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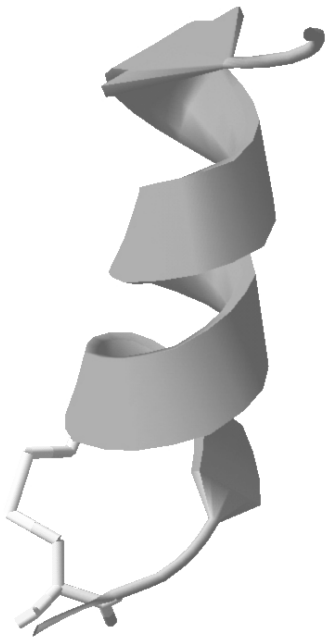
BACHEM

Product Monograph

CGRP

Calcitonin Gene-Related Peptide



Abstract

Calcitonin gene-related peptide (CGRP) is a 37 amino acid peptide which belongs to a family of related peptides including calcitonin, amylin, and adrenomedullin. It exists in two isoforms α-CGRP (or CGRP I) and β-CGRP (or CGRP II) which are very similar in their biological activities and are encoded by different genes. CGRP peptides are mainly localized in sensory and central neurons and have been implicated in a variety of physiological processes such as cardiovascular homeostasis, calcium metabolism, and control of fetoplacental vascular tone. Receptors for this family of peptides belong to the seven transmembrane G-protein-coupled receptors linked to the activation of adenylate cyclase. Their interaction with receptor activity modifying proteins (RAMPs) is essential for membrane trafficking and for conferring ligand specificity. In this monograph Bachem presents a selection of its products for CGRP research.

Introduction

α-CGRP and β-CGRP, also known as CGRP I and II, respectively, belong to the calcitonin family of peptides comprising such members as calcitonin, amylin, and adrenomedullin. Recently, the cloning of intermedin-1 added an additional member to this family. At their N-terminus, these peptides have in common a characteristic disulfide loop structure, generally formed by six to seven amino acids.

The 37 amino acid peptides α-CGRP and β-CGRP are encoded by different genes on chromosome 11. α-CGRP mRNAs are derived from the calcitonin/CGRP gene by alternative tissue specific splicing of the primary RNA transcripts whereas β-CGRP is encoded by a separate gene with high homology to the calcitonin/CGRP gene. The amino acid sequences of CGRP peptides are well conserved among species. In humans α- and β-CGRP differ by 3 amino acids, in rat, by one amino acid. In their biological activities they are very similar.

Distribution of CGRP

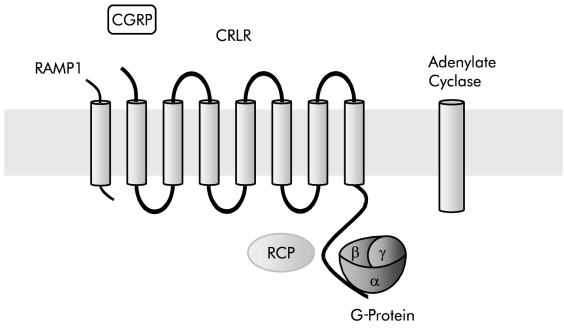
CGRP expression is widely distributed in the central and peripheral nervous system. In the brain, it is particularly concentrated in the hypothalamus and in certain nuclei of the brainstem. In the periphery, CGRP is mainly detected in sensory afferents projecting to the spinal cord, in motor neurons at the neuromuscular junctions and in nerve fibers associated with the vasculature. In capsaicin-sensitive sensory neurons CGRP co-localizes with substance P and other neuropeptides, in the motor endplate with acetylcholine.

Physiological Functions

On the basis of pharmacological studies several physiological functions of CGRP have been suggested. Due to its potent vasodilatory action and its ionotropic and chronotropic effects, CGRP is likely to play a role in cardiovascular homeostasis. Furthermore, it influences feeding and digestion since it has shown to decrease food intake, gastric secretion, and intestinal motility. Based on its ability to modulate substance P signaling, an additional function of CGRP in nociception has been proposed. Additionally, CGRP might also be important in processes such as control of fetoplacental vascular tone, regulation of calcium metabolism and insulin secretion, acetylcholine receptor synthesis, peripheral nerve regeneration, and neurogenic inflammation.

