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Effect of Dexamethasone and Cytochrome *P*450 Inhibitors on the Formation of 7α-Hydroxydehydroepiandrosterone by Human Adipose Stromal Cells

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7α-Hydroxydehydroepiandrosterone (7α-OHDHA) is a major metabolite of dehydroepiandrosterone (DHA) using adipose stromal cells. To gain a better understanding of the factors regulating DHA metabolism, we examined the effect of dexamethasone and cytochrome *P*450 inhibitors on the formation of 7α-OHDHA. Dexamethasone (10^{-9} to 10^{-7} M) stimulated 7α-OHDHA formation in a dose-dependent manner with a 2- to 5-fold stimulation at 10^{-7} M. The dexamethasone stimulated 7α-OHDHA formation was inhibited by RU486 in a dose-dependent manner with suppression to basal levels at 10^{-6} M. Progesterone (10^{-7} M) had no effect on 7α-OHDHA formation suggesting that the dexamethasone stimulation was acting through the glucocorticoid receptor. Conversion of DHA to 7α-OHDHA was inhibited by ketoconazole and metyrapone. An inhibition of 70–80% was obtained with ketoconazole and 25–60% with metyrapone at concentrations of 10^{-5} M. Aminoglutethimide phosphate was less effective than either ketoconazole or metyrapone in inhibiting 7α-OHDHA formation with <30% inhibition at 10^{-5} M. These studies indicate that 7-hydroxylation provides an alternative pathway for the metabolism of DHA in peripheral tissues. This pathway, which is regulated by glucocorticoids, may influence the amount of DHA available for conversion to androstenedione and its subsequent aromatization to estrone. The biological role of the 7-oxygenated metabolites and their effects on other steroidogenic pathways have not been established.

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INTRODUCTION

Metabolism of steroid hormones in peripheral tissues modifies the biological activity of secreted adrenal and gonadal steroids [1–4]. Adipose tissue is a major site for steroid metabolism and variability in the quantity of the adipose mass may significantly perturb the physiological hormonal balance. Adipose tissue has therefore been utilized for studies of the peripheral metabolism of androgens [5]. Using whole adipose tissue or mature isolated adipocytes is difficult because of the marked solubility of steroid hormones in lipids. Under these conditions, the dissolved steroids are not readily available to the cytoplasmic enzymes for further metabolism [6]. Stromal cells, obtained after collagenase digestion of adipose tissue [7–9], can be grown in culture and under appropriate conditions can differentiate into mature adipocytes. These adipose stromal cells have been found to be satisfactory for the study of the peripheral metabolism of androgens.

A focal area of peripheral steroid metabolism has been the conversion of androgens to estrogens by the process of aromatization. Peripheral estrogen formation from androgens is the sole source of estrogens in the postmenopausal female [10] and the amount of estrogen formed may have an impact on the progression of osteoporosis or the development of endometrial carcinoma [11]. Peripheral aromatization may also be significant as a local source of estrogens in patients with breast carcinoma [12, 13].

While androstenedione appears to occupy a pivotal position in the metabolism of androgens in peripheral tissues, other androgens of primarily adrenal origin also undergo extensive peripheral metabolism. The compounds which are of interest because of their large production rates are dehydroepiandrosterone (DHA) and its sulfate (DHAS) with production rates of approx. 5 and 15 mg per day, respectively [14, 15].

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Plasma levels of both DHA and DHAS peak between age 20 to 30 with a progressive decline to the postmenopausal state [16].

The overall conversion of DHAS and DHA to products with greater biological activity is relatively low [17]. With the large production rates of these steroids, however, small conversions can account for significant amounts of active steroids such as estrone (E_1), estradiol and testosterone. Haning *et al.* [3, 14] in *in vivo* studies found that the percent conversion of DHAS to E_1 averaged 0.08% and this accounted for 13–51% of the E_1 produced in four premenopausal females with high plasma DHAS levels. These calculations include E_1 formed from DHA as well as DHAS. Longcope *et al.* [18] found that the conversion of DHA to E_1 in postmenopausal women was 0.6% and this accounted for 48% of the E_1 produced.

Earlier studies of androgen metabolism in our laboratory focused mainly on the formation of estrogens and 5α-reduced androgens in adipose stromal cells obtained from different body sites. The conversion of androstenedione to E_1 was 6- to 10-fold greater in cells from lower body fat (fat from the flank, and buttock area) than in cells from upper body fat (cells from subcutaneous abdominal fat) [19, 20]. Formation of 5α-reduced androgens varied from individual to individual but there were no consistent differences in metabolism from different body sites from the same individual.

In continuing studies of androgen metabolism, 7α-hydroxydehydroepiandrosterone (7α-OHDHA) was identified as a polar metabolite of DHA produced by adipose stromal cells [21]. This metabolite of DHA had previously been identified in the urine after administering DHA to a normal subject [22], in the urine of a patient with adrenal carcinoma [23] and also after *in vitro* incubation of DHA with human fetal liver, adrenal and chorion [24], abdominal skin [25], testis and epididymis [26], placenta [27], normal and tumorous human mammary tissues [28, 29] and human lung [30]. 7α-Hydroxylation of DHA and pregnenolone occurs in rat brain microsomes [31, 32]. These findings that an additional metabolic pathway exists in adipose stromal cells for DHA in addition to its conversion to androstenedione (and subsequently to E_1) have prompted us to study the factors that may regulate this conversion.

