Localization of Estrogen-induced DNA Adducts and Cytochrome P-450 Activity at the Site of Renal Carcinogenesis in the Hamster Kidney

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ABSTRACT

Renal carcinoma in male Syrian hamsters, induced by chronic administration of estradiol for 5–7 months, is known to arise in the cortex at the cortico-medullary junction. In this in vivo model for hormonal carcinogenesis, estrogen-induced covalent DNA adducts have previously been observed in whole kidney and have been postulated to be involved in tumor induction. In the present study, the intrarenal distribution of estrogen-induced DNA modification and estrogen metabolizing enzymes were investigated in male Syrian hamsters to ascertain a role of metabolism and adduct formation in estrogen-induced carcinogenesis. The highest estrogen-induced DNA adduct concentrations as measured by 32P-postlabeling analysis were found in the renal cortex of hamsters treated with estradiol for 7 months. Total adduct levels in medullary DNA were approximately one-half of those found in cortex. Cytochrome P-450 enzymes were detected only in microsomes of kidney cortex (approximately 0.8 ± 0.6 nmol P-450/mg protein) but not medulla of untreated male Syrian hamsters. Prostaglandin endoperoxide synthase activity in kidney cortical microsomes was 1/3 of the activity found in medullary microsomes. Thus, microsomal cytochrome P-450 levels and estrogen-induced DNA adduct formation were highest in hamster kidney cortex, the origin of renal tumorigenesis. It is postulated that estrogen metabolism by cytochrome P-450 enzymes leading to covalent DNA modification plays a role in hormonal carcinogenesis in the hamster kidney.

INTRODUCTION

Estrogens induce tumors in laboratory animals and are increasingly associated with genital tract malignancies in humans. The mechanism of carcinogenesis is unknown. However, evidence is accumulating from several laboratories that estrogen-induced neoplastic cell transformation or carcogenic activity cannot be correlated with hormonal potencies of the substances tested (2, 3). Consequently, estrogen metabolism was postulated to play a key role in hormonal carcinogenesis (4–6). Several enzyme systems have been shown to oxidize or cooxidize estrogens and have been associated with hormonal carcinogenesis. Of the many metabolic hydroxylations of estradiol (E2) by cytochrome P-450-mixed function oxidase (for a review see Refs 7 and 8), aromatic hydroxylation (catechol estrogen formation) has been correlated with neoplastic transformation of BALB/c 3T3 cells (subclone A-31-1-13) (6, 9) and incidence of estrogen-induced renal carcinoma in Syrian hamsters (10, 11). Also, elevated 16α-hydroxyestradiol or -estrone formation has been associated with increased tumor incidence in mice and in humans (12, 13). Peroxidases isolated from various sources oxidize diethyldithilestrol and also catechol estrogens to their corresponding quinones (14, 15) and have therefore been postulated to mediate estrogen-induced carcinogenesis (16). Finally, prostaglandin endoperoxide synthase containing both cyclooxygenase and hydroperoxidase activity cooxidizes diethyldithilestrol to Z,Z-dienestrol (17, 18). This reaction has been demonstrated (19) to be a major metabolic pathway in Syrian hamster embryo cells and has been postulated (19) to be involved in neoplastic transformation of these cells. At present, it is not clear which of these enzyme systems is involved in induction of hamster renal carcinoma by estrogen, an accepted model for the study of hormonal carcinogenesis (20). Furthermore, the role of metabolism in the formation of covalent DNA adducts by estrogen specifically in the hamster kidney (21) has also not been elucidated. Covalently modified nucleotides of unknown structure (21, 22) were induced by estrogens specifically in the target organ of carcinogenesis and preceded hormonal cancer indicating their involvement in hormonal tumorigenesis. Estrogens elicited these DNA adducts, but were not a part of the modified nucleotide structure (21). Therefore, the role of metabolism in estrogen-induced DNA damage and subsequent tumor formation may have unique features, since the activation of unknown endogenous genotoxic factors is elicited by the estrogens. In the present study, cytochrome P-450 and prostaglandin endoperoxide synthase activities were therefore investigated in Syrian hamster renal tissue. Enzyme activities were measured in cortex and medulla and related to the intrarenal site of estrogen-induced covalent DNA adduct formation and carcinogenesis.