CGRP Receptors

CGRP receptors have been identified in several tissues, including brain, cardiovascular, endothelial, and smooth muscle tissue. Based on early pharmacological studies the existence of two classes, CGRP1 and CGRP2 receptors, has been described. According to this historical classification CGRP1 receptors are more sensitive to the antagonistic properties of α-CGRP (8-37) (H-9895, H-4924) whereas CGRP2 receptors are more responsive to the agonistic CGRP analogs, (Cys(Acm)^{2,7})-α-CGRP (human) (H-5766) and (Cys(Et)^{2,7})-α-CGRP (human) (H-5784). Recent studies have shown that the previously cloned G-protein-coupled orphan receptor named calcitonin receptor-like receptor (CRLR) can interact with members of a new family of three single-transmembrane domain receptor activity modifying proteins (RAMPs). Interaction with RAMP1 resulted in a CGRP receptor which is sensitive to α-CGRP (8-37) whereas binding to RAMP2 and RAMP3 led to receptors for adrenomedullin known as AM1 and AM2 receptors, respectively. The AM2 receptor showed considerable affinity for CGRP. Besides their essential role in regulating ligand specificity RAMPs are also required for membrane trafficking of CRLR. Recently, a receptor component protein (RCP) of the CRLR/RAMP1 complex was described. RCP is a intracellular protein which is highly conserved between species and might be required for G-protein-coupled signal transduction.



CGRP Receptor

CGRP binds to a receptor complex formed by the calcitonin receptor-like receptor (CRLR) and one of three single transmembrane receptor activity modifying proteins (RAMP1). RAMP1 is essential for membrane trafficking of CRLR and for regulation of ligand specificity. An intracellular receptor component protein (RCP) is required for coupling to the cellular signal transduction pathway.

Therapeutic Implications

Given the multitude of physiological and pathophysiological effects of CGRP, modulations of its properties represent potential therapeutic interventions in a variety of disease states including cardiovascular disorders and neurogenic inflammation. Clinical trials have indicated that the vasodilatory effect of CGRP might be beneficial in the treatment or prevention of Raynaud's disease, hypertension, angina pectoris and heart failure. Since CGRP is rapidly metabolized, longer acting CGRP ago-

nists are needed for long term treatment. CGRP antagonists, for their part, might be useful in the treatment of migraine which involves the activation of the trigeminal system and CGRP-evoked dilatation of cranial vessels. The non-peptidic CGRP antagonist BIBN-4096BS (Boehringer Ingelheim) is presently under clinical investigation to assess the importance of CGRP in migraine headache.

Prospects

CGRP has proven to be a molecule which is involved in diverse physiological processes. Future research will contribute to a better understanding of its various properties, the heterogeneity of its receptors, and its physiological interactions with other molecules.

For further details, please see the following literature references:

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Calcitonin Gene-Related Peptides (CGRP) and Fragments offered by Bachem

