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A primary adrenal steroid, 11β-hydroxyandrostenedione, has an osteotropic effect and little androgenic activity

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Abstract

The physiological role of 11β hydroxy-androstenedione (11βOHA), a primary adrenal steroid, remains unknown. In the present study, we investigated the effect of 11βOHA on bone metabolism in vitro and in vivo. Administration of 11βOHA enhanced the clonal growth of marrow osteoprogenitor cells cultured from normal rats. In ovariectomized rats, 11βOHA restored osteogesis and increased the bone mineral density at both the metaphyseal and diaphyseal regions of the femur. Bone histomorphometric study of ovariectomized rats demonstrated that the mineral apposition rate of both cortical bone and trabecular bone was increased by treatment with 11βOHA. In addition, 11βOHA increased alkaline phosphatase activity in cultured osteoblastic cells (MC3T3-E1 and SaOS-2). The androgenic and anabolic effects of 11βOHA were respectively estimated to be less than 1/100th and 1/10th–1/100th of those of testosterone, while the estrogenic action of 11βOHA was also very weak. These findings suggest an influence of 11βOHA on physiological bone metabolism and indicate that this steroid may be useful for stimulating of bone formation in the treatment of osteoporosis.

Keywords: 11β-Hydroxyandrostenedione; Androgenic activity; Primary adrenal steroid

1. Introduction

11β Hydroxy-androstenedione (4-androstene-11β-ol-3,17-dione:11βOHA) is a primary adrenal steroid that is chemically related to the adrenal androgen, androstenedione. Steroids with 19 carbon atoms (C^{19} steroids), which are synthesized in the adrenal glands, act as weak androgens or androgen precursors [1]. Dehydroepiandrosterone (DHEA) and its sulfate are the most abundant products of the adrenal glands. Androstenedione is produced by side-chain cleavage of 17α-hydroxyprogesterone or is formed from DHEA by 3β-hydroxy steroid dehydrogenase and Δ^5-Δ^4-isomerase, and is subsequently converted to testosterone mainly in the peripheral tissues [1–3]. On the other hand, androstenedione is converted to a third C^{19} steroid, 11βOHA by the enzyme 11β-hydroxylase in the zona reticularis. In contrast to DHEA or androstenedione, 11βOHA is also generated from cortisol via side-chain cleavage at the C^{17} position, a process that mainly occurs in the liver [4].

The physiological role of adrenal C^{19} steroids is still unclear. However, there have been a few reports suggesting that DHEA is involved in the regulation of bone maturation [5–7] and cytokine production by human lymphocytes [8]. Although the serum concentration of 11βOHA has been reported to range from 1 to 4 ng/ml [9], its actions have not been investigated.

The present study demonstrated that 11βOHA treatment increased both trabecular and cortical bone mass in rats after ovariectomy by stimulating bone formation, and showed that this steroid had a much weaker androgenic action than other C^{19} adrenal steroids. This is the first report on a possible role of 11βOHA in bone metabolism. Our findings also suggest that this steroid

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has the potential to be used as a bone stimulatory agent in the treatment of various types of osteoporosis.

2. Materials and methods

2.1. Steroids

11βOHA and DHEA were purchased from Sigma (St. Louis, MO).Testosterone propionate, and estradiol benzotate were purchased from Wako Pure Chemical Co. (Osaka, Japan).

2.2. Animals

2.2.1. Effect on normal animals

Seven-week-old Wistar rats were obtained from Japan SLC, Inc. (Shizuoka, Japan). All steroids were dissolved in sesame oil and administered via subcutaneous injection into the back daily for 7 days. Control animals received the vehicle (sesame oil) only. After the animals were killed, the bilateral femurs were resected for the preparation of bone marrow.

2.2.2. Ovariectomy experiments

The following two experiments were performed: (1) Comparison of the effect of subcutaneous administration of 11βOHA and DHEA on osteogenic potential and bone mineral density after ovariectomy in growing rats; and (2) assessment of the effect of oral 11βOHA and the mode of action of 11βOHA on bone by a bone histomorphometric study in older rats.

The first experiment involved four groups of rats: (1) a sham-operated group; (2) an ovariectomized group; (3) a DHEA group; and (4) an 11βOHA group. Seven-week-old female Wistar rats were ovariectomized under pentobarbital anesthesia via a dorsal approach. From 3 months after ovariectomy, the steroid-treated animals were administered either 11βOHA or DHEA (1 mg/kg) subcutaneously three times weekly for up to 2 months. Animals from the sham-operated and ovariectomized groups received the vehicle (sesame oil) only. After 2 months, all animals were killed, blood samples were collected, and the bilateral femurs were resected for the preparation of bone marrow or the measurement of bone mineral density.

Fourteen-week-old female rats were used for another experiment and after being assigned to the following five groups: (1) a sham-operated group; (2) an ovariectomized group; (3) an 11βOHA group treated at 1 mg/kg; (4) an 11βOHA group treated at 10 mg/kg; and (5) an 11βOHA group treated at 25 mg/kg. From 2 months after ovariectomy, animals were orally administered 1, 10, or 25 mg/kg of 11βOHA daily for 2 months. Animals from the sham-operated and ovariectomized groups received the vehicle (carboxymethyl-cellulose) only. All rats were injected with carcein (5 mg/kg) at 6 and 3 days before being killed after 2 months of treatment. Then the bilateral femurs were resected for the measurement of bone mineral density and the bilateral tibiae were harvested for bone histomorphometry.