MATERIALS AND METHODS

ABSTRACT

Renal carcinoma in male Syrian hamsters, induced by chronic administration of estradiol for 5–7 months, is known to arise in the cortex at the cortico-medullary junction. In this in vivo model for hormonal carcinogenesis, estrogen-induced covalent DNA adducts have previously been observed in whole kidney and have been postulated to be involved in tumor induction. In the present study, the intrarenal distribution of estrogen-induced DNA modification and estrogen metabolizing enzymes were investigated in male Syrian hamsters to ascertain a role of metabolism and adduct formation in estrogen-induced carcinogenesis. The highest estrogen-induced DNA adduct concentrations as measured by 32P-postlabeling analysis were found in the renal cortex of hamsters treated with estradiol for 7 months. Total adduct levels in medullary DNA were approximately one-half of those found in cortex. Cytochrome P-450 enzymes were detected only in microsomes of kidney cortex (approximately 0.8 ± 0.6 nmol P-450/mg protein) but not medulla of untreated male Syrian hamsters. Prostaglandin endoperoxide synthase activity in kidney cortical microsomes was 1/3 of the activity found in medullary microsomes. Thus, microsomal cytochrome P-450 levels and estrogen-induced DNA adduct formation were highest in hamster kidney cortex, the origin of renal tumorigenesis. It is postulated that estrogen metabolism by cytochrome P-450 enzymes leading to covalent DNA modification plays a role in hormonal carcinogenesis in the hamster kidney.

Received 8/25/86; revised 12/17/86; accepted 1/5/87.

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1. Financial support was provided by the NIH, National Cancer Institute (Grants CA-43232, CA-43233, CA-32157 and CA-10893) and National Institute of Neurological and Communicative Disorders and Stroke (Grants K04-NS00873 and PO1-NS-8494).

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3. The abbreviations used are: E2, 17β-estradiol; TXBβ, thromboxane B₂.
subtracted. For the calculation of the approximate adduct level of kidney cortex DNA, an estimated intensification factor of 10 (21) was used.

Cytochrome P-450 Activity. Three male Syrian hamsters, 8–10 weeks old, were killed by decapitation. Their kidneys were excised and sliced. The central slices (approximately 1 mm thickness) of each kidney were covered by an ice-cold solution of 50 mM potassium phosphate, pH 7.4, containing 10 mM sodium fluoride and 5 mM phenylmethylsulfonyl fluoride and were dissected into cortex and medulla. Pooled cortical or medullary tissue was homogenized using a Polytron homogenizer (Brinkmann Instruments, Westbury, NY; 2 x 30 s, at a setting of 5–6) in 4 volumes of 50 mM potassium phosphate, pH 7.4, containing 10 mM sodium fluoride and 5 mM phenylmethylsulfonyl fluoride. Cell debris was removed by centrifugation at 3,000 x g for 40 min. The supernatant was further centrifuged at 102,000 x g for 1 h at 4°C. The resultant pellet was homogenized in the same buffer in a volume equivalent to 1 ml/g of tissue. The cytochrome P-450 activity was determined by the difference in absorbance at 450 nm of the microsomal samples versus a carbon monoxide treated sample as described previously (27).

Prostaglandin Endoperoxide Synthase Assay. The prostaglandin endoperoxide synthase activities of the hamster kidney cortical and medullary microsomes were assayed by quantitating the synthesis of TXB2 from arachidonic acid in the presence of partially purified human platelet thromboxane synthase (28). The reaction mixture contained thromboxane synthase (200 µl; 1 mg protein), 300 µl of microsomal preparation (described above) and 500 µl of 50 mM potassium phosphate, pH 7.4. The reaction was initiated by the addition of 100 µg of arachidonic acid and incubated at room temperature for 1 h. The reaction was terminated by acidification with 25 µl of 2 N hydrochloric acid. The mixture was then neutralized to pH 7.3 with 50 µl of 1 M Tris-HCl, and diluted for TXB2 measurement by a sensitive and specific radioimmunoassay (29). TXB2 production exhibited a linear response to the volume of microsomes assayed. Protein concentrations of the samples were determined by the method of Bradford (30).

