0163-769X/03/$20.00/0
Printed in U.S.A.

Endocrine Reviews 24(2):152–182

doi: 10.1210/er.2001-0031

Endocrine and Intracrine Sources of Androgens in Women: Inhibition of Breast Cancer and Other Roles of Androgens and Their Precursor Dehydroepiandrosterone

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Serum androgens as well as their precursors and metabolites decrease from the age of 30–40 yr in women, thus suggesting that a more physiological hormone replacement therapy at menopause should contain an androgenic compound. It is important to consider, however, that most of the androgens in women, especially after menopause, are synthesized in peripheral intracrine tissues from the inactive precursors dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEA-S) of adrenal origin. Much progress in this new area of endocrine physiology called intracrinology has followed the cloning and characterization of most of the enzymes responsible for the transformation of DHEA and DHEA-S into androgens and estrogens in peripheral target tissues, where the locally produced sex steroids are exerting their action in the same cells in which their synthesis takes place without significant diffusion into the circulation, thus seriously limiting the interpretation of serum levels of active sex steroids. The sex steroids made in peripheral tissues are then inactivated locally into more water-soluble compounds that diffuse into the general circulation where they can be measured. In a series of animal models, androgens and DHEA have been found to inhibit breast cancer development and growth and to stimulate bone formation. In clinical studies, DHEA has been found to increase bone mineral density and to stimulate vaginal maturation without affecting the endometrium, while improving well-being and libido with no significant side effects. The advantage of DHEA over other androgenic compounds is that DHEA, at physiological doses, is converted into androgens and/or estrogens only in the specific intracrine target tissues that possess the appropriate physiological enzymatic machinery, thus limiting the action of the sex steroids to those tissues possessing the tissue-specific profile of expression of the genes responsible for their formation, while leaving the other tissues unaffected and thus minimizing the potential side effects observed with androgens or estrogens administered systemically. (*Endocrine Reviews* 24: 152–182, 2003)

I. Androgens and Their Role in Women

A. Introduction

THE MOST WIDELY recognized fact about menopause is that it is accompanied by a rapid arrest of estrogen secretion by the ovaries. The cessation of ovarian estrogen secretion is illustrated by the marked decline in circulating 17β-estradiol (E_2) levels. This easily measurable change in circulating E_2, coupled with the demonstrated benefits of estrogens on menopausal symptoms and bone resorption (1), has concentrated almost all of the efforts of hormone replacement therapy (HRT) on various forms of estrogens as well as combinations of estrogen and progestin to avoid the potentially harmful stimulatory effects of estrogens used alone on the endometrium, which can result in endometrial hyperplasia and cancer. It should be mentioned, however,

Abbreviations: ADT-G, Androsterone glucuronide; AR, androgen receptor(s); DHEA, dehydroepiandrosterone; DHEA-S, DHEA sulfate; DHT, dihydrotestosterone; 3α-diol-G, androstane-3α,17β-diol glucuronide; 3β-diol-G, androstane-3β,17β-diol glucuronide; 5-diol, androst-5-ene-3β,17β-diol; 4-dione, androstenedione; DMBA, dimethylbenz-(a)anthracene; E_1, estrone; E_2, 17β-estradiol; ERT, estrogen replacement therapy; HRT, hormone replacement therapy; HSD, hydroxysteroid dehydrogenase; MPA, medroxyprogesterone acetate; PRAP, prolactin receptor-associated protein.

that although progestins are well recognized to protect the endometrium, preclinical (2–4) and clinical (5–7) data strongly suggest that they have a negative impact on breast cancer. The recent data of the Women's Health Initiative Study show that the combination of Premarin and Provera (Prempro) causes a 26% increase in the risk of breast cancer at 5.2 yr of follow-up, thus seriously questioning the use of a progestin as part of HRT in postmenopausal women (8).

Despite the well known beneficial effects of estrogen therapy on menopausal symptoms (9–11) and their role in reducing bone loss and possibly coronary heart disease (12–17), compliance is low. The majority of women decide not to take estrogens or stop treatment early because of the fear of breast and uterine cancer (11) and of symptoms associated with this therapy, namely uterine bleeding, breast tenderness, and fluid retention.

The almost exclusive focus on the role of ovarian estrogens at menopause has removed the attention from the progressive and dramatic fall in circulating dehydroepiandrosterone (DHEA), which starts early at the age of 30–40 yr (18–23). Because DHEA is transformed into both androgens and estrogens in peripheral tissues, such a fall in the serum concentration of the steroid precursors DHEA and DHEA sulfate (DHEA-S) explains why postmenopausal women, as discussed later, are not only lacking estrogens but are also deprived from androgens. Moreover, women taking contraceptives or estrogen replacement therapy (ERT) have reduced ovarian androgen secretion attributable to inhibition of gonadotropin secretion, as well as reduced androgen bioavailability attibutable to increased SHBG levels (24).

B. Decrease of serum DHEA, androgens, and their metabolites with age

Until recently, because of assay difficulties, only a limited number of circulating adrenal and gonadal steroids have been measured during advancing age, thus limiting the evaluation of the relative role of different sources of sex steroids. This analysis is of special importance in postmenopausal women in whom the sex steroids of adrenal origin gain particular importance after the arrest of estrogen secretion by the ovaries at menopause (25). It is important to recall that in the 50- to 60-yr-old age group, serum DHEA has already decreased by 70%, compared with the 20- to 30-yr-old peak values (Ref. 23; Fig. 1). It is thus quite remarkable that most of the important decline in circulating DHEA, DHEA-S, androst-5-ene-3β,17β-diol (5-diol), 5-diol-G, androstenedione (4-dione), and the conjugated metabolites of androgens, namely androsterone glucuronide (ADT-G), androstane-3α,17β-diol glucuronide (3α-diol-G), and androstane-3β,17β-diol glucuronide (3β-diol-G), occurs between the age ranges of 20–30 and 50–60 yr, whereas relatively smaller changes occur after the age of 60 yr (23). It is important to realize, as illustrated in Fig. 2, not only that serum DHEA and DHEA-S decrease by 50% between the ages of 21 and 40 yr but also that a similar decrease is observed for serum testosterone (26). Such data could well suggest that HRT with androgens should start early at menopause to compensate for this early fall in the secretion of androgen precursors by the adrenals and the parallel decrease in serum testosterone.

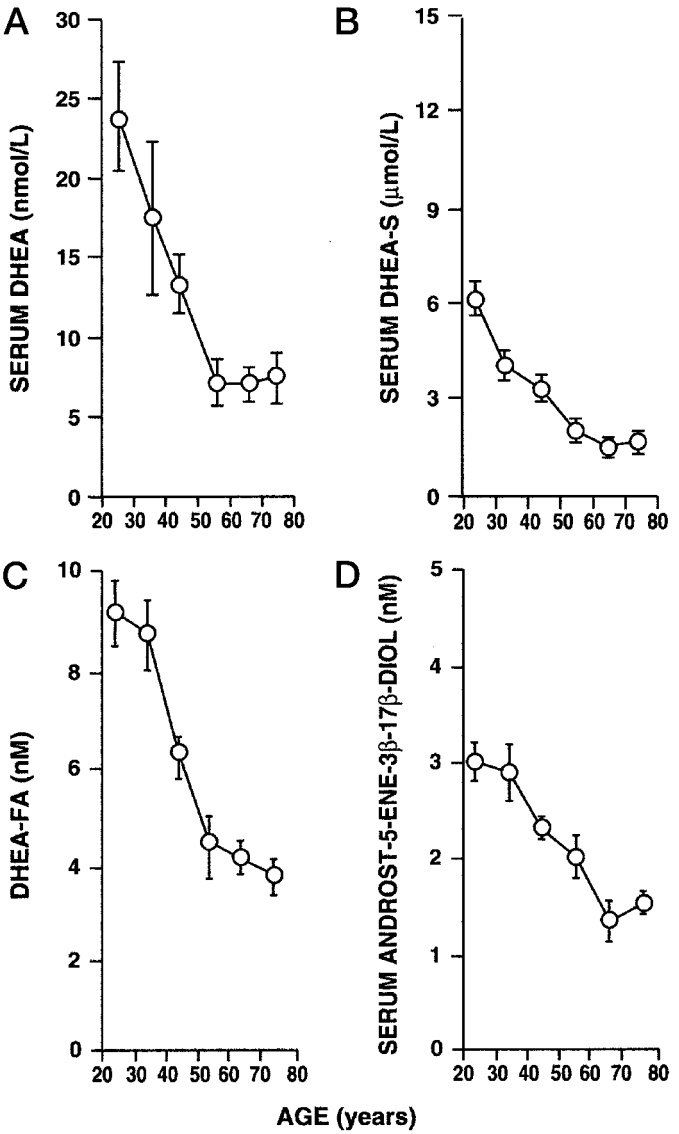

FIG. 1. Effect of age (20–30 to 70–80 yr old) on serum concentration of DHEA (A), DHEA-S (B), DHEA-fatty acid esters (DHEA-FA; C), and 5-diol (D) in women. A marked decline is shown in serum concentrations of adrenal C19 sex steroid precursors and conjugated androgen metabolites during aging (26). [Reproduced with permission from F. Labrie *et al.*: *J Clin Endocrinol Metab* 82:2396–2402, 1997 (23). © The Endocrine Society.]

Using the serum concentrations of ADT-G, 3α-diol-G, and 3β-diol-G as estimates of total androgens, the average sum of the serum concentrations of these conjugated metabolites of dihydrotestosterone (DHT) are 37.5, 8.47, and 30.2 nM in men compared with 32.5, 4.28, and 17.3 nM in women (23). The average serum concentrations of ADT-G, 3α-diol-G, and 3β-diol-G, measured in women between the ages 20 and 80 yr are thus 86.6% (ADT-G), 50.5% (3α-diol-G), and 57.2% (3β-diol-G), compared with those found in men of the same age (20–80 yr; Table 1; Ref. 23). Although the metabolic clearance rates of the three main androgen metabolites are likely to show differences between men and women, an estimate of the relative amount of total androgens in women and men calculated on the basis of the sum of the serum

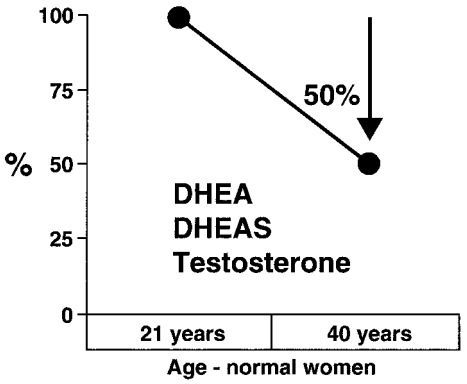

FIG. 2. Illustration of the 50% parallel decrease in serum DHEA, DHEA-S, and testosterone between the ages of 21 and 40 yr in normal women (26).

concentrations of these three metabolites suggests that total androgen production in women is more than two thirds, or 71%, of that observed in men (23, 27). Such an approach is based on the knowledge that active androgens are inactivated to glucuronide derivatives before their diffusion from the intracellular compartment into the circulation where they can be measured as ADT-G, 3α-diol-G, and 3β-diol-G.

Such data showing the presence of relatively high levels of androgens in normal women strongly suggest that the androgens play a major physiological role in women. The 44.5% fall that occurs in serum DHEA from 20–30 yr of age to the age of 40–50 yr in women could well explain the bone loss that precedes menopause (27–30). Age-related bone loss has been reported to begin in the fourth decade, and changes in bone turnover have been found well before menopause (28–30). In agreement with these findings, bone density was lower at all sites examined in women classified as perimenopausal compared with premenopausal (31). In fact, the changes in precursor androgen secretion by the adrenals precede by 10–20 yr the detectable decrease in ovarian steroidogenesis that occurs abruptly at menopause (23). In fact, serum FSH increases in premenopausal women even before serum E_2 shows a decline (32).

After the recognition that such a large proportion of androgens and estrogens in men and women originate from DHEA and DHEA-S of adrenal origin (25), we have studied the serum concentration of a large series of androgens and estrogens as well as their metabolites after percutaneous administration of DHEA in 60- to 70-yr-old men and women (27). We then observed that changes in serum DHEA within the physiological range of young adult men and women led only to small or nonsignificant changes in serum testosterone, DHT, or E_2, whereas, on the other hand, the concentration of the conjugated metabolites of DHT were markedly increased (27). Such data clearly indicate the poor value of measurements of serum androgens and estrogens as parameters of total androgenic and estrogenic activities in men and women.

As well demonstrated in a long series of preclinical studies, supplementation with physiological amounts of exogenous DHEA permits the biosynthesis of androgens (essentially testosterone and DHT) and estrogens only in the target tissues that contain the specific steroidogenic enzymes (25, 33). The widespread tissue distribution of steroidogenic enzymes is illustrated in Table 2 (34). In fact, in 22 peripheral tissues of the monkey, steroid sulfatase, 3β-hydroxysteroid dehydrogenase (HSD), androgenic 17β-HSD, estrogenic 17β-HSD, aromatase, and 5α-reductase are all present in 114 of 132 (86%) possible sites. Genomic studies are in progress to determine the identity of all families of steroidogenic enzymes in the various peripheral target tissues.

The active androgens and estrogens synthesized in peripheral target tissues exert their activity in the cells of origin, and very little diffusion of the active sex steroids occurs, thus resulting in very low levels in the circulation. In fact, the most striking effects of DHEA administration are seen on the circulating levels of the glucuronide derivatives of the metabolites of DHT, namely ADT-G, 3α-diol-G, and 3β-diol-G, these metabolites being produced locally in the peripheral intracrine tissues that possess the appropriate steroidogenic enzymes to synthesize DHT from the adrenal precursors DHEA and DHEA-S. These peripheral target tissues also contain the steroid-inactivating enzymes required to metabolize DHT into inactive and more water-soluble conjugates, especially glucuronide derivatives (25, 35). Such local biosynthesis and action of androgens in target tissues eliminates the exposure of other tissues to androgens and thus minimizes the risks of undesirable masculinizing or other androgen-related side effects. The same applies to estrogens, although we feel that a reliable parameter of total estrogen secretion (comparable to the glucuronides for androgens) has not yet been identified.

C. Androgens and bone physiology

1. Role of androgens and estrogens in bone physiology. A predominant role of androgens in bone physiology has already been suggested (36). In fact, both testosterone and DHT increased the transcription of α (I) procollagen mRNA in osteoblast-like osteosarcoma cells (37). Treatment with DHT has also been shown to stimulate endochondral bone development in the orchiectomized rat (38). Androgens stimulate osteoblast differentiation, these cells being known to contain androgen receptors (AR; Refs. 39–41). Moreover, bone mineral density measured in the lumbar spine, femoral trochanter, and total body was increased more by estrogen plus testosterone implants than by E_2 alone over a 24-month treatment period in postmenopausal women (42). In agreement with these data, biomarkers of bone formation were increased compared with estrogen alone when methyltestosterone was added to estrogen (43).

