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Original Article

Hylonome

The role of strain in the response of rapidly growing young male rat bones to parathyroid hormone

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Abstract

Human parathyroid hormone (hPTH 1-34) stimulates an anabolic response in human and animal skeletons; however, it is unclear if the effect is strain dependent. To determine if the anabolic response to hPTH (1-34) was dependent upon strain in rats we used 2 outbred strains (Sprague Dawley, Wistar), 2 inbred strains (Fischer 344, Wistar spontaneously hypertensive:SHR), and 2 mutant strains (Zucker obese, Zucker lean) of rats. Male rats, 5 weeks of age, from each strain were treated subcutaneously with 80ug/kg body weight hPTH (1-34) or vehicle for 12 days. The response to PTH was similar in all strains whereby PTH exerted an anabolic effect on femoral bone mass and cancellous bone histology that was independent of strain differences. Histomorphometric indices of bone volume, mineralized surface and bone formation in lumbar vertebrae increased in all PTH-treated rats. Additionally, femur bone mineral content and bone mineral density measured by dual energy X-ray absorptiometry (DEXA), and ash weight increased in all PTH-treated rats. These increases occurred regardless of strain. In summary, PTH exerted comparable anabolic effects on bone mass, bone mineral density and bone formation in all rat models tested demonstrating that the skeletal responsiveness to PTH was not dependent upon strain.

Keywords: Parathyroid Hormone, Rat Strains, Bone Mineral Density, Histomorphometry, Bone Formation

Introduction

Genetics play a significant role in the attainment of peak bone mass, bone size, and the regulation of bone turnover in humans[1,2] and mice[3-5]. While mice provide the model of choice for mammalian genetic studies[3], mouse strains vary tremendously in their response to hormones[6] and as such, caution is advised when using mice as a model for human skeletal studies. In contrast, rats are the model of choice for studies of human skeletal disorders and have traditionally been used to test safety and efficacy in drug development. Unlike mice, however, genetic, or strain, differences in response to various hormones and conditions have not been adequately addressed in rats. As the genetic component of osteoporosis is rapidly being defined, it would be very important to know and understand the influence of genetic differences and the effect on responses to hormones in different strains of rats.

While human parathyroid hormone (hPTH) 1-34 administered intermittently to humans[7-9], mice[10], rats[11-16], rabbits[17], dogs[18], and monkeys[19] of various ages stimulates formation of normal bone, and increases bone mass and strength, the effect of strain (genotype) on the response has not been formally addressed. In order to further our understanding of the effects of PTH on the skeleton, strain dependencies need to be more clearly defined.

To determine if genotype (strain) significantly modified the response of rat bone to PTH we evaluated inbred strains, in which the genetic characteristics are fixed, outbred strains, in which the phenotype is variable, and mutant rat strains. We chose a young, growing rat model as that is the model we developed[11], and others have validated, in which 12 days of PTH treatment allowed a predictable increase in bone mass, our major outcome measure. This model has reliably predicted the increase in cancellous bone in older intact and ovariectomized rats, intact rabbits, ovariectomized monkeys and osteoporotic humans. Young, immature rats were used in this study in an attempt to understand factors that affect attainment of peak bone mass during growth as well as to minimize confounding variables due to aging and

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environmental factors. Additionally, as many patients with osteoporosis may have co-existing diseases, we determined if mutant rat strains known to be genetically at risk for obesity might also vary in the skeletal responsiveness to PTH. This study was not designed to address the mechanism of the anabolic effect of PTH on bone; rather, our objective was to determine if genetic differences between rat strains would influence the skeletal response to PTH.

Materials and methods

Hormones and drugs

Synthetic human parathyroid hormone (hPTH) 1-34, (Bachem, Inc., Torrance, CA), at a dose of 80 ug/kg body weight (bw), was prepared in a vehicle (V) of acidified saline containing 2% heat-inactivated rat serum, and given once daily by subcutaneous (SC) injection. To compensate for rapid weight gain, the dose was adjusted for 50g increments in mean group bw every 4 days. To label mineralized surfaces subcutaneous injections of calcein (Sigma, St. Louis, MO), 20mg/kg were administered three and one days before euthanasia.

