Immunochrometric Detection and Quantitation of Microsomal Cytochrome P-450 and Reduced Nicotinamide Adenine Dinucleotide Phosphate: Cytochrome P-450 Reductase in the Rat Ventral Prostate

Tapio Haaparanta,1 2 James Halpert,1 Hans Glaumann,1 and Jan-Åke Gustafsson1

Departments of Medical Nutrition [T. H., J. H., J. A. G.] and Pathology [T. H., H. G.], Karolinska Institute, Huddinge University Hospital, S-141 86 Huddinge, Sweden

ABSTRACT

Treatment with β-naphthoflavone (BNF) was found to induce 7-ethoxyresorufin O-deethylase and aryl hydrocarbon hydroxylase activities about 500-fold in the microsomal fraction of the rat ventral prostate but had no effect on aminopyrine N-demethylase or reduced nicotinamide adenine dinucleotide phosphate:cytochrome c reductase activities. Phenobarbital (PB) treatment did not alter any of these enzyme activities. Antibodies raised in rabbits against rat liver cytochrome P-450 reductase (P-450 reductase) and against P-450 BNF-B2 and P-450 PB-B2, the major forms of P-450 isolated from liver microsomes of BNF- and PB-treated rats, respectively, were used to characterize the P-450-dependent monoxygenase system in the rat ventral prostate. Anti-P-450 reductase immunoglobulin G inhibited reduced nicotinamide adenine dinucleotide phosphate:cytochrome c reductase activity in prostatic microsomes, and anti-P-450 BNF-B2 but not anti-P-450 PB-B2 immunoglobulin G inhibited the P-450-induced prostatic microsomal 7-ethoxyresorufin O-deethylase and aryl hydrocarbon hydroxylase activities.

A highly sensitive immunoblotting method was used to quantitate P-450 BNF-B2, P-450 PB-B2, and P-450 reductase in prostatic microsomes. Using this technique, prostatic P-450 reductase with a molecular weight corresponding to that of purified liver P-450 reductase was detected at a level of 0.02 nmol/mg of microsomal protein. In the liver, the same enzyme amounts to 0.2 nmol/mg of microsomal protein. P-450 BNF-B2 was not detected in prostatic microsomes from control or PB-treated rats, whereas a protein band with a molecular weight corresponding to that of purified liver P-450 BNF-B2 was found in prostatic microsomes from BNF-treated rats at a level of 0.05 nmol P-450 per mg microsomal protein. P-450 PB-B2 was not detected in prostatic microsomes from either control, PB-treated, or BNF-treated animals.

INTRODUCTION

The etiology of prostatic cancer is still largely unknown despite its high frequency in the Western world. Hormonal and genetic factors as well as viral agents have been suggested to be associated with malignant changes in the gland. Although chemical carcinogenesis has been established for numerous cancer forms in humans including lung, skin, bladder, and liver cancer (2), only recently have chemical factors been associated with prostatic carcinoma (5, 12).

Many xenobiotics require metabolic activation before they exhibit any mutagenic activity. The major enzymes participating in the biotransformation of xenobiotics are cytochrome P-450, P-450 reductase,3 epoxide hydrolase, and conjugation enzymes such as glutathione S-transferase and sulfotransferase (1). Cytochrome P-450 catalyzes the initial step in this process, which may give rise to highly reactive intermediates of the parent compound. These intermediates, often consisting of epoxides, may reach the genetic material, bind to DNA, and in certain instances cause malignant transformation (3, 15).

Recent work from this laboratory has shown that a prostatic S-9 mix, which is the cell subfraction containing the enzymes necessary for biotransformation, is capable of activating a number of promutagens as assayed in the Ames’ Salmonella mutagenicity test (18). These findings initiated an investigation of the cytochrome P-450-dependent monoxygenase system in rat prostatic microsomes (19).

