0013-7227/05/$15.00/0
Printed in U.S.A.

Endocrinology 146(11):4577–4583
Copyright © 2005 by The Endocrine Society
doi: 10.1210/en.2005-0239

Chronic Elevation of Parathyroid Hormone in Mice Reduces Expression of Sclerostin by Osteocytes: A Novel Mechanism for Hormonal Control of Osteoblastogenesis

T. Bellido, A. A. Ali, I. Gubrij, L. I. Plotkin, Q. Fu, C. A. O'Brien, S. C. Manolagas, and R. L. Jilka

Division of Endocrinology and Metabolism, Center for Osteoporosis and Metabolic Bone Diseases, Central Arkansas Veterans Healthcare System, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205

Both chronic excess of PTH, as in hyperparathyroidism, and intermittent elevation of PTH (by daily injections) increase the number of osteoblasts; albeit, the former is associated with bone catabolism and the later with bone anabolism. Intermittent PTH increases osteoblast number by attenuating osteoblast apoptosis, an effect that requires the transcription factor Runx2. However, chronic elevation of PTH does not affect osteoblast apoptosis because it stimulates the proteasomal degradation of Runx2. Here, we studied the effects of PTH on Sost, a Runx2 target gene expressed in osteocytes (former osteoblasts embedded in the bone matrix), which antagonizes the pro-osteoblastogenic actions of bone morphogenetic proteins and Wnts. We report that continuous infusion of PTH to mice for 4 d decreased Sost mRNA expression in vertebral bone by 80–90%. This effect was accompanied by a comparable reduction of sclerostin, the product of Sost, in osteocytes, as determined by quantitative immunoblot analysis of bone extracts and by immunostaining. In contrast, a single injection of PTH caused a transient 50% reduction in Sost mRNA at 2 h, but four daily injections had no effect on Sost mRNA or sclerostin. PTH strongly decreased Sost expression in osteocytes formed in primary cultures of neonatal murine calvaria cells as well as in osteocytic MLO-A5 cells, demonstrating a direct effect of PTH on this cell type. These results, together with evidence that sclerostin antagonizes bone morphogenetic proteins and Wnts, strongly suggest that suppression of Sost by PTH represents a novel mechanism for hormonal control of osteoblastogenesis mediated by osteocytes. (*Endocrinology* 146: 4577–4583, 2005)

BOTH CHRONIC EXCESS of PTH, as in hyperparathyroidism, and intermittent elevation of PTH (by daily injections) increase the number of osteoblasts. However, although the former condition can lead to bone catabolism (1), intermittent administration of PTH causes bone anabolism (2, 3). In both situations, the rate of bone turnover is increased as evidenced by a simultaneous increase in osteoclast number as well as an elevation of serum markers of both formation and resorption. Whether the striking difference between bone loss and bone gain in the two conditions results from a negative *vs.* positive balance between bone formation and resorption within each bone remodeling unit, or from *de novo* bone formation not coupled to previous resorption in the case of intermittent PTH administration, remain unclear.

In any event, an increase in the number of osteoblasts, or osteoclasts for that matter, can be achieved by only two mechanisms: an increase in the rate of their production from progenitors or a decrease in the rate of their death by apoptosis, or a combination of the two (4). Recent studies of ours in mice indicate that chronic and intermittent PTH must increase osteoblast number by distinct mechanisms. Indeed, whereas the increase in osteoblast number and the anabolic effect of intermittent PTH can be accounted for by attenuation of osteoblast apoptosis (5, 6), chronic elevation of the endogenous hormone resulting from dietary calcium deficiency or continuous infusion of exogenous PTH had no effect on osteoblast survival (6). Hence, the increase in osteoblasts seen in chronic hyperparathyroidism must be the result of increased production.

An explanation for the different effects of chronic *vs.* intermittent PTH on osteoblast survival has been provided by the evidence that the osteoblast-specific transcription factor Runx2 is required for the antiapoptotic effect of PTH and that PTH also stimulates proteasomal proteolysis of Runx2 (6). Repeated injections of the hormone are therefore needed to inhibit osteoblast apoptosis because the duration of the PTH-induced survival signaling is self-limited by down-regulation of Runx2. In contrast, we have reasoned that chronic elevation of PTH is unable to attenuate osteoblast apoptosis because of the decrease of Runx2 below the threshold needed for hormone-mediated survival signaling. Direct effects on osteoblast production are unlikely because of extensive *in vitro* evidence that long-term exposure of osteoblast progenitors to PTH inhibits rather than stimulates their proliferation and differentiation (7).

PTH receptors are present in osteocytes (8), former osteoblasts embedded in bone. Osteocytes form a syncytium among themselves and with cells on the bone surface via processes (as many as 50 per cell) that radiate from each cell body and travel along canaliculi. This network is ideally suited to sense and respond to mechanical as well as systemic stimuli by generating signals that affect osteoblasts, oste-

First Published Online August 4, 2005

Abbreviations: b, Bovine; BMP, bone morphogenetic protein; Ct, threshold cycle; DMP1, dentin matrix protein 1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GFP, green fluorescent protein; h, human; L, lumbar; m, murine.

