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Pregnancy-associated plasma protein-A modulates the anabolic effects of parathyroid hormone in mouse bone

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ABSTRACT

Intermittent parathyroid hormone (PTH) is a potent anabolic therapy for bone, and several studies have implicated local insulin-like growth factor (IGF) signaling in mediating this effect. The IGF system is complex and includes ligands and receptors, as well as IGF binding proteins (IGFBPs) and IGFBP proteases. Pregnancy-associated plasma protein-A (PAPP-A) is a metalloprotease expressed by osteoblasts in vitro that has been shown to enhance local IGF action through cleavage of inhibitory IGFBP-4. This study was set up to test two specific hypotheses: 1) Intermittent PTH treatment increases the expression of IGF-I, IGFBP-4 and PAPP-A in bone in vivo, thereby increasing local IGF activity. 2) In the absence of PAPP-A, local IGF activity and the anabolic effects of PTH on bone are reduced. Wild-type (WT) and PAPP-A knock-out (KO) mice were treated with 80 μg/kg human PTH 1-34 or vehicle by subcutaneous injection five days per week for six weeks. IGF-I, IGFBP-4 and PAPP-A mRNA expression in bone were significantly increased in response to PTH treatment. PTH treatment of WT mice, but not PAPP-A KO mice, significantly increased expression of an IGF-responsive gene. Bone mineral density (BMD), as measured by DEXA, was significantly decreased in femurs of PAPP-A KO compared to WT mice with PTH treatment. Volumetric BMD, as measured by pQCT, was significantly decreased in femoral midshaft (primarily cortical bone), but not metaphysis (primarily trabecular bone), of PAPP-A KO compared to WT mice with PTH treatment. These data suggest that stimulation of PAPP-A expression by intermittent PTH treatment contributes to PTH bone anabolism in mice.

1. Introduction

The ability of intermittent parathyroid hormone (PTH) to increase bone formation in several animal models and in humans led to the approval of PTH as an anabolic treatment for osteoporosis [1–3]. However, the underlying mechanisms for the potent anabolic effect of PTH in bone are not fully understood. Early in vitro studies indicated that PTH treatment increased the production of insulin-like growth factor (IGF)-I by bone cells [4] Several in vivo studies followed that highlighted the importance of local IGF-I and IGF-I receptor (IGF-IR) in mediating the anabolic actions of PTH in bone [5–8]. Regulation of local IGF signaling is complex and can be modulated not only by changes in ligand or receptor levels, but also by expression and post-translational modification of IGF binding proteins (IGFBPs) (reviewed in [9]). IGFBP-4 is the most abundant IGFBP in bone, and has been shown to bind and prevent IGF-I from interacting with its receptor [10]. Furthermore, cleavage of IGFBP-4 by the zinc metalloprotease, pregnancy-associated plasma protein-A (PAPP-A), markedly deceases IGFBP-4 affinity for IGF-I, thereby increasing local IGF-I available for receptor activation (reviewed in [11]). Transgenic mice over-expressing PAPP-A in bone show increased bone formation, which can be blocked with simultaneous expression of protease-resistant IGFBP-4 [12,13].

PTH acts in part through increases in cyclic AMP to stimulate bone formation. In vitro, PTH increases IGFBP-4 and IGF-I expression in bone through a cyclic AMP-mediated pathway [14]. Agents that increase intracellular cyclic AMP also increase PAPP-A expression in cultured osteoblasts [15]. Thus, we hypothesize that PTH increases expression of IGF-I and IGFBP-4 in vivo, thereby creating a pericellular reservoir of IGF-I/IGFBP-4 in the bone microenvironment, and that concerted PTH stimulation of PAPP-A enhances IGF-I action in bone through proteolysis of IGFBP-4. If true, then in the absence of PAPP-A the anabolic effect of PTH on bone would be attenuated. In the study herein, we test this hypothesis in wild-type and PAPP-A knock-out mice.

Abbreviations: PTH, parathyroid hormone; PAPP-A, pregnancy-associated plasma protein-A; IGF-I, insulin-like growth factor-I; IGFBP, insulin-like growth factor binding protein; IGF-IR, IGF-I receptor; WT, wild-type; KO, knock-out; DEXA, dual energy X-ray absorptiometry; pQCT, peripheral quantitative computer tomography; BMC, bone mineral content; BMD, bone mineral density.

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2. Materials and methods

2.1. Mice

Mice heterozygous for *Pappa* (C57BL/6, 129) were bred as previously described to produce wild-type (WT) and PAPP-A knock-out (KO) littermates for these experiments [16]. Genotypes were determined before assignment to groups and confirmed at harvest. Three-month-old female WT and PAPP-A KO mice (N = 10 per group) were treated with human PTH 1-34 (Bachem Americas), at a dose of 80 μg/kg, or with vehicle (0.9% acidified saline with 2% heat-inactivated WT mouse serum) by subcutaneous injection five days per week for six weeks. This study was approved by the Institutional Animal Care and Use Committee of Mayo Clinic.

2.2. Bone morphometry

Dual Energy X-ray absorptiometry (DEXA) and peripheral quantitative computer tomography (pQCT) scans were taken at the start (day 0) and end (day 42) of the treatment period. DEXA measured bone mineral content (BMC) and areal bone mineral density (BMD) in the femur using a PIXImus densitometer (GE-Lunar, Madison, WI). Volumetric BMD was measured by pQCT at the midshaft and distal metaphysis of the femur using a Stratec XCT Research Plus scanner with v 5.40 software (Norland Medical Systems, Fort Atkinson, WI). Each bone at each site was scanned in triplicate using scanning parameters that have been described elsewhere [16]. pQCT was considered inadequate to distinguish trabecular and cortical bone in these small animals, so only total BMD is reported for each site.