Constitutive levels of aminopyrine N-demethylase, 7-ethoxyresorufin O-deethylase, and AHH activities were found to be very low in the rat ventral prostate. These activities were shown to be cytochrome P-450-dependent. AHH and 7-ethoxyresorufin O-deethylase activities were induced almost 1000-fold after a single i.p. injection of either BNF or 2,3,7,8-tetrachlorodibenzo-p-dioxin. These studies suggested a similarity between the cytochrome P-450 isozyme(s) induced in the prostate and in the liver.4 Studies on the cytochrome P-450 system in rat prostatic microsomes are hampered by the low levels of the enzyme (0.02 to 0.06 nmol/mg protein). In the present investigation, we have made use of a highly sensitive immunoblotting method (8, 21) to characterize the cytochrome P-450 and P-450 reductase in the rat ventral prostate.

MATERIALS AND METHODS

Materials. [125I]-Labeled Protein A (89 µCi/µg) was purchased from New England Nuclear, Dreieich, West Germany. [6,7,8,9H4]Benzo(a)pyrene (40 Ci/mmol) and [3H]aminopyrine (114 mCi/mmol) were purchased from Amersham International, Amersham, Buckinghamshire, United Kingdom. 7-Ethoxyresorufin and 7-hydroxyresorufin were purchased from Pierce Eurochemie B.V., Rotterdam, Holland. Protein A-Sepharose was obtained from Pharmacia, Uppsala, Sweden, and nitrocellulose filters (GSWP 304 F0; 0.22 µm) were from Millipore, Bedford, Massachusetts.

The abbreviations used are: P-450 reductase, NADPH:cytochrome P-450 P-450 reductase; AHH, aryl hydrocarbon hydroxylase; BNF, β-naphthoflavone (5,6-benzoflavone); SDS, sodium dodecyl sulfate; PB, phenobarbital; PBS, phosphate-buffered saline (10 mM potassium phosphate buffer, pH 7.4, containing 0.15 M NaCl); P-450 PB-B2, the major form of cytochrome P-450 isolated from liver microsomes of phenobarbital-treated rats; P-450 BNF-B2, the major form of cytochrome P-450 isolated from liver microsomes of β-naphthoflavone-treated rats; P-450 PB-B2, P-450 PB-B2, minor forms of cytochrome P-450 isolated from liver microsomes of phenobarbital-treated rats; T. Haaparanta, H. Glaumann, and J.Å. Gustafsson, Induction of cytochrome P-450 dependent reactions in the rat ventral prostate by β-naphthoflavone and 2,3,7,8-tetrachlorodibenzo-p-dioxin, submitted for publication.

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2 To whom requests for reprints should be addressed, at the Department of Medical Nutrition.

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ford, Mass. Reagents for SDS-polyacrylamide gel electrophoresis were purchased from Bio-Rad, Richmond, Calif. Bovine serum albumin (Fraction V) and BNF were obtained from Sigma Chemical Co., St. Louis, Mo. All other reagents were of analytical grade or of highest purity available.

Animals. Male Sprague-Dawley rats, 350 to 400 g (200 g in case of preparation of liver microsomes for cytochrome P-450 purification), were obtained from Anticimex (Stockholm, Sweden). They were housed in plastic cages with hardwood bedding in a room with controlled temperature and light (12-hr light cycle) and had free access to food and water. PB (80 mg/kg in 0.5 ml 0.9% NaCl solution) and BNF (40 mg/kg in 0.5 ml corn oil) were injected i.p. once daily for 3 days, if not otherwise indicated. The animals were starved overnight after the last injection and then killed by decapitation.

Isolation of Microsomes. Liver microsomes were prepared as described by van der Hoeven and Coon (22). Prostatic microsomes were isolated as described earlier (9). The microsomal pellets were suspended in 0.3 M sucrose and, if not used immediately, stored at −80°C.

