Asp at position 3 and all other CGRPs has Asn at the position 3. CGRP has an amphiphilic α-helical secondary structure in the amino acid sequence between 8-25. The region between residues 8-18 adopts an α-helical conformation (Breeze et al. 1991). The half-life of CGRP in mammalian plasma is about 10 min.

5. Synthesis

CGRP is produced by alternative splicing from the gene that originally shown to encode the hormone CT, whereas CT is synthesized predominantly in the thyroid gland. CGRP is secreted by primary afferents and causes primary hyperalgesia, and its expression increases in dorsal horn, under sensitization conditions. CGRP is present in a variety of central and peripheral neurons. In motoneurons, it is packaged into dense core vesicles, transported to motor nerve terminals, and released on stimulation (Uchida et al. 1990, Changeux et al. 1992). Application of CGRP to cultured myotubes stimulates AChR synthesis (New et al. 1986, Fontaine et al. 1986, 1987). In the central nervous system slicing of the α-CT/CGRP gene produces CGRP, whereas in the C cells of the thyroid gland, CT is predominantly formed.

Fischbach and colleagues isolated an acetylcholine receptor-inducing activity (ARIA) from brain, based on its ability to stimulate AChR accumulation in cultured myotubes (Usdin et al. 1986). The purified protein of 42 kD had no apparent AChR clustering activity, but did increase AChR subunit mRNA levels, suggesting a transcriptional effect (Martinou et al. 1991). Molecular

cloning revealed that ARIA was one of many alternatively spliced products of a gene now called neuregulin (Falls et al. 1993). Other products of the neuregulin gene had been isolated as ligands of the neu/erbB protooncogene (heregulin and neu differentiation factor) and as a glial growth factor (Fischbach et al. 1997). Neuregulin, like CGRP, is synthesized by many kinds of cells, including motoneurons. Moreover, it is transported down motor axons, and becomes incorporated into synaptic basal lamina, probably by binding to heparin sulfate proteoglycans (Goodearl et al. 1995, Loeb et al. 1995).

6. Distribution

CGRP and its receptors are widely distributed in the nervous system, including discrete brain areas and in the cardiovascular system (Yoshizaki et al. 1987). In the peripheral nervous system, CGRP is present in the sensory ganglia, often costored with substance P. Together with substance P, bradykinin and calcium-sensing receptor, CGRP-rich nerve fibers form part of the primary afferent nervous system, comprising capsaicin-sensitive A and C fiber afferent nerves, and "type B" medium-sized cells. In motor neurons, CGRP coexists with acetylcholine. In most neurons, the α- and β-CGRP coexist, but the β-form of the peptide is predominant in the enteric nervous system and in the human pituitary gland. The distribution of CT and CGRP-producing cells and pathways in the brain and other tissues suggests functions for the peptide in nociception, ingestive behaviour and modulation of the autonomic and endocrine systems.

Substance P is an 11 amino acid neuropeptide that is abundant in the periphery and the central nervous system, where it is colocalized with other neurotransmitters such as serotonin or dopoamine. Substance P is often co-localized with CGRP in perivascular sensory nerves (Katki et al. 2002) and influences coronary flow rate (Ma et al. 2002). CGRP is immunohistochemically co-localized with substance P in capsaicin-sensitive, varicose axons supplying the skin, viscera and cardiovascular system of the guinea pig. After treatment with colchicine in vitro, 82% of substance P neurons in the dorsal root ganglia contained CGRP-like immunoreactivity while 96% of CGRP neurons were immunoreactive for substance P (Gibbins et al. 1985).

7. Functions

CGRP has several important physiologic roles: (1) CGRP is a potent vasodilator, and can affect the force and rate of heart beat. (2) CGRP can modulate acetylcholine receptor function at the neuromuscular junction. (3) CGRP has been demonstrated to block tolerance to morphine. (4) CGRP can modulate antigen presentation in Langerhans cells in the skin. Despite these important physiologic functions, therapeutic strategies using CGRP have been impeded due to the lack of a cloned CGRP receptor with which ligands could be developed. CGRP results in a dose-dependent decrease in blood pressure and increase in heart rate. Furthermore, CGRP selectively changes regional organ blood flowers. CGRP gene knockout mice show lower coronary flow rate that supposes hypertension related (Ma et al. 2002). CGRP has similar role to that of substance P. CGRP gene knockout mice blood pressure increase (Gangula et al. 2000) and blood flow rate decrease (Ma et al. 2002). CGRP induced nitric oxide (NO) production in mouse peritoneal macrophages (Liu et al. 2001). This indicates that CGRP may involve animal oxidation-reduction system.

