11-ketotestosterone is a major androgen produced in human gonads

Yoshitaka Imamichi1,2, Koh-ichi Yuhki1, Makoto Orisaka3, Takeshi Kitano4, Kuniaki Mukai5, Fumitaka Ushikubi1, Takanobu Taniguchi6, Akihiro Umezawa7, Kaoru Miyamoto2, Takashi Yazawa6

1Department of Pharmacology and 6Department of Biochemistry, Asahikawa Medical University, Hokkaido 078–8510, Japan, 2Department of Biochemistry and 3Department of Obstetrics and Gynecology, Faculty of Medical Sciences, University of Fukui, Fukui 910–1193, Japan, 4Department of Materials and Life Science, Graduate School of Science and Technology, Kumamoto University, Kumamoto 860–8555, Japan, 5Department of Biochemistry and Medical Education Center, Keio University School of Medicine, Tokyo 160–8582, Japan, 7Department of Reproduction, National Research Institute for Child Health and Development, Tokyo 157–8535, Japan

Context: 11-ketotestosterone (11-KT) is a novel class of active androgen. However, the detail of its synthesis remains unknown for humans.

Objective: The objective of this study was to clarify the production and properties of 11-KT in human.

Design, Participants and Methods: Expression of CYP11B1 and HSD11B2 (key enzymes involved in the synthesis of 11-KT) were investigated in human gonads. The production of 11-KT was investigated in Leydig cells. Plasma concentrations of testosterone and 11-KT were measured in 10 women and 10 men of reproductive age. Investigation of its properties was performed using breast cancer-derived MCF-7 cells.

Results: CYP11B1 and HSD11B2 were detected in Leydig cells and theca cells. Leydig cells produced 11-KT, and relatively high levels of plasma 11-KT were measured in both men and women. There was no sexual dimorphism in the plasma levels of 11-KT, even though testosterone levels were more than 20-times higher in men than in women. It is noteworthy that the levels of testosterone and 11-KT were similar in women. In a luciferase reporter system, 11-KT activated human androgen receptor (AR)-mediated transactivation. Conversely, 11-KT did not activate estrogen receptor (ER)-mediated transactivation in aromatase-expressed MCF-7 cells, whereas testosterone did following conversion to estrogen. 11-KT did not affect the estrogen/ER-mediated cell proliferation of MCF-7 cells. Furthermore, it significantly inhibited cell proliferation when AR was transfected into MCF-7 cells.

Conclusions: The current study indicates that 11-KT is produced in the gonads and represents a major androgen in human. It can potentially serve as a non-aromatizable androgen.
Although androgens are traditionally viewed as male hormones, androgen/AR signaling is also important for optimal female reproduction and physiology (4, 5). Because androgen levels reduce with aging before menopause, postmenopausal women suffer various symptoms by androgen insufficiency (5, 6). On the other hand, androgen excess in women results in a variety of pathological conditions, including polycystic ovary syndrome (PCOS) and idiopathic hirsutism (7). Therefore, proper androgen signaling is important for the health of women. In addition to androgenic actions, testosterone is also a precursor for the production of 11-KT in human gonads J Clin Endocrinol Metab.

The production of 11-KT in human gonads involves the most potent estrogen, 17β-estradiol (E2). Aromatase (CYP19A1) converts testosterone and other weak androgens, such as dehydroepiandrosterone (DHEA), DHEA sulfate, androstenedione, 11β-hydroxysterone (11-OHA), and 11β-hydroxytestosterone (11-OHT), which are also precursors for stronger androgens in target tissues (8–10). In previous studies, we and others have reported that 11-ketosterone (11-KT, International Union of Pure and Applied Chemistry name is 17-Hydroxysterone-4-ene-3,11-dione) is another class of active androgen that can be converted from testosterone and other weaker precursors (9–11).