Purification of Enzymes. P-450 reductase and the major forms of cytochrome P-450 were purified from liver microsomes of PB- and BNF-treated rats according to the method of Guengerich and Martin (7), with the following modifications. After sample application, the octylamino-Sepharose column was washed with buffer containing 0.5% rather than 0.42% (w/v) sodium cholate. The cytochrome P-450 was then eluted with buffer containing 0.08% rather than 0.06% (w/v) Lubrol PX and 0.40% rather than 0.33% (w/v) sodium cholate. The P-450 reductase was eluted with buffer containing 0.40% rather than 0.35% (w/v) cholate and 0.20% rather than 0.15% (w/v) sodium deoxycholate. The cytochrome P-450 fraction from octylamino-Sepharose was chromatographed on a 2 × 90-cm column of DEAE-Sephadex. A 1.5-liter linear gradient of 25 to 100 mM NaCl in buffer was used to elute the various cytochrome P-450 forms. The altered conditions were necessary to separate the major cytochrome P-450 fraction (B2) from a later fraction of slightly higher molecular weight (B0) found in microsomes from both PB- and BNF-treated rats.

The B2 fractions and the P-450 reductase used in this study were >95% pure as judged by SDS-polyacrylamide gel electrophoresis according to the method of Laemmli (11). The specific content of these cytochrome P-450 preparations was 15 μmol/mg protein based on the protein concentration determined by the method of Lowry et al. (13) using bovine serum albumin as the standard. The specific activity of the P-450 reductase was 44 μmol cytochrome c reduced per min per mg protein as assayed in 300 mM potassium phosphate buffer, pH 7.7, at 25°C.

Preparation of Antibodies. Antibodies were raised against the purified enzymes in adult male New Zealand rabbits. Rabbits were bled prior to immunization to obtain preimmune sera. The enzymes were emulsified with Freund's incomplete adjuvant (1:1, v/v) and injected at 20 sites on the back of the rabbits. The first injection consisted of 100 μg protein per rabbit. Two booster injections were given in Freund's incomplete adjuvant (50 μg/rabbit) with an interval of 3 weeks between them, 3 weeks after the first injection. Two to 4 weeks after the last injection, the rabbits were bled, and serum was isolated. Serum was heated for 20 min at 56°C to inactive complement proteins. IgG fractions were isolated by affinity chromatography using a Protein A-Sepharose CL-4B column (4). The column (15.5 × 6.0 cm) was equilibrated in 50 mM potassium phosphate buffer, pH 7.4, containing 0.15 μM NaCl, and 0.5 ml of swelled gel was used per ml of serum. After a washing with 100 ml of the above buffer (1.5 ml/min), the IgG fraction was eluted with 0.1 M acetic acid at a flow rate of 0.4 ml/min. The IgG-containing fractions were pooled and dialyzed immediately against the washing buffer for 48 hr with 2 changes of the buffer. After dialysis, the IgG fractions were concentrated to 30 mg/ml using an Amicon ultrafiltration cell (PM-30 filter; Amicon Corp., Lexington, Mass.). The whole procedure was performed at 4°C. The antibodies were stored at −80°C.

Assays. Cytochrome P-450 contents were measured in prostatic microsomes from the dithionite-reduced minus oxidized carbon monoxide difference spectrum using an extinction coefficient of 104 mm⁻¹ cm⁻¹ (14) and in liver microsomes by the method of Omura and Sato (16) from the CO-reduced difference spectrum using an extinction coefficient of 91 mm⁻¹ cm⁻¹. 7-Ethoxyresorufin O-deethylase, NADPH:cytochrome c reductase, aminopyrine N-demethylase, and AHH activities were measured as described earlier. Protein concentrations were estimated by the method of Lowry et al. (13) as modified by Peterson (17) using bovine serum albumin as the standard.

SDS-Polyacrylamide Gel Electrophoresis and Electrophoretic Transfer of Protein to Nitrocellulose. SDS-polyacrylamide gel electrophoresis was performed using the system of Laemmli (11) with 8% acrylamide and 2.7-mm-thick slab gels. Proteins were transferred to nitrocellulose sheets essentially using the method of Towbin et al. (21). Shortly after completion of the electrophoretic run, the gel was placed onto a filter paper (Whatman No. 3MM) prerewetted in blotting buffer [25 mM Tris:192 mM glycine:20% (v/v) methanol, pH 8.3]. A nitrocellulose sheet was placed on the gel, care being taken to remove air bubbles and excess buffer. The nitrocellulose sheets were handled with gloves and forceps in order to minimize nonspecific background staining. A second filter paper, also prerewetted with blotting buffer, was placed on the nitrocellulose sheet. The “sandwich” was placed between 2 Scotch-Brite scouring pads, which were held together tightly with rubber bands, and put into a chamber containing 3 liters of ice-cold blotting buffer. A voltage gradient of 10 V/cm was applied for 16 hr. The chamber was chilled in an ice bath during the transfer.

