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The Effects of Programmed Administration of Human Parathyroid Hormone Fragment (1–34) on Bone Histomorphometry and Serum Chemistry in Rats*

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ABSTRACT

PTH treatment can result in dramatic increases in cancellous bone volume in normal and osteopenic rats. However, this potentially beneficial response is only observed after pulsatile treatment; continuous infusion of PTH leads to hypercalcemia and bone abnormalities. The purpose of these studies was to determine the optimal duration of the PTH pulses. A preliminary study revealed that human PTH-(1–34) (hPTH) is cleared from circulation within 6 h after sc administration of an anabolic dose of the hormone (80 μg/kg). To establish the effects of gradually extending the duration of exposure to hPTH without increasing the daily dose, we programmed implanted Alzet osmotic pumps to deliver the 80 μg/kg·day dose of the hormone during pulses of 1, 2, and 6 h/day, or 40 μg/kg·day continuously. Discontinuous infusion was accomplished by alternate spacing of external tubing with hPTH solution and sesame oil. After 6 days of treatment, we evaluated serum chemistry and bone histomorphometry. As negative and positive controls, groups of rats received pumps that delivered vehicle only and 80 μg/kg·day hPTH by daily sc injection, respectively. Dynamic and static bone histomorphometry revealed that the daily sc injection and 1 h/day infusion dramatically increased osteoblast number and bone formation in the proximal tibial metaphysis, whereas longer infusion resulted in systemic side-effects, including up to a 10% loss in body weight, hypercalcemia, and histological changes in the proximal tibia resembling abnormalities observed in patients with chronic primary hyperparathyroidism, including peritrabecular marrow fibrosis and focal bone resorption. Infusion for as little as 2 h/day resulted in minor weight loss and changes in bone histology that were intermediate between sc and continuous administration. The results demonstrate that the therapeutic interval for hPTH exposure is brief, but that programmed administration of implanted hormone is a feasible alternative to daily injection as a route for administration of the hormone. (*Endocrinology* **138:** 4607–4612, 1997)

PULSATILE treatment with PTH, which is usually accomplished by one to three daily sc injections, increases cancellous bone volume in normal and osteopenic rats (1–3). The beneficial effects of pulsatile PTH treatment do not require weight bearing (4) or gonadal hormones (5–9). GH was formally believed to be essential for the anabolic effects of PTH on bone (10), but recent studies suggest that this may not be the case (11). Although evidence for an increase in osteoblast activity is often observed (5, 11, 12), the increased bone mass is primarily due to a dramatic increase in osteoblast number (11, 12). Recent studies suggest that the PTH-induced increase in osteoblast number is mainly due to modulation of bone-lining cells to express the osteoblast phenotype (12, 13).

The potent anabolic actions of PTH on rat bone has stimulated considerable interest in the possible therapeutic use of the hormone for treating human skeletal disorders and has been recently reviewed by Mosekilde and Reeve (14). An especially important currently unresolved clinical problem that might be amenable to PTH therapy is the restoration of bone to the severely osteopenic skeleton. There are, however, serious questions regarding possible limitations of PTH therapy, including undesirable side-effects and parenteral mode of administration of the hormone.

Recent reviews document that continuous exposure to elevated PTH in humans and laboratory animals results in hypercalcemia and a variety of abnormalities in bone (14–16). The purpose of the present study was to more precisely determine the duration of exposure to PTH that results in increased bone formation without hypercalcemia. Additionally, we performed these studies to establish whether programmed administration of implanted hormone is a feasible alternative to daily injection.

Materials and Methods

Animals

All animal procedures received prior approval by the institutional welfare committee. The rats used in these studies were obtained from Harlan Sprague-Dawley (Madison, WI). The animals were maintained in group cages (three or four rats per cage) and given food and water *ad libitum*.