2.3. Measurement of biochemical parameters

Serum levels of glucose, sodium, potassium, chloride, calcium, phosphorus, total cholesterol, and alkaline phosphatase (ALP) were measured using an autoanalyser (RX-40 Model, Nihon Denshi Inc. Ltd). The serum osteocalcin level was determined by radioimmunoassay using a specific antibody to rat osteocalcin (Biochemical Technologies, Inc., Stoughton, MA). The urinary excretion of deoxypyridinoline was measured by EIA using a commercial kit. The serum level of 1α,25-dihydroxy-vitamin D [1,25$(OH)_2$D] was determined by radioreceptor assay using a Yamasa 1,25$(OH)_2$D assay kit (Yamasa Shouyu, Japan).

2.4. Formation of fibroblast-colony forming units (FCFU) in bone marrow culture

Marrow FCFU growth was investigated by the method described previously [1,10] as an indirect bioassay of osteogenic potential. Briefly, bone marrow cells were plated into 25 cm^2 culture flasks at a density of 10^6/ml (10^7/flask). Cells were grown in α-minimal essential medium (α MEM; Gibco BRL, Life Technologies, Inc., Grand Island, NY) supplemented with 15% fetal bovine serum (FBS) and a 1% penicillin-streptomycin mixture in a 5% CO_2 atmosphere. The medium was changed completely after 24 h of culture and every 3 days thereafter. Cells forming FCFU colonies were fixed in 2% glutaraldehyde or citrate-acetone-formaldehyde and were stained with Wright-Giemsa stain or were stained for ALP activity (Sigma kit, Sigma). Then all FCFU containing a minimum of 50 cells were counted, as well as the number of ALP-positive FCFU with 50 or more cells.

2.5. Measurement of bone mineral density (BMD)

The femoral BMD was determined by dual-energy X-ray absorptiometry (DXA) using an Aloka DCS-600 (Aloka Co., Mitaka, Japan). In some experiments, the BMD was determined with a DPX-L (Lunar Co., Madison, WI) using the small animal appendicular scanning program. BMD was measured in the proximal midshaft, and distal regions of femur. In some experiments, the BMD was examined in 20 femoral regions with equal longitudinal lengths.

2.6. Histomorphometry

The bilateral tibiae were prefixed for 24 h in phosphate-buffered formalin followed by fixation in 70% ethanol and embedding in methylmetacryate. The left tibia was cut longitudinally into 5-μm thick sections, while cross-sections of the right tibia were made at 0.6 mm from the tibiofibular junction. The sections were stained with Villanueva bone stain and subjected to histomorphometric analysis.

2.7. Assessment of the androgenic, anabolic, and estrogenic actions of 11βOHA

The androgenic and anabolic actions of 11βOHA were assessed by the method of Hershberger et al. [11]. Three-week-old male Wistar rats were subjected to bilateral orchiectomy. From 1 week after surgery, steroids in sesame oil were administered subcutaneously to the castrated rats for 10 days, while sham-operated and control rats received sesame oil only. Testosterone propionate (0.1–1 mg/day) was used as a positive control. All animals were killed and the androgenic action of each agent was assessed from the weight of the anterior lobe of the prostate, while the anabolic action was assessed from the weight of the levator ani muscle.

The estrogenic actions of 11βOHA were assessed by the method of Louson et al. [12] using 21-day-old female Wistar rats. Steroids in sesame oil were administered subcutaneously twice daily for 3 days and control animals received the vehicle only. Estradiol benzoate was used as a positive control. Then all animals were killed and the uterine weight was measured.

2.8. In vitro effect of 11βOHA on ALP activity in osteoblast-like cells

Two osteoblastic cell lines were used for the study. Mouse osteoblastic MC3T3-E1 cells [13] can differentiate into osteoblasts and osteocytes in vitro, while SaOS-2 is a clonal human osteosarcoma cell line with a mature osteoblastic phenotype. MC3T3-E1 cells were generously provided by Dr H. Kodama (Ohu University, Koriyama, Japan), and were seeded into 96-well plates at a density of 2×10^3/well in 200 μl of α-MEM containing 10% FBS. SaOS-2 cells were seeded at the same density and were cultured in McCoy's medium supplemented with 10% FBS. After 2 days, the concentration of FBS was decreased to 1%. After culture for 24 h, the medium was replaced with fresh serum-free medium containing 0.1% bovine serum albumin and culture was continued for another 24 h. Then the cells were incubated with various concentrations of steroids for a further 48 h. Finally, the ALP activity was analyzed biochemically by incubating the cells with a 0.1 M aminnoethanol solution (pH 10.5) containing *p*-nitrophenolphosphate for 1 h at 37°C, as described previously [14]. Production of *p*-nitrophenol was quantified by measuring the absorbance at 405 nm using an automatic microtiter plate reader.

2.9. Statistical analysis

Differences between the mean values of two groups were assessed by Student's unpaired *t*-test and $P < 0.05$ was considered to indicate significance.

3. Results

3.1. Bone marrow FCFU in normal rats (Fig. 1)

Marrow cultures from normal female rats treated with 11βOHA (0.1–1 mg/kg) or 11βOHA (1 mg/kg) for 7 days showed a significantly greater number of both total FCFU and ALP-positive FCFU than cultures from vehicle-treated control rats. Testosterone also increased the number ALP-positive FCFU, but its effect was weaker than that of 11βOHA or DHEA.