Immunological Detection and Quantitation of Proteins on the Nitrocellulose Sheets. The nitrocellulose sheets were shaken in PBS, 3% (w/v) bovine serum albumin, 0.1% (w/v) Nonidet P-40, and 0.01% (w/v) NaN₃ for 1 hr. The sheets were then incubated with the appropriate antibody in the above solution (0.1 mg IgG per ml) for 2 hr. The same antibody solution can be used several times if kept in a freezer (−20°C) between each use. The blots were washed in PBS containing 0.1% Nonidet P-40 and 0.01% NaN₃ for 2 hr with 4 changes and rinsed thoroughly with distilled water between each buffer change. The blots were incubated in 125I-labeled Protein A (500,000 cpm/ml) in PBS, 3% bovine serum albumin, 0.1% Nonidet P-40, and 0.01% NaN₃ for 1 hr; then washed in PBS, 0.1% Nonidet P-40, and 0.01% Na₂SO₄ for 1 hr with 3 changes; and then rinsed in water. After this final wash, the blots were air dried and exposed to LKB Ultradens, usually for 2 to 3 days at room temperature. The whole washing and incubation procedure was performed at room temperature. Incubations and washes were carried out on a rocking platform. Small plastic boxes with the same size as the blots were used to minimize the volumes.

After autoradiography, the labeled protein bands were cut out from the blots and counted for radioactivity in a gamma counter (LKB Mini-gamma). Quantitation was performed by running varying amounts of the purified antigens in parallel with the samples on the nitrocellulose sheets. The radioactivity was proportional to the amount of antigen (0.01 to 1.0 μg), which permitted the construction of standard curves for quantitation. The absolute amount of 125I-Protein A bound per given amount of antigen was found to vary by as much as ±50% between different nitrocellulose sheets. However, by running the standards and the samples on the same gels, we were able to keep the variation between duplicate determinations below ±15%.

Ouchterlony Immunodiffusion. This was performed in 1% agarose gels which contained 1 mM glucose, 0.2% (w/v) Lubrol PX, and 0.02% (w/v) NaN₃, pH 7.4. Small microscope slides were used (2.6 × 7.6 cm), and 10 μl of antigen and antibody were usually added to the wells. Plates were incubated at room temperature for 24 to 48 hr. They were first washed with at least 3 changes of 0.3 M NaCl for 2 to 3 days if stained for protein and were subsequently stained with 0.5% (w/v) Coomassie Brilliant Blue R-250 in ethanol:acetic acid:H₂O (45:10:45, by volume) and destained with the same solution but without the stain.

Quantitative Immunoprecipitation. This was performed by mixing varying amounts of the antigen (1 to 70 μg) with 0.25 mg IgG in a final volume of 0.2 ml of 20 mM potassium phosphate buffer (pH 7.4), 10% glycerol, 0.15 M NaCl, and 0.2% Lubrol PX and incubating at room temperature for 1 hr and then at 4°C for 24 hr. Immunoprecipitates (5 to
100 μg were centrifuged at 3000 × g for 30 min, washed twice with PBS, and analyzed for protein (17).

RESULTS

Antibodies. Antibodies against rat liver cytochromes P-450 PB-B2 and P-450 BNF-B2 and against NADPH-cytochrome P-450 reductase were raised in rabbits, and the IgG fractions were isolated by affinity chromatography on Protein A-Sepharose. The 3 antibodies gave visible precipitin bands on the Ouchterlony immunodiffusion plates against the respective antigen. The antibodies were specific, giving no precipitin bands against the 2 other antigens (not shown). Anti-P-450 BNF-B2 did not cross-react with P-450 PB-B2 (7), whereas a line of identity was obtained with P-450 PB-B2 (not shown). Using quantitative immunoprecipitation, each antibody was specific against its antigen (not shown). Finally, each antibody recognized only its own antigen also using the immunoblotting technique.