CYP11B1 and HSD11B2 play important roles in the 11-KT synthesis from testosterone (10, 11). It is well-known that these enzymes are involved in glucocorticoid synthesis and metabolism. CYP11B1 catalyzes the final step of glucocorticoid production in the adrenal gland (12, 13), while HSD11B2 converts active glucocorticoids into inactive 11-ketosteroid forms, which are abundantly expressed in the kidney and placenta (14). We have demonstrated that Cyp11b1 and Hsd11b2 are expressed in murine gonads and are involved in gonadotropin-induced 11-KT production (11). 11-KT can strongly activate mammalian AR-mediated transactivation. It was also reported that 11-KT can be detectable in human blood samples (9). Thus, it is conceivable that 11-KT is common androgen in mammals, although the detail of its synthesis remains unknown. In the present study, we evaluated the production and properties of 11-KT in humans.

**Materials and Methods**

**Cell culture and transfection**

Human ovarian granulosa cell tumor-derived KGN cells (kindly donated by Dr. Toshikiko Yanase, University of Fukuoka, Fukuoka, Japan) and MCF-7 cells were cultured in DMEM/Ham’s F-12 medium supplemented with 10% fetal bovine serum (FBS). Human adrenocortical tumor-derived H295R cells were cultured in Opti-MEM supplemented with 2% Nutserum IV (BD Biosciences, Franklin Lakes, NJ, USA). Human Leydig cells were purchased from ScienCell Research Laboratories (Carlsbad, CA), which are sourced within the USA under protocols that have obtained Institutional Review Board (IRB) approval. They were cultured in Leydig Cell Medium (ScienCell Research Laboratories). The research protocol using human materials was approved at the ethical committee of Ashikawa Medical University. H295R cells were transfected using an Amaxa Nucleofector Technology system (Lonza, Cologne, Germany) as described (15). KGN and MCF-7 cells were transfected using Lipofectamine LTX reagent (Life Technologies, Inc., Carlsbad, CA, USA) and HilyMax (Dojindo Laboratories, Kumamoto, Japan). One day before transfection, the cells were seeded on 24-well plates and cultured with phenol red-free medium supplemented with 10% charcoal/dextran stripped (CD)-FBS. After 24 hours transfection, the cells were treated with vehicle (EtOH) or steroid hormones for 24 hours. Luciferase assays were performed as described previously (15, 16). Each data point represents the mean of at least four independent experiments.

**Adenovirus production and infection**

Adenovirus vectors for LacZ, GFP, and Steroidogenic factor-1 (SF-1) were prepared using the Adeno-X Expression System 1 (Takara, Shiga, Japan), following the manufacturer’s instructions as described (16). Using these vectors, replication-defective recombinant adenoviruses were propagated and titered in HEK 293 cells. Then, they were used to infect KGN cells at MOI 10. GFP-expressing adenovirus used as a transduction control during infection. Transduction efficiency and optimal concentrations of virus were determined by investigating GFP expression using fluorescent microscopy. At 48 hours postinfection, cells were processed for RNA or protein extraction.

**DNA microarray**

Expression analysis by DNA microarray has been described elsewhere (17). Briefly, labeled cRNA was prepared from KGN cells with adenovirus-mediated expression of LacZ, GFP, or SF-1. After fragmentation of cRNA, hybridization was performed with a human U133 Plus 2.0 Affymetrix GeneChip (Affymetrix, Santa Clara, CA, USA). The arrays were scanned using a Gene-Array scanner and the data were generated by Affymetrix Microarray Suite 4.0. Data were analyzed using Subio Platform software (http://www.subio.jp/products/platform).

**RT-PCR and quantitative (Q)-PCR**

Total RNA from the cultured cells was extracted using TriPure Isolation Reagent (Roche, Carlsbad, CA, USA). Total RNA from human testis, ovary, adrenal, liver, and kidney was purchased from Takara Bio Inc. (Shiga, Japan) and Biochain Institute Inc. (Newark, CA). RT-PCR and Q-PCR was performed as described (16, 18). The RT-PCR products were electrophoresed on a 1.5% (w/v) agarose gel, and the resulting bands were visualized by staining with ethidium bromide. In Q-PCR, each gene expression was measured by real-time PCR and normalized with β-actin expression. The primers used for PCR are described in Supplemental Table 1. The primers used for other genes were as described (16, 18).
Western blotting analysis

