Parathyroid gland volume increases with postmaturational aging in the rat

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Halloran, Bernard, Per Udén, Quan-Yang Duh, Shoichi Kikuchi, Tracy Wieder, Jay Cao, and Orlo Clark. Parathyroid gland volume increases with postmaturational aging in the rat. Am J Physiol Endocrinol Metab 282: E557–E563, 2002; 10.1152/ajpendo.00261.2001.—To examine the pathophysiology of the age-related rise in the plasma concentration of parathyroid hormone (PTH), we studied the relationships among plasma immunoreactive PTH (iPTH), parathyroid gland volume, parathyroid cell proliferation rate, renal function, and blood Ca2+ in male Fischer 344 rats aged 6–28 mo. Plasma iPTH increased 2.5-fold between 6 and 28 mo and correlated with parathyroid gland volume (r = 0.87). Gland volume began to increase as early as 6–12 mo of age and by 28 mo was threefold greater than at 6 mo. Gland expansion was a consequence of hyperplasia stimulated in age and by 28 mo, the increase in parathyroid gland volume was threefold greater than at 6 mo. Unlike what has been observed in the human, these data suggest that the age-related increase in plasma iPTH in the rat is linked to parathyroid gland hyperplasia and that early gland growth does not appear to be associated with hypocalemia or renal insufficiency, but rather to developmentally related metabolic changes. Later in life (>24 mo), the increase in parathyroid cell proliferation rate, further hyperplastic expansion of the gland, and increase in iPTH secretion appear to be associated with renal insufficiency.

The serum concentrations of immunoreactive (3, 9, 11, 16, 23, 24, 28, 31) and bioactive (8) parathyroid hormone (PTH) increase with postmaturational aging in humans and rats. Bone turnover also increases with aging and correlates directly with the rise in circulating PTH, suggesting that the mild hyperparathyroidism of aging may contribute to bone loss in the elderly (6, 19). In the Fischer 344 (F344) rat, the serum concentration of PTH begins to increase as early as 12 mo of age, and by 24 mo of age (the mean life span for the F344 rat) is as much as 5 times higher than in 6-mo-old animals (3, 16, 28).

In the rat, the age-related increase in serum PTH reflects increased secretion. Fox and Mathew (10) measured the metabolic clearance rate of PTH in 6- and 25-mo-old animals and showed that clearance of PTH does not change with age.

The age-related rise in PTH secretion has been interpreted to reflect the decrease in renal function associated with aging (20), decreased intestinal calcium absorption (21), decreased renal conservation of calcium (19), and/or senescent changes intrinsic to the parathyroid secretory cell itself (4, 24, 27). Whatever the underlying pathology, a consistent feature of the change in PTH secretory dynamics with aging has been an increase in the PTH minimum and maximum secretory rates (9, 18, 24). Fox (9) reports that minimum and maximum PTH secretion rates are higher in older than in younger animals, and Ledger et al. (18) demonstrated that minimum and maximum serum concentrations of PTH are higher in elderly than in young women. We have shown that the minimum suppressible level of PTH during calcium infusion is two- to threefold higher in elderly than in young men (24). These findings are consistent with parathyroid gland enlargement (25) and suggest that the rise in circulating PTH associated with aging may in part be a consequence of gland hypertrophy. Despite this inference, however, data from autopsy studies indicate that parathyroid gland volume in human subjects remains nearly constant between the ages of 30 and 80 yr (1, 12). To test the hypothesis that parathyroid gland volume increases with postmaturational aging in the rat, and to determine whether the putative increase in gland volume is associated with hypocalemia or linked to renal insufficiency, we measured the volume of the parathyroid glands, the glomerular filtration rate...
(GFR), and whole blood Ca\(^{2+}\) in F344 rats aged 6–28 mo. Parathyroid cell proliferative activity was also measured. The data suggest that parathyroid gland volume increases with age in the rat and that the age-related rise in plasma iPTH is linked to gland enlargement.

**METHODS**

*Animals.* Thirty male F344 rats aged 6, 12, 18, 24, and 28 mo (n = 6/age group) were obtained through the National Institute of Aging (NIA colony, Harlan Sprague Dawley, Indianapolis, IN, Barrier 202B and 202C) and fed a standard laboratory rodent diet containing 1.0% calcium, 0.61% phosphorus, and 4.5 IU of vitamin D/g of diet (Purina Mills, Richmond, IN). Animals were maintained in accordance with the NIH Guide for the Care and Use of Laboratory Animals in certified animal care facilities and allowed 3–4 days of acclimatization before experimentation. All experimental protocols were approved by the Animal Studies Subcommittee at the Veterans Affairs Medical Center, San Francisco, CA.