3.2. Bone marrow osteogenic potential in ovariectomized rats

The number of FCFU developing in marrow cultures from ovariectomized rats was significantly smaller than in marrow cultures from sham-operated rats. Total and ALP-positive FCFU were increased significantly by treatment with 1 mg/kg of 11βOHA for 2 months (Fig. 2(a)). Treatment with DHEA at 1 mg/kg also increased both total and ALP-positive FCFU (Fig. 2(a)).

3.3. Effect of 11βOHA on biochemical parameters in ovariectomized rats

The serum osteocalcin level was higher in ovariectomized rats compared with sham-operated controls. Administration of 11βOHA at 10 mg/kg caused an increase of serum osteocalcin, but this was not significant (Table 1). Urinary excretion of deoxypyridinoline was increased by ovariectomy, but was unchanged by 11βOHA treatment. The serum level of $1,25(OH)_2D$ was also increased by ovariectomy, but was unchanged by treatment with 11βOHA. No significant changes of serum calcium, phosphate, and alkaline phosphatase were observed in any of the animals.

3.4. Effect of 11βOHA on bone loss after ovariectomy

Ovariectomized rats showed a significant decrease of BMD in the proximal and distal femur (metaphyseal regions), although the midshaft BMD was unchanged (Fig. 2(b)). After 2 months of treatment, there was a

significant increase of BMD in all three regions among rats given 11βOHA, while DHEA failed to significantly alter the BMD at any site (Fig. 2(b)).

To clarify the mode of action of 11βOHA, the femur was divided into 20 regions of equal length and the BMD of each region was measured in another experiment (Fig. 3(a, b)). Regions 1–2 corresponded to the proximal epiphysis and regions 3–4 were the femoral neck. Regions 5–9 corresponded to the proximal metaphysis composed of both cortical and trabecular bone, regions 10–14 were the diaphysis with mainly cortical bone, regions 15–18 corresponded to the distal metaphysis containing primary and secondary spongiosa rich in trabecular bone, and regions 19–20 were the distal epiphysis. The BMD in regions 5–11 (proximal metaphysis and diaphysis) was significantly higher in 11βOHA-treated rats than in ovariectomized (OVX) rats, as was the case in regions 1–4 (proximal epiphysis and femoral neck). 11βOHA did not affect the BMD in regions 15–20.

3.5. Effect of 11βOHA on bone histomorphometric parameters (Table 2)

Two months of treatment with 11βOHA restored bone that had been lost after ovariectomy. The cortical bone area was dose-dependently increased by 11βOHA treatment. A dose-dependent increase in the mineral apposition rate (MAR) at the periosteal surface suggested that the increase in cortical bone mass was due to enhanced bone formation at the periosteal surface. Although a dose-dependent increase of tibial metaphyseal MAR was also observed, the increase in trabecular bone volume was small and was only significant at a dose of 10 mg/kg. Thus, a favorable effect of 11βOHA treatment after ovariectomy was more evident on cortical bone.

3.6. Androgenic, anabolic, and estrogenic actions of 11βOHA

The highest dose of 11βOHA (100 mg/kg) increased the weight of the anterior lobe of the prostate, although the increase was less than that caused by 1 mg/kg of testosterone (Fig. 4(a)). At a dose of 10 mg/kg, 11βOHA also caused a slight but significant increase in the weight of the levator ani muscle (Fig. 4(b)). A very slight estrogenic action of 11βOHA (increase in uterine weight) was observed at 100 mg/kg (Fig. 4(c)).

3.7. Effect on ALP activity in cultured osteoblastic cells

11βOHA increased the ALP activity of MC3T3-E1 cells in a dose-dependent fashion at concentrations between 10^{-11} and 10^{-7}M. Higher concentrations of 11βOHA also increased the ALP activity of SaOS-2 human osteosarcoma cells (Fig. 5). A stimulatory effect on MC3T3-E1 cells was observed at lower concentrations when compared with SaOS-2 cells.

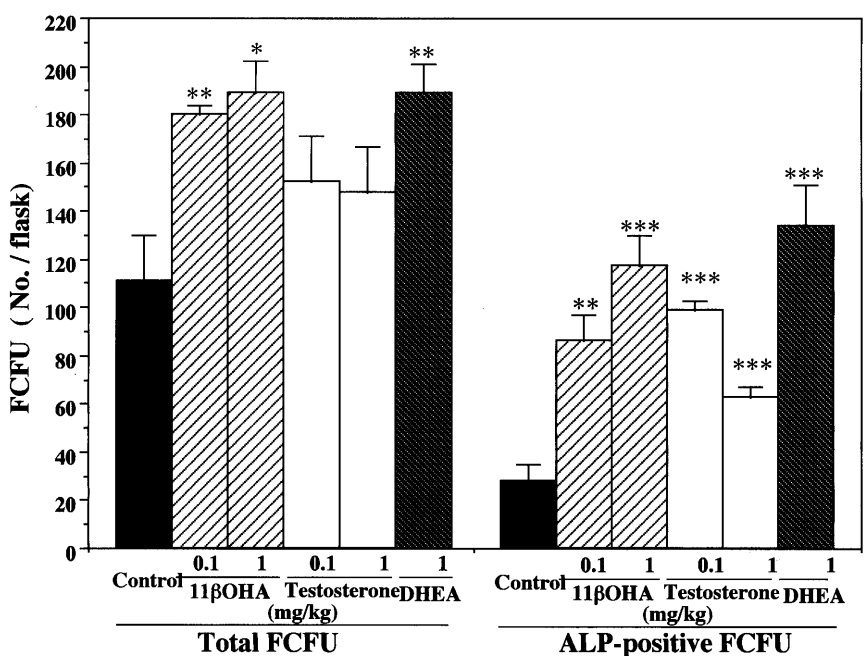

Fig. 1. Effect of 11βOHA, testosterone, and DHEA on total and ALP-positive marrow FCFU growth in normal rats. Results represent the mean ± S.E. from eight animals. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ vs. control rats.