Inhibition of Enzymatic Activities. The effect of anti-P-450 reductase antibodies on prostatic microsomal NADPH-cytochrome c reductase activity is shown in Chart 1. The antibodies inhibited the activity to 40% of the control activity (using 1 mg IgG in the assay mixture), whereas the preimmune IgG had no effect. In a similar experiment with liver microsomes or purified reductase, using the same antibody:enzyme activity ratio, these antibodies inhibited the activity to 32 and 10% of the control value, respectively.

Charts 2 and 3 display the effect of anti-P-450 PB-B2, anti-P-450 BNF-B2, anti-P-450 reductase, and preimmune IgG on the metabolism of 7-ethoxyresorufin and benz(a)pyrene (AHH) by prostatic microsomes isolated from BNF-treated animals (80 mg/kg for 24 hr). Both anti-P-450 PB-B2 and preimmune IgG had a slight stimulatory effect on the activities, whereas anti-P-450 reductase inhibited both 7-ethoxyresorufin O-deethylation and AHH by 60 and 50%, respectively, of the control values. Anti-P-450 BNF-B2 inhibited both activities to less than 10% of the control values.

Immunoblotting. The cross-reactivity of liver anti-P-450 reductase antibodies with prostatic microsomal NADPH-cytochrome c reductase is shown in Chart 1. The antibodies inhibited the activity by 75%. Chart 2 shows the autoradiogram of a nitrocellulose sheet from prostatic microsomes, whereas in liver microsomes PB increased the amount or apparent molecular weight of P-450 reductase. The different treatments of the rats did not affect the amount of P-450 reductase and of preimmune IgG on AHH activity in prostatic microsomes isolated from BNF-treated rats (24 hr). Antibody incubation was performed as in the legend to Chart 2. Control activity was 310 pmol per min per mg of microsomal protein, and 0.3 mg microsomal protein was used per assay. Values represent the mean of duplicate determinations for 2 separate microsomal preparations. Anti-P-450 BNF-B2 inhibited both activities to less than 10% of the control values.

Chart 2. Effect of antibodies against P-450 BNF-B2, P-450 PB-B2, and P-450 reductase and of preimmune IgG on 7-ethoxyresorufin O-deethylase activity in prostatic microsomes isolated from BNF-treated rats (24 hr). The antibodies were incubated for 30 min at room temperature with the microsomes and buffer (50 mM potassium phosphate, pH 7.4) in a total volume of 100 μl prior to assay. The amount of microsomal protein used was 0.07 mg/incubation, and the control activity was 275 pmol/min/mg. Assays were performed as described in "Materials and Methods." Values represent the mean of duplicate determinations for 2 separate microsomal preparations. Antibodies: anti-P-450 BNF-B2; O, anti-P-450 PB-B2; C, anti-P-450 reductase; •, preimmune IgG.

In Chart 3 shows an autoradiogram of a nitrocellulose sheet from an immunoblotting experiment with anti-P-450 reductase antibodies. A band was seen in both prostatic and liver microsomes isolated from untreated, PB-treated, or BNF-treated rats. The proteins were transferred to nitrocellulose filters and incubated with anti-P-450 reductase, anti-P-450 PB-B2, anti-P-450 BNF-B2, or preimmune IgG. No bands could be detected in prostatic microsomes isolated from untreated rats. The proteins were transferred to nitrocellulose filters and incubated with anti-P-450 reductase, anti-P-450 PB-B2, anti-P-450 BNF-B2, or preimmune IgG. The efficiency of protein transfer from the gel to the nitrocellulose was checked by staining the blotted gel with Coomassie Brilliant Blue R-250. Only faint bands were seen after transfer when 100 to 400 μg of microsomal protein were run on the polyacrylamide gels. No visible bands could be seen on the gels when less protein was used. It was necessary to use transfer times of at least 12 hr to achieve quantitative transfer of the proteins to the nitrocellulose.