Product	Prod. No.		References
CGRP (chicken) H-Ala-Cys-Asn-Thr-Ala-Thr-Cys-Val-Thr-His-Arg-Leu-Ala-Asp-Phe-Leu-Ser-Arg-Ser-Gly-Gly-Val-Gly-Lys-Asn-Asn-Phe-Val-Pro-Thr-Asn-Val-Gly-Ser-Lys-Ala-Phe-NH_2 (Disulfide bond)	**H-3352**	Solubility: 2 mg/ml in water $C_{165}H_{262}N_{52}O_{50}S_2$ M_r: 3838.35 [114679-42-4]	[1]
α-CGRP (human) H-Ala-Cys-Asp-Thr-Ala-Thr-Cys-Val-Thr-His-Arg-Leu-Ala-Gly-Leu-Leu-Ser-Arg-Ser-Gly-Gly-Val-Val-Lys-Asn-Asn-Phe-Val-Pro-Thr-Asn-Val-Gly-Ser-Lys-Ala-Phe-NH_2 (Disulfide bond) CGRP-I (human) **Trifluoroacetate salt**	**H-1470**	Solubility: in water $C_{163}H_{267}N_{51}O_{49}S_2$ M_r: 3789.36 [90954-53-3] net	[2,3]
([^{125}I]-Tyr^0)-α-CGRP (human)	**H-5354**		
Biotinyl-α-CGRP (human) Biotinyl-Ala-Cys-Asp-Thr-Ala-Thr-Cys-Val-Thr-His-Arg-Leu-Ala-Gly-Leu-Leu-Ser-Arg-Ser-Gly-Gly-Val-Val-Lys-Asn-Asn-Phe-Val-Pro-Thr-Asn-Val-Gly-Ser-Lys-Ala-Phe-NH_2 (Disulfide bond)	**H-5688**	Solubility: 1 mg/ml in water $C_{173}H_{281}N_{53}O_{51}S_3$ M_r: 4015.66	
(Cys(Acm)^{2,7})-α-CGRP (human) H-Ala-Cys(Acm)-Asp-Thr-Ala-Thr-Cys(Acm)-Val-Thr-His-Arg-Leu-Ala-Gly-Leu-Leu-Ser-Arg-Ser-Gly-Gly-Val-Val-Lys-Asn-Asn-Phe-Val-Pro-Thr-Asn-Val-Gly-Ser-Lys-Ala-Phe-NH_2	**H-5766**	Solubility: in water $C_{169}H_{279}N_{53}O_{51}S_2$ M_r: 3933.53 [125448-83-1]	
(Cys(Et)^{2,7})-α-CGRP (human) H-Ala-Cys(Et)-Asp-Thr-Ala-Thr-Cys(Et)-Val-Thr-His-Arg-Leu-Ala-Gly-Leu-Leu-Ser-Arg-Ser-Gly-Gly-Val-Val-Lys-Asn-Asn-Phe-Val-Pro-Thr-Asn-Val-Gly-Ser-Lys-Ala-Phe-NH_2	**H-5784**	(Cys(Et)^{2,7})-α-CGRP is a potent and selective CGRP2 receptor agonist. It had a high potency to inhibit the rat vas deferens twitch response (ED_{50} = 4-5 nM) whereas high concentrations (>1 µM) were required for inducing chronotropic and inotropic effects in the prototypical CGRP1 receptor guinea pig atrium in vitro bioassay. Solubility: in TFA $C_{167}H_{277}N_{51}O_{49}S_2$ M_r: 3847.48 [196413-73-7]	[4]
Tyr-α-CGRP (human) H-Tyr-Ala-Cys-Asp-Thr-Ala-Thr-Cys-Val-Thr-His-Arg-Leu-Ala-Gly-Leu-Leu-Ser-Arg-Ser-Gly-Gly-Val-Val-Lys-Asn-Asn-Phe-Val-Pro-Thr-Asn-Val-Gly-Ser-Lys-Ala-Phe-NH_2 (Disulfide bond) Tyr-CGRP I (human)	**H-3354**	Solubility: 2 mg/ml in water $C_{172}H_{276}N_{52}O_{51}S_2$ M_r: 3952. 54 [124756-98-5]	
α-CGRP (rat) H-Ser-Cys-Asn-Thr-Ala-Thr-Cys-Val-Thr-His-Arg-Leu-Ala-Gly-Leu-Leu-Ser-Arg-Ser-Gly-Gly-Val-Val-Lys-Asp-Asn-Phe-Val-Pro-Thr-Asn-Val-Gly-Ser-Glu-Ala-Phe-NH_2 (Disulfide bond)	**H-2265**	Induces elevation of cAMP and cGMP in rat aorta. Solubility: in water $C_{162}H_{262}N_{50}O_{52}S_2$ M_r: 3806.30 [83651-90-5]	[5,6]

