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DHEA and Its Transformation into Androgens and Estrogens in Peripheral Target Tissues: Intracrinology

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A new understanding of the endocrinology of menopause is that women, at menopause, are not only lacking estrogens resulting from cessation of ovarian activity but have also been progressively deprived for a few years of androgens and some estrogens originating from adrenal DHEA and androstenedione (4-dione). In fact, serum DHEA decreases by about 60% between the maximal levels seen at 30 years of age to the age of menopause. This decreased secretion of DHEA and DHEA-S by the adrenals is responsible for a parallel decrease in androgen and estrogen formation in peripheral tissues by the steroidogenic enzymes specifically expressed in each cell type in individual target tissues. This new field of endocrinology, called intracrinology, describes the local synthesis of androgens and estrogens made locally in each cell of each peripheral tissue from the adrenal precursors DHEA and 4-dione. These androgens and estrogens exert their action in the same cells where their synthesis takes place and they are released from these target cells only after being inactivated. To further understand the effect of DHEA in women, DHEA has been administered in postmenopausal women for 12 months. Such treatment resulted in increased bone formation and higher bone mineral density accompanied by elevated levels of osteocalcin, a marker of bone formation. Vaginal maturation was stimulated, while no effect was observed on the endometrium. Preclinical studies, on the other hand, have shown that, due to its predominant conversion into androgens, DHEA prevents the development and inhibits the growth of dimethylbenz(a)anthracene-induced mammary carcinoma in the rat, a model of breast cancer. DHEA also inhibits the growth of human breast cancer ZR-75-1 xenografts in nude mice. The inhibitory effect of DHEA on breast cancer is due to an androgenic effect of testosterone and dihydrotestosterone made locally from DHEA. When used as replacement therapy, DHEA is free of the potential risk of breast and uterine cancer, while it stimulates bone formation and vaginal maturation and decreases insulin resistance. The combination of DHEA with a fourth generation SERM, such as EM-652 (SCH 57068), a compound having pure and potent antiestrogenic activity in the mammary gland and endometrium, could provide major benefits for women at menopause (inhibition of bone loss and serum cholesterol levels) with the associated major advantages of preventing breast and uterine cancer. A widely used application of intracrinology is the treatment of prostate cancer where the testicles are blocked by an LHRH agonist while the androgens made locally in the prostate from DHEA are blocked by a pure antiandrogen. Such treatment,

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called combined androgen blockade, has led to the first demonstration of a prolongation of life in prostate cancer. KEY WORDS: intracrinology; DHEA; androgens; estrogens; menopause; osteoporosis; hormone replacement therapy; SCH 57068; EM-652; antiestrogen; breast cancer; uterine cancer; cholesterol.

INTRODUCTION

Man is unique, with some other primates, in having adrenals that secrete large amounts of the precursor steroids dehydroepiandrosterone sulfate (DHEA-S)[1] and DHEA, which are converted into androstenedione (4-dione) and then into potent androgens and/or estrogens in peripheral tissues (50, 40, 41) (Fig. 1). Adrenal secretion of DHEA and DHEA-S increases during adrenarche in children at the age of 6 to 8 years, and maximal values of circulating DHEA-S are reached between the ages of 20 and 30 years. Thereafter, serum DHEA and DHEA-S levels decrease markedly (78, 101, 83, 8). In fact, at 70 years of age, serum DHEA-S levels are at approximately 20% of their peak values, while they can decrease by 95% by the age of 85 to 90 years (78). The 70 to 95% reduction in the formation of DHEA-S by the adrenals during aging results in a dramatic reduction in the formation of androgens and estrogens in peripheral target tissues, which could well be involved in the pathogenesis of age-related diseases such as insulin resistance (16, 92) and obesity (81, 74). Low circulating levels of DHEA-S and DHEA have been found in patients with breast (105) and prostate (98) cancer and DHEA has been found to exert antioncogenic activity in a series of animal models (93, 34, 64). DHEA has also been shown to have immunomodulatory effects *in vitro* (99) and *in vivo* in fungal and viral diseases (89), including HIV (35). On the other hand, a stimulatory effect of DHEA on the immune system has been described in postmenopausal women (13).

It is thus remarkable that man, in addition to possessing very sophisticated endocrine and paracrine systems, has largely vested in sex steroid formation in peripheral tissues (50, 40, 41). In fact, while the ovaries and testes are the exclusive sources of androgens and estrogens in lower mammals, the situation is very different in man and higher primates, where active sex steroids are in large part or wholely synthesized locally in peripheral tissues, thus providing target tissues with controls which adjust the formation and metabolism of sex steroids to local requirements.

[1] Abbreviations used: 3α-diol-G, androstane-3α, 17β-diol glucuronide; 3β-diol-G, androstane-3β,17β-diol glucuronide; 3β-HSD, 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase; 4-dione, androstenedione; 5-diol, androst-5-ene-3β, 17β-diol; aa, amino acids; ACTH, adrenocorticotropin; A-dione, androstanedione; ADT-G, androsterone glucuronide; AR, androgen receptor; BMD, bone mineral density; BPH, benign prostatic hypertrophy; DHEA, dehydroepiandrosterone; DHEA-FA, DHEA–fatty acid esters; DHEA-S, dehydroepiandrosterone sulfate; DHT, dihydrotestosterone; DRT, DHEA replacement therapy; E_1, estrone; E_2, 17β-estradiol; ERT, estrogen replacement therapy; HRT, hormonal replacement therapy; HSD, hydroxysteroid dehydrogenase; LHRH, luteinizing hormone-releasing hormone; MHC, major histocompatibility complex; p, progesterone; PKD, polycystic kidney disease; PRAP, prolactin receptor-associated protein; RoDH1, type 1 retinol dehydrogenase; SERM, selective estrogen receptor modulator; T, testosterone.

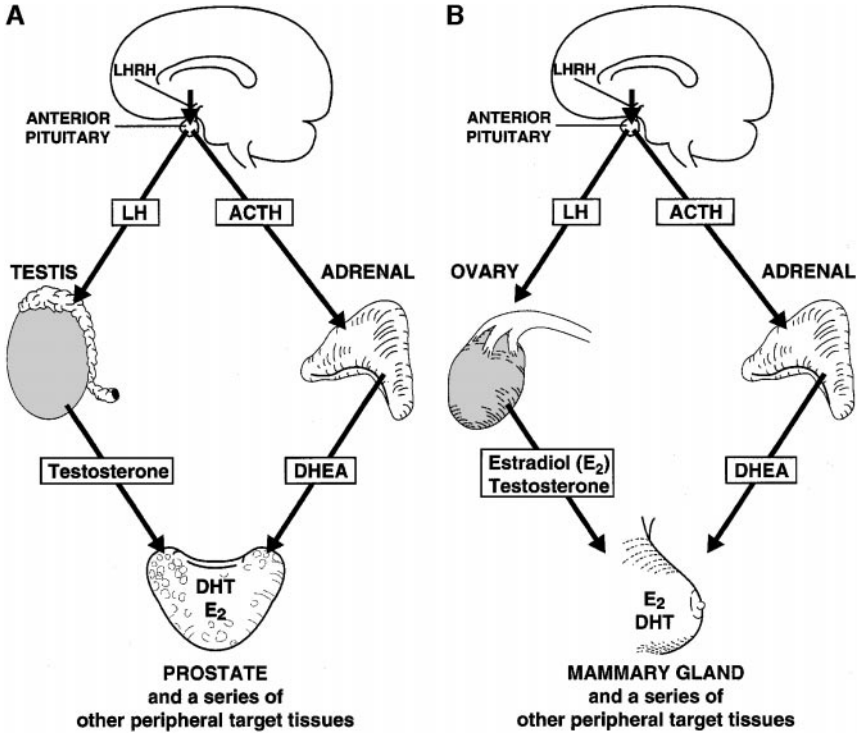

FIG. 1. Schematic representation of the role of gonadal (testicular and ovarian) and adrenal sources of sex steroids in men (A) and premenopausal women (B). After menopause, the secretion of estradiol by the ovaries ceases and almost 100% of sex steroids are made locally in peripheral target intracrine tissues. Abbreviations: ACTH, adrenocorticotropin; DHEA, dehydroepiandrosterone; DHT, dihydrotestosterone; E_2, 17β-estradiol; LH, luteinizing hormone; LHRH, LH-releasing hormone.

