
REVISIÓN

G Protein-Coupled Receptor Family C 6A (GPRC6A): Possible molecular target in Bone

Receptor acoplado a proteína G familia C6A: Posible blanco molecular en hueso

Prof. Dr. Negri, Armando Luis

Cátedra de Fisiología y Biofísica, Escuela de Medicina de la Universidad del Salvador

RESUMEN

El GPRC6A es un miembro recientemente identificado de la familia C de receptores acoplados a proteínas G (GPCRs) que está estrechamente emparentado con el receptor sensor de calcio (CASR). Se ha demostrado que este receptor es capaz de sensar cationes extracelulares y aminoácidos y que requiere tanto de los cationes extracelulares y de los aminoácidos para su óptima estimulación *in vitro*. El estudio del perfil de ligandos ha mostrado que la l-ornithine es el más potente eficaz l-aminoácido agonista seguido de varios otros aminoácidos alifáticos, neutros, y básicos. Algunos estudios han mostrado la activación por cationes del GPRC6A, pero comparado con el CASR, se necesitan concentraciones extracelulares más altas de calcio para activar este receptor. Es más, el Mg(2+) ha mostrado ser un modulador positivo de la respuesta a la l-ornithine. Se lo ha propuesto como el candidato para el elusivo mecanismo de sensado de calcio extracelular del osteoblasto, que se sabe responde a altas concentraciones locales de Ca²⁺. También se ha propuesto al GPRC6A como candidato a receptor de la osteocalcina, regulando el metabolismo energético y como blanco molecular para la acción del estroncio sobre el hueso. **Rev Argent Endocrinol Metab 47: 24-28, 2010**

Los autores declaran no poseer conflictos de interés.

Palabras clave: Receptor acoplado a proteína G C6A, hueso, estroncio, osteocalcina

ABSTRACT

GPRC6A is a recently identified member of family C of G protein-coupled receptors (GPCRs) that is closely related to the calcium-sensing receptor CASR. It has recently been shown that GPRC6A extracellular cations and amino acids and requires both extracellular cations and amino acids for optimal stimulation *in vitro*. The study of the ligand profile of GPRC6A has shown that l-ornithine is the most potent and efficacious l-amino acid agonist, followed by several other aliphatic, neutral, and basic amino acids. Some studies show cation-dependent activation of GPRC6A, but compared to CASR, much higher extracellular calcium concentrations are needed to activate this receptor. Furthermore, the divalent cation Mg(2+) was found to be a positive modulator of the l-ornithine response. GPRC6A may be a candidate for the elusive extracellular calcium-sensing mechanism known to be present in osteoblasts, which respond to high local Ca²⁺ concentrations. GPRC6A has also been proposed as a candidate receptor for osteocalcin, regulating energy metabolism and as a molecular target for the action of strontium on bone. **Rev Argent Endocrinol Metab 47: 24-28, 2010**

No financial conflicts of interest exist.

Key Words: G Protein-Coupled Receptor Family C 6A, Bone, Strontium, osteocalcin

INTRODUCTION

GPRC6A is a recently identified member of family C of G protein-coupled receptors (GPCRs) that is closely related to the calcium-sensing receptor CASR⁽¹⁻³⁾. Structural homologies and conservation of specific domains in members of this family of receptors suggest an evolutionary link between extracellular calcium and amino acid-sensing⁽⁴⁻⁶⁾.

Family C G protein-coupled receptors

Family C of G protein-coupled receptors (GPCRs) from humans is constituted by eight metabotropic glutamate (mGlu(1-8)) receptors, two heterodimeric gamma-aminobutyric acid(B) (GABA(B)) receptors, the calcium-sensing receptor (CaR), three taste (T1R) receptors, a promiscuous L-alpha-amino acid receptor (GPRC6A), and five orphan receptors. Aside from the orphan receptors, the family C GPCRs is characterized by a large extracellular amino-terminal domain, which binds the endogenous orthosteric agonists, a seven transmembrane domain and a carboxy terminus domain. Recently, a number of allosteric modulators binding to the seven transmembrane domains of the receptors have also been reported. Family C GPCRs regulate a number of important physiological functions and are thus intensively pursued as drug targets. So far, two drugs acting at family C receptors (the GABA B agonist baclofen and the positive allosteric CaR modulator cinacalcet) have been marketed. Cinacalcet is the first allosteric GPCR modulator to enter the market, which demonstrates that the therapeutic principle of allosteric modulation can also be extended to this important drug target class.