Product	Prod. No.		References
([^{125}I]-Tyr^{0})-α-CGRP (rat)	**H-4958**		
Biotinyl-α-CGRP (rat) Biotinyl-Ser-Cys-Asn-Thr-Ala-Thr-Cys-Val-Thr-His-Arg-Leu-Ala-Gly-Leu-Leu-Ser-Arg-Ser-Gly-Gly-Val-Val-Lys-Asp-Asn-Phe-Val-Pro-Thr-Asn-Val-Gly-Ser-Glu-Ala-Phe-NH_2 (Disulfide bond)	**H-5684**	Solubility: 1 mg/ml in 1 % acetic acid $C_{172}H_{276}N_{52}O_{54}S_3$ M_r: 4032.60	
α-CGRP (8-37) (human) H-Val-Thr-His-Arg-Leu-Ala-Gly-Leu-Leu-Ser-Arg-Ser-Gly-Gly-Val-Val-Lys-Asn-Asn-Phe-Val-Pro-Thr-Asn-Val-Gly-Ser-Lys-Ala-Phe-NH_2	**H-9895**	CGRP and calcitonin cross-react with each other at the levels of their distinct receptors. α-CGRP (8-37) has been described as a selective antagonist for CGRP receptors but not for calcitonin receptors. This fragment thus appears to be a valuable tool for receptor characterization studies. Solubility: in water $C_{139}H_{230}N_{44}O_{38}$ M_r: 784.98 [119911-68-1]	[7,8,9]
α-CGRP (8-37) (rat) H-Val-Thr-His-Arg-Leu-Ala-Gly-Leu-Leu-Ser-Arg-Ser-Gly-Gly-Val-Val-Lys-Asp-Asn-Phe-Val-Pro-Thr-Asn-Val-Gly-Ser-Glu-Ala-Phe-NH_2	**H-4924**	It has been demonstrated that this competitive CGRP1 receptor antagonist exhibited hypothermic and antinociceptive effects. This peptide antagonized the effects of amylin and amylin (1-8) on osteoblasts but was substantially less potent in this regard than amylin (8-37) (H-2746). Furthermore, it inhibited the evoked discharge frequency of wide dynamic range neurons in dorsal horn of the spinal cord in rats. Solubility: 1 mg/ml in water $C_{138}H_{224}N_{42}O_{41}$ M_r: 3127.56 [129121-73-9]	[10,11, 12,13, 14,15]
Acetyl-α-CGRP (19-37) (human) Ac-Ser-Gly-Gly-Val-Val-Lys-Asn-Asn-Phe-Val-Pro-Thr-Asn-Val-Gly-Ser-Lys-Ala-Phe-NH_2	**H-8890**	Solubility: 10 mg/ml in water $C_{88}H_{139}N_{25}O_{26}$ M_r: 1963.22 [145459-34-3]	[16]
α-CGRP (19-37) (human) H-Ser-Gly-Gly-Val-Val-Lys-Asn-Asn-Phe-Val-Pro-Thr-Asn-Val-Gly-Ser-Lys-Ala-Phe-NH_2	**H-8885**	Solubility: 10 mg/ml in 20 % acetic acid $C_{86}H_{137}N_{25}O_{25}$ M_r: 1921.19 [101233-12-9]	[16]
α-CGRP (23-37) (human) H-Val-Lys-Asn-Asn-Phe-Val-Pro-Thr-Asn-Val-Gly-Ser-Lys-Ala-Phe-NH_2	**H-8895**	Solubility: 10 mg/ml in water $C_{74}H_{117}N_{21}O_{20}$ M_r: 1620.87 [145459-33-2]	[16]
Tyr-α-CGRP (23-37) (rat) H-Tyr-Val-Lys-Asp-Asn-Phe-Val-Pro-Thr-Asn-Val-Gly-Ser-Glu-Ala-Phe-NH_2	**H-2270**	Solubility: 20 mg/ml in water $C_{82}H_{120}N_{20}O_{25}$ M_r: 1785.97 [198277-54-2]	[17]
(Tyr^{27})-α-CGRP (27-37) (rat) H-Tyr-Val-Pro-Thr-Asn-Val-Gly-Ser-Glu-Ala-Phe-NH_2	**H-5504**	Solubility: in water $C_{54}H_{79}N_{13}O_{17}$ M_r: 1182.30 [124501-79-7]	[2]
α-CGRP (29-37) (rat) H-Pro-Thr-Asn-Val-Gly-Ser-Glu-Ala-Phe-NH_2	**H-5746**	Solubility: in water $C_{40}H_{61}N_{11}O_{14}$ M_r: 919.99 [219991-19-2]	[18]

Calcitonin Gene-Related Peptides (CGRP) and Fragments offered by Bachem (continued)

Product	Prod. No.		References
α-CGRP (30-37) (rat) H-Thr-Asn-Val-Gly-Ser-Glu-Ala-Phe-NH_2	**H-5748**	Solubility: in water $C_{35}H_{54}N_{10}O_{13}$ M_r: 822. 87 [132917-49-8]	[18]
α-CGRP (31-37) (rat) H-Asn-Val-Gly-Ser-Glu-Ala-Phe-NH_2	**H-5752**	Solubility: in water $C_{31}H_{47}N_9O_{11}$ M_r: 721.77 [110953-70-3]	[18]
α-CGRP (32-37) (rat) H-Val-Gly-Ser-Glu-Ala-Phe-NH_2	**H-5742**	This hexapeptide is a potent stimulator of amylase secretion. In contrast to α-CGRP (rat) it did not increase cellular cyclic AMP, but did stimulate outflux of ^{45}Ca in guinea pig pancreatic acini. α-CGRP (32-37) (rat)-stimulated amylase secretion was inhibited by the CCK receptor antagonist L364,718. Solubility: in water $C_{27}H_{41}N_7O_9$ M_r: 607.66 [132917-48-7]	[18]
α-CGRP (33-37) (rat) H-Gly-Ser-Glu-Ala-Phe-NH_2	**H-5744**	Solubility: in water $C_{22}H_{32}N_6O_8$ M_r: 508.54 [132917-50-1]	[18]
β-CGRP (human) H-Ala-Cys-Asn-Thr-Ala-Thr-Cys-Val-Thr-His-Arg-Leu-Ala-Gly-Leu-Leu-Ser-Arg-Ser-Gly-Gly-Met-Val-Lys-Ser-Asn-Phe-Val-Pro-Thr-Asn-Val-Gly-Ser-Lys-Ala-Phe-NH_2 (Disulfide bond) CGRP-II (human)	**H-6730**	Solubility: 1 mg/ml in water $C_{162}H_{267}N_{51}O_{48}S_3$ M_r: 3793.41 [101462-82-2]	[19,20]