Chart 3. Effect of antibodies against P-450 BNF-B2, P-450 PB-B2, and P-450 reductase and of preimmune IgG on AHH activity in prostatic microsomes isolated from BNF-treated rats (24 hr). Antibody incubation was performed as in the legend to Chart 2. Control activity was 310 pmol per min per mg of microsomal protein, and 0.3 mg microsomal protein was used per assay. Values represent the mean of duplicate determinations for 2 separate microsomal preparations. Antibodies: anti-P-450 BNF-B2; O, anti-P-450 PB-B2; C, anti-P-450 reductase; •, preimmune IgG.
Anti-P-450 reductase

Chart 4. Autoradiogram of an immunoblot incubated with antibodies against rat liver P-450 reductase (Red). A SDS:gel was run with the following samples (left to right): P-450 reductase, P-450 PB-B2, and P-450 BNF-B2. 0.5 \mu g protein each; liver and prostatic microsomes from animals pretreated with BNF or PB and from untreated animals. Five \mu g of liver and 100 \mu g of prostatic microsomal protein were applied to the gel. The proteins were transferred to nitrocellulose sheets and incubated with antibodies. \textsuperscript{125}I-labeled Protein A and autoradiographed as described in “Materials and Methods.” A single immunoreactive band is seen in the microsomal preparations as well as for the purified P-450 reductase, whereas no reaction occurs with P-450 PB-B2 or P-450 BNF-B2.

from control or PB-treated rats, whereas a band is visible in the microsomes isolated from BNF-induced rats with the same apparent molecular weight as that of the purified liver P-450 BNF-B2. This band is also markedly induced in the liver microsomes from BNF rats. These antibodies also recognized other proteins, in addition to the P-450 BNF-B2 band. This is seen especially in the liver microsomes from control and PB-treated animals because these wells were loaded with 50 times more protein in order to facilitate detection of the P-450 BNF-B2 band.

In a similar experiment with antibodies against P-450 PB-B2 (Chart 6), no immunoreactive protein bands were detected in prostatic microsomes regardless of treatment (not shown in Chart 6). When liver microsomes from control, PB-treated, or BNF-treated rats were analyzed, a band corresponding to purified P-450 PB-B2 was seen. In addition to the P-450 PB-B2 band, a second band was seen in all 3 samples. This band is very faint in the PB-treated microsomes in Chart 6 due to the 50-fold lower amount of microsomal protein analyzed. The limit of detection of antigen in microsomes was approximately 0.4 pmol/mg microsomal protein.

Quantitation of the Antigens by Immunoblotting. Quantitation of the amount of cross-reacting antigen was possible by running known amounts of the purified antigens on SDS-polyacrylamide gels in parallel with unknown samples. The gel was blotted and stained with the appropriate antibody and \textsuperscript{125}I-labeled Protein A. The radioactivity labeled bands were visualized by autoradiography, cut out, and counted in a gamma counter. Chart 6 shows an autoradiogram of a typical immunoblot with 3 different amounts of purified P-450 PB-B2 (0.1, 0.3, and 1.0 \mu g). The radioactivity was found to be linear with the amount of protein over this range (Chart 7). However, due to varying staining intensities between different blots, it was necessary to run the standard curve on the same immunoblot as the samples.

Comparison of Enzymatic Activities with the Immunological and Spectral Contents of P-450 BNF-B2, P-450 PB-B2, and P-450 Reductase. Chart 8 demonstrates the time course of induction of 7-ethoxyresorufin O-deethylase activity and of immunodetectable P-450 BNF-B2 in prostatic microsomes after a single i.p. injection of BNF. The amount of protein and the enzymatic activity paralleled each other during the induction and the decay process. The maximal enzyme activity was achieved 24 hr after treatment, followed by a relatively rapid normalization of the activity. The amount of P-450 BNF-B2 was 50 pmol per mg of microsomal protein at 24 hr after treatment, and the activity of 7-ethoxyresorufin O-deethylase was 0.25 nmol product per mg of microsomal protein.

