

CGRP在骨稳态中的作用及研究进展

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摘 要

降钙素基因相关肽(Calcitonin gene-related peptide, CGRP)是一种由感觉神经纤维合成释放的生物活性肽类物质之一。通过与CGRP受体复合物结合发挥血管扩张、疼痛传导等生理功能。已证实的降钙素基因相关肽拮抗剂有缓解偏头痛的能力, 是目前偏头痛相关药物开发的热点。但近年来, 降钙素基因相关肽对骨稳态的调节作用已引起广泛关注, 本文就CGRP合成释放及其在骨稳态中的作用的研究进展进行作一综述。

关键词

降钙素基因相关肽, 骨稳态, 神经肽, 成骨诱导分化, 血管成骨耦联

Role and Research Progress of CGRP in Bone Homeostasis

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Abstract

Calcitonin gene-related peptide (CGRP) is one of the bioactive peptides released by sensory nerve fibers. It plays physiological functions such as vascular dilation and pain conduction by binding with the CGRP receptor complex. It has been proven that calcitonin gene-related peptide antagonists have

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the ability to relieve migraine and are currently hot spots for migraine-related drug development. However, in recent years, the regulatory effect of calcitonin gene-related peptides on bone homeostasis has attracted much attention. This paper summarizes the CGRP synthesis and its role in bone homeostasis.

Keywords

Calcitonin Gene-Related Peptide, Bone Homeostasis, Neuropeptide, Osteogenesis Induces Differentiation, Angiogenesis and Osteogenesis Coupling

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1. 引言

降钙素基因相关肽(CGRP)主要由感觉神经纤维表达, 如 C 和 A δ 感觉纤维, 这些纤维于全身广泛分布, 尤其是血管周围[1][2]。此外, 还有研究表明一些非神经性组织也可表达产生 CGRP, 但目前对此知之甚少[3]-[7]。降钙素基因相关肽主要由感觉神经纤维所分泌, 可以调节交感神经[8]。并且 CGRP 还具有强大的血管扩张活性, 当外源性 CGRP 注射到人和动物的皮肤上时, 这种活性得到了明显的体现[9], 并且通过啮齿动物模型上研究同样为 CGRP 的血管保护作用提供了充分的证据。此外, CGRP 所在的感觉纤维也与疼痛过程有关, CGRP 拮抗剂的研究揭示了 CGRP 在偏头痛中所起的关键作用, CGRP 受体拮抗剂可能是偏头痛的治疗靶点[10][11], 目前已开发了相关药物, 如治疗急性偏头痛的 CGRP 受体拮抗剂和用于预防偏头痛的靶向 CGRP 及 CGRP 的单克隆抗体。近年来, 有不少研究发现了 CGRP 通过调控成骨相关细胞、成骨微环境等途径参与骨稳态的维持与调控方面的作用, 本文就这方面进行一综述。

2. CGRP 的概述

2.1. CGRP 的合成与释放

已知降钙素基因相关肽的合成在神经损伤的模型中上调, 比如周围神经切断模型, 并且与在炎症模型中也可发现降钙素基因相关肽的合成增强[12]。有研究表明, 神经生长因子(nerve growth factor, NGF)可能参与了局部 CGRP 的调节释放。NGF 对于感觉神经的生长成熟及神经功能的维持至关重要[13]。NGF 参与神经末梢的感觉神经肽耗尽后, 新肽的合成[14]。有研究通过动物实验证明[15], NGF 参与背根神经节(dorsal root ganglia, DRG)内 CGRP 的产生释放及生理活性[16]。

背根神经节(dorsal root ganglia, DRG)内参与骨合成调节[17][18]。其中与疼痛刺激的传递有关[17]的大部分 DRG 神经元是中小型的, 而小部分 DRG 是初级传入神经元[17]。神经生长因子是 DRG 神经元合成 CGRP 所必不可少的[19]。CGRP 合成后储存在神经源轴突末梢的突触小泡中[20], 当神经元细胞受到热、低 pH 和辣椒素等因素刺激时, 突触小泡会向胞外方向迁移并与神经元胞膜的融合, 释放 CGRP [19]。

2.2. CGRP 的分布与亚型

降钙素基因相关肽与感觉神经元有联系, 尤其是无髓 C 纤维和稀髓 A δ 纤维, 对机械、化学和电刺激等敏感[17][21]-[25], 并且研究发现 CGRP 通常与 SP [26]共定位。此外, CGRP 在运动神经元中也与 ACh 共定位, 并可能参与乙酰胆碱受体的合成[27]。研究证明, 血浆 CGRP 的主要由血管周围神经合成

释放, 其缺乏可能是高血压[7][28]的潜在发病机制。此外, 还认为血浆 CGRP 是血管周围感觉神经元“过度溢出”的结果, 即血浆中 CGRP 的浓度与神经炎症的状态存在相关性, 神经元 CGRP 的释放与局部炎症有关, 并且局部炎症促进 CGRP 的释放[19][28]。

虽然神经是其主要来源, 但 CGRP 也来自于一些非神经细胞, 如免疫细胞、内皮细胞、脂肪细胞[29]-[34]等, 其中非神经源性的 CGRP 可能参与了相关细胞的重要生理功能, TRPV1 的激活参与了其调控释放。还有研究表明, CGRP 可能与参与了内皮祖细胞的衰老调控过程, 因为晚期内皮祖细胞存在着 CGRP 的高表达[33]。

α CGRP 和 β CGRP 是 CGRP 的两种亚型, 也有部分研究称之为 CGRP I 和 CGRP II, 是由人类 11 号染色体上不同位置的两个不同基因合成的[35], 它们具有相似的结构和生物学活性, 但由不同的基因形成[36]。 α CGRP 和 β CGRP 有 90% 的同源性[36]-[38]。 α CGRP 由 CALCI 基因选择性剪接可以产生[38][39], 分布于中枢和外周神经系统[40]; 而 β CGRP 由 CALC II 基因转录合成[39]肽, 主要存在于肠道神经系统[41]的。有研究表明, β CGRP 在肠道中检测到的表达是 α CGRP [42][43]的数倍。在血管系统, 有研究表明 β CGRP 已与 α CGRP 的释放是同步的[44]。但是 β CGRP 的免疫反应性显著低于 α CGRP [42][45], 且研究较少, 故本研究的以下讨论仅指 α CGRP, 除非另有说明。