Related Immunology Products offered by Bachem

Product	Prod. No.
α-CGRP (human) Immunology Products, Host: Guinea Pig	
α-CGRP (human) - Diluted Antiserum for RIA, Host: Guinea Pig	T-5026
α-CGRP (human) - Undiluted Antiserum for Immunohistochemistry, Host: Guinea Pig	T-5027
α-CGRP (human) - Immunofluorescence Kit, Host: Guinea Pig	S-3046
α-CGRP (human) - RIA Kit, Host: Guinea Pig	S-2099
α-CGRP Immunology Products, Host: Mouse	
α-CGRP - Clone CD8	T-1604
α-CGRP (human) Immunology Products, Host: Rabbit	
α-CGRP (human) - Diluted Antiserum for RIA, Host: Rabbit	T-4237
α-CGRP (human) - Purified Antiserum - IgG, Host: Rabbit	T-4238
α-CGRP (human) - Undiluted Antiserum for Immunohistochemistry, Host: Rabbit	T-4239
α-CGRP (human) - EIA Kit (H - sr, pl), Host: Rabbit, Extraction-free	S-1198
α-CGRP (human) - EIA Kit, Host: Rabbit, High Sensitivity	S-1199
α-CGRP (human) - Immunofluorescence Kit, Host: Rabbit	S-3047
α-CGRP (human) - Immunohistochemistry Staining Kit, Host: Rabbit	S-4016
α-CGRP (human) - RIA Kit, Host: Rabbit	S-2100

Product	Prod. No.
α-CGRP (rat) Immunology Products, Host: Rabbit	
α-CGRP (rat) - Diluted Antiserum for RIA, Host: Rabbit	T-4030
α-CGRP (rat) - Purified Antiserum - IgG, Host: Rabbit	T-4031
α-CGRP (rat) - Undiluted Antiserum for Immunohistochemistry, Host: Rabbit	T-4032
α-CGRP (rat) - EIA Kit, Host: Rabbit, High Sensitivity	S-1167
α-CGRP (rat) - Immunofluorescence Kit, Host: Rabbit	S-3006
α-CGRP (rat) - Immunohistochemistry Staining Kit, Host: Rabbit	S-4001
α-CGRP (rat) - RIA Kit (H - sr, pl), Host: Rabbit, Extraction-free	S-2020
α-CGRP (rat) - RIA Kit (R - sr, pl), Host: Rabbit, Extraction-free	S-2088
α-CGRP (rat) - RIA Kit, Host: Rabbit	S-2019
β-CGRP (human) Immunology Products, Host: Rabbit	
β-CGRP (human) - Diluted Antiserum for RIA, Host: Rabbit	T-4241
β-CGRP (human) - Purified Antiserum - IgG, Host: Rabbit	T-4242
β-CGRP (human) - Undiluted Antiserum for Immunohistochemistry, Host: Rabbit	T-4243
β-CGRP (human) - EIA Kit, Host: Rabbit, High Sensitivity	S-1200
β-CGRP (human) - Immunofluorescence Kit, Host: Rabbit	S-3107
β-CGRP (human) - RIA Kit, Host: Rabbit	S-2101
β-CGRP (rat) Immunology Products, Host: Rabbit	
β-CGRP (rat) - RIA Kit, Host: Rabbit	S-2102

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The cover shows human CGRP-1 residues 2-17. The disulfide bond between cysteine residues 2 and 7 is shown at the bottom.
The picture has been kindly provided by Prof. Dr. Iain Campbell, Department of Biochemistry, University of Oxford, U.K.

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