Extraction of total proteins from cultured cells and subsequent quantification were conducted as described previously (16). Protein extract samples from each human tissue were purchased from BioChain Institute Inc. (Hayward, CA, USA). Ovarian tissue donors were able to donate healthy organs and were of reproductive age (aged 30–34 years). Each tissue sample was collected under strict IRB ethical consenting practices. Equal amounts of protein (20 μg) were resolved using 12.5% SDS-PAGE and transferred to polyvinylidene difluoride membranes. Western blot analyses of HSD11B2, CYP11B1, AR, β-actin and GAPDH were performed with antibodies directed against HSD11B2 (H-145, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), CYP11B1 (13), AR (N-20, Santa Cruz Biotechnology, Inc.), β-actin (C4, Santa Cruz Biotechnology, Inc.) and GAPDH (6C5, Santa Cruz Biotechnology, Inc.), respectively. Enhanced chemiluminescence western blot reagents (Bio-Rad Laboratories Inc., Hercules, CA, USA) were used for detection.

Plasmids

The pcDNA3 expressing HSD11B2 was generated by cloning the open reading frame of HSD11B2 into a pcDNA3 vector (Invitrogen, Carlsbad, CA). A Slp-ARU/Luc reporter and pQCXIP/ GAPDH were performed with antibodies directed against HSD11B2 (H-145, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), CYP11B1 (13), AR (N-20, Santa Cruz Biotechnology, Inc.), β-actin (C4, Santa Cruz Biotechnology, Inc.) and GAPDH (6C5, Santa Cruz Biotechnology, Inc.), respectively. Enhanced chemiluminescence western blot reagents (Bio-Rad Laboratories Inc., Hercules, CA, USA) were used for detection.

Immunohistochemistry

Immunohistochemistry was performed as described previously (11, 19). Sections of human testis (from men aged 23 and 50 years) and ovary (from woman aged 19 and 34 years) were purchased from US Biomax Inc. (Rockville, MD) and Biochain Institute Inc. (Newark, CA). Sections were subjected to the antigen retrieval technique with Dako Target Retrieval Solution, pH 9 (Dako Denmark A/S, Glostrup, Denmark) and treated with anti-CYP11B1 (13) and anti-HSD11B2 (20). They were then developed using a Vectastain Elite ABC kit (Vector Laboratories, Burlingame, CA).

Human blood samples

Blood samples were collected in collection tubes containing heparin from the median cubital vein of two healthy women volunteers at University of Fukui Hospital in 2007. Plasma was separated by centrifugation at 1000 × g for 5 minutes. Other plasma samples were purchased from AllCells (Emeryville, CA) and ProMedDX (Norton, MA). All plasma samples were collected under IRB approved collection protocols and subject informed consent. The donors were 10 men (aged 32.1 ± 7.8 year) and 10 women (aged 31.7 ± 6.0 years). Plasma samples were stored at −80°C until assays. The research protocol for using human materials was approved by the Ethics Committee of the University of Fukui.

Measurements by enzyme immunoassays (EIA) and liquid chromatography-tandem mass spectrometry (LC-MS/MS)

Concentrations of testosterone, 11-KT and E2 in culture media of KGN, H295R and human Leydig cells were determined by competitive EIA (11, 19). Each sample was diluted with EIA buffer, and analyzed using progesterone, testosterone, 11-KT and E2 EIA Kit (Cayman Chemical Company, Ann Arbor, MI) following the manufacturer’s instructions in a microplate reader (Molecular Device SpectraMax M5; Molecular Device, LLC, Sunnyvale, CA). On the other hand, concentrations of steroid hormones in human plasma were measured by LC-MS/MS for optimal quantification of the clinical samples. Processing of human plasma samples and quantification of testosterone, 11-KT and E2 by LC-MS/MS are based on methods as described previously (9).

Retrovirus preparation and infection

The Phoenix packaging cell line was transiently transfected with the retroviral plasmids using the FuGENE 6 reagent (Promega). The supernatant was concentrated by centrifugation. The virus solution was stored at −80°C until use. MCF-7 cells were infected with the retrovirus in the presence of 8 μg/mL Polybrene (Sigma-Aldrich) for 48 hours. The cells were then replated and selected using puromycin.