3. CGRP 对骨稳态的影响

骨是一种高度血管化、神经化的器官, 含有丰富的血管和神经纤维, 并且参与了骨代谢, 被称之为“神经-血管-成骨耦联”[46][47]。在胚胎阶段, 血管神经系统发育早于骨骼系统, 极大的影响着胚胎发育阶段的骨量[48][49]。大神经束与负责骨供给的主要动脉伴行, 通过营养孔进入骨干[50]。骨膜、骨髓腔中也分布着密集的、与毛细血管相近的神经末梢[51]。

骨骼中的大部分神经是感觉神经纤维, 为感觉肽阳性神经[52]。有研究通过动物模型, 敲除 CGRP 后可以发现骨形成的减少和骨量的减少[53], 而成骨细胞特异性的过表达 CGRP 则会导致骨密度的增加[54], 说明 CGRP 影响了骨代谢, 参与了骨稳态的调节[52]-[54]。通过对老年雌性大鼠和绝经后妇女的研究发现, 激素替代疗法可以一定程度增加 CGRP 浓度, 并且与雌激素的调节控制有关[55][56], 说明 CGRP 可能参与了老年性和绝经后骨质疏松的发生。有研究通过骨折模型发现了, 较低浓度的血浆 CGRP 有利于的骨折愈合[57]。还有一些体外实验表明, CGRP 可促进多种细胞的成骨分化并且增强成骨功能, 包括骨膜干细胞(perosteum-derived stem cells, PDSCs)、牙周膜干细胞、骨细胞和骨髓间充质细胞(bone marrow mesenchymal stem cells, BMSCs) [36][58]-[60]等。同时, CGRP 在体内的成骨作用也被证明[36]。关于其具体的分子机制, 有研究表明 CGRP 促进骨合成的代谢主要通过上调 Wnt/ β -catenin 信号通路和 cAMP-CREB 信号通路[60][61]。CGRP 调控以上信号通路的上调的分子基础是 CGRP 与 CGRP 受体的结合, 主要由主要由降钙素受体样受体(calcitonin receptor-like receptor, CRLR)和受体活性修饰蛋白 1 (receptor activity modifying protein 1, RAMP1) [62]组成, 并且在广泛的体细胞细胞膜上表达, 如骨膜来源细胞、牙周膜干细胞、骨髓间充质细胞、和成骨细胞等[61][63][64], 这也是 CGRP 发挥骨代谢作用的基础。

3.1. CGRP 与成骨前体细胞及成骨细胞

BMSC 作为主要的成骨前体细胞, 是成骨细胞的主要来源[65]。CGRP 可以促进大鼠 BMSCs 的增殖和成骨分化[66]。CGRP 敲基因小鼠骨形成率降低, 骨质减少[52][53], 骨骼和牙胚的发育受影响[49]。而 CGRP 过表达小鼠骨形成率增加, 骨小梁密度和体积增加[53]。CGRP 通过多个信号通路参与调控骨生成[67]。有研究通过体外的人类成骨前体细胞培养实验探究具体的分子机制, 一方面, 通过提高 cAMP 水平[61], 另一方面上调 Wnt/ β -catenin 信号通路, 提高了 BMP-2 的表达[68], 抑制 BMSCs 的凋亡并且促进

成骨分化。通过加入外源性的 CGRP, BMSCs 的骨集落形成数目和大小均增加, 并且与 CGRP 外源性加入的浓度呈正相关[69]。大鼠闭合性股骨骨折早期, CGRP 阳性的神经纤维在局新生, 提高损伤局部的 CGRP 浓度, 促进了修复性骨组织形成和改建[70]。

3.2. CGRP 与破骨前体细胞和破骨细胞

破骨细胞形成及其骨吸收功能同样受 CGRP 的影响与调控。CGRP 可抑制骨髓来源巨噬细胞的趋化融合及破骨分化[71], 在动物实验中表现为牙周炎模型的小鼠牙槽骨吸收减少, 去卵巢大鼠的骨质疏松程度减轻[58]。CGRP 的受体复合物, 即 CRL/RAMP1 二聚体, 是 CGRP 发挥破骨调控功能的分子基础, 完整表达在小鼠骨髓来源巨噬细胞(bone marrow macrophages, BMMs)和破骨细胞(Osteoclasts)的细胞膜上。其潜在的分子机制为上调骨保护素(osteoprotegerin, OPG), 并且下调 RANKL (receptor activator of nuclear factor kappa-B ligand, RANKL)表达[72], 抑制 BMMs 的破骨分化和骨吸收的生物活性。NF- κ B 受体活化因子配体 (receptor activator of nuclear factor kappa-B ligand, RANK)与 OPG、RANKL 共同参与着破骨细胞的分化调控[73]。RANK 与 RANKL 结合激活了破骨前体细胞, 如骨髓来源巨噬细胞、单核细胞的破骨分化, 而 CGRP 则通过促进 OPG 表达导致其对 RANK/RANKL 结合的拮抗增强[74], 最终造成了破骨分化的过程受阻[58] [75]。有研究推测 CGRP 可增加破骨前细胞的比例[59] [76]。

