Neural modulation of bone anabolism: Role of the neuropeptide Y system in the direct control of osteoblast activity.

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Introduction
Control of osteoblast activity has historically been viewed as a balance between systemic endocrine and local mechanical influences. However, the importance of neural inputs has become increasingly apparent. In particular, central processes, mediated by efferent hypothalamic signaling, have revealed novel and marked bone anabolic responses. One important mediator of these central anabolic pathways to bone is the neuropeptide Y (NPY) system. Murine studies identified a generalized increase in anabolic activity following NPY, Y2 receptor deletion, which was recapitulated by deletion of hypothalamic Y2 receptors, with changes up to 7-fold (1). The central nature of this NPY pathway to bone was further confirmed by a similar decrease in anabolism following over expression of NPY solely in the hypothalamus (2).

While it is clear that central NPY inhibits osteoblast activity, the peripheral mediator of the pathway is unknown. However, Y1 receptors have been implicated in this process; Y1 WT mice display a bone anabolic phenotype similar to Y2-/- and Y1-/Y2-/- mice have no additive anabolic phenotype (3), and Y2 deletion decreases in Y1 expression in osteoblast like cultures.

In this study we aimed to investigate the response of osteoprogenitor and mature osteoblasts to loss of Y1 signaling using murine genetic models and pharmacological treatment of ex vivo cultures.

Hypotheses
Bone anabolic changes induced by altered central NPY signaling is mediated in the periphery by osteoblastic Y1 receptors.

Methods
Mesenchymal stem cell and Osteoprogenitor cultures
MSCs and OP cells were isolated from the femurs, tibias, and iliac crests of 12-18 week male mice. Bones were cleaned, flushed of marrow, crushed and incubated for 45 minutes in collagenase. Following digestion, haematopoietic cells were removed and MSCs and OP cells were selected by FACS using the surface markers Sca-1 and CD51, isolating the Sca-1+ MSCs and Sca-1-CD51+ OP cells were collected. Osteogenic media contained 50 mg/L ascorbic acid, 10 mM β-glycerophosphate and 100ng/mL BMP-2. Mineralization was visualized by von Kossa staining.

Bone marrow stromal cell cultures
Bone marrow stromal cells (BMSCs) were isolated from femurs and tibias of 5-9 week old male wt and Y1-/- mice were sacrificed, and marrow flushed. Cells were plated and the non-adherent population was collected and digested, haematopoietic cells were removed and MSCs and OP cells were collected.

In vivo studies
Y1 receptor floxed mice were crossed with mice expressing Cre under control of the collagen 1a1 promoter, thereby enabling lineage (Col 3.6) or cell specific (Col 2.3) deletion of Y1. Femurs of 16 week old were collected and processed in methyl methacrylate. Undecalcified 5μm sagittal sections were stained for mineralized tissue. Unstained sections were used to visualize fluorescent labels (calcine 30mg/kg) injected 10 and 3 days prior to collection, thereby enabling mineral apposition rate to be calculated.

Results
Mesenchymal stem cells and osteoprogenitors express Y1 receptor

Y1 deficiency increases CFU-F and mineralization capacity

Bone marrow stromal cells express Y1 receptor

Osteoblast-specific deletion of Y1 induces anabolic response in vivo

Conclusion
- Y1 receptors are expressed throughout the osteoblastic lineage, acting to decrease bone anabolism by altering osteoprogenitor cell proliferation and differentiated osteoblast activity.
- Attenuation of osteoblastic Y1 signaling increased anabolic activity.
- Osteoblastic Y1 receptors represent the terminal stage of the NPY mediated pathway form the hypothalamus to bone.
- Modulation of osteoblastic Y1 signaling may offer a novel mechanism for local control of anabolism.

References