J. Steroid Biochem. Molec. Biol. Vol. 37, No. 3, pp. 317–325, 1990
Printed in Great Britain.

0960-0760/90 $3.00 + 0.00

THE SIGNIFICANCE OF STEROID METABOLISM IN HUMAN CANCER

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Summary—Epidemiological and clinical evidence suggests that steroid hormones are intimately involved in the natural history of many cancers, including those of the breast, endometrium and prostate. However, it has been difficult to demonstrate that progressive changes in tumour development are related to circulating levels of steroids. This may be because further metabolism of steroids occurs locally within the tumour and its adjacent host tissue. Using the breast as an example, data has been reviewed that such local metabolism may (a) markedly change the biological potency of steroid hormones and (b) be associated with the risk, presence, pathology, stage and hormone sensitivity of cancer. The implications of these findings are discussed including the need to identify factors which regulate steroid metabolism in peripheral tissue and tumours. In this way the potential to influence the microenvironment around and within tumour cells may be realized in favour of the patient.

INTRODUCTION

Steroid hormones play an important part in regulating the growth, differentiation and function of a wide spectrum of normal tissues, notably the gonads, pituitary and secondary sex organs. That steroids also influence development of cancers in these tissues may be inferred from (i) analysis of aetiological factors for cancer which indicate that reproductive history may significantly modify risk, (ii) the presence of specific high-affinity steroid receptors in malignant tumours, (iii) response of the same tumours to endocrine manipulation (for review see Ref. [1]). The strength of evidence for steroid involvement is most convincing for cancers of the breast, prostate and endometrium. Of these, the depth of information is greatest for the breast and its tumours. This tissue system has therefore been taken as an illustrative example by which to evaluate the potential significance of steroid metabolism in human cancer.

The specific evidence linking steroid hormones with breast cancer is summarized in Table 1. Data relating to ovarian function is especially impressive. Thus breast cancer does not occur before puberty and an extended reproductive life caused by either early menarche or late menopause increases risk of breast cancer [2]. Conversely, an induced menopause protects against breast cancer. Overall castration is associated with 40% reduction in risk but if performed before the age of 35 yr, ovariectomy decreases incidence of breast cancer to one third of that in women with a natural menopause [3]. Ovarian ablation, whether by surgical or radiological means, will also produce tumour regression in about 30–40% of premenopausal women with metastatic breast cancer [4]. These effects seem to be mediated by oestrogens being reversed by administration of such steroids [5] and being more likely to occur in tumours possessing oestrogen receptors [6].

However, despite the evidence implicating ovarian function with breast cancer, it has been difficult to show that women either at high risk of cancer or with established breast cancer have abnormal circulating levels of ovarian hormones [7]. Additionally most breast cancers are to be found in postmenopausal women in whom the ovary is no longer the major source of active steroid hormones. Consideration has therefore to be given to the possibility that the further metabolism of steroid hormones at peripheral and target sites is also important. It is pertinent to draw attention to the steroidogenic capacity of tissues such as fat [8] and skin [9] and the similar potential in the breast and its tumours [10] (the same observation may be made for prostatic tissues in the male). Peripheral and local controls assume particular importance after the menopause and are, at least in part, independent of circulating steroids. The following review will therefore attempt to assess the significance of such steroid metabolism by

Proceedings of the XIV Meeting of the International Study Group for Steroid Hormones, Rome, Italy, 30 November–2 December 1989.

Table 1. Steroid hormones and breast cancer

A: Hormonal factors influencing risk	
Female sex	
Length of reproductive life	
Parity	
Age at first pregnancy	
B: Hormone receptors in breast cancer	
Oestrogen receptors	75–80%
Progestogen receptors	40–50%
Androgen receptors	30–40%
C: Endocrine therapies causing tumour regression	
Ovarian ablation	Antioestrogen
Adrenalectomy	Inhibitors of steroid metabolism
Hypophysectomy	Pharmacological doses of steroid

evaluating associations with (i) risk of cancer, (ii) presence of cancer, (iii) steroid levels in tumours, (iv) tumour pathology, (v) stage of cancer, (vi) tumour hormone-sensitivity and (vii) controlling factors. The implications of these observations will also be considered in terms of achieving the twin goals of preventing breast cancer and influencing the behaviour of established tumours by selectively regulating steroid metabolism.