2.3. Bone histomorphometry

Fluorescent bone labels (declomycin, calcein) were administered to mice 12 days, 5 days, and 2 days before sacrifice. Femurs were processed and embedded in methyl methacrylate. Analyses were performed by Mayo Clinic's Biomaterials Characterization and Quantitative Histomorphometry Core Laboratory.

2.4. Gene expression analyses

Tibias were rapidly harvested immediately after the second scan and frozen in liquid nitrogen. RNA extraction, reverse transcription, and quantitative real-time PCR have been described previously [16]. Target gene expression was normalized to ribosomal protein L19.

2.5. Statistical analyses

Results are presented as Mean ± SD. Mann-Whitney U test was used to compare results in WT versus PAPP-A KO mice. Significance was set at $P < 0.05$.

3. Results

3.1. PTH treatment regulates IGF system gene expression in vivo

IGF-I, IGFBP-4 and PAPP-A mRNA expression were significantly increased in bone from WT mice in response to intermittent PTH treatment 80 μg/kg for six weeks (Fig. 1). In particular, PTH increased PAPP-A expression three-fold. Based on in vitro data, coordinated up-regulation of IGF-I/IGFBP-4/PAPP-A would be predicted to create a situation of enhanced local IGF-I available for receptor activation through PAPP-A-induced cleavage of IGFBP-4. It is difficult to measure IGF-I receptor activation in vivo using the phosphorylation assays typically used in vitro due to the transient nature of phosphorylation events. However, IGFBP-5 is an IGF-I responsive gene and elevated mRNA levels have been used as an indicator of IGF-I signaling in vivo in several tissues, including bone [17–19]. PTH treatment of WT mice significantly increased IGFBP-5 mRNA levels in bone (Fig. 2). However, PTH treatment of PAPP-A KO mice did not increase IGFBP-5 expression, consistent with a blunting of local IGF action in the absence of PAPP-A.

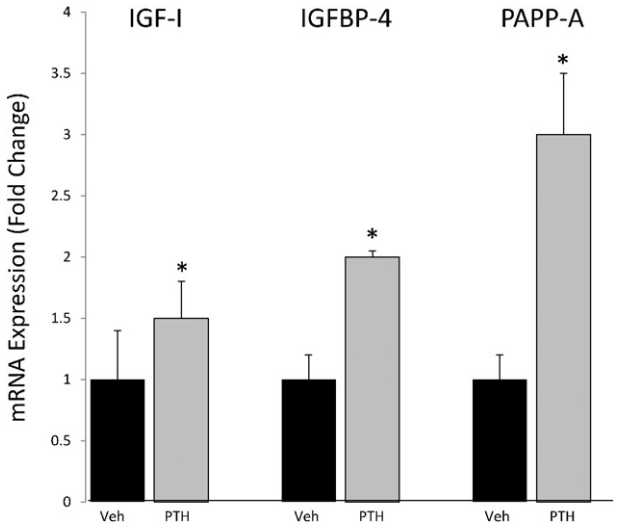

Fig. 1. IGF system gene expression in response to intermittent PTH treatment. Expression of IGF-I, IGFBP-4, and PAPP-A was assessed by real-time PCR of RNA extracted from the proximal tibia of wild-type mice. Fold change in PTH-treated mice is expressed relative to vehicle-treated mice of the same genotype. N = 10. *Significant effect of PTH treatment, $P < 0.05$.

3.2. PAPP-A deficiency attenuates the anabolic effects of PTH in the femur

DEXA of the femur was used to assess changes in BMC and areal BMD over the 42 day treatment period. PAPP-A KO mice are 40% smaller than their WT littermates and have proportionately smaller skeletons and lower BMD [16]. Thus, to directly compare the magnitude of PTH-induced changes in DEXA parameters between WT and PAPP-A KO mice, fold-increases for PTH-treated animals were calculated against vehicle-treated animals of the same genotype. Although femur area was not significantly different between groups due to high variability, BMC was significantly lower in PAPP-A KO than in WT mice, resulting in lower areal BMD (Fig. 3; Supplemental Table 1).

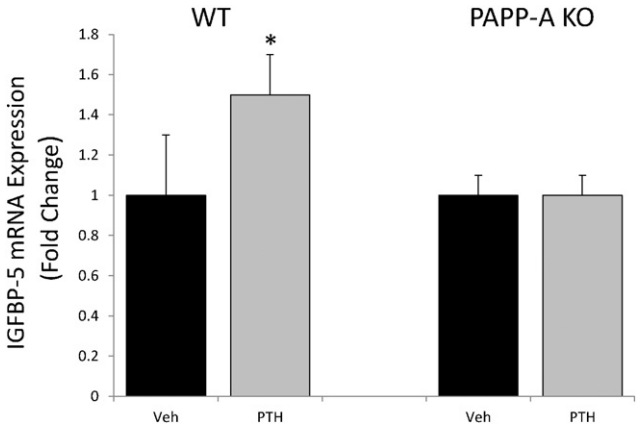

Fig. 2. In vivo biomarker of IGF receptor activation in response to intermittent PTH treatment. Expression of an IGF-responsive gene, IGFBP-5, was assessed by real-time PCR of RNA extracted from the proximal tibia of WT and PAPP-A KO mice. Fold change in PTH-treated mice is expressed relative to vehicle-treated mice of the same genotype. N = 10. *Significant effect of PTH treatment, $P < 0.05$.