Transformation of the adrenal precursor steroids DHEA-S and DHEA into androgens and/or estrogens in peripheral target tissues depends upon the level of expression of the various steroidogenic and metabolizing enzymes in each of these tissues. This sector of endocrinology that focuses on the intracellular hormone formation and action has been called intracrinology (40, 41) (Fig. 2). The term intracrinology was thus coined in 1988 (40) to describe the synthesis of active steroids in peripheral target tissues where the action is exerted in the same cells where synthesis takes place without release in the extracellular space and general circulation (41).

Proof of the role of estrogen formation in peripheral intracrine tissues is well illustrated in women by the important benefits on breast cancer observed in postmenopausal women treated by a series of aromatase inhibitors (12). Most convincingly, the recent observation that administration of the antiestrogen Raloxifene for only 3 years in postmenopausal women led to a 76% decrease in the incidence of breast cancer (19) is a clear demonstration of the role of extraovarian estrogens in the development and growth of breast cancer.

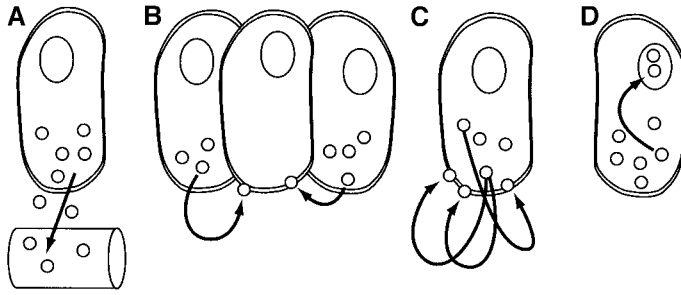

FIG. 2. Schematic representation of endocrine (A), paracrine (B), autocrine (C), and intracrine (D) secretion. Classically, endocrine activity includes the hormones secreted in specialized glands, called endocrine glands, for release into the general circulation and transport to distant target cells. On the other hand, hormones released from one cell can influence neighboring cells (paracrine activity) or can exert a positive or negative action on the cell of origin (autocrine activity). Intracrine activity describes the formation of active hormones which exert their action in the same cells where synthesis took place without release into the pericellular compartment. Reprinted from *Molecular and Cellular Endocrinology,* F. Labrie, Intracrinology, C113–C118, Copyright 1991, with permission from Elsevier Science.

HOW THE ROLE OF DHEA AS SOURCE OF ANDROGENS WAS DISCOVERED

In the course of studies on the hormonal therapy of prostate cancer, a surprising observation was that approximately 50% of DHT was left in the prostate in men who had their testicles removed or had complete blockade of testicular androgen secretion following treatment with a luteinizing hormone-releasing hormone (LHRH) agonist (52, 46) (Fig. 3). Thus, while blood levels of testosterone were reduced by 90–95% following castration, the intraprostatic concentration of DHT, the active intracellular androgen, was decreased by only 50%, thus suggesting that, in the absence of testicles, another source continued to provide androgens to the prostate.

This crucial observation led to the development of combined androgen blockade which uses a pure antiandrogen added to medical or surgical castration. The objective was to simultaneously block the androgens of both testicular and adrenal origin at the start of treatment of prostate cancer (51, 50). Combined androgen blockade was thus the first treatment demonstrated in prospective and randomized trials to prolong life in prostate cancer (18, 14, 9, 88). Recent metaanalyses of all the randomized studies using the pure antiandrogens flutamide and nilutamide show that the risk of death from any cause is reduced by 8 to 20% in men with advanced prostate cancer who received combined androgen blockade compared to the standard therapy, namely, castration alone (14, 9, 88). In analogy with any type of cancer, where treatment of early disease is known to be much more efficient than treatment of advanced cancer, the same combined androgen blockade applied at the localized stage of prostate cancer can even cure the disease in a large proportion of patients (48, 43).

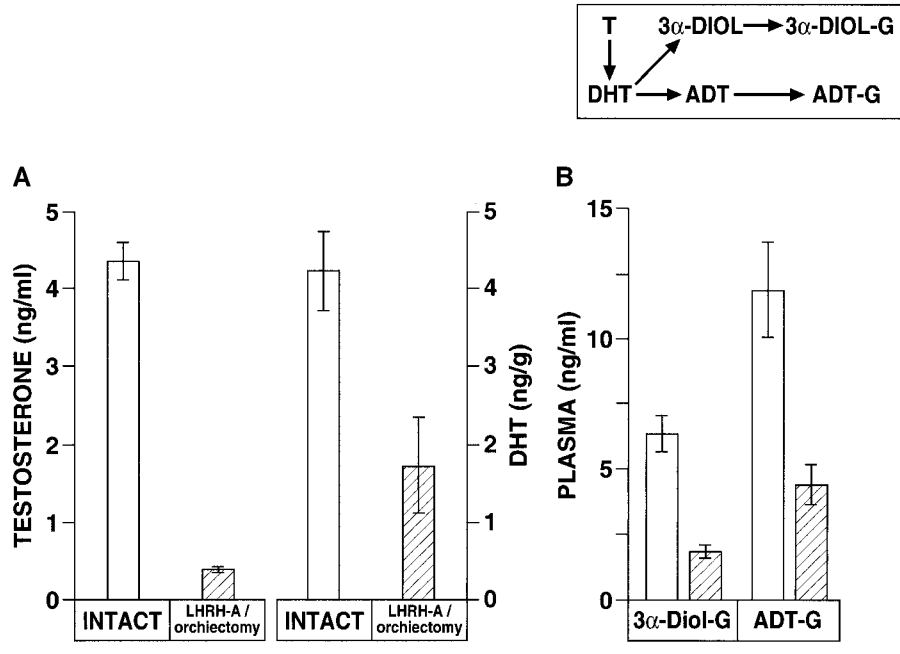

FIG. 3. (A) Effect of castration on the serum levels of testosterone (T) and on the concentration of the active androgen 5α-dihydrotestosterone (DHT) remaining in prostatic cancer tissue after castration. Note the relatively small effect (approximately 60%) of castration on intraprostatic DHT concentration compared to the 90% fall in serum T. LHRH-A, luteinizing hormone releasing hormone agonist. (B) Plasma concentrations of androstane-3α, 17β-diol glucuronide (3α-Diol-G), and androsterone glucuronide (ADT-G) in 20 intact (open bars) and 18 castrated (hatched bars) men with prostate cancer. Patients were of similar age [reproduced with permission: Labrie F. Endocrine therapy of prostate cancer: Optimal form and timing. *The Journal of Clinical Endocrinology and Metabolism*, 1995; **80:** 1066–1071. © The Endocrine Society.

STRUCTURE OF THE HUMAN STEROIDOGENIC ENZYMES

As mentioned above, transformation of the adrenal precursor steroids DHEA-S and DHEA into androgens and/or estrogens in peripheral target tissues depends upon the level of expression of the various steroidogenic and metabolizing enzymes in each of these tissues. Knowledge in this area has recently made rapid progress with the elucidation of the structure of most of the tissue-specific genes that encode the steroidogenic enzymes responsible for the transformation of DHEA-S and DHEA into androgens and/or estrogens in peripheral tissues (55, 59, 60, 73, 54) (Fig. 4). The particular importance of DHEA and DHEA-S is illustrated by the finding that approximately 50% of total androgens in the prostate of adult men derive from these adrenal precursors steroids (50, 7, 47). As mentioned above, our best estimate of the intracrine formation of estrogens in peripheral tissues in women is in the order of 75% before menopause and close to 100% after menopause (41).