Animals

Young male rats, 5 weeks of age, representing two outbred strains (Sprague Dawley and Wistar), two inbred strains (Fischer 344, and Wistar spontaneously hypertensive:SHR), and two mutant strains (Zucker obese and Zucker lean)[20] were purchased from Harlan Sprague Dawley (Indianapolis, IN). The rats were weighed and selectively distributed into groups so that the mean initial group body weight was comparable. Animals were housed in a facility maintained at 22°C with a 12 hour light/dark cycle and were fed chow (TD89222, Harlan Teklad, Madison, WI) containing 0.5% calcium and 0.4% phosphorus. Rats were labeled in a random number sequence to eliminate experimentor bias and this sequence was utilized for all procedures and analyses. These studies were approved by the Lilly Animal Care Assurance Committee and conducted under the guidance of a veterinarian.

A total of 72 male rats (N=6 rats/group) from each of the six strains was treated with either vehicle (V) or PTH by SC injection for 12 days. Approximately 3 hours after the last injection on day 12, rats were euthanized with CO_2, lumbar vertebrae (L4-6) were excised for histology and left femurs were excised and stored in 70% ethanol.

Femur bone mass

Left femurs were measured for maximal length, weighed to determine wet bone weight and then dehydrated in ether for 2 days. Femurs were air-dried in a fume hood for 24 hours, dry weight was recorded and bones were ashed in a muffle furnace at 850°C for approximately 16 hours and ash weights recorded. Cross-sectional bone area (BA: cm2), bone mineral content (BMC: g), and bone mineral density (BMD: g/cm2) were determined on the right femur by dual energy X-ray absorptiometry (DEXA) using a Hologic QDR-4500A (Waltham, MA) equipped with Small Animal Regional High Resolution software.

Cancellous bone histology

Lumbar vertebrae (L4-6) were fixed immediately in 70% ethanol, dehydrated in ascending concentrations of ethanol, and then embedded undecalcified in methyl methacrylate[21]. For cancellous bone histology, longitudinal sections were cut at 5 and 10-um with a Reichert-Jung Polycut S microtome (Leica Corp., Deerfield, IL). The 5-um sections were stained with von Kossa and mounted with Permount, while the 10-um sections were mounted unstained for fluorescent microscopy. Histologic parameters of bone structure and formation were measured on 2-4 sections per bone using semi-automatic image analysis software (KS400, Kontron Elektronik, Carl Zeiss, Thornwood, NY). Measurements were performed in the central vertebral body to exclude primary spongiosa and the growth plates. Structural parameters were measured at a magnification of 156x on the stained sections and indices were calculated as follows: cancellous bone volume (BV/TV: percent of tissue area) and trabecular thickness (Tb.Th). Dynamic parameters were measured on the unstained sections at a magnification of 625x and indices calculated as follows: mineralized surface (MS/BS: percent of bone surface with double calcein labels plus half single labels), mineral apposition rate (MAR) determined by dividing the interlabel distance by the interval labeling time, and bone formation rate referenced to bone surface (BFR/BS: MAR X MS/BS). Nomenclature and calculations of histomorphometric indices follow standards established by the American Society for Bone and Mineral Research[22].

Statistical analyses

In order to compare the effects of strain on the PTH-induced changes in bone, statistical significance was assessed by a two-way analysis of variance (ANOVA: JMP ver 3.0, SAS, Cary, NC)[23] with 1 interaction term and 2 main effect levels. The interaction term (Treatment X Strain) tests whether the changes in bone mass or histomorphometry induced by PTH were dependent upon strain. If the interaction term, which compared differences between all groups, was not significant by ANOVA, then main effect differences of Treatment or Strain were determined. The significance of difference between all groups was determined by the Tukey-Kramer Honest Significant Difference (HSD) multiple comparison test or when only 2 groups compared, the t-test was utilized[23]. Data are expressed as mean ± standard error of the mean (SEM).