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

Downloaded from https://academic.oup.com/endo/article/146/11/4577/2499717 by guest on 20 August 2022

oclasts, and their progenitors in the bone marrow (9). Osteocytes, but no other cells of the osteoblastic lineage, express sclerostin, a Runx2-dependent product of the Sost gene (10–12). Sclerostin is a potent antagonist of bone morphogenetic protein (BMP)-2, -4, -5, -6, and -7 (10, 11) and also binds to LRP5/LRP6 preventing canonical Wnt signaling (13). Both BMPs and Wnts are critical for osteoblastogenesis because they provide the initial and essential stimulus for commitment of multipotential mesenchymal progenitors to the osteoblast lineage (14, 15). Importantly, it has been recently elucidated that loss of Sost in humans causes the high-bone mass disorders Van Buchem's disease (OMIM 239100) (16) and sclerosteosis (OMIM 269500) (17), and administration of an antisclerostin antibody increases bone formation in adult mice (18). Conversely, transgenic mice overexpressing Sost exhibit low bone mass (10). Taken together, these lines of evidence have led to the conclusion that sclerostin derived from osteocytes, the ultimate progeny of the osteoblast differentiation pathway, must be exerting a negative feedback control at the earliest step of mesenchymal stem cell differentiation toward the osteoblast lineage (10, 11).

Here, we show that chronic elevation of PTH dramatically down-regulates expression of Sost in osteocytes both *in vivo* and *in vitro*. Hence, the pro-osteoblastogenic effects of chronic PTH excess may be due, at least in part, to this previously unappreciated action of the hormone on osteocytes, which in turn unleashes the actions of BMPs and Wnts on mesenchymal progenitors.

Materials and Methods

Animals

Adult (5–6 months old) female Swiss Webster mice (n = 6–8 per group) were given human (h) PTH(1–84) (Bachem California, Inc., Torrance, CA) or vehicle (0.9% saline, 10 μM β-mercaptoethanol, and 0.01% acetic acid) either continuously at 500 ng/h using a microosmotic pump (Durect Corp., Cupertino, CA) or by daily injection at 230 ng/g for up to 4 d, concentrations previously shown to increase bone remodeling and induce bone anabolism in mice (6). Secondary hyperparathyroidism was induced in weanling female (23 d old) mice by feeding a calcium-deficient diet as we have previously described (19). At the end of the experiment, serum was obtained by retroorbital bleeding for measurement of osteocalcin (Biomedical Technologies, Soughton, MA). Effective delivery of the hormone was documented by measurement of circulating hPTH(1–84) or endogenous murine (m) PTH(1–84) by ELISA (Immunotopics, Inc., San Clemente, CA). Vertebral bone was dissected for analysis of gene and protein expression as described below. All animal protocols were approved by the Institutional Animal Care and Use Committees of the University of Arkansas for Medical Science and the Central Arkansas Veterans Health Care System.

Cell culture

Osteocytic MLO-A5 cells (20) were obtained from L.F. Bonewald (University of Missouri, Kansas City, MO) and cultured on collagen I-coated plates at an initial density of $10^4/cm^2$ for 12 d in α-MEM supplemented with 2.5% fetal bovine serum and 2.5% fetal calf serum. Osteoblastic cells from Swiss Webster mice or from dentin matrix protein 1 (DMP1)-green fluorescent protein (GFP) mice (21) (provided by David Rowe, University of Connecticut, Farmington, CT) were obtained from neonatal murine calvaria as previously described (6) and cultured at an initial density of $5 \times 10^4/cm^2$ for 6 d in the presence of α-MEM supplemented with 10% fetal bovine serum and 50 μg/ml ascorbic acid. Cultures were treated with bovine (b) PTH(1–34) (Bachem California, Inc.).

Immunocytochemistry

Lumbar (L) vertebrae (L3 and L4) were fixed in neutral buffered formalin, decalcified with 5% EDTA (pH 7.0) for 5–7 d, and embedded in paraffin. Sections were deparaffinized, and endogenous peroxidase activity was inhibited by 3% H_2O_2 treatment for 15 min. Subsequently, slides were blocked with 10% rabbit serum for 1 h in PBS and then incubated for 2 h with goat polyclonal antimouse Sost antibody (R&D Systems, Minneapolis, MN) diluted 1:100 in 2% rabbit serum. Nonimmune goat IgG was used as a negative control. After extensive rinsing, sections were incubated for 1 h with rabbit antigoat-horseradish peroxidase-conjugated secondary antibody (Santa Cruz Biotechnologies, Santa Cruz, CA) diluted 1:200 in 2% rabbit serum and developed with a 3,3′-diaminobenzidine substrate-chromogen system (Dako Corp., Carpinteria, CA) for up to 5 min. Sections were washed and counterstained with methyl green. Alternatively, tetramethylrhodamine B isothiocyanate-labeled rabbit antigoat secondary antibody was used to detect antisclerostin antibody binding in calvaria-derived cell cultures.

Western-blot analysis

Bone lysates were prepared from vertebral bone (L5 and L6) in 2% sodium dodecyl sulfate, 2 M urea, 10% glycerol, 10 mM Tris-HCl (pH 6.8), 10 mM dithiothreitol, and 1 mM phenylmethylsulfonylfluoride. Protein of lysates from individual mice (100 μg) was separated on a 10% sodium dodecyl sulfate gel and transferred to an Immobilon membrane (Millipore, Billerica, MA). The membrane was blocked with 3% BSA and 5% nonfat dry milk in Tris buffer saline containing 0.05% Tween 20 for 1 h at room temperature and then incubated with goat polyclonal antimouse Sost antibody (1:100 in 5% nonfat milk) overnight at 4 C. After rinsing, membranes were incubated with rabbit antigoat-horseradish peroxidase secondary antibody (1:2000 in 5% milk) for 1 h at room temperature and then developed with enhanced chemiluminescence (Pierce Biotechnology, Inc., Rockford, IL).