Because the molecular structure of most of the key non-P-450-dependent enzymes required for sex steroid formation had not been elucidated and

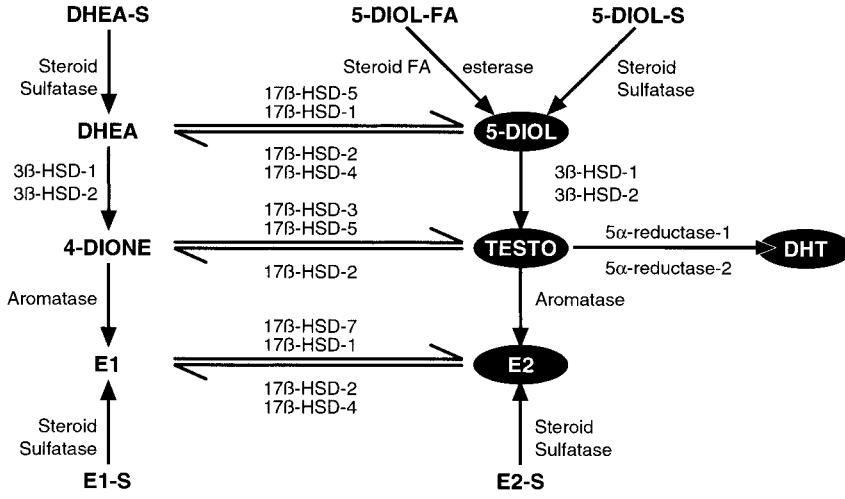

FIG. 4. Human steroidogenic enzymes in peripheral intracrine tissues.

knowing that local formation of sex steroids is most likely to play a major role in both normal and tumoral hormone-sensitive tissues, an important proportion of our research program and that of other groups has been devoted to this exciting and therapeutically promising area (53–56, 58, 84).

Human 3β-HSD Isoenzymes and Their Genes

Despite its essential role in the biosynthesis of all classes of hormonal steroids, the structure of the 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4-isomerase gene family, hereafter called 3β-HSD, was elucidated only relatively recently (71, 61, 62, 90, 55).

The membrane-bound enzyme 3β-HSD catalyzes an essential step in the transformation of all 5-pregnen-3β-ol and 5-androsten-3β-ol steroids into the corresponding Δ^4-3-ketosteroids, namely, progesterone as well as the precursors of all androgens, estrogens, glucocorticoids, and mineralocorticoids. In addition, 3β-HSD is responsible for the interconversion of 3β-hydroxy- and 3-keto-5α-androstane steroids. Not only is 3β-HSD found in the classical steroidogenic tissues (placenta, adrenal cortex, ovary, and testis), but also in several peripheral tissues, including the skin, adipose tissue, breast, lung, endometrium, prostate, liver, kidney, epididymis, and brain (55, 84, 79, 75), thus catalyzing the first step in the intracrine transformation of DHEA into androstenedione (4-dione), the precursor of both androgens and estrogens.

Following purification of 3β-HSD from human placenta and development of antibodies against the enzyme in rabbits, we isolated and characterized a first 3β-HSD cDNA type (71) and its corresponding gene (61). The second 3β-HSD cDNA type, which corresponds to the almost exclusive mRNA species expressed in the adrenals and gonads, was chronologically designated human

type 2 3β-HSD (90). The structure of the corresponding human type 2 3β-HSD gene has also been elucidated (62). The human 3β-HSD genes corresponding to human cDNAs types 1 and II contain four exons and three introns within a total length of 7.7 to 7.8 kbp. These genes were assigned by *in situ* hybridization to the p13.1 region of chromosome 1 and are closely linked to D1S514 located at 1–2 cM of the centromeric marker D1Z5 (80).

We have demonstrated that mutations in the type 2 3β-HSD gene are responsible for classic 3β-HSD deficiency, a form of congenital adrenal hyperplasia that impairs steroidogenesis in both the adrenals and gonads (91, 97, 96). However, the absence of mutations in the type 1 gene provided the long-awaited molecular explanation for the persistence of peripheral steroidogenesis in these patients.

In addition to the characterization of the structure of the types of 3β-HSD expressed in the macaque and bovine ovary, the nucleotide sequence of four types of rat and four types of mouse 3β-HSD cDNAs and their corresponding deduced amino acid sequences have recently become available (95, 55, 94, 58). The existence of multiple members of the 3β-HSD gene family offers the unique possibility of tissue- and/or cell-specific expression of this enzymatic activity.

Human 17β-Hydroxysteroid Dehydrogenases

The synthesis from DHEA of the most potent natural androgen, dihydrotestosterone (DHT), and of the most potent natural estrogen, 17β-estradiol (E_2), involves several enzymes, namely, 3β-hydroxysteroid dehydrogenase/Δ^5-Δ^4 isomerase (3β-HSD), 17β-HSD, 5α-reductase, and/or aromatase (Fig. 3).

Type 1 17β-HSD

The molecular structure of the gene and mRNA for human type 1 17β-HSD cDNA, which encodes a predicted protein of 327 amino acids, was the first to be elucidated (85, 70, 69, 86) (Fig. 4). This enzyme is a member of the short-chain alcohol dehydrogenase superfamily. The type 1 17β-HSD enzyme is a cytosolic protein that exists in a homodimeric form that catalyses predominantly the interconversion of E_1 to E_2 using NADP(H) as cofactor (25, 65).

To perform the structure–function analysis of type 1 17β-HSD, the protein was rapidly purified from the placenta, thus yielding a highly active preparation, which was then crystallized (65, 104). The protein was also overproduced in bacillovirus and crystals were obtained (11). This crystallization led to the elucidation of the three-dimensional structure (33) of human type 1 17β-HSD, thus achieving the first X-ray structure determination of a mammalian steroidogenic enzyme. The structure of type 1 17β-HSD from human placenta was determined at 2.2 Å resolution by a combination of isomorphous replacement (with a single mercury derivative) and molecular replacement techniques (Fig. 5).

Type 2 17β-HSD

The structure of a cDNA encoding a second type of 17β-HSD cDNA was then reported (103, 3). This cDNA encodes a predicted protein of 387 amino acids with a molecular weight of 42,782 (Fig. 4). This protein is most likely associated with the membranes of the endoplasmic reticulum. The enzyme catalyzes the conversion of E_2 to E_1, testosterone to 4-dione, and androst-5-ene-3β, 17β-diol (5-diol) to DHEA. This enzyme, chronologically designated type 2 17β-HSD, is also a member of the short-chain alcohol dehydrogenase superfamily but it shares only about 20% sequence identity with the type 1 17β-HSD cytoplasmic enzyme (70). This enzyme uses NAD(H) as a cofactor (103) and is less specific than type 1 17β-HSD, both estrogens and androgens acting as substrates.

Type 3 17β-HSD

A third type of human 17β-HSD cDNA encoding a predicted protein of 310 amino acids with a molecular weight of 34,513 was then characterized (32). Type 3 17β-HSD, a microsomal isozyme, using NADP(H) as a cofactor, is expressed predominantly in the testes, with an equilibrium of the reaction favoring testosterone production from 4-dione. This enzyme, which shares 23% sequence identity with the two other 17β-HSD enzymes, is the site of the mutations responsible for male pseudohermaphroditism resulting from 17β-HSD deficiency (32).

Type 4 17β-HSD

Human type 4 17β-HSD is a 736-amino-acid protein with a molecular mass of 80 kDa which transforms E_2 to E_1 and 5-diol to DHEA (63, 1). The human type 4 17β-HSD mRNA is expressed in virtually all human tissues examined by Northern blot, including the liver, heart, prostate, testis, lung, skeletal muscle, kidney, pancreas, thymus, ovary, intestine, placenta, and several human breast cancer cell lines. Thus, this enzyme is likely to play a role in the inactivation of estrogens in a large series of peripheral tissues, although its activity is low and its physiological importance remains to be established in the human.