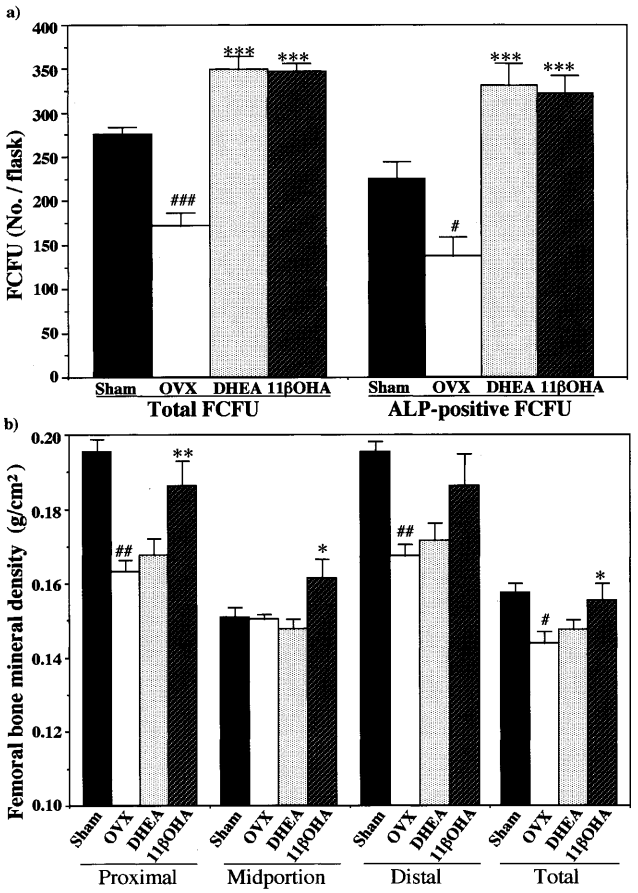

Fig. 2. Effect of treatment with 11βOHA or DHEA for 2 months (a, b) on marrow FCFU growth and femoral BMD in OVX rats. BMD was measured in the proximal, midshaft, and distal regions of the femur. # $P<0.05$, # # $P<0.01$, # # # $P<0.001$ vs. sham-operated control rats (sham). * $P<0.05$, ** $P<0.01$, *** $P<0.001$ vs. OVX rats.

4. Discussion

Three C^{19} steroids (DHEA, androstenedione, and 11βOHA) are normally synthesized by the adrenal glands. There are two different synthetic pathways for 11βOHA, one that produces it from androstenedione as a final product of adrenal steroidogenesis and one that produces it from cortisol. In this respect, 11βOHA is unique among C^{19} steroids. The daily production of 11βOHA is estimated to about 2 mg based on the level of urinary metabolites [15], but daily adrenal production may be lower because this figure includes hepatic synthesis via the cortisol pathway. The serum concentration of 11βOHA in normal women has been reported to range from 0.5 to 4 ng/ml, which is similar to that of androstenedione [9].

The physiological role of adrenal C^{19} steroids is still unclear, except for mild intrinsic androgenic activity. The relative potency of the androgenic actions of both androstenedione and DHEA has been estimated to be 1/10th to 1/20th of the activity of testosterone [16]. A recent study of androgen-sensitive parameters in hamsters demonstrated that DHEA and androstenedione both exhibit potent androgenic activity, about 50 and 80%, respectively, of that for the active gonadal androgen, dehydrotestosterone [17]. The present study demonstrated that the androgenic activity of 11βOHA was only 1/100th of that shown by testosterone, while the anabolic activity was between 1/10th and 1/50th of that for testosterone. These data indicate that 11βOHA has much less androgenic and anabolic activity compared with DHEA and androstenedione.

There is evidence suggesting that gonadal steroids are important in maintaining skeletal integrity [16], but little is known about the role of adrenal C^{19} steroids in this context. Secretion of DHEA and its sulfate is known to decline gradually with advancing age [18] and a correlation between decreased vertebral BMD and the circulating DHEA level has been found in postmenopausal women [19,20]. In addition, the plasma DHEA level is reduced after ovariectomy in rats, while administration of DHEA ameliorates osteopenia of cancellous bone in ovariectomized rats [21]. However,

Table 1
Biochemical and bone metabolic parameters[a]

	Sham-operated	OVX	11βOH A (10 mg/kg)
Sodium (mEq/l)	147 ± 1	148 ± 1	148 ± 1
Potassium (mEq/l)	5.1 ± 0.5	5.1 ± 0.3	5.2 ± 0.1
Calcium (mg/dl)	9.9 ± 0.1	9.7 ± 0.1	9.7 ± 0.2
Phosphorus (mg/dl)	5.4 ± 0.4	5.1 ± 0.2	6.6 ± 0.3
Alkaline phosphatase (I.U./l)	305 ± 19	270 ± 11	276 ± 57
Osteocalcin (ng/ml)	27.9 ± 1.6	34.3 ± 2.2*	39.7 ± 3.3
Urinary deoxypyridinoline (pmol/μmol Cr)	8.7 ± 0.5	11.1 ± 1.2	10.7 ± 0.5
1α,25(OH)$_2$D (pg/ml)	8.8 ± 0.5	17.1 ± 3.2	16.6 ± 2.5

[a] From 3 months after ovariectomy, treated rats were given 11β-hydroxyandrostenedione orally for 2 months. Then blood samples were obtained for the measurement of biochemical and bone metabolic parameters. Data represent the mean ± S.E.