Strain	Treatment	Ash Wt [a, b, c] (mg)	BMC [a, b] (mg)	BMD [a, b,c] (mg/cm^2)
Sprague Dawley	Vehicle	122 (3)	74 (4)	105 (2)
Sprague Dawley	PTH	137 (2)	96 (2)	119 (2)
% Change from Vehicle		11	23	12
Fischer	Vehicle	102 (2)	56 (2)	102 (1)
Fischer	PTH	122 (1)	82 (4)	120 (2)
% Change from Vehicle		16	32	15
Wistar	Vehicle	132 (3)	89 (4)	110 (2)
Wistar	PTH	158 (5)	123 (6)	136 (3)
% Change from Vehicle		16	28	19
Zucker obese	Vehicle	127 (3)	81 (4)	114 (2)
Zucker obese	PTH	149 (6)	110 (7)	132 (4)
% Change from Vehicle		15	26	14
Zucker lean	Vehicle	120 (2)	79 (2)	109 (1)
Zucker lean	PTH	127 (4)	89 (1)	119 (2)
% Change from Vehicle		5	11	8
SHR	Vehicle	114 (2)	78 (5)	109 (2)
SHR	PTH	139 (3)	104 (3)	125 (2)
% Change from Vehicle		18	25	13

Data are expressed as mean (SEM). PTH = hPTH(1-34) 80µg/kg as a once daily subcutaneous injection for 12 days. N=6/grp, N=5/Zucker obese. BMC=bone mineral content, BMD=bone mineral density.
[a] Significant Strain effect, $P < 0.001$. [b] Significant Treatment effect, $P < 0.001$. [c] Significant Interaction Effect (Strain X Treatment), $P < 0.05$.

Table 1. Femoral bone mass in different strains of rats treated with vehicle or PTH for 12 days.

Results

Femoral bone mass

PTH exerted an anabolic effect on femoral bone mass measures of ash weight, BMC and BMD in which all PTH-treated rats had significantly greater bone mass values than all vehicle-treated rats (Table 1). A significant Treatment effect by ANOVA showed that ash weight, BMC and BMD increased 14%, 24%, and 14%, respectively, after PTH treatment. Within each individual strain, BMD was significantly greater in PTH-treated rats than in the respective vehicle-treated rats. Ash weight was significantly greater in PTH-treated rats than in vehicle-treated rats for every strain except the Zucker lean strain. The differences for the Zucker lean strain ash weights did not achieve statistical significance. Strain differences were also noted with Fischer rats having the lowest and Wistar rats having the greatest values for all femoral bone mass measures with the exception of the Zucker lean strain.

Cancellous bone histomorphometry

All rats treated with PTH had significantly elevated measures of cancellous bone histomorphometry when compared to all rats treated with vehicle as shown by a significant Treatment effect by ANOVA (Table 2). Bone volume (BV/TV), trabecular thickness (Tb.Th), mineralized surface (MS/BS) and bone formation rate (BFR/BS) significantly increased 19%, 17%, 23%, and 30% in all PTH-treated rats when compared to all vehicle-treated rats. When evaluated by strain, mineralized surface and bone formation rates were highest in Fischer rats and lowest in spontaneously hypertensive rats (SHR).

