ANDROGEN METABOLISM IN MALE AND FEMALE BREAST TISSUE

E. Perel, S. Davis and D.W. Killinger

Department of Medicine, University of Toronto
and The Wellesley Hospital, Toronto, Canada M5S 1A8

Received 12-15-80

ABSTRACT

Incubation studies have been carried out using normal breast tissue and breast tissue from patients with gynecomastia, mammary dysplasia and breast carcinoma to determine the pattern of androstenedione metabolism. All tissues formed estrone (E_1) and testosterone (T) in all incubations. Estradiol (E_2) was isolated in incubations of tissue from 1 of 6 patients with mammary dysplasia, 5 of 6 patients with gynecomastia and in all incubations with normal and carcinoma tissue. Estrone formation was lowest in mammary dysplasia and gynecomastia, and higher in apparently normal breast tissue. The greatest E_1 formation was found in incubations with breast carcinoma tissue, although there was considerable variation within this tissue group. Estradiol formation was low in all tissues, with the highest conversion rates in carcinoma tissue. Testosterone formation in carcinoma tissue was greater than in mammary dysplasia or gynecomastia, but similar to apparently normal tissue. These results indicate that breast tissue from different pathological states varies in its capacity to aromatize androstenedione (A) to estrogenic products and to convert it to other androgens. They have also shown that the pattern of metabolism is distinctive for the nature of the pathological abnormality.

INTRODUCTION

Breast tissue undergoes marked changes in growth in response to fluctuations in hormone stimulation. This response suggests that there may be a relationship between hormone stimulation and the development or maintenance of both benign and malignant pathological states. The concept that local steroid metabolism can affect the hormonal environment in target tissue has led to the examination of the metabolic capabilities of breast tissue from a variety of sources.

The ability of breast carcinoma tissue to metabolize testosterone (T) has been well established. The aromatization of T has been reported by several laboratories as well as the conversion of T to androstenedione (A) and 17β-hydroxy-5α-androstan-3-one (DHT) (1-9).

The metabolism of A to E_1, E_2 and T by breast carcinoma tissue has also been documented (10,11). Other investigators have reported the conversion of dehydroepiandrosterone sulfate (DHAS) to A, T, DHT and estrone (E_1) (12).

In studies with normal breast tissue from patients undergoing mastectomy for breast carcinoma the conversion of T to estradiol (E_2) could not be demonstrated (5). Aromatization has however been demonstrated in parenchymal tissue from patients with mammary dysplasia (11). The aromatization of androgens has also been reported in parenchymal tissue from patients with gynecomastia (13,14).

In a previous study from our laboratory (11), breast adipose tissue from patients with mammary carcinoma and mammary dysplasia showed similar metabolic capabilities in the aromatization of A to E_1 and E_2 and in the conversion of A to T. Breast carcinoma tissue, however, was more active than parenchymal tissue from patients with mammary dysplasia in both the aromatization of A to E_1 and the conversion of A to T.

The present study was designed to compare A metabolism under similar conditions, in parenchymal tissue from patients with gynecomastia, mammary dysplasia and breast carcinoma, and in normal breast tissue removed during mastectomy for breast carcinoma.

MATERIALS AND METHODS

Chemicals

Reference steroids were obtained from Makor Chemicals Ltd., Jerusalem, Israel and the Sigma Chemical Company, St. Louis, Missouri and were recrystallized prior to use. Radioactive steroids (1,2,6,7 ^{3}H)-A (SA 114.0 Ci/mmol), (4-^{14}C)-T (SA 50 mCi/mmol), (4-^{14}C)-E_1 (SA 50 mCi/mmol), and (4-^{14}C)-E_2 (SA 50 mCi/mmol), were obtained from New England Nuclear Corporation, Boston, Massachusetts and were purified by paper chromatography prior to use. Precoated

TLC plates, SIL-G-25 UV 254, were purchased from Brinkmann Instruments, Westbury, New York.