4. CGRP 对成血管的影响

CGRP 是一种有效的血管扩张剂, 发挥血管扩张作用, 保护器官免受缺血损伤[4] [77]。还可以通过内皮细胞、内皮祖细胞表达的 CGRP 受体结合, 促进内皮细胞、内皮祖细胞的增殖, 并且抑制其凋亡[78] [79]。其潜在分子机制为 CGRP 激活腺苷环化酶后使胞内 cAMP 含量增高激活蛋白激酶 A, 导致内皮细胞内一氧化氮和钙离子的滞留[4]。有研究表明, 血管内皮生长因子(vascular endothelial growth factor, VEGF)是诱导血管生成的主要趋化因子[80] [81]。CGRP 缺失可导致 CD31 和 VEGF 的表达降低, 延缓小鼠创面愈合[4]。在大鼠膝关节模型中, 关节内注射 CGRP 可通过激活 CGRP 受体增加内皮细胞的增殖直接刺激体内血管生成[82], 而抑制 CRLR/RAMP1 可减少内皮细胞的增殖[83]。有研究通过体外实验说明, CGRP 通过促进 VEGF 的表达, 促进了内皮细胞的增殖和血管形成功能[82] [84] [85]。这一机制在肿瘤相关血管生成中得到了进一步验证[85]。血管生成和成骨作用的耦合越来越受到人们的关注[46] [47] [86] [87], 一方面, 血管内皮生长因子是不可或缺的桥梁。另一方面, 血管扩张效应和增加的血管生成可能影响血液流动, 也有助于成骨[88] [89]。在成骨细胞和内皮细胞共培养体系中, 与单纯成骨细胞相比, CGRP 可以进一步促进成骨作用, 这表明 CGRP 的对骨稳态的调节作用不仅是直接的, 而且还有间接的, 即通过对内皮细胞的作用[90]。另外, H 型血管对偶联成骨具有重要作用, 但 CGRP 是否能唯一地调节 H 型血管的形成还有待检验。考虑到血管内皮生长因子和 H 型血管之间的已知作用[91], CGRP 和 CD31+血管之间的正相关[92], 这一方面有很大的研究潜力。

5. 结语

骨稳态是由多种细胞事件共同维持的, 包括成骨、破骨细胞形成、血管生成、脂肪生成和骨免疫等。血管为骨骼生长和重塑所必须的持续提供氧气、营养物质和骨祖细胞, 是维持健康骨组织的关键[93]。CGRP 不仅通过影响成骨、破骨细胞形成, 还通过上调内皮细胞中一些血管生成标志物的表达, 在促进成血管功能中发挥作用[94]。综上所述, CGRP 可能是神经 - 血管 - 骨网络的上游调节因子, 但其在成骨成血管中的作用的现有信息有限。进一步研究其分子调节机制与相互作用可能有助于深入了解其 CGRP 的骨稳态调节机制, 为骨组织修复提供更好的策略。

参考文献

- [1] Maggi, C.A. (1995) Tachykinins and Calcitonin Gene-Related Peptide (CGRP) as Co-Transmitters Released from Peripheral Endings of Sensory Nerves. *Progress in Neurobiology*, **45**, 1-98. [https://doi.org/10.1016/0301-0082\(94\)e0017-b](https://doi.org/10.1016/0301-0082(94)e0017-b)
- [2] Rosenfeld, M.G., Mermod, J., Amara, S.G., Swanson, L.W., Sawchenko, P.E., Rivier, J., *et al.* (1983) Production of a Novel Neuropeptide Encoded by the Calcitonin Gene via Tissue-Specific RNA Processing. *Nature*, **304**, 129-135. <https://doi.org/10.1038/304129a0>
- [3] Bell, D. and McDermott, B.J. (1996) Calcitonin Gene-Related Peptide in the Cardiovascular System: Characterization of Receptor Populations and Their (Patho)Physiological Significance. *Pharmacological Reviews*, **48**, 253-288.
- [4] Brain, S.D. and Grant, A.D. (2004) Vascular Actions of Calcitonin Gene-Related Peptide and Adrenomedullin. *Physiological Reviews*, **84**, 903-934. <https://doi.org/10.1152/physrev.00037.2003>
- [5] Deng, P. and Li, Y. (2005) Calcitonin Gene-Related Peptide and Hypertension. *Peptides*, **26**, 1676-1685. <https://doi.org/10.1016/j.peptides.2005.02.002>
- [6] Watson, R.E., Supowit, S.C., Zhao, H., Katki, K.A. and DiPette, D.J. (2002) Role of Sensory Nervous System Vasoactive Peptides in Hypertension. *Brazilian Journal of Medical and Biological Research*, **35**, 1033-1045. <https://doi.org/10.1590/s0100-879x2002000900004>
- [7] Wimalawansa, S.J. (1996) Calcitonin Gene-Related Peptide and Its Receptors: Molecular Genetics, Physiology, Pathophysiology, and Therapeutic Potentials. *Endocrine Reviews*, **17**, 533-585. <https://doi.org/10.1210/edrv-17-5-533>
- [8] Fisher, L.A., Kikkawa, D.O., Rivier, J.E., Amara, S.G., Evans, R.M., Rosenfeld, M.G., *et al.* (1983) Stimulation of Noradrenergic Sympathetic Outflow by Calcitonin Gene-Related Peptide. *Nature*, **305**, 534-536. <https://doi.org/10.1038/305534a0>
- [9] Brain, S.D., Williams, T.J., Tippins, J.R., Morris, H.R. and MacIntyre, I. (1985) Calcitonin Gene-Related Peptide Is a Potent Vasodilator. *Nature*, **313**, 54-56. <https://doi.org/10.1038/313054a0>