STEROID METABOLISM

Over the last 30–40 yr, the metabolism of steroid hormones has been the subject of intensive research. Whilst the major pathway of steroidogenesis from cholesterol via C21 progestogens and C19 androgens to C18 oestrogens is common to all endocrine glands, there are a variety of routes and a plethora of interconversions between metabolic products in individual classes of steroid. More recently, it has become evident that most of these aspects of steroid metabolism may also be identified in peripheral tissues including cancers (for reviews see Refs [11–13]). A text of this size therefore cannot be comprehensive and it is proposed to consider only the conversions depicted in Fig. 1. These

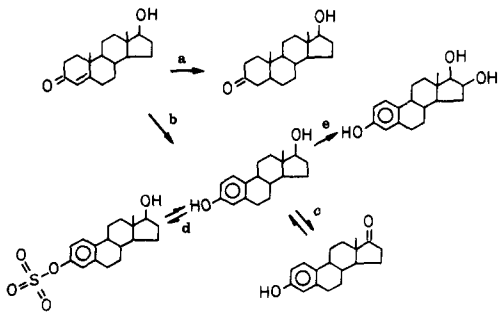

Fig. 1. Pathways of steroid metabolism associated with changes in biological activity between substrate and product. (a) 5α-Reductase, (b) aromatase, (c) 17β-hydroxysteroid dehydrogenase, (d) sulphatase/sulphokinase, (e) 16α-hydroxylase.

have been selected on the basis that the transformations are associated with marked changes in biological activity between substrate and product. The specific enzymes are listed below.

5α-Reductase. This activity is associated with the conversion of testosterone to 5α-dihydrotestosterone and then to 5α-androstanediols. There are potent androgenic steroids and natural ligands for the androgen receptor. Androgen-sensitive tissues invariably show high 5α-reductase activity [14].

Aromatase. This key enzyme complex irreversibly transforms C19 androgens into C18 oestrogens with the concomitant aromatization of the steroid A-ring. The activity is classically associated with the endocrine glands of the gonads, placenta and adrenal cortex but is also found in peripheral tissues such as adipose tissue [8], skin [9] and some cancers [10, 15].

17β-Hydroxysteroid dehydrogenase. This pivotal enzyme activity may be responsible for the interconversion of both androgen and oestrogens. Reduced steroids are in general more biologically active than the oxidized hormones. The position of the equilibrium reaction is therefore important and may vary between different tissues [16, 17].

Sulphatase/sulphokinase. These two enzymes are concerned with the cleavage of sulphated steroids to unconjugated hormones and the sulphation of free steroids respectively. Since unconjugated steroids are more likely to enter cells and be available for cellular processes [18], they are regarded as more active. In contrast sulphated steroids are biologically less potent and more readily excreted.

16α-Hydroxylase. The activity results in 16α-hydroxylated steroids and is classically thought of as a mechanism of steroid deactivation. For example, oestriol is the major urinary derivative of oestradiol [19]. However, the conversion of oestrone to 16α-hydroxyoestrone may have the reverse effect. Despite its low affinity for the oestrogen receptor, 16α-hydroxyoestrone has similar oestrogenicity to oestradiol when given continuously [20], perhaps because of its property of irreversibly binding to proteins [21] so prolonging cellular retention [22].

Steroid metabolism and risk of breast cancer

The most convincing data relating steroid metabolism to risk of breast cancer comes from the work of Bradlow and his co-workers on 16α-hydroxylation [23]. This group used a novel method to determine whole body metabolism

by employing [16α^{3}H]oestrone and measuring ^{3}H found in body water [24]. It was possible to show that the extent of 16α-hydroxylation was enhanced by 50% in postmenopausal patients with breast cancer as compared with healthy control subjects [25]. From this data alone it is impossible to distinguish between the possibility that the difference in 16α-hydroxylation preceded and predisposed for cancer or whether it reflects tumour burden within cancer patients. However, further studies support the former concept. Thus enhanced 16α-hydroxylation was also detected in women at high risk for breast cancer for familial reasons but without overt disease at the time of study [25]. Furthermore, in strains of mice with varying incidence of spontaneous mammary tumours, extent of 16α-hydroxylation was positively correlated with extent of tumour formation [26]. Lastly 16α-hydroxylation was higher in terminal duct lobular units obtained from mastectomy specimens (from cancer patients) compared with reduction mammoplasties (derived from women without cancer) [25].