Subjects

Incubation studies were carried out on breast tissue from 5 patients with gynecomastia, 6 patients with mammary dysplasia, 17 patients with breast carcinoma, and 3 patients having apparently normal breast tissue removed during mastectomy for breast carcinoma. The patients with mammary dysplasia (ages 13-30) and gynecomastia (ages 15-22) were younger than the carcinoma patients (ages 40-86).

In the group of patients with gynecomastia, one patient had Klinefelter's syndrome with a typical XXY karyotype. In the others, the gynecomastia had begun with the onset of puberty. In all of the subjects with mammary dysplasia, surgery was performed because of painful, excessive enlargement of the breasts. No hormonal abnormalities were noted on routine investigation and no hormone therapy had been given for at least one month prior to surgery.

The patients with mammary carcinoma had been newly diagnosed and had undergone either mastectomy or removal of the mass as initial therapy. None of the patients had been treated by hormonal manipulation. Estrogen receptor assays were carried out on 14 of the 17 tumours, where 3 were receptor positive, 8 were receptor negative and 3 were equivocal. Normal breast tissue, obtained from patients undergoing mastectomy for breast carcinoma, was dissected from an area remote from the tumour and was judged by inspection to be separate from the tumour tissue. The data from two patients with mammary dysplasia and two with carcinoma were included in a previous publication (11).

Incubation and Isolation Procedure

Tissue was obtained at the time of surgery after representative sections had been removed for pathological study. Following removal, the tissue was kept at $0^{o}C$ and the parenchymal tissue was manually dissected from the surrounding adipose tissue. The material selected for incubation was washed in cold Krebs-Ringer bicarbonate buffer, pH 7.4, to remove adherent blood, and homogenized in the same buffer. The volume of buffer required for satisfactory homogenization varied with the weight and texture of the tissue. Tissue concentration was 0.2 - 0.6 g/ml for both gynecomastia and mammary dysplasia; 0.1 - 0.3 g/ml for carcinoma and 0.1 - 0.7 g/ml for normal tissue. After centrifugation at 300 g, the supernatant fraction was incubated at $37^{o}C$ for 90 min with cofactors under 95% O_2: 5% CO_2. ^{3}H-A (20×10^{6} dpm) was used at substrate concentrations ranging from 10 to 50 ng/ml.

As previously reported (15), the isolation procedure included phenolic partition, acetylation and TLC. In all purifications a constant ^{3}H:^{14}C ratio was achieved in the final 2 chromatograms.

RESULTS

Figure 1 summarizes the results of the 31 incubations. Final recoveries ranged from 25% to 35% for both estrone and estradiol, and