- [10] Negro, A., Lionetto, L., Simmaco, M. and Martelletti, P. (2012) CGRP Receptor Antagonists: An Expanding Drug Class for Acute Migraine? *Expert Opinion on Investigational Drugs*, **21**, 807-818. <https://doi.org/10.1517/13543784.2012.681044>
- [11] Olesen, J., Diener, H., Husstedt, I.W., Goadsby, P.J., Hall, D., Meier, U., *et al.* (2004) Calcitonin Gene-Related Peptide Receptor Antagonist BIBN 4096 BS for the Acute Treatment of Migraine. *New England Journal of Medicine*, **350**, 1104-1110. <https://doi.org/10.1056/nejmoa030505>
- [12] Donnerer, J. and Stein, C. (1992) Evidence for an Increase in the Release of CGRP from Sensory Nerves during Inflammation. *Annals of the New York Academy of Sciences*, **657**, 505-506. <https://doi.org/10.1111/j.1749-6632.1992.tb22814.x>
- [13] Terenghi, G. (1999) Peripheral Nerve Regeneration and Neurotrophic Factors. *Journal of Anatomy*, **194**, 1-14. <https://doi.org/10.1046/j.1469-7580.1999.19410001.x>
- [14] Donnerer, J., Schuligoi, R. and Stein, C. (1992) Increased Content and Transport of Substance P and Calcitonin Gene-Related Peptide in Sensory Nerves Innervating Inflamed Tissue: Evidence for a Regulatory Function of Nerve Growth Factor *in Vivo*. *Neuroscience*, **49**, 693-698. [https://doi.org/10.1016/0306-4522\(92\)90237-v](https://doi.org/10.1016/0306-4522(92)90237-v)
- [15] Supowit, S.C., Zhao, H. and DiPette, D.J. (2001) Nerve Growth Factor Enhances Calcitonin Gene-Related Peptide Expression in the Spontaneously Hypertensive Rat. *Hypertension*, **37**, 728-732. <https://doi.org/10.1161/01.hyp.37.2.728>
- [16] Salio, C., Averill, S., Priestley, J.V. and Merighi, A. (2007) Costorage of BDNF and Neuropeptides within Individual Dense-Core Vesicles in Central and Peripheral Neurons. *Developmental Neurobiology*, **67**, 326-338. <https://doi.org/10.1002/dneu.20358>
- [17] Hoheisel, U., Mense, S. and Scherotzke, R. (1994) Calcitonin Gene-Related Peptide-Immunoreactivity in Functionally Identified Primary Afferent Neurons in the Rat. *Anatomy and Embryology*, **189**, 41-49. <https://doi.org/10.1007/bf00193128>
- [18] Lawson, S.N., Crepps, B. and Perl, E.R. (2002) Calcitonin Gene-Related Peptide Immunoreactivity and Afferent Receptive Properties of Dorsal Root Ganglion Neurons in Guinea-Pigs. *The Journal of Physiology*, **540**, 989-1002. <https://doi.org/10.1113/jphysiol.2001.013086>
- [19] Russell, F.A., King, R., Smillie, S.-J., Kodji, X. and Brain, S.D. (2014) Calcitonin Gene-Related Peptide: Physiology and Pathophysiology. *Physiological Reviews*, **94**, 1099-1142. <https://doi.org/10.1152/physrev.00034.2013>
- [20] Matteoli, M., Haimann, C., Torri-Tarelli, F., Polak, J.M., Ceccarelli, B. and de Camilli, P. (1988) Differential Effect of Alpha-Latrotoxin on Exocytosis from Small Synaptic Vesicles and from Large Dense-Core Vesicles Containing Calcitonin Gene-Related Peptide at the Frog Neuromuscular Junction. *Proceedings of the National Academy of Sciences*, **85**, 7366-7370. <https://doi.org/10.1073/pnas.85.19.7366>
- [21] Li, J., Kreibergs, A., Bergström, J., Stark, A. and Ahmed, M. (2007) Site-Specific CGRP Innervation Coincides with

