Immunohistochemical Localization of Cytochrome P-450 and Reduced Nicotinamide Adenine Dinucleotide Phosphate: Cytochrome P-450 Reductase in the Rat Ventral Prostate

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ABSTRACT

Rabbit antibodies raised against the major isozymes of cytochrome P-450 isolated from hepatic microsomes of β-naphthoflavone (BNF) and phenobarbital-treated rats (cytochrome P-450 BNF-B2 and cytochrome P-450 PB-B2, respectively) and against rat liver NADPH-cytochrome P-450 reductase were used to localize these enzymes immunohistochemically in the rat ventral prostate.

Using the unlabeled antibody peroxidase-antiperoxidase technique, NADPH-cytochrome P-450 reductase was detected exclusively in the epithelial cells of the gland to the same magnitude in untreated, phenobarbital-, and BNF-treated rats. Cytochrome P-450 BNF-B2-like immunoreactivity was exclusively present in the glandular epithelium in BNF-treated rats, whereas staining could not be visualized in untreated or in phenobarbital-treated rats. The staining for NADPH-cytochrome P-450 reductase was more uniformly distributed within the epithelium than was the cytochrome P-450 BNF-B2-like immunoreactivity. Cytochrome P-450 PB-B2-like immunoreactivity was not found, regardless of animal pretreatment.

These findings support our previous results (Haaparanta, T., Halpert, J., Glaumann, H., and Gustafsson, J-Å., Cancer Res. 43: 5131–5137, 1983) demonstrating the presence of constitutive NADPH-cytochrome P-450 reductase in the prostate and that an isozyme of cytochrome P-450 is highly inducible by BNF in this gland. The significance of these findings are discussed in view of the essentially unknown etiology of human prostatic cancer.

INTRODUCTION

The oxidative metabolism of a variety of xenobiotics and endogenous compounds in living organisms is mediated by the monoxygenase system, which consists of the membrane-bound enzymes NADPH-cytochrome P-450 reductase and cytochrome P-450 (4, 8, 16). P-450 reductase is thought to be present in only one form in a single species, whereas cytochrome P-450 constitutes a family of hemoproteins with capacity to metabolize a variety of substrates. Several isozymes of P-450 are inducible by various compounds, and the expression of P-450 isozymes is also tissue specific and species specific (11). Polycyclic aromatic hydrocarbons and barbiturates represent 2 major classes of P-450 inducers, each displaying characteristic properties of inducing specific isozymes. Highly reactive intermediates are frequently generated in the P-450-mediated detoxification process, which thus, in fact, becomes a process of toxification. These intermediates, which often consist of epoxides or radicals, may reach the genetic material, bind to DNA, RNA, and proteins, and thereby possibly initiate cell transformation (4, 8, 16).

Next to lung cancer, prostatic cancer is the most common malignant disease among men in the Western world (7). Recently, chemicals have been associated with the etiology of prostatic cancer (10, 15). The reason why certain tissues and cells are more prone to develop cancer than are others and exhibit a selective sensitivity toward specific chemicals may partly be answered by differences in absorption and transport of various xenobiotics within the body to the target cell or tissue (9). The distribution of drug-metabolizing enzymes, their inducibility, and varying levels of the so-called 2,3,7,8-tetrachlorodibenzop-dioxin receptor could also contribute to such differences (3).

Histochimical techniques based on specific antibodies against drug-metabolizing enzymes are of special interest when revealing their distribution (1, 2, 5, 6, 17, 19). In the present immunohistochemical study using the unlabeled antibody peroxidase-antiperoxidase technique, we used specific antibodies against rat liver cytochrome P-450 reductase, against cytochrome P-450 PB-B2, and against cytochrome P-450 BNF-B2, in order to determine the intralobular localization of the prostatic monoxygenase system.

MATERIALS AND METHODS

Materials. The following immunochemicals were obtained from Dakopatts a/s, Copenhagen, Denmark: normal swine serum; swine anti-rabbit IgG; and the peroxidase rabbit-antiperoxidase complex. BNF and 3,3′-diaminobenzidine tetrahydrochloride were purchased from Sigma Chemical Co., St. Louis, MO; p-benzoquinone was from Fluka AG, Buchs, Switzerland; and PB was from Apoteksbolaget, Stockholm, Sweden. All other chemicals used were of analytical grade.

Methods. Male Sprague-Dawley rats weighing 300 to 350 g (obtained from Anticimex, Stockholm, Sweden) were given injections i.p. once daily with PB [75 mg/kg in 0.5 ml 0.9% (w/v) NaCl solution] for 3 days or with BNF [80 mg/kg in 0.5 ml corn oil] i.p. 24 hr before sacrifice by decapitation.