Preliminary study: clearance of PTH

The time course for clearance of PTH from the circulation was estimated after sc administration of human PTH-(1–34) (hPTH; Bachem, Torrance, CA; 80 μg/kg BW) to 22 14-month-old virgin female rats. hPTH was dissolved in a solution containing 150 mM NaCl, 1 mM HCl, and 2% heat-inactivated rat serum. The rats were anesthetized with ethrane and killed with exsanguination via the abdominal aortic artery. Serum was frozen at −70 C until assayed for immunoreactive PTH (iPTH).

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Study 1: effects of programmed administration of hPTH for 6 h/day

The purpose was to compare programmed infusion of hPTH for 6 h/day with sc administration of the hormone. Three groups of virgin 6-month-old female rats with six or seven animals in each group were studied. One group received hPTH by sc injection (80 μg/kg·day) each day for 6 days. The two other groups received osmotic pumps with a tubing system external to the pump that was designed to deliver vehicle alone or hPTH (80 μg/kg·day) for 6 h/day for 6 days.

The reservoir volume of the pumps was filled with saline solution, and the pumps were primed at 37 C for 6 h sitting in a microcentrifuge tube also filled with saline before the delivery portal of the pump was connected to a coiled external tubing system consisting of PE60 polyethylene tubing (Becton Dickinson, Sparks, MD). The tubing itself was prepared in the following way; a regular straight piece of tubing material was wrapped around a pencil with both ends fixed to the pencil, then submerged in boiling water for 3 min and chilled in an ice-cold water bath. The coil was then loaded with sesame oil alone (vehicle pump group) or interspaced with 6 μl hPTH solution to produce a regular pulsatile release of 6 h "on hormone" followed by 18 h "off hormone," respectively. A methylene blue dye mark of 2 μl was positioned at the end of the coil facing the outlet of the pump. This marker migrated toward the opening end of the tubing system during the course of the study and indicated proper emptying of the vehicle or hormone at the time of death.

A calcein (20 mg/kg BW; Sigma Chemical Co., St. Louis, MO) and tetracycline (20 mg/kg BW; Sigma) label was given by perivascular tail injection on days 0 and 5 to all animals.

Study 2: effects of programmed (1 and 2 h/day) and continuous administration of hPTH

The purpose was to further refine the optimal duration of infusion of hPTH by programmed administration of the hormone. Five groups of 4-month-old male rats, with 4–10 animals in each group, were studied. One group received hPTH daily (80 μg/kg·day) for 6 days by sc injection. The remaining 4 groups were implanted with Alzet osmotic pumps as described for study 1 (Alza Corp., Palo Alto, CA), which were designed to deliver for 6 days vehicle solution only, continuous hPTH (40 μg/kg·day), and intermittent hPTH at 1 and 2 h/day (80 μg/kg·day) at a nominal pumping rate of 1 μl/h.

Serum chemistry

Total serum calcium, serum phosphorous, and alkaline phosphatase activity were determined using a Hitachi 717 (Hitachi, Hialeah, FL).

Serum iPTH was measured using an immunoradiometric assay for rat PTH (Nichols Institute, San Juan Capistrano, CA) that exhibits cross-reactivity for hPTH of approximately 100%. Rat serum samples with expected high values of hPTH were diluted up to 20-fold with the 0 pg/ml rat PTH standard and assayed. In rats that were not injected or infused with hPTH, the measured concentrations reflect endogenously secreted "rat" PTH. For measurements of 1,25-dihydroxyvitamin D (1,25D), serum samples were dilipidated, extracted with a highly specific solid phase monoclonal anti-1,25D, and quantitated by RIA (IDS, Boldon, UK). High 1,25D concentrations were measured after a 10-fold dilution of the samples with the zero calibrator.

Histomorphometry

Histomorphometric procedures were carried out using an imaging system that has been described in detail previously (11).

Cancellous bone measurements made on stained sections

The metaphysis was dehydrated, embedded without demineralization, and sectioned as previously described (11).

