Hormonal Regulation of the Metabolism of Carcinogens in Renal Tissue of BALB/c Mice

Suresh Mohla, F. R. Ampy, K. J. Sanders, and Wayne E. Criss

Departments of Oncology [S. M., W. E. C.], Genetics and Human Genetics [K. J. S.], Human Nutrition and Food [W. E. C.], and Zoology [F. R. A.], Howard University Cancer Center, Washington, D. C. 20059

Abstract

The role of androgens in the regulation of carcinogen metabolism in the renal tissue of BALB/c mice was investigated. Kidney microsomal enzyme preparations from mature and immature animals were used in mutagenic studies using the Ames test. Androgen receptors (cytosolic and nuclear) were also evaluated. The results show that the microsomal enzymes from mature males had greater potential to biotransform dimethylnitrosamine than did the microsomal enzymes from mature females or immature animals. Testosterone treatment of mature females or immature animals resulted in a significant increase in the mutagenic ability of their renal microsomal enzymes. Androgen receptors were detected in kidney cytosols of mature and immature animals (both males and females); however, nuclear androgen receptors were detected only in the mature males. Testosterone treatment resulted in a significant accumulation of nuclear androgen receptors in the kidneys of mature females and immature animals. The relationships among mutagenic activity, androgen receptors, the levels of N-demethylase (an enzyme responsible for conversion of dimethylnitrosamine to its active metabolite), dietary fat, and the carcinogen metabolism are discussed.

Introduction

Epidemiological and clinical observations have led to the hypothesis that human renal cancer is a hormone-dependent cancer. This concept is supported by the following observations: (a) renal carcinoma is twice as common in men as in women; (b) administration of progestins causes greater regression of metastatic renal cancer in men than in women; and (c) estrogens and androgens promote the growth of renal carcinoma (5, 6, 14, 15). Thus, steroid hormones have been implicated in renal cancer. In addition, recent evidence indicates that these hormones also play an important role in regulating biosynthetic activities of normal renal tissue (8, 15, 16).

There is ample evidence to indicate that obesity or high intake of dietary fat can elevate plasma steroid hormone levels, especially plasma androgens and estrogens in both women and men (9–11, 17, 20, 22). This increase in the circulating levels is partially due to increased adrenal activity that results in an increased conversion of adrenal steroids to estrogens and androgens. Thus, it would appear that obesity in humans could render the renal tissue more susceptible to elevated circulating androgens.

The susceptibility of male renal tissue to tumorigenesis (perhaps as a result of androgen sensitivity) can also be evidenced in laboratory animals. Several studies have indicated that species- and sex-related differences in drug metabolism play an important role in the response of individuals to the effects of chemical carcinogens, especially those chemicals which require metabolic biotransformation to ultimate carcinogenic forms (13). The results indicate that male rodents may be more susceptible to renal cancer than are females. Furthermore, DMN2 and 2-acetylaminofluorene metabolism in the mouse kidney is under androgenic control. The androgen-regulated microsomal activity in the kidneys of male and female mice may be a factor in the etiology of certain chemically induced renal cancers (3).

These differences strongly suggest a role for sex hormones in the metabolism of certain chemical carcinogens and, hence, differential susceptibility to renal tumorigenesis in males and females of the same species.

Although steroids per se are not transformed to bacterial mutagens by microsomal preparations, they appear to have a highly specific regulatory action on the metabolic capacity of organs at work (12, 19). Clearly, if the sex hormones play a role in the conversion of a procarcinogen into a mutagen (or carcinogen), the tissue so implicated must contain receptors for those steroids. It has been clearly demonstrated that steroid hormones act on their target cells by interacting first with specific cytoplasmic receptors. The hormone-receptor complex is then activated and translocated into the nucleus, where it associates with chromatin at specific acceptor sites (12).

Although 5α-dihydrotestosterone (17 β-hydroxy-5α-androstan-3-one) is the biologically active metabolite of testosterone in male reproductive tissues, the biologically active hormone in the kidney is testosterone (4). Specific testosterone receptors have been identified in the kidney, while 5α-dihydrotestosterone receptors have been observed in normal, benign, and malignant prostate tissue. Thus, it is possible that the increased ability of the kidney microsomal fraction (in the males) to convert procarcinogens to mutagens may be due to (among other factors) modification of the metabolism of chemical mutagens (carcinogens). It is also possible that androgens may regulate the metabolic activity of these microsomal enzymes.

The androgenic modulation of androgen receptors in male and female renal tissues and the alterations of N-demethylase, an enzyme postulated to be responsible for the conversion of DMN to its mutagenic form, are briefly presented in this current report. A preliminary report of this work has been published (2).

1 Presented at the Workshop on Fat and Cancer, December 10 to 12, 1979, Bethesda, Md. Supported by National Cancer Institute Grant CA-14718 and NIH Grant 2800 RR08016-10.

2 The abbreviations used are: DMN, dimethylnitrosamine; R1881, methyltrienolone [17β-hydroxy-17α-methylstra-4,9,11-triene-ethylenbis(oxy)pregna-1,4-diene, 3,20-dione].
Materials and Methods

Animals and frozen tissues used in this study were purchased from Flow Laboratories, Rockville, Md., and Pel-Freeze Biologicals, Rogers, Ark., respectively. Mutagenesis assays were performed as described by Ames et al. (1).