MATERIALS AND METHODS

Solvents obtained from BDH, Baker or Canadian Laboratory Supplies, Toronto, Canada were either American Chemical Society or HPLC grade. Radioactive steroids [1,2,6,7-^{3}H]DHA (sp. act. 86.6 Ci/mmol) and [1,2,6,7-^{3}H]androstenedione (sp. act. 86.1 Ci/mmol) were obtained from New England Nuclear (Montreal, PQ, Canada). Unlabeled steroids used as standards were purchased from Steraloids Inc. (Wilton NH, U.S.A.) and Sigma Chemical Co. (St Louis, MO, U.S.A.). 7α-OHDHA was a gift from Dr J. I. Raeside (University of Guelph, Guelph, Ontario, Canada). C_{18} Cartridges (S.P.E. Cartridges) obtained from Scientific Products and Equipment Ltd (Concord, Ontario) were washed twice with 10 ml methanol and 10 ml H_2O before use. Metyrapone was purchased from Sigma. Ketoconazole was a generous gift of Dr H. Vanden Bossche (Jannsen Laboratories, Beerse, Belgium), while aminoglutethimide phosphate was kindly donated by Dr C. A. Brownley (Ciba-Geigy Corporation, Summit, NJ, U.S.A.). RU486 (Roussel-Uclaf) was kindly provided by Dr D. T. Armstrong (Department of Obstetrics and Gynecology, University of Western Ontario). Tissue culture flasks (75 and 25 cm^2) and 6-well plates (Falcon) were purchased from Johns Scientific (Toronto, Canada).

Equipment

HPLC equipment consisted of a model 6000A and model 600E pumps, a model 700 and 710B automatic injectors, and models 441 and 486 UV spectrophotometers all from Waters Associates. Radiolabeled steroid metabolites were detected and quantified by an in-line Beckman model 171 radioactivity detector (Beckman Instruments, Toronto, Ontario, Canada). Data from the UV and radioactivity detectors were analyzed by computer using the MAXIMA 820 Chromatographic Software package from Waters Associates.

Preparation and incubation of cells

Adipose tissue obtained from patients during mammoplasty or abdominal surgery was washed with Hanks Balanced Salt Solution (HBSS), minced with scissors and incubated at 37°C with a 1 mg/ml collagenase solution (5 ml/g of tissue) in HBSS in a trypsinizing flask (Bellco Glass Inc., Vineland, NJ) for 30 min with constant stirring. After digestion, the tissue was allowed to stand for 10 min and separated into three fractions. The upper layer consisted of mature adipocytes and released lipid, the middle layer of cells liberated from the stromal-vascular fraction, and the lower layer of undigested material.

Cells were then prepared for culture by procedures described previously [20]. The studies described in this report were carried out on cells up to the third subculture, and the results were obtained from duplicate or triplicate incubations. Cells were grown for up to 15 days in culture and then used for steroid metabolism studies.

Metabolism of DHA by adipose stromal cells. Dexamethasone (10^{-9}–10^{-7} M), ketoconazole (10^{-9}–10^{-5} M), metyrapone (10^{-8}–10^{-4} M), aminoglutethimide phosphate (10^{-6}–10^{-4} M) and RU486 (10^{-8}–10^{-6} M) were added as indicated. Incubations were carried out in 6-well plates, 25 or 75 cm^2 flasks when the cells were approaching confluence. The medium was removed, and replaced with fresh medium containing 1 μCi [^{3}H]DHA/ml. The substrate concentration was adjusted to range from 10 nM to 1 μM by adding non-radioactive DHA. The incubation proceeded for 24 h, after which the medium was removed

and stored at −20°C until analyzed. The cells were trypsinized and counted in a hemocytometer and averaged 4.0×10^6, 5×10^5 and 2.8×10^5 cells for 75 cm^2, 25 cm^2 flasks and 6-well plates, respectively. The medium was thawed, and passed through a C_{18} SPE cartridge (between 2–10 ml medium/cartridge), and the retained steroids were eluted with methanol (5 ml per cartridge). An aliquot (approx. 10%) of the eluate was dried under vacuum, the residue was dissolved in 70% methanol and analyzed on HPLC.

HPLC analysis

Radiolabeled steroid metabolites were analyzed by HPLC on a C_{18} Spherisorb ODS-2 25 × 0.46 cm (5 μm) column (Jones Chromatography) eluted isocratically with 70% methanol–H_2O, flow rate 1 ml/min. Metabolites were detected by an in-line radioactivity detector and were identified on the basis of their retention times compared to authentic standards, and quantified using [1,2,6,7-^{3}H]androstenedione as an external standard.

Non-radioactive 3-oxo-4-ene steroids and 5-ene-3β-hydroxysteroids were detected at 254 and 215 nm, respectively, using 0.10 absorbance units full scale deflection.