*Renal function.* GFR was measured using continuous infusion of nonradioactive iothalamate by osmotic minipump (2, 5). Undiluted meglumine iothalamate (Conray 60; Mallinckrodt Medical, St. Louis, MO) was infused subcutaneously from Alza osmotic minipumps (model 2ML1; Alza, Palo Alto, CA) implanted on the back. Blood samples were collected between 9:00 and 11:00 AM from the orbital plexus 48, 72, and 96 h after pump implantation, and serum was isolated. Twenty microliters of an aqueous solution containing 200 μg/ml of barbital were added to each serum sample (100 μl) for purposes of measuring recovery, and protein was precipitated with 500 μl of methanol. After centrifugation, the supernatants were removed and dried under nitrogen. The samples were reconstituted in a mobile phase of acetonitrile-methanol-water (2.5:22.5:75) containing potassium phosphate (10 mM) and dodecyl triethylammonium phosphate (Q12IP) (1.75 mM); they were filtered and submitted to reverse-phase high-pressure liquid chromatography (room temperature, 1.5 ml/min) with a Rainin C8, 4.6 × 250-mm, 5-μg column. Recovery ranged from 70 to 80%. The serum concentration of iothalamate was calculated by comparing the area under the curve for absorbance at 236 nm for each sample to a set of standards. The GFR was calculated as the clearance of iothalamate

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GFR = \frac{C_{io}}{\text{infusion rate}_{io}} \text{ (mg/min)}
\]

\[
+ \text{ serum concentration}_{io} \text{ (mg/ml)}
\]

The mean of three determinations of \(C_{io}\) for each animal was taken as an estimate of the GFR.

*Cell proliferation and gland volume.* Parathyroid cell proliferative activity was estimated using bromodeoxyuridine (BrdU). At the same time that the osmotic pumps containing iothalamate were implanted, a separate Alza osmotic minipump (model 2001) containing BrdU (250 mg/ml) in DMSO-propylene glycol (50:50, vol/vol) was implanted. Four days later, animals were weighed and anesthetized by intraperitoneal injection of 0.1–0.2 g ketamine/kg body wt. Articular blood was collected from the dorsal aorta, and the concentration of ionized calcium was measured immediately. The remaining blood was maintained on ice and centrifuged, and plasma was harvested for later determination of the plasma concentration of immunoreactive PTH (iPTH). After collection of the blood, the tracheal/thyroid/parathyroid complex was quickly removed and placed in phosphate-buffered formalin (10%), and the parathyroid glands were dissected free of extraneous tissue with a dissecting microscope. Both parathyroid glands were harvested in all animals except one, where only one gland was found. The glands were embedded in paraffin, and 5- to 10-μm-thick sections were prepared by sequentially sectioning through the entire thickness of the gland. The sections were mounted in consecutive order on glass slides and either stained with hematoxylin-eosin or set aside for BrdU immunocytochemistry. The stained slides were mounted in a microscope fitted with a videocamera interfaced to a video monitor (Panasonic Wv-5470) and a MacIntosh Ilf computer. The complete image of each glandular section was brought to focus on the video monitor, and the outline of the gland was traced and area calculated using a modification of the NIH Image program (15). Parathyroid gland volume was calculated by multiplying the sum of the areas of each glandular section by its thickness (10 μm).

*Laboratory analyses.* The concentration of ionized calcium in whole blood was measured using a Ciba-Corning calcium ion/pH analyzer (model 234 Ca\(^{2+}\)/pH analyzer, Ciba-Corning Diagnostics, Medfield, MA). The intra- and interassay coefficients of variation for this assay are 6.9 and 12.4%, respectively. Plasma inorganic phosphate was measured as previously described (28). Plasma iPTH was measured using a commercially available kit (INS-PTH, Nichols Institute Diagnostics, San Juan Capistrano, CA), with rat PTH-(1–84) as standard (7, 25). The intra- and interassay coefficients of variation for this assay are 6.9 and 12.4%, respectively.