The essential role of androgens in bone mineralization is illustrated by the reduced bone mineral density in patients with the androgen insensitivity syndrome (44–46). In such

TABLE 1. Comparison of serum androgen metabolites (20–80 yr of age; nM)[a]

	Men	Women
ADT-G	37.5	32.5 (87%)
3α-Diol-G	8.5	4.3 (51%)
3β-Diol-G	30.2	17.3 (57%)
Total	76.2	54.1 (71%)

[a] Data from Ref. 23.

TABLE 2. Distribution of intracrine steroidogenic enzymes in the monkey

Tissue	Steroid sulfatase	3β-HSD	17β-HSD androgen	17β-HSD estrogen	Aromatase	5α-Reductase
Adrenal	√	√	√	√	√	√
Testis	√	√	√	√	√	√
Ovary	√	√	√	√	√	
Prostate	√	√	√	√	√	√
Seminal vesicle	√	√	√	√	√	√
Oviduct	√	√	√	√	√	
Cervix	√	√	√	√	√	
Endometrium	√	√	√	√	√	
Myometrium	√	√	√	√	√	
Small intestine	√	√	√	√	√	√
Large intestine	√	√	√	√	√	√
Mammary gland	√	√	√	√	√	
Kidney	√	√	√	√	√	√
Liver	√	√	√	√	√	√
Lung	√	√	√	√	√	√
Heart	√	√	√	√	√	
Spleen	√		√	√	√	
Mesenteric fat	√	√	√	√	√	√
Skin	√	√	√	√		√
Muscle	√	√	√	√	√	
Salivary gland		√	√	√	√	
Pituitary	√	√	√	√		
Cerebral cortex	√		√	√	√	
Cerebellum	√		√	√	√	√
Cerebrum	√		√	√	√	√

patients having an inactive AR, estrogens are unable to increase bone mineral density (44, 45). Thus, at doses of estrogen able to restore bone mineral density in hypogonadal women, estrogens could not exert a similar effect in patients with androgen insensitivity. Such data suggest that both estrogens and androgens are required to acquire normal bone mineral density. In fact, a correlation has been found between androgens and bone mineral density in premenopausal women (31, 47).

In established osteoporosis, anabolic steroids have been reported to help prevent bone loss (48). Moreover, androgen therapy, as observed with nandrolone decanoate, increases vertebral bone mineral density in postmenopausal women (49). Similarly, sc E_2 and testosterone implants have been found to be more efficient than oral estrogen in preventing osteoporosis in postmenopausal women (50). Although the difference has been attributed to the different routes of administration of the estrogen, the cause of the difference could well be the action of testosterone. Studies have convincingly shown that androgen plus estrogen was more efficient than estrogen in improving bone mineral density in postmenopausal women (42, 43, 50, 52–55).

Although androgens are gaining increasing support because of their unique actions in postmenopausal women, virilizing effects are observed with the use of supraphysiological doses of testosterone (56, 57). The availability of a compound such as DHEA, an inactive precursor that is transformed into active androgens only in specific target tissues, would be an important advantage over androgens exerting systemic effects in all tissues possessing AR.

D. Other roles of androgens in women

1. General. It is likely that the androgens produced from DHEA have other beneficial effects in postmenopausal women. The detailed benefits of androgens added to ERT or HRT have been described on general well-being, energy, mood, and general quality of life (58, 59). Improvements in the major psychological and psychosomatic symptoms, namely irritability, nervousness, memory, and insomnia, have been reported after addition of androgens to ERT (60). In addition, androgenic compounds have been found to be beneficial for the treatment of the mastalgia frequently caused by HRT (61). In fact, ERT may result in severe breast pain that may lead to discontinuation of therapy.

2. Libido and sexual satisfaction. Loss of libido and/or sexual satisfaction are common in early postmenopause. The addition of androgens to HRT is known to have beneficial effects on these problems (42, 53, 57, 58, 62–64). Moreover, a series of studies have shown the beneficial effects of androgens on libido in postmenopausal women (42, 65–67). In women who have undergone oophorectomy and hysterectomy, transdermal testosterone improves sexual function and psychological well-being (68). Similar findings have been observed with DHEA administered to women with adrenal insufficiency, this steroid being the most important precursor of androgens in postmenopausal women (69). On the other hand, mood and fatigue were significantly improved after DHEA replacement therapy in Addison's disease (70).

3. Hot flashes. The addition of androgens has been found to be effective in relieving hot flashes in women who had unsatisfactory results with estrogen alone (71). Androgen therapy is also successful in reducing hot flashes in hypogonadal men (72). In agreement with its transformation into androgens (27), DHEA has been found useful in reducing hot flashes (73, 74). In fact, marked improvements in the vasomotor symptoms were observed in early postmenopausal women who received 50 mg DHEA orally daily from an

average score of 18.4 before treatment to a score of 4.5 at 6 months (74).

4. Cardiovascular function and lipids. There is also evidence that androgens may improve endothelium-dependent and -independent vasodilation in postmenopausal women (75). In fact, parenteral testosterone therapy improved brachial artery vasodilatation in postmenopausal women using long-term estrogen therapy. It is also of great interest that the addition of parenteral testosterone does not negate the favorable effects of estrogen on low-density lipoprotein cholesterol (76).

II. DHEA Is Predominantly Converted into Androgens in Women

A. Intracrinology

Man is unique, with some other primates, in having adrenals that secrete large amounts of the precursor steroids DHEA and DHEA-S, which are converted into 4-dione and then into potent androgens and/or estrogens in peripheral tissues (Refs. 25, 77, and 78; Fig. 3). Adrenal secretion of DHEA and DHEA-S increases during adrenarche in children at the age of 6–8 yr, and maximal values of circulating DHEA-S are reached between the ages of 20 and 30 yr. Thereafter, serum DHEA and DHEA-S levels decrease markedly (Fig. 1; Refs. 18 and 20–22). In fact, as mentioned earlier, at 70 yr of age, serum DHEA-S levels are decreased to approximately 20% of their peak values, whereas they can decrease by 95% by the age of 85–90 yr (22). The 70–95% 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. Such a marked decrease in the formation of sex steroids in peripheral tissues could well be involved in the pathogenesis of diseases associated with aging.

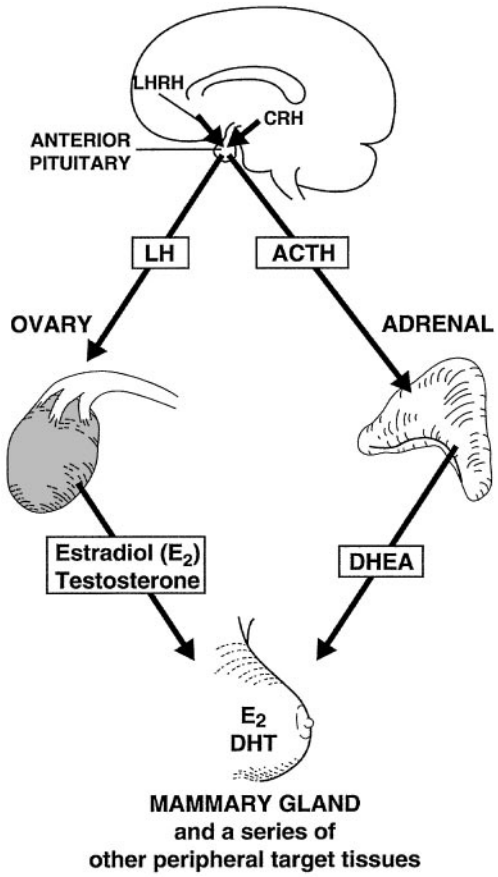

FIG. 3. Schematic representation of the role of ovarian and adrenal sources of sex steroids in premenopausal women. After menopause, the secretion of estradiol by the ovaries ceases, and almost 100% of sex steroids are made locally in peripheral target intracrine tissues.

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 (25, 27, 77, 78). In fact, although 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 wholly synthesized locally in peripheral tissues, thus providing target tissues with controls that adjust the formation and metabolism of sex steroids to local requirements. This situation is well illustrated in women by the absence of significant difference in the intracellular levels of E_2 in breast cancer tissue between premenopausal and postmenopausal women (79). Because the postmenopausal ovary does not secrete estrogens, intratumoral E_2 is necessarily made from adrenal precursor steroids (25).

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 (Refs. 25 and 78; Fig. 4). This situation of a high secretion rate of adrenal precursor sex steroids in men and women is thus completely different from all animal models used in the laboratory, namely rats, mice, guinea pigs, and all others (except monkeys) in which the secretion of sex steroids takes place exclusively in the gonads (77, 80). A major problem that is at least partially responsible for the delayed progress in the recognition of the formation of a major proportion of sex steroids in peripheral tissues or intracrinology is the fact that the animal models usually used in the laboratory do not secrete significant amounts of adrenal precursor sex steroids, thus focusing all attention on the testes and ovaries as the exclusive sources of androgens and estrogens. The term intracrinology was thus coined (78) to describe the synthesis of active steroids in peripheral target tissues in which the action is exerted in the same cells where synthesis takes place without release of the active steroids in the extracellular space and general circulation (25).

Proof of the role of estrogen formation in peripheral intracrine tissues is particularly well illustrated in women by the important benefits on breast cancer observed in postmenopausal women treated by a series of aromatase inhibitors (81). Most convincingly, because the postmenopausal ovaries do not secrete estrogens, the recent observation that administration of the antiestrogen raloxifene for only 3 yr in postmenopausal women led to a 76% decrease in the incidence of breast cancer (82) is a clear demonstration of the role

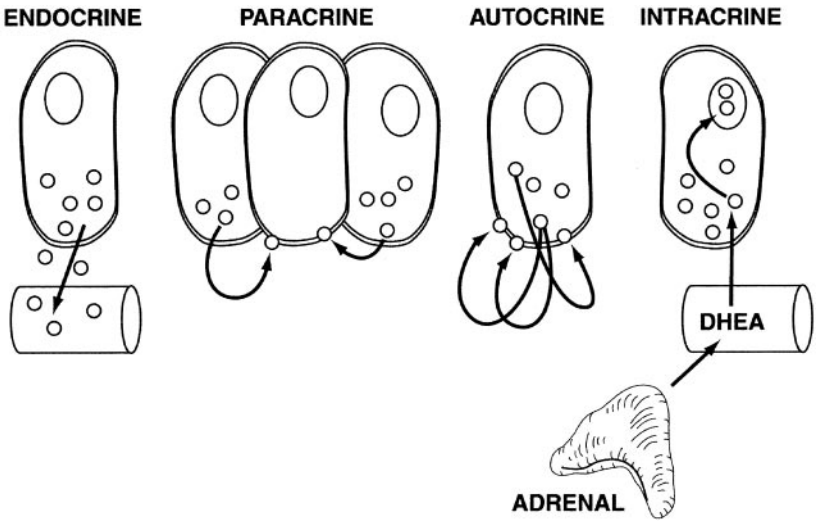

FIG. 4. Schematic representation of endocrine, paracrine, autocrine, and intracrine secretion. Classically, endocrine activity includes the hormones secreted in specialized glands, called endocrine glands, for release into the general circulation and transport to distant target cells. On the other hand, 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 that exert their action in the same cells in which synthesis took place without release into the pericellular compartment. [Reprinted with permission from F. Labrie: *Mol Cell Endocrinol* 78:C113–C118, 1991 (25).]

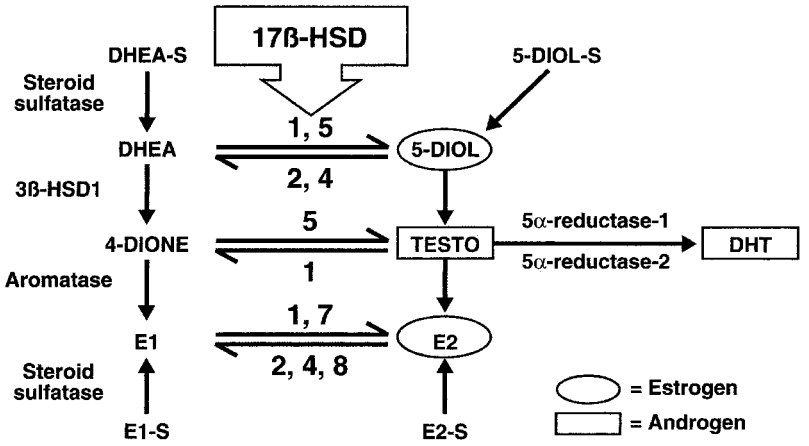

FIG. 5. Human steroidogenic enzymes in peripheral intracrine tissues.

of extraovarian estrogens in the development and growth of breast cancer.

B. Structure of the human steroidogenic enzymes

As mentioned above, transformation of DHEA and DHEA-S into active androgens and/or estrogens in peripheral target tissues depends on the level of expression of the various steroidogenic and metabolizing enzymes in each cell type. Elucidation of the structure of most of the tissue-specific genes that encode the steroidogenic enzymes responsible for the transformation of DHEA and DHEA-S into androgens and/or estrogens has permitted rapid progress in this area (Refs. 33 and 83–86; Fig. 5). The major 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 precursor steroids (77, 87, 88). 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 (25). Although testosterone of ovarian and adrenal origin can act directly in peripheral tissues, its transformation into estrogens requires the action of the peripheral or intracrine steroidogenic enzymes, especially aromatase (89).

Because the molecular structure of most of the key non-P-450-dependent enzymes required for sex steroid formation had not been elucidated, and knowing that local formation of sex steroids is most likely to play a major role in the control of activity of 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 (33, 35, 84, 90–92). The synthesis from DHEA of the most potent natural androgen, DHT, and of the most potent natural estrogen, E_2, involves several enzymatic activities, namely 3β-HSD, 17β-HSD, 5α-reductase, and/or aromatase (Fig. 5).

1. 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-HSD/Δ^5-Δ^4 -isomerase gene family, hereafter called 3β-HSD, was only elucidated relatively recently (84, 93–96). 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-keto-steroids, namely progesterone as well as the precursors of all androgens, estrogens, glucocorticoids, and mineralocorticoids.