Type 5 17β-HSD

Although type 3 17β-HSD synthesizes testosterone from 4-dione in the Leydig cells of the testes, thus providing approximately 50% of the total amount of androgens in men, the same enzymatic reaction is catalyzed in peripheral target tissues by a different enzyme, namely, type 5 17β-HSD. This

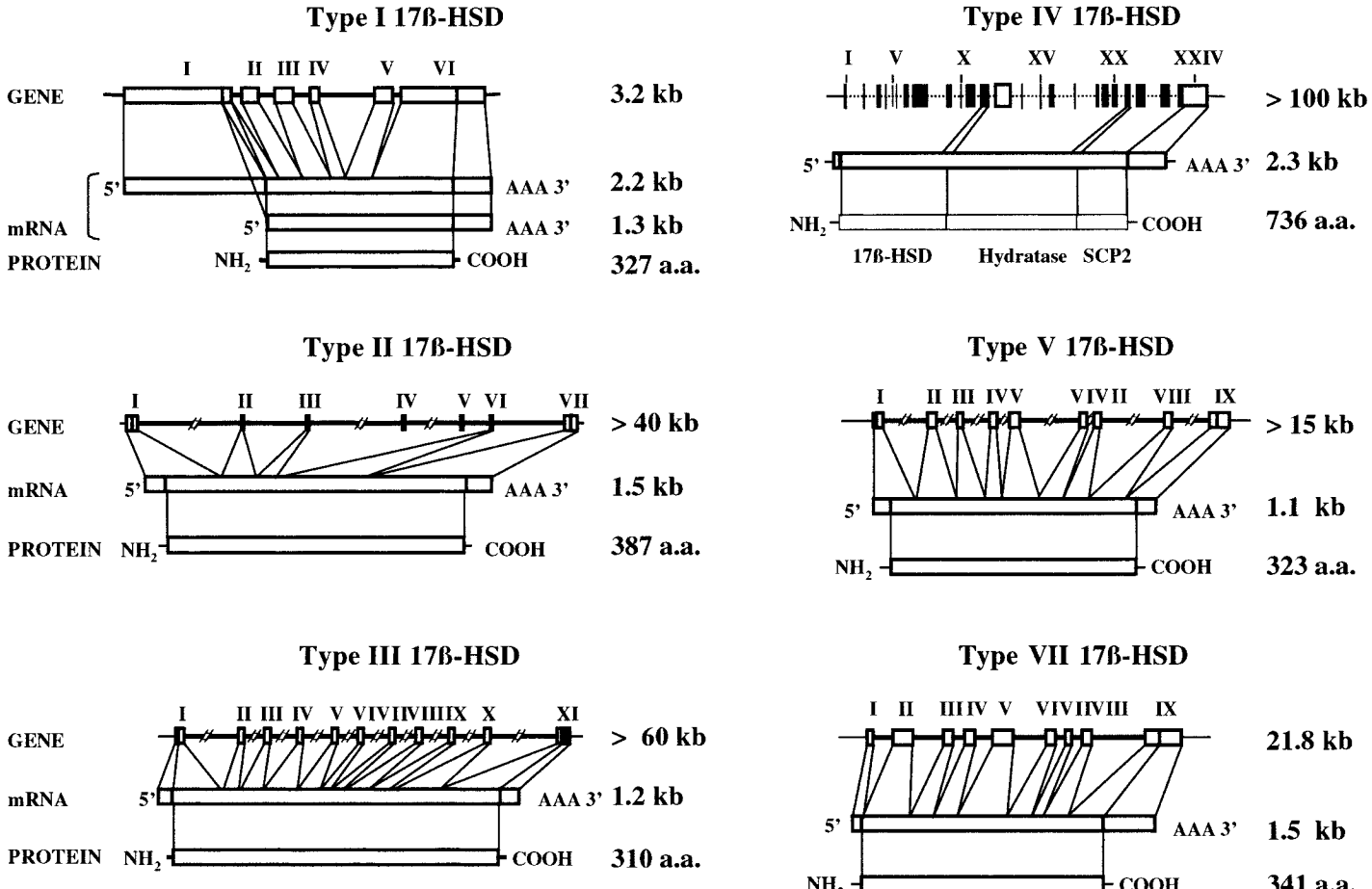

FIG. 5. Structure of the genes and mRNAs encoding human types 1, 2, 3, 4, 5, and 7 17β-HSD and the corresponding proteins. Abbreviations: aa, amino acids; HSD, hydroxysteroid dehydrogenase (54).

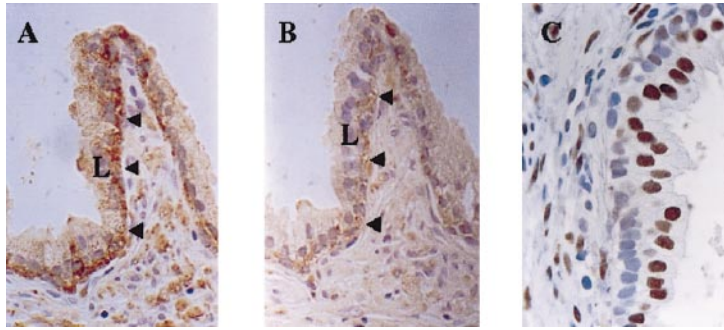

FIG. 6. Paraffin-embedded sections of benign prostatic hypertrophy (BPH) tissue, immunostained with antibodies for (A) type 5 17β-HSD, (B) 3β-HSD, and (C) the AR. Consecutive sections immunostained for type 5 17β-HSD (A) and 3β-HSD (B). Although the reaction for 3β-HSD is somewhat weaker than that obtained for type 5 17β-HSD, the distribution of the two enzymes in basal (arrowheads) and luminal (L) cells is similar (27). (C) AR immunoreactivity was found to be exclusively localized in the nuclei. Abbreviations: AR, androgen receptor; HSD, hydroxysteroid dehydrogenase. (A) and (B) scale bars, 100 μm; (C) scale bar, 65 μm (54).

enzyme is highly homologous with types 1 and 3 3α-HSDs as well as 20α-HSD (24) and thus belongs to the aldo-keto reductase family.

The human prostate can be used to illustrate the potential role of type 5 17β-HSD as the local source of androgens in a peripheral target tissue. As mentioned above, about 50% of androgens in the human prostate are synthesized locally from the inactive adrenal precursors DHEA and DHEA-S of adrenal origin.

The stratified epithelium lining the tube alveoli of the human prostate is divided into two layers, namely, the basal layer of low cuboidal cells located underneath a layer of columnar secretory cells (luminal cells) (Fig. 6). Type 5 17β-HSD enzyme is expressed in the basal cells, whereas luminal cells show a much lower and variable amounts of the enzyme (Fig. 6A). Similar results are obtained when the cellular distribution of 3β-HSD is examined (Fig. 6B). The *in situ* hybridization results obtained with a [^{3}H]UTP-labeled type 5 17β-HSD riboprobe are in agreement with the immunostaining data obtained with the specific antibody to the enzyme (28). However, androgen receptor (AR) immunoreactivity shows a different distribution because, in the prostatic epithelium, the nuclei of basal cells are negative, whereas the nuclei of the luminal cells show intense positive staining (Fig. 6C).

In conclusion, human type 5 17β-HSD and 3β-HSD are found at high levels in the basal epithelial cells of the human prostate. By contrast, the AR is found in high amounts in the luminal cells. Such data suggest that DHEA of adrenal origin is transformed in the basal cells of the glandular epithelium to 4-dione by 3β-HSD and then to testosterone by type 5 17β-HSD, whereas DHT appears to be synthesized from testosterone by 5α-reductase in both the basal and luminal cells (27).

Two cell types have been shown to involved in the biosynthesis of steroids in the ovary. In fact, in the ovary, C19 steroids (4-dione and testosterone) syn-

thesized by theca interna cells are transferred to granulosa cells, where they are aromatized to estrogens (77). The present data strongly suggest the possibility of a similar two-cell mechanism of androgen information in the human prostate: testosterone synthesized from DHEA in the basal cells diffuses into the luminal cells, where transformation into DHT occurs. It is of interest to mention that the basal cells of the human prostate are themselves unresponsive to androgens.

Type 6 17-HSD

Using a rat prostate cDNA obtained by expression cloning, Biswas and Russell (10) have isolated cDNA clones which metabolize 3α-diol. Among the many clones obtained, one type, termed type 6 17β-HSD, catalyzes selectively the oxidation of 3α-diol to androsterone. The transformation of other C19-steroids—namely, DHT to androstanedione (A-dione) and testosterone to 4-dione—also occurs, but at an approximately 50- to 100-fold lower rate.

Type 6 17β-HSD shares 65% homology with rat type 1 retinol dehydrogenase (RoDH1) and thus belongs to the retinol dehydrogenase family. The human counterpart has not yet been described, and its role remains to be established.

Type 7 17β-HSD

Type 7 17β-HSD was first cloned from a rat corpus luteum cDNA library and was identified as prolactin receptor-associated protein (PRAP) (23). With the use of expression cloning of a mouse mammary epithelial (HC11) cell cDNA library, a clone that shares 89% identity with rat PRAP and catalyzes selectively the transformation of E_1 to E_2 has been isolated (82). After transfection into HEK-293 cells, Nokelainen *et al.* (82) also found that rat PRAP catalyzes efficiently and selectively the transformation of E_1 to E_2, while the transformation of C19 steroids was much weaker.

