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TISSUE ANDROGENS AND THE ENDOCRINE AUTONOMY OF BREAST CANCER

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Summary—To evaluate whether a tumour-directed gradient in androgen levels in fatty tissue can account for the maintenance of intra-tissue oestradiol levels, androstenedione (Adione), dehydroepiandrosterone (DHEA), testosterone (Testo) and androstenediol (Adiol) were assayed in breast tumour tissues and in fatty tissue taken at different distances from the tumour. The concentration of Adione was significantly lower in tumour tissue (5.6 ± 1.5 pmol/g tissue; mean $\pm$ SEM; $n = 14$) than in the adjacent fatty tissue (20.4 ± 2.2; $P < 0.005$). Testo, by contrast, occurred in equal concentrations in tumour (0.80 ± 0.11) and in adjacent fatty tissue (0.70 ± 0.07). Adione levels tended to be lower after the menopause only in fatty tissue, not in the tumour tissue; for Testo no differences were observed between samples from pre- and postmenopausal patients. Tumour DHEA levels (57 ± 12 pmol/g tissue) were lower than those in fatty tissue (117 ± 17; $P < 0.02$). As with Adione, fatty tissue DHEA concentrations tended to be higher in pre- than in postmenopausal patients. Adiol showed a similar pattern as Testo. For none of the aromatase substrates nor their precursors a tumour-directed gradient was observed. The concentration of Adione in breast cancer tissue is much lower than the reported K_m of the aromatase system for Adione. We have concluded, therefore, that the maintenance of oestradiol concentrations in tumour tissues is not substrate-driven.

INTRODUCTION

Multiple factors, environmental, biometric as well as endocrine, play a role in the aetiology of breast cancer [1] and it has long been known that oestrogens are particularly important in the process of breast carcinogenesis. Based on the model of operationally defined stages in carcinogenesis of Hecker [2], Ekeris [3] recently reviewed that the role which hormonal steroids play in carcinogenesis is that of a promoter of growth of a previously initiated cell. Oestrogens thus would not be carcinogenic themselves, but would act to stimulate expression of oncogenes. Such action of oestrogens would be mediated by the oestrogen receptor. It has been reported that, in spite of the massive decrease in circulating oestradiol associated with menopause, oestradiol levels in tumour tissue from pre- and postmenopausal patients are not different [4, 5]. Unlike normal breast tissue and peripheral and abdominal fatty tissue, breast cancer tissue thus appears to be autonomous in the maintenance of its intra-tissue oestradiol level. Moreover, Miller and O'Neill [6, 7] have reported that aromatase activity in breast adipose tissue was higher in quadrants harbouring breast cancer tissue than in adipose tissue from unaffected quadrants. This would imply that a tumour directed gradient might exist in aromatase activity, which could explain the apparent autonomy of human breast cancer tissue with respect to the maintenance of its oestradiol level. Alternatively, tumour-directed gradients in aromatase substrate(s) might be important in this respect.

In previous papers we have reported that, based on the results of our experiments, the contribution of remote (i.e. abdominal) fat to the intra-tissue concentration of oestradiol in tumour tissue is not substantial [8] and that tumour-directed gradients in aromatase [9] and oestrogens [10] do not exist. The aim of the present paper is to report on the levels of the androgen precursors in fatty tissue taken at different distances from the tumour in an attempt to evaluate whether the maintenance of breast cancer tissue oestradiol levels is substrate-driven.

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EXPERIMENTAL

Tissues

The origin of the tissue samples used in this study has been described in a previous communication [9]. Briefly, breast tumour and fatty tissue were obtained at mastectomy from 6 pre- and 10 postmenopausal Polish breast cancer patients. The fatty tissue was obtained from the quadrant containing the tumour, either from the vicinity of the tumour (designated fat close, FC) or from a distance of 5–7 cm from the tumour (designated fat distant, FD) or from the quadrants not affected by the tumour. The specimens from the nonaffected quadrants were taken from the periphery of these quadrants, which were identified by a three letter code, i.e. FUI, FUL, FLI and FLL. The first letter indicates the type of tissue, i.e. fat, the second letter (U or L) designates the vertical position of the quadrant, i.e. upper or lower, and the third letter (I or L) gives the horizontal position of the quadrant, i.e. inner or lateral. Tissue was frozen immediately after preparation and stored at −80°C or lower until processing. At that time, tissue was minced at 0°C and the mince was divided into several aliquots for estimation of steroid receptors and endogenous steroids, aromatase, and other parameters which will not be dealt with in this communication. The aliquots were frozen again and stored at −80°C.