Experiments performed using microsomes and purified enzymes show that 3β-HSD can catalyze the interconversion of 3β-hydroxy- and 3-keto-5α-androstane steroids (97). On

the other hand, experiments performed under more physiological conditions (*i.e.*, in intact transfected cells in culture without added cofactor) indicate that 3β-HSD catalyzes almost exclusively the oxidation of 3β-hydroxy- into 3-keto-5α-androstane steroids (98). The reverse reductive reaction is catalyzed by another enzyme, namely 3(α→β)-hydroxysteroid epimerase [3(α→β)-HSE; Refs. 98 and 99] and type 7 17β-HSD (our unpublished data).

3β-HSD is found not only 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 (34, 84, 91, 100), thus catalyzing the first step in the intracrine transformation of DHEA into 4-dione, the precursor of both androgens and estrogens. 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.

After purification of 3β-HSD from human placenta and development of antibodies against the enzyme in rabbits (101), we have isolated and characterized a first 3β-HSD cDNA type (93) and its corresponding gene (94). 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 (95). The structure of the corresponding human type 2 3β-HSD gene has also been elucidated (96). The human 3β-HSD genes corresponding to human cDNAs type 1 and 2 contain four exons and three introns within a total length of 7.7–7.8 kb. 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 (102).

We have observed 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 (103–105). However, the absence of mutations in the type 1 gene provided the long-awaited molecular explanation for the persistence of peripheral steroidogenesis in these 3β-HSD type 2-deficient patients, thus demonstrating the importance of peripheral sex steroid formation or intracrinology.

2. Human 17β-HSDs. The 17β-HSDs are responsible for the formation and inactivation of all active androgens and estrogens. As discussed above for 3β-HSD, until recently, 17β-HSDs as well as almost all other dehydrogenases were considered to be reversible enzymes that catalyze the interconversion of substrates and products, mainly because the enzymatic activity was first characterized using tissue homogenates, subfractions, or purified proteins with added oxidized (NAD+, NADP+) or reduced (NADH, NADPH) cofactors. These exogenous cofactors drive the reaction in the oxidative or reductive direction depending on their oxidized or reduced state, respectively. However, using a more physiologically relevant method of enzymatic activity analysis, namely intact transfected cells in culture without the addition of exogenous cofactors, the transfected enzyme catalyzes the reaction in a unidirectional manner (85, 98, 99, 106, 107). These findings agree with the isolation of multiple types of 17β-HSDs in which approximately half catalyze the reductive reaction (types 1, 3, 5, and 7) and half catalyze the oxidative reaction (types 2, 4, 6, and 8).

a. Type 1 17β-HSD. The molecular structure of the human type 1 17β-HSD gene and mRNA, which encode a predicted protein of 327 amino acids, was the first of the 17β-HSDs to be elucidated (Refs. 108–111; Fig. 6). 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 catalyzes predominantly the interconversion of estrone (E_1) to E_2 using NADP(H) as cofactor (112, 113).

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 (113, 114). The protein was also overproduced in baculovirus, and crystals were obtained (115). This crystallization led to the elucidation of the three-dimensional structure of human type 1 17β-HSD (116), 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.

b. Type 2 17β-HSD. The structure of a cDNA encoding a second type of 17β-HSD cDNA was then reported (117, 118). This cDNA encodes a predicted protein of 387 amino acids with a molecular weight of 42,782 (Fig. 6). 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 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 (109). This enzyme uses NAD(H) as a cofactor (117) and is less specific than type 1 17β-HSD, both estrogens and androgens acting as substrates. This enzyme inactivates the estrogens and androgens made after the reductive action of type 2, 3, and 5 17β-HSDs.

c. 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 (119). Type 3 17β-HSD, a microsomal isozyme, using NADP(H) as a cofactor, is expressed predominantly in the testes, where it synthesizes testosterone 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 (119).

d. Type 4 17β-HSD. Human type 4 17β-HSD is a 736-amino-acid protein of molecular mass 80 kDa that can transform E_2 to E_1 and 5-diol to DHEA (120, 121). 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. This enzyme possibly plays a role in the inactiva-

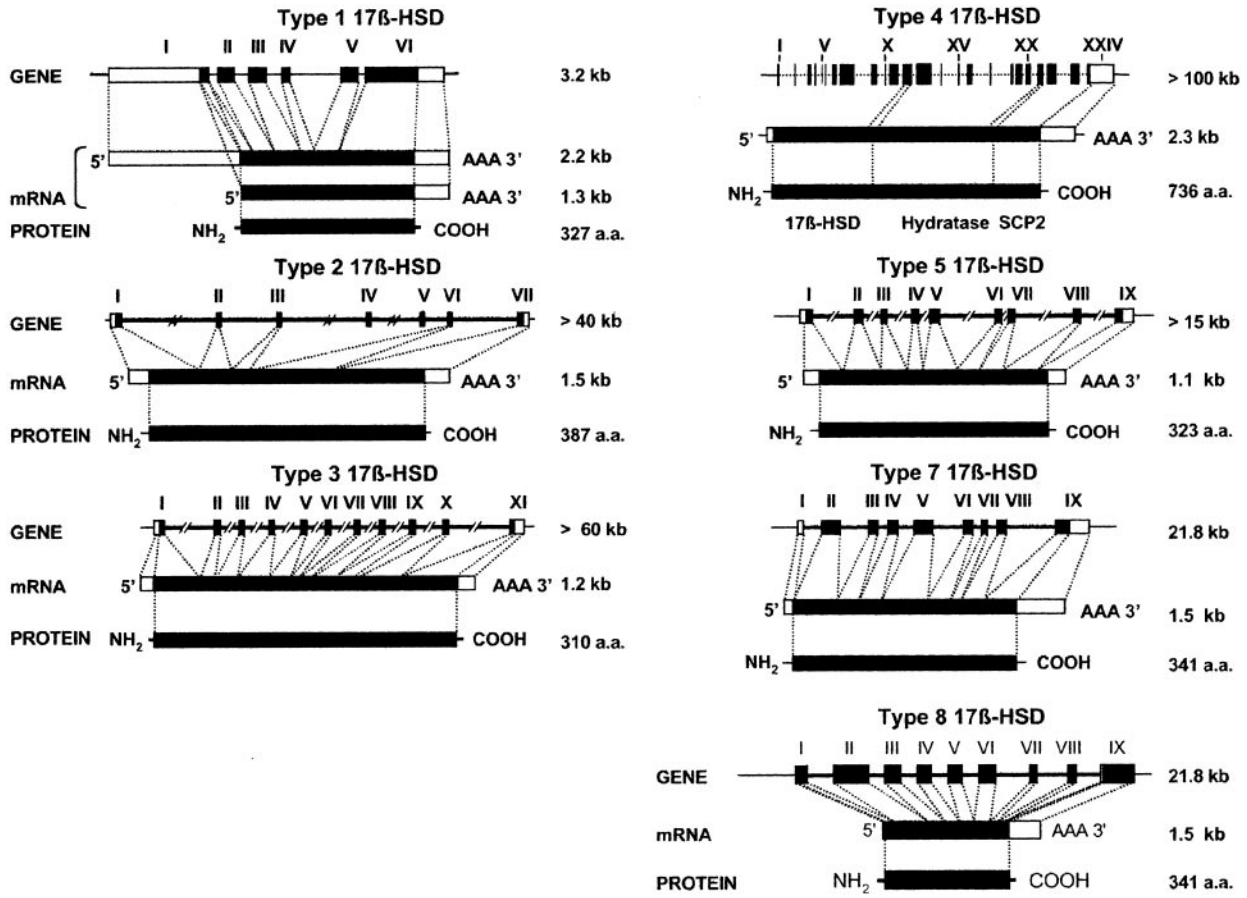

FIG. 6. Structure of the genes and mRNAs encoding human types 1–5, 7, and 8 17β-HSD and the corresponding proteins. aa, Amino acids. [Reproduced by permission of the Society for Endocrinology (33).]

tion of estrogens in a large series of peripheral tissues, although its activity is low and its importance in steroid formation in the human remains to be established. Indeed, mutations in type 4 17β-HSD gene lead to a fatal form of Zellweger syndrome (122).

e. 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 the peripheral target tissues in both men and women as well as in the ovary by a different enzyme, namely type 5 17β-HSD (106). This enzyme is highly homologous with types 1 and 3 3α-HSD as well as 20α-HSD (106) and thus belongs to the aldo-keto reductase family.

In the postmenopausal ovary, hypertrophied stromal cells are localized mainly at the periphery and hilus (123). These stromal cells contain both 3β-HSD and type 5 17β-HSD, thus permitting the transformation of DHEA into 4-dione and then into testosterone. The amount of stromal hyperplasia in postmenopausal ovaries is correlated with the ovarian vein levels of 4-dione and testosterone (124). These hyperplastic stromal cells are thus responsible for the synthesis of 4-dione and testosterone in the postmenopausal ovary.

Type 5 17β-HSD is not only expressed in the ovary but is also present in a large series of peripheral tissues including the mammary gland. The epithelium lining the acini and ducts of the mammary gland is composed of two layers, an inner epithelial layer and an outer discontinuous layer of myoepithelial cells. By immunocytochemistry, 3β-HSD is seen in the epithelial cells of acini and ducts as well as in stromal fibroblasts (Fig. 7A). Immunostaining is also observed in the walls of blood vessels, including the endothelial cells. In the positive cells, the labeling is mainly cytoplasmic. No significant labeling could be detected in the myoepithelial cells. As shown in Fig. 7B, immunostaining for type 5 17β-HSD gives results almost superimposable to those obtained for 3β-HSD, the cytoplasmic labeling being observed in both epithelial and stromal cells and blood vessel walls (125). Studies performed at the electron microscopic level revealed that in sections stained for 3β-HSD or type 5 17β-HSD, labeling was not associated with any specific membrane-bound organelles in the different reactive cell types (126).

f. Type 6 17-HSD. Using a rat prostate cDNA obtained by expression cloning, Biswas and Russell (127) have isolated cDNA clones that metabolize 3α-diol. Among the many clones obtained, one type, named type 6 17β-HSD, catalyzes selectively the oxidation of 3α-diol to androsterone. The transformation of other C19-steroids, namely DHT to androstanedione 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 and thus belongs to the retinol de-

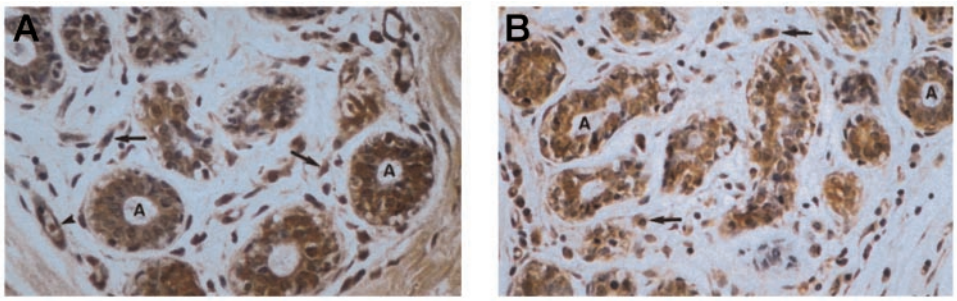

FIG. 7. Human mammary gland immunostained for 3β-HSD (A) and type 5 17β-HSD (B). Staining can be observed in the secretory epithelial cells of acini (A). Stromal cells (*arrows*) and capillaries (*arrowheads*) are also labeled. Magnification, ×430.

hydrogenase family. The human counterpart has not yet been described, and its role remains to be established.

g. 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; Ref. 128). 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 (129). After transfection into HEK-293 cells, Nokelainen *et al.* (129) also found that rat PRAP catalyzes efficiently and selectively the transformation of E_1 to E_2, whereas 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 (130). 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 (130). It is noteworthy that type 5 17β-HSD is also mapped to human chromosome 10 (bands 10p15→14). The importance of this enzyme remains to be established.

h. 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 (131). This area is well known to contain genes encoding the human major histocompatibility complex. This complex is thought to be involved in polycystic kidney disease because aberrant gene expression has been found in two different models of polycystic kidney disease mice (132). Recently, Fomitcheva *et al.* (133) 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. Using HEK-293 cells stably transfected with human type 8 17β-HSD, we have shown recently that this enzyme selectively converts E_2 to E_1, the transformation of E_1 as well as of androgen substrates being negligible (134).

3. Human 5α-reductase isoenzymes. The enzyme 5α-reductase catalyzes the 5α-reduction of 4-dione, testosterone, 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 testosterone 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 human prostatic cDNA libraries (135, 136). The structure of the human type 1 5α-reductase gene was first elucidated (137). This gene is not responsible for 5α-reductase deficiency and is relatively insensitive to the inhibitor finasteride (136). Type 2 5α-reductase, on the other hand, is the isozyme responsible for male pseudohermaphroditism from 5α-reductase deficiency and is sensitive to finasteride (136, 138).

Considering the crucial role of type 2 5α-reductase, we have elucidated the structure of its corresponding gene (83). 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 the type 1 5α-reductase gene. Type 1 5α-reductase is the predominant form expressed in human skin (139).

C. Women produce about two thirds of the androgens synthesized in men

1. Decline in serum androgen precursors and metabolites occurs well before menopause. To gain a better knowledge of the role of DHEA and DHEA-S transformation in both men and women, we have analyzed the serum levels of 18 conjugated C21- and C19-steroids (23). The data obtained show a dramatic decline in the circulating levels of DHEA, DHEA-S, 5-diol, and 5-diol fatty acid esters between the ages of 20 and 80 yr (Fig. 1). As mentioned earlier, in the 50- to 60-yr-old group, serum DHEA has already decreased by 70% from its 20- to 30-yr-old peak values in women (Fig. 1). It should be added that between the ages of 21 and 40 yr, mean serum testosterone in normal women decreases from approximately 1.3 to 0.61 nM (Ref. 26; Fig. 2). A parallel decrease is observed for serum DHEA and DHEA-S, thus suggesting the role of DHEA in the progressive decline in serum testosterone between the ages of 21 and 40 yr in normal women.

The serum concentrations of the conjugated metabolites of DHT, namely ADT-G, 3α-diol-G, and 3β-diol-G, are the most reliable parameters of the total androgen pool in women, whereas serum testosterone is mostly a measure of direct secretion of testosterone by the ovaries and/or adrenals. In fact, although the vast majority of testosterone and DHT is synthesized in the peripheral tissues in women, only a small proportion, estimated at 10–15% of the intracellular content of these androgens, diffuses out of the intracellular compartment without prior metabolism and can be measured as active androgen in the circulation. This is because testoster-

one and DHT, instead of being almost quantitatively released in the circulation, are rapidly glucuronidated into ADT-G, 3α-diol-G, and 3β-diol-G (Fig. 8). Because the individual glucuronosyltransferases responsible for the inactivation of androgens in the human mammary gland have not yet been identified, the human prostate is used as an example of the types of glucuronosyltransferases involved (140, 141). These metabolites are much more water soluble than DHT and thus easily diffuse into the general circulation where they can be measured en route for their elimination mainly by the kidneys (Figs. 9 and 10). The serum concentration of the above-indicated conjugated androgen metabolites decreases by 47.5–72.7% between the 20- to 30- and 70- to 80-yr age groups in women, thus suggesting a parallel decrease in the total androgen pool with age (23).