Human type 7 17β-HSD cDNA is 1.5 kb long and encodes a protein of 37 kDa or 341 amino acids (39). With the use of RT–PCR, this enzyme is detected in the ovary, breast, placenta, testis, prostate, and liver. Comparison with other 17β-HSDs indicates that it shares less than 20% identity, a typical percentage for the other members of the 17β-HSD family. The human type 7 17β-HSD gene spans 21.8 kb and consists of nine exons and eight introns. The gene is assigned to human chromosome bands 10p11.2 (39). It is noteworthy that type 5 17β-HSD is also mapped to human chromosome 10 (bands 10p15 $\rightarrow$ 14).

Type 8 17β-HSD

Type 8 17β-HSD is also known as the product of the Ke6 gene, which is found in the HLA region (38). This area is well known to contain genes encoding the

human major histocompatibility complex (MHC). This complex is thought to be involved in polycystic kidney disease (PKD) because aberrant gene expression has been found in two different models of PKD mice (5). Recently, Fomitcheva *et al.* (29) have found that the overproduced protein fused with GST catalyzes efficiently the transformation of E_2 to E_1. The transformation of testosterone to 4-dione is about 25% of that of E_2 into E_1.

Although the human Ke6 gene has been known for a few years (38), the cDNA clone and thus the protein itself have not been isolated and the activity of the enzyme is unknown. The gene encodes a protein of 274 amino acids. Whether human Ke6 protein possesses 17β-HSD activity thus remains to be determined.

Human 5α-Reductase Isoenzymes

The enzyme 5α-reductase catalyzes the 5α-reduction of 4-dione, T, and other 4-ene-3-keto-steroids to the corresponding 5α-dihydro-3-keto-steroids. The best known role of this enzyme is the transformation of T into DHT, the most potent androgen, which is responsible for the differentiation of the male external genitalia and prostate as well as virilization at puberty. The major impact of 5α-reductase in men, however, is its role in prostate cancer and benign prostatic hyperplasia. Two types of human steroid 5α-reductases, chronologically identified as type 1 and type 2, were isolated from a human prostatic cDNA library (4, 2). The structure of the human type 1 5α-reductase gene has been elucidated (36). This gene is not responsible for 5α-reductase deficiency, and it is relatively insensitive to the inhibitor finasteride (2). Type 2 5α-reductase is the isozyme responsible for male pseudohermaphroditism from 5α-reductase deficiency (2, 102).

Considering the crucial role of type 2 5α-reductase, we have elucidated the structure of its corresponding gene (59). The type 2 5α-reductase gene contains five exons and four introns and shows splicing sites identical to those of the type 1 gene. Its coding region shares 57% homology with that of type 1 5α-reductase gene. Type 1 5α-reductase is the predominant form expressed in human skin (72).

MOST OF THE DECLINE IN CIRCULATING DHEA OCCURS BEFORE THE AGE OF 60 YEARS

To gain a better knowledge of the role of DHEA and DHEA-S transformation in both men and women, we analyzed the serum levels of 18 conjugated C21 and C19 steroids (45). We thereby wanted to assess precisely the changes occurring in the serum concentration of these steroids of particular interest over a range of ages from the peak value of adrenal secretion of DHEA and DHEA-S (20–30 years) to the near lowest values found at 70–80 years.

The data obtained show a dramatic decline in the circulating levels of DHEA, DHEA-S, androst-5-ene-3β,17β-diol (5-diol), 5-diol-S, 5-diol fatty acid

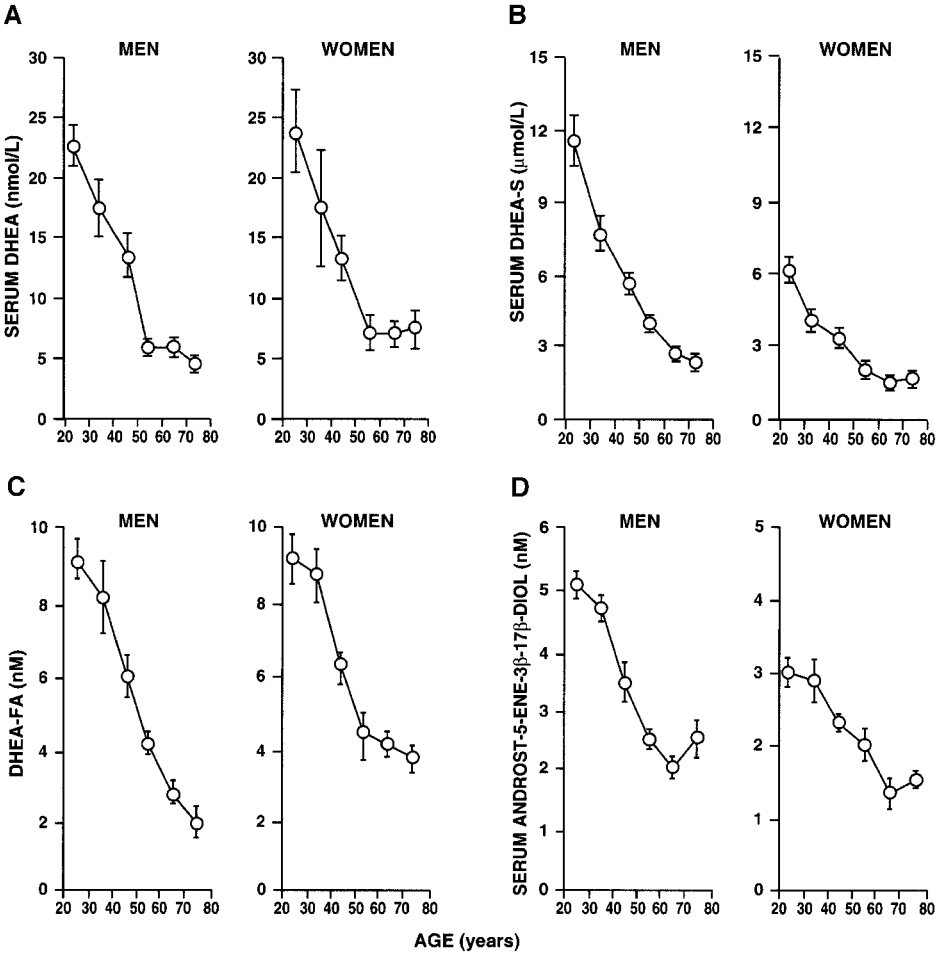

FIG. 7. Effect of age (20–30 to 70–80 years old) on serum concentration of DHEA (A), DHEA-S (B), DHEA–fatty acid esters (DHEA-FA) (C), and 5-diol (D) in men and women [reproduced with permission: Labrie F, Bélanger A, Cusan L, Gomez JL, Candas B. Marked decline in serum concentrations of adrenal C19 sex steroid precursors and conjugated androgen metabolites during aging. *The Journal of Clinical Endocrinology and Metabolism;* 1997; **82:** 2396–2402. © The Endocrine Society.

esters, and androstenedione in both men and women between the ages of 20 and 80 years. In the 50- to 60-year-old group, serum DHEA has already decreased by 74 and 70% from its 20- to 30-year-old peak values in men and women, respectively (Fig. 7).

The serum concentrations of the conjugated metabolites of DHT, namely, androsterone (ADT)-G, androstane-3α,17β-diol (3α-diol-G), and androstane-3β,17β-diol (3β-diol-G), are the most reliable parameters of the total androgen pool in both men and women, while serum testosterone and dihydrotestoster-