Proliferation assay

MCF-7 cells or AR-introduced cells were seeded with DMEM/F-12 supplemented with 10% or 2% CD-FBS at 1 × 10^3 cells/well in 96-well plates. At 24 hours after seeding, the cells were treated with the media containing various concentrations of DHT, testosterone, 11-KT, or E2. Six days (parental cells) or nine days (AR-introduced cells) after incubation, cell proliferation was evaluated using a CellTiter 96 Aqueous One Solution Kit (Promega) following the manufacturer’s instructions. To evaluate the effect of an aromatase inhibitor, fadrozole (Sigma, St. Louis, MO) or an estrogen receptor antagonist, fulvestrant (Sigma), on the growth of MCF-7 cells, a proliferation assay was performed with or without these agents.

Statistics

Data are presented as the mean ± SEM. Differences between groups (P < .05) were assessed by the Student’s t test or one-way ANOVA followed by Tukey’s multiple comparison tests using EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan) (21), which is a graphical user interface (GUI) for R (The R Foundation for Statistical Computing, Vienna, Austria).

Results

HSD11B2 is induced by SF-1 and involved in the 11-KT production

Human ovarian granulosa cell tumor-derived KGN cells have low steroidogenic capacity under basal conditions. However, they can be transformed to produce a range of steroid hormones by infection with an adenovirus expressing SF-1 and its coactivator (16). To examine changes in gene expression in this transformation, we used a DNA microarray with GFP- or SF-1-introduced KGN cells. SF-1 introduction induced a number of genes, including steroidogenic enzymes such as CYP11A1, HSD3B2, CYP17A1, and CYP19A1 (Supplemental Table 2), which are known SF-1 targets (22). In addition to these
genes, HSD11B2 was a strong SF-1-inducible candidate gene (Supplemental Table 2) and was almost undetectable in GFP-introduced cells (Figure 1 A and B). Consistent with DNA microarray data, Q-PCR and immunoblotting analyses revealed that introduction of SF-1 strongly induced HSD11B2 mRNA and proteins (Figure 1 A and B). In previous studies, it was demonstrated that HSD11B2 is involved in 11-KT production from testosterone with CYP11B1 (Supplemental Figure 1) in murine gonads (11, 19) or from adrenal androgens by in vitro analysis (10). The DNA microarray analyses suggest that CYP11B1 is an SF-1-inducible gene, as well as other steroidogenic genes that are involved in testosterone and E2 synthesis (Figure 1C, Supplemental Table 2, Supplemental Figure 1). In fact, the introduction of SF-1 induced not only the production of testosterone and E2, but also the CYP11B1 expression and 11-KT production (Figure 1 C and D).

To verify the role of HSD11B2 during 11-KT production in human steroidogenic cells, it was ectopically expressed in human adrenocortical H295R cells. H295R cells expressed CYP11B1 and other steroidogenic enzymes for testosterone synthesis (Supplemental Figure 2), whereas endogeneous HSD11B2 was undetectable (Figure 2A). Even though H295R cells produce testosterone at relatively high levels, its conversion to 11-KT was marginal (Figure 2 C and D). Transient transfection of the HSD11B2 expression vector (Figure 2B) markedly increased 11-KT production compared with the control group (Figure 2D), whereas testosterone concentrations were similar in both groups (Figure 2C). These results indicate that the expression of human HSD11B2 could be an important factor for the production of 11-KT in steroidogenic cells.

11-KT is produced in gonads and is one of the major androgens in human

To elucidate the 11-KT synthesis pathway, we investigated the expression of CYP11B1 and HSD11B2 in human gonads (Figure 3 A and B). Q-PCR and western blot analyses showed that both genes were detectable in the

Figure 1. SF-1 induces HSD11B2 expression and 11-KT production in KGN cells. KGN cells were infected with adenoviruses expressing GFP or SF-1. Induction of HSD11B2 mRNA (A) and protein (B) by SF-1. A, mRNA expression of HSD11B2 gene was analyzed by Q-PCR and normalized to β-actin expression. Data represent the mean ± SEM of at least three independent experiments. B, Western blot analyses were performed with antibodies against HSD11B2 and GAPDH using the same lysates. C, Induction of the enzymes for the synthesis of testosterone, 11-KT and E2 was confirmed by Q-PCR. Data represent the mean ± SEM of at least three independent experiments. D, 11-KT, testosterone and E2 levels in each group were measured by EIA. Data represent the mean ± SEM of at least three independent experiments. Differences between groups are indicated by **P < .01.
testis and ovary at the mRNA and protein levels (Figure 3 A and B). Immunohistochemical analysis showed that both proteins are localized on testicular Leydig cells and ovarian theca cells, even though HSD11B2 is also detectable in some populations of ovarian granulosa cells (Figure 3C). These results strongly suggest that 11-KT is produced in testicular Leydig cells and ovarian theca cells. To confirm this hypothesis, we investigated the production of 11-KT in human Leydig cells. In support of the immunohistochemical analyses, Leydig cells expressed CYP11B1 and HSD11B2 genes (Supplemental Figure 3A). They can produce progesterone, testosterone and 11-KT under basal conditions (Figure 3D, Supplemental Figure 3B). cAMP-treatment moderately increased the production of these steroid hormones.