* $P<0.05$ vs. sham-operated rats.

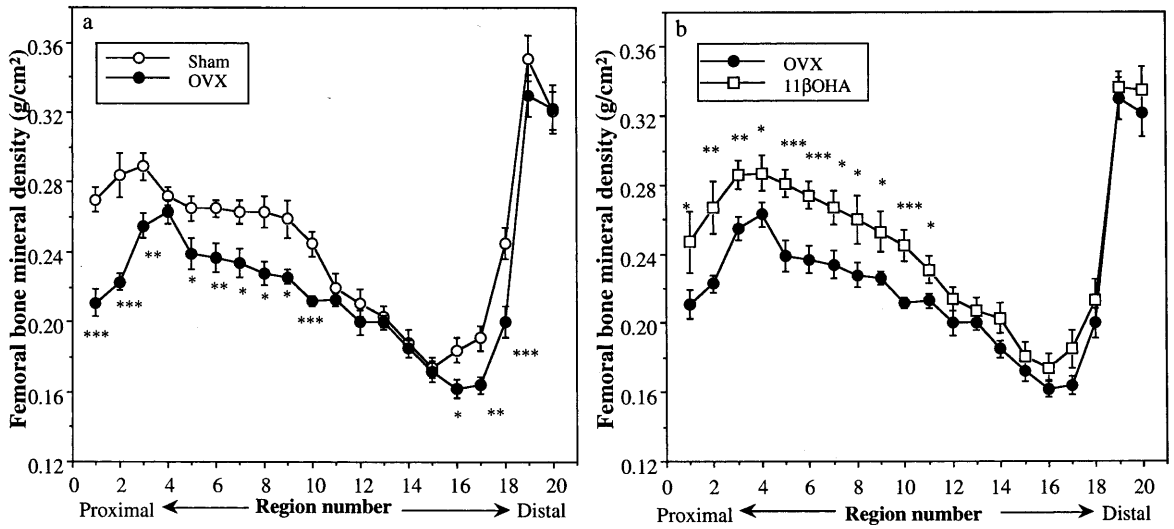

Fig. 3. Detailed analysis of femoral BMD. The femur was divided into 20 regions of equal length, and the BMD of each region was measured by DXA. (a) Sham-operated group versus OVX group. (b) OVX group versus 11βOHA (10 mg/day) group. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. sham-operated group (a) or OVX group (b).

Table 2
Effect of 11βOHA on bone mineral density and histomorphometric parameters in OVX rats[a]

Group	Total femoral bone mineral density (g/cm²)	Tibial diaphysis			Tibial metaphysis	
		MAR (μm/day)		Cortical bone area (mm²)	MAR (μm/day)	BV/TV (%)
		Endosteal	Periosteal			
Sham-operated	0.251 ± 0.006	0.199 ± 0.029	0.327 ± 0.119	4.74 ± 0.26	0.498 ± 0.084	26.04 ± 2.14
OVX						
Control	0.228 ± 0.004	0.225 ± 0.038	0.135 ± 0.054	4.91 ± 0.25	0.413 ± 0.055	15.32 ± 2.74
11βOHA						
1 mg/kg	0.235 ± 0.005	0.265 ± 0.042	0.268 ± 0.079	5.06 ± 0.12	0.489 ± 0.076	16.63 ± 2.22
10 mg/kg	0.250 ± 0.004**	0.192 ± 0.023	0.357 ± 0.087*	5.20 ± 0.22	0.660 ± 0.073*	21.76 ± 2.74
25 mg/kg	0.240 ± 0.003*	0.284 ± 0.026	0.446 ± 0.102**	5.78 ± 0.44	0.785 ± 0.053***	16.70 ± 1.82

[a] From 3 months after ovariectomy, treated rats received 11βOHA orally for 2 months. Then the femoral BMD was analyzed by DXA and the tibiae were subjected to bone histomorphometry. Results represent the mean ± S.E. Abbreviations: BV/TV, bone volume/tissue volume; MAR, mineral apposition rate.

* $P < 0.05$ vs. OVX rats.

** $P < 0.01$ vs. OVX rats.

*** $P < 0.001$ vs. OVX rats.

despite a significant improvement of marrow FCFU growth by DHEA, the increase of BMD was not significant in the present study. This might be explained by differences in the DHEA dosage and duration of treatment compared with previous studies.

