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COMMENTARIES

A Skeletal Role for Gq/11 Signaling by the PTH Receptor

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Commentary on: Guo J, Liu M, Yang D, Bouxsein ML, Thomas CC, Schipani E, Bringhurst FR, Kronenberg HM. Phospholipase C signaling via the parathyroid hormone (PTH)/PTH-related peptide receptor is essential for normal bone responses to PTH. *Endocrinology*. 2010 Aug;151(8):3502-13.

The PTH/PTHrP receptor (PTHR) is known to have the ability to signal through two G proteins – Gs and Gq. The physiological role of Gs activation is well-documented, but the extent to which Gq activation contributes to PTH action is unclear. Guo *et al*. (1) recently reported that mice harboring PTHRs deficient in activating Gq are defective in their ability to increase bone formation in models of hyperparathyroidism. These results provide direct evidence that Gq signaling contributes meaningfully to PTH action in bone.

The PTH/PTHrP receptor (PTHR) is a G protein-coupled receptor that is capable of activating at least two G proteins: Gs leading to the activation of adenylyl cyclase (AC) and increased cyclic AMP production, and Gq/11 leading to the activation of phospholipase C (PLC), intracellular calcium mobilization, and increased activity of protein kinase C (2;3). The Gs/AC response to PTHR activation accounts for many of the actions of PTH on target cells, but there is evidence that Gq/11/PLC activation participates in some cases (4-8). Much of the evidence in this regard has involved the use of pharmacological tools *ex vivo*, and the relevance of Gq/11/PLC signaling to the biological actions of PTH *in vivo* has been unclear.

Several years ago, Guo *et al*. (9) used a genetic strategy to generate mice with targeted alterations in the PTHR such that the receptor responded to PTH with normal activation of Gs/AC without activation of Gq/11/PLC. This was achieved by a knock-in mutation of the sequence DSEL in place of EKKY in the second cytoplasmic loop of the receptor, an alteration previously shown to selectively block PTH-induced activation of PLC presumably by preventing PTHR coupling to Gq/11 (10). DSEL mice were shown to display abnormalities in endochondral bone development presumed to reflect the lack of PTHR signaling to Gq/11 in cartilage. These effects included delayed ossification and increased chondrocyte proliferation, suggesting that PTHR-induced Gq/11/PLC signaling normally limits the chondrogenic response to endogenous PTHrP.

Guo *et al*. (1) have now utilized this mouse model to assess the role of Gq/11 signaling by the PTHR in maintaining skeletal homeostasis under normal conditions and in models of primary and secondary hyperparathyroidism. Ten-week-old DSEL mice were found to display a mild cancellous bone phenotype with decreased fractional bone volume and decreased serum P1NP consistent with reduced bone formation. Interestingly, these mice had serum PTH levels that were nearly twice as high as wild-type mice despite having normal levels of serum calcium and phosphate. Although the increase in serum PTH was not statistically significant, the results nonetheless suggest that DSEL mice display an element of target cell resistance to PTH. This was more dramatically evident in the models of hyperparathyroidism. In wild-type mice, maintenance on a low calcium diet (model of secondary hyperparathyroidism) resulted in a catabolic effect in cortical bone and an increase in cancellous bone formation with peritrabecular stromal cell expansion. DSEL mice on a low calcium diet also displayed a

catabolic effect in cortical bone (consistent with an up-regulation of cyclic AMP-dependent RANKL expression), but the increase in cancellous bone formation and expansion of peritrabecular stromal cells were attenuated. These findings suggest that the long bones of DSEL mice are resistant to the anabolic but not the catabolic actions of PTH. Similar results were obtained in mice infused with PTH (model of primary hyperparathyroidism).

Serum phosphate levels were low in wild-type mice on the low calcium diet, presumably due to the action of elevated PTH on the proximal renal tubule. Strikingly, DSEL mice maintained on a low calcium diet were hyperphosphatemic. The reasons for this are not entirely clear, but the results are again consistent with resistance to the target cell actions of PTH (in this case in the proximal renal tubule). Indeed, previous studies have implicated PLC activation (presumably via PTHR-mediated activation of Gq/11) in PTH-induced down-regulation of NaPi2 transporters in the apical membrane of proximal renal tubular cells (5). Guo *et al*. (1) did not report $1,25(OH)_2D$ levels, but it would be of great interest to know whether DSEL mice were also resistant to this aspect of PTH action. If so, one would need to consider the possibility that some of the skeletal resistance to the anabolic action of PTH in DSEL mice could be secondary to reduced circulating levels of $1,25(OH)_2D$ (11).

Previous studies have indicated that the anabolic action of PTH is at least in part due to paracrine signals initiated in relatively mature osteoblast lineage cells that promote expansion of the osteoprogenitor pool (12;13). This is associated with activation of canonical Wnt signaling in osteoprogenitors (13). DSEL mice may be deficient in this activity as suggested by the failure of the low calcium diet to increase the number of osteoprogenitors. On the other hand, osteoblasts from DSEL mice also failed to respond to PTH with an increase in proliferation or cyclin D1 expression *in vitro*, suggesting a cell-autonomous component to the skeletal phenotype.

Taken together, these results demonstrate that mice harboring PTHRs lacking the ability to couple to Gq/11 display evidence of selective target cell resistance to PTH. These findings provide the most compelling evidence to date that this G protein pathway contributes importantly to the physiological actions of PTH and to the skeletal response to states of PTH excess. Understanding how Gq/11 signaling integrates with Gs signaling and other pathways such as arrestin-based signaling (14;15) will be essential for defining the mechanism of action of PTH physiologically and as an anabolic therapy for osteoporosis.

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