TaqMan PCR

Total RNA was extracted from vertebral bone (L1 and L2) using Ultraspec reagent (Biotecx Laboratories, Inc., Houston, TX). RNA (2 μg) was reverse-transcribed using a High Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA). The transcripts of interest and that of the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were amplified from first strand cDNA by real-time PCR using TaqMan Universal PCR Master Mix and Assay on Demand or Assay by Design primer and probe sets (Applied Biosystems). Amplification and detection were carried out with an ABI Prism 7300 Sequence Detection System (Applied Biosystems) as follows: 5-min denaturation at 95 C for 10 min, 40 cycles of amplification including denaturation at 94 C for 15 sec, and annealing/extension at 60 C for 1 min. Gene expression was quantified by subtracting the GAPDH threshold cycle (Ct) value from the Ct value of the gene of interest and expressed as $2^{-\Delta Ct}$, as described by the protocol of the manufacturer.

Statistics

Data were analyzed using SigmaStat (SPSS Science, Chicago, IL) or SAS software (SAS Institute Inc., Cary, NC). All values are reported as the mean ± SD. Differences in group means were evaluated with Student's *t* test or ANOVA.

Results

Adult Swiss-Webster mice were infused with hPTH(1–84) at 500 ng/h with an osmotic pump, which resulted in a circulating level of approximately 1 ng/ml. Endogenous PTH(1–84) in vehicle controls was approximately 15 pg/ml. Vertebral bone of mice receiving PTH exhibited a progressive decline in the level of Sost mRNA to as low as 10% of that found in contemporaneous vehicle controls after 24–96 h (Fig. 1A). The level of Sost mRNA was also suppressed by feeding mice a calcium-deficient diet for 7 d. In this model

Downloaded from https://academic.oup.com/endo/article/146/11/4577/2499717 by guest on 20 August 2022

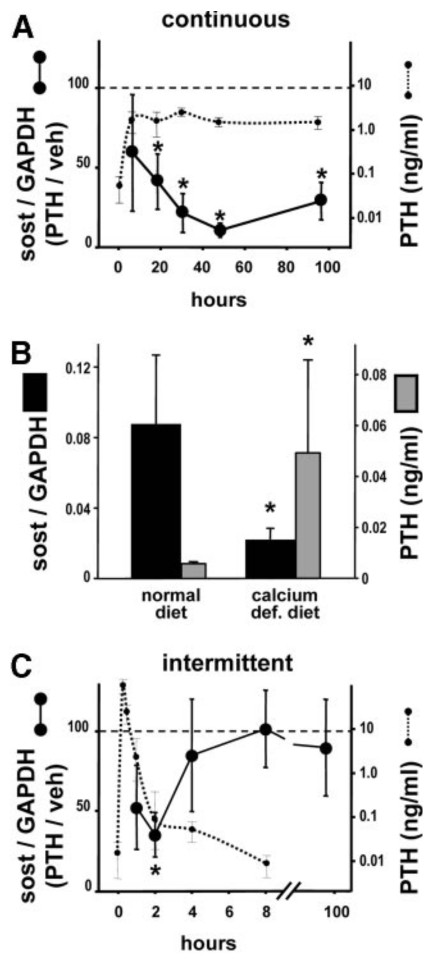

FIG. 1. Continuous administration of PTH induces a progressive and sustained decline in Sost mRNA in bone. Mice (six to eight per group) were given hPTH(1–84) or vehicle continuously with an osmotic pump (A), were fed a normal or a calcium-deficient diet for 7 d (B), or given hPTH(1–84) or vehicle by daily injections (C). Circulating levels of hPTH(1–84) (A and C) or mPTH(1–84) (B) were determined by ELISA. Total RNA was obtained from vertebral bones and the level of Sost transcripts quantified by real-time PCR, as indicated in *Materials and Methods*. *, $P < 0.05$ *vs.* contemporaneous vehicle control or *vs.* normal diet, by Student's t test.

of secondary hyperparathyroidism (19), endogenous PTH was increased to approximately 50 pg/ml (Fig. 1B). In contrast to continuous PTH, a single injection of 230 ng/g hPTH(1–84) caused a transient decline in Sost transcripts at 2 h after injection, corresponding with a transient increase in PTH that reached a peak of 40 ng/ml at 30 min (Fig. 1C). Sost mRNA levels were indistinguishable from controls by 4 h, corresponding to the disappearance of the injected PTH from the circulation. After four daily injections of PTH, the level of Sost mRNA, measured 24 h after the last injection, was indistinguishable from that of control animals.

The reduction in Sost mRNA caused by infused PTH resulted in a decrease in sclerostin protein as evidenced by Western-blot analysis of bone lysates obtained after 4 d of administration (Fig. 2A). In contrast, sclerostin levels did not significantly change in bones of animals receiving intermittent administration of the hormone. The sclerostin detected by Western blotting reflected protein expressed exclusively in osteocytes. This was established by positive immunocytochemical staining of vertebral bone sections with the same antibody used for Western blotting and lack of immunostaining in the osteoblasts seen in the same sections (Fig. 2, B and C, *lower panels*). There was some immunostaining in the bone marrow, but this was nonspecific, as indicated by its presence in serial sections stained with either nonimmune IgG or antisclerostin antibody (Fig. 2, B and C, *upper panels*). As expected for an osteocyte-derived secreted protein, high levels of sclerostin were also detected in canaliculi when the plane of section was parallel to the canalicular system (Fig. 2D). In agreement with the results of real-time PCR and Western blotting, continuous elevation of PTH for 4 d practically eliminated sclerostin immunostaining of osteocytes in bone sections, whereas intermittent administration of the hormone for the same period of time had minimal effect, if any (Fig. 2, E–G).

