In Vivo Metabolism of Testosterone-$^3$H in R-3327, an Androgen-sensitive Rat Prostatic Adenocarcinoma

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SUMMARY

A spontaneous prostatic adenocarcinoma (R-3327) of a Copenhagen rat has been maintained for 14 generations by s.c. transplantations to Copenhagen and F$_1$ hybrid hosts. The tumor remains histologically a well-differentiated adenocarcinoma. In order to study the role of gonadal hormones in this system, tumor explants were inoculated into intact males, castrated males, and intact females, and the growth of the explants was followed for 5 months. The tumor grew well only in intact males; it grew moderately well in intact females and did not grow in castrates. The metabolism of testosterone-$^3$H by this tumor was studied by injecting 0.5 mCi of the labeled steroid (specific activity, 45 Ci/mnmole) into tumor-bearing males 1 hr before decapitation. Samples of tumor tissue, liver, muscle, and prostate were obtained and the distribution of radioactive metabolites of testosterone-$^3$H was analyzed by thin-layer chromatography. 5a-Dihydrotestosterone and 3a-androstanediol were the major metabolites found in both the tumor and the prostate. These metabolites were absent or were found in smaller amounts in the nontarget tissues, liver and muscle. The preferred metabolism of testosterone via 5a-reduction, together with the better growth of the tumor in intact males, indicates that this is an androgen-responsive tumor.

INTRODUCTION

It is well established that androgens play a role in the etiology of prostatic neoplasia; however, the biochemical mechanisms involved are still poorly understood. The lack of suitable experimental models of prostatic carcinomas that exhibit androgen dependency has always been a major obstacle to the design of meaningful experiments in this area of research. Although squamous cell carcinomas of rat prostate have been induced by implantation of carcinoargent pellets in the ventral prostate (5, 10), no adenocarcinoma has been reported to have resulted from this or any other method of induction.

In 1963, Dunning (4) described a spontaneous tumor in the prostate of a Copenhagen rat, which had the histological appearance of a functional adenocarcinoma. The tumor (R-3327) has since been successfully transplanted to genetically compatible hosts and is now in its 14th generation. In this paper we report our studies of the metabolism of testosterone-$^3$H by this tumor. These results, together with other observations, indicate that R-3327 is an androgen-sensitive tumor and is responsive to endocrinological manipulations.

MATERIALS AND METHODS

Animals and Tumors. The prostatic adenocarcinomas have been maintained by s.c. transplantation from the original Copenhagen male host to either Copenhagen or F$_1$ hybrids (from Copenhagen and Fischer rats). Inoculations were made with a trocar needle on both flanks of the animals. Approximately 1 to 2 cu mm of tumor tissue were injected in each site. The tumors became palpable after 60 days and thereafter their average diameters were recorded at various times. Three groups of 10 adult male animals each were inoculated with tumor tissue: (a) intact males, (b) intact females, and (c) castrated males.

Administration of Testosterone-$^3$H. In order to study the in vivo metabolism of testosterone by the tumor, the labeled steroid was injected s.c. into intact males bearing large tumors. Each animal received 0.25 mCi of testosterone-1,2-$^3$H (New England Nuclear Corp., Boston, Mass.; specific activity, 52.2 Ci/mnmole) dissolved in 10 µl of ethanol in 0.12 ml of 0.9% NaCl solution, and was killed by decapitation 1 hr after the injection.

Extraction and Analysis of Steroids. Samples of tumor tissue, liver, musculus quadriceps, and ventral prostate weighing between 300 and 400 mg were excised, weighed, and minced in 25 ml of ethanol. To each flask, 100 µg of testosterone, 17β-hydroxy-5a-androstan-3-one, 5α-androstan-3α, 17β-diol, and 5α-androstan-3,17-dione were added. After overnight extraction, the ethanol was decanted and the tissue was homogenized in a TenBroeck homogenizer with another 25 ml of ethanol plus 10 ml of water. The homogenate was centrifuged and the ethanol extracts were pooled and evaporated to dryness. The evaporating flasks were rinsed with 25 ml ethyl acetate plus 15 ml of water with shaking. The phases were separated in a separatory funnel with the aid of 3 to 4 ml of a saturated solution of sodium sulfate. The aqueous phase was further extracted with 20 ml...
of ethyl acetate, and the organic phases were pooled and evaporated to dryness. In order to eliminate some non-steroidal lipids which often interfere with chromatographic separation, the flasks were rinsed with 60 ml of 70% methanol:petroleum ether (3:1 v/v).

To the residue from the methanol extract, 200 µg each of testosterone, androst-4-ene-3, 17-dione, 17β-hydroxy-5α-androstan-3-one, 5α-androstan-3, 17-dione, and 3α-androstan-3α, 17β-diol were added and the mixture was dissolved in ethanol and assayed by thin-layer chromatography as described previously (15).

**Histology.** Samples of tumor tissue were fixed in Carnoy's fixative, embedded in paraffin and sectioned in slices 6 µm thick. The resulting slides were stained in hematoxylin and eosin.