*Data analysis.* Data are presented as means ± SE and are analyzed using ANOVA and Dunnett’s post hoc test, with use of the 6-mo-old animals as the control group where appropriate. Linear regression analysis was used to examine relationships between variables. Parathyroid gland volume is expressed as volume per body weight to compensate for animal growth between 6 and 12 mo and loss of lean body mass later in life. Parathyroid cell birth rate was calculated as LI (% cells labeled) × 30 days/labeling interval (4 days) = LI × 7.5 (%/mo).

**RESULTS**

The plasma concentration of iPTH increased with postmaturational aging (Fig. 1). Between 6 and 28 mo, plasma iPTH increased from 20 ± 2 to 51 ± 11 pg/ml (\(P < 0.05\)). The mean plasma level of iPTH correlated with age (\(r = 0.56, P < 0.001\)). Parathyroid gland volume also increased with age (Fig. 2). By 12 mo, gland volume was increased significantly above that of 6-mo-old animals (\(P < 0.03\, Dunnett’s test\)). By 28 mo, parathyroid gland volume was nearly threefold greater than in 6-mo-old animals (+289%). Although gland volume increased, there were no apparent histological differences between glands from young and aged animals (Fig. 3). The relationship between individual...
gland volumes within an animal did not change with age. For animals aged 6, 12, 18, 24, and 28 mo, the range and mean of the ratios of the larger to the smaller gland were 1.07–2.54 (1.55), 1.03–1.09 (1.05), 1.02–1.92 (1.22), 1.05–1.49 (1.18), and 1.13–1.63 (1.38), respectively. Both glands within a given animal increased in volume at approximately the same rate.

Regression of the plasma concentration of iPTH onto gland volume demonstrated a highly significant correlation (r = 0.87, P < 0.001; Fig. 4).

The LI, or the percentage of cells positive for BrdU, is shown in Fig. 5. There was no significant change in LI between 6 and 18 mo. The mean cell birthrate during this period was 9.75%/mo or 117%/yr. Between 24 and 28 mo, the LI increased dramatically. At 28 mo the mean cell birthrate reached 23.25%/mo or 279%/yr.

No difference in the total number of cells per unit area could be detected across age. For animals aged 6, 12, 18, 24, and 28 mo, cell numbers averaged 1,355 ± 64, 1,502 ± 229, 1,424 ± 73, 1,247 ± 31, and 1,262 ± 35 cells/unit area, respectively.

Although differences in body weight between age groups did not reach significance (Table 1), there was a trend for weight to increase between 6 and 18 mo and then to decrease between 18 and 28 mo of age. To ensure that changes in gland volume did not simply reflect changes in body mass, we normalized gland volume to body weight.

Whole blood ionized Ca (Ca^{2+}) tended to increase between 6 and 18 mo, but the change did not reach significance (P < 0.07) (Table 1). Between 18 and 28 mo, blood Ca^{2+} tended to decrease, but again the difference did not reach significance. Overall, there were no significant differences in whole blood Ca^{2+} among age groups. Power analysis showed a minimum detection limit between age groups in a one-way ANOVA of 0.11 mmol/l (α = 0.05, 1-β = 0.90). Regression of either plasma iPTH or gland volume onto blood Ca^{2+} produced no significant correlations. Plasma inorganic phosphate concentrations did not change significantly with age (Table 1).

The GFR did not change between 6 and 18 mo of age (Table 1). Between 18 and 24 mo, the GFR tended to decrease, but the change did not reach significance. By 28 mo, however, GFR was significantly lower than in 6-mo-old animals (P < 0.05). Regression of the LI onto GFR is shown in Fig. 6. LI correlated negatively with GFR (r = -0.76, P < 0.001).

DISCUSSION

Our results confirm that the plasma concentration of iPTH increases with postmaturational aging in the rat (3, 9, 16, 28). The increase is gradual and similar to that seen in the human (11, 23, 24, 31).
The data also indicate that parathyroid gland volume increases with postmaturational aging. Gland volume appears to increase linearly from 6 to 24 mo and then more dramatically near the end of life. The mean life span of the male F344 rat is 24 mo. Thus the gland volume-to-body weight ratio (gland volume/body weight) increases relatively early in life. In studies by Wang et al. (29), parathyroid gland volume was measured in male Sprague-Dawley rats during their growth period (6–24 mo). Gland volume/body weight expressed as mm$^3$/g body wt $\times 10^{-6}$ decreased from 1,140 at 8 wk to 610 at 14 wk, and finally to 590 at 22 wk. The largest decrease in relative volume occurred between 2 and 4 mo, a time during which growth is slowing. Collectively, our data and those of Wang et al. suggest that relative gland volume decreases during growth and then increases during postmaturational aging.