We next investigated in the same experiment whether other Runx2 target genes expressed by osteocytes were down-regulated by chronic or intermittent administration of PTH. Like Sost, osteocalcin and DMP1 are strongly regulated by Runx2 (22, 23), but unlike Sost, these proteins are highly expressed in both osteoblasts and osteocytes. During the first 48 h of PTH infusion, there was a progressive decline in osteocalcin mRNA to less than 10% of that found in vehicle controls, which was accompanied by a significant decrease in the amount of osteocalcin present in the circulation (Fig. 3A). On the other hand, a single injection of PTH caused only a modest and transient decline in osteocalcin mRNA at 2 h after injection, and there was no impact on circulating osteocalcin up to 8 h after the injection (Fig. 3B). The inhibitory effect of PTH on DMP1 transcripts exhibited a similar pattern (Fig. 3). Hence, the PTH-induced changes in osteocalcin and DMP1 expression temporally coincided with the changes in Sost during the first 48 h of administration. After this time, however, the effects of chronic or intermittent administration of PTH on osteocalcin and DMP1 diverged from those on Sost. Specifically, at 96 h, osteocalcin mRNA and circulating osteocalcin were increased, and DMP1 returned to control levels (Fig. 3). We previously demonstrated an increase in osteoblast number in mice after 96 h of continuous or intermittent administration of PTH (6). Therefore, the rebound of osteocalcin and DMP1 at 96 h likely reflects an increase in osteoblasts expressing these transcripts. On the other hand, Sost remained decreased at this time point, consistent with the fact that it is exclusively expressed in osteocytes. It is extremely unlikely that the number of these cells changed during this period of time.

The decreased expression of the three Runx2 target genes occurred in the absence of a significant decrease in Runx2 mRNA by either continuous or intermittent administration of the hormone (Fig. 3), consistent with PTH regulation of Runx2 levels by inducing proteasomal degradation of the protein (6). In fact, Runx2 expression was increased after 96 h of continuous administration of PTH, again reflecting the increase in osteoblast number at this time (6).

Downloaded from https://academic.oup.com/endo/article/146/11/4577/2499717 by guest on 20 August 2022

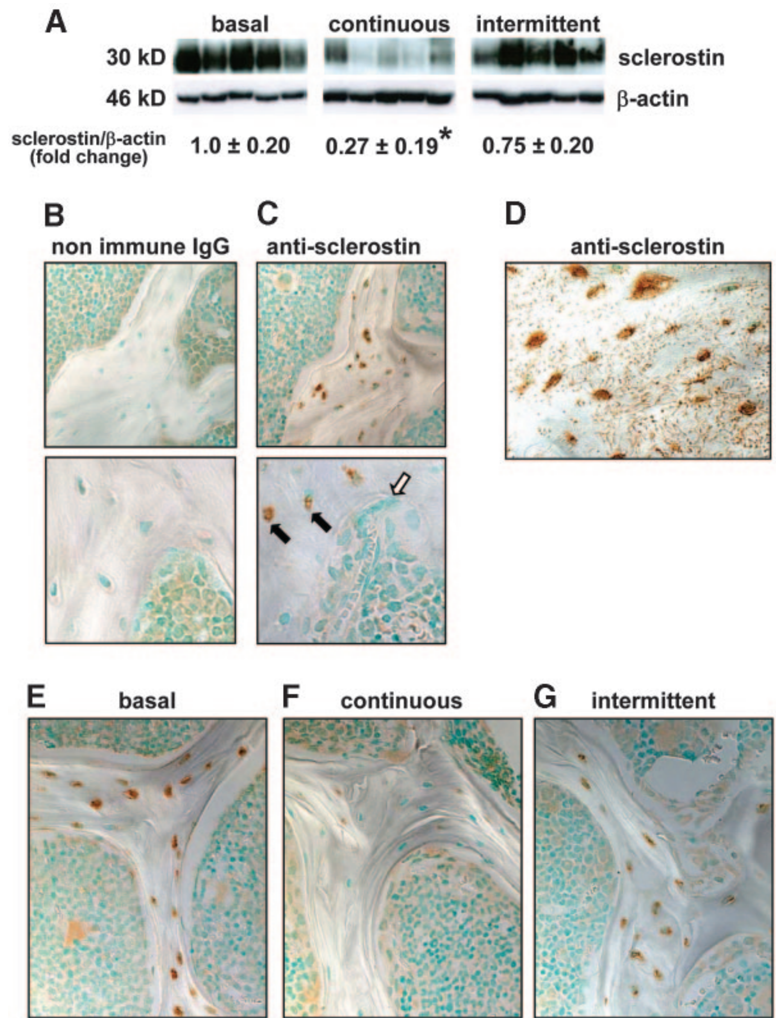

FIG. 2. Expression of sclerostin protein by osteocytes is markedly decreased by continuous but not intermittent elevation of PTH. Sclerostin levels were determined by Western-blot analysis of vertebral bone lysates (A) or by immunostaining of vertebral bone sections (B–G) obtained from animals receiving vehicle (basal) or hPTH(1–84) administered continuously for 4 d (continuous) or four daily injections of the hormone (intermittent). The same goat anti-mouse sclerostin antibody was used for the Western blotting and the immunohistochemistry. In A, each lane corresponds to a vertebral lysate derived from one mouse. Serial sections were stained with nonimmune IgG (B) or antisclerostin antibody (C). Low- and high-power images are shown in *upper* and *lower panels*, respectively, of B and C. Images were acquired using differential interference contrast optics and are representative of those obtained from a minimum of three vertebral sections from three to four animals per each treatment group in three independent experiments. *Black arrows*, Sclerostin-positive osteocytes; *white arrow*, row of sclerostin-negative osteoblasts. *, $P < 0.05$ *vs.* basal and intermittent PTH by ANOVA.

