LETTER TO THE EDITOR

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RE: "The Influence of RAMP1 Overexpression on CGRP-Induced Osteogenic Differentiation in MG-63 Cells In Vitro: An Experimental Study"

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TO THE EDITOR:

In a recent issue of Journal of Cellular Biochemistry, we read with great interest the article by Zhao et al. [2013] showing the molecular mechanisms governing CGRP-induced MG-63 differentiation. However, we found that the authors neglected a key concept regarding CGRP; it is the two subtypes of CGRP, αCGRP, and βCGRP.

In this article, the authors constructed a eukaryotic expression vector containing human RAMP1 and stably transfected it into MG-63 cells. Results showed that RAMP1 overexpression can promote CRLR expression, localization on the cell membrane, and enhanced CGRP-mediated differentiation of MG-63 cells. In the MATERIALS, authors presented that the investigated human CGRP was purchased from Sigma–Aldrich Corp. (Sigma, St. Louis, MO). However, which subtype of CGRP was investigated, αCGRP or βCGRP, was not pointed out in this article.

The human calcitonin (CT)/calcitonin gene-related peptide (CGRP) gene family, based on their nucleotide sequence homologies, is composed of five separate genes: calcitonin-I (CALC-I), -II, -III, -IV, and -V. The CALC-I gene encodes either CT or αCGRP by tissue-specific alternative processing of the primary RNA transcript [Amara et al., 1982]. CALC-II, considered to have arisen from gene duplication, encodes βCGRP. The CALC-III gene is not transcribed and is considered a pseudogene. CALC-IV and -V encode amylin (AMY) and the vasodilator peptide, adrenomedullin (AM), respectively.

CT can be found in at least three categories of species: teleost/avian, artiodactyl, and primate/rodent. CT is capable of lowering serum calcium and stimulating urinary cyclic adenosine monophosphate excretion (cAMP) after administration. Human CT is a 32-amino-acid peptide hormone with a molecular mass of 3,418 Da, which is mainly synthesized from CT-producing cells (i.e., C cells) within the thyroid gland.

CGRP is one of the most abundant peptides in the nervous system, being found in sensory, motor, and autonomic nerves, as well as in the hypothalamus, thalamus, and hippocampus. CGRPs also are located in cardiovascular system, C cells of the thyroid, and a variety of tumors (i.e., medullary thyroid carcinoma) [Wimalawansa, 1996]. CGRPs exhibit wide biological activities. They are potent endogenous vasodilators, and involved in bone metabolism, regulation of gastric vascularity, gastric acid secretion, and migraine attacks [Olesen et al., 2004].

αCGRP (also termed CGRP-I; molecular mass, 3,789 Da) is a 37-amino-acid peptide. In humans, βCGRP (or CGRP-II; molecular mass, 3,794 Da), the product of the CALC-II gene, differs from αCGRP in three of the 37 amino acids [Steenbergh et al., 1985].

CGRP receptors are formed by heterodimerization of the seven transmembrane domain CT receptor or calcitonin receptor-like receptor (CRLR) with a receptor activity-modifying protein (RAMP) [McLatchie et al., 1998]. CT receptor belongs to the type II seven transmembrane G protein-coupled receptors, and specific CGRP binding requires the coexpression of two proteins: CRLR and RAMP1. CRLR shares about 55% amino acid sequence homology with CT receptor. RAMP1 belongs to a family of proteins with a single transmembrane domain, which includes three members: RAMP1, RAMP2, and RAMP3. Further studies established that different combinations of CT receptor and CRLR with RAMPs produce receptors with different affinities for peptides of the CT family. The αCGRP and βCGRP peptides have equipotent affinity for CRLR. Most of the previous studies focused on αCGRP. Some people hold the viewpoint that the two CGRPs exhibit nearly identical

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biological activities so they may be collectively referred to as CGRP [Bilezikian et al., 2008; Wang et al., 2010].

However, Antje et al. analyzed the spine and tibia sections of 6 months old Calcb$^{-/-}$ mice via von Kossa-staining. Results revealed no obvious changes compared to wildtype littermates. Evidences showed that βCGRP did not have an important physiological function in the regulation of bone remodeling [Antje et al., 2008]. Hence, the biological function of βCGRP and its difference with αCGRP is still to be elucidated.

In any case, when investigating CGRP, it should be pointed out clearly which subtype is being discussed.

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