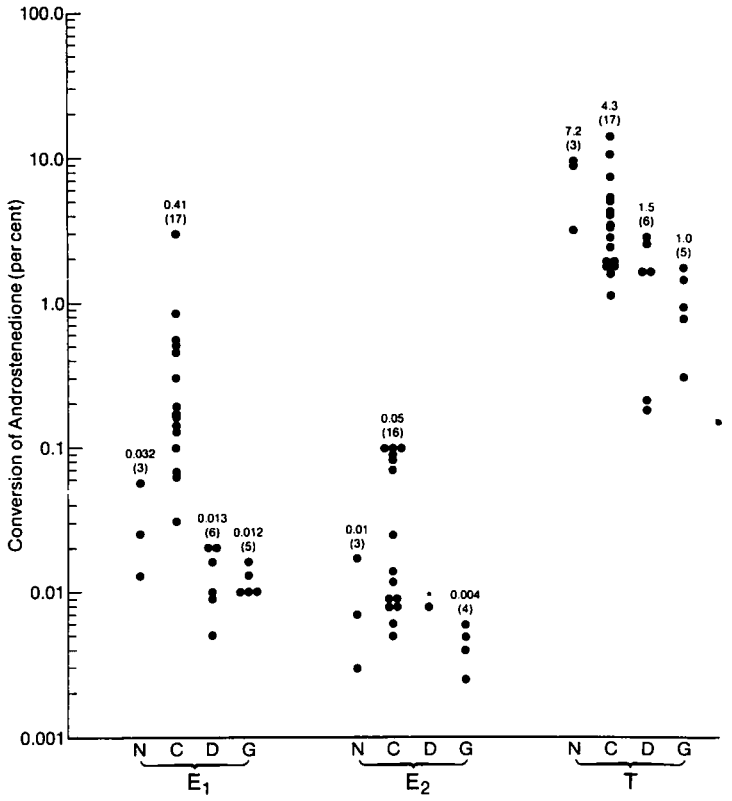

Figure 1. Comparison of conversion of androstenedione (A) to estrone (E_1), estradiol (E_2) and testosterone (T) in different breast tissues: normal (N), carcinoma (C), mammary dysplasia (D) and gynecomastia (G). Mean values are reported with the number of incubations in which product could be detected given in parentheses. * indicates that E_2 was not detected in 5 of 6 incubations.

from 40% to 50% for testosterone. All tissues formed E_1 and T in all incubations. E_2 was isolated in incubations of tissue from 1 of 6 patients with mammary dysplasia, 5 of 6 patients with gynecomastia and in all incubations with normal and carcinoma tissue.

Estrone formation in both gynecomastia and mammary dysplasia tissues was very low, with mean conversion values of 0.012% and 0.013% respectively. By contrast, the carcinoma tissue showed greater ability to aromatize to estrone, with the mean value of 0.4%. In one case, E_1 formation was as great as 3.0% while the lowest conversion was 0.01%. Most of the conversions, however, tended to range between 0.1% and 1.0%. In apparently normal tissue, E_1 formation was slightly higher than in gynecomastia and mammary dysplasia tissues, but even the highest conversion was lower than that found in most carcinoma incubations.

Estradiol formation was low in all tissues studied. The highest rates of conversion were found with carcinoma tissue, having a mean conversion value of 0.05%. The spectrum of conversion was broad with overlap among all the tissues.

Differences in testosterone formation between tissues were less pronounced. Testosterone formation was lowest in gynecomastia and mammary dysplasia tissues, with mean conversions of 1.0% and 1.5% respectively. Normal breast tissue and carcinoma tissue showed higher levels of T formation, having mean conversion values of 7.2% and 4.3% respectively. In all tissues T formation was about 100-fold greater than estrone formation under corresponding conditions.

DISCUSSION

It has been established that androgen metabolism occurs in breast tissue from patients with gynecomastia (13,14), mammary dysplasia

(11), breast carcinoma (1-10) and in normal breast tissue (5,7). The present study was designed to compare androgen metabolism in these tissues under the same set of experimental conditions.

These incubations have shown that, given the same substrate concentration, tissue from different subjects with the same breast disorder varies in its ability to form E_1, E_2 and T. When the incubations are considered according to tissue source, however, they indicate that tissue from different types of breast pathology may have specific patterns of hormone metabolism. This pattern may result in differences, both in the absolute amount and in the ratio of E_1 and T to which the cell is exposed.

There appears to be a metabolic heterogeneity in the group of carcinomas incubated, with the variation in E_1 formation being greater than that in T formation. There was no apparent correlation between receptor content and metabolic activity, but such a correlation might be detected in a larger series. Reports on the correlation between estrogen receptor and the clinical response to hormone manipulation indicate a response in about 50% of patients with estrogen receptor positive tumours, and 80% of patients positive for both estrogen and progesterone receptors (16). It is possible that some assessment of metabolic activity may also have value in predicting the outcome of such therapy. Perhaps, using a larger series, criteria for separating tumours within the heterogeneous population might be defined on the basis of their metabolic activity, histological appearance and receptor content.

Studies on the metabolism of androgens in breast tissue have tended to focus on the capabilities of these tissues to aromatize

androgens to estrogens. The formation of androgens with altered biological activity may, however, be another factor in the biological response of the tissue. The formation of T from A was always approximately 100-fold greater than the formation of E_1 in these incubations. In addition, A was converted to a group of reduced metabolites which migrated with DHT during the isolation procedure. Recrystallization studies have shown that this fraction contains small amounts of DHT, along with androsterone, etiocholanolone and other as yet unidentified metabolites. The conversion to this fraction was of a similar magnitude as the conversion of A to T. This metabolism of a relatively weak androgen such as A to compounds with altered biological activity such as T and a group of reduced metabolites may exert significant effects on a tissue. For instance, Zava and McGuire (17) have presented data showing that, whereas physiological levels (10^{-8}) of DHT affect only androgen receptors, high concentrations (10^{-6}M) translocate both estrogen and androgen receptors into the cell nuclei and stimulate growth of the MCF-7 cell line. Testosterone and 3β-androstanediol displayed similar estrogenic effects.