- Bone Formation during Fracture Healing and Modeling: A Study in Rat Angulated Tibia. *Journal of Orthopaedic Research*, **25**, 1204-1212. <https://doi.org/10.1002/jor.20406>
- [22] Lau, Y.-C., Qian, X., Po, K.-T., Li, L.-M. and Guo, X. (2014) Electrical Stimulation at the Dorsal Root Ganglion Preserves Trabecular Bone Mass and Microarchitecture of the Tibia in Hindlimb-Unloaded Rats. *Osteoporosis International*, **26**, 481-488. <https://doi.org/10.1007/s00198-014-2866-3>
- [23] Lau, Y., Lai, Y., Po, K., Qian, X., Hao, H., Zhao, H., *et al.* (2014) Dorsal Root Ganglion Electrical Stimulation Promoted Intertransverse Process Spinal Fusion without Decortications and Bone Grafting: A Proof-of-Concept Study. *The Spine Journal*, **14**, 2472-2478. <https://doi.org/10.1016/j.spinee.2014.04.001>
- [24] Yuen-Chi Lau, R., Qian, X., Po, K., Li, L. and Guo, X. (2017) Response of Rat Tibia to Prolonged Unloading under the Influence of Electrical Stimulation at the Dorsal Root Ganglion. *Neuromodulation: Technology at the Neural Interface*, **20**, 284-289. <https://doi.org/10.1111/ner.12488>
- [25] Naot, D., Musson, D.S. and Cornish, J. (2019) The Activity of Peptides of the Calcitonin Family in Bone. *Physiological Reviews*, **99**, 781-805. <https://doi.org/10.1152/physrev.00066.2017>
- [26] Gibbins, I.L., Furness, J.B., Costa, M., MacIntyre, I., Hillyard, C.J. and Girgis, S. (1985) Co-Localization of Calcitonin Gene-Related Peptide-Like Immunoreactivity with Substance P in Cutaneous, Vascular and Visceral Sensory Neurons of Guinea Pigs. *Neuroscience Letters*, **57**, 125-130. [https://doi.org/10.1016/0304-3940\(85\)90050-3](https://doi.org/10.1016/0304-3940(85)90050-3)
- [27] New, H.V. and Mudge, A.W. (1986) Calcitonin Gene-Related Peptide Regulates Muscle Acetylcholine Receptor Synthesis. *Nature*, **323**, 809-811. <https://doi.org/10.1038/323809a0>
- [28] Smillie, S. and Brain, S.D. (2011) Calcitonin Gene-Related Peptide (CGRP) and Its Role in Hypertension. *Neuropeptides*, **45**, 93-104. <https://doi.org/10.1016/j.npep.2010.12.002>
- [29] Cai, W., Bodin, P., Loesch, A., Sexton, A. and Burnstock, G. (1993) Endothelium of Human Umbilical Blood Vessels: Ultrastructural Immunolocalization of Neuropeptides. *Journal of Vascular Research*, **30**, 348-355. <https://doi.org/10.1159/000159017>
- [30] Gupta, S., Mehrotra, S., Villalón, C., de Vries, R., Garrelds, I., Saxena, P., *et al.* (2006) Effects of Female Sex Hormones on Responses to CGRP, Acetylcholine, and 5-HT in Rat Isolated Arteries. *Headache: The Journal of Head and Face Pain*, **47**, 564-575. <https://doi.org/10.1111/j.1526-4610.2006.00596.x>
- [31] Ozaka, T., Doi, Y., Kayashima, K. and Fujimoto, S. (1997) Weibel-Palade Bodies as a Storage Site of Calcitonin Gene-Related Peptide and Endothelin-1 in Blood Vessels of the Rat Carotid Body. *The Anatomical Record*, **247**, 388-394. [https://doi.org/10.1002/\(sici\)1097-0185\(199703\)247:3<388::aid-ar10>3.0.co;2-l](https://doi.org/10.1002/(sici)1097-0185(199703)247:3<388::aid-ar10>3.0.co;2-l)
- [32] Hu, R., Li, Y. and Li, X. (2016) An Overview of Non-Neural Sources of Calcitonin Gene-Related Peptide. *Current Medicinal Chemistry*, **23**, 763-773. <https://doi.org/10.2174/0929867323666160210125416>
- [33] Fang, L., Chen, M., Xiao, Z., Liu, Y., Yu, G., Chen, X., *et al.* (2011) Calcitonin Gene-Related Peptide Released from Endothelial Progenitor Cells Inhibits the Proliferation of Rat Vascular Smooth Muscle Cells Induced by Angiotensin II. *Molecular and Cellular Biochemistry*, **355**, 99-108. <https://doi.org/10.1007/s11010-011-0843-0>
- [34] Ma, W., Dumont, Y., Vercauteren, F. and Quirion, R. (2010) Lipopolysaccharide Induces Calcitonin Gene-Related Peptide in the RAW264.7 Macrophage Cell Line. *Immunology*, **130**, 399-409. <https://doi.org/10.1111/j.1365-2567.2009.03239.x>
- [35] Wimalawansa, S.J., Morris, H.R., Etienne, A., Blench, I., Panico, M. and MacIntyre, I. (1990) Isolation, Purification and Characterization of B-hCGRP from Human Spinal Cord. *Biochemical and Biophysical Research Communications*, **167**, 993-1000. [https://doi.org/10.1016/0006-291x\(90\)90621-s](https://doi.org/10.1016/0006-291x(90)90621-s)
- [36] Amara, S.G., Arriza, J.L., Leff, S.E., Swanson, L.W., Evans, R.M. and Rosenfeld, M.G. (1985) Expression in Brain of a Messenger RNA Encoding a Novel Neuropeptide Homologous to Calcitonin Gene-Related Peptide. *Science*, **229**, 1094-1097. <https://doi.org/10.1126/science.2994212>
- [37] Morris, H.R., Panico, M., Etienne, T., Tippins, J., Girgis, S.I. and MacIntyre, I. (1984) Isolation and Characterization of Human Calcitonin Gene-Related Peptide. *Nature*, **308**, 746-748. <https://doi.org/10.1038/308746a0>
- [38] Steenbergh, P.M., Höppener, J.W.M., Zandberg, J., Visser, A., Lips, C.J.M. and Jansz, H.S. (1986) Structure and Expression of the Human Calcitonin/CGRP Genes. *FEBS Letters*, **209**, 97-103. [https://doi.org/10.1016/0014-5793\(86\)81091-2](https://doi.org/10.1016/0014-5793(86)81091-2)
- [39] Alevizaki, M., Shiraishi, A., Rassool, F.V., Ferner, G.J.M., MacIntyre, I. and Legon, S. (1986) The Calcitonin-Like Sequence of the β CGRP Gene. *FEBS Letters*, **206**, 47-52. [https://doi.org/10.1016/0014-5793\(86\)81338-2](https://doi.org/10.1016/0014-5793(86)81338-2)
- [40] Aiyar N., Daines R.A., Disa J., *et al.* (2001) Pharmacology of SB-273779, a Nonpeptide Calcitonin Gene-Related Peptide 1 Receptor Antagonist. *Journal of Pharmacology and Experimental Therapeutics*, **296**, 768-775.
- [41] Mulderry, P.K., Ghatei, M.A., Bishop, A.E., Allen, Y.S., Polak, J.M. and Bloom, S.R. (1985) Distribution and Chromatographic Characterisation of CGRP-Like Immunoreactivity in the Brain and Gut of the Rat. *Regulatory Peptides*, **12**,