There have been no previous reports regarding the physiological actions of 11βOHA, although one study demonstrated that the serum concentration of 11βOHA was significantly lower in osteoporotic women than in normal women and the decrease was not age-related [9]. The present study clearly demonstrated that 11βOHA ameliorated bone loss after ovariectomy in rats. Interestingly, the effect of this steroid on BMD was apparent in both the metaphyseal and diaphyseal regions of the femur. Despite the fact that diaphyseal bone mass was not affected by ovariectomy, there was an increase of BMD at the midshaft of the femur with 11βOHA therapy, suggesting that this steroid may influence the turnover of cortical bone, as has been reported for insulin-like growth factor I [22–24]. In contrast to the evidence that the serum estrogen level is an important factor influencing age-related bone loss, the serum

DHEAS level has been correlated with the bone mineral content of the midradius, which consists of cortical bone [25]. Taken together with our results, it seems possible that adrenal androgens is one of the regulators of cortical bone mass.

The mechanism of action of 11βOHA on bone is still unclear. Unchanged urinary excretion of deoxypyridinoline suggests that this steroid does not have an antiresorptive action in OVX rats. The stimulation of marrow FCFU growth by administration of this steroid to both normal and ovariectomized rats suggests that osteogenic activity was increased by 11βOHA under both normal and pathological conditions since marrow FCFU growth can be used as an indirect bioassay of osteogenesis [10]. The increased mineral apposition rate in both trabecular and cortical bone shown by histomorphometry is also consistent with this conclusion. However, it remains unclear whether 11βOHA itself is involved in the regulation of bone turnover or whether other active metabolite(s) exist.

Furthermore, we cannot exclude the possibility that 11βOHA influences bone metabolism through other calcium-regulating hormones, such as parathyroid hormone, calcitonin, vitamin D metabolites, gonadal steroids, and insulin-like growth factors. Although decreased marrow FCFU growth in ovariectomized rats was reported to be reversed by administration of dihydrotachysterol, an analog of vitamin D [26], treatment

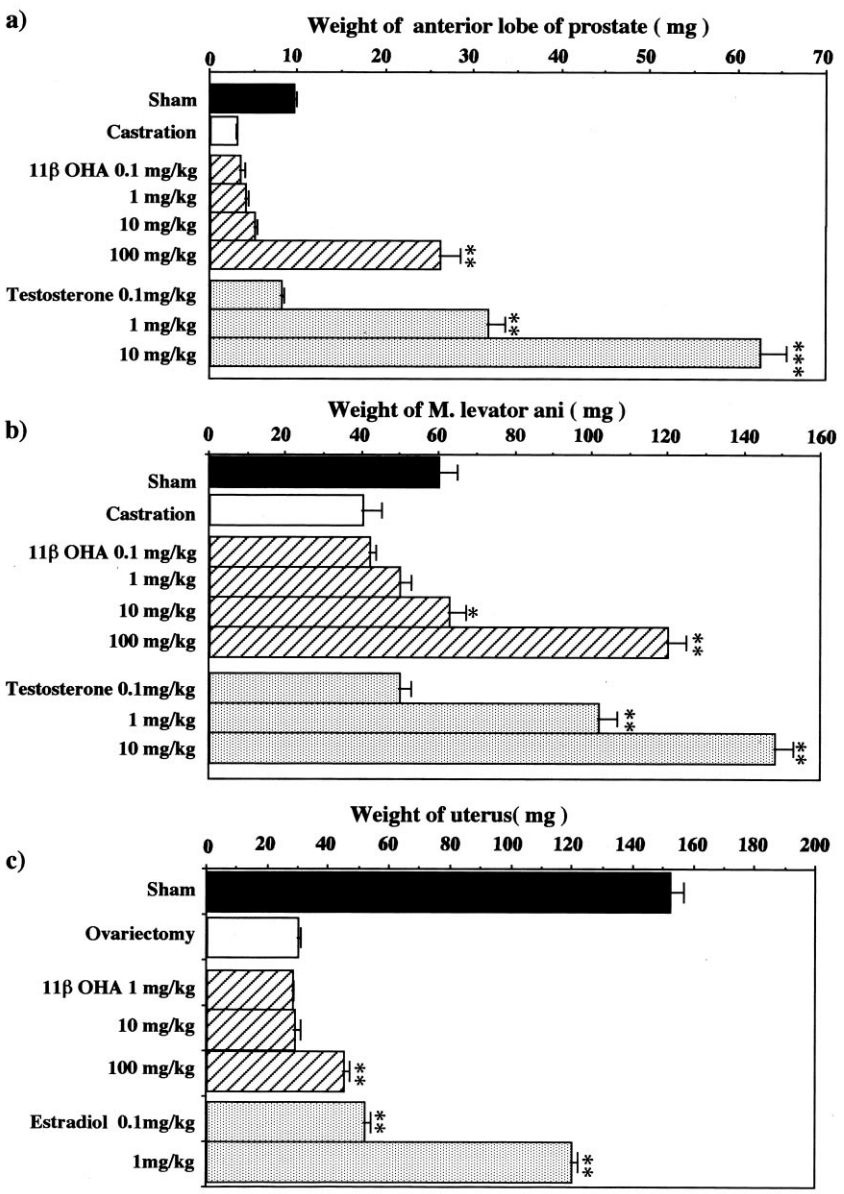

Fig. 4. Androgenic (a), anabolic (b), and estrogenic(c) actions of 11βOHA. Androgenic and anabolic actions were determined by the method of Hershberger and the estrogenic action was determined by the method of Louson. Results represent the mean ± S.E. from ten animals. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. castrated or ovariectomized controls.