One point of interest at the onset of this study was whether local hormone formation was a factor in the initiation or maintenance of benign hormone responsive disorders such as mammary dysplasia and gynecomastia. These incubations have demonstrated low local formation of both estrogens and androgens in both tissues and hence would not support the hypothesis that increased local estrogen formation was implicated in either disorder. Whether the local androgen formation relative to estrogen formation could be a factor is still open to speculation.

ACKNOWLEDGEMENTS

The authors would like to express their appreciation to Dr. Betty Mobbs for performing the assays of estrogen receptors. We are also grateful to Mrs. Ann McCully for her assistance with the manuscript. This work was supported by grants from the Medical Research Council of Canada and the National Cancer Institute of Canada.

REFERENCES

The following trivial names and abbreviations have been used:

Androstenedione (A): 4-Androstene-3,17-dione
Androsterone: 3α-hydroxy-5α-androstan-17-one
3β-Androstanediol: 5α-androstane-3β,17β-diol
Dehydroepiandrosterone sulfate (DHAS): 17-oxo-5-androsten-3β-yl sulfate
Dihydrotestosterone (DHT): 17β-hydroxy-5α-androstan-3-one
Estradiol (E_2): 1,3,5(10)-estratriene-3,17β-diol
Estrone (E_1): 3-hydroxy-1,3,5(10)-estratrien-17-one
Etiocholanolone: 3α-hydroxy-5β-androstan-17-one
Testosterone (T): 17β-hydroxy-4-androsten-3-one

1. Miller, W.R. and Forrest, A.P.M. LANCET ii, 966 (1974).
2. Adams, J.B. and Li, K. BR. J. CANCER 31, 429 (1975).
3. Li, K., Chandra, D.P., Foo, T., Adams, J.B. and McDonald, D. STEROIDS 28, 561 (1976).
4. Miller, W.R. and Forrest, A.P.M. BR. J. CANCER 33, 116 (1976).
5. Miller, W.R., Shivas, A.A. and Forrest, A.P.M. CLIN. ONCOL. 4, 77 (1978).
6. Jones, D., Cameron, E.H.D. and Griffiths, K. BIOCHEM. J. 116, 919 (1970).
7. Miller, W.R., Telford, J. and Shivas, A.A. BR. J. SURG. 63, 153 (1976).
8. MacIndoe, J.H. J. CLIN. ENDOCRINOL. METAB. 49, 272 (1979).
9. Abul-Hajj, Y.J., Iverson, R. and Kiang, D.T. STEROIDS 33, 205 (1979).
10. Varela, R.M. and Dao, T.L. CANCER RES. 38, 2429 (1978).
11. Perel, E., Wilkins, D. and Killinger, D.W. J. STEROID BIOCHEM. 13, 89 (1980).
12. Abul-Hajj, Y.J. STEROIDS 26, 488 (1975).
13. Miller, W.R., McDonald, D., MacFadhey, I., Roberts, M.M. and Forrest, A.P.M. CLIN. ENDOCRINOL. 3, 123 (1974).
14. Rajendran, K.G., Shah, P.N., Bagli, N.P., Mistry, S.S. and Ghosh, S.N. HORMONE RES. 6, 329 (1975).
15. Perel, E. and Killinger, D.W. J. STEROID BIOCHEM. 10, 623 (1979).
16. McGuire, W.L., Horwitz, K.B., Zava, D.T., Garola, R.E. and Chamness, G.C. METABOLISM 27, 487 (1978).
17. Zava, D.T. and McGuire, W.L. ENDOCRINOLOGY 103, 624 (1978).