Intra-tissue androgens

One tissue aliquot was used for the assessment of the concentration of endogenous steroids which was carried out basically as described by van Landeghem *et al.* [11] and described in detail previously [10]. Briefly, the tissue was pulverized at −196°C; and suspended in phosphate buffer pH 7.4; the tissue suspension was extracted twice with ethanol–acetone (1:1, v/v); the extract is concentrated and delipidated with 70% methanol. The liquid phase is concentrated and acidified and steroids are extracted from it with ether. The extract is evaporated to dryness and the residue is dissolved in 2.2 ml absolute ethanol. Androgens were estimated by specific and sensitive radioimmunoassays in 1.0 ml of this solution, following celite chromatography. Columns were eluted with a discontinuous gradient of 2.5 to 20% iso-octane in toluene (v/v). Performance of the radioimmunoassays was assessed with plasma pools containing different concentrations of the androgens. The following coefficients of variation were observed in the assays employed for the tissue samples studied here: for testosterone (Testo): 3.6; 1.2 and 5.4% at 0.5; 3.0 and 7.7 nmol/l; for androstenedione (Adione): 4.5; 7.6 and 8.4% at 2.7; 8.1 and 15.7 nmol/l; for androstenediol (Adiol): 3.1 and 6.8% at 1.8 and 6.2 nmol/l and for DHEA: 11.7; 5.7 and 3.3% at 11.6; 19.1 and 36.7 nmol/l, respectively ($n = 4$–5).

Statistical procedures

The significance of differences between groups of patients was evaluated with Wilcoxon's two-sample rank sum test, whereas for differences within groups Wilcoxon's sign rank test was used. The existence of relationships between parameters was evaluated with Spearman's correlation coefficient. P-values < 0.05 were considered to reflect statistical significance.

RESULTS

Testo and Adione

The concentrations of the aromatase substrates Testo and Adione in tumour and fatty tissue are shown in Fig. 1. It was striking to see that the levels of Adione and Testo in the different fatty tissues from each patient showed a rather homogeneous pattern. For Testo, for instance, the variation (SD) in the different fatty tissue samples per patient was $10 \pm 4\%$ (mean $\pm$ SD; $n = 15$). A similar pattern was observed for Adione ($11 \pm 5\%$). The mean Adione concentration was higher in fatty tissues from pre- than from postmenopausal patients, although statistical significance was only reached in the FUL tissue. For Testo no differences between pre- and postmenopausal patients were found. Adione levels were lower in tumour tissue than in fatty tissue ($P < 0.005$), whereas no differences were found for Testo. A significant correlation between levels in tumour tissue and adjacent fatty tissue (FC) was observed both for Adione ($R_s = 0.7901$; $P < 0.01$; $n = 12$) and Testo ($R_s = 0.6996$; $P < 0.02$; $n = 12$). The concentrations of Adione and Testo in tumour tissue showed a significant correlation ($R_s = 0.8084$; $P < 0.01$; $n = 14$). In adjacent fat this relation did not reach statistical significance ($R_s = 0.4097$; NS; $n = 14$).