RESULTS

Metabolism of DHA by human adipose stromal cells in the presence of dexamethasone

HPLC analysis of total unconjugated steroids from stromal cells from human breast adipose tissue is shown in Fig. 1. Elution was carried out using the 70% methanol–water system. Figure 1a shows the metabolites isolated under basal conditions. 7α-OHDHA, with a relative retention time of 0.61 (androstenedione = 1), was the major metabolite with 9.7% of the total radioactivity detected in this peak. Figure 1b

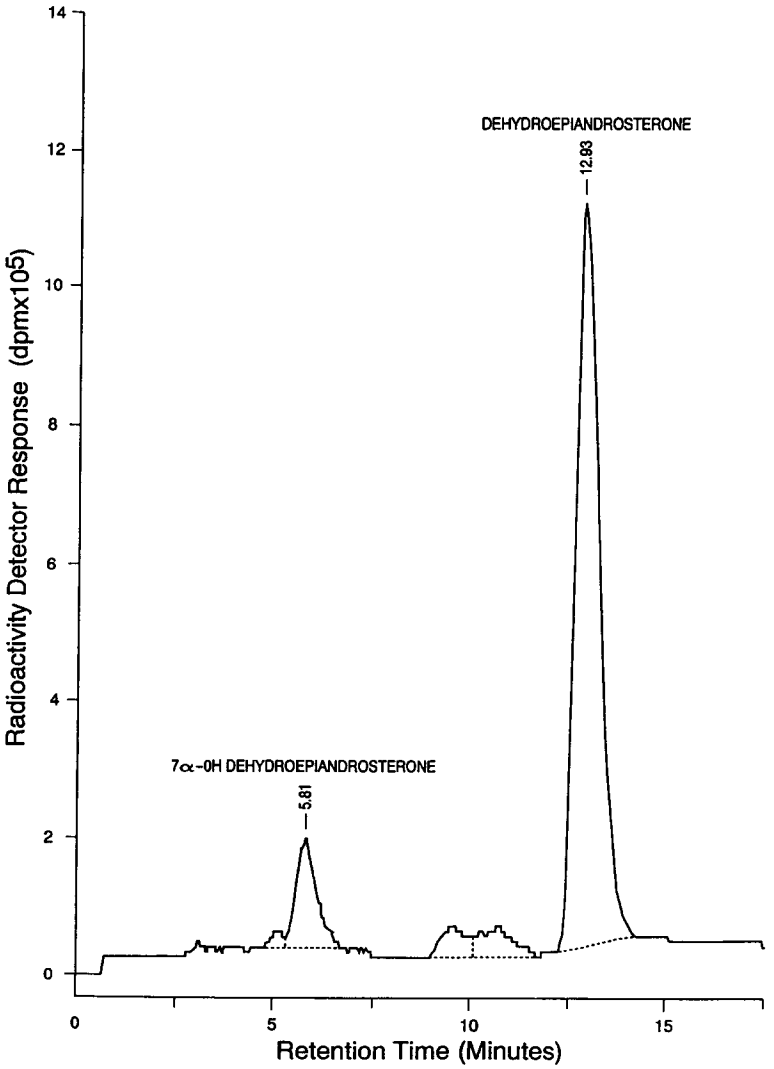

Fig. 1a. HPLC analysis of the steroid metabolites from the culture medium of stromal cells of breast adipose tissue (Patient 3) incubated with [1,2,6,7-^{3}H]DHA and fetal calf serum. Steroids were separated on a C_{18} Spherisorb ODS2 column (250 × 4.6 mm) eluted with 70% MeOH/H_2O. Flow rate was 1 ml/min. Steroids were identified on the basis of their relative retention times relative to androstenedione = 1. These steroids included a polar metabolite (0.53), 7α-OHDHA (0.61), and the substrate DHA (1.37). Steroid metabolites were detected by an in-line radioactivity detector.

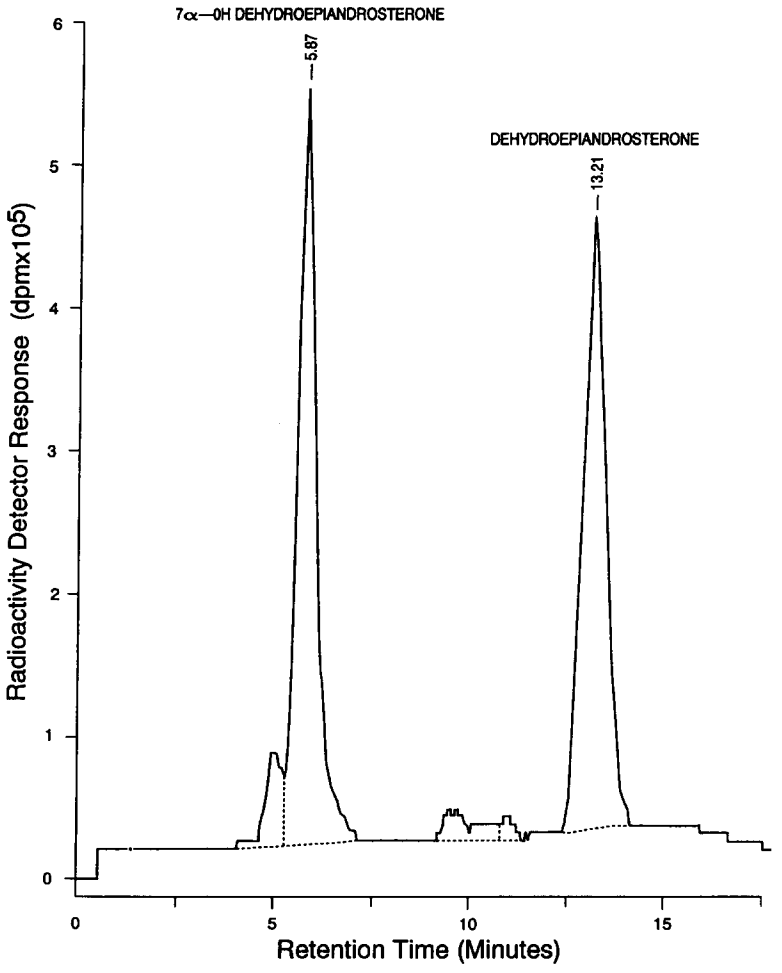

Fig. 1b. HPLC analysis of the steroid metabolites from the culture medium of stromal cells of breast adipose tissue (Patient 3) incubated with [1,2,6,7-^{3}H]DHA and fetal calf serum, in the presence of dexamethasone (10^{-7} M) and separated on a C_{18} Spherisorb ODS2 column (250 × 4.6 mm) eluted with 70% MeOH/H_2O. Flow rate was 1 ml/min. Steroids identified on the basis of their relative retention times were: a polar metabolite (0.53), 7α-OHDHA, and the substrate DHA. Steroid metabolities were detected by an in-line radioactivity detector.