Table 1 summarizes the effects of PB and BNF on prostatic and liver microsomes with respect to cytochrome P-450 and P-
450 reductase content in comparison with the activities of AHH, 7-ethoxyresorufin O-deethylase, and aminopyrine N-demethylase. The treatments did not markedly affect the yield of microsomal protein obtained from the prostate gland, whereas the yield of liver microsomes was increased after PB treatment.

Unfortunately, the spectral determination of prostatic cytochrome P-450 content is very imprecise due to a large interfering peak at about 430 nm. This peak is especially dominant when the carbon monoxide difference spectrum of Omura and Sato (16) is used. We have found the dithionite difference spectrum as described by Matsubara et al. (14) to be more suitable for prostatic microsomes due to less interference of the 430 nm peak. The spectral P-450 values are, however, a crude estimation of the real cytochrome P-450 content in prostatic microsomes due to these difficulties. The immunologically determined amount of the BNF-induced P-450 was 0.05 nmol/mg of microsomal protein. The amount was below the detection limit in untreated or PB-treated rats. Anti-P-450 PB-B2 did not cross-react with the prostatic microsomes regardless of the pretreatment of the animals.

The activities of NADPH:cytochrome c reductase in both the liver and the prostatic microsomes correspond well to the immunodetectable content. PB induced the liver P-450 reductase about twice, and the immunological content was also elevated almost twice. The prostatic microsomal reductase activity and the immunological content of the P-450 reductase were one-tenth of the liver P-450 reductase activity and content, respectively. The NADPH:cytochrome c reductase activity was not significantly affected by PB or BNF treatments.

The AHH and 7-ethoxyresorufin O-deethylase activities were induced by BNF about 500-fold in the prostate and about 100-fold in the liver. However, PB treatment did not affect the activity of 7-ethoxyresorufin O-deethylase as much in the liver or the prostate. Aminopyrine N-demethylase was increased in the prostate by both PB and BNF treatment, whereas PB induced aminopyrine N-demethylase about 4-fold, and BNF had no effect in the liver.

**DISCUSSION**

In the present investigation, we have used antibodies against rat liver cytochromes P-450 PB-B2 and P-450 BNF-B2 and against NADPH:cytochrome P-450 reductase to characterize the prostatic cytochrome P-450-dependent monoxygenase system. Anti-P-450 BNF-B2 and anti-P-450 reductase were found to cross-react with prostatic proteins as confirmed by enzyme inhibition and immunoblotting. Anti-P-450 reductase inhibited the prostatic NADPH:cytochrome c reductase activity by 60%, which can be compared with the 70% inhibition of liver microsomal...
NADPH:cytochrome c reductase activity obtained with the same antibodies. The anti-P-450 reductase gave 50% inhibition of prostastic microsomal AHH and 7-ethoxyresorufin O-deethylase activity. The only partial inhibition of the P-450 reductase in prostate or liver microsomes, as judged from the effects of the antibodies on the NADPH:cytochrome c reductase or monoxygenase activities, could reflect partial shielding of the protein by the microsomal membrane. The purified liver P-450 reductase was inhibited by 90% by the same antibodies. As judged by immunoblotting, the anti-P-450 reductase recognized only a single protein in either liver or prostastic microsomes, which had the same molecular weight as did the purified liver P-450 reductase. The prostastic microsomes were found to contain only one-tenth as much P-450 reductase as did liver microsomes, measured either by quantitative immunoblotting or by assay of NADPH:cytochrome c reductase activity. Neither PB nor BNF treatment affected the amount of the P-450 reductase in the prostate, whereas PB gave a 2-fold induction in the liver.

