A Reassessment of the Role of Breast Tumor Aromatization

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Abstract

While the role of estrogens in the maintenance of human breast carcinoma has been firmly established for many years, the sources of this estrogen remain unresolved. Input-output analysis of steroid uptake by breast carcinomas showed no specific uptake of estrogens from blood. Based on the detection of the necessary enzyme systems, Adams and Wong proposed that breast tumors could function as "paraendocrine organs" capable of producing sufficient estradiol (E$_2$) to stimulate their own growth (Adams, J. B., and Wong, M. S. F. Lancet, 2: 1163, 1968). Using the reported values for the concentration of C$_19$ steroid precursors and the $p_k^{E_2}$ values one can estimate that the contribution of in situ aromatization to the tumor estrogen pool is quite small.

Introduction

A role for estrogens as promoters of human breast tumor has been accepted since Beatson (11) demonstrated a beneficial response to ovariectomy in women with breast cancer. Subsequent studies on animal and human breast tumors (12, 16, 19, 21, 33, 36, 37, 44) have shown that estrogens directly stimulate the growth of breast tumors. The still unfolding story of estrogen receptors originating in the work of Jensen et al. (20) and Gorski and Gannon (18) has provided a mechanism for the effects of estrogens in promoting tumor growth. Only minimal consideration was given, however, to the source of the hormone. Until the mid-1960's, the source of the estrogen was considered to be external to the breast. Support for this view came from the favorable response to medical or surgical adrenalectomy, hypophysectomy, or ovariectomy. This concept was challenged when Adams and others (3, 6-8, 13) demonstrated the presence of all of the enzyme systems required for the transformation of cholesterol to estrogens (Chart 1) in breast tumors and proposed that these tumors should be regarded as "paraendocrine organs" (7). Subsequent to these initial findings, attention was focused almost entirely on firmly establishing aromatization in breast tumors as outlined below. Any consideration of the quantitative importance of "in situ" aromatization in breast tumors requires examination of 2 items: how much estrogen is required to maintain a biological response; and the steady-state contribution of in situ aromatization to the tumor estrogen pool.

Examination of the scheme for steroid receptor action (Chart 2) permits us to estimate the former. Although the results clearly show that the extent of the early responses to estrogen (water imbibition, glucose uptake, etc.) parallels the degree of saturation of the nuclear receptors (38), the same does not appear to be true when the long-term effects of estrogen, in particular true growth as exemplified by an increase in uterine dry weight (5, 34), are studied. Under conditions of continuous stimulation, maximum responses are observed with occupancy of as little as 30% of the maximum level of nuclear receptors. Since we are primarily concerned with the extent of growth of breast tumors, it seems logical to use the requirements for long-term action in calculating hormonal requirements. Since maximal response was obtained at the 30% receptor occupancy level, it seems reasonable that some response will be obtained at 20% receptor occupancy, although the data in Ref. 9 suggest that little or no response will be obtained at levels much below this.

Hormone Requirements

Binding of estradiol (E$_2$) to the receptor (E$_n$) follows classic Michaelis-Menton kinetics

$$[E_2] + [E_n] \rightarrow [E_2,E_n]$$

(A)

and

$$K_d = \frac{[E_2][E_n]}{[E_2,E_n]}$$

(B)

As Anderson et al. (10) have shown, the $K_d$ at 37° does not differ greatly from that at 4° and is $\sim 1 \times 10^{-8}$ M. The 37° value is the appropriate value for our calculations, since we are interested in approximating in vivo conditions.

To calculate the steroid concentration required to achieve 20% saturation, we can rearrange Equation B

$$E_2 = \frac{[E_2,E_n]}{[E_2,E_n] + [E_n]} = \frac{1 \times 10^{-8} \times 2}{8} = 2.5 \times 10^{-10} M$$

If we go to 10% saturation and a corresponding lower level of response, the required steroid concentration will be only slightly lower.

$$\frac{1 \times 10^{-9} \times 1}{9} = 1.1 \times 10^{-10} M$$

Estradiol Production by Breast Tumors

Estradiol production by breast tumors has been studied using stably labeled tracers (e.g., $[7\alpha-^3H]$testosterone, $[7\alpha^{-}\text{H}]$dehydroisoandrosterone) (a) and by the tritium water release method of Siiteri using $[1\beta^{-3H}]$testosterone (39, 40) (b). The mean conversion for each of the 2 procedures will be computed separately.

Although the earliest study with confirmed estradiol formation reported an almost trivial $p_k^{T}$ value of 0.0005% (22), subsequent studies by Abul-Hajj et al. (1-4), Adams et al. (6-8), Miller (28-32) and others (23-25, 35, 43) with stably labeled tracers have established a small but very real conversion of androgenic precursors to estradiol. In an effort to critically validate the formation of estradiol, Adams and Li (6) used a...
highly specific derivatization of estradiol to estradiol 3-sulfate. Using method a, 173 tumors were studied of which 115 were positive for estradiol formation. The mean conversion was 0.02% for all of the tumors and 0.029% for the positive tumors not correcting for the blank reaction.