The ventral prostatic lobes were fixed with 0.95% (w/v) para-benzoquinone:0.02 N CaCl2 in 0.2 N sodium cacodylate buffer, pH 7.4, for 4 hr at +4° as described by Baron et al. (1). Serial sections of 5 μm were prepared after dehydration and embedding in paraffin. The unlabeled antibody peroxidase-antiperoxidase immunohistochemical staining tech-
nique of Stemberger et al. (18) was used with modifications at room temperature, except where noted. The antibodies used in this investigation have been characterized earlier (14). Prior to immunohistochemical staining, the tissue sections were exposed to 0.3% (v/v) H2O2 in 95% methanol for 30 min to block endogenous peroxidase activity. After exposure to 3% (v/v) normal swine serum for 30 min, the sections were incubated for 16 hr at +4°C with the appropriate antibody (IgG fraction, 25 mg/ml) or preimmune IgG in dilutions ranging from 1:500 to 1:2500. All dilutions were prepared in 20 mM potassium phosphate buffer, pH 7.4, containing 0.15 M NaCl and 1% (v/v) normal swine serum. In control experiments, where the antibodies were preabsorbed with the purified enzymes, a 1:200 dilution of the IgG fractions was incubated for 16 hr at +4°C together with the purified enzymes, which were present at a concentration of 1 mg/ml. The antigen-antibody complex was removed by centrifugation at 3000 x g for 15 min. After incubation with the antibodies, preimmune IgG, or preabsorbed antibodies, the sections were exposed to swine anti-rabbit IgG diluted 1:20 and finally to the peroxidase-antiperoxidase complex at a dilution of 1:50. Subsequent exposure of the sections to 3.3'-diaminobenzidine (0.3 mg/ml) and H2O2 (0.05%, v/v) in phosphate-buffered saline (10 mM potassium phosphate buffer, pH 7.4, containing 0.15 mM NaCl) made the reaction product visible.

RESULTS AND DISCUSSION

Fig. 1, a to c, shows tissue sections of the ventral prostate from BNF-treated rats incubated with either preimmune IgG, anti-P-450 reductase, or anti-P-450 BNF-B2. Fig. 1d displays a tissue section of prostate from an untreated rat incubated with anti-P-450 BNF-B2. The positive immunoreactivity in Fig. 1, b and c can be compared with the tissue section incubated with preimmune IgG (Fig. 1a) representing the background staining. Cytochrome P-450 BNF-B2-like immunoreactivity was found in the epithelial cells of the prostatic gland from BNF-treated animals (Fig. 1c), whereas no staining was obtained with these antibodies in untreated (Fig. 1d) or PB-treated rats (not shown). P-450 reductase-like immunoreactivity was found in untreated, PB-treated, and BNF-treated rats and again exclusively in the epithelial cells of the gland (a section of prostatic tissue from a BNF-treated rat is shown in Fig. 1b).

The positive immunoreactivity in Fig. 1, b and c could be blocked by preabsorption of the antibodies with the respective purified antigen prior to immunostaining (not shown). Similarly, the positive immunostaining of tissue sections from untreated or PB-treated rats with anti-P-450 reductase was blocked by preabsorption of the antibody with purified P-450 reductase. The different treatments did not affect the distribution or intensity of the P-450 reductase staining. No cytochrome P-450 BNF-B2-like immunoreactivity could be found in the prostate, regardless of pretreatment of the rats (not shown). The results were reproducible on unfixed or p-benzoquinone fixed frozen sections, suggesting that the fixative or embedding in paraffin did not alter the antigenicity.

We have shown previously that rat prostatic microsomal P-450 is highly inducible by BNF or 2,3,7,8-tetrachlorodibenzo-p-dioxin treatment but not by PB treatment (12, 14). The cytochrome P-450-dependent enzyme activities, aryl hydrocarbon hydroxylase and 7-ethoxyresorufin O-deethylase, are almost undetectable in prostatic microsomes from untreated rats but are induced about 500-fold within 24 hr after a single i.p. injection of BNF or 2,3,7,8-tetrachlorodibenzo-p-dioxin. Antibodies against rat liver cytochrome P-450 BNF-B2 (anti-P-450 BNF-B2, but not anti-P-450 PB-B2), cross-react with the induced prostatic enzyme, as shown by enzyme inhibition and by immunoblotting of prostatic microsomes. The BNF-induced cytochrome P-450 species is thus immunologically similar to the major BNF-induced cytochrome P-450 from the liver. Also, the prostatic and liver P-450 reductase are immunologically similar. The amount of cross-reacting cytochrome P-450 BNF-B2 was 50 pmol/mg of microsomal protein in BNF-treated animals, whereas the level was below the detection limit (0.4 pmol/mg) in microsomal fractions from prostates of untreated or PB-treated rats. The amount of P-450 reductase was 0.02 nmol/mg of microsomal protein in untreated animals and was not changed by treatment with BNF or PB. The apparent molecular weights of both cytochrome P-450 BNF-B2 and P-450 reductase were similar to those of the liver enzymes (12, 14).