GPRC6A Receptor

GPRC6A has recently been shown to sense extracellular cations and amino acids and to require both extracellular cations and amino acids for optimal stimulation *in vitro*⁽³⁾. This dual sensitivity of GPRC6A to both divalent cations and amino acids is analogous to the related receptor CASR⁽⁷⁾. Some studies show cation-dependent activation of GPRC6A, but compared to CASR, much higher extracellular calcium concentrations are needed to activate this receptor^(3,8). Other studies suggest that cations may only be allosteric modulators of GPRC6A⁽⁹⁾. The calcimimetic NPS-R578, an allosteric modulator of CASR⁽¹⁰⁻¹²⁾ and osteocal-

cin, a bone derived calcium binding protein, both enhance the functional responses of GPRC6A to extracellular calcium *in vitro*⁽³⁾.

The physiologically relevant ligands for and biological function of GPRC6A remain to be determined⁽¹³⁾. Utilizing co-expression of rat orthologue of GPRC6A (rGPRC6A) and the promiscuous Galpha(q)(G66D) protein in an engineered cell-based inositol phosphate turnover assay, Wellendorph et al⁽⁹⁾ were able to study the ligand profile of this receptor. They found that l-ornithine is the most potent and efficacious l-amino acid agonist with an EC⁽⁵⁰⁾ value of 264 microM, followed by several other aliphatic, neutral, and basic amino acids. Furthermore, the divalent cation Mg(2+) was found to be a positive modulator of the l-ornithine response. Christiansen tried to further characterize the ligand preferences of the GPRC6A receptor and to elucidate structural requirements for activity. They previously generated a functional chimeric receptor construct, h6A/5.24, containing the ligand-binding amino-terminal domain of the human GPRC6A and the seven-transmembrane domain and carboxy terminus of the homologous goldfish receptor 5.24⁽²¹⁾. Based on knowledge that this chimera prefers basic L-alpha-amino acids such as arginine, lysine and ornithine, they searched for commercially available analogues of these and other L-alpha-amino acids, and tested them for activity in a fluorescence-based calcium assay. The majority of the tested compounds are involved in the regulation of nitric oxide synthase (NOS) and arginase enzymes. Altogether they profiled 30 different analogues and found that a structurally wide range of L-alpha-amino-acid analogues of arginine, lysine, and ornithine are agonists at h6A/5.24, whereas no antagonists were identified. From the profiling they concluded that L-alpha-amino acids containing a highly basic side chain confer the highest activity, although the most potent compound was only twice as potent as L-arginine, which has an EC₅₀ value of 23.5 microM. The reported agonism of NOS- and arginase-active compounds at GPRC6A has obvious implications in interpretation of experiments involving the NOS and arginase systems, and interfering effects at GPRC6A should be regarded of relevance, especially as the physiological function of the receptor is not yet understood.

GPRC6A is broadly expressed in many tissues and organs, including lung, liver, spleen, heart, kidney, skeletal muscle, testis, brain and bone⁽¹⁻⁴⁾. The L amino acid, osteocalcin, and divalent calcium li-

gand specificity of this receptor and its wide tissue distribution implicate GPRC6A multiple processes. For example, GPRC6A may be a candidate for the elusive extracellular calcium-sensing mechanism known to be present in osteoblasts^(3,15-18), which respond to high local Ca^{2+} concentrations (in the range of 8 to 40 mM)⁽¹⁹⁾, amino acids and osteocalcin in the bone microenvironment. GPRC6A is also a candidate for the putative osteocalcin receptor regulating energy metabolism⁽²⁰⁾.

GPRC6A Null mice

In a recent study Chen et al⁽²²⁾ created and characterized the phenotype of GPRC6A Null mice (GPRC6A^(-/-) mice). They observed complex metabolic abnormalities in GPRC6A^(-/-) mice involving multiple organ systems that express GPRC6A, including bone, kidney, testes, and liver. GPRC6A^(-/-) mice exhibited hepatic steatosis, hyperglycemia, glucose intolerance, and insulin resistance. In addition, they observed high expression of GPRC6A in Leydig cells in the testis. Ablation of GPRC6A resulted in feminization of male GPRC6A^(-/-) mice in association with decreased lean body mass, increased fat mass, increased circulating levels of estradiol, and reduced levels of testosterone. GPRC6A was also highly expressed in kidney proximal and distal tubules, and GPRC6A^(-/-) mice exhibited increments in urine Ca/Cr and PO_4/Cr ratios as well as low molecular weight proteinuria. Finally, GPRC6A^(-/-) mice exhibited a decrease in bone mineral density (BMD) in association with impaired mineralization of bone. So the GPRC6A^(-/-) mice have a metabolic syndrome characterized by defective osteoblast-mediated bone mineralization, abnormal renal handling of calcium and phosphorus, fatty liver, glucose intolerance and disordered steroidogenesis. These findings suggest the overall function of GPRC6A may be to coordinate the anabolic responses of multiple tissues through the sensing of extracellular amino acids, osteocalcin and divalent cations.