Osteocytes express PTH receptors (8). We therefore attempted to demonstrate whether our *in vivo* findings were the result of a direct effect of PTH on this cell type. Consistent with this contention, addition of 50 nM bPTH(1–34) to cultured osteocyte-like MLO-A5 cells reduced Sost mRNA expression by approximately 50% after 3 h and to less than 1% of control values after 24 h (Fig. 4A). Similarly, down-regulation of Sost mRNA by PTH was observed in cultures of MLO-Y4 osteocytic cells (not shown); however, basal levels of Sost were much lower than in MLO-A5 cells.

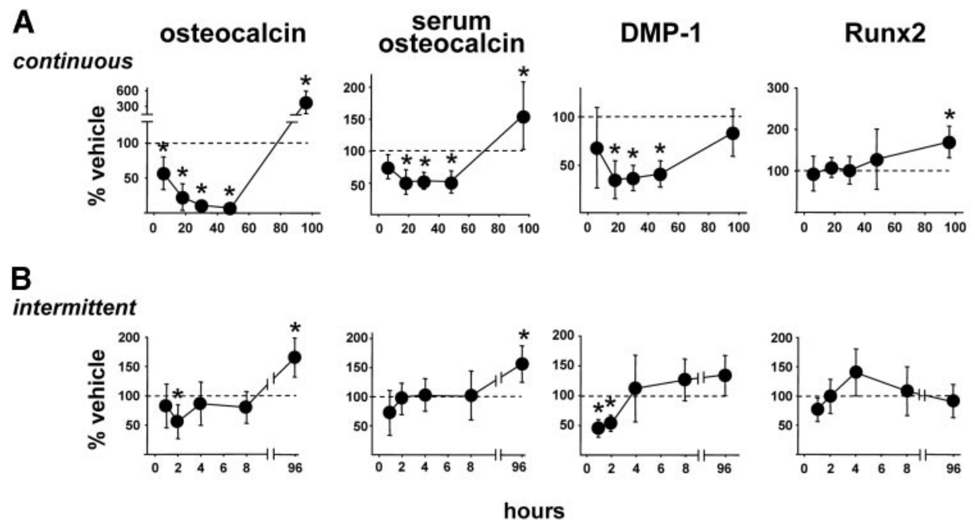

FIG. 3. PTH regulates the expression of the Runx2 target genes osteocalcin and DMP1 without affecting Runx2 mRNA. Expression of osteocalcin, DMP1, and Runx2 mRNA was quantified by real-time PCR in the same RNA samples analyzed for Sost expression in Fig. 1. Serum osteocalcin was determined by RIA. *, $P < 0.05$ *vs.* contemporaneous vehicle control by Student's *t* test.

Downloaded from https://academic.oup.com/endo/article/146/11/4577/2499717 by guest on 20 August 2022

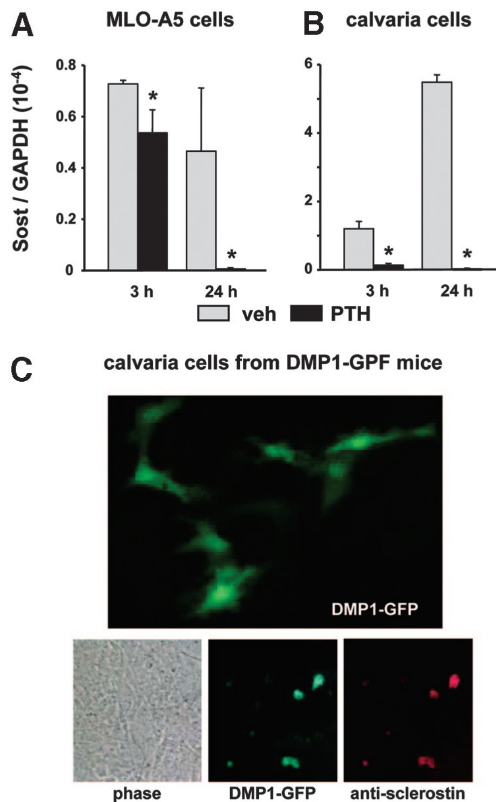

FIG. 4. Sost expression is down-regulated by PTH in cultured osteocytic and osteoblastic cells. Sost transcripts were quantified by real-time PCR in MLO-A5 cells (A) or neonatal murine calvaria cells (B) cultured for 6 d in the presence of 50 μg/ml ascorbic acid and then treated with vehicle or 50 nM bPTH(1–34) for the indicated times. *, $P < 0.05$ *vs.* contemporaneous vehicle controls by Student's *t* test. C, Presence of osteocytic cells expressing sclerostin in cultures of calvaria cells obtained from DMP1-GFP mice maintained as described above. *Green fluorescence* reflects GFP expression in osteocytes (21). Note the dendritic morphology of fluorescent cells in the high power image of the *top panel*. The *lower three panels* depict photomicrographs of the same microscopic field obtained with phase contrast optics or epifluorescence with a *green* filter set to detect GFP-expressing cells or a *red* filter set to detect cells stained by antisclerostin/tetramethylrhodamine B isothiocyanate-labeled rabbit antigoat secondary antibody.