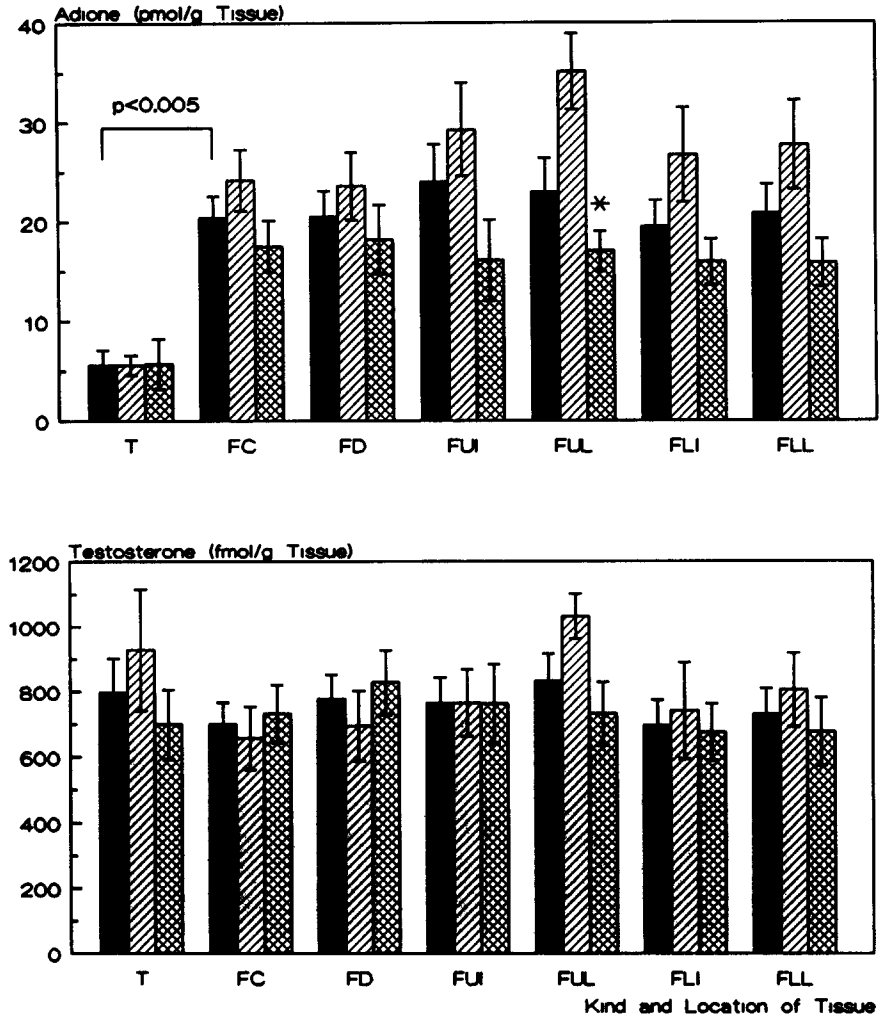

Fig. 1. Adione and Testo levels in breast cancer tissue and fatty tissue taken at different distances from the tumour. Results are given as means ± SEM for tissues from all patients (solid bars; $n = 9$–14); premenopausal patients (hatched bars; $n = 3$–6) and postmenopausal patients (cross-hatched bars; $n = 4$–8). The designation of the tissue type is given in the Experimental section. The asterisk reflects $P < 0.02$ between tissues from pre- and postmenopausal patients.

DHEA and Adiol

As shown in Fig. 2, the steroids from which Adione and Testo can be derived, DHEA and Adiol, respectively, showed a similar tissue distribution as their derivatives. DHEA was lower in tumour than in fat ($P < 0.02$) and tended to be lower in fatty tissues from postmenopausal women. Tumour and fatty tissue Adiol levels were comparable and mean Adiol levels were lower in tissues from postmenopausal patients. In tumour tissue, the concentrations of DHEA and Adiol were found to be highly correlated ($R_s = 0.9758$; $P < 0.001$). No other correlations were found in tumour tissue. In adjacent fatty tissue, significant correlations were observed between the concentrations of DHEA and Adione ($R_s = 0.6733$; $n = 14$; $P < 0.02$); Adiol and Testo ($R_s = 0.5969$; $P < 0.05$); Adiol and Adione ($R_s = 0.6220$; $P < 0.05$) and Adiol and DHEA (0.9593; $P < 0.001$). Tumour and adjacent fatty tissue concentrations of DHEA and Adiol respectively did not show significant correlations ($R_s = 0.5618$ and 5640; $0.05 < P < 0.01$), As with Adione and Testo, a remarkable consistency was observed in the DHEA and Adiol levels in the different fatty tissue specimens of individual patients, although with DHEA and Adiol the variation was somewhat larger than with the other two hormones (%SD = 14 ± 13 and 19 ± 12; mean ± SD, respectively).