- 133-143. [https://doi.org/10.1016/0167-0115\(85\)90194-6](https://doi.org/10.1016/0167-0115(85)90194-6)
- [42] Mulderry, P.K., Ghatki, M.A., Spokks, R.A., Jonhs, P.M., Pierson, A.M., Hamid, Q.A., *et al.* (1988) Differential Expression of α -CGRP and β -CGRP by Primary Sensory Neurons and Enteric Autonomic Neurons of the Rat. *Neuroscience*, **25**, 195-205. [https://doi.org/10.1016/0306-4522\(88\)90018-8](https://doi.org/10.1016/0306-4522(88)90018-8)
- [43] Somasundaram, C., Diz, D.I., Coleman, T. and Bukoski, R.D. (2006) Adventitial Neuronal Somata. *Journal of Vascular Research*, **43**, 278-288. <https://doi.org/10.1159/000092765>
- [44] Li, D., Chen, B., Peng, J., Zhang, Y., Li, X., Yuan, Q., *et al.* (2009) Role of Anandamide Transporter in Regulating Calcitonin Gene-Related Peptide Production and Blood Pressure in Hypertension. *Journal of Hypertension*, **27**, 1224-1232. <https://doi.org/10.1097/hjh.0b013e328329bbd7>
- [45] Schütz, B., Mauer, D., Salmon, A., Changeux, J. and Zimmer, A. (2004) Analysis of the Cellular Expression Pattern of β -CGRP in α -CGRP-Deficient Mice. *Journal of Comparative Neurology*, **476**, 32-43. <https://doi.org/10.1002/cne.20211>
- [46] Marrella, A., Lee, T.Y., Lee, D.H., Karuthedom, S., Sylva, D., Chawla, A., *et al.* (2018) Engineering Vascularized and Innervated Bone Biomaterials for Improved Skeletal Tissue Regeneration. *Materials Today*, **21**, 362-376. <https://doi.org/10.1016/j.mattod.2017.10.005>
- [47] Kusumbe, A.P., Ramasamy, S.K. and Adams, R.H. (2014) Coupling of Angiogenesis and Osteogenesis by a Specific Vessel Subtype in Bone. *Nature*, **507**, 323-328. <https://doi.org/10.1038/nature13145>
- [48] Ramasamy, S.K., Kusumbe, A.P., Wang, L. and Adams, R.H. (2014) Endothelial Notch Activity Promotes Angiogenesis and Osteogenesis in Bone. *Nature*, **507**, 376-380. <https://doi.org/10.1038/nature13146>
- [49] Maeda, Y., Miwa, Y. and Sato, I. (2019) Distribution of the Neuropeptide Calcitonin Gene-Related Peptide-A of Tooth Germ during Formation of the Mouse Mandible. *Annals of Anatomy—Anatomischer Anzeiger*, **221**, 38-47. <https://doi.org/10.1016/j.aanat.2018.09.001>
- [50] Lerner, U.H. (2006) Deletions of Genes Encoding Calcitonin/ α -CGRP, Amylin and Calcitonin Receptor Have Given New and Unexpected Insights into the Function of Calcitonin Receptors and Calcitonin Receptor-Like Receptors in Bone. *Journal of Musculoskeletal and Neuronal Interactions*, **6**, 87-95.
- [51] Chen, B., Pei, G.X., Jin, D., *et al.* (2007) Distribution and Property of Nerve Fibers in Human Long Bone Tissue. *Chinese Journal of Traumatology*, **10**, 3-9.
- [52] Nencini, S. and Ivanusic, J.J. (2016) The Physiology of Bone Pain. How Much Do We Really Know? *Frontiers in Physiology*, **7**, Article 157. <https://doi.org/10.3389/fphys.2016.00157>
- [53] Schinke, T., Liese, S., Priemel, M., Haberland, M., Schilling, A.F., Catala-Lehnen, P., *et al.* (2004) Decreased Bone Formation and Osteopenia in Mice Lacking A-Calcitonin Gene-Related Peptide. *Journal of Bone and Mineral Research*, **19**, 2049-2056. <https://doi.org/10.1359/jbmr.040915>
- [54] Ballica, R., Valentijn, K., Khachatryan, A., Guerder, S., Kapadia, S., Gundberg, C., *et al.* (1999) Targeted Expression of Calcitonin Gene-Related Peptide to Osteoblasts Increases Bone Density in Mice. *Journal of Bone and Mineral Research*, **14**, 1067-1074. <https://doi.org/10.1359/jbmr.1999.14.7.1067>
- [55] Petraglia, V., degli Uberti, E.C., *et al.* (1996) Changes of Plasma Calcitonin Gene-related Peptide Levels in Postmenopausal Women. *American Journal of Obstetrics and Gynecology*, **175**, 638-642. <https://doi.org/10.1053/ob.1996.v175.a74287>
- [56] Peroni, R.N., Abramoff, T., Neuman, I., Podestá, E.J. and Adler-Graschinsky, E. (2012) Phytoestrogens Enhance the Vascular Actions of the Endocannabinoid Anandamide in Mesenteric Beds of Female Rats. *International Journal of Hypertension*, **2012**, 1-10. <https://doi.org/10.1155/2012/647856>
- [57] Gao, F., Lv, T., Zhou, J. and Qin, X. (2018) Effects of Obesity on the Healing of Bone Fracture in Mice. *Journal of Orthopaedic Surgery and Research*, **13**, Article No. 145. <https://doi.org/10.1186/s13018-018-0837-7>
- [58] He, H., Chai, J., Zhang, S., Ding, L., Yan, P., Du, W., *et al.* (2016) CGRP May Regulate Bone Metabolism through Stimulating Osteoblast Differentiation and Inhibiting Osteoclast Formation. *Molecular Medicine Reports*, **13**, 3977-3984. <https://doi.org/10.3892/mmr.2016.5023>
- [59] Wang, L., Shi, X., Zhao, R., Halloran, B.P., Clark, D.J., Jacobs, C.R., *et al.* (2010) Calcitonin-Gen-Related Peptide Stimulates Stromal Cell Osteogenic Differentiation and Inhibits RANKL Induced NF-Kb Activation, Osteoclastogenesis and Bone Resorption. *Bone*, **46**, 1369-1379. <https://doi.org/10.1016/j.bone.2009.11.029>
- [60] Zhou, R., Yuan, Z., Liu, J. and Liu, J. (2016) Calcitonin Gene-Related Peptide Promotes the Expression of Osteoblastic Genes and Activates the WNT Signal Transduction Pathway in Bone Marrow Stromal Stem Cells. *Molecular Medicine Reports*, **13**, 4689-4696. <https://doi.org/10.3892/mmr.2016.5117>
- [61] Zhang, Y., Xu, J., Ruan, Y.C., Yu, M.K., O'Laughlin, M., Wise, H., *et al.* (2016) Implant-Derived Magnesium Induces Local Neuronal Production of CGRP to Improve Bone-Fracture Healing in Rats. *Nature Medicine*, **22**, 1160-1169. <https://doi.org/10.1038/nm.4162>