Then, we measured plasma concentrations of 11-KT, testosterone and E2 in both sexes (Figure 3 E-G). Testosterone levels in men were about 22-fold higher than those in women, whereas 11-KT levels were similar between the sexes (Figure 3 E and F). It is noteworthy in women that 11-KT concentrations were similar to testosterone concentrations and about 5-fold higher than E2 concentrations (Figure 3H). Human AR-mediated transactivation was significantly increased by 11-KT at concentrations > 10^-9 M in KGN cells (Figure 4). This level is lower than that for the induction by other androgens, although transactivation was increased to similar levels by DHT and testosterone at 10^-8 and 10^-7 M, respectively. These results suggest that 11-KT may play some roles in human as one of the major androgens.

**11-KT is not convertible to estrogenic hormones**

In female individuals, testosterone acts as both an androgen and a precursor for estrogen. To determine whether 11-KT is a precursor for estrogen, we performed a luciferase assay using a reporter plasmid containing the estrogen response element (ERE) in human breast cancer derived MCF-7 cells, which endogenously express aromatase and ERα. In contrast to E2, androgens had no effect on luciferase activity at low concentrations (10^-11 and 10^-10 M) (Figure 5A). However, testosterone activated ER-dependent transcription at high concentrations and at 10^-7 M testosterone was effective as E2. This activity was completely suppressed by an aromatase inhibitor, fadrozole (Figure 5B). DHT weakly activated ER-mediated transactivation in an aromatase-independent manner at 10^-7 M. In contrast, 11-KT had no effect on ER-mediated transactivation, even at 10^-6 M (Figure 5A, data not shown). These results indicate that 11-KT is a nonaromatizable androgen, and does not convert to a compound that activates ER-mediated transactivation.

Then, we assessed the effect of 11-KT on cell proliferation in MCF-7 cells. The proliferation of MCF-7 cells is highly dependent on estrogens. Consistent with previous studies, MCF-7 cells were increased by > 10^-11 M E2 (Figure 6A). In support of the reporter assays result, testosterone significantly stimulated cell proliferation at higher concentrations (Figure 6A), and fadrozole and the ER antagonist fulvestrant completely inhibited testosterone-induced cell proliferation (Figure 6B and C). Conversely, 11-KT had no effect on cell growth at any concentration, similar to DHT. These results strongly suggest that 11-KT acts as a stable androgen in both AR- and aromatase-positive cells. To further test this hypothesis, AR was stably transfected into MCF-7 cells (Figure 6D) and we measured the AR-mediated growth inhibition of each androgen. Consistent with previous reports (23), DHT strongly suppressed cell proliferation in AR-introduced MCF-7 cells (Figure 6E). Similarly, 11-KT also inhibited proliferation, though to a lesser extent than DHT. In contrast, testosterone did not significantly inhibit cell growth.
Discussion

11-KT is one of the active androgens, which was originally characterized as a teleost-specific hormone. However, we and others have since noted that it is also present in mammals (9–11). Here, we found evidence that 11-KT is produced in human gonads and is one of the major androgens. It can potentially act as a nonaromatizable androgen. Testicular Leydig cells and ovarian theca cells expressed the enzymes for producing 11-KT from testosterone, CYP11B1 and HSD11B2. In fact, Leydig cells produced 11-KT, autonomously.

