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Endogenous Oestrogens and Androgens in Normal and Malignant Endometrial and Mammary Tissues

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Abstract—*Because of a well-established mechanism of action, tissue concentrations of steroid hormones are thought to be more closely related than blood levels to the biological effects exerted by these hormones.*

The results of studies on oestrogen and androgen concentrations in malignant and normal breast tissues are presented. Normal fatty and epithelial breast tissues and malignant tumour samples which had been obtained from pre- and postmenopausal women of two countries (Poland and The Netherlands) differing in the incidence of this malignancy were studied. In both countries highly comparable oestradiol *concentrations in the breast were found. The median hormone levels in tumour tissue of* 0.65 pmol/g *tissue did not change with age. They were significantly higher than in normal epithelial* (0.48 *and* 0.25 pmol/g *in pre- and postmenopausal women) and fatty tissues* (0.54 *and* 0.19 pmol/g *respectively). Particularly in postmenopausal women, hormone levels in tumour tissue were much higher than plasma concentrations, which are comparable in both populations.* Oestrone *levels decreased with age in normal and malignant breast tissues. In both countries median levels in normal and fatty tissues of premenopausal women were similar* (1.10 pmol/g *tissue) but higher than those in postmenopausal patients* (0.45 pmol/g *tissue. Significantly lower levels were found in the malignant tissue samples of Polish premenopausal women* (0.70 pmol/g) *than in Dutch women* (1.05 pmol/g); *similarly, after menopause the tissue concentrations were higher in Dutch* (0.55 pmol/g) *than in Polish* (0.31 pmol/g) *patients. Thus lower oestrone tissue levels were observed in tumours from the country with the lower incidence for breast cancer.*

In a comparable study of uterine tissues, obtained from pre- and postmenopausal women, higher oestradiol concentrations than in the breast were found, whereas estrone levels were very similar. The levels in the uterus did not correlate with those in the plasma; no relation with histology was observed.

The results of androgen measurements in breast tissues were in agreement with the concept that, particularly, androstenedione and testosterone could play a role as substrates for local aromatization. Lower concentrations were observed in the tumours than in the normal and fatty tissues.

More extensive investigations will be needed to clarify the role of local formation (aromatization, hydrolysis by sulphatase) of oestrogens in tissues and of the interconversion of less active (oestrone) to more active (oestradiol) oestrogens.

INTRODUCTION

EPIDEMIOLOGICAL STUDIES on the incidence of breast and endometrial cancer have demonstrated that large differences exist between countries. In general the incidence of breast cancer is highest in countries with a Western life-style, particularly in postmenopausal women. The development of clinically detectable tumours is a very long and complex process and a number of factors can have an influence on either the induction or the growth promotion of the tumours. Especially in postmenopausal women follow-up studies have provided evidence that a Western life-style is related to the growth rate of cancers [1]. Several studies indicate that weight, overweight and/or nutritional factors are related to growth stimulation of malignant cells in the breast [1, 2].

The incidence of endometrial cancer also shows large variations between different populations [3], and correlates with environmental factors including diet. The strongest association was found with total fat consumption, which is highest in Western countries.

Biologically, an increase in fat consumption is thought to result in higher body weight and thus, via

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the mechanism of peripheral conversion of androgen precursors to oestrogens [4], in enhanced oestrogen production. In addition, evidence has been presented [5] that the biological availability of oestrogens will be stimulated by the competition of fatty acids and oestrogens for binding sites on the transport protein in blood.

However, although there is much evidence for a relation between risk factors and changes in the oestrogenic environment of tumours [6], the results of measurements of oestrogen excretion in the urine [7], plasma concentrations [8–10] and production rates [4, 11] have not revealed consistent differences between women with and without tumours or women belonging to high risk versus low risk populations. Relevant differences have been found in the metabolism of oestradiol to metabolites with or without intrinsic oestrogenic activity [12] related to a number of risk factors for breast cancer. In addition, the role of binding proteins [5, 13, 14], especially sex hormone binding globulin (SHBG), in the biological activity of plasma oestrogens has not yet been settled conclusively.

In order to exert their biological activity oestrogens must enter cells, bind to specific intracellular binding proteins, called receptors, and be retained in the nuclei of the responsive cells [15]. Therefore, it seems more appropriate to compare concentrations of oestrogens in the cells of the target tissues than in peripheral blood. Additional arguments for measurements in tissue are based on studies showing no relations between plasma and tissue oestrogen concentrations [16] and on the capability of breast cells to influence their hormonal milieu by synthesizing active hormones or by metabolizing steroids into less active forms [17].