Bone architecture measurements and calculations

Cancellous bone area was determined in unstained 5-μm thick sections as the area of total cancellous bone per mm^2 metaphyseal tissue within the sampling site and expressed as a percentage (tissue referent). Cancellous bone perimeter was expressed as the perimeter of cancellous bone per mm^2 metaphyseal sampling area (tissue referent). Calculations relating to cancellous bone perimeter and area (17) were the following: 1) trabecular thickness, calculated as the cancellous bone perimeter (millimeters) divided by the cancellous bone area (square millimeters per mm^2), then dividing that number by 2 and multiplying by 1000 (millimeters); 2) trabecular number, defined as the cancellous bone area (square millimeters per mm^2) divided by the trabecular thickness (microns); and 3) trabecular separation, calculated as the trabecular thickness (millimeters) divided by the cancellous bone area (square millimeters per mm^2).

Bone cell measurements

The cancellous bone perimeters lined by osteoblasts and osteoclasts were measured and expressed as percentages (perimeter referent). Briefly, undemineralized 5-μm thick sections were stained with toluidine blue. Osteoclast perimeter was determined as the cancellous perimeter lined by multinucleated cells. These cells usually had other characteristics of osteoclasts, including a foamy cytoplasm and location in a pronounced lacuna. Osteoblasts were identified as a palisade of large basophilic cuboidal cells directly lining a bone perimeter. The peritrabecular fibrotic marrow perimeter was identified as the bone perimeter lined by multiple layers of fibroblasts (see Fig. 3).

Bone formation measurements and calculations

The bone formation rate (perimeter referent) was calculated as the sum of the double labeled perimeter (microns) plus half of the single labeled perimeter multiplied by the mineral apposition rate (microns per day) and divided by the total bone perimeter (millimeters per mm^2). The mineral apposition rate was the mean distance between the calcein label and the tetracycline label divided by the interlabel period of 5 days. The calcein label perimeter (tissue area referent) was measured as the length of label (millimeters) per mm^2 cancellous tissue area.

Results

The time course for clearance of iPTH from circulation is shown in Fig. 1. The maximum concentration of iPTH (>15,000 pg/ml) was observed 15 min after hormone ad-

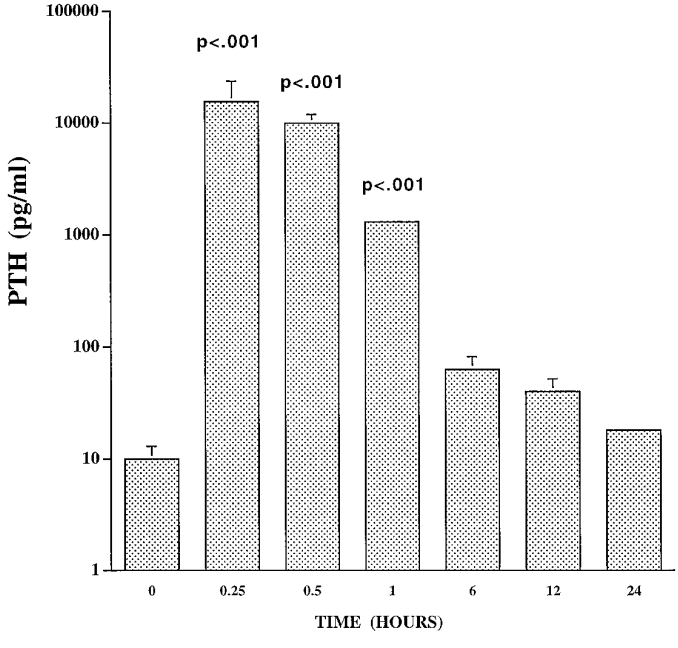

FIG. 1. hPTH was rapidly cleared from the circulation after sc administration of 80 μg/kg. Values are the mean ± SE (n = 2–4).

ministration. The serum levels of the hormone declined rapidly; they were less than 10% of the peak value at 1 h and did not differ from the pretreatment value at 6 h. The calculated $t_{1/2}$ for the disappearance of serum iPTH was 11 min. Subsequent studies designed to determine the optimal duration of programmed infusion of hPTH focused on pulses of 6 h in duration or less.

