Extragonadal synthesis of sex steroids: intracrinology

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INTRODUCTION

Humans, along with the other primates, are unique among animal species in having adrenals that secrete large amounts of the inactive precursor steroids dehydroepiandrosterone (DHEA) and especially DHEA sulfate (DHEA-S), which are converted into potent androgens and/or estrogens in peripheral tissues [1-6] (fig. 1). In fact, plasma DHEA-S levels in adult men and women are 100-500 times higher than those of testosterone and 1,000-10,000 times higher than those of estradiol, thus providing a large reservoir of substrate for conversion into androgens and/or estrogens in the peripheral intracrine tissues which possess the enzymatic machinery necessary to transform DHEA into active sex steroids.

Adrenal secretion of DHEA and DHEA-S increases during adrenarche in children at the age of 6 to 8 years, and maximal values of circulating DHEA-S are reached between the ages of 20 and 30 years. Thereafter, serum DHEA and DHEA-S levels decrease markedly (fig. 2) [3, 7-10]. In fact, as mentioned earlier, at 70 years of age, serum DHEA-S levels are decreased by 95% by the age of 85 to 90 years [7].

The marked reduction in the formation of DHEA-S by the adrenals during aging [7-12] results in a dramatic fall in the formation of an-
drogens and estrogens in peripheral target tissues, a situation that has been proposed to be associated with age-related diseases such as insulin resistance [13, 14] and obesity [15-17]. On the other hand, much attention has been given to the benefits of DHEA administered to postmenopausal women, especially on the bone, skin, vagina and well being after oral [18, 19] and percutaneous [20, 21] administration.

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 [1, 22-24]. In fact, while the ovaries and testes are the exclusive sources of androgens and estrogens in lower mammals, the situation is very different in man and higher primates, where active sex steroids are in large part or wholly synthesized locally in peripheral tissues, thus providing target tissues with controls which adjust the formation and metabolism of sex steroids to local requirements.

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 metabolism of sex steroids in peripheral tissues is known as intracrine endocrinology.
formation and action has been called intracrinology [1, 24] (fig. 3, 4, 5). 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), where the secretion of sex steroids takes place exclusively in the gonads [22, 25]. A major problem which can explain the delayed progress in the field of formation of sex steroids in peripheral target 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 [24] to describe the synthesis of active hormones which exert their action in the same cells where synthesis takes place without release into the pericellular compartment [1].

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 [26]. Most convincingly, since the postmenopausal ovaries do not secrete estrogens, the recent observation that administration of the antiestrogen Raloxifene for only 3 years in postmenopausal women led to a 76% decrease in the incidence of breast cancer [27] is a clear demonstration of the major role of extraovarian estrogens in the development and growth of breast cancer.

CHARACTERISTICS OF THE HUMAN STEROIDOGENIC ENZYMES IN INTRACRINE TISSUES

As mentioned above, transformation of the adrenal precursor steroids DHEA and DHEA-S into androgens and/or estrogens in peripheral target tissues depends upon the level of expression of the various steroidogenic and metabolizing enzymes in each of these tissues. Knowledge in this area has recently made rapid progress with the elucidation of the structure of most of the tissue-specific genes that encode the steroidogenic enzymes responsible for the transformation of DHEA and DHEA-S into androgens and/or estrogens in peripheral intracrine tissues [5, 28-31] (fig. 4, 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 precursors steroids [22, 32, 33]. On the other hand, 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 [1].

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 [3-6, 29, 34, 35].
SOURCES, IMPORTANCE AND ROLE OF ANDROGENS IN WOMEN

The most widely recognized fact concerning menopause is that there is a progressive decrease and finally an arrest of estrogen secretion by the ovaries. The cessation of ovarian estrogen secretion is illustrated by the marked decline in circulating 17β-estradiol (E2) levels. This easily measurable change in circulating E2 levels coupled with the demonstrated beneficial effects of estrogens on menopausal symptoms and bone resorption [36] has concentrated most of the efforts of hormone replacement therapy on various forms of estrogens as well as to combinations of estrogen and progestin in order 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 that recent data suggest that progestins have a negative impact on breast cancer [37-39], with reports indicating an increased risk of this cancer [40-43].

Despite the well-known beneficial effects of estrogen therapy on menopausal symptoms [44-46] and their role in reducing bone loss and potential coronary heart disease [47-52], compliance is low. Women decide not to take estrogens and stop treatment early because of the fear of breast and uterine cancer [46] and of symptoms associated with their therapy, namely uterine bleeding, breast tenderness, and fluid retention.

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 [12]. The data obtained show a dramatic decline in the circulating levels of DHEA, DHEA-S, androstenediol (5-diol), and 5-diol fatty acid esters, between the ages of 20 and 80 years. As mentioned earlier, in the 50- to 60-year-old group, serum DHEA has already decreased by 70% from its 20-30-year-old peak values (fig. 2).