shows the metabolites from parallel incubations in which 10^{-7} M dexamethasone was added to the incubation medium at the time of the addition of the [^{3}H]DHA substrate. The formation of 7α-OHDHA was stimulated 5-fold by dexamethasone and 46% of the total radioactivity was detected in this fraction. The formation of a smaller peak with a relative retention time of 0.53 eluting just before the 7α-OHDHA peak and incompletely resolved from it also appeared to be stimulated by dexamethasone (from 0.9 to 5.4% of total radioactivity).

Figure 2 shows the effect of increasing concentrations of dexamethasone from 10^{-9} to 10^{-7} M on the formation of 7α-OHDHA in four experiments using adipose stromal cells from three patients. The studies were carried out when the cells were in passage 2 or 3 in culture. The amount of 7α-OHDHA formed under basal conditions varied with stromal cells from different patients and in cells from different passages from the same patient. The formation of 7α-OHDHA increased progressively with increasing concentrations of dexamethasone; maximum stimulation was obtained in these studies with 10^{-7} M dexamethasone. Stimulation could be detected with concentrations as low as 10^{-9} M.

The effect of RU486 on dexamethasone stimulated 7α-OHDHA formation (Fig. 3)

To establish whether the effect of dexamethasone in stimulating 7α-OHDHA formation was mediated through the glucocorticoid receptor, studies were carried out with RU486. Since this compound blocks both the glucocorticoid and the progesterone receptors, studies were carried out using 10^{-7} M dexamethasone or 10^{-7} M progesterone with increasing concentrations of RU486 from 10^{-8} to 10^{-6} M. Progesterone had no effect on the formation of 7α-OHDHA. Dexamethasone resulted in a much greater stimulation of 7α-OHDHA formation in Patient 9 than in Patient 10 but in each case there was inhibition of 7α-OHDHA formation toward basal levels as the concentration of RU486 increased from 10^{-8} to 10^{-6} M.

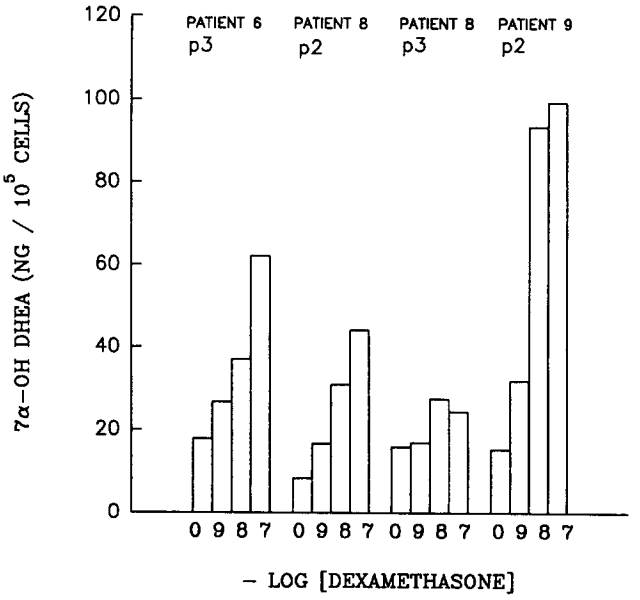

Fig. 2. The formation of 7α-OHDHA (ng/10^5 cells) from [1,2,6,7-^{3}H]DHA in human adipose stromal cells from breast tissue in the presence of increasing concentrations of dexamethasone (10^{-9} to 10^{-7} M). Radiolabeled metabolites were separated by C_{18} reverse phase HPLC. The patient numbers indicate different patients and p indicates the passage number of cells in culture.

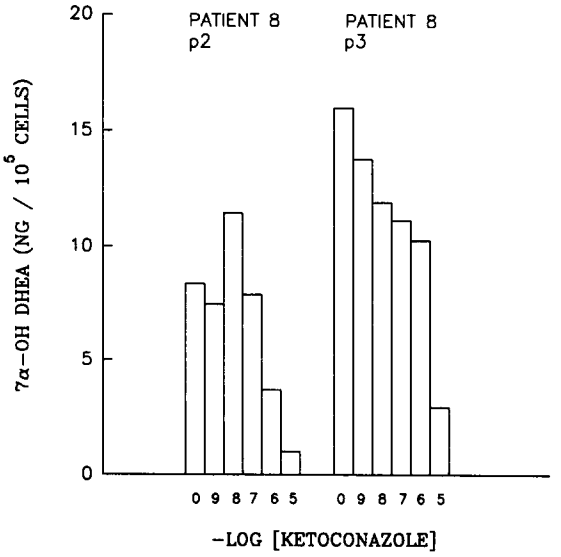

Fig. 4. The formation of 7α-OHDHA (ng/10^5 cells) from [1,2,6,7-^{3}H]DHA in human adipose stromal cells from breast tissue in the presence of increasing concentrations of ketoconazole (10^{-9} to 10^{-5} M). Radiolabeled metabolites were separated by C_{18} reverse phase HPLC. The patient numbers indicate different patients and p indicates the passage number of cells in culture.