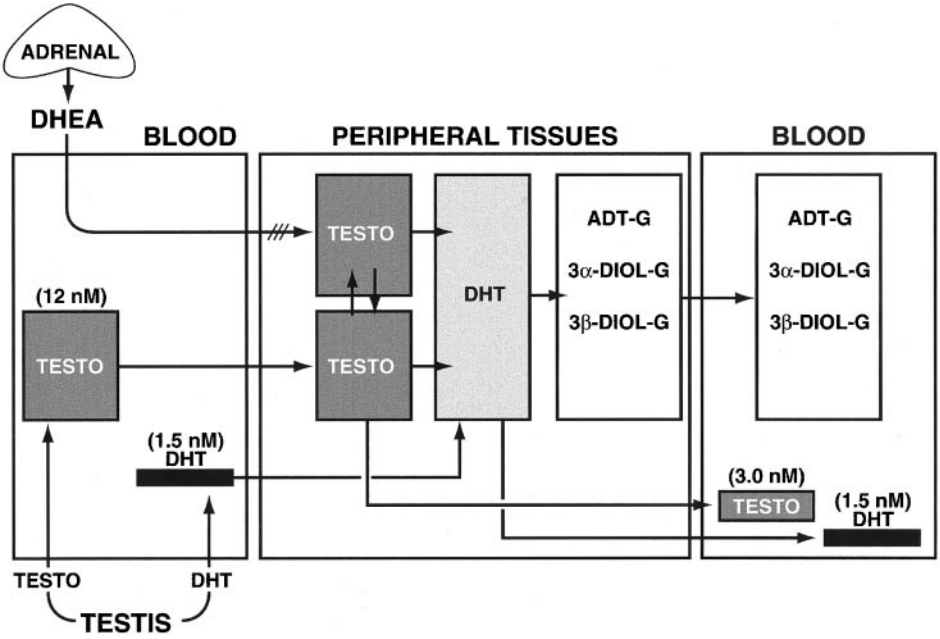

FIG. 8. Distribution in men of the active androgens T and DHT, the sex steroid precursor DHEA, and the main metabolites of androgens (ADT-G, 3α-diol-G, and 3β-diol-G) in the circulation and peripheral intracrine tissues. The height of the bars is proportional to the concentrations of each steroid or its derivatives in individual compartments.

one can be used as markers of testicular secretion in men and interstitial ovarian secretion in women, respectively. The serum concentration of the above-mentioned conjugated androgen metabolites decreased by 40.8 to 72.8% between the 20–30 and 70–80 age groups in men and women, thus suggesting a parallel decrease in the total androgen pool with age. As estimated by measurement of the circulating levels of these conjugated metabolites of DHT, it is noteworthy that women produce approximately 71% or two thirds of the total androgens found in men: in women, most of these androgens originate from the transformation of DHEA and DHEA-S into testosterone and DHT in peripheral intracrine tissues while, in men, the testes and DHEA + DHEA-S provide approximately equal amounts of androgens at the age of 50 to 60 years. An additional potentially highly significant observation is that the majority of the marked decline in circulating adrenal C_{19} steroids and in their resulting androgen metabolites takes place between the age groups of 20–30 and 50–60 years, with smaller changes observed after the age of 60 years (45).

It seems appropriate, at this stage, to represent schematically the distribution of androgen precursors, active androgens (T and DHT), and their metabolites in blood and peripheral tissues (Figs. 8 and 9).

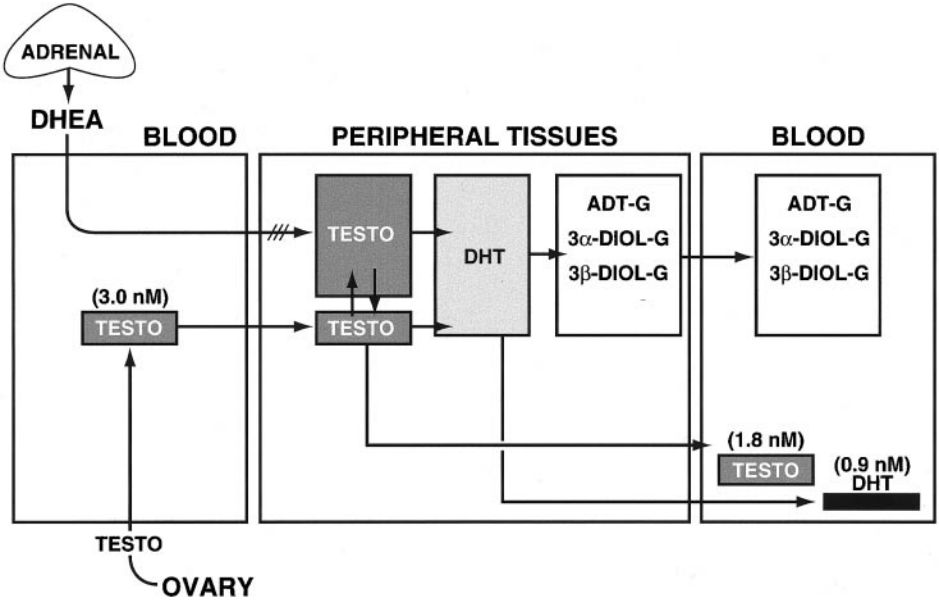

FIG. 9. Distribution in women of the active androgens T and DHT, the sex steroid precursor DHEA, and the main metabolites of androgens (ADT-G, 3α-diol-G, and 3β-diol-G) in the circulation and in peripheral intracrine tissues. The height of the bars is proportional to the concentrations of each steroid or its derivatives in individual compartments.

EFFECTS OF DHEA IN POSTMENOPAUSAL WOMEN

As mentioned above, the 70 to 95% reduction in the formation of DHEA and DHEA-S by the adrenals during aging results in a dramatic reduction in the formation of androgens and estrogens in peripheral target tissues, which could well be involved in the pathogenesis of age-related diseases such as insulin resistance (16, 92), obesity (81, 74, 100), and bone loss. Low circulating levels of DHEA-S and DHEA have, in fact, been found in patients with breast cancer (105).

The potential use of DHEA in postmenopausal women is based upon the recent progress achieved in our understanding of sex steroid physiology in men and women (41, 53–58, 60, 68, 44, 45, 49) and the recognition that women, at menopause, are not only deprived from estrogen activity due to a declining ovarian function, but have already been submitted for a few years to a decreasing exposure to androgens due to the reduced secretion of DHEA by the adrenals.

As mentioned above, osteoporosis is a major problem among aging women, causing morbidity and mortality, mainly through increased fracture rates (37). In order to avoid the limitations of standard estrogen (ERT) or hormonal replacement therapy (HRT), we have studied the effect of DHEA administration to 60- to 70-year-old women for 12 months on bone mineral density, parameters of bone formation and turnover, serum lipids, glucose and insulin,

TABLE 1

Effect of Percutaneous Administration of DHEA for 6 and 12 Months on Bone Mineral Density in Total Hip, Ward's Triangle, and Lumbar Spine ($n = 14$) (49)

		DHEA	
Site	Pretreatment	6 months	12 months
Total hip	0.744 ± 0.021	0.753 ± 0.023*	0.758 ± 0.025*
Ward's triangle	0.486 ± 0.026	0.500 ± 0.026	0.494 ± 0.026
Lumbar spine	0.829 ± 0.030	0.835 ± 0.032	0.839 ± 0.033

Note. Values are expressed as grams per square centimeter.
* $p < 0.05$, DHEA treatment vs pretreatment value.

adipose tissue mass, muscular mass, energy, and well-being as well as on vaginal and endometrial histology (22, 49). DHEA was administered percutaneously. We have thus evaluated the effect of chronic replacement therapy with a 10% DHEA cream applied once daily for 12 months in 60- to 70-year-old women ($N = 15$).

Bone Mineral Density

As presented in Table 1, total hip bone mineral density (BMD) increased significantly from 0.744 ± 0.021 to 0.753 ± 0.023 g/cm^2 (1.2%) after 6 months of treatment ($p < 0.05$) and to 0.759 ± 0.025 g/cm^2 (2.0%) after 12 months of DHEA administration ($p < 0.05$). On the other hand, after the 12 months of DHEA treatment, the femoral Ward's triangle BMD increased from 0.486 ± 0.026 to 0.494 ± 0.026 g/cm^2 (1.6%, NS), while the lumbar spine BMD increased from 0.829 0.030 to 0.839 ± 0.033 g/cm^2 (1.2%, NS), although these last changes did not reach the level of statistical significance.

Biochemical Markers of Bone Physiology

The serum concentration of osteocalcin, a marker of bone formation, increased from 1.16 ± 0.30 μg/L at pretreatment to 1.95 ± 0.59 (N.S.), 2.28 ± 0.53 ($p < 0.05$), 2.49 ± 0.58 ($p < 0.01$, 115% over control), and 2.44 ± 0.47 ($p < 0.01$, 110% over control) after 3, 6, 9, and 12 months of treatment, respectively (Fig. 10). Following cessation of DHEA administration, the serum levels of osteocalcin returned to pretreatment values of 1.52 ± 0.33 and 1.14 ± 0.58 μg/L at 3 and 6 months under placebo, these values being not statistically different from pretreatment values (data not shown).