The serum concentrations of the conjugated metabolites of dihydrotestosterone (DHT), namely androsterone glucuronide (ADT-G), androstane-3α, 17β-diol glucuronide (3α-diol-G), and androstane-3β, 17β-diol glucuronide (3β-diol-G), are the most reliable parameters of the total androgen pool in both men and women while serum testosterone and DHT can be used as markers of direct or interstitial ovarian secretion. In fact, while the vast majority of testosterone and close to 100% of DHT are synthesized in the peripheral tissues in women, only a small proportion estimated at 10 to 15% diffuses out of the intracellular compartment and can be measured as active androgen in the circulation. This is due to the fact that DHT is metabolized locally into the metabolites ADT, 3α-diol and 3β-diol which are rapidly glucuronidated into ADT-G, 3α-diol-G and 3β-diol-G which are much more water soluble and thus diffuse into the general circulation where they can be measured en route for their elimination mainly by the kidneys (fig. 6, 7). The serum concentration...
of the above-indicated conjugated androgen metabolites decreases by 47.5% to 72.7% between the 20-30 and 70-80 age groups in women, thus suggesting a parallel decrease in the total androgen pool with age [12].

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: 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 upon the serum concentration of androgen metabolites can be influenced by possible differences in the metabolic clearance rates of these metabolites in men and women.

**BENEFICIAL EFFECTS OF DHEA IN POSTMENOPAUSAL WOMEN**

We feel that the increased understanding of androgen and estrogen formation and action in peripheral target tissues called intracrinology [1, 3, 4, 12, 21, 29, 31, 34, 53-55] as well as our recent observations indicating the predominant role of androgens over that of estrogens in the prevention of bone loss after ovariectomy in the rat [56] and the observation of a similar situation in post-menopausal women [21] have paved the way for a timely and potentially highly significant progress in the field of hormone replacement therapy and aging. Such a possibility is well supported by our observations and that of others of a series of beneficial effects of DHEA observed in postmenopausal women [19-21].

The use of DHEA at menopause is thus based upon the recent progress achieved in our understanding of sex steroid physiology in women [1, 3, 12, 21, 29, 31, 34, 53-55] and the recognition that women, at menopause, are not only deprived from estrogen due to the arrest of estrogen secretion by the ovaries, but have already been submitted for a few years to a decreasing exposure to androgens. In fact, as mentioned above, normal women produce an amount of androgens equivalent to two thirds of the androgens secreted in men [55]. The pool of androgens in women decreases progressively from the age of 30 years in parallel with the decrease in the serum concentration of DHEA and DHEA-S [12]. Consequently, it appears logical to use both androgenic and estrogenic replacement therapy at peri- and post-menopause, thus maintaining a physiological balance between these two classes of sex steroids in each cell and tissue,
a goal which can only be met by the local formation of androgens and estrogens in peripheral tissues from a steroid precursor such as DHEA.

The 70 to 95% reduction in the formation of DHEA and DHEA-S by the adrenals during aging results in a dramatic reduction in the formation of androgens and estrogens in peripheral target tissues, which could well be involved in the pathogenesis of age-related diseases such as insulin resistance [13, 14] and obesity [15-17]. Low circulating levels of DHEA-S and DHEA have, in fact, been found in patients with breast cancer [57] and DHEA has been found to exert antioncogenic activity in a series of animal models [58-60]. DHEA has also been shown to have immuno modulatory effects in vitro [61] and in vivo in fungal and viral diseases [62], including HIV [63]. On the other hand, a stimulatory effect of DHEA on the immune system has been described in postmenopausal women [64].

As mentioned above, osteoporosis is a major problem among aging women, causing morbidity and mortality, mainly through increased fracture rates [65]. The use of estrogen replacement therapy (ERT), either with or without progestins, is the standard of care for postmenopausal women. An increased risk of breast cancer [40], cardiovascular disease [41], and deep vein thrombosis [42] has been observed. ERT also increases the risk of endometrial cancer [43], and studies have shown a significant decrease in bone mass density at the hip and lumbar spine [44].

We have thus evaluated the effect of chronic replacement therapy with a 10% DHEA cream applied once daily for 12 months in 60- to 70-year-old women. Anthropometric measurements showed no change in body weight but a 9.8% decrease in subcutaneous skin fold thickness at 12 months (p<0.05) [20]. Bone mass density was increased by 2.3% at the hip, 3.75% at the humeral shaft, and 2.2% at the lumbar spine level (all p<0.05) [21]. 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.

Measurements of mid-thigh 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 muscle mass, indicating a decrease in body fat and an increase in lean body mass.
femoral muscular area at 12 months [20]. 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% to 10% decrease in total cholesterol and its lipoprotein fractions. 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 out of 10 women who had a maturation value of zero at the onset of therapy while a stimulation was also seen in the three women who had an intermediate vaginal maturation before therapy. Most importantly, the estrogentic 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 [21].