Androgen Receptor Assays

Cytosol Receptors. Tissue homogenization, cytosol preparation, and incubation of cytosol with hormones were essentially as described by Jensen et al. (12). Briefly, kidney cytosols were preincubated with 10^{-6} M triamcinolone acetonide to rule out nonspecific binding of [^3]HJR1881 to progesterone receptors. Cytosols were then incubated with 10^{-8} M [^3]HJR1881 in the presence or absence of 200-fold excess, nonradioactive R1881. After incubation at 20° for 1 hr or 0° for 18 hr, Dextrancoated charcoal was added to remove the unbound radioactivity. Results are expressed as fmol of receptors per mg of tissue.

Nuclear Exchange Assay. The procedure as described by Menon et al. (18) was used with minor modifications (21). Briefly, the KCl extract was diluted and incubated with [^3]HJR1881 in the presence or absence of excess R1881. Triamcinolone acetonide (10^{-7} M) was present in all tubes. After incubation, aliquots of samples were precipitated with protamine sulfate and processed as described (18). N-Demethylase activity of the kidney microsomal fractions was assayed as described by Brodie and Axelrod (7).

Results and Discussion

The in vitro activation of DMN by the microsomal fractions (S-9) from various tissues of mice, rats, and monkeys is shown in Table 1. The results indicate that microsomal fractions from male and female liver tissues have similar capacity to biotransform DMN.

However, apparent differences exist among the animal species. Mouse liver S-9 fractions were more active than rat liver S-9 fractions. In contrast, kidney S-9 fractions from adult male BALB/c mice demonstrated a greater potential to activate DMN than did kidney S-9 fractions from adult female, immature male, or female BALB/c mice (data not shown). The S-9 fractions from adult rat and adult female kidneys showed little potential to activate DMN. These data indicate that androgenic hormones may influence the ability of kidney microsomal enzymes to transform DMN into its active metabolite(s). Experiments were therefore designed to test this hypothesis.

The kidney S-9 fractions from immature mice (2 to 5 weeks) exhibited minimal activity in the in vitro activation of DMN. In this regard, there were no differences between male and female mice. With advancement of puberty, the S-9 fractions from male kidneys showed a significant increase in biotransforming ability as evidenced by the Ames test. In the mature males, the biotransforming potential of the kidney S-9 fractions was 8- to 10-fold higher compared to the activity of S-9 fractions from immature mice (of both sexes) or mature females. Testosterone treatment (1 mg/day for 7 days) of immature mice or mature female mice resulted in a significant increase (8- to 12-fold) in the biotransforming potential of kidney S-9 fractions, reaching the levels observed in mature males. Testosterone treatment of mature males caused no significant increase in the activity of the kidney S-9 fractions when compared to that in noninjected adult males. Thus, androgenic hormones appear to play a role in modulating the biotransforming potential of kidney S-9 fractions in BALB/c mice.

The modulations of cytoplasmic and nuclear androgen receptors were next analyzed in attempts to explore the relationship between the potential ability of kidney to activate DMN (in vitro). One question to be asked is whether there is receptor-mediated regulation of enzymes which are responsible for biotransformation of procarcinogen to mutagen (carcinogen).

Our data on androgen receptors in the kidney cytosol indicate the presence of specific cytoplasmic receptors (as measured by [^3]HJtestosterone or [^3]HJR1881 binding) in both male and female renal tissue (data not shown). In contrast, nuclear
androgen receptors are detected only in the kidney tissue of male mice. Administration of testosterone to mature female mice (2 mg/day for 10 days) resulted in a significant accumulation of translocation of androgen receptors in kidney nuclei (Chart 1). The data in Chart 1 also show absence of nuclear androgen receptors in immature renal tissue from untreated mice.

Our preliminary data on the levels of N-demethylase activity (7) indicate a much higher level of enzyme activity in the liver tissues of adult males and females compared to the enzyme activity of kidney tissues. Further, the N-demethylase activity in kidney tissue is higher in male compared to that in female BALB/c mice.

Thus, a correlation exists between in vitro mutagenic ability of kidney microsomal fractions and the levels of N-demethylase, the enzyme which activates the carcinogen DMN. Thus, it is possible that the increased ability of the kidney microsomal fraction, in males and testosterone-treated females, to convert the procarcinogen to mutagen (carcinogen) may partially be due to modification of the metabolism of certain chemical carcinogens. Androgen receptors could regulate this metabolism by modulating the activity of the enzyme(s) responsible for the biotransformation. It has been clearly demonstrated that androgens elicit their biochemical responses by first interacting with and then activating their specific cytoplasmic receptors, which are subsequently translocated to the nucleus. Whether androgen receptors regulate the activity of the biotransforming enzymes via transcription and/or posttranscription-translational mechanisms or whether the synthesis or activation of steroid hormone receptors may be under some common or related control point which is affected by the tumorigenic process remains to be elucidated. The BALB/c mouse kidney system can serve as a useful experimental model to study the mechanism(s) by which steroid hormones augment or inhibit biotransformation of procarcinogens to mutagens. The role of dietary fat in the biotransformation process remains to be evaluated in this system.

References


Hormonal Regulation of the Metabolism of Carcinogens in Renal Tissue of BALB/c Mice

Suresh Mohla, F. R. Ampy, K. J. Sanders, et al.


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/41/9_Part_2/3821

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.