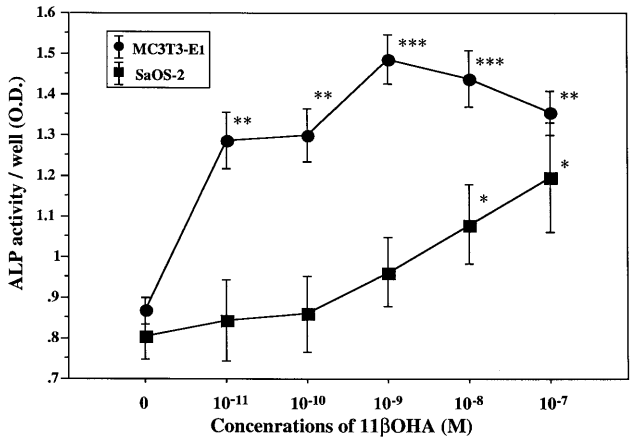

Fig. 5. In vitro effect of 11βOHA on ALP activity in mouse osteoblastic cells (MC3T3-E1) and human osteoblastic osteosarcoma cells (SaOS-2). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ vs. basal ALP activity in the absence of 11βOHA.

of ovariectomized rats with 11βOHA did not alter the serum concentration of 1,25-dihydroxyvitamin D in the present study. Gonadal and adrenal androgens are converted to estrogen by aromatase, but 11βOHA showed a very low estrogenic action. In addition, it was recently reported that androstenedione, an adrenal androgen, reduced bone loss after ovariectomy and that this effect was not mediated by estrogen [27].

Stimulation of ALP activity by gonadal and adrenal androgens has previously been demonstrated in osteoblastic cells [28,29]. In the present study, 11βOHA stimulated the ALP activity of both mouse osteoblastic cells and human osteoblastic osteosarcoma cells in a concentration-dependent fashion. The effect was more evident in MC3T3-E1 cells, which show the capacity to differentiate in vitro. Such in vitro stimulation of osteoblasts suggests that 11βOHA may be an active osteotropic hormone, which influences the differentiation, proliferation, and activation of osteoblasts. However, further studies are necessary to determine whether this steroid acts on bone cells through other hormones, such as insulin-like growth factors, since this factor has been reported to have a beneficial effect on cortical bone like 11βOHA [22–24].

In conclusion, an intrinsic adrenal steroid, 11βOHA restored bone loss in rats after ovariectomy by stimulating bone formation. Although the dose of oral 11βOHA that caused a significant increase of BMD in ovariectomized rats was high, androgenic and anabolic activities were negligible even at this dose. Therefore, its administration for the treatment of osteoporosis may be well tolerated. Various antiresorptive agents have been used for the treatment of postmenopausal osteoporosis and have been shown to maintain bone mass by preventing bone resorption, but such therapy is inadequate to achieve an increase of bone mass in patients with established severe osteoporosis. Furthermore, senile osteoporosis, characterized by reduced bone formation, affects both aged men and women and causes long bone fractures. Since a decrease of cortical bone mass plays an important role in the loss of bone strength and is not effectively prevented by antiresorptive agents, an increase of both cancellous and cortical bone mass is needed to treat such osteoporosis and to prevent long bone fractures. Thus, 11βOHA has the potential to be used as a bone-stimulating agent with or without antiresorptive agents for the treatment of various types of osteoporosis, including postmenopausal, senile, and glucocorticoid-induced osteoporosis. However, further studies are necessary to elucidate pharmacokinetics and bioavailability of 11βOHA after oral or subcutaneous administration in large animals.

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References

[1] E.R. Simpson, M.R. Waterman, Steroid hormone biosynthesis in adrenal cortex and its regulation by adrenocorticotropin, in: L.J. DeGroot (Ed.), Endocrinology, Vol. 2, Saunders, Philadelphia, PA, 1989, p. 1543, pp. 1543–1545.

[2] R. Horton, J.F. Tait, In vivo conversion of DHEA to plasma androstenedione and testosterone in man, J. Clin. Endocrinol. Metab. 27 (1967) 79–88.

[3] R. Horton, J.F. Tait, Androstenedione production and interconversion rates measured in peripheral blood and studies on the possible site of its conversion to testosterone, J. Clin. Invest. 45 (1966) 301–313.

[4] D.N. Orth, W.J. Kovacs, C.R. Debol, The adrenal cortex. Steroid hormone biosynthesis, in: J.D. Wilson, D.W. Foster (Eds.), Williams Textbook of Endocrinology, Saunders, Philadelphia, PA, 1992, p. 469, pp. 495–499.

[5] E. Howard, Steroids and bone maturation in infant mice: relative activities of dehydroepiandrosterone and testosterone, Endocrinology 70 (1960) 131–141.

[6] B.E.C. Nordin, A. Robertson, R.F. Seamark, A. Bridge, J.C. Philcox, A.G. Need, M. Horowitz, H.A. Morris, S. Deam, The relation between calcium absorption, serum dehydroepiandrosterone, and vertebral mineral density in postmenopausal women, J. Clin. Endocrinol. Metab. 60 (1985) 651–657.

[7] J.E. Nestler, C.O. Barascini, J.N. Clare, W.G. Blackard, Dehydroepiandrosterone reduces serum low density lipoprotein levels and body fat but does not alter insulin sensitivity in normal men, J. Clin. Endocrinol. Metab. 66 (1988) 57–69.