- [62] Moad, H.E. and Pioszak, A.A. (2013) Selective CGRP and Adrenomedullin Peptide Binding by Tethered Ramp-Calci-
tonin Receptor-Like Receptor Extracellular Domain Fusion Proteins. *Protein Science*, **22**, 1775-1785.
<https://doi.org/10.1002/pro.2377>
- [63] Villa, I., Mrak, E., Rubinacci, A., Ravasi, F. and Guidobono, F. (2006) CGRP Inhibits Osteoprotegerin Production in
Human Osteoblast-Like Cells via cAMP/PKA-Dependent Pathway. *American Journal of Physiology-Cell Physiology*,
291, C529-C537. <https://doi.org/10.1152/ajpcell.00354.2005>
- [64] Uzan, B., de Vernejoul, M. and Cressent, M. (2004) Ramps and CRLR Expressions in Osteoblastic Cells after Dexamethasone Treatment. *Biochemical and Biophysical Research Communications*, **321**, 802-808.
<https://doi.org/10.1016/j.bbrc.2004.07.037>
- [65] Wang, L., Zhao, R., Shi, X., Wei, T., Halloran, B.P., Clark, D.J., *et al.* (2009) Substance P Stimulates Bone Marrow
Stromal Cell Osteogenic Activity, Osteoclast Differentiation, and Resorption Activity *in Vitro*. *Bone*, **45**, 309-320.
<https://doi.org/10.1016/j.bone.2009.04.203>
- [66] Liang, W., Zhuo, X., Tang, Z., Wei, X. and Li, B. (2015) Calcitonin Gene-Related Peptide Stimulates Proliferation and
Osteogenic Differentiation of Osteoporotic Rat-Derived Bone Mesenchymal Stem Cells. *Molecular and Cellular Biochemistry*, **402**, 101-110. <https://doi.org/10.1007/s11010-014-2318-6>
- [67] Chen, W., Ma, L., Sun, W., Xiao, W., Guo, H., Xiu, J. and Jiang, X. (2024) CGRP Promotes Osteogenic Differentiation
by Regulating Macrophage M2 Polarization through HDAC6/AKAP12 Signaling Pathway. *Regenerative Medicine*, 1-
13. <https://doi.org/10.1080/17460751.2024.2370697>
- [68] Vignery, A. and McCarthy, T.L. (1996) The Neuropeptide Calcitonin Gene-Related Peptide Stimulates Insulin-Like
Growth Factor I Production by Primary Fetal Rat Osteoblasts. *Bone*, **18**, 331-335.
[https://doi.org/10.1016/8756-3282\(96\)00017-8](https://doi.org/10.1016/8756-3282(96)00017-8)
- [69] Tian, G., Zhang, G. and Tan, Y. (2013) Calcitonin Gene-Related Peptide Stimulates BMP-2 Expression and the Differ-
entiation of Human Osteoblast-Like Cells *in Vitro*. *Acta Pharmacologica Sinica*, **34**, 1467-1474.
<https://doi.org/10.1038/aps.2013.41>
- [70] Shih, C. and Bernard, G.W. (1997) Calcitonin Gene Related Peptide Enhances Bone Colony Development *in Vitro*.
Clinical Orthopaedics and Related Research, **334**, 335-344. <https://doi.org/10.1097/00003086-199701000-00043>
- [71] Sample, S.J., Hao, Z., Wilson, A.P. and Muir, P. (2011) Role of Calcitonin Gene-Related Peptide in Bone Repair After
Cyclic Fatigue Loading. *PLoS ONE*, **6**, e20386. <https://doi.org/10.1371/journal.pone.0020386>
- [72] Valentijn, K., Gutow, A.P., Troiano, N., Gundberg, C., Gilligan, J.P. and Vignery, A. (1997) Effects of Calcitonin Gene-
Related Peptide on Bone Turnover in Ovariectomized Rats. *Bone*, **21**, 269-274.
[https://doi.org/10.1016/s8756-3282\(97\)00142-7](https://doi.org/10.1016/s8756-3282(97)00142-7)
- [73] Yoo, Y., Kwag, J., Kim, K. and Kim, C. (2014) Effects of Neuropeptides and Mechanical Loading on Bone Cell Resorp-
tion *in Vitro*. *International Journal of Molecular Sciences*, **15**, 5874-5883. <https://doi.org/10.3390/ijms15045874>
- [74] Khosla, S. (2001) Minireview: The OPG/RANKL/RANK System. *Endocrinology*, **142**, 5050-5055.
<https://doi.org/10.1210/en.142.12.5050>
- [75] Maruyama, K., Takayama, Y., Kondo, T., Ishibashi, K., Sahoo, B.R., Kanemaru, H., *et al.* (2017) Nociceptors Boost the
Resolution of Fungal Osteoinflammation via the TRP Channel-CGRP-Jdp2 Axis. *Cell Reports*, **19**, 2730-2742.
<https://doi.org/10.1016/j.celrep.2017.06.002>
- [76] Xie, H., Cui, Z., Wang, L., Xia, Z., Hu, Y., Xian, L., *et al.* (2014) PDGF-BB Secreted by Preosteoclasts Induces Angio-
genesis during Coupling with Osteogenesis. *Nature Medicine*, **20**, 1270-1278. <https://doi.org/10.1038/nm.3668>
- [77] Aubdool, A.A., Thakore, P., Argunhan, F., Smillie, S., Schnelle, M., Srivastava, S., *et al.* (2017) A Novel A-Calcitonin
Gene-Related Peptide Analogue Protects against End-Organ Damage in Experimental Hypertension, Cardiac Hypertro-
phy, and Heart Failure. *Circulation*, **136**, 367-383. <https://doi.org/10.1161/circulationaha.117.028388>
- [78] Zhang, Q., Guo, Y., Chen, H., Jiang, Y., Tang, H., Gong, P., *et al.* (2018) The Influence of Receptor Activity-Modifying
Protein-1 Overexpression on Angiogenesis in Mouse Brain Capillary Endothelial Cells. *Journal of Cellular Biochemistry*,
120, 10087-10096. <https://doi.org/10.1002/jcb.28292>
- [79] Wu, J., Liu, S., Wang, Z., Ma, S., Meng, H. and Hu, J. (2018) Calcitonin Gene-Related Peptide Promotes Proliferation
and Inhibits Apoptosis in Endothelial Progenitor Cells via Inhibiting MAPK Signaling. *Proteome Science*, **16**, Article
No. 18. <https://doi.org/10.1186/s12953-018-0146-4>
- [80] Maeda, Y., Miwa, Y. and Sato, I. (2017) Expression of CGRP, Vasculogenesis and Osteogenesis Associated Mrnas in
the Developing Mouse Mandible and Tibia. *European Journal of Histochemistry*, **61**, Article 2750.
<https://doi.org/10.4081/ejh.2017.2750>
- [81] Barleon, B., Sozzani, S., Zhou, D., Weich, H., Mantovani, A. and Marme, D. (1996) Migration of Human Monocytes in
Response to Vascular Endothelial Growth Factor (VEGF) Is Mediated via the VEGF Receptor Flt-1. *Blood*, **87**, 3336-
3343. <https://doi.org/10.1182/blood.v87.8.3336.bloodjournal8783336>