Our own studies have investigated the role of steroid metabolism in adipose tissue within the breast [27, 28]. Compared with other glands, the adult breast is unusual in being invested in an abundance of adipose tissue, particularly in older women [29]. Circumstantial evidence also links breast fat with risk to breast cancer. Thus the proportion of adipose tissue in the breast increases in parallel with that of risk of breast cancer in certain populations of women [30, 31]. Furthermore, data from animal studies also indicate that only species with well-developed mammary fat pads have a high susceptibility of spontaneous mammary tumours [32]. Conversely animals with rudimentary or experimentally cleared fat pads rarely develop mammary cancer [33]. It is therefore of interest that such adipose tissue is a site of steroid metabolism. We have compared aromatase activity in adipose tissue derived from the breasts of women with breast cancer and those with benign conditions [27]. The results showed a significant difference in enzyme activity between the populations, the median value in the cancer group being 2-fold higher than that of the benign group. Again this design of study does not distinguish between predisposition to cancer or the response to tumour presence. But in contrast to 16α-hydroxylation, the more likely explanation is that the phenomenon is caused by the tumour itself.

Thus a series of risk factors for breast cancer, including family history (Fig. 2), obesity (Fig. 3), age at menarche, parity, age at first pregnancy and previous benign breast disease, were not significantly associated with level of aromatase activity in breast fat. In contrast it was noticeable that, in each sub-group analysis, adipose tissue obtained from patients with cancer tended to possess higher aromatase activity than that obtained from breasts without malignant change. Further circumstantial evidence

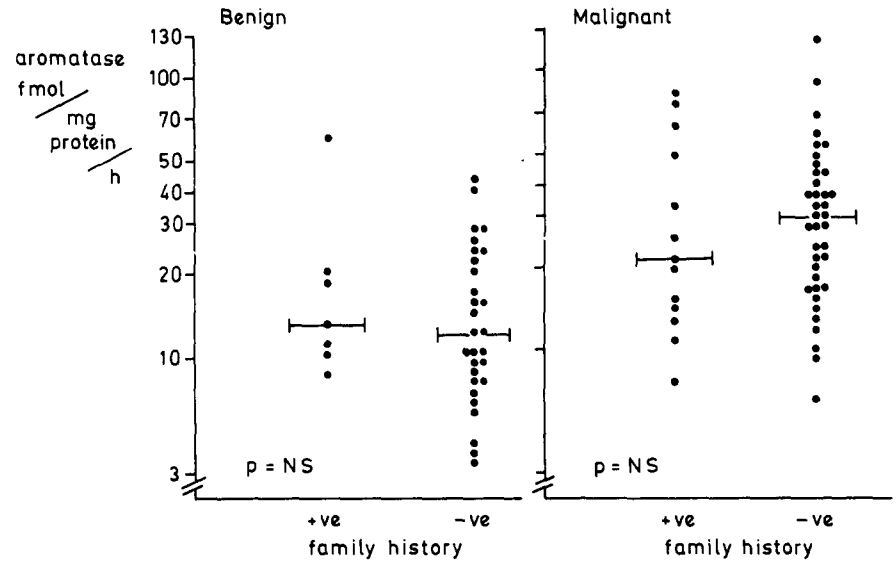

Fig. 2. Aromatase activity in breast adipose tissue from women with and without a family history of breast cancer, the tissue being obtained from patients undergoing surgery for either benign or malignant breast disease. Bar represents median value.

that the presence of carcinoma within the breast may influence steroid metabolism in adipose tissue has come from studies in which variation in aromatase within mastectomy specimens was related to location of cancer within the breast [28]. In all 12 mastectomy specimens examined, the quadrant displaying the highest level of aromatase activity always contained palpable tumour and conversely the quadrant with the lowest activity never contained tumour. Furthermore, in those breasts in which tumour was present in more than one quadrant, tumour-bearing areas always had higher aromatase activity than those without tumour. This variation in aromatase activity was not a simple reflection of increased steroid metabolism, 17β-hydroxysteroid dehydrogenase activity showing no relation with tumour site in the same mastectomy specimens. Whilst not representing definite proof, these observations would be consistent with breast tumours secreting factors into their local environment which either induce or stimulate aromatase activity, a concept that will be discussed in more detail later.

