Effect of adrenal steroids on bone resorption in rats

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YASUMURA, SEICHI. Effect of adrenal steroids on bone resorption in rats. Am. J. Physiol. 230(1): 90-93. 1976.—Rats labeled with strontium-85 (85Sr) were injected with adrenergic steroids for 2 wk. The urinary-to-tibial (U/T) 85Sr ratio was used as an index of bone resorption. The glucocorticoids caused an inhibition of skeletal resorption, as judged by the 50% reduction in the U/T ratio, and decreased excretion of hydroxyproline. Thyroidal calcitonin levels were slightly elevated in glucocorticoid-treated animals, suggestive of a possible retardation of calcitonin release. The U/T ratios of thyroparathyroidectomized (TPTX) rats injected with corticosteroids were 50% of control values. The results indicate that glucocorticoids inhibit bone resorption independent of the action of calcitonin. Cortisol treatment increased the tibial density as measured by a radiographic technique. However, bone density was decreased and the U/T ratio increased in glucocorticoid-treated rats fed a low-calcium diet. In TPTX cortisol-treated rats, parathyroid extract (PTE) increased the U/T ratio and serum calcium but not to the degree observed in TPTX PTE-injected control animals. These experiments indicate that in rats glucocorticoids inhibit the rate of bone resorption but this effect can be overcome in part by PTE.

calcitonin; parathyroid extract; thyroparathyroidectomy; low-calcium diet; hydroxyproline; strontium 85

GLUCOCORTICOIDS ARE KNOWN TO HAVE AN EFFECT ON BONE metabolism. High blood levels of these hormones whether of endogenous or exogenous origin can result in a negative skeletal balance, but the mechanism by which an excess of corticoids affects bone is unclear. There are conflicting estimates of osteoblastic and osteolytic activity in human beings treated with glucocorticoids. However, during actively progressing Cushings syndrome with osteoporosis, the bulk of evidence favors the interpretation that there is a normal or diminished rate of bone formation combined with an accelerated rate of bone resorption (14, 16, 23). In rabbits, Storcy (22) and Thompson and Urist (24) have presented evidence of a cortisone-induced inhibition of bone formation and an increased bone resorption. In rats, however, steroids with glucocorticoid activity appear to decrease total skeletal turnover so that both formation and resorption are reduced (6, 23, 27).

Since calcitonin appears to prevent hypercalcemia by its inhibitory action on the bone resorption process (11), it was of interest to determine whether calcitonin was involved in any of the changes in skeletal resorption produced by cortisol or the synthetic anti-inflammatory steroid dexamethasone. Also, since corticoids have been implicated in increasing bone resorption by inducing secondary hyperparathyroidism (8, 13, 21), it was of interest to examine the effects of parathyroid extract (PTE) in rats in the presence of excessive amounts of cortisol.

MATERIALS AND METHODS

85Sr excretion studies. Twenty-four-day-old male Holtzman rats were injected with 15 µCi of carrier-free 85Sr, and adrenal steroid injections were started 16–24 days later. After 14 days of steroid treatment the rats were placed in individual metabolic cages without food or water for collection of 16-h urine samples. At the end of this period the rats were anesthetized with sodium pentobarbital, and their tibias were removed, cleaned, and weighed. Five milliliters of 0.1 N HCl were used to wash any remaining urine from the funnel of the metabolic cage. The wash and any urine remaining in the bladder were added to the urine sample so that total 85Sr excreted in the 16-h period could be determined. The 85Sr activity in tibia and urine was determined by standard scintillation spectrometry, and the data have been expressed as the ratio of total urinary-to-tibial (U/T) 85Sr. Plasma calcium levels were determined by atomic absorption spectrometry. Urinary hydroxyproline levels were measured by the method of Bergman and Loxley (1).

A standard mammography X-ray unit was used to obtain tibial radiographs with settings at 100 mA, 3/20 s, 30 kV, at a distance of 28 inches. Tibias from control and treated rats were x-rayed on the same plate to eliminate errors caused by changes in exposure time or differences in film sensitivity. A scanning densitometer with a 1-mm aperture size was used to quantify the relative bone density on the radiographs.

Adrenal steroid administration. In each of the experiments the animals were divided into groups and injected intramuscularly each day with 2.5 mg or 3.5 mg of cortisol acetate (21), 0.2 mg dexamethasone, 0.5 mg deoxycorticosterone acetate, or physiological saline. Deoxycorticosterone, an adrenal steroid with low glucocorticoid activity, was used as a control. The animals were fed a normal Purina chow diet or a synthetic diet (General Biochemicals) deficient in calcium during the entire period of steroid treatment.