The effects of PTH on body weight are shown in Table 1 (A and B). Infusion of hPTH at concentrations of 2 h/day and higher led to a significant decrease in body weight, with a mean reduction of up to 11% in the group with continuous hPTH infusion. Unexpectedly, programmed infusion of hPTH for 6 h/day led to a mortality rate of 43% (three of seven rats died). In the subsequent study, the dose rate in the group continuously infused with hPTH was reduced to decrease the risk of life-threatening hypercalcemia; none of the animals in the other groups died during the course of the study.

The effects of hPTH on serum chemistry are shown in Table 2 (A and B) and Fig. 2. Daily sc hPTH administration for 7 days to female rats (Table 2A) had no effect on serum chemistry, which consisted of measurements of calcium, phosphorous, alkaline phosphatase activity, and iPTH. In contrast, programmed infusion of hPTH for 6 h/day resulted in severe hypercalcemia, no change in phosphorous, depressed alkaline phosphatase activity, and greatly elevated iPTH. In male rats (Table 2B), sc PTH had no effect on calcium or alkaline phosphatase, and increased phosphorous and $1,25D_3$ levels (Fig. 2). Programmed infusion of hPTH for either 1 or 2 h/day had no effect on serum chemistry. In contrast, continuous infusion of hPTH resulted in hypercalcemia, no change in phosphorous, depressed alkaline phosphatase activity, increased iPTH, and decreased $1,25D_3$.

As illustrated in Fig. 3, continuous (male rats) and programmed infusion of hPTH for 6 h/day (female rats) resulted in similar marked histological changes in the proximal tibia, consisting of multiple layers of fibroblastic cells encircling trabecular profiles. Additionally, focal increases in resorption lacunae with osteoclasts were observed. However, equal or larger doses of hPTH administered sc or by programmed infusion for up to 2 h/day did not result in abnormal bone histology.

The effects of hPTH on cancellous bone histomorphometry were evaluated in male rats and are summarized in Tables 3–5. Short term sc treatment, continuous infusion, and programmed infusion for 2 h/day with hPTH had no effect on indexes of cancellous bone architecture, consisting of bone area, trabecular number, trabecular thickness, and trabecular separation (Table 3). In contrast, programmed infusion of hPTH for 1 h/day resulted in increases in bone area and trabecular number, no change in trabecular thickness, and a decrease in trabecular separation. Subcutaneous administration and programmed infusion of hPTH for 1 h/day resulted in increases in dynamic bone measurements and calculated bone values, consisting of double label perimeter, mineral apposition rate, and bone formation rate (Table 4). Continuous infusion of hPTH had no effect on dynamic bone histomorphometry, whereas programmed administration of hPTH for 2 h/day increased the mineral apposition rate, but did not influence the double label perimeter or bone formation rate Table 5. The effects of hPTH on cancellous perimeter lined by osteoblasts, osteoclasts, and intertrabecular fibrotic marrow in male rats are shown in Table 4. Osteoblast perimeter was increased in rats treated with hPTH sc and with programmed infusion of PTH for 1 h/day. Continuous treatment and programmed infusion of the hormone for 2 h/day had no effect on osteoblast perimeter. The only change in osteoclast number in males was an increase after continuous infusion with hPTH. Similarly, peritrabecular fibrotic marrow was only observed in male rats after continuous infusion with hPTH.