As assessed by measurement of the circulating levels of these conjugated metabolites of DHT, it can be estimated that women produce approximately 71% or two thirds of the total androgens synthesized in men (Table 1); in women, most of these androgens originate from the transformation of DHEA and DHEA-S into testosterone and DHT in peripheral intracrine tissues. Such an estimate of the androgen pools in men and women based on the serum concentration of androgen metabolites can be influenced by possible differences in the

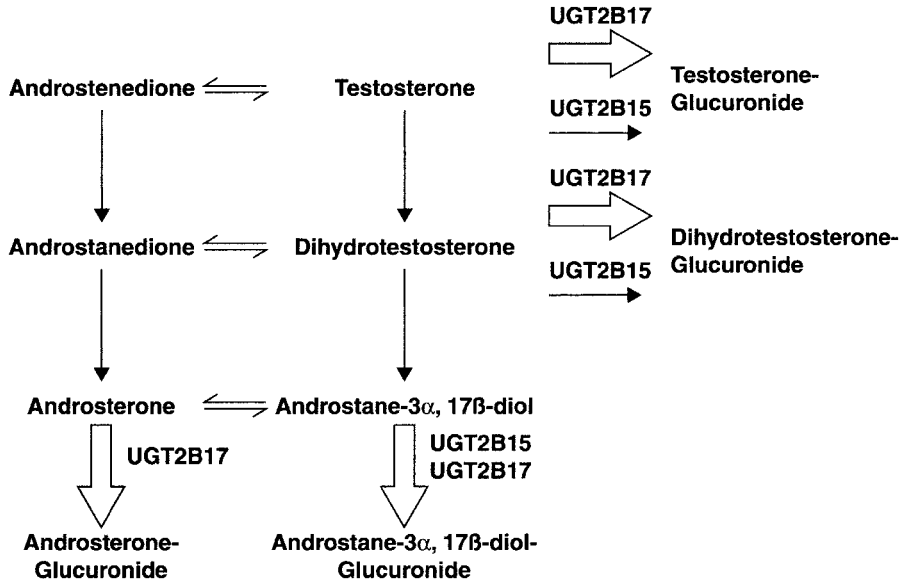

FIG. 8. Enzymes involved in the peripheral metabolism or inactivation of androgens in peripheral tissues.

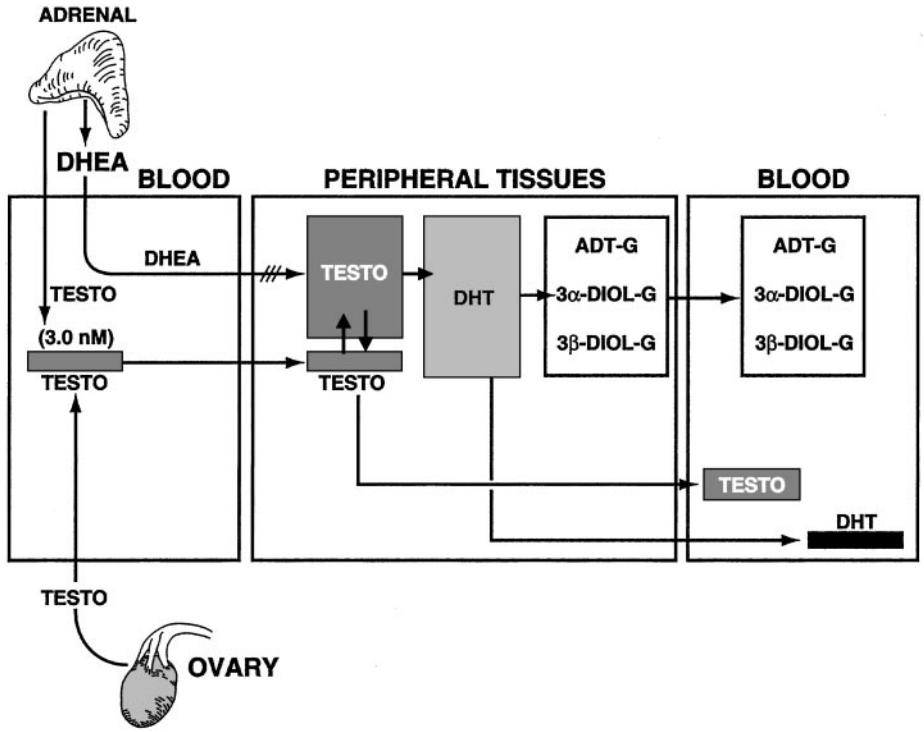

FIG. 9. Distribution in women of the active androgens testosterone and DHT, the sex steroid precursor DHEA, and the main metabolites of androgens (ADT-G, 3α-diol-G, and 3β-diol-G) in the circulation, and in peripheral intracrine tissues. The *height of the bars* is proportional to the concentration of each steroid or its derivatives in individual compartments (336).

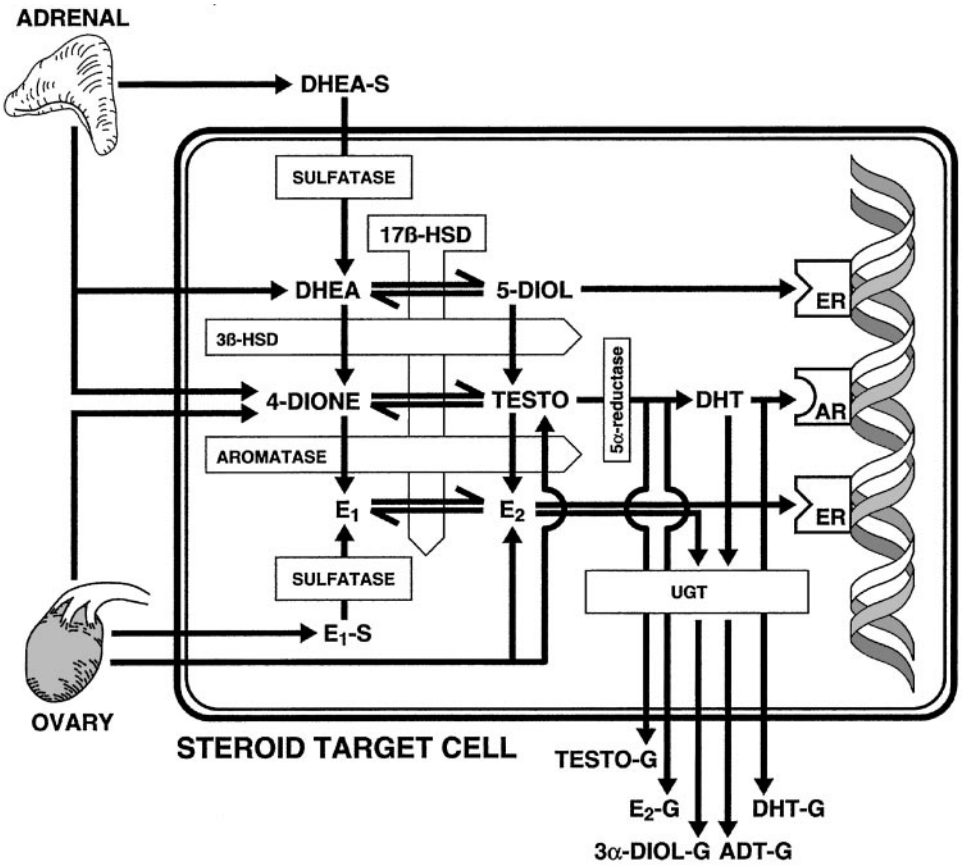

FIG. 10. Schematic representation of the secretion of DHEA, DHEA-S, and 4-dione by the adrenals and E_2, 4-dione, and testosterone by the ovaries as well as the intracellular metabolism of these steroids in the peripheral intracrine tissues. Especially after menopause, the level of androgens active in peripheral tissues is best estimated by the serum concentration of the metabolites of DHT, namely ADT-G, 3α-diol-G, and 3β-diol-G.

metabolic clearance rates of these metabolites in men and women.

2. Plasma sex steroid levels are not a valid parameter of the intracellular situation in women. Proof that changes of the intracellular concentration of sex steroids cannot be estimated by the measurement of testosterone and E_2 in the circulation has been obtained in a study performed in postmenopausal women (23). This study analyzed in detail the serum concentrations of the active androgens and estrogens, as well as a series of free and conjugated forms of their precursors and metabolites, after daily application for 2 wk of a 10-ml 20% DHEA solution on the skin to avoid first passage of DHEA through the liver.

After daily administration of a single dose of DHEA percutaneously, serum DHEA, DHEA-S, and DHEA fatty acid esters increased approximately 175%, 130%, and 250% above control, respectively (Fig. 11), whereas serum 4-dione and testosterone increased by about 100% and 50% over control, respectively (Fig. 12). In parallel with the changes in serum DHEA, DHEA-S, and DHEA fatty acids, the most important effects (Fig. 13) were seen on the glucuronidated metabolites of ADT, 3α-diol, and 3β-diol. In fact, treatment with DHEA caused an increase in serum ADT-G, 3α-diol-G, and 3β-diol-G of approximately 125% (Fig. 13A), 140% (Fig. 13B), and 120% (Fig. 13C), respectively. No significant effect was observed on serum E_1, E_2, or DHT.

The present data show that elevations in serum DHEA within the physiological range found in young adult women led to only small or even no significant changes in serum testosterone, DHT, or E_2, whereas, by contrast, the concentrations of the conjugated metabolites of DHT are markedly elevated, in parallel with the changes in serum DHEA, DHEA-S, and 5-diol. Such data obtained in normal postmenopausal women offer unique proof that the serum levels of androgens and estrogens are poor indicators of total androgenic and estrogenic activities in women. In fact, as mentioned earlier, serum testosterone and E_2 reflect almost exclusively the contribution of the small and direct sex steroid secretion by the ovaries and/or adrenals.

The 50% increase in serum testosterone of approximately 0.8 nM (from 1.5–2.3 nM) observed in women during DHEA treatment corresponds to a much larger increase of approximately 20 nM in serum DHEA. These data are in agreement with the information obtained in men after medical or surgical castration in which the serum levels of testosterone decreased from 15 nM to about 1.5 nM after elimination of testicular androgens. Thus, after castration, the serum levels of testosterone in 60- to 70-yr-old men became comparable to those observed in intact postmenopausal women. The 1.5 nM serum testosterone remaining after castration in men originates essentially from adrenal DHEA (77, 87). The present data thus offer an independent measure of the amount of testosterone that diffuses into the circulation from the androgens synthesized from DHEA and DHEA-S in peripheral intracrine tissues (25).

In a recent study, daily oral administration of 50 mg DHEA

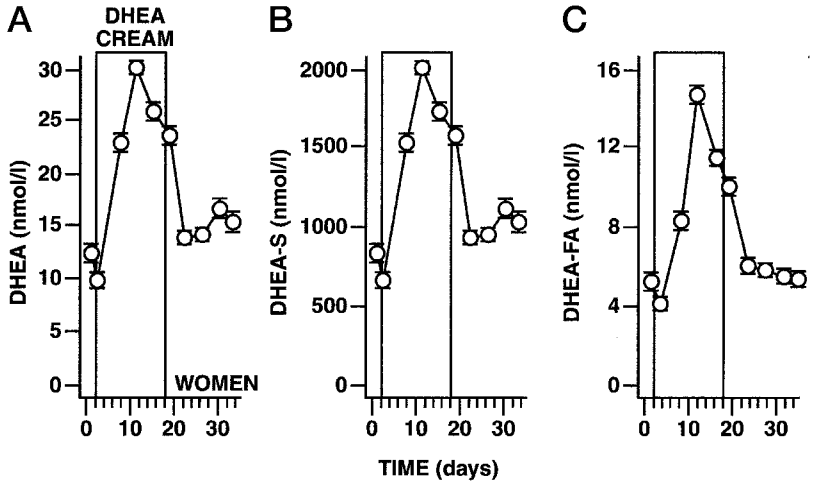

FIG. 11. Effect of daily percutaneous administration of a 10 ml 20% solution of DHEA in 50% ethanol-50% propylene glycol for 2 wk in 60- to 70-yr-old women on serum levels of DHEA (A), DHEA-S (B), and DHEA-fatty acid esters (C; Ref. 26).

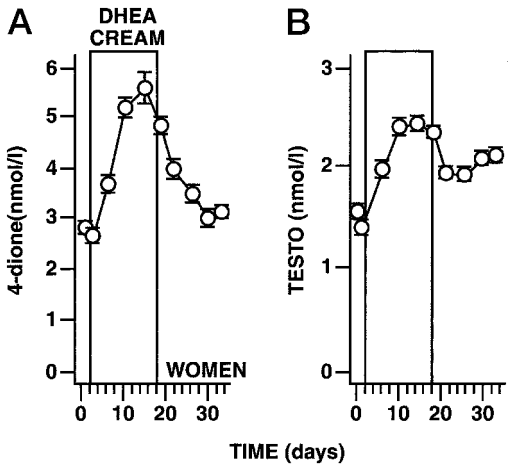

FIG. 12. Effect of daily percutaneous administration of 10 ml 20% solution of DHEA in 50% ethanol-50% propylene glycol for 2 wk in 60- to 70-yr-old women on serum levels of 4-dione (A) and testosterone (B; Ref. 26).

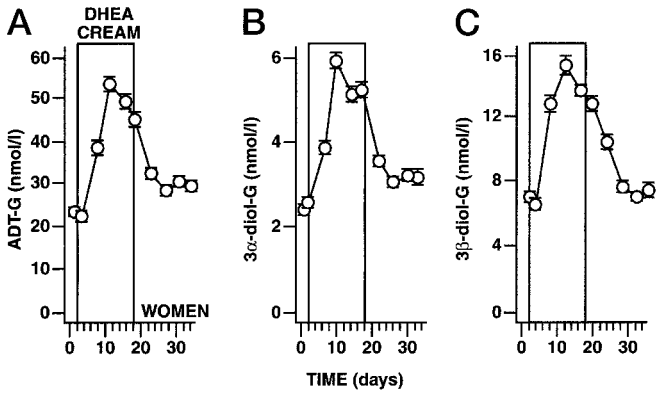

FIG. 13. Effect of daily percutaneous administration of 10 ml 20% solution of DHEA in 50% ethanol-50% propylene glycol for 2 wk in 60- to 70-yr-old women on serum levels of ADT-G (A), 3α-diol-G (B), and 3β-diol-G (C; Ref. 26).

had no significant effect on serum testosterone or DHT, whereas DHEA and ADT-G were increased to a similar extent (80–90%; Ref. 142). In another study, predosing serum levels of DHEA-S in postmenopausal women were increased from 0.55 μg/ml to about 1.4 μg/ml (143) after daily oral administration of 25 mg DHEA for 6 months. Serum DHEA and testosterone levels, however, measured 23 h after the last administration of DHEA, were not changed significantly. Similarly, the 50-mg/d oral dose of DHEA was found to lead to serum androgen levels in the premenopausal range (144).