The effect of cytochrome P450 inhibitors on 7α-OHDHA formation by human adipose stromal cells

Ketoconazole. The effect of ketoconazole (10^{-9}–10^{-5} M) on the formation of 7α-OHDHA in adipose stromal cells in Patient 8 in passages 2 and 3 is shown in Fig. 4. There was 30% inhibition at 10^{-6} M and over 75% inhibition with 10^{-5} M ketoconazole.

Metyrapone. Figure 5 shows the effect of metyrapone on 7α-OHDHA formation in cells in passages 2 and 3 from Patient 8 and passage 2 from Patient 9. There was a progressive decrease in 7α-OHDHA formation with concentrations of metyrapone from 10^{-6} to 10^{-4} M. At 10^{-5} M, inhibition varied between 25 and 60%.

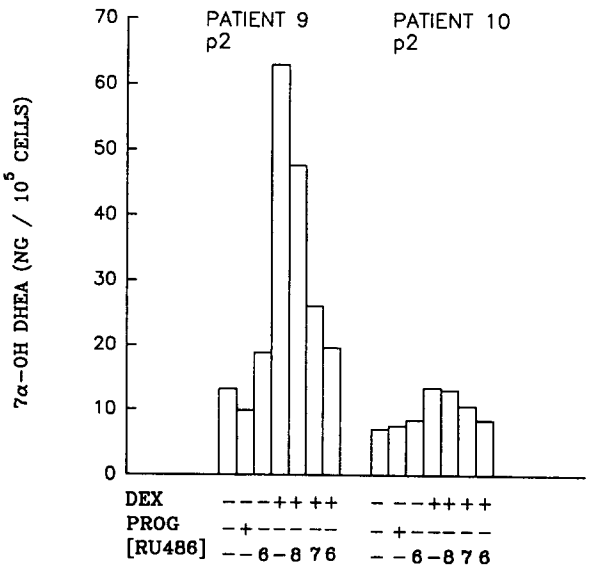

Fig. 3. The formation of 7α-OHDHA (ng/10^5 cells) from [1,2,6,7-^{3}H]DHA in human adipose stromal cells from breast tissue cultured with progesterone alone, and increasing concentrations of RU486 (10^{-8} to 10^{-6} M) in the presence of 10^{-7} M dexamethasone. Radiolabeled metabolites were separated by C_{18} reverse phase HPLC. The patient numbers indicate different patients and p indicates the passage number of cells in culture.

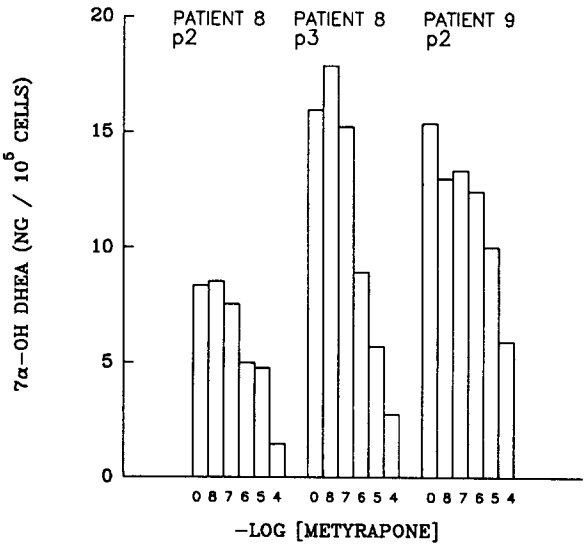

Fig. 5. The formation of 7α-OHDHA (ng/10^5 cells) from [1,2,6,7-^{3}H]DHA in human adipose stromal cells from breast tissue in the presence of increasing concentrations of metyrapone, a cytochrome *P*450 inhibitor. Radiolabeled metabolites were separated by C_{18} reverse phase HPLC. The patient numbers indicate different patients and p refers to the passage number of cells in culture.

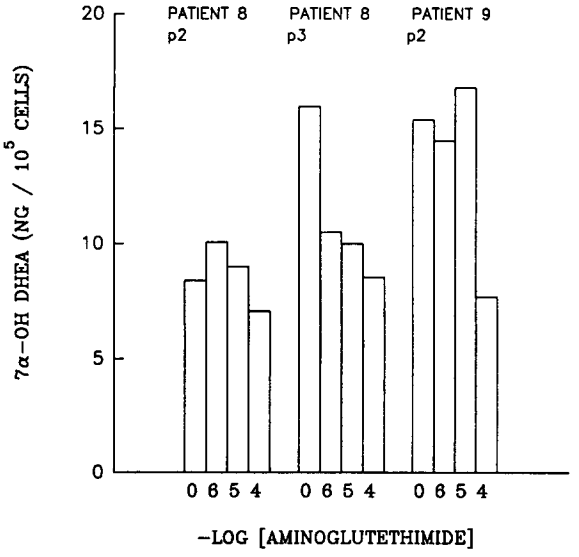

Fig. 6. The formation of 7α-OHDHA (ng/10^5 cells) from [1,2,6,7-^{3}H]DHA in human adipose stromal cells from breast tissue in the presence of increasing concentrations of aminoglutethimide phosphate (10^{-6} to 10^{-4} M). Radiolabeled metabolites were separated by C_{18} reverse phase HPLC. The patient numbers indicate different patients and p indicates the passage number of cells in culture.