With regard to cytochrome P-450, a protein which cross-reacted with anti-P-450 BNF-B2 was found in the prostastic microsomes, but only after BNF treatment. No protein which cross-reacted with anti-P-450 PB-B2 was detected in the prostastic microsomes regardless of treatment of the animals. The results were somewhat more complicated for the liver microsomes, in which more than a single band was seen using anti-P-450 BNF-B2 and anti-P-450 PB-B2. When the spectral and the specific immunological contents of the 2 investigated isozymes P-450 PB-B2 (measured as the sum of P-450 PB-B2 and P-450 PB-B2) and P-450 BNF-B2 are compared, it can be seen that these forms constitute about 1 to 2% of the spectrally detected total P-450 in the untreated liver and increase to account for approximately 80 to 85% of the total P-450 in PB- or BNF-treated liver microsomes, respectively. Our results can be compared with those of other investigators who have shown that the major rat liver BNF or 3-methylcholanthrene-induced cytochrome P-450 isozyme constitutes about 70% and the major PB-induced form about 50% of the total cytochrome P-450 content (8, 10, 20).

These results are, however, based on the spectral determination of total cytochrome P-450 content, which does not measure the apo-P-450 in microsomes. The actual total cytochrome P-450 content may therefore be underestimated by the spectral method, consequently causing the contents of the individual isozymes measured using immunological techniques to be overestimated. Recently, Guengerich et al. (6), using antibodies against 8 cytochrome P-450 isozymes, provided evidence that the total immunological content of cytochrome P-450 may exceed the spectral cytochrome P-450 content by as much as 100%. Thus, the major BNF-induced form constituted 45% and the major PB-induced form constituted 28% of the total immunologically determined liver microsomal cytochrome P-450 in BNF- and PB-treated rats, respectively.

Several bands with lower molecular weight were recognized by anti-P-450 BNF-B2 IgG. No attempts were made to elucidate the identity of these bands. However, none of the unidentified bands were identical with P-450 PB-B2. Anti-P-450 PB-B2 IgG recognized a polypeptide with a lower molecular weight than that of P-450 PB-B2 in liver microsomes. The identity of this band was not determined. The presence of extra bands did not affect the results obtained because only the bands of interest (P-450 PB-B2 or P-450 BNF-B2) were cut out and quantified. It is, however, interesting to note these immunochemo similarities between P-450 BNF-B2 and P-450 PB-B2 and other polypeptides in liver microsomes, probably other cytochrome P-450 isozymes. Anti-BNF-B2 inhibited both AHH and 7-ethoxyresorufin O-deethylase activities in the prostastic microsomes by more than 90%. As judged by immunoblotting, the anti-P-450 BNF-B2 recognized a single protein only (M, 54,000) in prostacic microsomes from BNF-treated animals. In microsomes from control rats, the level of this protein was below the limit of detection (0.4 pmol/mg) of the immunoblotting method whereas, 24 h after a single i.p. injection of BNF, prostacic microsomes contained 50 pmol/mg, which represents at least a 125-fold induction. The fact that the time course of induction and decay of 7-ethoxyresorufin O-deethylase activity and the content of P-450 BNF-B2 in prostacic microsomes paralleled each other after a single i.p. injection of BNF suggests that the P-450 form which cross-reacts with the antibodies is the isozyme responsible for the induced metabolism of 7-ethoxyresorufin in the prostate.

The turnover numbers of prostacic microsomes from untreated rats for the substrates aminopyrine, benzo(a)pyrene, and 7-ethoxyresorufin are very low. Such microsomes are, however,
capable of hydroxylation a number of steroids. Whether or not the steroid hydroxylases are responsible for the low xenobiotic-metabolizing activity in prostatic microsomes from control rats remains to be established.

Previous work from this laboratory has shown that a prostatic S-9 mix from BNF-treated rats is capable of activating the promutagens benzo(a)pyrene and 2-aminofluorene as assayed in the Ames' Salmonella mutagenicity test (18). The results of the present investigation suggest that a highly inducible cytochrome P-450 isozyme identified in the rat prostatic microsomes using antibodies to rat liver cytochrome P-450 B6 may be responsible for the metabolic activation of the above compounds. Whether or not the human prostate also possesses the ability to activate promutagens and contains highly inducible cytochrome P-450 isozymes remains to be established and may have important consequences for the understanding of the etiology of human prostatic cancer.

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REFERENCES

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