TABLE 1. Effects of hPTH-(1–34) on body weight in male and female rats

Group	n	Starting wt (g)	Ending wt (g)	Change in wt (%)
Female rats				
VEH pump	7	293 ± 3	300 ± 4	2.5 ± 4
PTH sc	6	293 ± 4	302 ± 5	3.2 ± 1.0
PTH pump (6 h)	4	301 ± 6	273 ± 4[a]	−9.3 ± 1.4[a]
Male rats				
VEH pump	10	420 ± 4	436 ± 4	3.7 ± 0.9
PTH sc	7	429 ± 4	430 ± 5[b]	0.2 ± 0.6[b]
PTH pump (1 h)	4	421 ± 4	425 ± 4[b]	1.2 ± 0.4[b]
PTH pump (2 h)	6	429 ± 6	418 ± 4[b]	−2.7 ± 1.1[b]
PTH pump (continuous)	8	426 ± 6	378 ± 15[a]	−10.9 ± 3.3[a]

Values are the mean ± SE.
[a] $P < 0.001$ *vs.* VEH pump.
[b] $P < 0.05$ *vs.* VEH pump.

Discussion

Continuous infusion of hPTH into rats was shown in this and earlier studies (1) to result in severe hypercalcemia. Additionally, continuous hPTH resulted in fibrotic bone similar to that sometimes observed in patients with chronically elevated iPTH levels (16). These detrimental effects of the hormone contrast with the well established dramatic increases in cancellous bone volume, osteoblast perimeter, and bone formation observed in rats after daily sc administration of similar or greater amounts of the hormone (2–10).

Intermittent and programmed administration of hPTH for 1 h/day resulted in large increases in the label perimeter and calculated bone formation rate and small increases in the mineral apposition rate. The dynamic measurements may underestimate the calculated mineral apposition rate and bone formation rate, because the initial fluorochrome label was administered before initiating hormone treatment.

The noted differential effects of chronic and intermittent exposure to hPTH are well recognized, but the precise mechanisms that mediate the respective responses have not been identified. Daily sc hPTH treatment increases bone mass over a wide dose range, without resulting in hypercalcemia. Additionally, the hormone can be administered as often as three times per day (3). In contrast, continuous infusion of hPTH, although leading to much lower peak blood levels of the hormone (compare Fig. 1 and Table 2) resulted in severe pathology (Fig. 3). Thus, it is reasonable to assume that the critical variable is duration

TABLE 2. Effects of hPTH-(1–34) on serum chemistry in female and male rats

Group	n	Calcium (mmol/l)	Phosphorus (mg/dl)	Alkaline phosphatase (U/liter)	PTH (pg/ml)
Female rats					
VEH pump	7	2.54 ± 0.02	5.8 ± 0.2	199 ± 19	10 ± 2
PTH sc	6	2.60 ± 0.04	5.5 ± 0.2	166 ± 13	26 ± 7
PTH pump (6 h)	3	3.63 ± 0.54[a]	6.4 ± 0.5	108 ± 17[b]	829 ± 411[c]
Male rats					
VEH pump	10	2.50 ± 0.03	6.9 ± 0.2	194 ± 9	11 ± 1
PTH sc	7	2.60 ± 0.01	7.7 ± 0.2[c]	201 ± 12	10 ± 1
PTH pump (1 h)	4	2.67 ± 0.04	6.4 ± 0.1	236 ± 16	70 ± 17
PTH pump (2 h)	6	2.71 ± 0.06	7.1 ± 0.3	161 ± 10	56 ± 13
PTH pump (continuous)	8	3.11 ± 0.20[a]	6.5 ± 0.3	150 ± 20[c]	602 ± 146[a]

Values are the mean ± SE. In rats that were not injected or infused with hPTH-(1–34), measured concentrations reflect endogenously secreted rat PTH.

[a] $P < 0.001$ *vs.* VEH pump.

[b] $P < 0.01$ *vs.* VEH pump.

[c] $P < 0.05$ *vs.* VEH pump.