Our data obtained after percutaneous administration of DHEA in normal postmenopausal women offer the first direct analysis of the correlation between the serum levels of DHEA and DHEA-S with the serum concentration of active androgens and estrogens and their corresponding glucuronidated and sulfated metabolites. It can be concluded that measurements of serum testosterone and E_2 mainly reflect ovarian and/or adrenal steroid secretion, whereas the major contribution of the adrenals is not accurately represented in the circulating levels of active sex steroids. The present data clearly demonstrate that DHEA and DHEA-S are converted in a series of intracrine tissues into the active androgens and/or estrogens that exert their biological effects at their site of synthesis. These steroids are then metabolized in the same cells into inactive glucuronidated and sulfated metabolites, which finally diffuse in the extracellular compartment and can be measured in the circulation. Measurement of the conjugated metabolites of androgens is the only approach that permits an accurate estimate of the total androgen pool in women. It is likely that a similar situation exists for estrogens, although a precise evaluation of the pharmacokinetics of estrogen metabolism and identification of their metabolites remains to be completed.

3. Contribution of the postmenopausal ovary to serum 4-dione and testosterone. It is well recognized that the postmenopausal ovary is a steroid-secreting gland (145, 146). In fact, the postmenopausal ovary is well known to secrete testosterone, and most authors agree that it also secretes some 4-dione (147, 148). In fact, a correlation has been observed between the degree of ovarian stromal hyperplasia and the secretion of androgens by the ovary (124, 149). Moreover, lowering serum gonadotropins with a GnRH agonist has been shown to result in decreased serum androgen levels, thus indicating that the stromal cells of the ovary are under gonadotropin control (150, 151). In agreement with these data, receptors for LH and FSH have been described in the ovarian stromal cells.

It should be mentioned that Couzinet *et al.* (152) have reported that the postmenopausal ovary does not contribute significantly to serum androgen levels. This observation is unique and, if confirmed, will bring even more emphasis on the importance of the adrenals in sex steroid physiology after menopause.

Despite the above-described limitations of the interpretation of serum levels of sex steroids, it is of interest to provide the best available estimate of the contribution of the ovaries and adrenals to the serum levels of 4-dione and testosterone. The majority of studies show declining levels of serum testosterone and 4-dione with age (149, 153–156). Testosterone concentration in the ovarian venous blood is 15 times higher than in peripheral blood (147). In fact, the production of testosterone by the ovary has been estimated to decrease from 250 to 180 μg/d after menopause (157).

As illustrated in Fig. 14A, although the ovaries and adrenals contribute about equally to the serum levels of 4-dione in premenopausal women (158, 159), the contribution of the ovaries decreases to about 20% after menopause (158, 159), despite a progressive fall in the contribution of the adrenals through transformation of declining amounts of DHEA into 4-dione, thus leading to lower total serum concentration of 4-dione after menopause. Similarly, the serum levels of testosterone in premenopausal women originate in approximately equal amounts from the ovaries and adrenals (Refs. 158 and 159; Fig. 15). Peripheral serum testosterone decreases by 50% after ovariectomy in postmenopausal women, thus indicating that the approximately equal contribution of the ovaries and adrenals to serum testosterone remains after menopause. In another study, human chorionic gonadotrophin stimulation and dexamethasone suppression tests in postmenopausal women have suggested that the ovary contributes about 50% of testosterone and 30% of 4-dione in the peripheral circulation (160).

To take into account the low degree of diffusion of the active androgens synthesized intracellularly from adrenal DHEA in peripheral target tissues, we estimate that the serum levels of testosterone should be multiplied by about 10 to compare with the testosterone of direct ovarian and adrenal origins. In other words, as mentioned above, only about 10% of intracellular testosterone synthesized from DHEA leaks into the general circulation. The remaining 90% of locally produced testosterone is mostly converted locally into DHT, which is then converted into ADT-G, 3α-diol-G, and 3β-diol-G (Figs. 8–10). Some testosterone and DHT are also glucuronidated and are found in the circulation as Testo-G and DHT-G. One can thus estimate, as illustrated schematically in Fig. 16, that after menopause the contribution of the ovaries to the intracellular concentration of testosterone is only about 10%. This estimate is based on the observation that serum levels of testosterone are reduced by

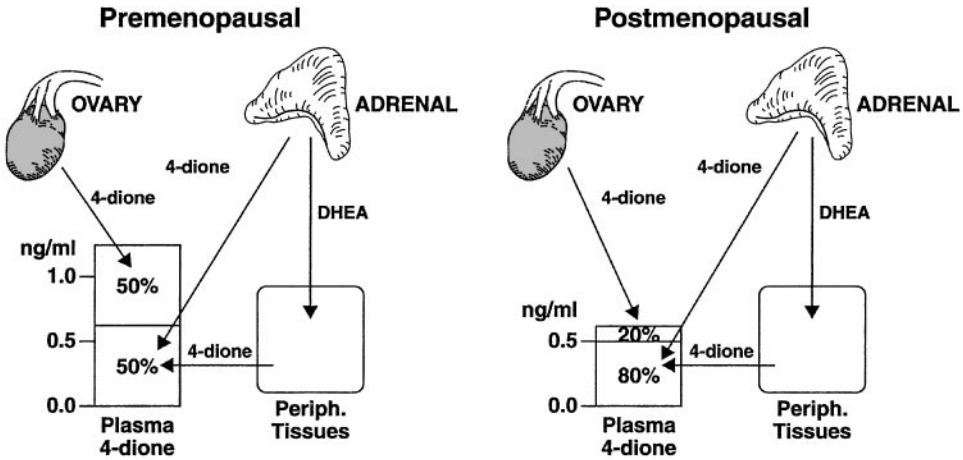

FIG. 14. Contribution of the ovaries and adrenals to the serum levels of 4-dione in pre- and postmenopausal women, respectively (158, 159).

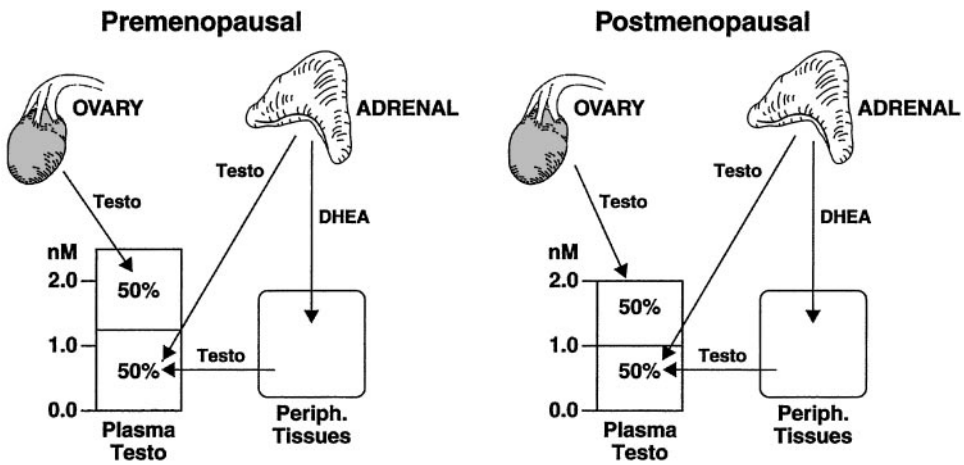

FIG. 15. Contribution of the ovaries and adrenals to the serum levels of testosterone in pre- and postmenopausal women, respectively (158, 159).

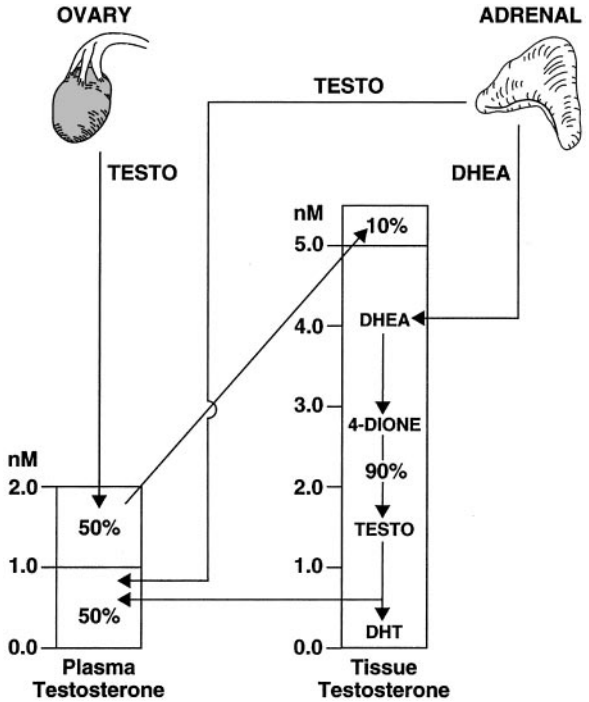

FIG. 16. Schematic representation of the contribution of the ovaries and adrenals to the serum and intratissular concentrations of testosterone. DHEA is transformed in a series of peripheral intracrine tissues into testosterone, which acts locally on the AR directly or after transformation into the more active androgen DHT. Only a small fraction (estimated at 10%) of the active androgens diffuse into the extracellular space and reach the general circulation, whereas the majority of testosterone and DHT is inactivated by glucuronosyltransferases and released as ADT-G, 3α-diol-G, 3β-diol-G, Testo-G, and DHT-G. These are estimates based on the steroid measurements performed in prostatic tissue of intact and castrated men (77, 80).

90% from 15.0 nM to about 1.5 nM after castration in men, whereas the intraprostatic concentration of DHT is reduced only by 50% to about 2.5 ng/g tissue or about 7.5 nM (77, 80). Thus, whereas 7.5 nM intratissular DHT of testicular origin corresponds to 13.5 nM serum testosterone, 1.5 nM serum testosterone of adrenal origin corresponds to the same 7.5 nM intratissular DHT, thus requiring a multiplication factor of 9 to compensate for the poor diffusion of testosterone synthesized intracellularly from DHEA compared with the efficacy of entry of circulating testosterone in the prostatic tissue. Such calculations are in agreement with other data showing that serum ADT-G levels reflect essentially adrenal androgen secretion (161). In fact, Giagulli *et al.* (161) have concluded that DHEA-S accounts for 70–80% of serum ADT-G levels.

III. Androgens Inhibit Breast Cancer

Androgens have been suspected for many decades of being estrogen antagonists and have been used to treat or prevent estrogen-sensitive mammary cancer (162, 163).

A. Clinical data

Estrogens have long been known to play a predominant role in the development and growth of human breast cancer (164–166). On the other hand, well recognized observations have shown that androgens such as testosterone propionate (162, 167–169), fluoxymesterone (170, 171), and calusterone (172) used in the adjuvant therapy of breast cancer have an efficacy comparable to that achieved with other types of endocrine manipulations (165, 169, 173, 174).

Most importantly, a higher response rate and a longer time to disease progression have been observed when androgens were combined with an antiestrogen, compared with an antiestrogen alone (171, 175). The benefits of combined treatment with fluoxymesterone and tamoxifen *vs.* tamoxifen alone were observed in postmenopausal women with metastatic breast cancer (175), both in terms of response rate and time to progression of disease.

As summarized later, such additive inhibitory effects of an antiestrogen and androgen on breast cancer have been clearly demonstrated in a series of experimental models. The above-mentioned clinical data are also well supported by the observation of a synergistic effect of DHEA and of the pure antiestrogen EM-800 on prevention of the development of dimethylbenz(a)anthracene (DMBA)-induced mammary tumors in the rat (176). Moreover, the almost exclusive androgenic component in the action of DHEA on the histomorphology and structure of the rat mammary gland has recently been shown (177), thus supporting such an inhibitory effect of DHEA.

It should also be mentioned that androgens have been shown to induce an objective remission after failure of antiestrogen therapy and hypophysectomy. These clinical observations indicate that the benefits obtained with androgen therapy in breast cancer cannot be due solely to a suppression of pituitary gonadotropin secretion but must result, at least in part, from a direct effect on tumor growth in women. The role of androgens as direct inhibitors of breast cancer growth is well supported by the presence of AR in a large proportion of human breast cancers (178–181). In fact, in primary breast cancer, AR has been found in 54% of premenopausal and 48% of postmenopausal patients (180, 182). The presence of AR has also been described in MCF-7 cells (183, 184).

The overwhelming clinical evidence for tumor regression observed in 20–50% of pre- and postmenopausal breast cancer patients treated with various androgens (173) favors the view that naturally occurring androgens might constitute an as yet overlooked, direct inhibitory control of mammary cancer cell growth. It is thus reasonable to suggest that an imbalance between androgenic and estrogenic influences could modify the overall growth rate of breast tumors in much the same way as that suggested for progestins in estrogen target tissues (185). There is also genetic evidence in agreement with a protective role of androgens against breast cancer (186, 187). Interestingly, the observation that an increased response rate can be obtained by combining androgens and an antiestrogen therapy in breast cancer patients (171, 175) is in agreement with our observations summarized later that the mechanisms of the inhibition exerted by the two types of agents are different, whereas their effects, at least in part, are additive.

In this context, it has been found that Western women having a low excretion of adrenal androgenic metabolites respond more poorly to endocrine therapy and have a shorter survival time (188–190). Possibly because of the small

number of cancer cases in many studies, the methodology used, the low predictive value of measurements of serum sex steroid levels, and the association in case-control studies between serum androgen levels and breast cancer risk have led to contradictory data. Thus, subnormal levels of serum androgens have been found in women with increased risk of breast cancer (191–193), whereas opposite data have also been reported (194–197).

It is of interest that suppression of androgens in men is associated with breast growth (198). Moreover, mutations in AR have been linked with breast cancer in men (199).