Pi et al investigated whether the osseous abnormalities found in GPRC6A^(-/-) mice were a direct function of GPRC6A in osteoblasts⁽²³⁾. They examined the function of primary osteoblasts and bone marrow stromal cell cultures (BMSCs) in GPRC6A^(-/-) mice⁽²³⁾. They confirmed that GPRC6A^(-/-) mice exhibited a decrease in bone mineral density (BMD) associated with reduced expression of osteocalcin, ALP, osteoprotegerin,

and Runx2-II transcripts in bone. Osteoblasts and BMSCs derived from GPRC6A^(-/-) mice exhibited an attenuated response to extracellular calcium-stimulated extracellular signal-related kinase (ERK) activation, diminished alkaline phosphatase (ALP) expression, and impaired mineralization *ex vivo*. In addition, siRNA-mediated knockdown of GPRC6A in MC3T3 osteoblasts also resulted in a reduction in extracellular calcium-stimulated ERK activity. To further explore the potential relevance of GPRC6A function in humans, they looked for an association between GPRC6A gene polymorphisms and BMD in a sample of 1000 unrelated American Caucasians. They found that GPRC6A gene polymorphisms were significantly associated with human spine BMD. These data indicate that GPRC6A directly participates in the regulation of osteoblast-mediated bone mineralization and may mediate the anabolic effects of extracellular amino acids, osteocalcin, and divalent cations in bone.

Wellendorph et al generated another GPRC6A knockout mice to study their phenotype with particular focus on bone homeostasis⁽²⁴⁾. The generated GPRC6A knockout mice were viable and fertile, developed normally, and exhibited no significant differences in body weight compared with wild-type littermates. Assessment of bone mineral density, histomorphometry, and bone metabolism demonstrated no significant differences between 13-week-old knockout and wild-type mice. These authors concluded that based on their GPRC6A knockout mice there was no support for a role of GPRC6A in normal bone physiology.

GPRC6A receptor as the molecular target for strontium in osteoblasts

Strontium has anabolic effects on bone and it is currently being used for the treatment of osteoporosis. The molecular target for strontium in osteoblasts has not been determined, but the existence of CASR, a G-protein-coupled receptor calcium-sensing receptor, raises the possibility that strontium actions on bone may be mediated through this or a related receptor. Pi et al⁽⁸⁾ used activation of a transfected serum response element (SRE)-luciferase reporter in HEK-293 cells to determine if CASR is activated by strontium. In addition, they examined strontium-mediated responses in MC3T3-E1 osteoblasts and osteoblasts derived from wild-type and CASR null mice to determine if other cation-sensing mechanisms were present in osteoblasts. They found that strontium stimu-

lated SRE-luc activity in HEK-293 cells transfected with full-length CASR but not in cells expressing the alternatively spliced CASR construct lacking exon 5. In contrast, they found that MC3T3-E1 osteoblasts that lack CASR as well as osteoblasts derived from CASR null mice respond to millimolar concentrations of strontium. The response to strontium in osteoblasts was nonadditive to that of a panel of extracellular cations, including aluminum, gadolinium, and calcium, suggesting a common mechanism of action. In contrast, neither the CASR agonist magnesium nor the calcimimetic NPS-R568 activated SRE activity in osteoblasts, but the response to these agonists was imparted by transfection of CASR into these osteoblasts, consistent with the presence of distinct cation-sensing mechanisms. Co-expression of the dominant negative Galphaq(305-359) minigene also inhibited cation-stimulated SRE activity in osteoblasts lacking known CASR. These findings are consistent with strontium activation of a novel Galphaq-coupled extracellular cation-sensing receptor in osteoblasts with distinct cation specificity.