Likewise, the level of Sost transcripts was dramatically reduced by 50 nM PTH to approximately 10% after 3 h and to 0.01% at 24 h, compared with vehicle control values in calvaria cell cultures maintained in the presence of ascorbic acid for up to 6 d (Fig. 4B). Previous evidence indicated that these culture conditions promote the development of osteocyte-like cells as visualized by transmission electron microscopy (24). Consistent with this, Sost expression increased progressively during culture in our experiment as manifested by the 5-fold increase in Sost transcripts between 6 and 7 d (Fig. 4B). Finally, to establish whether these Sost transcripts originated in osteocytes, we cultured calvaria cells from a mouse in which the expression of GFP is driven by an 8-kb region of the DMP1 promoter. In these mice, GFP expression is limited to osteocytes in bone and in mineralizing bone marrow-derived cell cultures (21). We found that GFP-positive cells were present at 6 d of culture and that many of them exhibited the dendritic morphology that characterizes osteocytes (Fig. 4C, *upper panel*). Moreover, the majority of GFP-labeled cells stained positive with the antisclerostin antibody (Fig. 4C, *lower panels*). Therefore, PTH-induced reduction in Sost expression in calvaria cells shown in Fig. 4B reflects actions of the hormone on osteocytes.

Discussion

We have previously demonstrated *in vitro* and *in vivo* that osteocytes are direct targets of systemic hormones like estrogens, androgens, and glucocorticoids (25, 26). The findings described in this report demonstrate that chronic elevation of PTH in mice dramatically decreases the expression of Sost in osteocytes. Osteocytes, therefore, are also targets of PTH actions. This conclusion, taken together with evidence that Sost antagonizes the pro-osteoblastogenic actions of BMPs and Wnts (10, 11, 14, 15), strongly supports the contention that a PTH-induced suppression of Sost secretion by osteocytes unleashes the actions of these proosteoblastogenic cytokines to cause commitment of multipotential mesenchymal progenitors to the osteoblast lineage (Fig. 5). Hence, the increased production of osteoblasts needed for the increased bone turnover characteristic of hyperparathyroidism may result from such an indirect effect of PTH on the earliest stage of osteoblastogenesis. It has been shown before that PTH acts on stromal/osteoblastic cells to stimulate the production of IGFs and other growth factors (7) and that PTH-stimulated osteoclastic bone resorption releases TGFβ from the bone matrix (27). It is therefore possible that the Sost-mediated effects of PTH on osteoblastogenesis are amplified by these other actions.

In contrast to the effects of continuous PTH elevation, we found that intermittent PTH elevation only transiently affected Sost mRNA levels, consistent with a recent report in rats (28). However, intermittent PTH did not significantly affect the expression of Sost mRNA or sclerostin protein in our 4-d-long studies. In the absence of definitive evidence for decreased sclerostin protein expression in response to intermittent PTH, we tentatively conclude that decreased sclerostin expression does not substantially contribute to the increase in osteoblast number resulting from daily injections of PTH. However, it remains possible that small transient de-

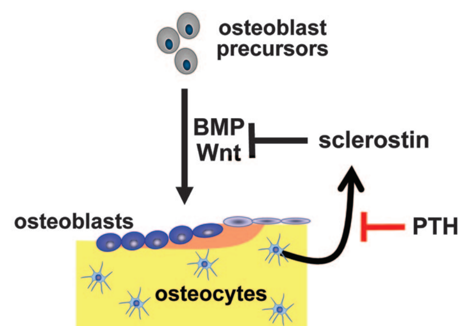

FIG. 5. Regulation of osteoblastogenesis via actions of PTH on osteocytes. The model depicts the sclerostin-mediated negative feedback loop by which osteocytes suppress bone formation (10) (*black lines*) and the inhibitory effect of PTH on sclerostin secretion by osteocytes (*red line*), which unleashes the prodifferentiating actions of BMP and Wnt signaling on osteoblast progenitors.

Downloaded from https://academic.oup.com/endo/article/146/11/4577/2499717 by guest on 20 August 2022

creases in sclerostin secretion after each PTH injection cause bursts of BMP and/or Wnt signaling which stimulate osteoblastogenesis.

In view of recent evidence that sclerostin stimulates apoptosis of cultured human osteoblastic cells (29), it is conceivable that transient reductions in sclerostin levels with daily injections of PTH could contribute to the antiapoptotic signaling triggered by this regimen (5, 6). However, this seems unlikely because, if this were the case, sustained elevation of PTH should also decrease osteoblast apoptosis, but it does not (6).

In the present studies, PTH infusion strongly down-regulated the synthesis of two other Runx2 target genes, osteocalcin and DMP1. These findings are consistent with the evidence that PTH suppresses osteocalcin synthesis in cultured osteoblastic cells (for review, see Ref. 7). Our findings are also consistent with a transient decrease in circulating osteocalcin in response to infusion of PTH or PTH-related peptide in humans (30). Decreased Runx2 target gene expression could also result from decreased synthesis of Runx2 or a decrease in its transcriptional activity. However, we did not observe a decrease in Runx2 transcript levels, and rather than a decrease, other investigators have shown that PTH can cause a transient increase in Runx2 activity (31). These observations support the notion that the dramatic decrease in Sost expression caused by chronically elevated PTH is mediated in part by the ability of the hormone to stimulate Runx2 proteasomal degradation (6).