Steroid metabolism and tumour steroid levels

The concentrations and relative proportions of steroids may differ markedly between the circulation and breast cancers [34–37]. Disparities amongst oestrogens are especially striking [35–37]. Thus, whilst plasma levels of oestrogen fall markedly after the menopause, concentrations of oestrogen in breast cancers tend to be similar in pre- and postmenopausal women. As a result, in tumours from postmenopausal patients levels of oestrogens are significantly higher than those in the circulation. This is particularly so for oestradiol and whereas oestrone predominates over oestradiol in the circulation, levels of oestradiol are similar to or higher than oestrone in breast cancer.

The major precursors of active oestrogen in postmenopausal women are androgens [38] and oestrone sulphate [39] (as synthesized peripherally from androgen [40]). These precursors give rise to two major routes whereby oestradiol may be synthesized locally within tumours—(a) by aromatization of androgens and (b) by hydrolysis and reduction of oestrone sulphate. It has therefore been of interest to look for quantitative correlations with activity of the enzymes involved (aromatase sulphatase and 17β-hydroxysteroid dehydrogenase). Disappointingly, however, there have been no consistent reports on positive relationships between any enzyme conversion and concentrations of any individual oestrogen (or combination of oestrogen). Examples of the lack of correlation between aromatase, sulphatase and 17β-hydroxysteroid dehydrogenase and tumour oestradiol are shown in Fig. 4.

With the provisos that *in vitro* assays of enzymes may not precisely reflect *in vivo* activity and radioimmunoassay techniques may not accurately measure tumour oestrogens, there is little to support the concept that these particular enzymes in breast cancers are responsible for endogenous oestrogens.

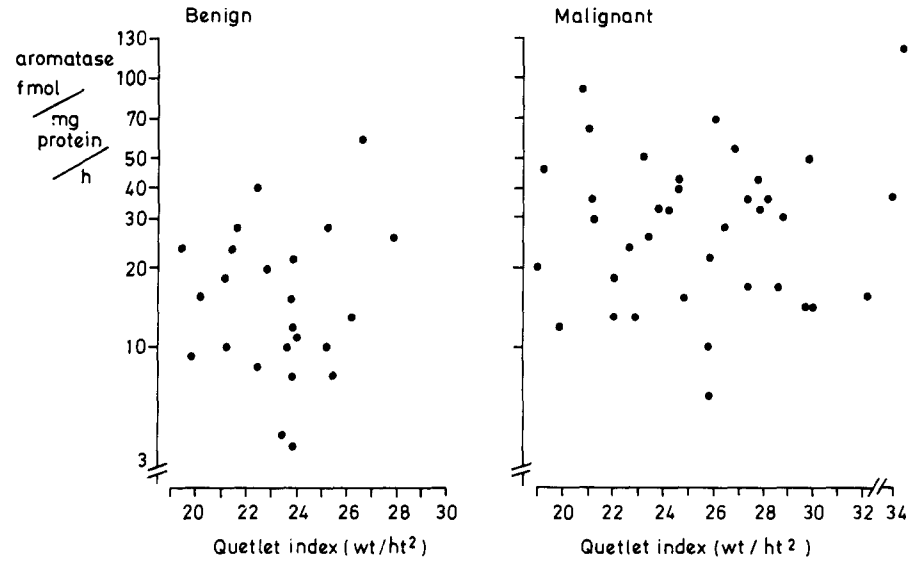

Fig. 3. The relationship between aromatase activity in breast adipose tissue and degree of obesity (Quetlet index) in women with either benign or malignant breast disease.

Steroid metabolism and tumour pathology

Although breast cancer may show a diverse pathology there are few substantial data to suggest that steroid metabolism is influenced by tumour histopathology or vice versa. The single exception to this is the association between tumour apocrine differentiation and the 5α-reduction of testosterone [41]. Thus breast cancers in which apocrine characteristics are a marked feature metabolize significantly more testosterone by conversion to 5α-reduced products such as 5α-dihydrotestosterone and 5α-androstanediols than tumours in which apocrine features do not predominate.