Gene transfer of prepro-CGRP restores erectile function in the aged rat. There is a significant decrease in CGRP concentrations and in cAMP and cGMP levels in aged rat cavernosal tissue compared to younger rats. Aged rats also have significantly lower erectile function as determined by cavernosal nerve stimulation compared to younger rats. Five days after transfection with adenoviral-mediated gene transfer of prepro-CGRP, these aged rats have an approximately threefold increase in cavernosal CGRP levels compared to animals transfected with adenoviruses encoding nuclear-targeted β-galactosidase. Five days after administration of adenoviral-mediated gene transfer of prepro-CGRP, a significant increase is observed in the erectile response to cavernosal nerve stimulation in the aged rat, similar to the response observed in younger rats. These data suggest that in vivo adenoviral gene transfer of CGRP can physiologically improve erectile function in the aged rat (Bivalacqua et al. 2001).

Increased levels of ovarian hormones during pregnancy may elevate the synthesis of CGRP and nerve growth factor receptors in dorsal root ganglia. CGRP levels in rat dorsal root ganglia are significantly higher during pregnancy than at Day 2 postpartum or in ovariectomized rats (Lanlua et al. 2001).

In primary headaches, there is a clear association between head pain and release of CGRP. Furthermore, when triptan antimigraine agents are administered, headache subsides and the neuropeptide release normalises, in part via a presynaptic effect. The central role of CGRP in primary headaches has led to the search for suitable antagonists of the receptors for this neuropeptide, which it is hoped will have less cardiovascular adverse effects than the triptans. These compounds are small molecules with high selectivity for human CGRP receptors. Hypothetically, these agents will be efficacious in the relief of migraine headaches via blockade of the effects of CGRP (Edvinsson 2001).

CGRP is a potent inhibitor of gastric acid secretion in the rat as well as in the dog. Its inhibitory action could be demonstrated against various stimuli and appears to

be independent of prostaglandin or vagal pathways. CGRP (2.6 nmol/kg) causes a 63%-78% inhibition of gastric acid secretion with all secretagogues tested. CGRP circulates at five times the concentration of CT, suggesting that it may be an important physiological regulator of vascular tone and blood flow.

hCGRP (545 pmol/min) causes human diastolic pressure to fall from 64 to 55 mmHg, heart rate to increase from 61 to 87 beats/min and skin temperature to increase from 34 to 35 ºC. Plasma noradrenaline increases from 481 to 835 pg/ml and plasma adrenaline from 57 to 82 pg/ml. There are no significant changes in the albumin-corrected plasma calcium. hCGRP is thus a potent endogenous vasodilator in man and is in fact more potent than any other known vasodilator. Together with the observations that CGRP circulates in normal subjects at relatively high concentration (25 pmol/l) and that CGRP is present in perivascular nerves, this study suggests a possible role for CGRP in controlling peripheral vascular tone in man (Struthers et al. 1986).

8. Receptors

CGRP and its receptors are widely distributed in the nervous system and cardiovascular system. Most neuropeptide receptors are members of the superfamily of proteins characterized by the presence of seven hydrophobic, putative membrane-spanning domains. These receptors are linked to intracellular G-proteins for signal transduction, and can participate in multiple signal transduction pathways, depending on which G-protein is present.

CT receptor-like receptor (CRLR) is a seven-transmembrane domain (7TM) protein that requires the receptor activity-modifying protein 1 (RAMP1) to be expressed at the cell surface as a functional CGRP receptor. When expressed alone RAMP1 is retained inside the cells where it is found in the endoplasmic reticulum and the Golgi predominantly as a disulfide-linked homodimer. In contrast, when expressed with CRLR, it is targeted to the cell surface as a 1:1 heterodimer with the 7TM protein. Although heterodimer formation does not involve intermolecular disulfide bonds, RAMP-CRLR association promotes the formation of intramolecular disulfide bonds within RAMP1. CGRP binding and receptor activation lead to the phosphorylation of CRLR and the internalization of the receptor as a stable complex. The internalization is found to be both dynamin- and β-arrestin-dependent, indicating that the formation of a ternary complex between CRLR, RAMP1, and β-arrestin leads to clathrin-coated pit-mediated endocytosis. These results therefore indicate that although atypical by its heterodimeric composition and its targeting to the plasma membrane, CGRP receptor shares endocytotic mechanisms that are common to most classical 7TM receptors (Hilairet et al. 2001).

9. Measurement

CGRP can be measured by standard immunohistochemical and radioimmunoassay techniques. Such as Western Blotting and ELISA, etc. Both CGRP and CT peptides are commercially available by Sigma Co., and both anti-CGRP and anti-CT monoclonal antibodies are commercially available by Sigma Co. also.

10. Therapeutics

Long term therapy with salmon CT does not affect any sympathetic skin response and heart rate variability parameters. It can be speculated that though human CGRP and CT have discrete functions in the human autonomic nervous system, replacement therapy with salmon CT does not interfere normal autonomic functions (Sahiner et al. 1999).

Evidently manual and electro-acupuncture have different effects, whereas electro-acupunctureand physical exercise has more similar effects on CGRP production and/or release (Wyon et al. 1998). Administration of CGRP may be a therapy treatment method on the high blood pressure cases. As CGRP half life is only 10 min, it will be better to discover similar molecules or some other method related CGRP.

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