The present data 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. 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 on bone formation, while ERT and HRT can only reduce the rate of bone loss.

**COMBINATION OF DHEA AND AN ANTIESTROGEN**

Androgen therapy, as observed with nandrolone decanoate, has been found to increase vertebral bone mineral density as well as cortical bone mineral content in postmenopausal women [67]. Androgenic side-effects, however, were recorded in 50% of patients. Such data are of interest since while almost all present therapies are limited to a reduction of bone loss, an increase in bone mass was found with the use of the anabolic steroid nandrolone. A similar stimulation of bone formation by androgens has been suggested in a hypogonadal male [68]. A stimulation of bone formation in postmenopausal women treated with DHEA for 12 months is reported in Labrie et al. [21].

Most importantly, it has been observed that androgens exert a direct antiproliferative activity on the growth of ZR-75-1 human breast cancer cells in vitro and that such an inhibitory effect of androgens is additive to that of an antiestrogen [69, 70]. Similar inhibitory effects have been observed in vivo on ZR-75-1 xenografts in nude mice [71]. Androgens have also been shown to inhibit the growth of DMBA-induced mammary carcinoma in the rat, this inhibition being reversed by the simultaneous administration of the pure antiandrogen Flutamide [72]. Taken together, these data indicate the involvement of the androgen receptor in the inhibitory action of DHEA on breast cancer.

Since antiestrogens and sex steroid precursors exert inhibitory effects on breast cancer via different mechanisms, it was possible that the combination of a SERM (EM-800) and a sex steroid precursor (DHEA) could exert more potent inhibitory effects than each compound used alone on the development of DMBA-induced mammary carcinoma. As well illustrated in figure 8, no DMBA-induced tumor was found at the end of the experiment in animals that had received both DHEA and EM-800.

We have shown that DHEA exerts beneficial effects on bone in both the female rat [73], and postmenopausal women [21]. In fact, in intact female rats, treatment with DHEA increases bone mineral density (BMD) of total skeleton, lumbar spine and femur [73]. Moreover,
we have found that the combination of a sex steroid precursor (DHEA) and a SERM (EM-800) not only maintained the stimulatory effect of DHEA on bone formation, but potentiated the inhibitory effect of the SERM (EM-800) alone on bone turnover and resorption as demonstrated by the further decreases in urinary hydroxyproline and calcium excretion when both compounds were combined [73].

Estrogens are known to lower serum cholesterol but to increase or to have no effect on serum triglycerides levels [74-80]. Figure 9 shows that EM-800 possesses both hypocholesterolemic and hypotriglyceridemic effects in the rat, thus showing its unique action on the serum lipid profile which is apparently different from other SERMs, such as tamoxifen [79-82], droloxifene [80], and raloxifene [78] which do not exert an inhibitory effect on serum triglycerides. The combination of DHEA and EM-800 preserved the hypocholesterolemic and hypotriglyceridemic effects of EM-800, thus suggesting that such a combination could exert beneficial effects on serum lipids.

In brief, the above-described data clearly demonstrate the beneficial effects of the combination of a SERM (EM-800) and a sex steroid precursor (DHEA) on the development of mammary carcinoma induced by DMBA as well as the protective effects of such a combination on bone mass and serum lipids. Such data clearly suggest the additional beneficial effects of such a combination for the treatment and prevention of osteoporosis while improving the lipid profile and preventing breast and endometrial cancer.

It is particularly important to indicate that the combination of DHEA and EM-800 exerted unexpected beneficial effects on important biochemical parameters of bone metabolism. In fact, DHEA alone did not affect the urinary hydroxyproline/creatinine ratio, a marker of bone resorption. Moreover, no effect of DHEA alone could be detected on daily urinary calcium or phosphorus excretion [73]. EM-800, on the other hand, decreased the urinary hydroxyproline/creatinine ratio by 48% while, similarly to DHEA, no effect of EM-800 was seen on urinary calcium or phosphorus excretion. EM-800, moreover, had no effect on serum alkaline phosphatase activity, a marker of bone formation, while DHEA increased the value of the parameter by about 75% [73].

One of the unexpected effects of the combination of DHEA and EM-800 relates to the urinary hydroxyproline/creatinine ratio, a marker of bone resorption, which was reduced by 69% when both DHEA and EM-800 were combined, this value being statistically different (p<0.01) from the 48% inhibition achieved by EM-800 alone while DHEA alone did not show any effect. Thus, the addition of DHEA to EM-800 increases by 50% the inhibitory effect of EM-800 on bone reabsorption. Most importantly, another unexpected effect of the addition of DHEA to EM-800 was the approximately 84% decrease in urinary calcium (from 23.17±1.55 to 3.71±0.75 µmol/24h/100g (p<0.01) and the 55% decrease in urinary phosphorus (from 132.72±6.08 to 59.06±4.76 µmol/24h/100g (p<0.01) respectively [73].