In conclusion, the results of the present report demonstrate that osteocytes are targets for the actions of PTH and that PTH acts to decrease Sost/sclerostin expression in these cells, probably as a consequence of its ability to increase proteasomal degradation of Runx2. Considering this, the evidence that sclerostin is a paracrine factor (a fact highlighted by the demonstration of its presence in the canalicular system in this report, Fig. 2D), and that it is a potent antagonist of the actions of BMPs and Wnts, we propose that suppression of Sost/sclerostin by PTH represents a novel mechanism for hormonal control of osteoblastogenesis. Further work will be needed to determine whether changes in osteocyte-derived sclerostin are involved in the control of bone formation by other hormones, locally produced growth factors, or mechanical stimuli; and whether regulation of other osteocyte products by PTH are involved in the profound effects of the hormone on bone.

Acknowledgments

The authors thank X. Liu, R. Wynne, and K. Vyas for their technical assistance and Robert S. Weinstein for valuable discussions.

Received February 28, 2005. Accepted July 26, 2005.

Address all correspondence and requests for reprints to: Teresita Bellido, Ph.D., Division of Endocrinology and Metabolism, Slot 587, University of Arkansas for Medical Sciences, 4301 West Markham, Little Rock, Arkansas 72205. E-mail: tmbellido@uams.edu.

This work was supported by the National Institutes of Health (Grants K02 AR02127 to T.B., R01 AR049794 to C.A.O., R01 AR046823 to R.L.J., and P01 AG013918 to S.C.M.) and by the Department of Veterans Affairs (Merit Reviews to R.L.J. and S.C.M. and Research Enhancement Award Program to S.C.M.).

References

1. **Parisien M, Dempster DW, Shane E, Bilezikian JP** 2001 Histomorphometric analysis of bone in primary hyperparathyroidism. In: Bilezikian JP, Marcus R, Levine MA, eds. The parathyroids. Basic and clinical concepts. San Diego: Academic Press; 423–436
2. **Neer RM, Arnaud CD, Zanchetta JR, Prince R, Gaich GA, Reginster JY, Hodsman AB, Eriksen EF, Ish-Shalom S, Genant HK, Wang O, Mitlak BH** 2001 Effect of parathyroid hormone (1–34) on fractures and bone mineral density in postmenopausal women with osteoporosis. N Engl J Med 344:1434–1441
3. **Dempster DW, Cosman F, Parisien M, Shen V** 1993 Anabolic actions of parathyroid hormone on bone. Endocr Rev 14:690–709
4. **Manolagas SC** 2000 Birth and death of bone cells: basic regulatory mechanisms and implications for the pathogenesis and treatment of osteoporosis. Endocr Rev 21:115–137
5. **Jilka RL, Weinstein RS, Bellido T, Roberson P, Parfitt AM, Manolagas SC** 1999 Increased bone formation by prevention of osteoblast apoptosis with parathyroid hormone. J Clin Invest 104:439–446
6. **Bellido T, Ali AA, Plotkin LI, Fu Q, Gubrij I, Roberson PK, Weinstein RS, O'Brien CA, Manolagas SC, Jilka RL** 2003 Proteasomal degradation of Runx2 shortens parathyroid hormone-induced anti-apoptotic signaling in osteoblasts: a putative explanation for why intermittent administration is needed for bone anabolism. J Biol Chem 278:50259–50272
7. **Aubin JE, Heersche JNM** 2001 Cellular actions of parathyroid hormone on osteoblast and osteoclast differentiation. In: Bilezikian JP, Marcus R, Levine MA, eds. The parathyroids. Basic and clinical concepts. San Diego: Academic Press; 199–211
8. **Fermor B, Skerry TM** 1995 PTH/PTHrP receptor expression on osteoblasts and osteocytes but not resorbing bone surfaces in growing rats. J Bone Miner Res 10:1935–1943
9. **Nijweide PJ, Burger EH, Klein-Nulend J** 2002 The osteocyte. In: Bilezikian JP, Raisz LG, Rodan G, eds. Principles of bone biology. 2nd ed. San Diego: Academic Press; 93–107
10. **Winkler DG, Sutherland MK, Geoghegan JC, Yu C, Hayes T, Skonier JE, Shpektor D, Jonas M, Kovacevich BR, Staehling-Hampton K, Appleby M, Brunkow ME, Latham JA** 2003 Osteocyte control of bone formation via sclerostin, a novel BMP antagonist. EMBO J 22:6267–6276
11. **Van Bezooijen RL, Roelen BA, Visser A, Wee-Pals L, De Wilt E, Karperien M, Hamersma H, Papapoulos SE, Ten Dijke P, Lowik CW** 2004 Sclerostin is an osteocyte-expressed negative regulator of bone formation, but not a classical BMP antagonist. J Exp Med 199:805–814
12. **Sevetson B, Taylor S, Pan Y** 2004 Cbfa1/RUNX2 directs specific expression of the sclerosteosis gene (SOST). J Biol Chem 279:13849–13858
13. **Li X, Zhang Y, Kang H, Liu W, Liu P, Zhang J, Harris SE, Wu D** 2005 Sclerostin binds to LRP5/6 and antagonizes canonical Wnt signaling. J Biol Chem 280:19883–19887
14. **Canalis E, Economides AN, Gazzerro E** 2003 Bone morphogenetic proteins, their antagonists, and the skeleton. Endocr Rev 24:218–235
15. **Kolpakova E, Olsen BR** 2005 Wnt/[β]-Catenin: a canonical tale of cell-fate choice in the vertebrate skeleton. Dev Cell 8:626–627
16. **Balemans W, Ebeling M, Patel N, Van Hul E, Olson P, Dioszegi M, Lacza C, Wuyts W, Van Den Ende J, Willems P, Paes-Alves AF, Hill S, Bueno M, Ramos FJ, Tacconi P, Dikkers FG, Stratakis C, Lindpaintner K, Vickery B, Foernzler D, Van Hul W** 2001 Increased bone density in sclerosteosis is due to the deficiency of a novel secreted protein (SOST). Hum Mol Genet 10:537–543
17. **Brunkow ME, Gardner JC, Van Ness J, Paeper BW, Kovacevich BR, Proll S, Skonier JE, Zhao L, Sabo PJ, Fu Y, Alisch RS, Gillett L, Colbert T, Tacconi P, Galas D, Hamersma H, Beighton P, Mulligan J** 2001 Bone dysplasia sclerosteosis results from loss of the SOST gene product, a novel cystine knot-containing protein. Am J Hum Genet 68:577–589
18. **Warmington K, Morony S, Sarosi I, Gong G, Stephens P, Winkler DG, Sutherland MK, Latham JA, Kirby H, Moore A, Robinson M, Kostenuik PJ, Simonet S, Lacey DL, Paszty C**2004 Sclerostin antagonism in adult rodents, via monoclonal antibody mediated blockade, increases bone mineral density and implicates sclerostein as a key regulator of bone mass during adulthood. J Bone Miner Res 19:S56 (Abstract)
19. **O'Brien CA, Jilka RL, Fu Q, Stewart S, Weinstein RS, Manolagas SC** 2005 IL-6 is not required for parathyroid hormone stimulation of RANKL expression, osteoclast formation, and bone loss in mice. Am J Physiol Endocrinol Metab 10.1152/ajpendo.00029.2005
20. **Kato Y, Boskey A, Spevak L, Dallas M, Hori M, Bonewald LF** 2001 Establishment of an osteoid preosteocyte-like cell MLO-A5 that spontaneously mineralizes in culture. J Bone Miner Res 16:1622–1633
21. **Kalajzic I, Braut A, Guo D, Jiang X, Kronenberg MS, Mina M, Harris MA, Harris SE, Rowe DW** 2004 Dentin matrix protein 1 expression during osteoblastic differentiation, generation of an osteocyte GFP-transgene. Bone 35:74–82
22. **Ducy P, Zhang R, Geoffroy V, Ridall AL, Karsenty G** 1997 Osf2/Cbfa1: a transcriptional activator of osteoblast differentiation. Cell 89:747–754
23. **Feng JQ, Zhang J, Dallas SL, Lu Y, Chen S, Tan X, Owen M, Harris SE,**