We have also reported a highly significant correlation between presence of apocrine features within breast cancers and degree of immunohistochemical staining using an antibody against androgen-conjugates (the staining being particularly localized within the apocrine cells) [42]. It is therefore interesting that the enhanced 5α-reduction is classically associated with androgen sensitive tissues such as prostate and increases in prostate cancer with degree of differentiation [43].

Steroid metabolism and stage of cancer

Whilst there is little evidence that steroid metabolism within tumours changes as the cancer becomes more advanced circumstantial data suggests that stage of disease may influence metabolism in the surrounding tissue. Thus 17β-hydroxysteriod dehydrogenase in breast fat is positively and significantly related to the size of the primary tumour in the breast [44]. We have also shown that levels of the same enzyme activity are significantly higher in breast fat from women whose cancer have been proven histologically to have spread to axillary lymph nodes, as compared with activity in women who were pathologically node-negative [45].

Steroid metabolism and hormone sensitivity

Approximately 30–40% of breast cancers appear to be hormone dependent and will respond to endocrine manipulation; the others display a hormone-independent phenotype. It is therefore of interest to determine whether endocrine-sensitive cancers display different patterns of steroidogenesis as compared with autonomous tumours. As oestrogen receptors are the single best parameter by which to predict hormone sensitivity, correlations with receptor status are also relevant.

Studies on oestrogen sulphurylation have identified two subgroups of breast cancer which relate to oestrogen receptor status [46]. High sulphating activity is associated with receptor-positive tumours whereas receptor-negative cancers have low activity. Interestingly, an earlier study found a correlation between tumour sulphation and response to adrenalectomy in patients with advanced breast cancer [47]. Remissions were in patients with tumours having sulphating activity and conversely patients whose tumours lacked steroid sulphation were treatment failures. These results suggest that steroid sulphurylation is a marker of hormone dependency.

Our own studies have focused on tumour aromatase. We have asked whether tumours which possess both oestrogen receptors and the ability to synthesize oestrogen from androgens are more likely to respond to anti-aromatase therapy than tumours without the biosynthetic capacity. To address this question, aromatase

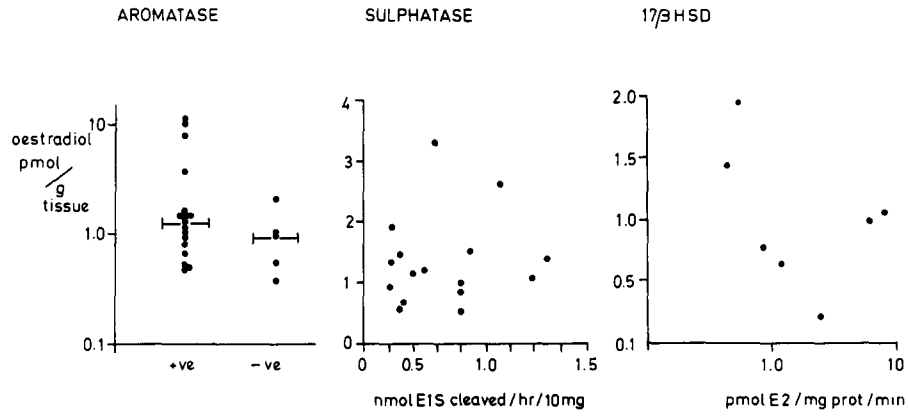

Fig. 4. Levels of oestradiol in breast cancers correlated with (a) tumour aromatase status, (b) levels of sulphatase activity and (c) levels of 17β-hydroxysteroid dehydrogenase activity. Bars represent the median value for tumours with and without aromatase activity.

has been measured in tumours from postmenopausal patients with oestrogen receptor-positive advanced breast cancer before treatment with aminoglutethimide-hydrocortisone. All 5 tumours without aromatase activity failed to respond to treatment whereas 11 of 18 with oestrogen biosynthesis responded [48]. While the presence of aromatase activity in a tumour is not an absolute marker for response to aminoglutethimide, the drug seems more likely to produce beneficial effects in this group.

Implications

If steroid hormones are involved in the risk and development of cancer and local metabolism of steroids may influence or be influenced by the behaviour of tumours, there are important implications for the prevention, therapy and biological understanding of cancer.