Strain	Treatment	BV/TV[b] (%)	Tb.Th[b] um	MS/BS[a,b] (%)	BFR/BS[a,b] (u3/u2/d)
Sprague Dawley	Vehicle	20.4 (1.4)	56.9 (3.1)	46.1 (1.5)	178.6 (13.6)
Sprague Dawley	PTH	27.8 (2.1)	74.3 (6.9)	56.8 (3.5)	268.3 (33.6)
% Change from Vehicle		27	23	19	33
Fischer	Vehicle	21.2 (1.7)	65.2 (3.2)	52.9 (2.5)	247.9 (24.3)
Fischer	PTH	26.8 (2.9)	72.2 (5.3)	62.0 (0.8)	293.7 (16.6)
% Change from Vehicle		21	10	15	16
Wistar	Vehicle	21.4 (3.1)	54.1 (5.4)	45.9 (2.2)	194.6 (16.9)
Wistar	PTH	24.9 (2.5)	66.9 (5.5)	60.9 (1.7)	291.9 (7.7)
% Change from Vehicle		14	19	25	33
Zucker obese	Vehicle	25.4 (2.4)	66.5 (4.5)	48.8 (2.3)	190.8 (15.2)
Zucker obese	PTH	25.1 (1.5)	72.2 (2.1)	56.5 (4.5)	233.8 (27.8)
% Change from Vehicle		-1	8	14	18
Zucker lean	Vehicle	21.8 (3.1)	60.8 (5.1)	48.2 (1)	212.5 (15.8)
Zucker lean	PTH	30.2 (2.3)	77.2 (2.3)	63.9 (1)	319.2 (10.4)
% Change from Vehicle		28	21	24	33
SHR	Vehicle	22.9 (2.7)	61.7 (3.9)	31.2 (2.4)	135 (14)
SHR	PTH	28.8 (2.3)	76.7 (3.6)	56.3 (2.6)	261.5 (16.7)
% Change from Vehicle		20	20	44	48

Data are expressed as mean (SEM) . PTH = hPTH(1-34) 80mg/kg as a once daily subcutaneous injection for 12 days. N=6/grp.
[a]Significant Strain effect, P<0.001. [b]Significant Treatment effect, P<0.001.

Table 2. Cancellous histomorphometry of lumbar vertebrae in different strains of rats treated with vehicle or PTH for 12 days.

Discussion

In this study, we show that the response of bone to PTH in rats is not dependent upon strain, and, moreover, the skeletal responsiveness to PTH is not modified in mutant strains of rats. When given once daily for 12 days, PTH increased bone volume, bone formation, and bone mass in lumbar vertebrae and femurs of young male rats of different strains. This indicates that the PTH stimulated changes in bone mass, volume, and formation were not dependent upon strain and therefore, males rats of any strain evaluated in this study could be utilized in further studies to evaluate the mechanism of the PTH anabolic effect.

The results of the present study extend our earlier studies in young and aged Sprague Dawley, Brown Norway and Brown Norway/Fischer344 rats[24] by demonstrating an anabolic effect of PTH in young outbred (Sprague Dawley, Wistar), inbred (Fischer, spontaneously hypertensive) and mutant (Zucker obese, Zucker lean) rat strains. There have been no systematic, published studies to determine if the anabolic effect of PTH on bones may have a genetic component, although the various properties of bones and the skeleton are known to have significant genetic determinants[25]. The toxicology literature cites many examples of tests where the treatment responsiveness and outcome were governed by the strain or gender of the animal used[26]. Additionally, type of cage conditions, diet and strain are important factors in space flight studies[27]. The published literature on the skeletal responsiveness to PTH has reported data for primarily Sprague Dawley males, ovariectomized females, and Fischer and Wistar ovariectomized females, but the possibility of confounding variables due to strain have not been adequately considered. Our results show that the anabolic effect of PTH on the skeleton is independent of strain.

There are inherent, genetic differences in skeletal morphology, bone mass, bone structure, and bone histomorphometry between the different strains but these

differences are independent of the PTH anabolic effect. Our data from males of 2 outbred, 2 inbred, and 2 mutant strains showed significant independent effects of strain. For example, Wistar rats exhibited the greatest values for bone mass while Fischer rats had the lowest values. Bone formation rate was also highest in Fischer rats and lowest in SHR rats but these differences were independent of the PTH effect. Despite these effects of strain alone, all rats responded to intermittently administered PTH with increased bone mass. Other studies of aged virgin and multiparous female rats have also shown no significant differences in the skeletal response to PTH in Brown-Norway, Brown-Norway/F344 and Sprague Dawley rats[11, 28].

As there is comorbidity of other common diseases with osteoporosis, we wanted to determine if mutant strains at high risk for spontaneous hypertension might differ in the response of their bones to PTH. Previous studies have shown that while cancellous bone volume is decreased in male[29], and increased in female[30] spontaneously hypertensive rats, the response to estrogen is similar in ovariectomized spontaneously hypertensive rats[31]. Zucker obese male rats have been shown to have shorter femurs but equivalent distal femoral cancellous bone volume[32]. In our study PTH elicited an anabolic response in the skeletons of mutant strains of rats: the bone mass of all mutant strains increased in response to intermittent administration of PTH, irrespective of their disease risk background.