It should be added that treatment of ovariectomized monkeys with testosterone decreased by about 40% the stimulation of mammary epithelial proliferation induced by E_2 (200). It is possible that part of the increased risk of breast cancer in BRCA-1 mutant patients is associated with the decreased efficiency of the mutated BRCA-1 gene to interact with the AR (201). It is also pertinent to mention that female athletes and transsexuals taking androgens show atrophy of mammary gland epithelial tissue (202, 203).

B. Preclinical data

Lacassagne (204) first observed in 1936 that treatment of mice with testosterone propionate delayed the occurrence of E_1-stimulated mammary tumors. In DMBA-induced tumors, high doses of DHT (0.5–4.0 mg/d) for several weeks caused the regression of 60% of established tumors (163). Similar effects were observed with testosterone propionate (205) and dromostanolone propionate (206, 207).

In support of the early clinical data mentioned above, our previous studies have clearly demonstrated that androgens exert a direct inhibitory effect on the proliferation of human breast cancer cells (208–213). In fact, the first demonstration of a potent and direct inhibitory effect of androgens on human breast cancer growth was obtained in the estrogen-sensitive human breast cancer cell line ZR-75-1 (208). In that study, as shown in Fig. 17A, DHT not only completely blocked the stimulatory effect of E_2 on cell proliferation but also reduced cell growth in the absence of estrogens. At low cell density (Fig. 17B), it can be seen that DHT completely prevented breast cancer cell growth.

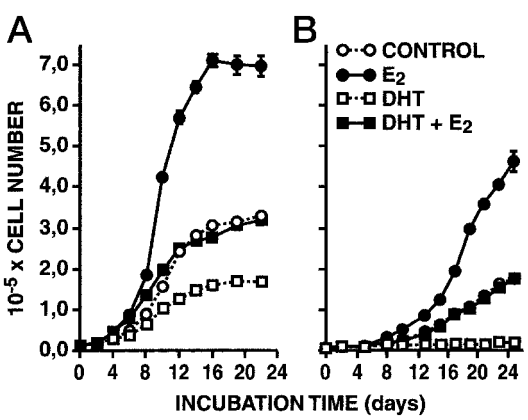

FIG. 17. Time course of the effect of DHT and/or E_2 on the proliferation of ZR-75-1 cells. A, Cells were plated at 1×10^4 cells/2.0-cm^2 well; 48 h later (zero time), 1 nM E_2 (●), 10 nM DHT (□), or both steroids (■) were added, and cell numbers were determined at the indicated time intervals. Control cells received the ethanol vehicle only. B, Same as A, except that the initial density was 5.0×10^3 cells/2.0-cm^2 well (208).

DHT has been shown to be formed from testosterone and 4-dione in human breast cancer tissue both *in vitro* in tissue pieces and *in vivo* (214). Such data indicate the presence of 5α-reductase in breast cancer tissue, an enzyme thought to be specific for androgen-dependent tissues. In ZR-75-1 cells, concentrations of DHT in the incubation medium similar to the plasma levels found in normal women (215–217) and breast cancer patients (Ref. 218; 0.3–0.7 nM) are potent inhibitors of the mitogenic effect of E_2 and even inhibit growth in the absence of estrogens (208). Furthermore, testosterone, at concentrations observed in adult women (1–3 nM; Refs. 215–218), is also a potent inhibitor of cell growth. 4-Dione also led to significant growth inhibition in ZR-75-1 cells, although the active concentrations (IC_{50}, 15 nM) are in the upper range of the plasma concentrations (1–10 nM) found in women (215–218).

Several lines of evidence show that the potent growth-inhibitory effect of androgens observed in ZR-71-1 cells is mediated through their specific interaction with the AR. First, the potency of DHT and testosterone to induce antiproliferative effects (IC_{50}, ~0.10 and 0.50 nM, respectively) is in agreement with their relative binding affinity for androgen specific binding sites in intact ZR-75-1 cells as well as in other human breast cancer cells (219, 220). Such values compare well with the potency of DHT to specifically stimulate the secretion of the Zn-α_2-glycoprotein (221) and the GCDFP-15 glycoprotein (221, 222) in T47-D human breast cancer cells. The ability of 4-dione to induce an antiproliferative effect (IC_{50}, ~15 nM) most likely results from its metabolic transformation into testosterone and DHT (223–225) than from its direct interaction with the AR (K_D, ~200 nM). Secondly, the antiandrogen OH-flutamide competitively reversed the effect of DHT and 4-dione with an apparent dissociation constant (K_i, ~110 nM) consistent with its known affinity for the AR (226, 227).

Because the benefits of combined treatment with an androgen and an antiestrogen have already been observed in women with breast cancer, (171, 175), in agreement with the *in vitro* data mentioned above (208–212), a more precise understanding of the mechanisms of action of androgens and antiestrogens in breast cancer cells becomes important. After a 12-d incubation of ZR-75-1 cells in the presence of 0.1 nM E_2 in phenol red-free medium, cell number was increased 2.8-fold above control ($P < 0.01$; Fig. 18A). The addition of 1 nM DHT, on the other hand, caused a 78% blockade ($P < 0.01$) of E_2-induced ZR-75-1 cell growth, whereas the pure steroidal antiestrogen EM-139 (228), on the other hand, not only completely reversed the effect of E_2 but further inhibited cell number by 30% below control values ($P < 0.01$; Fig. 18B). It can also be seen in Fig. 4B that, in the absence of E_2, EM-139 and DHT alone caused 21% ($P < 0.01$) and 43% ($P < 0.01$) inhibitions of basal cell growth, respectively. It can also be seen in Fig. 18B that the inhibitory effect of DHT is completely prevented by the addition of the pure antiandrogen OH-flutamide. Most interestingly, in another study, it was found that the growth-inhibitory effect of DHT is clearly additive to that induced by maximally effective concentra-

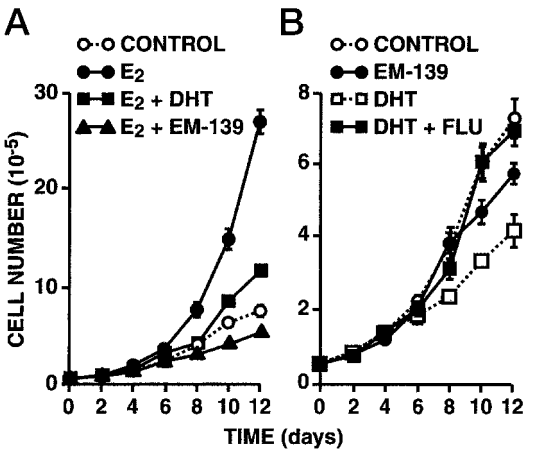

FIG. 18. A, Time course of the effect of 0.1 nM E_2, 1 nM DHT + E_2, 0.3 μM EM-139 + E_2, or control medium on the proliferation of ZR-75-1 cells during a 12-d incubation period. B, Time course of the effect of 1 nM DHT, 0.3 μM EM-139, DHT + EM-139, DHT + 0.3 μM OH-FLU, or control medium on the proliferation of ZR-75-1 cells. Three days after plating at an initial density of 5×10^5 cells/10 cm^2 per well, cells were incubated with the indicated concentrations of the compounds with medium changes every 48 h for the indicated time periods. At the end of the indicated incubation periods, cell number was determined with a Coulter counter. Data are expressed as means ± SEM of quadruplicate wells (263).

tions of the antiestrogen LY156758, thus indicating an action mediated by a mechanism different from interaction with the estrogen receptor (ER; 229). Accordingly, the evidence obtained leaves little doubt that the antiproliferative effect of androgens does not result from competition for binding to the ER, but rather is caused by an AR-mediated mechanism that is additive to blockade of the ER by an antiestrogen.

After our demonstration of the inhibitory effect of DHT and antiestrogens on ZR-75-1 cell proliferation *in vitro* (208–212, 229), we extended our study *in vivo* to ovariectomized athymic mice using the same human breast cancer cells to more closely mimic the clinical situation in women. We thus examined the effect of DHT on tumor growth stimulated by physiological doses of E_2 administered by SILASTIC-brand (Dow Corning Corp., Midland, MI) implants.

As illustrated in Fig. 19, E_2 caused a progressive increase in total tumor area from 100% (which corresponds to an average of 0.23 ± 0.08 cm^2) at the start of the experiment to 226 ± 31% after 100 d of treatment. Treatment with DHT, on the other hand, not only completely reversed the stimulatory effect of E_2 on tumor growth but also decreased total tumor area to 48 ± 10% of its original size. The androgen DHT is thus a potent inhibitor of the stimulatory effect of E_2 on ZR-75-1 human breast carcinoma growth in *in vivo* athymic mice. Similar inhibitory effects on E_2-stimulated tumor growth were achieved with medroxyprogesterone acetate [MPA (Provera); Ref. 230], a compound having progestational, androgenic, and glucocorticoid activities (231). Because ovariectomized animals supplemented by exogenous estrogen were used in these studies, such data provide further support for a direct inhibitory action of androgens at the tumor cell level under *in vivo* conditions, thus adding to the well known inhibitory effect androgens exerted on the pituitary gonadal axis in intact women (232).

Considering the potential importance of androgens in breast cancer therapy, and to better understand the molecular mechanisms responsible for the antagonism between androgens and estrogens, we have investigated the effect of androgens on ER expression in the ZR-75-1 human carcinoma cell line. The specific uptake of [^{3}H]E_2 in intact ZR-75-1 cell monolayers was decreased by as much as 88% after a 10-d preincubation with increasing concentrations of DHT (Fig. 20). A half-maximal effect of DHT on [^{3}H]E_2 uptake was observed at 70 pM (209). Preincubation with dexamethasone and R5020 (100 nM each) had no effect on the specific uptake

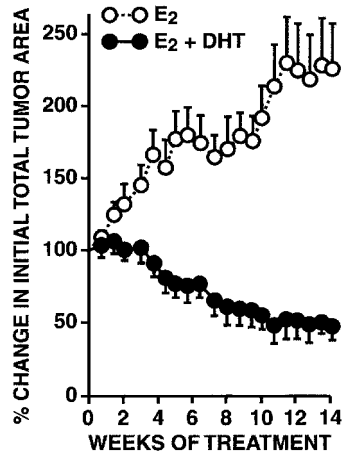

FIG. 19. Effect of 100-d treatment of ovariectomized athymic mice with Silastic brand implants of E_2 (1:3000, E_2/cholesterol, wt/wt) alone or in combination with SILASTIC-brand implants of DHT (1:5, DHT/cholesterol, wt/wt) on average total ZR-75-1 tumor area in nude mice. Results are expressed as percentage of pretreatment values (means ± SEM of 11 tumors in the E_2 group, 9 tumors in the E_2 group, and 9 tumors in the E_2 + DHT group; Ref. 230).

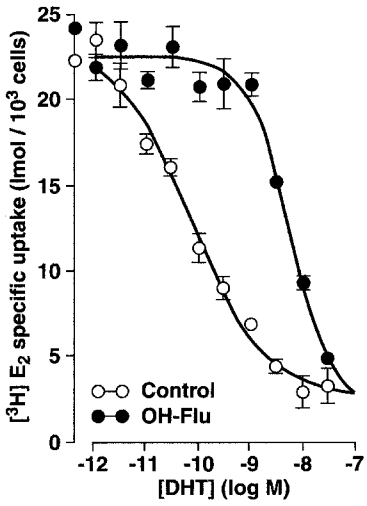

FIG. 20. Effect of preincubation with increasing concentrations of DHT on [^{3}H]E_2-specific binding in ZR-75-1 human breast cancer cells, hydroxylapatite exchange assay of [^{3}H]E_2-specific binding of cytosol and nuclear (cytosol + nuclear = total) extracts obtained from ZR-75-1 cells preincubated for 11 d with the indicated concentrations of DHT. E_2 specific uptake of [^{3}H]E_2 in intact ZR-75-1 cells preincubated for 10 d with the indicated concentrations of DHT alone (○, control) or in the presence of 3 μM antiandrogen hydroxyflutamide (●, OH-FLU). Values are given as means ± SE from triplicate determinations (209).

of [^{3}H]E_2 (data not shown). The addition of hydroxyflutamide, a nonsteroidal antiandrogen devoid of agonistic activity and with no significant affinity for receptors other than the AR (226, 227) competitively reversed inhibition of [^{3}H]E_2 specific uptake by DHT. The inhibition constant (K_i) value for the reversal of DHT action by hydroxyflutamide was estimated at 39 nM (233), in agreement with the affinity of the antagonist for the AR (227). Thus, the primary site of action of DHT on [^{3}H]E_2-specific binding was clearly consistent with a specific interaction with the AR, rather than a direct activation and processing of the ER by DHT (234–239). Similar results were observed on progesterone receptor levels, thus showing a direct inhibitory effect of DHT in human breast cancer cells (209).

This study showed for the first time that androgens strongly suppress ER content in the human breast cancer cell line ZR-75-1, as measured by radioligand binding and anti-ER monoclonal antibodies. Similar inhibitory effects were observed on the levels of ER mRNA measured by ribonuclease protection assay (209). The androgenic effect was observed at subnanomolar concentrations of the nonaromatizable androgen DHT, regardless of the presence of estrogens, and was competitively reversed by the antiandrogen hydroxyflutamide. Such data on ER expression provide an explanation for at least part of the antiestrogenic effects of androgens on breast cancer cell growth and provide an explanation for the observations showing that the specific inhibitory effects of androgen therapy are additive to the standard treatment limited to blockade of estrogens by antiestrogens (229). Another possible clue to the mechanism of action of DHT in breast cancer cells is provided by the observation that androgens and estrogens exert opposite effects on progesterone receptor levels (240).

The data summarized above clearly support the hypothesis that at least part of the antagonism observed between the action of androgens and estrogens in breast cancer cells (208, 211, 215, 240) may be explained by the heterologous down-regulation of the ER by an AR-mediated mechanism. The concentration of DHT needed to exert a half-maximal suppression of ER binding activity (0.07–0.1 nM) is lower than the concentration known to induce binding and nuclear retention of the ER (215, 234). Moreover, the inhibitory effect of DHT on ER content was competitively reversed by the antiandrogen hydroxyflutamide (226, 227). Such data clearly show that AR mediates the down-regulation of the ER by DHT observed in ZR-75-1 cells.

The effect of androgens on ZR-75-1 cell proliferation, however, cannot be solely explained by the suppression of ER expression, because androgens still exert very potent inhibitory effects on growth in the absence of estrogens, even after prolonged periods of estrogen deprivation before exposure to androgens (208, 211). Moreover, the antiproliferative activity of androgens in estrogen-deprived ZR-75-1 cells is more pronounced and is additive to that exerted by antiestrogens (208, 241).