RESULTS

Estrogen-induced DNA Adducts. In E2- or diethylstilbestrol-treated male Syrian hamsters, estrogen-induced covalent adducts had previously been identified by 32P-postlabeling analysis of DNA from whole kidney homogenates (21). In contrast to the distribution of polycyclic aromatic hydrocarbon-induced DNA adducts in various tissues (target and nontarget organs) (31), estrogen-induced DNA modifications were observed (21) specifically in the hamster kidney preceding renal tumor formation. In the present study, this target organ specificity of estrogen-induced DNA modification was further investigated to identify the intrarenal region in which DNA damage and subsequent carcinoma arose.

When DNA was isolated from sections of hamster kidney exposed to E2 for 7 months and analyzed by the adduct intensification version (24) of a 32P-postlabeling assay (24), the fingerprints shown in Fig. 1 were obtained. Adducts a, b, d, and e displayed by kidney cortex DNA were the same as found earlier for DNA from estrogen-exposed whole hamster kidney (21). The highest intensities of DNA adducts were observed in the cortex and the lowest intensities in the inner stripe of the outer zone of medulla. Furthermore, a greater number of adducts were detected in the cortex than in either of the two medulla preparations (Fig. 1). In addition to these adducts, the fingerprints (Fig. 1, especially A) exhibited several radioactive spots that were not seen in control cortex DNA (Fig. 1D).

Since these spots have not been observed previously for DNA preparations from estrogen-exposed whole kidneys that were frozen immediately after excision, it appears likely that they represent decomposition products of the adducts formed during dissection of the kidneys.

Fig. 1. Covalent DNA adducts induced in sections of male Syrian hamster kidney by chronic E2 treatment for 7 months: A, cortex; B and C, outer and inner stripe, respectively, of the outer zone of medulla; D, untreated control cortex. DNA was isolated by a solvent extraction procedure and digested to deoxyribonucleoside 3'-monophosphates, which were subsequently 32P-postlabeled by the adduct intensification version of 32P-postlabeling assay (see "Materials and Methods"). Labeled DNA adducts were separated by two-dimensional polyethyleneimine-cellulose thin-layer chromatography using a solvent combination described previously for estrogen-induced DNA adducts (Fig. 2 of Ref. 21) and visualized by screen-enhanced autoradiography for 4.5 days at ~80°C. The major estrogen-induced DNA adducts have been designated a, b, d, and e (21). radioactive background spots were present on all fingerprints (compare A–C with D). The nature of additional spots, seen particularly in A, was not clear.

Table 1. Intensities of E2-induced DNA adducts in various regions of male Syrian hamster kidney

<table>
<thead>
<tr>
<th>Kidney section</th>
<th>Adduct intensities (cpm)</th>
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<tr>
<td></td>
<td>a</td>
</tr>
<tr>
<td>Cortex</td>
<td>28</td>
</tr>
<tr>
<td>Outer stripe</td>
<td>23</td>
</tr>
<tr>
<td>Inner stripe</td>
<td>7</td>
</tr>
</tbody>
</table>

* ND, not determinable.

Cerenkov counting of the adduct spots seen in Fig. 1 yielded the data shown in Table 1, which lists intensities for individual and total adducts. All adduct intensities declined sharply from cortex towards the inner stripe. Using an approximate intensification factor of 10 (21, 24), we estimated that the total adduct levels were approximately 1 adduct in 1.5 x 10⁵, 3.1 x 10⁵, and 18.6 x 10⁵ nucleotides in cortex, outer and inner stripe DNA, respectively. The ratios of adduct intensities in cortex versus outer and inner stripe show that intensities of individual adducts in cortex were 1.2–2.5 and ~4 times higher than those in outer and inner stripe, respectively. The corresponding numbers for total adducts were 2.1 and 12.4. Similar values for adduct levels and ratios of adduct intensities were also obtained when DNA preparations were analyzed from kidney sections exposed to E2 for 5 months or when another highly sensitive version of the
DNA ADDUCT AND P-450 LOCALIZATION IN HAMSTER KIDNEY

32P-postlabeling assay, i.e., a nuclease P1-mediated adduct enrichment procedure (32), was used for DNA analysis (data not shown).

Enzyme Activities in Cortex and Medulla. Microsomes, prepared from cortex or medulla of untreated male Syrian hamsters, were analyzed for cytochrome P-450 and prostaglandin endoperoxide synthase activities. Cytochrome P-450 could be identified only in cortical microsomes (Table 2). In cortex, a cytochrome P-450 content of approximately 0.8 nmol P-450/mg protein was detected, which is in the range of activity observed previously (33) for rat or rabbit kidney. Medullary microsomes did not contain any detectable cytochrome P-450 concentrations. Similar findings in untreated rabbits were reported by Davis et al. (34).