Aminoglutethimide phosphate. Aminoglutethimide phosphate (AGP) was a less effective inhibitor of 7α-OHDHA formation than either metyrapone or ketoconazole in studies using adipose stromal cells from Patient 8 (passages 2 and 3) and in Patient 9 (passage 2). Up to 50% inhibition was obtained with 10^{-4} M AGP in two of the three experiments (Fig. 6), compared with >60% inhibition with 10^{-4} M metyrapone and >75% inhibition with 10^{-5} M ketoconazole.

DISCUSSION

Adipose tissue is an important source of estrogens in the postmenopausal female and the rate of conversion of plasma androstenedione to E_1 in humans is a function of obesity and aging [2]. Androstenedione is generally accepted as the major precursor for estrogen formation in adipose tissue. DHA can be converted to androstenedione by 3β-hydroxysteroid dehydrogenase (3βHSD) and can therefore contribute to the androstenedione pool available for aromatization to E_1 or reduction to testosterone. DHA can also be converted to 7α-OHDHA [21] and, in some preparations of adipose stromal cells, is the major metabolite of DHA. The cells used in this study were cultured in the presence of fetal calf serum and are maintained primarily as preadipocytes. The variation in the formation of 7α-OHDHA found in cells under different culture conditions suggests that the culture medium or the state of differentiation of the cells may influence 7α-hydroxylation. Under conditions in which 7α-OHDHA is the major metabolite less substrate is available locally for conversion to androstenedione.

The factors controlling 3βHSD and 7-hydroxylase activities in peripheral tissues are, at present, poorly understood. As was shown in Fig. 1, stimulation of the conversion of DHA to 7α-OHDHA by dexamethasone occurred in a situation in which there was no significant conversion of DHA to androstenedione. This suggests that the regulation of each of these pathways is independent of the other. In the present study, it has been demonstrated that the formation of 7α-OHDHA is stimulated by dexamethasone. The degree of stimulation varied with the culture conditions and up to 5-fold stimulation was observed at a dexamethasone concentration of 10^{-7} M. The stimulation of aromatase activity with glucocorticoids under similar conditions resulted in a 20- to 100-fold increase in E_1 formation [33, 34]. With the data currently available it is difficult to know whether a relationship exists between stimulation of 7α-OHDHA and E_1 formation by glucocorticoids. The inhibition of the stimulatory effect of dexamethasone on 7α-OHDHA formation by RU486 suggests that it is mediated by the glucocorticoid receptor. This compound also blocks the progesterone receptor, but progesterone did not influence 7α-OHDHA formation in these studies. The stimulation of aromatase activity in adipose stromal cells by dexamethasone is also inhibited by a glucocorticoid receptor antagonist [33]. Glucocorticoids can therefore stimulate both 7-hydroxylase and aromatase activities, and the observation that dexamethasone can inhibit 3βHSD activity in rat Leydig cells may suggest an additional mechanism for regulating DHA metabolism in adipose stromal cells [35]. The possibility that sex steroids may also influence the peripheral metabolism of DHA was suggested by the results of Faredin *et al.* [25]. Using slices of human skin obtained at the time of surgery, they found a higher ratio of 7α-OHDHA to androst-5-ene-3β, 17β-diol after incubating DHA with skin from an adult male when compared with skin from an agonadal male.

Metyrapone, which is used clinically as an 11β-hydroxylase inhibitor to block the conversion of 11-deoxycortisol to cortisol [36], was found to be an effective inhibitor of 7α-OHDHA formation at concentrations of 10^{-5} M or greater. Ketoconazole, which also inhibits cytochrome *P*450 hydroxylations and is used clinically to inhibit adrenal and gonadal steroidogenesis [37, 38] also decreased 7α-OHDHA formation by >75% at a concentration of 10^{-5} M.

The role of 7α-hydroxylation in the peripheral metabolism of steroid hormones remains to be established. In a previous study, the 7α-hydroxylation of androstenedione in human adipose stromal cells could not be demonstrated [21] and Akwa *et al.* [31] have shown that rat brain microsomes were able to 7α-hydroxylate DHA and pregnenolone but not testosterone and androstenedione. Li *et al.* [29] reported the conversion of DHA to 7α-hydroxy, 7β-hydroxy and 7-keto DHA in human mammary carcinoma tissue, but were unable to detect the conversion of cholesterol to 7-hydroxycholesterol. These observations suggest that there are separate enzymes responsible for the 7-hydroxylation of these substrates.

In vitro studies of the metabolism of DHA to 7-hydroxylated metabolites in human mammary tumors have been reported [28, 29]. Both groups reported the conversion of DHA to 7α- and 7β-hydroxy DHA with the 7α-hydroxy isomer as the major metabolite. Using *in vivo* studies, Van Landeghem *et al.* [39] were unable to detect an increase in the concentration of 7α-OHDHA in the mammary vein relative to mammary artery in patients undergoing mastectomy for breast carcinoma. The difficulties in obtaining blood draining only tumor tissue may have influenced the sensitivity of these studies. Using a radioimmunoassay, Skinner *et al.* [40] measured plasma levels of 7α-OHDHA in normal hospitalized subjects, patients with benign breast disease, breast cancer patients and pregnant women. The groups were not entirely comparable in size or age but there did not appear to be any differences among the normal subjects, the patients with benign breast disease or the pregnant subjects. The range of values in patients with breast cancer was much wider but it is not known if this is of biological significance.