Downloaded from https://academic.oup.com/endo/article/146/11/4577/2499717 by guest on 20 August 2022

MacDougall M 2002 Dentin matrix protein 1, a target molecule for Cbfa1 in bone, is a unique bone marker gene. J Bone Miner Res 17:1822–1831
24. **Herbert B, Lecouturier A, Masquelier D, Hauser N, Remacle C** 1997 Ultrastructure and cytochemical detection of alkaline phosphatase in long-term cultures of osteoblast-like cells from rat calvaria. Calcif Tissue Int 60:216–223
25. **Kousteni S, Bellido T, Plotkin LI, O'Brien CA, Bodenner DL, Han L, Han K, DiGregorio GB, Katzenellenbogen JA, Katzenellenbogen BS, Roberson PK, Weinstein RS, Jilka RL, Manolagas SC** 2001 Nongenotropic, sex-nonspecific signaling through the estrogen or androgen receptors: dissociation from transcriptional activity. Cell 104:719–730
26. **O'Brien CA, Jia D, Plotkin LI, Bellido T, Powers CC, Stewart SA, Manolagas SC, Weinstein RS** 2004 Glucocorticoids act directly on osteoblasts and osteocytes to induce their apoptosis and reduce bone formation and strength. Endocrinology 145:1835–1841
27. **Pfeilschifter J, Mundy GR** 1987 Modulation of type β transforming growth factor activity in bone cultures by osteotropic hormones. Proc Natl Acad Sci USA 84:2024–2028
28. **Keller H, Kneissel M** 2005 SOST is a target gene for PTH in bone. Bone 37:148–158
29. **Sutherland MK, Geoghegan JC, Yu C, Turcott E, Skonier JE, Winkler DG, Latham JA** 2004 Sclerostin promotes the apoptosis of human osteoblastic cells: a novel regulation of bone formation. Bone 35:828–835
30. **Horwitz MJ, Gundberg CM, Tedesco M, Sereika S, Garcia-Ocana A, Bisello A, Marshall G, Rosen CJ, Stewart AF**2004 The temporal profile in humans of the subacute skeletal anabolic response to continuous infusion of PTHrP and PTH. J Bone Miner Res 19:S18 (Abstract)
31. **Krishnan V, Moore TL, Ma YL, Helvering LM, Frolik CA, Valasek KM, Ducy P, Geiser AG** 2003 PTH bone anabolic action requires Cbfa1/Runx2-dependent signaling. Mol Endocrinol 17:423–435

***Endocrinology* is published monthly by The Endocrine Society (http://www.endo-society.org), the foremost professional society serving the endocrine community.**

Downloaded from https://academic.oup.com/endo/article/146/11/4577/2499717 by guest on 20 August 2022