Down-regulation of ER expression by androgens might be of crucial importance in their physiological mode of action, *i.e.*, when estrogens are simultaneously present in normal as well as cancerous mammary gland tissue. In the specific case of human breast cancer, endogenous androgens may reduce the tumor cell sensitivity to estrogens by decreasing ER levels. Thus, in normal breast tissue, endogenous as well as locally produced androgens are likely to contribute to the regulation of the level of ER, thus modulating the sensitivity to estrogens. This inhibitory effect of androgens on intracellular ER concentrations may be expected to leave the relative effectiveness of the competitive blockade of estrogen action by antiestrogens unaffected, while decreasing the efficiency of any residual estrogenic stimulation of cell growth.

In agreement with the *in vitro* data, Dauvois *et al.* (242) have shown that constant release of the androgen DHT in ovariectomized rats bearing DMBA-induced mammary carcinoma caused a marked inhibition of tumor growth induced by E_2 (Fig. 21). That DHT acts through interaction with the AR in DMBA-induced mammary carcinoma is well supported by the finding that simultaneous treatment with the antiandrogen flutamide completely prevented DHT action. Such data demonstrated, for the first time, that androgens are potent inhibitors of DMBA-induced mammary carcinoma growth by an action independent from inhibition of gonadotropin secretion and suggested an action exerted directly at the tumor level, thus further supporting *in vitro* data obtained with the human ZR-75-1 breast cancer cell line (208, 209).

It should be mentioned that *in vivo* studies have demonstrated that controlled release of low-dose MPA, a compound having androgenic activity, also exerts a potent inhibitory effect on the development and growth of DMBA-induced mammary carcinoma in the rat (243, 244). MPA has in fact been clearly shown to exert androgenic inhibitory effects on the growth of human breast cancer cells *in vitro* (209, 231,

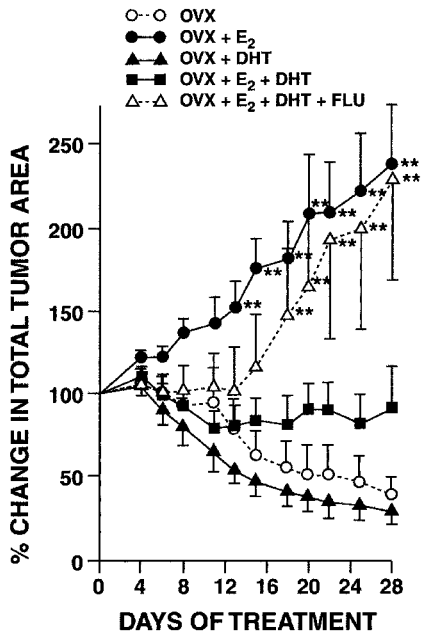

FIG. 21. Effect of 28-d treatment of ovariectomized (OVX) rats with Silastic brand implants of E_2, DHT, E_2 + DHT, or E_2 + DHT + twice daily injections of flutamide (FLU) on average total DMBA-induced mammary tumor area in the rat. Results are expressed as percentage of pretreatment values as means ± SEM of 22–26 tumors per group. **, $P < 0.01$ OVX rats treated with the indicated steroid *vs.* OVX animals at the same time interval (242).

245), thus suggesting the role of AR in the beneficial effects of MPA in breast cancer in women (246, 247). As previously described, MPA is a compound having a complex series of activities, namely progestational, glucocorticoid, and androgenic (231). Despite the beneficial androgenic effects of this compound observed on human breast cancer cells *in vitro* (209, 231, 245) and in clinical studies (246, 247), the recent results of the Women's Health Initiative Study (8) clearly indicate that this compound is not recommended for long-term use in normal women where the stimulatory progestational component could well be predominant. A recent study in rats has shown that the addition of methyltestosterone inhibits the marked proliferation of the mammary gland epithelium induced by a low-dose oral contraceptive (248).

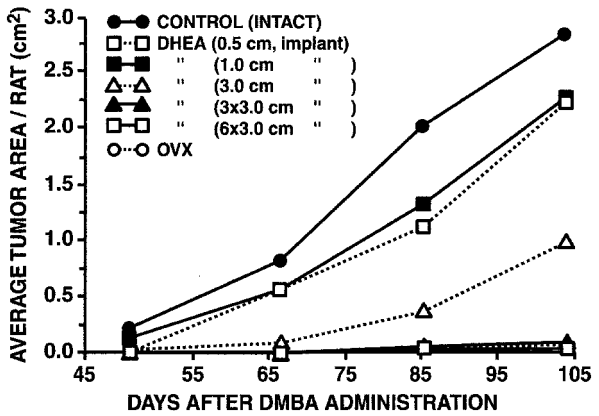

FIG. 22. Effect of increasing doses of DHEA constantly released from SILASTIC-brand implants and administered 7 d before the intragastric administration of 20 mg of DMBA in intact 50- to 52-d-old female rats on average tumor area (cm^2) per rat at the indicated time intervals (258).

IV. DHEA Inhibits Breast Cancer

A. Preclinical studies

1. Introduction. Labrie and colleagues (78, 249) first demonstrated that DHEA possesses relatively potent androgenic activity and stimulates androgen-dependent gene expression in the rat ventral prostate. As mentioned earlier, the first androgen successfully used in the treatment of advanced breast cancer was testosterone propionate (250). Many studies subsequently confirmed the beneficial effects of androgens on breast cancer (165, 167–174, 251, 252). Moreover, *in vitro* studies have provided the first demonstration of the direct antiproliferative activity of androgens on the growth of human mammary carcinoma cells using the cell line ZR-75-1 as model (208, 253). Interestingly, Poulin *et al.* (208) have found that the inhibitory effect of androgens on the growth of ZR-75-1 human breast carcinoma cells is additive to that of an antiestrogen. The additive inhibitory effects of an androgen and an antiestrogen on the growth of human breast carcinoma cell line ZR-75-1 have also been observed *in vivo* in nude mice (230).

2. Inhibitory effect of DHEA on breast cancer

a. Prevention of mammary tumor development by DHEA. As described above, the human adrenals secrete large amounts of the precursor steroids DHEA and DHEA-S, both of which are converted into androgens in target intracrine tissues (25, 35, 78, 92, 249, 254, 255). To investigate the possibility that DHEA and its metabolites could have a preventive effect on the development of mammary carcinoma, we have studied the effect of increasing circulating levels of DHEA constantly released from Silastic brand implants on the development of mammary carcinoma induced by DMBA in the rat. The DMBA-induced mammary carcinoma in the rat has been widely used as a model of hormone-sensitive breast cancer in women (242, 256, 257).

Treatment with increasing doses of DHEA delivered constantly by SILASTIC-brand implants of increasing length and number caused a progressive inhibition of tumor development (258). It is of interest to see that tumor size in the group of animals treated with the highest dose (6 × 3.0-cm long implants) of DHEA was similar to that found in ovariectomized animals (Fig. 22), thus showing a complete blockade of estrogen action by DHEA. Such data clearly demonstrate that circulating levels of the precursor adrenal steroid DHEA comparable to those observed in normal adult premenopausal women (259) exert a potent inhibitory effect on the development of mammary carcinoma induced by DMBA in the rat. It is of special interest to see that serum levels of DHEA of 7.09 ± 0.64 nM and 17.5 ± 1.1 nM led to a dramatic inhibition of tumor development to 22% and 11% of animals bearing mammary carcinoma compared with 68% in control intact animals. At the highest dose of DHEA used, which corresponds to serum DHEA values of 27.2 ± 2.2 nM, the incidence of tumors was reduced to only 3.8%. It should be mentioned that the serum DHEA levels in normal 20- to 30-yr-old women ranges between 8.3 and 17.3 nM (259).

With the previous knowledge of the potent inhibitory effect of androgens on the growth of human breast cancer as well as on the development and growth of DMBA-induced mammary carcinoma in the rat, it is reasonable to suggest that the present data showing a potent inhibitory effect of DHEA on the development of DMBA-induced mammary carcinoma can be at least partially explained by the androgenic action of the steroids synthesized by the enzymes present in the peripheral target tissues, an action exerted through intracrinology. Although the rat adrenals do not secrete significant amounts of DHEA (80), the enzymes required for the formation of androgens and estrogens are expressed in rat peripheral tissues (260, 261). Such data also suggest a potential use of DHEA as a physiological approach for the prevention of breast cancer in women.

b. Inhibitory effects of DHEA on the growth of human breast cancer xenografts. Because, as mentioned above, androgens have been clearly demonstrated to inhibit the growth of human breast cancer in women as well as in laboratory studies *in vitro* (167–170, 172, 175, 208–213, 230, 242, 262–264) and DHEA is predominantly transformed into androgens in the mammary gland, we have studied the possibility that DHEA could inhibit the growth of the human ZR-75-1 breast cancer cell line *in vivo* in nude mice. To avoid the inhibitory effects of DHEA-derived steroids on gonadotropin secretion, we have used ovariectomized animals supplemented with E_1.

As illustrated in Fig. 23, the size of the ZR-75-1 tumors increased by 9.4-fold over a 291-d period (9.5 months) in ovariectomized nude mice supplemented with E_1; in contrast, in control ovariectomized mice that received the vehicle alone, tumor size decreased to 36.9% of the initial value during the course of the study (265). On the other hand, treatment with increasing doses of percutaneous DHEA caused a progressive inhibition of E_1-stimulated ZR-75-1 tumor growth. Inhibitions of 50.4%, 76.8%, and 80.0% were achieved at 9.5 months of treatment with the daily doses of DHEA of 0.3, 1.0, or 3.0 mg per animal, respectively (Fig. 23). In agreement with the decrease in total tumor load, treatment with DHEA led to a marked decrease in the average weight of the tumors remaining at the end of the experiment. To our knowledge, these data provide the first demonstration of the inhibitory effect of DHEA on the growth of human breast cancer xenografts in nude mice.

In the ovariectomized mouse, exogenous DHEA represents the only source of sex steroids in peripheral tissues, including the mammary gland. Moreover, by itself, DHEA does not possess any significant androgenic or estrogenic activity, its activity being dependent upon its transformation into androgens and/or estrogens in peripheral target intracrine tissues (25). Consequently, the inhibition of tumor growth seen after DHEA treatment in ovariectomized animals results from its intracrine *in situ* conversion into androgens in the mammary gland (25, 35, 78, 92, 255). In fact, we have recently shown that DHEA exerts an almost exclusively androgenic effect in the rat mammary gland (177). Moreover, DHEA is well known to be converted into androgens, and treatment with DHEA is known to induce androgen-sensitive gene expression in the rat ventral prostate (78, 249). Taken together, these data strongly suggest that DHEA exerts its inhibition of breast cancer development and growth through its conversion to androgens and activation of the AR.

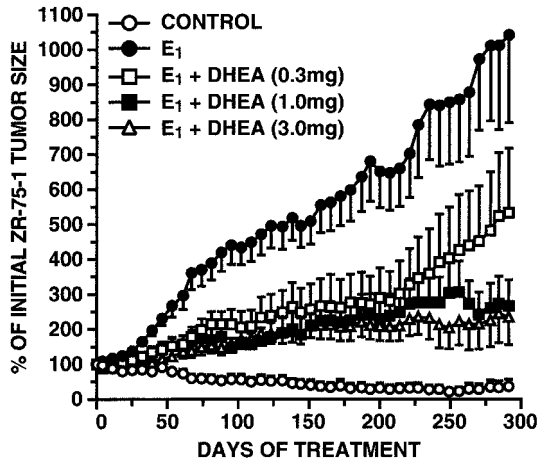

FIG. 23. Effect of increasing doses of DHEA (a total dose of 0.3, 1.0, or 3.0 mg) administered percutaneously in two doses daily on average ZR-75-1 tumor size in ovariectomized nude mice supplemented with 0.5 μg E_1 daily. Ovariectomized mice receiving the vehicle alone were used as additional controls. The initial tumor size was taken as 100%. DHEA (0.3, 1.0, or 3.0 mg per animal/d) was administered percutaneously on the dorsal skin in a 0.02-ml solution of 50% ethanol-50% propylene glycol. [Reproduced by permission of Oxford University Press (265).]

One proposed mechanism for the inhibitory action of DHEA has been the antagonism of DHEA-derived 5-diol on the ER (79, 236, 266). In fact, DHEA can be converted *in vivo* and *in vitro* into the weak estrogen 5-diol, which interacts with the ER and can exert weak estrogen-like effects independent from aromatase (253, 267–269). That this hypothesis of competition with 5-diol is most unlikely to apply is supported by the observation that increasing doses of diethylstilbestrol, a highly potent estrogen, do not interfere with the inhibitory effect of DHEA on human breast cancer MCF-7 cell proliferation (270). The argument is made even stronger by the finding that tamoxifen did not interfere with the antiproliferative action of DHEA. Moreover, despite the fact that human ZR-75-1 breast cancer cells do not express 3β-HSD and are thus unable to synthesize androgens, thus explaining the stimulatory effect of DHEA on the growth of these cells under *in vitro* conditions (253), DHEA exerts an inhibitory effect on the growth of the same cancer cells under *in vivo* conditions in nude mice, the androgens originating from neighboring or distant cells that possess the required mechanisms to transform DHEA into androgens in sufficient amounts to affect other cells after diffusion from their site of synthesis (271).

A group of researchers have reported that DHEA is inhibitory on breast cancer growth in the presence of estrogens, whereas it can be stimulatory on experimental models in which estrogens are absent (197, 270). It should be mentioned, however, that an absence of estrogens does not exist in women where comparable levels of E_2 are found in breast cancer tissue in pre- and postmenopausal women (272). In fact, such a hypothetical situation of an absence of estrogens does not exist in normal women, even after menopause.

Although DHT exerts a potent inhibitory effect on breast cancer cell proliferation in ZR-75-1 human breast cancer cells (208, 210), DHT has not always been found to inhibit the growth of MCF-7 cells. The lack of inhibitory action of DHT in some MCF-7 cell lines is most likely due to the presence of a high level of 3α-HSD activity in these cells, thus preventing DHT from exerting its inhibitory effect before its transformation into 3β-diol, a compound having intrinsic estrogenic activity (our unpublished data; and Ref. 273). That the inhibitory effect of DHEA on breast cancer MCF-7 cell growth is due to interaction with AR is supported by the finding that the antiandrogen flutamide reversed the inhibitory effect of DHEA on MCF-7 human breast cancer cell proliferation, whereas the antiestrogen tamoxifen had no effect (274).

c. Additive inhibitory effects of DHEA and the antiestrogen EM-652 on the growth of DMBA-induced mammary tumors. Because antiestrogens (230, 275–278) as well as DHEA (258) can independently inhibit the development of DMBA-induced mammary carcinoma, we have studied the potential benefits of combining the new antiestrogen EM-800 with DHEA on the development of mammary carcinoma induced by DMBA in the rat. As illustrated in Fig. 24, 95% of control animals developed palpable mammary tumors by 279 d after DMBA administration. Treatment with DHEA or EM-800 alone partially prevented the development of DMBA-induced mammary carcinoma, the incidence being thus re-

duced to 57% ($P < 0.01$) and 38% ($P < 0.01$), respectively. Interestingly, combination of the two compounds led to a significantly greater inhibitory effect than that achieved by each compound administered alone ($P < 0.01$ *vs.* DHEA or EM-800 alone). In fact, the only two tumors that developed in the group of animals treated with both compounds disappeared before the end of the experiment (279).