The conversion of DHA to 7α-OHDHA has been demonstrated in a variety of peripheral tissues. The present study has shown that this conversion can be stimulated by glucocorticoids and inhibited by cytochrome *P*450 inhibitors. The formation of 7α-OHDHA exceeds the conversion of DHA to other metabolites including androstenedione in some culture conditions. Understanding the factors which modulate the formation of 7α-OHDHA may therefore help to clarify the biological effects of DHA.

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REFERENCES

1. MacDonald P. C., Rombaut P. and Siiteri P. K.: Plasma precursors of estrogen. 1. Extent of conversion of plasma 4-androstenedione to estrone in normal males and non-pregnant, normal, castrate and adrenalectomized females. *J. Clin. Endocr. Metab.* **60** (1967) 1103–1111.
2. Hemsell D. L., Grodin J., Brenner P. F., Siiteri P. K. and MacDonald P. C.: Plasma precursors of estrogen II: correlation of extent of conversion of plasma androstenedione to estrone with age. *J. Clin. Endocr. Metab.* **38** (1974) 476–480.
3. Haning R. V., Carlson I. H., Flood C. A., Hackett R. J. and Longcope C.: Metabolism of dehydroepiandrosterone sulfate (DS) in normal women and women with high DHAS concentrations. *J. Clin. Endocr. Metab.* **73** (1991) 1210–1215.
4. Edman C. D. and MacDonald P. C.: Effect of obesity on conversion of plasma androstenedione to estrone in ovulatory and anovulatory young women. *Am. J. Obstet. Gynec.* **130** (1978) 456–462.
5. Perel E. and Killinger D. W.: The interconversion and aromatization of androgens by human adipose tissue. *J. Steroid Biochem.* **10** (1979) 623–628.
6. Cleland W. H., Mendelson C. R. and Simpson E. R.: Aromatase activity of membrane fractions of human adipose tissue stromal cells and adipocytes. *Endocrinology* **113** (1983) 2155–2161.
7. Poznanski W., Waheed I. and Van R.: Human fat cell precursors. Morphologic and metabolic differentiation in culture. *J. Lab. Invest.* **29** (1973) 570–576.
8. Van R., Bayliss C. and Roncari D.: Cytological and enzymological characterization of adult human adipocyte precursors in culture. *J. Clin. Invest.* **58** (1976) 699–704.
9. Roncari D. and Van R.: Promotion of human adipocyte precursor replication by 17β estradiol in culture. *J. Clin. Invest.* **62** (1978) 503–508.
10. Grodin J. M., Siiteri P. K. and MacDonald P. C.: Source of estrogen production in postmenopausal women. *J. Clin. Endocr. Metab.* **36** (1973) 207–214.
11. MacDonanld P. C., Edman C. D., Hemsell D. L., Porter Y. C. and Siiteri P. K.: Effect of obesity on conversion of plasma androstenedione to estrone in postmenopausal women with and without endometrial cancer. *Am. J. Obstet. Gynec.* **130** (1978) 448–455.
12. Perel E., Davis S. and Killinger D. W.: Androgen metabolism in male and female breast tissue. *Steroids* **37** (1981) 345–352.
13. Perel E., Daniilescu D., Kharlip L., Blackstein M. E. and Killinger D. W.: The relationship between growth and androstenedione metabolism in four cell lines of breast carcinoma cells in culture. *Molec. Cell. Endocr.* **41** (1985) 197–203.
14. Haning R. V., Chabot M., Flood C. A., Hacket R. and Longcope C.: Metabolic clearance rate (MCR) of dehydroepiandrosterone sulfate (DS), its metabolism to dehydroepiandrosterone, androstenedione, and dihydrotestosterone and the effect of increased plasma DHAS concentration on DHAS MCR in normal women. *J. Clin. Endocr. Metab.* **69** (1989) 1047–1052.
15. Haning R. V., Flood C. A., Hackett R. J., Loughlin J. S., McLure N. and Longcope C.: Metabolic clearance rate of dehydroepiandrosterone sulfate, its metabolism to testosterone, and its intrafollicular metabolism to dehydroepiandrosterone, androstenedione, testosterone and dihydrotestosterone *in vivo*. *J. Clin. Endocr. Metab.* **72** (1991) 1088–1095.
16. Liu C. H., Laughlin G. A., Fischer U. G. and Yen S. S. C.: Marked attenuation of ultradian and circadian rhythms of dehydroepiandrosterone in postmenopausal women: evidence for reduced 17,20-desmolase enzymatic activity. *J. Clin. Endocr. Metab.* **71** (1990) 900–906.
17. MacDonald P. C., Edman C. D., Kerber I. J. and Siiteri P. K.: Plasma precursors of estrogen III conversion of plasma dehydroepiandrosterone to estrogen in young nonpregnant women. *Gynec. Invest.* **7** (1976) 165–175.
18. Longcope C., Bourget C. and Flood C.: The production and aromatization of dehydroepiandrosterone in postmenopausal women. *Maturitas* **4** (1982) 325–332.
19. Killinger D. W., Perel E., Daniilescu D., Kharlip L. and Lindsay W. R. N.: The relationship between aromatase activity and body fat distribution. *Steroids* **50** (1987) 61–72.
20. Killinger D. W., Perel E., Daniilescu D., Kharlip L. and Lindsay W. R. N.: Influence of adipose tissue distribution on the biological activity of androgens. *Ann. N.Y. Acad. Sci.* **595** (1990) 199–211.
21. Khalil M. W., Strutt B., Vachon D. and Killinger D. W.: Metabolism of dehydroepiandrosterone by cultured human adipose stromal cells: identification of 7α-hydroxydehydroepiandrosterone as a major metabolite using high performance liquid chromatography and mass spectrometry. *J. Steroid Biochem. Molec. Biol.* **46** (1993) 585–595.
22. Schneider J. J. and Lewbart M. L.: Fractionation and isolation of steroid conjugates. *Recent Prog. Hormone Res.* **15** (1959) 201–230.
23. Okada M., Fukushima D. K. and Gallagher T. F.: Isolation and characterization of 3β-hydroxy-Δ^5steroids in adrenal carcinoma. *J. Biol. Chem.* **234** (1959) 1688–1692.
24. Sulcova J., Capkova A., Jirasek J. E. V. and Starka L.: 7-Hydroxylation of dehydroepiandrosterone in human fetal liver, adrenals and chorion *in vitro*. *Acta. Endocr.* **59** (1968) 1–9.
25. Faredin I., Fazekas A. G., Toth I., Kokai K. and Julesz M.: Transformation *in vitro* of [4-^{14}C]-dehydroepiandrosterone into 7-oxygenated derivatives by normal human male and female skin tissue. *J. Invest. Derm.* **52** (1969) 357–361.
26. Sulcova J. and Starka L.: 7α-Hydroxylation of dehydroepiandrosterone in human testis and epididymis *in vitro*. *Experimentia* **28** (1972) 1361–1362.
27. Mirhom Y. W. and Szontagh F. E.: Transformation *in vitro* of [4-^{14}C]dehydroepiandrosterone to 7-oxygenated derivatives by normal early human placental tissue. *J. Endocr.* **50** (1971) 301–306.
28. Couch R. A. F., Skinner S. K. M., Tobler C. J. P. and Doouss T. W.: The *in vitro* synthesis of 7-hydroxydehydroepiandrosterone by human mammary tissues. *Steroids* **26** (1975) 1–15.
29. Li K., Foo T. and Adams J. B.: Products of dehydroepiandrosterone metabolism by human mammary tumors and their influence on estradiol receptor binding. *Steroids* **31** (1978) 113–127.