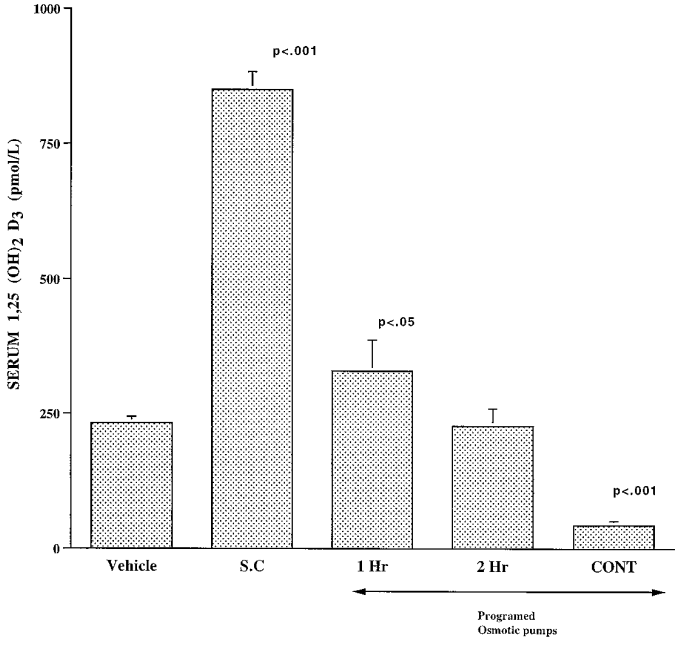

FIG. 2. Programmed infusion of hPTH resulted in treatment-dependent changes in serum $1,25D_3$ levels. The corresponding serum calciums are shown in Table 2B. Values are the mean ± SE (n = 4–6).

of exposure to elevated levels of PTH rather than peak levels of the hormone.

The time-course study revealed that the vast majority of administered hPTH was cleared from circulation within 1 h after sc administration of the hormone. This finding suggested that programmed infusion of hPTH over a short time frame should result in changes in bone and serum chemistries similar to those after sc administration of the hormone. This was found to be the case. On the other hand, prolonging the infusion of the same total dose of hPTH for 2 and 6 h/day resulted in progressive increases in undesirable changes that more closely emulated continuous hPTH infusion.

Our findings clearly demonstrate that the therapeutic duration of exposure to hPTH is approximately 1 h. Failure to clear the hormone from the circulation in less than 2 h due to excessive dose, retarded absorption, reduced degradation, or decreased clearance is likely to result in undesired side-effects. Although there may be species differences in the clearance of biologically active PTH (bPTH), the $t_{1/2}$ for the disappearance of serum iPTH in this study of about 11 min in old female rats was very similar to that reported in humans (18). We did not measure bPTH, but studies in humans have demonstrated that bPTH declines more rapidly than iPTH (18).

The rapid clearance of hPTH is the likely explanation for the reported optimal response of the rat skeleton to a three times per day sc dose regimen. Although theoretically possible to mimic using osmotic pumps, this regimen would be technically more difficult to achieve and is unlikely to have pronounced additional beneficial effects.

The increase in serum $1,25D_3$ after sc administration of hPTH was expected, and was probably due to up-regulation of 25-hydroxyvitamin D_3:1α-hydroxylase in the kidney (19). The mechanism for the remarkable decrease in $1,25D_3$ in the rats made hypercalcemic by continuous infusion of hPTH is less clear, but may have been due to a direct inhibition of the 1α-hydroxylase by calcium, as reported in previous investigations (19–21).

Serum alkaline phosphatase was insensitive to the large increase in cancellous bone formation in the proximal tibial metaphysis and was decreased in male and female rats after continuous infusion. This finding indicates that alkaline phosphatase activity is not a good marker for hPTH-induced changes in bone turnover in rats.

Intermittent PTH resulted in a small increase in serum phosphorous in male, but not female, rats. We have no explanation for this difference, but Wronski *et al.* reported a transient increase in serum phosphorous in PTH-treated female rats, so it is unlikely that the differences observed between male and female rats in the present studies reflect an inherent sex difference (5).