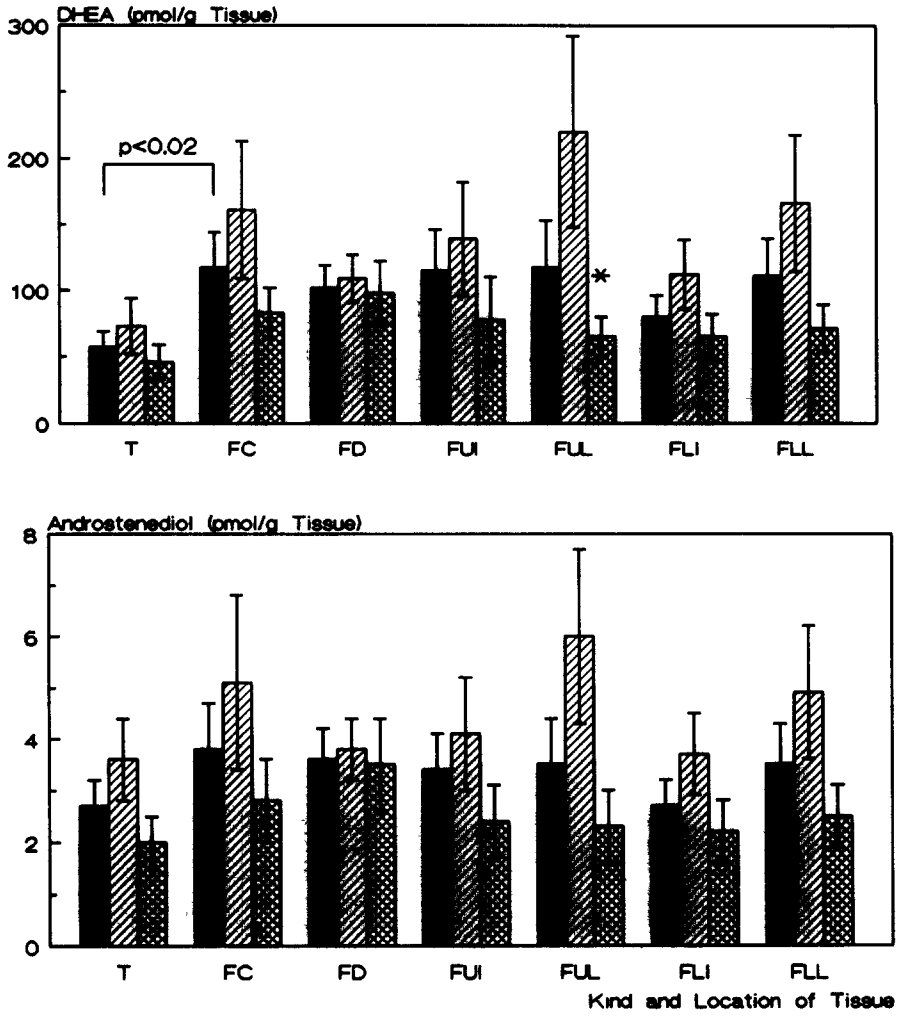

Fig. 2. DHEA and Adiol levels in breast cancer tissue and fatty tissue taken at different distances from the tumour. Results are given as means ± SEM for tissues from all patients (solid bars; $n = 9$–14); premenopausal patients (hatched bars; $n = 3$–6) and postmenopausal patients (cross-hatched bars; $n = 4$–8). The designation of the tissue type is given in the Experimental section. The asterisk reflects $P < 0.05$ between tissues from pre- and postmenopausal patients.

DISCUSSION

The observation that tumour-directed gradients in aromatase substrates or their precursors do not exist has led us to the conclusion that the maintenance of the intra-tumour oestradiol levels, or the endocrine autonomy of breast cancer cells, is not substrate-driven. The androgen concentrations reported here are expressed per gram of tissue. Even with this way of expressing, Adione and DHEA levels are higher in fat than in tumour tissue. It is very likely that when results would be expressed per mg of protein or DNA this would be even more pronounced. Then also the concentrations of Adiol and Testo would be much higher in fatty tissue than in tumour. A similar consideration applies for aromatase activity [9]. It is not clear which way of expressing the results is the best. When one considers that there is usually much more adipose tissue than tumour tissue in a breast one could argue that there is enough substrate available to the aromatase activity. The most important relationship, however, may be the ratio between the *in vivo* concentration and the K_m for the particular precursor. In this respect, expression of the results per gram of tissue may not be that inconvenient. Upon inspection of the literature, large variations in the reported K_m values for aromatase activity are found. As discussed previously [9] differences in experimental procedures may account for differences in enzyme activities and this also applies for K_m values. For conversion of Adione by human placental microsomes, for instance, K_m values between 1.4 nmol/l [12] and

4 μmol/l [13] can be found. For human adipose tissue values from 25 [14] to 250 nmol/l [15] have been reported. For breast cancer tissue, a K_m of 80 nmol/l has been reported [16] and in a recent review [17] a K_m value of 30 nmol/l was quoted for stromal cells from adipose tissue. As most of the reported values are low in the nanomolar range, it is not unrealistic to consider the K_m value for andostenedione to be around 30 nmol/l. This would imply that the concentration of Adione in breast tumour tissue is much lower than the K_m of the aromatase system for this substrate. It has been reported that within the cells responsible for the conversion of Adione to oestrone locally higher substrate concentrations may prevail [18]. For adipose tissue, the cytoplasmic concentration in the cells may even be much higher, since fat accounts for a large part of the tissue weight. In adipose tissue, the Adione concentration may then be around the K_m or even higher. The fact that the oestradiol concentration in breast adipose tissue decreases with menopause [8, 10], however, is an argument that uptake from the circulation is more important for the adipose tissue than *in situ* production. Thus, the factor(s) determining the tumour oestradiol level remain(s) to be identified.

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