REFERENCES

- Wellendorph P, Brauner-Osborne H. Molecular cloning, expression, and sequence analysis of GPRC6A, a novel family C G-protein-coupled receptor. *Gene*. 2004; 335: 37-46.
- Kuang D, Yao Y, Lam J, Tsushima RG, Hampson DR. Cloning and characterization of a Family C orphan G-protein coupled receptor. *J Neurochem*. 2005; 93: 383-391.
- Pi M, Faber P, Ekema G, Jackson PD, Ting A, et al. Identification of a novel extracellular cation-sensing G-protein-coupled receptor. *J Biol Chem*. 2005; 280: 40201-40209.
- Folkers K, Bowers CY, Tang PF, Kubota M. Decapeptides as effective agonists from L-amino acids biologically equivalent to the luteinizing hormone-releasing hormone. *Proc Natl Acad Sci U S A*. 1986; 83: 1070-1074.
- Conigrave AD, Quinn SJ, Brown EM. L-amino acid sensing by the extracellular Ca²⁺-sensing receptor. *Proc Natl Acad Sci U S A*. 2000; 97: 4814-4819.
- Tabata T, Araiishi K, Hashimoto K, Hashimoto-dani Y, van der Putten H, et al. Ca²⁺ activity at GABAB receptors constitutively promotes metabotropic glutamate signaling in the absence of GABA. *Proc Natl Acad Sci U S A*. 2004; 101: 16952-16957.
- Tfelt-Hansen J, Brown EM. The calcium-sensing receptor in normal physiology and pathophysiology: a review. *Crit Rev Clin Lab Sci*. 2005; 42: 35-70.
- Pi M, Quarles LD. A novel cation-sensing mechanism in osteoblasts is a molecular target for strontium. *J Bone Miner Res*. 2004; 19: 862-869.
- Wellendorph P, Burhenne N, Christiansen B, Walter B, Schmale H, et al. The rat GPRC6A: Cloning and characterization. *Gene*. 2007.
- Hu J, Reyes-Cruz G, Chen W, Jacobson KA, Spiegel AM. Identification of acidic residues in the extracellular loops of the seven-transmembrane domain of the human Ca²⁺ receptor critical for response to Ca²⁺ and a positive allosteric modulator. *J Biol Chem*. 2002; 277:46622-46631.
- Petrel C, Kessler A, Dauban P, Dodd RH, Rognan D, et al. Positive and negative allosteric modulators of the Ca²⁺-sensing receptor interact within overlapping but not identical binding sites in the transmembrane domain. *J Biol Chem*. 2004; 279: 18990-18997.
- Miedlich SU, Gama L, Seuwen K, Wolf RM, Breitwieser GE. Homology modeling of the transmembrane domain of the human calcium sensing receptor and localization of an allosteric binding site. *J Biol Chem*. 2004; 279: 7254-7263.
- Christiansen B, Hansen KB, Wellendorph P, Brauner-Osborne H. Pharmacological characterization of mouse GPRC6A, an L-alpha-amino-acid receptor modulated by divalent cations. *Br J Pharmacol*. 2007; 150: 798-807.
- Wellendorph P, Hansen KB, Balsgaard A, Greenwood JR, Egebjerg J, et al. Deorphanization of GPRC6A: a promiscuous L-alpha-amino acid receptor with preference for basic amino acids. *Mol Pharmacol*. 2005; 67: 589-597.
- Pi M, Garner SC, Flannery P, Spurney RF, Quarles LD. Sensing of extracellular cations in CasR-deficient osteoblasts. Evidence for a novel cation-sensing mechanism. *J Biol Chem*. 2000; 275: 3256-3263.
- Quarles LD, Gitelman HJ, Drezner MK. Induction of de novo bone formation in the beagle. A novel effect of aluminum. *J Clin Invest*. 1988; 81: 1056-1066.
- Martin TJ, Sims NA. Osteoclast-derived activity in the coupling of bone formation to resorption. *Trends Mol Med*. 2005; 11: 76-81.
- Zaidi M, Shankar VS, Tunwell R, Adebajo OA, Mackrill J, et al. A ryanodine receptor-like molecule expressed in the osteoclast plasma membrane functions in extracellular Ca²⁺ sensing. *J Clin Invest*. 1995; 96: 1582-1590.
- Cohen A, Silverberg SJ. Calcimimetics: therapeutic potential in hyperparathyroidism. *Curr Opin Pharmacol*. 2002; 2: 734-739.
- Lee NK, Sowa H, Hinoi E, Ferron M, Ahn JD, et al. Endocrine regulation of energy metabolism by the skeleton. *Cell*. 2007; 130: 456-469.
- Christiansen B, Wellendorph P, Bräuner-Osborne H. Known regulators of nitric oxide synthase and arginase are agonists at the human G-protein-coupled receptor GPRC6A. *Br J Pharmacol*. 2006; 147(8): 855-63.
- Pi M, Chen L, Huang MZ, Zhu W, Ringhofer B, Luo J, Christenson L, Li B, Zhang J, Jackson PD, Faber P, Brunden KR, Harrington JJ, Quarles LD. GPRC6A null mice exhibit osteopenia,

- feminization and metabolic syndrome. *PLoS One*. 2008; 3(12): e3858.
23. **Pi M, Zhang L, Lei SF, Huang MZ, Zhu W, Zhang J, Shen H, Deng HW, Quarles LD.** Impaired osteoblast function in GPRC6A null mice. *J Bone Miner Res*. 2010; 25(5): 1092-102.
24. **Wellendorph P, Johansen LD, Jensen AA, Casanova E, Gassmann M, Deprez P, Clément-Lacroix P, Bettler B, Bräuner-Osborne H.** No evidence for a bone phenotype in GPRC6A knockout mice under normal physiological conditions. *J Mol Endocrinol*. 2009; 42(3): 215-23.