A major source of error in all assays using stably labeled tracer compounds is the purity of the compounds used. Almost all of the commercially available labeled steroids are at best 96 to 98% pure, and even after careful laboratory repurification the amount of impurities remaining is far greater than the p values obtained in these aromatization studies. As MacInnes (27) has shown, the blank conversion of [3H]androstenedione to estradiol amounted to 0.001 and 0.005%/hr when [6,7-3H]testosterone was used as the precursor. If we use these figures to correct the results obtained in the studies described above where no blank correction was carried out, then as much as 20% of the reported p values may represent this kind of blank conversion.

When the tritium water release technique was used, the blank reaction is relatively small (50 to 60 cpm/hr) and was readily corrected for in carrying out the incubation studies. Using the figures obtained by Siiteri (40) or Santen et al. (39), the mean p value was 0.026% in close agreement with the stable labeled tracer technique. The methods agree quite well, and the combined mean value is 0.023%.

**Availability of Precursors**

If we accept these mean conversion values without correction as a reasonable upper limit for the p value, the other critical limiting value is the amount of precursor present in breast tumors. Recent studies by Thijssen (41) and VanLandeghem et al. (42) have shown an absence of any significant uptake or release of 9 of the 10 steroids studied in human mammary tumors in vivo. Both uptake and release of compounds in a random manner were observed. In the case of the tenth compound, Δ^4-androstenedione, a small but measurable uptake was observed. Significantly, no correlation was observed between uptake of testosterone or androstenedione and the release of estrone or estradiol.

Maximal values for testosterone and Δ^4-androstenedione in peripheral blood were reported to be 2 and 7 nm, respectively, both pre- and postmenopausally. In view of the lack of specific uptake or release, we can assume that the level for these compounds in tumor tissue should be no greater than plasma values. The other potential precursor, dehydroisoandrosterone, has been reported to be present in considerably greater amounts. Adams et al. (5) reported 14.4 ng/g in tumors obtained from premenopausal subjects and 7.2 ng/g in tumors obtained from postmenopausal subjects, while Maynard et al. (26) reported a mean level of 35 ng/g using a gas chromatography-mass spectrometry assay procedure. Both are well above the plasma levels (4.5 ng/g) which have been reported (42).

If we take the testosterone and Δ^4-androstenedione, the proximate precursors in the aromatization sequence, as the quantitatively important sources for in situ estradiol formation and the mean p values computed above, we can estimate the mean estradiol production per g of tumor

\[ 9 \text{ nm} \times 0.00023 = 1.8 \text{ pM or } 0.5 \text{ pg/g/hr} \] (C)

If we take the more abundant steroid, dehydroisoandrosterone, as the critical precursor even though it is several more steps removed from estradiol than using the mean value reported by Adams, we can calculate 43 nm × 0.0023 = 9.9 pM or 2.6 pg/g. Using the higher value reported by Maynard, the yield is 28 pm or 2.5 pg/g of tumor. There is thus a 15-fold range in the possible production of estradiol in tumors.

In light of studies on the metabolism of estradiol in breast tumors (17), it seems likely that these levels represent the steady-state contribution of in situ biosynthesis. Moreover, while these calculations are based on in vitro estimates of p values which may be lower than the in vivo values, we also do not have the losses by diffusion and blood transport occurring under the latter conditions. Thus, on net balance the in vitro values may well approximate the actual in vivo production values.

As calculated above for 20% saturation, we need a concentration of 250 pm, while the contribution from testosterone and androstenedione was only 1.8 pm or 0.7% of the amount needed for a minimal biological response. Using the highest values of 28 pm based on Maynard’s estimate of dehydroisoandrosterone, we obtain a value which is still only 11% of the amount required for a biological response. Even if we accept a still lower 10% receptor occupancy as sufficient to elicit a significant response, the highest of the production values is still only 25% of the amount required.

In light of these estimates of in situ production of estradiol relative to the amounts required to achieve a biological response, the role of the breast tumor as a quantitatively impor-
tant “paraendocrine organ” supplying sufficient estradiol to have a biological impact seems doubtful. Moreover, measurement of the estradiol content of breast tumor tissue has shown quite high values for estradiol (250 to 450 pg/g) according to the paper of Fishman et al. (15) and a mean value of 1500 pg/g according to the results of Edery et al. (14). The probability that the low synthesis rates which we have estimated can contribute to these levels is low. These findings based on the mean conversion rate for all tumors do not preclude meaningful conversion of C19 precursors to estradiol in that small subset of tumors with high (>0.15%) p values. Both Abul-Hajj (2, 4) and Miller (28–32) have reported a small number of such cases. In these cases, more than enough estradiol is formed in situ to saturate sufficient receptors to generate a significant biological response.

Aside from these considerations, consideration of the other compounds present in breast tumors raises questions about the need to consider estrogen as an agonist in breast tumors. High levels of dehydroisoandrosterone and androstenediol have been found in breast tumors (5, 26). The latter has been shown to function as a partial agonist-antagonist capable of translocating the estrogen receptor to the nucleus and effecting a biological response.

In light of all of these considerations, the probability that the aromatase system present in breast tumors plays a quantitatively important role as a source for the estrogen present in breast tumors in more than a small subset of tumors seems highly unlikely. These results do not, however, exclude the possibility that in situ aromatization could serve as a marker for tumor behavior such as responsiveness to hormonal therapy or growth rate.

References

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