- [82] Mapp, P.I., McWilliams, D.F., Turley, M.J., Hargin, E. and Walsh, D.A. (2012) A Role for the Sensory Neuropeptide Calcitonin Gene-Related Peptide in Endothelial Cell Proliferation *in Vivo*. *British Journal of Pharmacology*, **166**, 1261-1271. <https://doi.org/10.1111/j.1476-5381.2012.01848.x>
- [83] Walsh, D.A., Mapp, P.I. and Kelly, S. (2015) Calcitonin Gene-Related Peptide in the Joint: Contributions to Pain and Inflammation. *British Journal of Clinical Pharmacology*, **80**, 965-978. <https://doi.org/10.1111/bcp.12669>
- [84] Zheng, S., Li, W., Xu, M., Bai, X., Zhou, Z., Han, J., *et al.* (2010) Calcitonin Gene-Related Peptide Promotes Angiogenesis via Amp-Activated Protein Kinase. *American Journal of Physiology-Cell Physiology*, **299**, C1485-C1492. <https://doi.org/10.1152/ajpcell.00173.2010>
- [85] Toda, M., Suzuki, T., Hosono, K., Hayashi, I., Hashiba, S., Onuma, Y., *et al.* (2008) Neuronal System-Dependent Facilitation of Tumor Angiogenesis and Tumor Growth by Calcitonin Gene-Related Peptide. *Proceedings of the National Academy of Sciences*, **105**, 13550-13555. <https://doi.org/10.1073/pnas.0800767105>
- [86] Xu, R., Yallowitz, A., Qin, A., Wu, Z., Shin, D.Y., Kim, J., *et al.* (2018) Targeting Skeletal Endothelium to Ameliorate Bone Loss. *Nature Medicine*, **24**, 823-833. <https://doi.org/10.1038/s41591-018-0020-z>
- [87] Liu, W.C., Chen, S., Zheng, L. and Qin, L. (2017) Angiogenesis Assays for the Evaluation of Angiogenic Properties of Orthopaedic Biomaterials—A General Review. *Advanced Healthcare Materials*, **6**, Article 1600434. <https://doi.org/10.1002/adhm.201600434>
- [88] Ramasamy, S.K., Kusumbe, A.P., Schiller, M., Zeuschner, D., Bixel, M.G., Milia, C., *et al.* (2016) Blood Flow Controls Bone Vascular Function and Osteogenesis. *Nature Communications*, **7**, Article No. 13601. <https://doi.org/10.1038/ncomms13601>
- [89] Tomlinson, R.E. and Silva, M.J. (2013) Skeletal Blood Flow in Bone Repair and Maintenance. *Bone Research*, **1**, 311-322. <https://doi.org/10.4248/br201304002>
- [90] Bo, Y., Yan, L., Gang, Z., Tao, L. and Yinghui, T. (2012) Effect of Calcitonin Gene-Related Peptide on Osteoblast Differentiation in an Osteoblast and Endothelial Cell Co-Culture System. *Cell Biology International*, **36**, 909-915. <https://doi.org/10.1042/cbi20110562>
- [91] Ji, G., Xu, R., Niu, Y., Li, N., Ivashkiv, L., Bostrom, M.P.G., *et al.* (2019) Vascular Endothelial Growth Factor Pathway Promotes Osseointegration and CD31hiEMCNhi Endothelium Expansion in a Mouse Tibial Implant Model. *The Bone & Joint Journal*, **101**, 108-114. <https://doi.org/10.1302/0301-620x.101b7.bjj-2018-1473.r1>
- [92] Ohno, T., Hattori, Y., Komine, R., Ae, T., Mizuguchi, S., Arai, K., *et al.* (2008) Roles of Calcitonin Gene-Related Peptide in Maintenance of Gastric Mucosal Integrity and in Enhancement of Ulcer Healing and Angiogenesis. *Gastroenterology*, **134**, 215-225. <https://doi.org/10.1053/j.gastro.2007.10.001>
- [93] Schmid, J., Wallkamm, B., Hämmerle, C.H.F., Gogolewski, S. and Lang, N.P. (1997) The Significance of Angiogenesis in Guided Bone Regeneration. A Case Report of a Rabbit Experiment. *Clinical Oral Implants Research*, **8**, 244-248. <https://doi.org/10.1034/j.1600-0501.1997.080311.x>
- [94] Leroux, A., Paiva dos Santos, B., Leng, J., Oliveira, H. and Amédée, J. (2020) Sensory Neurons from Dorsal Root Ganglia Regulate Endothelial Cell Function in Extracellular Matrix Remodelling. *Cell Communication and Signaling*, **18**, Article No. 162. <https://doi.org/10.1186/s12964-020-00656-0>