The immunoreactivity in the epithelial cells was localized over the cell cytoplasm, with less apparent staining of the cell nucleus (see inset, Fig. 1, b and c). No immunoreactivity was noted in the glandular lumen or in the fibromuscular stroma. More careful examination of the tissue sections revealed that, although most epithelial cells stained with different intensities for cytochrome P-450 BNF-B2, occasional cells appeared unstained (Fig. 1b), whereas the staining was more uniform for anti-P-450 reductase (Fig. 1c). We can only speculate about the significance of this difference. P-450 reductase, which is also constitutively present in the prostatic epithelium, may have other functions than to participate in drug metabolism. It may, e.g., play a role in the metabolism of steroid hormones. In fact, we have recently demonstrated that cytochrome P-450-dependent hydroxylation of 5a-androstan-3β,17β-diol occurs in prostatic microsomes (13). The hydroxylase activity is constitutive in the gland, in contrast to the drug-inducible cytochrome P-450. Antibodies against rat liver P-450 reductase inhibited the hydroxylase, indicating that it is dependent on reducing equivalents from P-450 reductase. Also, in contrast to the BNF-induced cytochrome P-450 species, it was not markedly affected by pretreatment of the rats with BNF. It was, however, affected by endocrine factors.

A series of reports have described a heterogeneous distribution in the rat and rabbit liver of a number of cytochrome P-450 forms and P-450 reductase (1, 5, 6, 19). Cytochrome P-450 was localized preferentially in the hepatocytes pertaining to central veins with a gradient of less immunostaining towards periportal hepatocytes. Varying degrees of staining were also observed between different liver lobes. On the other hand, P-450 reductase was more uniformly distributed between central veins and periportal regions. Heterogeneous distribution of several cytochrome P-450 forms and P-450 reductase in various extrahepatic tissues of rats, rabbits, minipigs, and humans has also been reported (2, 5, 6, 17).

Baron et al. have reported recently that the human prostate contains antigens related to 4 rat hepatic cytochrome P-450 isozymes, including cytochrome P-450 PB-B2 (2). This isozyme does not seem to be expressed in the rat ventral prostate, as is evident from this and previous studies (12, 14). This difference may be due to exposure of humans to environmental factors with capacity to induce this isozyme.

In conclusion, we have shown here that the epithelial cells represent the principal site for drug metabolism in the rat ventral prostate. The induced cytochrome P-450 isozyme cannot be detected constitutively, whereas P-450 reductase is a constitutive enzyme in the gland. These results are in line with a possible
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connection between metabolism of xenobiotics and prostatic cancer in humans, since this cancer form usually originates in the glandular epithelial cells.

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REFERENCES

Fig. 1. Localization of cytochrome P-450 BNF-B2 and NADPH-cytochrome P-450 reductase in the rat ventral prostate. The tissue sections were processed and incubated using the unlabeled antibody peroxidase-antiperoxidase method described in "Materials and Methods." All sections were incubated with IgG fractions of the primary antiserum diluted to contain 12.5 μg protein/ml (1:2000). x 140. Bar, 50 μm. Insets, higher (x 630) magnifications of the epithelium. a, tissue section from a BNF-treated rat incubated with preimmune IgG. b, tissue section from a BNF-treated rat incubated with anti-P-450 reductase. A relatively uniform immunostaining of the epithelium is evident (arrows), whereas the glandular lumen (Lu) or stroma (S) are not stained. c, tissue section from a BNF-treated rat incubated with anti-P-450 BNF-B2. Immunostaining of several epithelial cells is clearly visible (arrows), with no staining of glandular lumen (Lu) or stroma (S). Note the relatively heterogeneous distribution of the reaction product in the epithelium. d, tissue section (from an untreated rat) incubated with anti-P-450 BNF-B2. Staining is similar to the background staining obtained with preimmune IgG in a.
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