The present results obtained in the rat clearly demonstrate that DHEA can provide the beneficial effects which are lacking with the use of a selective estrogen receptor modulator (SERM) alone such as EM-800, Raloxifene, etc. While a SERM has effects limited to inhibition of bone resorption, the addition of DHEA is believed to stimulate bone formation (an effect not

![Figure 9: 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 serum triglyceride (A) and cholesterol (B) levels in the rat. Data are expressed as the means ± SEM. **: P<0.01 experimental versus respective control.](image-url)
found with a SERM, an estrogen or a bisphophonate) and further reduce bone resorption above the effect achieved with EM-652 alone.

Importantly, the combination of EM-800 and DHEA in ovariectomized rats treated for 12 months had beneficial effects on bone morphometry. Trabecular bone volume is particularly important for bone strength and to prevent bone fractures. Thus, in the above-mentioned study, trabecular bone volume of the tibia increased from $4.1\pm0.7\%$ in ovariectomized rats to $11.9\pm0.6\%$ ($p<0.01$) with DHEA alone while the addition of EM-800 to DHEA further increased trabecular bone volume to $14.7\pm1.4\%$, a value similar to that found in intact controls.

The proposed novel approach not only eliminates the negative effects of estrogens on the breast, uterus and ovary but it also eliminates the need to use a progestin to protect against endometrial proliferation, thus avoiding the recently demonstrated stimulatory effect of progestins on breast cancer [37-43, 83-85]. EM-652, due to its high affinity for the estrogen receptor, should also avoid the risk of ovarian cancer recently reported to be associated with long-term use of estrogens [86, 88]. In fact, two recent studies have observed that the use of estrogen replacement therapy for 10 years or more increased the risk of ovarian cancer [86, 88]. Moreover, a recent Swedish study has reported that estrogen used alone and estrogen plus progestin used sequentially may be associated with an increased risk of ovarian cancer while no such risk was associated with the continuous use of the combination pill [87].

Due to its highly potent and pure antiestrogenic activity, EM-652 should not only eliminate the risk of breast, uterine and ovarian cancer associated with estrogen use [43, 86-88], but it should also reduce the spontaneous incidence of these cancers which are diagnosed in $13.3\%$ (breast cancer), $2.7\%$ (endometrial cancer) and $1.7\%$ (ovarian cancer) of women during their lifetime.

The approach proposed (fig. 10) is based upon the recent progress achieved in our understanding of sex steroid physiology in women and the recognition that women, at menopause, are not only deprived from estrogen due to the arrest of estrogen secretion by the ovaries, but have already been submitted for a few years to a decreasing exposure to androgens. In fact, as mentioned above, normal women produce an amount of androgens equivalent to two thirds of the androgens secreted in men [55]. The pool of androgens in women decreases progressively from the age of 30 years in parallel with the decrease in the serum concentration of DHEA and DHEA-S [12]. Consequently, it appears logical to use both androgenic and estrogenic replacement therapy at peri- and post-menopause, thus maintaining a physiological balance between these two classes of sex steroids in each cell and tissue, a goal which can only be met by the local formation of androgens and estrogens in peripheral tissues from a steroid precursor such as DHEA.

Previous data 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. 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 on bone formation.

In fact, our data obtained in the rat clearly demonstrate that DHEA can provide the beneficial effects which are lacking with the use of a SERM alone. While a SERM has effects limited to inhibition of bone resorp-
tion, the addition of DHEA is believed to stimulate bone formation (an effect not found with a SERM, an estrogen or a bisphosphonate) and further reduce bone resorption above the effect achieved with EM-652 alone. In addition to increased bone formation, DHEA has also been shown in postmenopausal women to stimulate vaginal maturation, and decrease skin dryness.

It thus appears logical to take advantage of the estrogen-like activity in the bones and lipid metabolism provided by EM-652 and the tissue-specific androgenic activity of DHEA and examine the possibility that DHEA and EM-652 could further improve the spectrum of benefits for menopausal women. Additive or synergistic effects of the two compounds have been observed in preclinical studies while the addition of E₂ should control hot flushes and add positive effects on brain functions, namely cognition and memory [89] and decrease of cognitive functions and memory. It therefore appears logical to take advantage of the estrogen-like activity of DHEA and the tissue-specific androgenic activity of EM-652 to achieve the spectrum of benefits for women at menopause, namely control of hot flushes, potential improvement of cognitive functions and memory [89] and decrease of disease. J. Mol. Endocrinol. 2000; 25(1) : p. 167-171.

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