In terms of treatment, recent developments in endocrine therapy have seen a move away from the irreversible ablation of endocrine organs by surgery or irradiation to the use of pharmacological agents which either antagonize steroid hormones or inhibit their synthesis. The latter have obvious advantages in being reversible, allowing endocrine function to return if treatment is discontinued (an important consideration if therapy is effective in only the minority of tumours). Inhibitors of steroid metabolism may also be targeted against individual conversions producing more specific effects and producing less toxicity than more general ablative procedures. Additionally, in view of the realization that multiple sites of steroid biosynthesis exist and that, peripheral tissues (which are not susceptible to surgery or radiotherapy) are important, the concept of using inhibitors which would be effective irrespective of the site of biosynthesis is attractive. This has encouraged the pharmaceutical industry to invest considerable effort into developing selective inhibitors of steroid metabolizing enzymes. Because of the central role of oestrogens in breast cancer, attention has focused on the last step in their biosynthetic sequence, that of aromatization of androgens. There are now a host of putative aromatase inhibitors which have been laboratory tested and are now entering clinical practice [49]. It is thus pertinent to comment on the screening and use of such drugs.

Aromatase inhibitors are most likely to be used in postmenopausal patients (the majority of cancers occur after the menopause and the use of inhibitors in premenopausal women is complicated by the higher levels of aromatase in the ovary and the compensatory feedback loop by which additional enzyme and substrate may be synthesized). In postmenopausal patients, the major sites of aromatase activity are in peripheral tissues. It is therefore important in screening for the efficacy of aromatase inhibitors to include some peripheral test system. Using breast cancers as an *in vitro* model for peripheral aromatase we have uncovered some interesting disparities with regard to the relative sensitivity of breast cancer and placental test systems. Thus Fig. 5 shows the effects of aminoglutethimide and analogues against aromatase in breast cancers and placenta. All are effective agents but in placenta 30972 is more potent than 30991 whereas in breast cancers 30991 is more potent to 30972. The effects of another aromatase inhibitor, 4-hydroxyandrostenedione may vary markedly between different tumours. Thus in the majority of breast cancers 4-hydroxyandrostenedione markedly inhibits *in vitro* aromatase activity at concentrations of 10^{-8} and 10^{-7} M, whereas in about 15% of tumours, the drug is much less effective despite other inhibitors such as aminoglutethimide and CGS16949, showing consistent effects. Interestingly 4-hydroxyandrostenedione also show variable effects on aromatase in peripheral tissues *in vivo* [50]. These observations may have implications if aromatase inhibitors are to be targeted against peripheral tissues.

The physiological control of ovarian steroidogenesis by gonadotrophins has been well documented and its elucidation has led to the development of agonist analogues of gonadotrophin releasing hormones as a form of medical castration for the treatment of metastatic breast cancer [51]. In contrast the factors which regulate steroid metabolism in peripheral tissues are relatively undefined and pituitary hormones may play a comparatively minor role. The most illuminating work has come from research on adipose tissue performed by Simpson and coworkers [52]. They have shown that factors such as dibutyryl cyclic AMP, phorbol esters and dexamethasone may induce aromatase activity in stromal cells from human adipose tissue. Conversely a variety of growth factors have inhibitory effects. This may be relevant to local control of steroid metabolism in relation to cancers. For example, it has been shown that breast cancer cells may produce growth factors [53] which influence steroid metabolism [54]. The potential for autocrine and