HSD11B2 protects mineralocorticoid receptor (MR) from glucocorticoid by inactivating cortisol to corticosterone in aldosterone-sensitive tissues (14). The gonads are target organs for mineralocorticoids, which express MR (24, 25). Mineralocorticoids can stimulate the production of testosterone and progesterone in testicular Leydig cells (24) and ovarian granulosa cells (26), respectively. Conversely, glucocorticoids inhibit testicular and ovarian steroidogenesis (27). Because HSD11B2 proteins are expressed in these cells, it is reasonable to assume that gonadal HSD11B2 plays a role in the protection of steroidogenic cells from the adverse effects of glucocorticoids. In addition to this classical role, the involvement of HSD11B2 in 11-KT production in Leydig cells and theca cells may represent another important role (10, 11). Our results strongly suggest that the gonads are main organs for 11-KT production in humans by the expression of HSD11B2.

Figure 3. Expression of 11-KT synthetic enzymes in human gonads and plasma concentrations of 11-KT. A-B, Expression of CYP11B1 and HSD11B2 in human testis and ovary. A, mRNA levels of each gene analyzed by Q-PCR and normalized to β-actin expression. The adrenal gland and kidney were used as a positive control for each analysis. Q-PCR data represent the mean ± sem of at least three independent samples. B, Western blot analyses were performed with antibodies against CYP11B1, HSD11B2, and GAPDH using lysates from each human tissue (20 μg protein). Western blot analysis is representative of the two experiments. C, Localization of CYP11B1 and HSD11B2 proteins in human gonads. Positive staining both for CYP11B1 and HSD11B2 was observed in testicular Leydig cells and ovarian theca cells. No staining was observed in control sections incubated with nonimmune serum. An, Antrum. D, Production of testosterone and 11-KT by human Leydig cells with or without 8br-cAMP for 48 hours. Each androgen levels in each group were measured by EIA. Data represent the mean ± SEM of at least three independent experiments. E-H, Levels of plasma testosterone, 11-KT and E2 in humans. Plasma testosterone (E), 11-KT (F) and E2 (G) levels in men and women were measured by LC-MS/MS. In the box and whisker plots, boxes show 75th and 25th percentiles. Horizontal lines in the boxes represent the medians. Whiskers show the lowest values and the highest values. Differences between groups are indicated by *P < .05 and **P < .01. H, Comparison of plasma testosterone, 11-KT and E2 levels within each sex. Data represent the mean ± SEM (n = 10 men, n = 10 women).
In mammals, CYP11B1 is thought to be an adrenal-specific enzyme that is essential for the final step of glucocorticoid synthesis (12). However, it is also expressed in the testicular Leydig cells and ovarian theca cells to a lesser extent. There is also evidence that CYP21 is expressed in the gonads (25, 28). Thus, a contribution of gonadal CYP11B1 to local glucocorticoid synthesis cannot be ruled out, although there are no studies documenting high levels of cortisol in the gonads. Rather, it is often reported that cortisol levels are lower in ovarian follicular fluid (FF) than in the blood (29, 30). Therefore, it is conceivable that the production of precursors for 11-KT could be one of the most important functions of CYP11B1 in the gonads. Indeed, testosterone and androstenedione are efficiently converted to the 11-KT precursors (11-OHT and 11-OHA) by ectopic expression of human CYP11B1 with ferredoxin in COS-1 cells (10). Consistent with this in vitro study, high levels of these steroids are produced in the CYP11B1-abundant adrenal gland, even though they are rarely converted to 11-keto products by low-level expression of HSD11B2 in the adrenal. However, it is probable that adrenal-derived 11-OHT and 11-OHA could be another sources of human 11-KT by the conversion in HSD11B2 expressing organs, including gonads.

There was no sexual dimorphism of human plasma 11-KT levels, even though testosterone levels were much higher in men. Similar phenomena were also observed in rodents (11). This indicates that the dominance of 11-KT production in female is conserved between rodents and humans. In mice, the abundant expression of ovarian HSD11B2 is likely responsible for this dominance (11). In addition, the testicular expression of Hsd11b1 was much higher than in the ovary (11). Because this 11β-HSD isoform preferentially catalyzes the reduction of 11-ketosteroid (31), the difference in its expression between sexes also results in ovarian dominance of 11-KT production. Such sexual differences in the expression of both HSD11B1 (unpublished data) and HSD11B2 were unclear in human gonads. In addition to the level of expression, the activity of 11β-HSD enzymes is regulated by a number of factors (32, 33). It is interesting that human ovarian FF contains the selective inhibitor for HSD11B1 (34). Additionally, it was also reported that ovarian FF contains much higher concentrations of 11-OHA than plasma levels, which is converted from cortisol in granulosa cells (35). This might be another pathway for ovarian 11-KT production. Fur-
ther studies are needed to evaluate the sexual dimorphism of 11-KT production in humans.