Such data obtained *in vivo* support our previous findings that the inhibitory effects of androgens and antiestrogens on mammary carcinoma are exerted at least in part by different mechanisms and that the combination of an androgenic compound with a pure antiestrogen has improved efficacy compared with each compound used alone in the prevention and treatment of breast cancer in women. The antagonism between androgens and estrogens on breast cancer growth is illustrated schematically in Fig. 25. DHEA, secondary to its predominant transformation into androgens in mammary gland tissue, exerts an inhibitory effect on mammary carcinoma development and growth, an effect that counteracts and can even completely neutralize the stimulatory effect of estrogens.

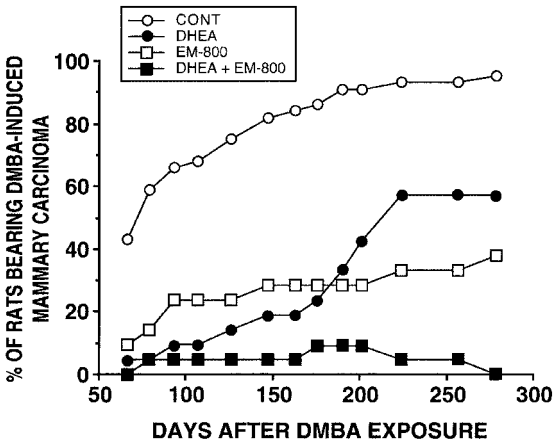

FIG. 24. Effect of treatment with DHEA (10 mg, percutaneously, once daily) or EM-800 (75 μg, orally, once daily), alone or in combination for 9 months, on the incidence of DMBA-induced mammary carcinoma in the rat throughout the 279-d observation period. Data are expressed as percentage of the total number of animals in each group (279).

It should be mentioned that recent data suggest that progestins exert a negative impact on breast cancer (2–5), with recent reports indicating an increased risk of the disease in women (6, 7). It is important to indicate that the absence of a stimulatory effect of DHEA on the human endometrium (73, 280) eliminates the need to administer a progestin to neutralize the stimulatory effect of estrogens in this tissue.

Although the above-mentioned data demonstrate the direct inhibitory effects of androgens and DHEA on breast cancer growth, it is likely that endogenous androgens and DHEA play an important physiological role in the control of normal breast tissue growth and function and that this antagonism between androgens and estrogens is also operative in breast cancer.

B. Epidemiological studies

Epidemiological studies have generally observed a protective effect of DHEA on breast cancer, especially in Western women (191, 193, 281, 282). In fact, low serum DHEA levels have been associated with breast cancer in women (281), whereas women with breast cancer were found to have low urinary levels of androsterone and etiocholanolone, two metabolites of DHEA (283, 284). Moreover, women with primary operable breast cancer had urinary levels of 11-deoxy-17-ketosteroids (derived mainly from DHEA-S and DHEA) lower than normal, thus suggesting that a low secretion rate of DHEA and DHEA-S could precede the development of breast cancer. It might be relevant to mention that treatment with DHEA markedly delayed the appearance of breast tumors in C3H mice that were genetically bred to develop breast cancer (285).

C. DHEA and other cancers

A series of studies performed in experimental animals have shown the anticarcinogenic activity of DHEA (258, 286, 287). In fact, DHEA has been found to inhibit progression of the cell cycle of pancreatic, breast, and colon cancer cells (274,

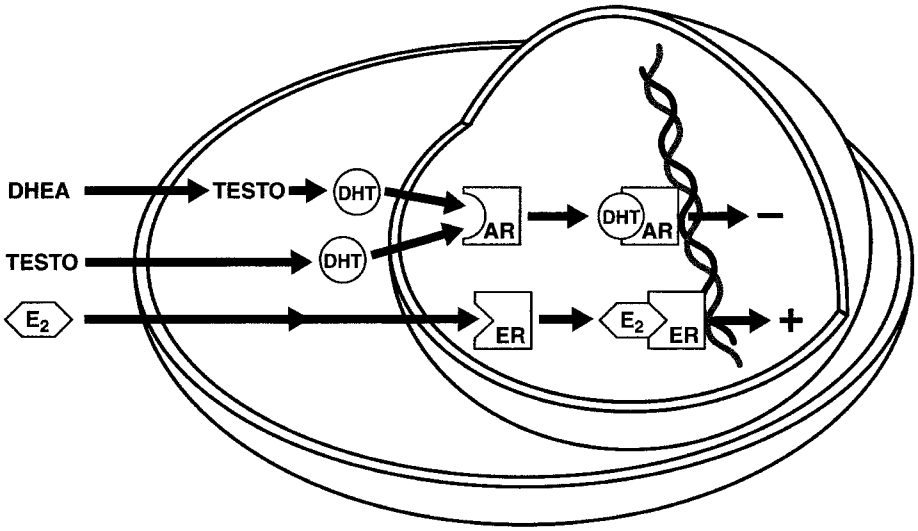

FIG. 25. Antagonism between the inhibitory effects of androgens and DHEA and the stimulatory effects of estrogens on breast cancer proliferation.

288, 289). Moreover, a series of epidemiological studies suggest an inhibitory effect of DHEA on various types of cancers. These epidemiological data pertain to breast cancer (281), prostate cancer (290), and ovarian cancer (291).

V. Rationale for the Use of DHEA as a Source of Androgens in Postmenopausal Women

A. Tissue-specific androgenic and/or estrogenic activity of DHEA

The use of DHEA is based on the recent progress achieved in our understanding of sex steroid physiology in men and women (23, 25, 27, 33, 84, 86, 90, 92, 261, 280, 292, 294) and the recognition that women, at menopause, are not only deprived from estrogens because of a rapid loss of ovarian activity but also have been deprived from androgens for a longer period because of a progressive decrease of serum DHEA levels starting quite a few years before menopause. In fact, as mentioned earlier, normal women produce androgens in amounts equivalent to two thirds of the total amount of androgens synthesized in men (Ref. 26; Table 1). Consequently, the pool of androgens in women decreases progressively from the age of 30 yr in parallel with the decrease in the serum concentration of DHEA and DHEA-S (23). It thus appears logical to include an androgenic component to HRT at peri- and postmenopause, thus maintaining a physiological balance between estrogens and androgens in each cell and tissue, a goal that can only be achieved by the local formation of androgens and estrogens in peripheral tissues from the steroid precursor DHEA.

An additional reason to use DHEA, the physiological precursor of androgenic steroids, is the recent finding that estrogen therapy, by increasing the concentration of SHBG, which reduces free testosterone, may accelerate lean mass loss among postmenopausal women receiving ERT (295).

We feel that the increased understanding of androgen and estrogen formation and action in peripheral target tissues, called intracrinology (23, 25, 27, 33, 35, 84, 86, 90, 92, 261, 280, 292, 294), as well as our recent observations indicating the predominant role of androgens in the prevention of bone loss after ovariectomy in the rat (296) and the observation of a similar situation in postmenopausal women (280) have paved the way for timely and potentially highly significant progress in the field of sex steroid replacement therapy and protection of women's health during aging. Such a possibility is well supported by our observations and that of others of a series of beneficial effects of DHEA in postmenopausal women (73, 74, 280, 297–300).

B. Benefits of DHEA in postmenopausal women

A series of clinical studies have consistently shown beneficial effects of DHEA on physical and psychological well-being as well as on bone mineral density (73, 74, 298, 299, 301–306). DHEA replacement in Addison's disease is associated with an improvement in psychological well-being, mood, and fatigue (70). Most importantly, all these benefits, including improved libido, have been obtained without significant side effect (73, 74).

The 70–95% 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 (307, 308) and obesity (309–311). In fact, DHEA has been found to improve glucose tolerance (312). Moreover, DHEA has been shown to have immunomodulatory effects *in vitro* (313) and *in vivo* in fungal and viral diseases (314), including HIV (315), and a stimulatory effect of DHEA on the immune system has been described in postmenopausal women (316).

As mentioned above, osteoporosis is a major problem among aging women, causing morbidity and mortality mainly through increased fracture rates (317). The use of ERT requires the addition of progestins to counteract the endometrial proliferation induced by estrogens, whereas both estrogens and progestins could increase the risk of breast cancer (5, 318). To avoid the limitations of ERT or HRT, we have studied the effect of 12 months of DHEA administration to 60- to 70-yr-old women on bone mineral density, parameters of bone formation and turnover, serum lipids, glucose and insulin, adipose tissue mass, muscular mass, energy, and well-being, as well as on vaginal and endometrial histology (280, 297). DHEA was administered percutaneously to avoid first passage of the steroid precursor through the liver.

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-yr-old women (n = 15). Anthropometric measurements showed no change in body weight but a 9.8% decrease in sc skin fold thickness at 12 months ($P < 0.05$; Ref. 297). Bone mass density was increased by 2.3% at the hip, 3.75% at the hip Ward's triangle, and 2.2% at the lumbar spine level (all $P < 0.05$; Ref. 280). These changes in bone mineral density were accompanied by significant decreases at 12 months of 38% and 22% in urinary hydroxyproline and in plasma bone alkaline phosphatase, respectively (all $P < 0.05$). An increase of 135% over control ($P < 0.05$) in plasma osteocalcin was concomitantly observed, thus suggesting increased bone formation in agreement with our preclinical data (296). Such data are in agreement with the finding that the remaining adrenal androgens play an essential role in the maintenance of bone mass in postmenopausal women with Addison's disease (319).

Testosterone administration to elderly men increases the fractional synthetic rate of muscle protein as well as muscle strength (320). The decline of testosterone and DHEA (23, 26, 321, 322) with age could be at least partially responsible for sarcopenia in older men and women. In fact, an age-related loss of muscle mass has been observed in women, this loss being particularly important at menopause (323, 324). Loss of muscle mass, especially in the lower extremities, could well increase the risk for fall-related injuries, fractures, and significant loss of independence and quality of life (325, 326).

Measurements of midthigh fat and muscle areas by computed tomography have shown a 3.8% decrease ($P < 0.05$) of femoral fat and a 3.5% increase ($P < 0.05$) in femoral muscular area at 12 months of treatment with DHEA (297). There was no significant change in abdominal fat measurements. These changes in body fat and muscular surface areas were

associated with a 12% decrease ($P < 0.05$) of fasting plasma glucose and a 17% decrease ($P < 0.05$) in fasting plasma insulin levels. Treatment with DHEA had no undesirable effect on the lipid or lipoprotein profile. In fact, there was an overall trend for a 3–10% decrease in total cholesterol. Plasma triglycerides were not affected.

The index of sebum secretion was 79% increased after 12 months of DHEA therapy, with a return to pretreatment values 3 months after cessation of treatment. DHEA administration stimulated vaginal epithelium maturation in 8 of 10 women who had a maturation value of zero at the onset of therapy, whereas a stimulatory effect was also seen in the three women who had an intermediate vaginal maturation before therapy. Most importantly, the estrogenic stimulatory effect observed in the vagina was not found in the endometrium, which remained completely atrophic in all women after 12 months of DHEA treatment (280).

As mentioned above, at the daily 50-mg dose orally, DHEA administered to women with adrenal insufficiency led to significant improvements in well-being, mood, and sexuality in subjects of both sexes (69, 70). Similarly, DHEA treatment in glucocorticoid-treated patients with systemic lupus erythematosus (327, 328) led to significant improvement in overall performance and activity. On the other hand, scores of activity of daily living were improved by DHEA in patients with myotonic dystrophy (329), whereas no change was observed in healthy elderly men (330). A significant improvement in mood and well-being was observed in patients with major depression (331) and midlife asthenia (332), whereas no effect was detected in perimenopausal women (333).

The data obtained after administration of DHEA clearly indicate the beneficial effects of DHEA therapy in postmenopausal women through its transformation into androgens and/or estrogens in specific intracrine target tissues without significant side effects. The absence of stimulation of the endometrium by DHEA eliminates the need for progestin replacement therapy, thus avoiding the fear of progestin-induced breast cancer added to the well known stimulatory effect of estrogens. The observed stimulatory effect of DHEA on bone mineral density and the increase in serum osteocalcin, a marker of bone formation, are of particular interest for the prevention and treatment of osteoporosis and indicate a unique activity of DHEA on bone physiology, namely a stimulation of bone formation, whereas ERT and HRT can only reduce the rate of bone loss.

The first studies with DHEA used supraphysiological doses of the compound going up to 800–1600 mg/d (298, 309, 334). The oral daily dose of 50 mg, however, has been found as the one providing physiological concentrations of androgens and estrogens (73, 74, 300, 335). We have also determined that the serum levels of DHEA using a 10% cream (280) were comparable to the ones obtained after daily oral administration of 100 mg of DHEA (our unpublished data).

The known specificity of the effect of DHEA in women is summarized in Table 3. Although bone formation, inhibition of mammary gland proliferation, stimulation of sebaceous glands, muscle mass increase, and improved libido are attributed to the formation of androgens in the corresponding target tissues, the decreased insulin resistance and vaginal maturation are best explained by the local formation of estrogens. Most importantly, at physiological replacement doses, DHEA does not stimulate the endometrium, thus removing the need to use a progestin to counteract the stimulation of the endometrium by estrogen. In summary, at physiological replacement doses, DHEA has been found in clinical studies to induce a series of beneficial effects closely associated with the protection of women's health, whereas no negative effects have been observed.

TABLE 3. Tissue-specific androgenic and estrogenic effects of DHEA

A. Androgenic	
Bone formation[a]	
Sebaceous gland stimulation[a]	
Mammary gland inhibition[a]	
Muscle mass increase[a]	
Improved libido[a,b,c]	
B. Estrogenic	
Vaginal mucosa maturation[a]	
Insulin resistance decreased[a,b]	
C. No effect	
Endometrium[a]	

[a] Demonstrated in postmenopausal women.
[b] Possibly also androgenic.
[c] Possibly also estrogenic.

Acknowledgments

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