In summary, the results of these studies demonstrate that the therapeutic duration of exposure to hPTH to initiate increases in osteoblast number and bone formation is critical and very brief. Nevertheless, programmed administration of implanted hPTH is feasible as an alternative to sc injection as a route for administration of the hormone.

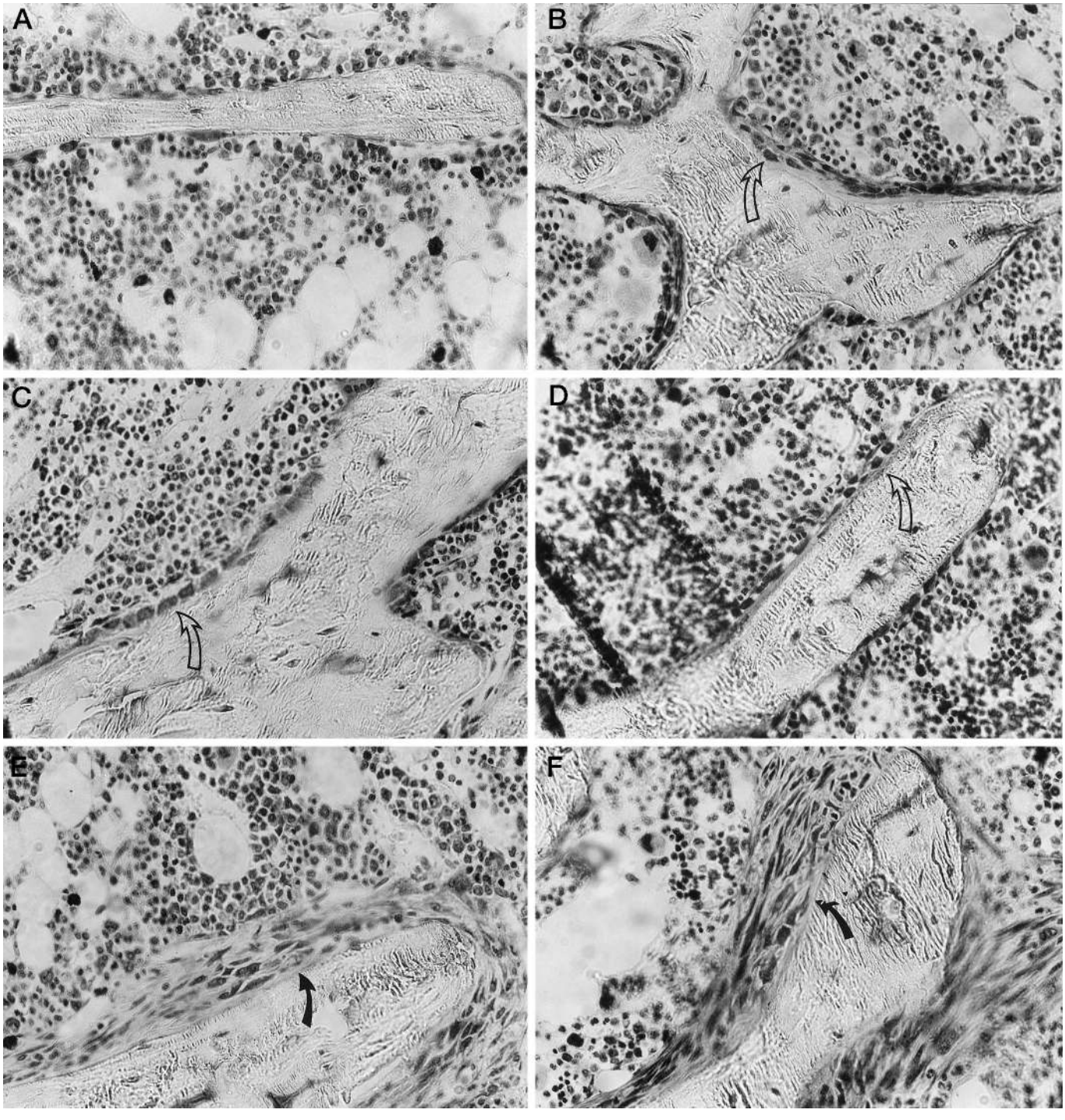

FIG. 3. Subcutaneous and programmed infusion of hPTH for 1 h/day increased osteoblast number, whereas administration of the hormone for 6 h/day and continuous infusion resulted in histological changes in the proximal tibial metaphysis resembling abnormalities reported in patients with chronic hyperparathyroidism. The photomicrographs are representative of rats infused with saline only (a), hPTH sc (b), hPTH for 1 h/day (c), hPTH for 2 h/day (d), hPTH for 6 h/day (e), and continuous hPTH (f). The *open curved arrows* point to trabecular perimeters bounded by osteoblasts. The *closed curved arrows* point to trabecular perimeters bounded by peritrabecular marrow fibrosis.

TABLE 3. Effects of hPTH-(1–34) on cancellous bone architecture in male rats

Treatment	n	Bone area/tissue area (%)	Trabecular no. (mm^{-1})	Trabecular thickness (μm)	Trabecular separation (μm)
Vehicle	10	16.5 ± 2.2	4.55 ± 0.28	75.4 ± 7.6	164 ± 15
PTH (SQ)	7	14.1 ± 3.2	4.53 ± 0.40	70.4 ± 8.2	161 ± 22