That the age-related rise in plasma iPTH correlates with the increase in gland volume suggests that the gradual rise in circulating PTH may in part be a consequence of an increase in parathyroid gland volume and secretory potential. This observation is consistent with previous reports that minimum and maximum plasma PTH concentrations are higher in older than in younger animals (9). A larger gland mass would be expected to produce a higher minimum suppressible plasma PTH level and a higher maximum stimulated release of hormone.

The increase in parathyroid gland volume in the rat with postmaturational aging is symmetrical. Both
glands increase in volume at the same rate. This suggests that the underlying mechanism driving gland expansion is systemic or metabolic in nature. A metabolic change influencing gland function would be expected to affect both glands equally. Alternatively, a senescence-induced intrinsic change in cellular function would be expected to influence all cells and thus would also be expected to affect both glands equally. That both glands increase in volume at approximately the same rate clearly indicates that the increase in volume is not a consequence of clonal expansion of one or two aberrant cells. If clonal expansion of a subset of cells does occur, it must involve a sufficiently large number of cells as to obscure differences between the two glands.

Cell number per unit area did not change with age, suggesting that the increase in gland volume is a consequence of an increase in total cell number and not cell hypertrophy. Despite the increase in cell number, the LI, or cell birthrate, did not change until after 24 mo. Thus gland expansion appears to be a consequence of clonal hyperplasia, but the hyperplasia does not appear to be linked to an increase in LI. Apoptosis in our animals could not be measured because of the relatively low turnover rate of parathyroid cells (29). Thus it is possible that cell death rate decreases with age and that this contributes to the increase in cell mass. Recent studies in humans, however, indicate that, in patients with primary and secondary hyperparathyroidism, apoptosis is increased (32).

That LI is not significantly increased at 12 and 18 mo, when gland volume is increasing, is puzzling. It seems to suggest that the rate of cell renewal exceeds the rate of cell death at the time the animal reaches full maturity (6 mo). Lean body mass stabilizes; however, because of an apparent imbalance between cell proliferation and death, the gland continues to expand. This expansion cannot be driven by renal insufficiency, because GFR remains unchanged through ≥18 mo of age. Later in life (18–24 mo), renal function begins to deteriorate, and secondary hyperparathyroidism develops. This increases the LI and growth of the gland. Regression of the LI onto GFR shows a highly significant correlation \( r = 0.76 \), \( P < 0.001 \). Thus parathyroid gland expansion and the gradual development of age-related hyperparathyroidism appear to have at least two phases: an early phase independent of renal function and a later phase associated with renal insufficiency.

In the human, minimum-suppressible and maximum-stimulated plasma iPTH concentrations are also higher in older than in younger subjects (18, 24). This seems to suggest that, like the rat, gland volume may also increase in humans with age. However, measurement of total gland weight at autopsy in healthy subjects ranging in age from 9 to 95 yr did not show a significant change in mass until late in life (≥80 yr), at which time gland weight tended to decrease (1, 12). The discrepancy in the human between the absence of change in gland weight and the increase in secretory minima and maxima is confusing, as is the discrepancy in the age-related changes in gland mass in rats and humans. That gland mass increases with age in the rat but not in the human suggests that the rat may not be a good model for at least certain aspects of the age-related rise in serum PTH in humans.

Hypocalcemia does not appear to contribute to gland expansion. Mean whole blood ionized calcium concentrations ranged from 1.37 ± 0.01 to 1.44 ± 0.02 mmol/l. The minimum detectable difference among the age groups was calculated to be 0.11 mmol/l. If blood calcium did change with age, it would be on the order of 2% to 8%. Furthermore, neither gland volume nor plasma iPTH correlates with whole blood Ca\(^{2+}\). These findings are consistent with metabolic studies in humans. Por-tale et al. (24) showed that blood ionized Ca measured hourly throughout the day was not different in young (39 yr) and elderly (74 yr) healthy men. There was also no relationship in either the young or elderly men between blood ionized Ca and plasma PTH. These observations do not support the thesis that hypocalcem-ia is causative in gland hypertrophy.