DHT, testosterone, and 11-KT can strongly activate AR-mediated transactivation, even though there are some differences, especially at lower levels. Testosterone acts not only as an androgen, but also as a precursor for estrogens. It can be converted to E₂ in aromatase-expressing cells, and induces estrogen-dependent phenomena. Conversely, it is probable that 11-KT is not converted to estrogen by aromatase. Although DHT is also a nonaromatizable androgen, it can be converted to 3β-diol by HSD17B7, which can bind to ERs (36). Therefore, 11-KT likely represents extremely difficult androgen to convert to an estrogenic steroid. In addition, plasma 11-KT levels are similar to testosterone levels in women at reproductive age, and in menopausal women (9). Thus, it is possible that 11-KT plays important roles in female, especially in AR-and aromatase-expressing tissues such as ovary and breast. In a previous study, we demonstrated using mice that testosterone and 11-KT are elevated at ovulatory LH/hCG-stimulation, and are involved in the expression of ovulation-related genes, such as Cyclooxygenase-2 and Amphiregulin, in granulosa cells (19). This issue could be a subject of further investigation in human, because pre-ovulatory follicles express CYP19A1 gene at high levels (37).

In summary, we demonstrated that 11-KT is a major androgen and produced in gonads. Because androgens are essential for reproduction and physiology, their excess and deficiency often induce pathogenesis. Then, it is possible that 11-KT could be responsible for, and the novel target of therapies against, such diseases. In addition, it

Figure 6. The effects of each androgen on the proliferation in parental and AR-introduced MCF-7 cells. A-C, The effects of each steroid hormone on the proliferation of parental MCF-7 cells. A, Cells were cultured with or without each steroid hormones at various concentrations for 6 days. Data represent the mean ± sem of three independent experiments. Values marked by different letters are significantly different (P < .05). B–C, Effects of the aromatase inhibitor fadrozole (B) and the ER-antagonist fulvestrant (C) at 1 μM on testosterone-induced (10⁻⁷ M) cell proliferation. Data represent the mean ± sem of three independent experiments. Values marked by different letters are significantly different (P < .05). D-E, Cell growth inhibition by androgens in AR-introduced MCF-7 cells. D, Western blot analyses were performed with the antibodies against AR and GAPDH using lysates derived from parental and AR-introduced MCF-7 cells. E, The effects of each androgen on the inhibition of cell growth in AR-introduced MCF-7 cells. Cells were cultured with or without each androgen at 10⁻⁷ M for 9 days. Data represent the mean ± sem of five independent experiments. Values marked by different letters are significantly different (P < .05).
might provide novel insights for elucidating ambiguous AR-mediated phenomena.

Acknowledgments

We are grateful to Dr. T. Yanase for providing reagents. This work was supported in part by JSPS KAKENHI Grant Number 23 590 329 (to T.T.), Grant-in-Aid for Scientific Research (C), 15K10654 (to T.Y., Grant-in-Aid for Scientific Research (C)) and 25 861 482 (to Y.I., Grant-in-Aid for Young Scientist (B)) granted by Japan Society for the Promotion of Science, the Smoking Research Foundation (to T.T.), Yamaguchi Endocrine Research Foundation (to T.Y.) and the fund for Asahikawa Medical University Creative Research Foundation (to T.Y.).

Address all correspondence and requests for reprints to: Takashi Yazawa, PhD, Department of Biochemistry, Asahikawa Medical University, Midorigaoka Higashi 2–1–1–1, Asahikawa, Hokkaido 078–8510, Japan, Phone: +81–166–68–2342, Fax: +81–166–68–2342, e-mail: yazawa@asahikawa-med.ac.jp.

Disclosure statement; all authors have nothing to disclose. This work was supported by .

References