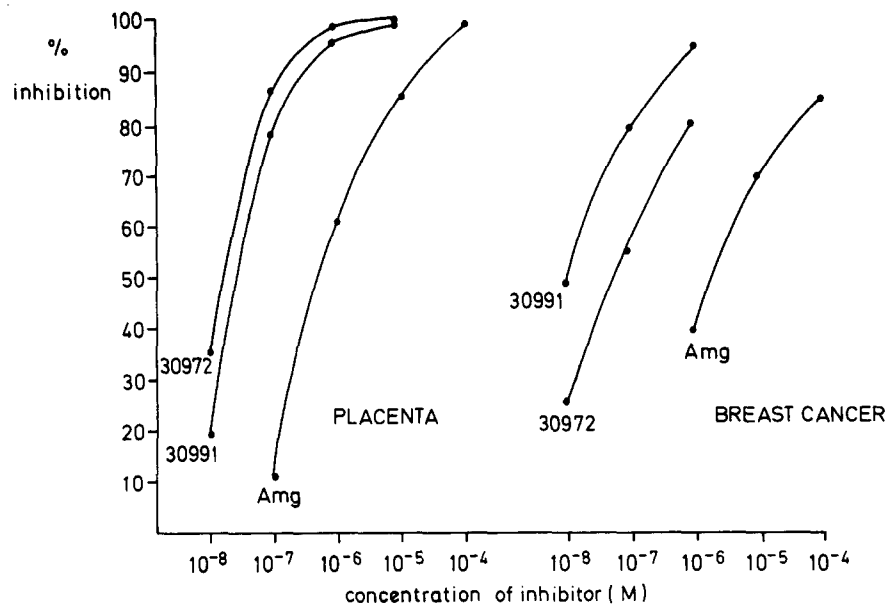

Fig. 5. The effects of aminoglutethimide and two synthetic analogues (30972, 30991) on aromatase activity in either placenta or breast cancers.

paracrine effects exists. Indeed, we have shown that both EGF and breast cancer cytosol have similar effects on aromatase activity in stromal cells derived from breast fat, being stimulatory in the absence of dexamethasone and inhibitory in its presence (Fig. 6). This observation is anecdotal but is compatible with the intimate relationship between aromatase activity in adipose tissue and the presence of tumour [28]. Similar effects have been reported by others investigating the effects of growth factors and tumour extracts on 17β-hydroxysteroid dehydrogenase activity in adipose tissues [55]. Conversely the addition of media conditioned by growth of fibroblasts apparently influences the direction of the interconversion between oestrone and oestradiol in cancer cells [56]. Thus the conversion to oestradiol is stimulated

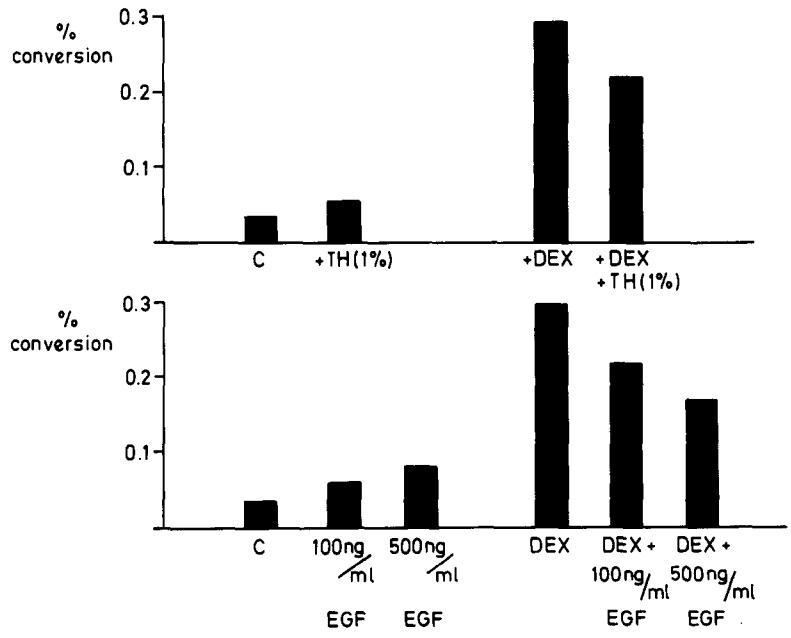

Fig. 6. Aromatase activity (expressed as percentage conversion of androstenedione to oestrogen) in human breast fat fibroblasts cultured in the absence of hormone additions (C), with a homogenate (1%) of breast cancer (TH), with dexamethasone (DEX), with both dexamethasone and the breast cancer homogenate (DEX + TH), with varying concentrations of epidermal growth factor (EGF) and with both dexamethasone and epidermal growth factor (DEX + EGF).

whereas that to oestrone is inhibited. This would be expected to increase the oestrogenic environment within the cells. Interestingly, the conditioned media which modified oestrogen metabolism was also capable of stimulating growth in the same cells.

In summary different aspects of steroid metabolism may be related to risk, presence, stage, histology and hormone sensitivity of breast cancer. Pathways of activation and deactivation co-exist and the concept of manipulating the local enviroment of steroid hormones to the advantage of the patient and the disadvantage of the tumour is attractive and should be achievable.

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