30. Milewich L., Winters A. J., Stephens P. and MacDonald P. C.: Metabolism of dehydroisoandrosterone and androstenedione by the human lung *in vitro*. *J. Steroid Biochem.* **8** (1977) 277–284.
31. Akwa Y., Young J., Kabbadj K., Sancho M. J., Zucman D., Vourc'h C., Jung-Testas I., Hu Z. Y., Le Goascogne C., Jo D. H., Corpéchot C., Simon P., Baulieu E. E. and Robel P.: Neurosteroids: Biosynthesis, metabolism and function of pregnenolone and dehydroepiandrosterone in the brain. *J. Steroid Biochem. Molec. Biol.* **40** (1991) 71–81.
32. Akwa Y., Morfin R. F., Robel P. and Baulieu E. E.: Neurosteroid metabolism: 7α-hydroxylation of dehydroepiandrosterone and pregnenolone by rat brain microsomes. *Biochem. J.* **288** (1992) 959–964.
33. Simpson E. R., Ackerman G. E., Smith M. E. and Medelson C. R.: Estrogen formation in stromal cells of adipose tissue of women: induction of glucocorticoids. *Proc. Natn. Acad. Sci. U.S.A.* **78** (1981) 5690–5694.
34. Perel E., Daniilescu D., Kindler S., Kharlip L. and Killinger D. W.: The formation of 5α-reduced androgens in stromal cells from human breast adipose tissue. *J. Clin. Endocr. Metab.* **62** (1986) 314–318.
35. Agular B.-M., Vinggaard A. M. and Vind C.: Regulation by dexamethasone of the 3β-hydroxysteroid dehydrogenase activity in adult rat Leydig cells. *J. Steroid Biochem. Molec. Biol.* **43** (1992) 565–571.
36. Vanden Bossche H., DeCoster R. and Amery W. K.: Pharmacology and clinical uses of ketoconazole. In *Pharmacology and Clinical Uses of Inhibitors of Hormone Secretion and Action* (Edited by B. J. A. Furr and A. E. Wakeling). Bailliere Tindall, London, Chapter 15 (1986) p. 288.
37. Somimo N.: The use of ketoconazole as an inhibitor of steroid production. *New Engl. J. Med.* **317** (1987) 812–818.
38. Vanden Bossche H., Willemsens G., Marichal P., Cools W. and Lauwers W.: The molecular basis for the antifugal activities of N-substituted azole derivatives, focus on R51211. In *Mode of Action of Antifugal Agents* (Edited by A. P. J. Trince and J. F. Ryley). Cambridge University Press, Cambridge (1984) pp. 321–341.
39. Van Landeghem A. A. J., Poortman J., Deshpande N., Di Martino L., Tarquini A., Thijssen J. H. H. and Schwarz F.: Plasma concentration gradient of steroid hormones across mammary tumours *in vivo*. *J. Steroid Biochem.* **14** (1981) 741–747.
40. Skinner S. K. M., Tobler C. J. P. and Couch R. A. F.: A radioimmunoassay for 7α-hydroxy-dehydroepiandrosterone in human plasma. *Steroids* **30** (1977) 315–330.