The mechanism of osteocalcin control of energy metabolism

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Introduction
There is accumulating evidence that bone, adipose tissue, the endocrine system, digestive system, and neurological systems cross communicate to regulate the processes of energy metabolism. The surprise is that there are no novel hormones, neurotransmitters, or cytokines that accomplish the communication: the communication and control is effected through already well established hormone-receptor systems. We propose that osteocalcin (OCN) regulates energy metabolism through insulin and adiponectin release by regulating OT action. OT receptors are present in bone, endothelial, beta islet, fat cells but the breadth of functions of OT is not fully known. In this cell culture study we examine Vitamin D (VitD) as a stimulus for OCN production and the effects of OCN and OT on insulin secretion with and without glucose.

Materials and Methods
The mouse bone cells MC3T3 (ATCC) were cultured in the presence and absence of VitD. Mouse β-islet cells cultured in medium (containing 50µg/ml of gentamicin, 50ng/ml amphotericin-B, 6% fetal bovine serum) were purchased from ATCC (BTC6). Near confluent cultures of cells were plated in 48-well culture plates. They were allowed to adhere overnight, then washed twice with serum-free medium and changed to a minimal growth basal medium containing 0.5% FBS. After 24 h, cells were washed twice with serum-free medium and incubated with basal medium supplemented with tested concentrations of OT or OCN. Each experiment had internal negative controls (basal medium alone), and positive controls (2-5% FBS). After 24, 48, or 72 h, the cell number was estimated by Celltiter 96 Aqueous One Solution cell proliferation assay from Promega Corp (Madison, WI) and verified by hemocytometer. Each experimental condition was evaluated from a minimum of three replicate cultures. Medium from cultures were collected and assayed for insulin levels by ELISA. (+) glucose indicates 100mg/dl glucose in medium, (-) glucose is glucose free medium. VitD was used at 100µM concentration, OCN at 3ng/ml and OT at 10^−6 M concentrations. Differences from control were established by ANOVA and TTests at significance of p=0.05.

Results

![Figure 1. Osteocalcin produced by MC3T3-E1 osteoblasts in the presence and absence of VitD (mean ± StdDev; n= 3).](image)

OCN used in the experiments was collected from 3T3 osteoblasts. Figure 1 shows VitD increasing the amount of osteocalcin secreted by the osteoblasts, particularly after 48 hours. Compared to the control group, OCN increases insulin secretion from β cells, as shown in Figure 2. After 6 hours, OCN caused a significant (75%) increase in insulin production.

![Figure 2. Insulin produced by β-islet cells in the presence and absence of OCN at three time points (mean ± SEM; n= 3). *Significantly different from 2 hours.](image)

Figure 2 shows that insulin produced by β-islet cells is significantly increased by OCN in the presence and absence of OT. The increase in insulin production by β-islet cells almost 75% six hours after treatment. As seen in figure 3 OT also significantly increased the amount of insulin secreted by OCN in the presence and absence of OT.

![Figure 3. Insulin produced by β-islet cells in the presence and absence of VitD, OCN, or OT (mean ± SEM; n= 4). *Significantly different from Cells only at same glucose level. VitD does not cause any significant increase in insulin secretion from the β cells. However, OCN and OT caused equal increases in the amounts of insulin secreted.](image)

Discussion
Our results show that OCN secreted by 3T3 bone cells significantly increases the insulin production by β-islet cells almost 75% six hours after treatment. As seen in figure 3 OT also significantly increased the amount of insulin secreted by the β-islet cells indicating that the cells have receptors for OT. We hypothesized that OCN is using the OT receptor on the β-islet cells. These data support this claim, as similar amounts of OT and OCN caused similar increases in insulin production. VitD is known to be related to insulin regulation and in our experiments, VitD inhibited the increased insulin secretion effects of both OT and OCN.

Conclusion
These in vitro results indicate that OCN plays a significant role in energy metabolism. Preliminary data from other research groups is showing a similar increase in insulin production caused by OCN in human β-cells, both in vitro and in vivo. Understanding the actions of the bone protein OCN could potentially have a significant impact on our understanding of the regulation of energy metabolism and lead to advances in the treatments of diabetes, obesity and diseases involving bone mass.