In parallel with the above-indicated effects on serum osteocalcin, the serum concentration of bone alkaline phosphatase decreased from 16.5 ± 1.3 (pretreatment) to 14.4 ± 0.9 ($p < 0.05$), 14.9 ± 0.7 (N.S.), 13.0 ± 0.9 ($p < 0.01$), and 13.2 ± 1.0 μg/L ($p < 0.01$) at the same time intervals. The values

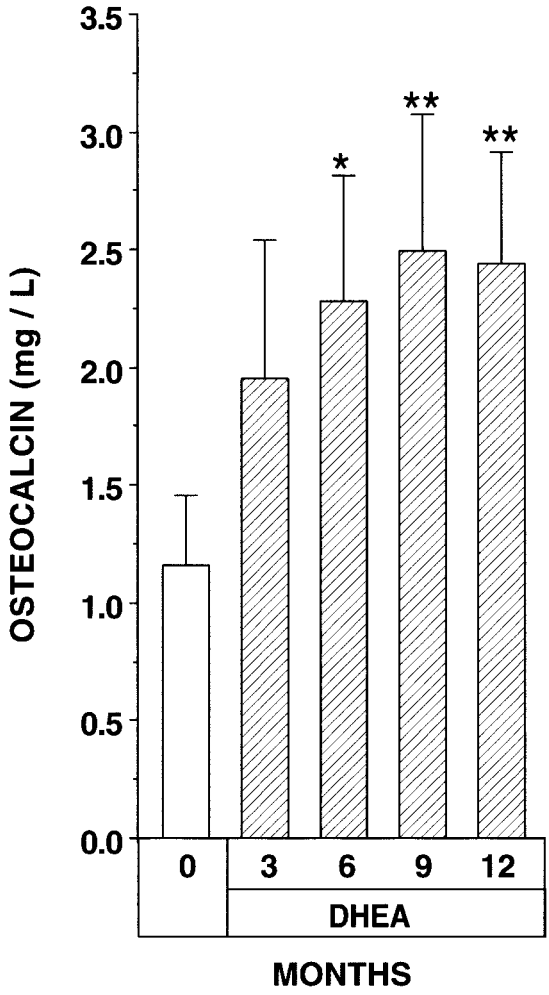

FIG. 10. Effect of DHEA administered percutaneously up to 12 months on serum concentrations of osteocalcin. Reproduced by permission: Labrie F, Diamond P, Cusan L, Gomez JL, Bélanger A. Effect of 12-month DHEA replacement therapy on bone, vaginum, and endometrium in postmenopausal women. *The Journal of Clinical Endocrinology and Metabolism;* 1997; **82:** 3498–3505. © The Endocrine Society.

observed 3 and 6 months after cessation of DHEA treatment were not significantly different from those measured at pretreatment or at 6 months of DHEA treatment, although they suggest a trend toward a return to pretreatment values. The urinary hydroxyproline/creatinine ratio, a marker of bone resorption, decreased from 24.0 ± 1.7 μmol/mmol creatinine at pretreatment to 20.6 ± 1.4 ($p < 0.05$) and 17.2 ± 0.93 μmol/mmol creatinine ($p < 0.01$) at 3 and 6 months of DHEA treatment, respectively. At 9 and 12 months of treatment, the urinary hydroxyproline/creatinine ratio was decreased at 18.4 ± 0.9 ($p < 0.01$) and 19.0 ± 1.3 ($p < 0.01$) μmol/mmol, respectively (data not

TABLE 2

Effect of 12-Month Percutaneous Administration of DHEA to 60- to 70-Year-Old Women on Plasma Alkaline Phosphatase, SHBG, and DHEA Concentration (49)

	Months of treatment				
Parameter	0	3	6	9	12
Plasma alkaline phosphatase (U/L)	92.4 ± 6.9	84.6 ± 4.1	82.6 ± 4.1*	74.9 ± 4.1**	77.1 ± 4.8**
SHBG (nmol/L)	65.0 ± 9.8	52.7 ± 5.6*	51.7 ± 5.3*	51.9 ± 7.1**	53.5 ± 6.2*
DHEA (nmol/L)	3.9 ± 0.5	31.6 ± 2.6	30.8 ± 2.4	31.5 ± 3.0	31.2 ± 3.0

* $p < 0.05$.
** $p < 0.01$.

shown). Total serum alkaline phosphatase decreased from 92.4 ± 6.9 units/L at pretreatment to 84.6 ± 4.1 (p = NS), 82.6 ± 4.1 ($p < 0.05$), 74.9 ± 4.1 ($p < 0.01$), and 77.1 ± 4.8 U/L ($p < 0.01$) at 3, 6, 9, and 12 months, respectively (Table 2).

Vaginal Cytology

Vaginal cytology was examined as a specific parameter of the estrogenic action of DHEA. Before treatment, 10 women had a completely atrophic vaginal smear exclusively composed of parabasal cells (Figs. 11A and 11B, as examples). In 8 of these 10 women under DHEA treatment, the vaginal cytology was converted into a pattern typical of normal cycling women showing mainly the presence of superficial pyknotic cells (Figs. 12A and 12B, as examples). In 2 of the 10 women having a zero maturation index value at start of treatment, no significant change in the cytological maturation value was observed up to 12 months of treatment (data not shown). In the 3 women who had a maturation value between 1 and 40 at start of treatment, stimulation was also observed and the cytology became typical of the normal reproductive range in all of them at 3 months, the first time interval measured after the start of DHEA administration (data not shown). In the last 2 women, the maturation value was already in the normal range of reproductive age at pretreatment and it remained unchanged during treatment (49).

Endometrial Histology

Considering the major concern related to the stimulatory effect of estrogens on endometrial proliferation with the related risk of endometrial carcinoma (30, 31), an endometrial biopsy was performed before starting treatment and after 12 months of DHEA administration. As can be seen in Fig. 13, the

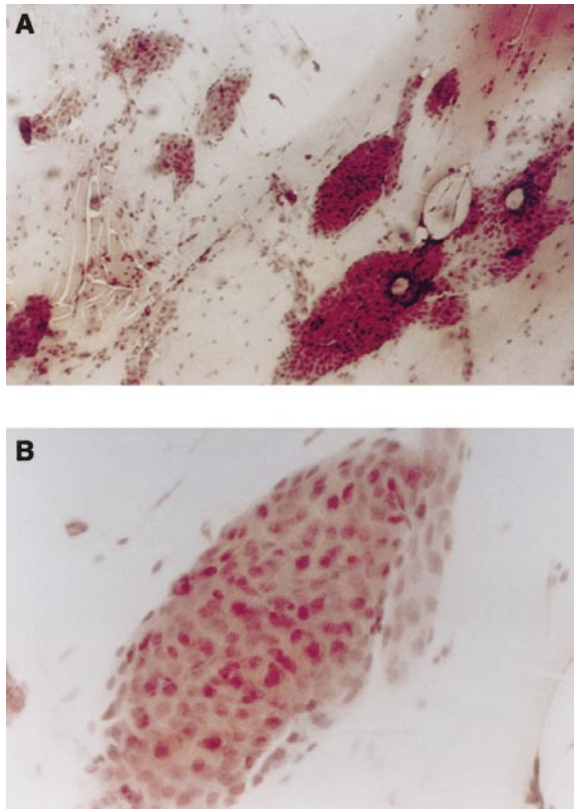

FIG. 11. (A) Atrophic vaginal smear with numerous parabasal cells in a 65-year-old woman before starting treatment with DHEA (×100). (B) Atrophic vaginal smear with numerous parabasal cells in a 65-year-old woman before starting treatment with DHEA at higher magnification (×300). Reproduced by permission: Labrie F, Diamond P, Cusan L, Gomez JL, Bélanger A. Effect of 12-month DHEA replacement therapy on bone, vaginum, and endometrium in postmenopausal women. *The Journal of Clinical Endocrinology and Metabolism;* 1997; **82:** 3498–3505. © The Endocrine Society.

endometrial atrophy seen in all women at the start of treatment remained unaffected by 12 months of DHEA administration.