PTH (1 h/day)	4	23.7 ± 1.3[a]	6.05 ± 0.39[a]	76.0 ± 5.6	107 ± 12
PTH (2 h/day)	6	15.8 ± 2.2	4.76 ± 0.30	79.9 ± 5.7	160 ± 11
PTH (continuous)	8	14.1 ± 2.9	4.61 ± 0.31	84.2 ± 8.5	168 ± 17

Values are the mean ± SE.
[a] $P < 0.05$ compared to vehicle.

TABLE 4. Effects of hPTH-(1–34) on dynamic bone measurements in male rats

Treatment	n	Label perimeter (%)	Mineral apposition rate (μm/day)	Bone formation rate ($mm^2 \times 10^{-3}$/day)
VEH pump	10	8.5 ± 2.6	1.21 ± 0.09	47 ± 17
PTH (sc)	7	18.7 ± 2.3[a]	2.03 ± 0.14[b]	142 ± 44[a]
PTH pump (1 h/day)	4	29.7 ± 4.8[b]	1.76 ± 0.17[c]	186 ± 26[b]
PTH pump (2 h/day)	6	15.4 ± 2.6	1.51 ± 0.04[c]	86 ± 16
PTH pump (continuous)	8	5.7 ± 1.9	1.31 ± 0.26	27 ± 9

Values are the mean ± SE.
[a] $P < 0.05$ *vs.* VEH pump.
[b] $P < 0.001$ *vs.* VEH pump.
[c] $P < 0.01$ *vs.* VEH pump.

TABLE 5. Effects of hPTH-(1–34) on osteoblast osteoclast and fibrotic marrow perimeter in male rats

Treatment	n	Osteoblast perimeter (%)	Osteoclast perimeter (%)	Fibrotic marrow perimeter (%)
VEH pump	10	8.8 ± 2.4	2.6 ± 0.8	0
PTH (sc)	7	16.7 ± 2.2[a]	4.8 ± 2.8	0
PTH pump (1 h/day)	4	26.3 ± 4.3[b]	2.8 ± 1.7	0
PTH pump (2 h/day)	6	14.6 ± 2.7	3.6 ± 1.2	0
PTH pump (continuous)	8	9.7 ± 2.6	18.2 ± 4.7[c]	62.9 ± 14.2[c]

Values are the mean ± SE.
[a] $P < 0.05$ *vs.* VEH pump.
[b] $P < 0.01$ *vs.* VEH pump.
[c] $P < 0.001$ *vs.* VEH pump.

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