The same microsomal preparations were then used to determine cortical or medullary prostaglandin endoperoxide synthase activities. Enzyme activities were determined with a newly developed assay, in which purified thromboxane synthase was added to synthesize TXB2 from any prostaglandin H2 formed by prostaglandin endoperoxide synthase. The TXB2 is then accurately measured by a highly specific and sensitive radioimmuno assay (29). In Table 2, prostaglandin endoperoxide synthase activities are therefore expressed as ng TXB2/mg protein/h. The highest concentration of prostaglandin endoperoxide synthase was found in kidney medulla. Enzyme activity in this region was five times that found in kidney cortex (P = 0.015 using Student's t test).

These enzyme measurements demonstrate that the inverse relationship, observed previously in rabbit (34, 35) for the cytochrome P-450 and prostaglandin endoperoxide synthase distribution within the kidney cortex and medulla, is also found in male Syrian hamsters. The cytochrome P-450 concentrations in kidney cortex and medulla correlate well with the overall estrogen-induced DNA adduct concentrations in these intrarenal regions. An inverse relationship exists between overall estrogen-induced DNA modifications and prostaglandin endoperoxide synthase activities in the renal tissues examined.

DISCUSSION

Estrogen-induced renal carcinoma in male Syrian hamsters is known (36, 37) to arise in the cortex near the corticomedullary junction. The origin of the tumors is still debated. The concentration of estrogen-induced DNA adducts was elevated in the cortex of estrogen-treated hamster kidney (Fig. 1), the site where tumor foci initially appear (36). Since DNA adduct levels declined sharply from the cortex towards the inner stripe, the presence of adducts in the outer zone could conceivably be due to migration of DNA-reactive metabolites from the cortex. The target organ specificity (21) and the further specificity with respect to intrarenal location described here (Fig. 1 and Table 1) strongly support a role of estrogen-induced covalent DNA alteration as a crucial event preceding hormone-associated malignancy.

The enzyme assays were carried out with hamster kidney medullary and cortical microsomes to identify the metabolizing enzyme system associated with DNA adduct formation and, ultimately, tumor genesis. Both cytochrome P-450 and prostaglandin endoperoxide synthase have previously been considered possible estrogen metabolizing systems involved in carcinogenesis in the hamster kidney (5, 11, 17, 18). The enzyme system most clearly associated with DNA damage and carcinoma induction in the kidney cortex has now been identified (Table 2) to be cytochrome P-450-mediated function oxidase. Alterations in estrogen-induced tumor incidence obtained by using inhibitors (38, 39) or by using modified estrogens (10) also support a contributing role of cytochrome P-450-mediated metabolism in hamster kidney tumorigenesis. Furthermore, this enzyme system may participate in the activation of the unknown endogenous electrophile(s) leading to DNA adduction. A role of prostaglandin endoperoxide synthase in estrogen-induced DNA damage and tumor induction in the hamster is less likely because of the distribution of this enzyme between cortex and medulla in hamster (Table 2) and other species less sensitive to hormonal carcinogenesis (34, 35).

Peroxidase activity in the hamster kidney has not been investigated here, since peroxidizing activity of cytochrome P-450 enzymes has now also been established for diethylstilbestrol or catechol estrogens as substrates (40). Thus, the action of peroxidase in estrogen oxidation in vivo may be indistinguishable from that of cytochrome P-450 enzymes.

Since cytochrome P-450-mediated function oxidase has been identified here as the enzyme system most closely associated with estrogen-induced DNA damage preceding carcinoma, the mechanism of formation of DNA-reactive substances by this enzyme system must be assessed in future experiments.

ACKNOWLEDGMENTS

The authors wish to thank Donald J. Garcia for isolating DNA from kidney tissue, Wei-Ming Tuan for the determination of protein concentrations, R. Scott Beyer and Dr. Henry W. Strobel for help with the cytochrome P-450 measurements, and Dr. Ruth E. Bulger for her help in separating the various zones of hamster kidney. The colleagues mentioned above are at the University of Texas Medical School, Houston, TX 77225.

REFERENCES

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