Alternatively, other systemic factors whose concentrations change with growth and development may

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Table 1. Body weight, whole blood ionized calcium, inorganic phosphate, and glomerular filtration rate in male F344 rats at 6, 12, 18, 24, and 28 mo of age

<table>
<thead>
<tr>
<th>Age, mo</th>
<th>Body Wt, g</th>
<th>Blood Ca(^{2+}), mmol/l</th>
<th>( P_\text{cre} ), mmol/l</th>
<th>GFR, ml/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>405 ± 11</td>
<td>1.38 ± .02</td>
<td>1.41 ± .03</td>
<td>2.9 ± 0.2</td>
</tr>
<tr>
<td>12</td>
<td>441 ± 9</td>
<td>1.41 ± .02</td>
<td>1.47 ± .06</td>
<td>3.1 ± 0.1</td>
</tr>
<tr>
<td>18</td>
<td>444 ± 9</td>
<td>1.44 ± .02</td>
<td>1.34 ± .06</td>
<td>3.0 ± 0.2</td>
</tr>
<tr>
<td>24</td>
<td>431 ± 18</td>
<td>1.41 ± .01</td>
<td>1.31 ± .05</td>
<td>2.6 ± 0.2</td>
</tr>
<tr>
<td>28</td>
<td>401 ± 18</td>
<td>1.37 ± .01</td>
<td>1.82 ± .32</td>
<td>1.6 ± 0.2*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Ca\(^{2+}\), blood ionized calcium; \( P_\text{cre} \), inorganic phosphate; GFR, glomerular filtration rate; F344, Fischer 344. * \( P < 0.05 \) vs. 6-mo age group, by one-way ANOVA and Dunnett’s test.
play a role. For example, it is well established that 1,25-dihydroxyvitamin D \([ {1,25(\text{OH})_2\text{D}} ]\) inhibits both PTH secretion and parathyroid cell proliferation (17, 22). Serum 1,25(\text{OH})_2\text{D} is high during growth (120 pg/ml at 6 wk of age) and then decreases to adult levels (30–40 pg/ml) between 2 and 4 mo of age as the rat reaches maturity (13, 28). The decrease in serum 1,25(\text{OH})_2\text{D} that occurs between 2 and 4 mo of age may release the parathyroid cell from tonic inhibition of proliferative activity. The subsequent proliferative activity level in the postmaturational months may exceed cell turnover and result in a gradual increase in gland volume. Although not measured in these studies, serum 1,25(\text{OH})_2\text{D} does not change significantly with postmaturational aging in healthy rats [at ages 6, 12, 18, and 24 mo, mean serum 1,25(\text{OH})_2\text{D} concentrations are 29 ± 4, 25 ± 4, 31 ± 3, and 35 ± 4 pg/ml (28); thus further decreases in 1,25(\text{OH})_2\text{D} with age (6–24 mo) do not contribute to the putative imbalance between proliferation and turnover established soon after maturity is reached. Serum phosphate may also play a role in modulating PTH secretion and gland growth. Serum phosphate is high during growth (6.3 mg/100 ml at 6 wk), falls to adult levels (4.0–4.5 mg/100 ml) by 6 mo of age, and then remains relatively constant to 24 mo of age (13, 28). We observed no change in the plasma concentration of inorganic phosphate in our rats until 28 mo of age, at which time the mean concentration increased. The change in plasma inorganic phosphate, however, did not reach significance because of the large increase in variance, a result presumably of the non-uniform decrease in renal function late in life.

The changes in parathyroid gland function with aging may be related to senescence-associated intrinsic changes in parathyroid secretory cell metabolism. Decrease expression of the calcium receptor with age, for example, could conceivably influence gland sensitivity to calcium and trigger gland expansion. Interestingly, calcium receptor concentration in the parathyroid gland has been shown to increase with age, not decrease, suggesting that if changes in the calcium receptor pathway are changing with age, it is not the receptor that is changing but rather downstream signaling events (4). Studies on the 1,25(\text{OH})_2\text{D} receptor are underway.

In conclusion, we show that parathyroid gland volume increases with postmaturational aging. The correlation between plasma iPTH and gland volume suggests that they are linked. That neither gland expansion nor plasma iPTH appears to be associated with hypocalcemia suggests that other factors underlie gland hyperplasia and increased PTH secretion. The developmentally related fall in serum 1,25(\text{OH})_2\text{D}, and the eventual loss of renal function later in life, are likely candidates.

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