In conclusion, PTH elicited an anabolic response in the skeletons of young, male and female rats of Sprague Dawley, Fischer 344, Wistar, and Zucker strains, and mutants based on these backgrounds that was independent of strain. The anabolic effect of PTH on the skeleton was not altered in strains at risk for hypertension. In summary, PTH exerted anabolic effects on bone mass, bone mineral density and bone formation in all rat models tested, demonstrating that the skeletal responsiveness to PTH was not dependent upon strain.

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References

1. Eisman JA. Genetics of osteoporosis. Endo Rev 1999; 20:788-804.
2. Pocock NA, Eisman JA, Hopper JL, Yeates MG, Sambrook PN, Ebert S. Genetic determinants of bone mass in adults: a twin study. J Clin Invest 1987; 80:706-710.
3. Klein RF, Shea M, Gunness ME, Pelz G, Belknap JK, Orwoll ES. Phenotypic characterization of mice bred for high and low peak bone mass. J Bone Miner Res 2000; 16:63-71.
4. Sheng MH-C, Baylink DJ, Beamer WG, Donahue LR, Rosen CJ, Lau K-HW, Wergedal JE. Histomorphometric studies show that bone formation and bone mineral apposition rates are greater in C3H/HeJ (high-density) than C57BL/6J (low-density) mice during growth. Bone 1999; 25:421-429.
5. Beamer WG, Donahue LR, Rosen CJ, Baylink DJ. Genetic variability in adult bone density among inbred strains of mice. Bone 1996; 18:397-403.
6. Zheng GQ, Cole HW, Smith SJ, Bryant HU, Sato M. Bone effects in mice of ovariectomy and recombinant human PTH (1-34) are highly strain dependent. Bone 1998; 23:S448.
7. Hodsman AB, Fraher LJ, Watson PH, Ostbye T, Stitt LW, Adachi JD, Taves DH, Drost D. A randomized controlled trial to compare the efficacy of cyclical parathyroid hormone versus cyclical parathyroid hormone and sequential calcitonin to improve bone mass in postmenopausal women with osteoporosis. J Clin Endocrinol Metab 1997; 82:620-628.
8. Neer RM, Slovik M, Daly M, Potts TJ, Nussbaum SR. Treatment of postmenopausal osteoporosis with daily parathyroid hormone plus calcitriol. Osteoporos Int (Suppl) 1993; 1:S204-205.
9. Lindsay R, Nieves J, Formica C, Henneman E, Woelfert L, Shen V, Dempster D, Cosman F. Randomised controlled study of effect of parathyroid hormone on vertebral bone mass and fracture incidence among postmenopausal women on oestrogen with osteoporosis. Lancet 1997; 350:550-555.
10. Rihani-Bisharat S, Maor F, Lewinson D. *In vivo* anabolic effects of parathyroid hormone (PTH) 28-48 n-terminal fragments of PTH and PTH-related protein on neonatal mouse bones. Endocrinol 1998; 139:974-981.
11. Gunness-Hey M, Hock JM. Increased trabecular bone mass in rats treated with synthetic parathyroid hormone. Metab Bone Dis Relat Res 1984; 5:177-181.
12. Liu CC, Kalu DN. Human parathyroid hormone-(1-34) prevents bone loss and augments bone formation in sexually mature ovariectomized rats. J Bone Miner Res 1990; 5:973-982.
13. Sato M, Zeng GQ, Turner CH. Biosynthetic human parathyroid hormone (1-34) effects on bone quality in aged ovariectomized rats. Endocrinol 1997; 138:4330-4337.
14. Shen V, Birchman R, Liang XG, Wu DD, Dempster DW, Lindsay R. Accretion of bone mass and strength with parathyroid hormone prior to the onset of estrogen deficiency can provide temporary beneficial effects in skeletally mature rats. J Bone Miner Res 1998; 13:883-890.
15. Sogaard CH, Wronski TJ, McOsker JE, Mosekilde L. The positive effect of parathyroid hormone on femoral neck bone strength in ovariectomized rats is more pronounced than that of estrogen or bisphosphonates.