Therefore, in the following contribution results will be presented on estimations of oestrogens and their potential precursors in normal and abnormal breast and uterine tissues. If local factors are related to the incidence or the course of breast cancer, attention must be given to the large variations in the incidence of this tumour. Thus results of tissue concentration measurements in normal and malignant breast tissues from two countries with a difference in breast cancer incidence are in order.

MATERIAL AND METHODS

Patients

Malignant breast tissue was obtained from 52 patients in Utrecht (The Netherlands) and from 39 in Wroclaw (Poland), while normal epithelial breast tissues were obtained from 28 Dutch and 16 Polish women. In all Polish patients a specimen of fatty tissue was also procured by the pathologist. All samples were chilled immediately after operation, tumours were trimmed free of fat and connective tissue. All samples were stored at −80°C until analysis. Tissue collection was done under identical conditions in both countries.

Normal uterine samples were obtained from 20 normal premenopausal women at well-defined times in their menstrual cycle and from 53 postmenopausal women, as described previously [18, 19]. Abnormal uterine tissues were studied as obtained from 10 postmenopausal patients with hyperplastic endometrium and from 13 women with endometrial cancer [20].

Hormone assays

All determinations were done using identical methods, carried out in the same laboratory, as described previously [21]. Essentially, they consist of homogenization of the tissues at −196°C, extraction of all steroids with acetone/ethanol 1:1, defatting in 70% methanol at −20°C, evaporation of the methanol, dilution with buffer and extraction of the non-conjugated steroids. The steroids remaining in the buffer are hydrolysed enzymatically and the free steroids are extracted; this second extract contains the conjugated steroids. Oestrogens and androgens are separated using chromatography on Sephadex LH-20 columns with toluene/methanol 92/8 for oestrogens and Celite/ethylene glycol 2/1 columns with a discontinuous gradient of increasing amounts of ethyl acetate in iso-octane for the androgens. Quantitation was performed by radio-immunoassay using highly specific antisera. Results have been corrected for losses during the purification procedures and expressed in pmol per g tissue. The amounts of breast tissue used for quantitation varies between 0.5 and 1.0 g, the amounts of endometrial tissue of postmenopausal women were smaller, varying between 0.01 and 0.28 g of normal tissues and between 0.03 and 0.40 g for abnormal endometrium.

Because the individual data were usually not normally distributed, median values will be given. Differences between groups of data were tested by using the Wilcoxon rank sum test. Correlations between parameters were calculated using Spearman's rank test.

RESULTS

Oestrogen concentrations in breast tissues

The median concentrations of the oestrogens measured in normal fatty, normal epithelial and malignant breast tissues are summarized in Table 1 for the two populations studied. In all groups large variations were observed within each of the groups studied. These variations will not be discussed here, since details have been given in the original descriptions of the various studies [16, 22].

Table 1. Median values of concentrations of oestrone, oestradiol and their sulphates in normal fatty, normal epithelial and abnormal breast tissues obtained from pre- and postmenopausal patients with breast tumours living in two countries with a high (The Netherlands) and a lower (Poland) breast cancer incidence. Values have been expressed in pmol/g *tissue*

	Poland						The Netherlands			
	Premenopausal			Postmenopausal			Premenopausal		Postmenopausal	
	Fat	Normal	Tumour	Fat	Normal	Tumour	Normal	Tumour	Normal	Tumour
n	17	8	17	21	8	21	22	19	6	33
E_1	1.13	1.14	0.65	0.50	0.38	0.29	1.03	1.04	0.38	0.61
E_2	0.38	0.48	0.57	0.18	0.19	0.60	0.44	0.67	0.32	0.77
E_1S	0.08	0.44	0.35	0.05	0.14	0.17	—	—	—	—
E_2S	0.04	0.07	0.08	0.05	0.04	0.06	—	—	—	—

Abbreviations: *n*: number of tissues in group; E_1: oestrone; E_2: oestradiol; E_1S: oestrone sulphate and E_2S: oestradiol disulphate. — no determinations.

As expected, median *oestrone* levels decrease with age, postmenopausal women having lower levels than premenopausal ones in the individual tissues investigated. No significant differences between the tissues were found for oestrone concentrations within the two Dutch age groups and within the postmenopausal Polish group; in premenopausal Polish women oestrone levels in tumours were significantly lower than in the two non-malignant tissues. Comparison of the results in the two populations showed that in premenopausal as well as in postmenopausal women oestrone concentrations in the Polish groups were significantly lower ($P < 0.02$ in both age groups) than in the Dutch groups.