[8] R.A. Daynes, D.J. Dudley, B.A. Araneo, Regulation of murine lymphokine production in vivo. II Dehydroepiandrosterone is a natural enhancer of interleukin-2 synthesis by helper T cells, Eur. J. Immunol. 20 (1990) 793–799.
[9] R. Schlaghecke, H. Beuscher, K. Kley, E. Juli, Age-related changes in 11β-hydroxyandrostenedione concentration in normal and osteoporotic women, J. Steroid Biochem. Molec. Biol. 40 (1991) 731–733.
[10] D.J. Scimmonds, K. Loeffelman, R. Frier, R. McCoy, B. Friedman, S. Melville, A.J. Kahn, Circadian changes in osteogenic competence of marrow stromal cells, in: E. Haus, H.F. Kabat (Eds.), Chronobiology, Karger, Basel, 1984, p. 37.
[11] L.G. Hershberger, E.G. Shipley, R.K. Meyer, Myotrophic activity of 19-nortestosterone and other steroids determined by modified levator ani muscle method, Proc. Soc. Exp. Biol. Med. 83 (1953) 175–180.
[12] H.D. Louson, C.G. Heller, J.B. Golden, E.L. Serringhaus, Immature rat uterus in assay of estrogenic substances, and comparison of estradiol, estrone, and estriol, Endocrinology 24 (1939) 35–40.
[13] H. Sudo, H. Kodama, Y. Amagai, S. Yamamoto, S. Kasai, In vitro differentiation and calcification in a new clonal osteogenic cell line derived from newborn mouse calvaria, J. Cell. Biol. 96 (1983) 191–198.
[14] B.A.A. Scheven, M.J. van der Veen, C.A. Damen, F.P.J.G. Lafeber, H.J.M. Van Rijn, J.W.J. Bijlsma, S.A. Duursma, Effects of methotrexate on human osteoblasts in vitro: modulation by 1,25-dihydroxyvitamin D_3, J. Bone Miner. Res. 10 (1995) 874–880.
[15] H.L. Bradlow, T.F. Gallagher, Metabolism of 11β-hydroxy-Δ4-androstene-3,17-dione in man, J. Biol. Chem. 229 (1957) 505–518.
[16] A.D. Mooradian, J.E. Morley, S.G. Korenman, Biological actions of androgens, Endocrine Rev. 8 (1987) 1–28.
[17] C. Chen, A. Belanger, F. Labrie, Adrenal steroid precursors exert potent androgenic action in the hamster sebaceous glands of flank organs and ears, Endocrinology 137 (1996) 1752–1757.
[18] N. Orentreich, J.L. Brind, R.L. Rizer, J.H. Vogelman, Age changes and sex differences in serum dehydroepiandrosterone sulfate concentrations throughout adulthood, J. Clin. Endcrinol. Metab. 59 (1984) 551–555.
[19] S. Brody, K. Carlström, A. Lagrelius, N.O. Lunell, L. Rosenborg, Adrenocortical steroids, bone mineral content and endometrial condition in post-menopausal women, Maturitas 4 (1982) 113–122.
[20] R.T. Wild, J.R. Buchanan, C. Myers, L.M. Demers, Declining adrenal androgens: an association with bone loss in aging women, Proc. Soc. Exp. Biol. Med. 186 (1987) 355–360.
[21] R.T. Turner, E.T. Lifrak, M. Beckner, G.K. Walkley, K.S. Hannon, L.N. Parker, Dehydroepiandrosterone reduces cancellous bone osteopenia in ovariectomized rats, Am. J. Physiol. 258 (1990) E673–E677.
[22] C.M. Bagi, E. DeLeon, R. Brommage, S. Adams, D. Rosen, A. Sommer, Systemic administration of rhIGF-I or IGF-1/IGFBP-3 increases cortical bone and lean body mass in ovariectomized rats, Bone 16 (suppl.) (1995) 263S–269S.
[23] P. Ammann, R. Rizzoli, K. Muller, D. Slosman, J.-P. Bonjour, IGF-I and pamidronate increase bone mineral density in ovariectomized adult rats, Am. J. Physiol. 265 (1993) E770–E776.
[24] E.M. Spencer, C.C. Liu, E.C.C. Si, G.A. Howard, In vivo actions of insulin-like growth factor-1 (IGF-I) on bone formation and resorption in rats, Bone 12 (1991) 21–26.
[25] S. Rozenberg, H. Ham, D. Bosson, A. Pretz, C. Robyn, Age, steroids and bone mineral content, Maturitas 12 (1990) 137–143.
[26] C. Tabuchi, D.J. Simmonds, A. Fausto, J.E. Russell, I. Binderman, L.V. Avioli, Bone deficit in ovariectomized rats. Functional contribution of the marrow stromal cell population and the effect of oral dihydrotachysterol treatment, J. Clin. Invest. 78 (1986) 637–642.
[27] C.K. Lea, V. Moxham, M.J. Reed, A.M. Flanagan, Androstenedione treatment reduces loss of cancellous bone volume in ovariectomized rats in a dose-responsive manner and the effect is not mediated by estrogen, J. Endocrinol. 156 (1998) 331–339.
[28] C.H. Kasperk, J.E. Wergedal, J.R. Farley, T.A. Linkart, R.T. Turner, D.J. Baylink, Androgens directly stimulate proliferation of bone cells in vitro, Endocrinology 124 (1989) 1576–1578.
[29] C.H. Kasperk, G.K. Wakley, T. Hierl, R. Ziegler, Gonadal and adrenal androgens are potent regulators of human bone cell metabolism in vitro, J. Bone Miner. Res. 12 (1997) 464–471.