Parathyroid extract administration. Rats were surgically thyro-parathyroidectomized (TPTX) 3 days prior to the start of the steroid injections. Each rat received a dose of 15 U (USP) of parathyroid extract (PTE) (Eli Lilly and Company) injected subcutaneously every 12 h.
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The last PTE injection was given just prior to placing of the rats in metabolic cages (16 h before they were killed).

Calcitonin assay. Thyroid glands were quickly removed and cleaned with the aid of a dissecting microscope, weighed to 0.1 mg, and homogenized in cold 0.1 N HCl. The method of calcitonin bioassay used in the present studies was similar to that reported by Hirsch et al. (12) and described in detail previously (28). The calcitonin standard was thyroid calcitonin standard A, Medical Research Council (MRC) of Great Britain. The bioassay data were processed by computer, with the use of a bioassay analysis program based on statistical methods described by Finney (5).

RESULTS

The effect of adrenal steroids on the plasma calcium level and on U/T ratios of rats maintained on a standard diet is shown in Table 1. Cortisol and dexamethasone caused a significant decrease in the U/T ratio. Plasma calcium levels remained within the normal range for all groups. Terminal body weights and tibia weights were significantly lower than in controls but the tibia-to-body weight ratios were increased, which would imply that the glucocorticoids have a more pronounced effect on soft tissue than on bone.

Thyroid calcitonin levels (Table 2) appeared to be slightly increased by glucocorticoid treatment, but this change was not statistically significant. It is unclear why the calcitonin concentration of saline-injected rats increased from 20 to 47 MRC mU/mg thyroid between the 1st and 2nd wk of the study. However, in this laboratory a 10-fold difference was measured in the thyroid concentration of calcitonin of 5-day-old rats and 60-day-old rats (7). Therefore, the change in calcitonin levels noted in control animals in this study may be a reflection of the increase that normally parallels aging during the early growth period.

By restriction of the intake of dietary calcium during the 14 days of steroid administration (Table 3), the U/T ratios were elevated in the steroid-treated groups. All other variables were similar to those seen in animals fed a normal diet (Table 1).

Table 4 shows the effect of steroids in rats in the absence of endogenous calcitonin. The last injection of PTE given 16 h before the animals were killed maintained the plasma calcium at higher levels in the smaller glucocorticoid-treated rats, but the U/T ratios of these rats were significantly lower than in the saline- or deoxycorticosterone-treated animals.

Less hydroxyproline was excreted by rats injected with cortisol (Table 5). However, more hydroxyproline was excreted by cortisol-treated animals fed a low-calcium diet than by cortisol-treated rats maintained on a normal diet. The bone density judged by radiographs of tibias (Fig. 1) was increased in rats that received corti-
TABLE 5. Effect of cortisol on urinary hydroxyproline and bone density of rats maintained on normal and low-calcium diets

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Wt, g</th>
<th>Tibia Wt, g</th>
<th>Relative Integrated Density of Tibial X Rays, cm²</th>
<th>Urinary Hydroxyproline, µg/15 h</th>
<th>Plasma Calcium, mg/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline control</td>
<td>256±7</td>
<td>1.12±0.06</td>
<td>2.64±0.38</td>
<td>281±31</td>
<td>10.4±0.1</td>
</tr>
<tr>
<td>Cortisol, 3.5 mg x 14</td>
<td>196±11*</td>
<td>1.60±0.53</td>
<td>3.88±0.24</td>
<td>141±12</td>
<td>10.9±0.3</td>
</tr>
<tr>
<td>Saline control, low-calcium diet</td>
<td>177±30</td>
<td>0.99±0.34</td>
<td>1.76±0.31</td>
<td>268±25</td>
<td>10.4±0.4</td>
</tr>
<tr>
<td>Cortisol, 3.5 mg x 14 days, low-calcium diet</td>
<td>148±30</td>
<td>0.78±0.03*</td>
<td>1.24±0.22</td>
<td>134±15*</td>
<td>10.6±0.3</td>
</tr>
</tbody>
</table>

Values are means ± SE for 6 rats. * P < 0.01 for treated vs. control on same dietary regimen. † P < 0.05 for treated vs. control on same dietary regimen.

TABLE 6. Effect of cortisol on thyroparathyroidectomized rats given parathyroid extract

<table>
<thead>
<tr>
<th>Group</th>
<th>Body Wt, g</th>
<th>Urinary ⁸⁶Sr/Tibial *Sr x 100</th>
<th>Plasma Calcium, mg/100 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>130 ± 4</td>
<td>0.45 ± 0.08</td>
<td>5.1 ± 0.2</td>
</tr>
<tr>
<td>PTE</td>
<td>140 ± 6</td>
<td>3.80 ± 0.54*</td>
<td>11.0 ± 0.6*</td>
</tr>
<tr>
<td>PTE + cortisol, 2.5 mg x 14 days</td>
<td>120 ± 7</td>
<td>1.47 ± 0.18*</td>
<td>7.4 ± 0.5*</td>
</tr>
<tr>
<td>Cortisol, 2.5 mg x 14 days</td>
<td>156 ± 11</td>
<td>1.00 ± 0.16*</td>
<td>4.3 ± 0.3†</td>
</tr>
</tbody>
</table>

Values are means ± SE for 7 rats. Parathyroid extract (15 U) was injected twice daily. * P < 0.001 for treated vs. saline control. † P < 0.05 for treated vs. saline control.

