

## The many roles of the extracellular calcium-sensing receptor, CaR, in osteoblast biology

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**INTRODUCTION:** Fluctuations in extracellular free ionized calcium concentration ( $[Ca^{2+}]_o$ ) occur naturally during bone remodeling and contribute to systemic  $Ca^{2+}$  homeostasis [1]. In tissue culture models, elevation in  $[Ca^{2+}]_o$  induces osteoblast chemotaxis and proliferation [2,3] and alter the levels of expression of several osteoblast differentiation markers [4,5]. Whether these effects are mediated by a functional calcium-sensing receptor, CaR [6] expressed in bone is controversial.

**METHODS:** Immunohistochemistry was performed to detect CaR expression in freshly frozen, undecalcified preparations of human mandible and rat femur. In addition, we have used primary and established models of osteoblasts, fetal rat calvarial (FRC) and the murine clonal cell line, 2T3 cells, to investigate the expression of the CaR and to study the effects of known CaR agonists ( $Ca^{2+}$ ,  $Gd^{3+}$  and the known anti-osteoporotic agent strontium,  $Sr^{2+}$ ) on acute, mitogenic outcomes (ERK1/2 phosphorylation), and chronic, differentiation-dependent cellular responses (expression of osteoblast differentiation markers, core binding factor  $\alpha 1$  [cbfa1] and osteopontin, and mineralization). The ability of the negative allosteric modulator (NPS 89636) to affect such responses was also investigated.

**RESULTS:** CaR mRNA and protein were detected in both human and rat osteoblasts and osteocytes and in primary and established models of osteoblasts, FRC and 2T3 cells. Elevating  $[Ca^{2+}]_o$  and treatment with non-permeant CaR agonists,  $Gd^{3+}$  and  $Sr^{2+}$ , resulted in activation of pro-proliferation and pro-survival signals. Expression of the osteoblast differentiation markers cbfa1, osteocalcin, osteopontin and collagen I mRNA and/or protein were increased by high  $[Ca^{2+}]_o$  and/or by treatment with  $Sr^{2+}$ , as was mineralized nodule formation. The calcilytic 89636

prevented  $Ca^{2+}$ -,  $Gd^{3+}$ - and  $Sr^{2+}$ -dependent responses in both FRC and 2T3 cells.

**DISCUSSION & CONCLUSIONS:** Small deviations of  $[Ca^{2+}]_o$  from physiological values directly and profoundly affect osteoblast function, through the CaR and independently of systemic calciotropic hormones. In addition, the beneficial effects of strontium ranelate as an anti-osteoporotic agent can be ascribed, at least in part, through stimulation of the osteoblast CaR. Pharmacological modulators of CaR function are currently available on the market for the treatment of hyperparathyroidism secondary to kidney failure. The possibility to use positive and negative allosteric modulators of the CaR for bone-related disease is currently being explored.

**REFERENCES:** <sup>1</sup>Parfitt, A. M. (1987) *Bone* 8 Suppl 1, S1-8; <sup>2</sup>Godwin, S. L. & Soltoff, S. P. (1997) *J Biol Chem* 272, 11307-12; <sup>3</sup>Yamaguchi, T., Chattopadhyay, N., Kifor, O., Butters, R. R., Jr., Sugimoto, T. & Brown, E. M. (1998) *J Bone Miner Res* 13, 1530-8; <sup>4</sup>Eklou-Kalonji, E., Denis, I., Lieberherr, M. & Pointillart, A. (1998) *Cell Tissue Res* 292, 163-71; <sup>5</sup>Nakade, O., Takahashi, K., Akuma, T., Aoki, T. & Kaku, T. (2001) *J Bone Miner Metab* 19, 13-9; <sup>6</sup>Brown, E., M., Gamba, G., Riccardi, D., Lombardi, M., Butters, R., Kifor, O., Sun, A., Hediger, M., A., Lytton, J. & Hebert, S., C. (1993) *Nature* 366, 575-580.

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