Oestradiol tissue concentrations showed a different behaviour. Whereas its levels in fatty and normal epithelial tissues decreased with advancing age, its concentration in the tumours seemed to be completely independent of menopause. In the Polish and in the Dutch patients, oestradiol levels were similar and they remained stable with age. Furthermore, the oestradiol concentrations in the malignant tissues were significantly higher ($P< 0.05$ in each of the four groups) than in the normal and/or fatty tissues studied. The increased levels of oestradiol were most pronounced in postmenopausal women of both countries.

The concentrations of the two *oestrogen sulphates* were lower than those of the unconjugated steroids. The influence of menopausal status was less clear for the sulphates; only oestrone sulphate decreases significantly with age.

Androgen concentrations in breast tissue

Median concentrations of the five androgens studied are presented in Table 2. Unfortunately, not all androgens have been measured in all available tissue samples [23]. Therefore a comparison of the levels in the tissue of the two populations of women can only be made for 5-androstene-3,17-diol (Adiol) and for dehydroepiandrosterone (DHEA). The concentrations of these steroids in the comparable tissues were shown to be very similar in the two populations.

The tissue concentrations of the androgens, with the exception of DHEA (data not shown), were lower or comparable to those in the plasma, the tissue/plasma gradient for DHEA clearly being higher than 1. Thus differences exist in the tissue/plasma gradients for the different androgens. In general, the concentrations of the androgens in breast tissues decrease with age, although not all differences reach formal statistical significance. The main exception is Adiol, which showed only a small decrease with age. Comparison of steroid concentrations in tumour tissue with those in fatty or normal tissues revealed significantly lower tumour levels for androstenedione (Adion) and for DHEAS.

Oestrogens and androgens in uterine tissues

Because the uptake and retention of steroids in target tissues is a receptor mediated process, additional studies were carried out to measure the same steroids in uterine tissues. The results of these measurements are presented in Table 3.

The concentrations of oestrone in the endometrium and myometrium of premenopausal women showed only slight variations during the menstrual cycle, whereas levels in the follicular and secretory phase were comparable. After the menopause a small decrease in oestrone was found. The tumours did not show different levels of oestrone. Similarly the hyperplastic endometria studied (data not shown) had no abnormal oestrone levels [20].

Oestradiol concentrations showed much larger variations in normal tissue: endometrial as well as myometrial concentrations decreased clearly after ovulation with much smaller changes in simultaneously measured plasma concentrations [18].

Table 2. Median concentrations of testosterone (Testo), androstenedione (Adion), 5-androstene-3β,17β-diol (Adiol), dehydroepiandrosterone (DHEA) and DHEA sulphate (DHEAS) in normal fatty, normal epithelial and abnormal breast tissues obtained from patients living in countries with a high (The Netherlands) and a lower (Poland) breast cancer incidence rate. All values are given in pmol/g *tissue*

	Poland						The Netherlands			
	Premenopausal			Postmenopausal			Premenopausal		Postmenopausal	
	Fat	Normal	Tumour	Fat	Normal	Tumour	Normal	Tumour	Normal	Tumour
n	14	7	13	10	10	10	22	18	7	25
Testo	0.74	1.03	0.54	0.59	0.37	0.63	—	—	—	—
Adion	2.03	0.78	0.57	1.30	0.58	0.38	—	—	—	—
Adiol	2.50	14	3.40	4.10	3.70	3.10	5.50	3.80	2.70	2.50
DHEA	101	175	90	108	70	40	130	70	52	38
DHEAS	—	—	—	—	—	—	3450	730	1790	430

— no determinations.
n: number of determinations.

Table 3. Median concentrations of oestrogens and androgens in normal and abnormal endometrium and in myometrium of premenopausal and postmenopausal women. All values have been expressed in pmol/g *tissue*

	Premenopausal				Postmenopausal		
	Endometrium		Myometrium		Endometrium		Myometrium
	Foll	Secr	Foll	Secr	Normal	Tumour	
n	10	10	10	10	53	13	11
E_1	1.44	0.96	1.11	0.74	1.00	0.67	0.81
E_2	10.86	2.32	5.15	2.02	1.55	1.29	0.77
N	23		23		11	12	8
Testo	3.5		3.6		3.4	1.5	2.1
Adion	7.2		12.6		5.4	2.3	5.5
Adiol	23.4		13.3		15.3	12.5	10.1
DHEA	275		200		130	101	125
DHEAS	1855		1245		502	245	775

n: number of observations (different samples were used for oestrogens and androgens);
Foll: follicular phase of the cycle; Secr: secretory phase of the cycle.