Sebum Secretion

As skin sebaceous glands are known to contain all the steroidogenic enzymes which catalyze the transformation of DHEA into the androgen DHT (87, 26, 72, 15) and androgens are the main stimulators of sebaceous gland activity (6, 20, 21, 15), we have measured the effect of DHEA treatment on sebum secretion. As measured by the Sebutape technique on six facial sites, percutaneous administration of DHEA led to a comparable 66 to 79% stimulation ($p < 0.01$) of sebum secretion measured after 3, 6, 9, and 12 months of treatment (data

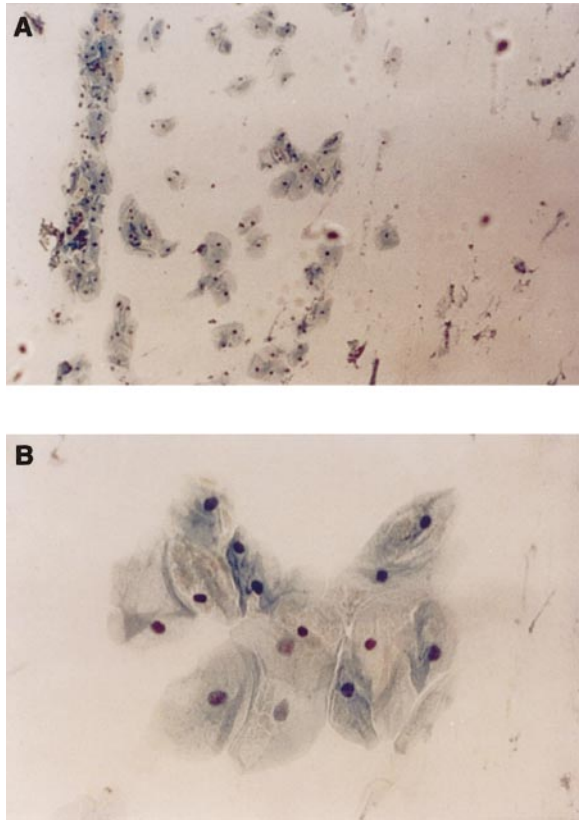

FIG. 12. (A) Vaginal smear from the same patient as Fig. 11A after 12 months of DHEA treatment showing superficial pyknotic cells (×100). (B) Vaginal smear from the same patient as Fig. 11B after 12 months of DHEA administration showing superficial pyknotic cells at higher magnification (×300). Reproduced by permission: Labrie F, Diamond P, Cusan L, Gomez JL, Bélanger A. Effect of 12-month DHEA replacement therapy on bone, vaginum, and endometrium in postmenopausal women. *The Journal of Clinical Endocrinology and Metabolism;* 1997; **82:** 3498–3505. © The Endocrine Society.

not shown). Sebum secretion had already returned to pretreatment values 3 months after cessation of DHEA therapy (49).

Potential of DHEA as Hormone Replacement Therapy

The present data clearly suggest the interest of a new approach to hormone replacement therapy having potentially improved efficacy and tolerance. It is possible that DHEA replacement therapy (DRT) could not only correct but also prevent the multiple problems associated with menopause, a phenomenon preceded and accompanied by a decreased formation of both androgens and estrogens during aging in women.

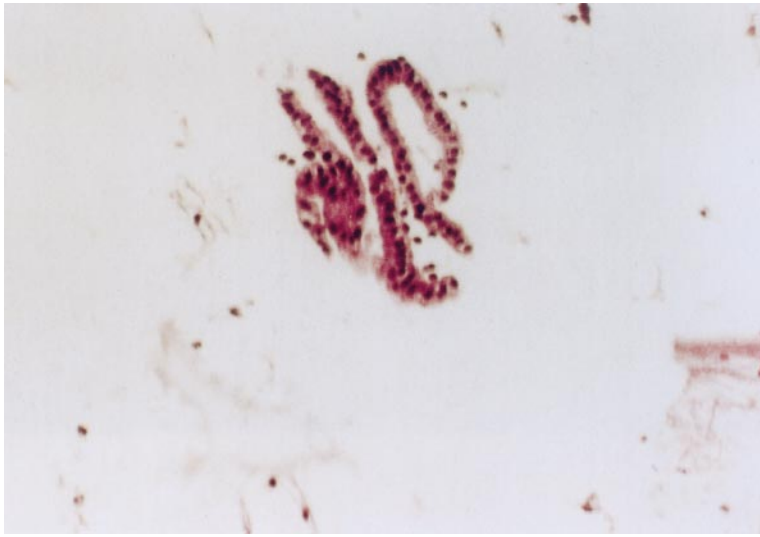

FIG. 13. Atrophic endometrium after 12 months of DHEA treatment in a representative 65-year-old woman (×50). Reproduced by permission: Labrie F, Diamond P, Cusan L, Gomez JL, Bélanger A. Effect of 12-month DHEA replacement therapy on bone, vaginum, and endometrium in postmenopausal women. *The Journal of Clinical Endocrinology and Metabolism;* 1997; **82:** 3498–3505. © The Endocrine Society.

The present data certainly suggest that DHEA treatment has no deleterious effects but rather indicate a trend toward positive effects on the serum lipid and lipoprotein profile, although a larger cohort of subjects is needed to reach definitive conclusions. On the other hand, it should be mentioned that conjugated equine estrogens raise triglyceride levels and show a deterioration of the insulin response (76, 66). In fact, our data show an inhibitory effect of DHEA treatment on fasting blood glucose and insulin levels (22), thus suggesting an advantage over equine estrogens on glucose metabolism.

In this context, it is important to indicate that the absence of a stimulatory effect of DHEA on the human endometrium (49) eliminates the need to administer a progestin to neutralize the potential effect of estrogens on the endometrium. Concerning the breast, DHEA is known to prevent the development (67) and to inhibit the growth (64) of dimethylbenz(a)anthracene mammary tumors in the rat. DHEA, in addition, inhibits the growth of human breast cancer xenografts in nude mice (17). Thus, contrary to estrogens and progestins, which exert stimulatory effects, DHEA is expected to inhibit both the development and the growth of breast cancer in women.

The inhibitory effect of DHEA on the growth of human breast cancer xenografts supports the use of DHEA as hormone replacement therapy in women. Moreover, since the administration of DHEA does not interfere with the inhibitory effect of EM-800 on ZR-75-1 tumor growth, combined treatment with DHEA and SCH 57068 (EM-652) could possibly be a convenient therapy in symptomatic postmenopausal women with breast cancer.

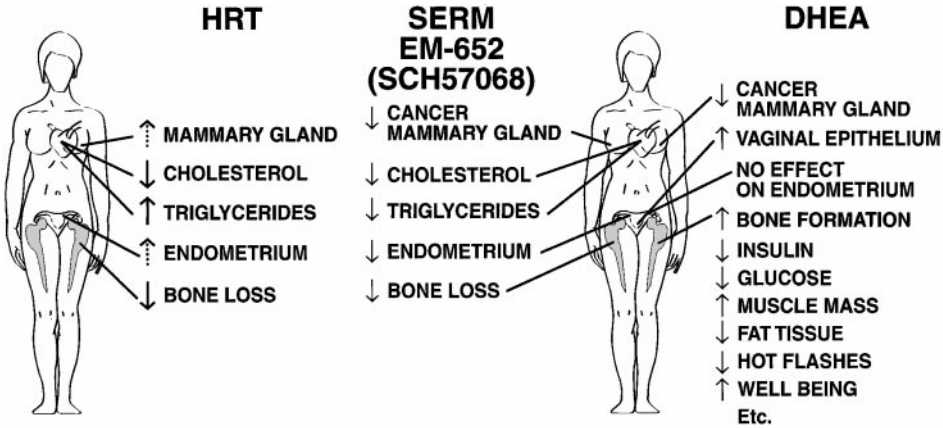

FIG. 14. Comparison of the potential benefits of combining DHEA with EM-652 (SCH 57068), a SERM having pure antiestrogenic activity in the mammary gland and endometrium, compared to standard HRT. Benefits (plain arrow) and negative effects (dotted arrow); from available evidence, there are no negative effects expected from the combination EM-652 + DHEA, while potential stimulation of breast and endometrial cancers are the risks associated with estrogen therapy. The combination EM-652 + DHEA has a series of potential benefits not achievable with standard ERT.

EM-652 (SCH 57068) is a fourth generation SERM having pure and potent antiestrogenic activity in the mammary gland and endometrium while decreasing serum cholesterol and triglyceride levels and preventing bone loss. The potential benefits of such combination can be seen in Fig. 14. Most importantly, such a combination appears to possess the ideal characteristics for prevention of breast and uterine cancer while preventing bone loss, cardiovascular risks, and other problems associated with menopause.

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