Endocrinol 1994; 134:650-657.
16. Wronski TJ, Yen CF. Anabolic effects of parathyroid hormone on cortical bone in ovariectomized rats. Bone 1994; 15:51-58.
17. Hirano T, Burr DB, Turner CH, Sato M, Cain RL, Hock JM. Anabolic effects of human biosynthetic parathyroid hormone fragment (1-34), LY333334, on remodeling and mechanical properties of cortical bone in rabbits. J Bone Miner Res 1999; 14:536-545.
18. Boyce RW, Paddock CL, Franks AF, Jankowsky ML, Eriksen EF. Effects of intermittent hPTH (1-34) alone and in combination with 1,25(OH)2D3 or risedronate on endosteal bone remodeling in canine cancellous and cortical bone. J Bone Miner Res 1996; 11:600-613.
19. Jerome CP, Johnson CS, Lees CJ. Effect of treatment for 3 months with human parathyroid hormone 1-34 peptide in ovariectomized cynomolgus monkeys (*Macaca fascicularis*). Bone 1995; 17:415S-420S.
20. Zucker LM, Zucker TF. Fatty, a new mutation in the rat. J Hered 1961; 52:275-278.
21. Baron R, Vignery A, Neff L, Silverglate A, Santa Maria A. Processing of undecalcified bone specimens for bone histomorphometry. In: Recker RR (ed) Bone histomorphometry: Techniques and interpretation. CRC Press, Boca Raton, USA; 1983:13-35.
22. Parfitt AM, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, Ott SM, Recker RR. Bone histomorphometry: standardization of nomenclature, symbols, and units. J Bone Miner Res 1987; 2:595-610.
23. SAS. JMP Statistics and Graphics Guide. Cary NC: SAS Institute Inc; 1995.
24. Gunness M, Hock JM. Anabolic effect of parathyroid hormone is not modified by supplementation with insulin-like growth factor I (IGF-I) or growth hormone in aged female rats fed an energy-restricted ad libitum diet. Bone 1995; 16:199-207.
25. Sambrook PN, Kelly PJ, White CP, Morrison NA, Eisman JA. Genetic determinants of bone mass. In: Marcus R, Feldman D, Kelsey J (eds) Osteoporosis. Academic Press, San Diego, USA; 1996:477-482.
26. Kacew S, Festing MFW. Role of rat strain in the differential sensitivity to pharmaceutical agents and naturally occurring substances. J Toxicol Environ Health 1996; 47:1-30.
27. Zernicke RF, Vailas AC, Grindeland RE, Li K-C, Salem GJ. Interactive effects of nutrition, environment, and rat-strain on cortical and vertebral bone geometry and biomechanics. Aviat Space Environ Med 1990; 61:640-647.
28. Hock JM, Wood R. Growth hormone does not enhance the anabolic effect of human parathyroid hormone (1-34) on bone in aging multiparous and virgin rats. Mech Ageing Dev 1995; 85:183-197.
29. Wang TM, Hsu JF, Jee WSS, Matthews JL. Evidence for reduced cancellous bone mass in the spontaneously hypertensive rat. Bone Miner 1993; 20:251-264.
30. Ma YF, Stimpel M, Liang H, Pun S, Jee WSS. Impact of antihypertensive therapy on the skeleton: effects of moexipril and hydrochlorothiazide on osteopenia in spontaneously hypertensive ovariectomized rats. J Endocrinol 1997; 154:467-474.
31. Liang H, Ma Y, Pun S, Stimpel M, Jee WSS. Aging- and ovariectomy-related skeletal changes in spontaneously hypertensive rats. Anat Rec 1997; 249:173-180.
32. Foldes J, Shi MS, Levy J. Bone structure and calcium metabolism in obese Zucker rats. Int J Obes Relat Metab Disord 1992; 16:95-102.