FIG. 1. Radiograph of tibias from representative rats included in this study. Letter under each bone refers to experimental group: A, normal control; B, cortisol; C, low-calcium diet plus cortisol; D, low-calcium diet control.

Sol. In contrast, a decrease in density was noted in rats fed a low-calcium diet that was decreased further when cortisol was administered to calcium-deprived animals.

In TPTX rats (Table 6) exogenous PTE caused an elevation of the U/T ratio and the plasma calcium. Cortisol treatment increased the U/T ratio, an observation that cannot be explained by this study since the plasma calcium was decreased slightly. When both PTE and cortisol were administered the full effect of PTE was blunted, but the U/T ratio and plasma calcium were significantly higher than the levels observed in TPTX rats that received only cortisol.

DISCUSSION

The finding that the administration of cortisol or dexamethasone for 14 days resulted in a depression of the urinary hydroxyproline levels and the U/T ratio indicates that the rate of bone resorption was decreased but does not exclude the possibility that bone formation was affected as well. The rate of resorption was not quantitatively determined; however, since virtually all the ⁸⁶Sr is in bone before the start of the treatment, any ⁸⁶Sr appearing in the urine can be assumed to be derived from bone (25). Recirculation of the isotope with a subsequent increase in the uptake by the skeleton may account for the depressed U/T ratio but is unlikely, since Clark et al. (2) have shown a reduced ⁴⁰Ca uptake by the skeleton of rats receiving glucocorticoids. In addition, histological evidence of decreased osteolytic activity in rats given glucocorticoids has been provided by Folli et al. (6) and Storey (23). In studies in vitro, Stern (20) reported that glucocorticoids directly inhibit bone resorption and Raisz et al. (18) have shown that cortisol blocks 25-hydroxyvitamin D₃ or PTE-induced bone resorption. These results taken together are compatible with the marked increase in bone density in animals treated with cortisol shown in this study. However, these findings are in sharp contrast with the effects of glucocorticoids in rabbits, birds, and humans (15, 16, 22, 23), where increased resorption is associated with osteoporosis.

Whether the steroid inhibition of bone resorption in the rat was due to a secondary stimulation of calcitonin release was explored as well. The amount of calcitonin in the thyroid may be inversely related to the secretion rate. Gittes et al. (9) reported a 35% decline in thyroid content of calcitonin after a 2-h infusion of a solution high in calcium; conversely, they noted a 12-fold increase 14 wk after parathyroidectomy. In the present studies thyroid calcitonin levels of glucocorticoid-treated rats appeared elevated (albeit not significantly), which suggests that the rate of calcitonin release may have been slightly inhibited. The experiments on thyroparathyroidectomized rats support the view that resorption, judged by the U/T ratio, is inhibited by the glucocorticoids even in the absence of endogenous calcitonin. These studies indicate that the glucocorticoids inhibit resorption by an effect on bone that is not mediated through the action of calcitonin.

The results obtained in the steroid-treated rats maintained on a calcium-deficient diet were of special interest. Under these conditions, the glucocorticoids caused an increase in the U/T ratio, indicating enhanced bone resorption, a conclusion similar to that reached by Clark et al. (2) and Storey (23). Deprivation of calcium in the diet is known to result in secondary hyperparathyroidism (15), but if the hypersecretion of parathyroid hormone is the major cause of the high U/T ratios in the present study, it must do this by overcoming the inhibitory effects of steroids on bone. Since PTE increased the U/T ratio and the serum calcium of cortisol-treated TPTX rats, the inhibitory effect of corticoids can be
reversed in part by PTE. Stoerk et al. (21) observed a dose-response relationship between the PTE dose and an increase in the serum calcium level in cortisol-treated TPTX rats. However, 3.5 times more PTE had to be administered to cortisol-treated TPTX animals to achieve an equivalent concentration of calcium in the plasma of TPTX controls. Thus, it appears that PTE stimulates bone resorption, but much larger doses of PTE are necessary in cortisol-treated rats.

Unlike other species, the rat does not develop osteoporosis when fed a normal diet. This fact can be attributed to the remarkably efficient calcium absorption from the intestine (4). Although cortisone impedes calcium absorption in the intestinal mucosa of rats fed a normal diet, this effect is not evident in the plasma of TPTX controls. Thus, it appears that PTE are necessary in cortisol-treated rats.

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REFERENCES


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