**RESULTS**

**Metabolism of Testosterone-3H.** One hr after the animals received injections of testosterone-3H, the distribution of radioactivity into the various metabolites of testosterone is shown for various tissues (Charts 1 and 2). The shaded areas indicate the location of various steroid standards added prior to chromatography and made visible by exposure to iodine vapors (Areas II to V). Area I does not represent a specific steroid, but is probably due to polar lipids extracted from the tissues. Some polar steroids are regularly found coinciding with these polar lipids in Area I. It can be seen (Chart 1) that the pattern of testosterone metabolism in the normal prostate gland is very similar to that in the transplanted R-3327 tumor tissue. The major metabolites in both tissues have been identified, after crystallization to constant specific activity, as 17β-hydroxy-5α-androstan-3-one and 5α-androstan-3α, 17β-diol, both 5α-reduced metabolites of testosterone. These 2 metabolites account for 28.0 and 36.6% of the total radioactivity in the chromatograms from the prostate and tumor tissue, respectively. Polar metabolites account for 10.3 and 12.5% of the radioactivity in prostate and tumor, respectively. Relatively small amounts of 4-androsten-3,17-dione were found in the above-mentioned tissues. In contrast, the radioactive metabolites from muscle and liver (Chart 2) contain little 5α-reduced metabolites, such as 17β-hydroxy-5α-androstan-3α, 17β-diol. The somewhat large peak of radioactivity found adjacent to Area II (5α-androstan-3α, 17β-diol) in the chromatogram from liver contain less than 30% of the 3α-diol as shown after repeated crystallization with testosterone; Area IV, 17β-hydroxy-5α-androstan-3-one; Area V, 4-androstene-3, 17-dione. Values are given per 100 mg of tissue. Percentages of the various metabolites are given in the text after consideration of the peaks purities.
carrier 3α-diol. The radioactive material did not cocrystallize either with 3β-diol standard (less than 5%). The largest zone of radioactivity in the liver chromatogram was Area 1, corresponding to polar metabolites (72.5% of total radioactivity).

**Tumor growth.** The growth of R-3327 tumors following transplantation to intact males, intact females, and castrated males is shown in Chart 3. It can be seen that the average tumor mass increases at a higher rate in males than in females or castrates, in which growth is almost nil. Since each point of the curves in Chart 3 represents the mean diameters of all 12 tumors implanted in a given group (6 animals, 2 tumors/animal), and since some tumor implants frequently do not take at all on one side of the animal, while the contralateral implant grows well, the data thus expressed tend to diminish the difference between the growth rates of those tumors that did take, in either males or females. In the latter case, up to a 5-fold difference in growth rate can be observed between males and females. The incipient tumors observable in castrates have remained stationary 90 days after the last measurement reported in Chart 3.

**Histology.** The tumor remains an obvious adenocarcinoma exhibiting hyperplastic epithelium lining many of the acini. The basal membrane of the glandular epithelium is not generally observed, indicating invasiveness. Abundant acinar secretion is also readily observed. Stromal tissue is present in a much higher degree than in normal rat prostatic tissue. Mitotic figures are rare as would be expected in a slow-growing tumor such as R-3327.

**DISCUSSION**

The metabolism of testosterone in the prostate gland and other androgen-sensitive organs of the genital tract of mammals, including man, has been well-documented (1, 3, 6, 7, 13). A common feature in the metabolic pathway of testosterone in these organs is the 5α-reduction of the steroid ring to yield mainly 17β-hydroxy-5α-androstan-3-one and the 5α-androstan-diol. The 5α-reductase enzyme responsible for this reduction has been characterized in our laboratories (15, 16) as well as in others (11, 12, 14). The importance of the 5α-reduced metabolites of testosterone as mediators of androgen action in androgen-dependent tissues has been suggested by many investigators (for a review, see Ref. 17). From the above data, the concept has emerged that a characteristic feature of androgen-sensitive tissues is a predominance of 5α-reduction pathway in the metabolism of testosterone by these tissues, instead of the oxidative pathway leading to 4-androsten-3,17-dione exhibited by muscle or hydroxylative pathways as in liver. Although these other tissues, especially the liver, contain active 5α-reductases (8, 12), their role in testosterone metabolism is relatively minor compared with that in typically androgen-dependent tissues. Recently, this fact has been emphasized by Yamaguchi et al. (18) while studying testosterone metabolism in the androgen-dependent mammary Shionogi carcinoma 115 (9).

Our observations in the present study, showing extensive in vivo conversion of testosterone-4H to 5α-reduced metabolites by the prostatic carcinoma R-3327 and by normal prostate gland, and not by either muscle or liver tissue, are in harmony with the view that R-3327 is an androgen-sensitive tumor. The fact that indeed R-3327 grows better when transplanted to male rats than when transplanted to females or castrated males, substantiates this view.

As mentioned above, we believe that the data in Chart 3 somewhat minimized the true differences in growth rates between males and females, since the average values include tumors that did not take at all. That these occurrences are not hormonally related is demonstrated by the fact that usually the contralateral implant grew well. However, for statistical accuracy, the present form of presentation of the data was chosen.

It is somewhat puzzling that tumors grew better in female animals than in castrated males since neither have testicular androgens, and their adrenal steroidogenesis should be similar. Still, it is possible that androgen levels are higher in female rats than in castrated males and that this accounts for the difference. Experiments to measure these values are currently in progress. Alternatively, it could be that estrogen has an effect on R-3327 growth, but this duality of hormonal sensitivity is uncertain.

In summary, we believe that R-3327, by its demonstrated androgenic sensitivity and its resemblance to normal prostate tissue, both by histological observations and by its pattern of testosterone metabolism, affords an excellent tissue model for further studies of the hormonal and other biochemical mechanisms in prostatic carcinoma.

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