After menopause tissue oestradiol was present in larger amounts per g uterine tissue than per g (ml) of plasma. Furthermore, its tissue level was higher than that of estrone although estrone levels in plasma were higher. The results show that also after menopause oestradiol remains the most important estrogen at the cellular level.

The androgens have higher uterine tissue concentrations than the corresponding plasma concentrations, except for DHEAS which is present in smaller amounts in the uterus. Calculation of the tissue/plasma gradient [24] for the individual androgens revealed that this gradient is different for each of the steroids and that the gradient is stable with age, since equal concentrations were found in premenopausal and postmenopausal women. The highest gradient was calculated for DHEA, its concentrations in uterine tissues being more than 10 times higher than in plasma.

Comparison of steroids in tissues

As mentioned already under oestrogen levels in the uterus, only relatively small variations were seen in oestrone levels, both in the breast and the uterus. In contrast, oestradiol concentrations showed much larger variations, behaving independently of the amounts found in plasma. Particularly, the changes in uterine tissues during the cycle and the relatively high levels in the uterus after menopause were striking. At a lower level, breast tumour oestradiol behaved almost autonomously, pointing to the fact that local factors must be involved in the maintenance of these concentrations.

Large differences have also been found between the concentrations of several androgens in uterine and breast tissues. In general, uterine tissues contain higher androgen levels than breast tissues, the tissue/plasma gradient for both uterine tissues being more than one whereas the gradient for the breast samples proved smaller.

DISCUSSION

The results of our studies on endogenous concentrations of androgens and oestrogens in steroid sensitive tissues used in this overview [16, 18, 19, 20, 22–24] are comparable to those of other authors [25–30] when the same steroids have been measured.

Thus the conclusion about oestrogen concentrations in malignant breast tumours has been drawn from women from various countries, that oestradiol concentrations in particular are higher than in normal glandular tissues. Furthermore, the stability of the amounts of oestradiol present in tumours from pre- and postmenopausal women is a rather consistent observation. These results prove that the local oestradiol concentration is not a reflection of the plasma levels in these women. The most likely explanation is that local factors are involved in the accumulation of oestradiol. Among these factors, local synthesis from precursors as androgens [28, 30, 31] and/or oestrogen sulphates [31] has been suggested. Differences in local oestradiol metabolism have also been suggested, although the enzymatic activities investigated [17] do not simply explain the high oestradiol concentrations. Results showing relatively low oestrogen sulphate levels in normal and abnormal breast tissues (Table 1, [30]) are not in favour of an important role for these conjugates as a precursor.

A surprising finding of our investigations in malignant breast tumours from women living in a country with a low breast cancer incidence is the highly significant lower concentration of oestrone in those tumours but not in normal breast samples. As the concentrations of androgens (as far as available) do not point to differences between the tissues of both countries, one could speculate that the local aromatase activity in the tumours is lower in Polish women than that in women living in an affluent country such as The Netherlands. No factors are known which are able to influence the aromatase activity so strongly. Inhibition by adrenal androgens [29, 30] is an unlikely explanation as the tissue levels of DHEA are very similar in both populations.

In the study on Polish breast cancer patients [32], a number of correlations were found between oestrogen levels and parameters of body weight. Significant correlations between the two oestrogens were found in fatty and epithelial tissues in pre- and in postmenopausal women, the hormone concentrations in tumours were correlated in the premenopausal women only, whereas no relation was observed in the postmenopausal ones. In view of epidemiological data, an interesting finding was the positive correlation between body mass and oestrone sulphate in the fatty tissue of postmenopausal women but not in the younger age group.

Regarding the androgen levels, remarkable differences were found between uterine and breast tissues: generally the uterus proved capable of concentrating androgens more extensively than the breast. The most abundant androgen in both tissues was DHEAS, its concentrations being 10 to more than 100 times higher than that of the other androgens (Tables 2 and 3). The reasons for the different behaviour of different tissues is not well understood because only for testosterone could a receptor mediated process play a role. The regulation of the cellular uptake of DHEAS is as yet unknown. Intracellular metabolism to other androgens is possible and thus DHEA and Adiol in tissues can result from both uptake and local formation. The importance of each of these reactions remains unclear at this moment.

The overall picture which emerges from these investigations is that the uterus as well as the human breast are both able to influence their own hormonal milieu, and therefore plasma hormone levels are not really reflecting the circumstances occurring within the cells. In combination with the growing insight that local synthesis of so-called growth factors is also important in the process of cellular proliferation in these organs, the relation between risk hormonal factors for breast and endometrial tumours is still far from being understood.

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