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Expression and Zonal Distribution of *CYP2D16* in the Guinea Pig Adrenal Cortex: Relationship to Xenobiotic Metabolism

QINSHI JIANG, YAJUE HUANG, JEFFREY M. VOIGT, B. KRISTINE DEBOLT, SHIRO KOMINAMI, SHIGEKI TAKEMORI, YOSHIHIKO FUNAE, and HOWARD D. COLBY

Department of Pharmacology and Toxicology, Philadelphia College of Pharmacy and Science, Philadelphia, Pennsylvania 19104 (Q.J., Y.H., J.M.V., B.K.D., H.D.C.), Faculty of Integrated Arts and Sciences, Hiroshima University, Higashi-Hiroshima 724, Japan (S.K., S.T.), and Laboratory of Chemistry, Osaka City University Medical School, Abeno-ku, Osaka 545, Japan (Y.F.)

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SUMMARY

We recently cloned a *CYP2D* subfamily member (*CYP2D16*) from a guinea pig adrenal cDNA library and investigated the expression of *CYP2D16* in the guinea pig adrenal cortex and its relationship to adrenal xenobiotic metabolism. A modified sodium dodecyl sulfate-polyacrylamide gel electrophoresis technique revealed three major bands in the molecular mass range of cytochromes P450 in guinea pig adrenal microsomes. Two of the bands were immunoreactive with anti-*CYP17* (54 kDa) or anti-*CYP21* (52 kDa) antibody. The third band (50 kDa) was immunoreactive with antibody raised against *CYP2D1* and with anti-*CYP1A1/1A2* antibody. Microsequencing of the 50-kDa band yielded an amino-terminal sequence of 38 amino acids identical to that deduced from the *CYP2D16* cDNA. In addition, Northern blot analyses indicated the *CYP1A1* was not expressed in the adrenal gland, suggesting that only *CYP2D16* composed the microsomal 50-kDa band. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blot analyses demonstrated greater expression of *CYP2D16* in microsomes from the inner zone (zona reticularis) of the adrenal cortex than from the outer zones, coinciding with the major site of adrenal xenobiotic metabolism. Bufuralol-1′-hydroxylase activity, a marker for *CYP2D* isozymes, was also greater in inner- than in outer-zone microsomal preparations and was highly correlated with *CYP2D16* concentrations. Northern blot analyses with a full-length *CYP2D16* cDNA as the probe gave strong bands with adrenal inner zone RNA preparations and relatively weak bands with outer zone RNA. *CYP2D16* mRNA was also detectable in liver and kidney RNA preparations, but at lower levels than in the adrenal inner zone, and it was not detectable in testes, lung, intestines, or heart. Overall, the results demonstrate that *CYP2D16* is expressed at highest levels in the inner zone of the guinea pig adrenal cortex and suggest a major role for this isozyme in adrenal xenobiotic metabolism.

The adrenal cortex contains an abundance of cytochromes P450, principally those required for steroid hormone synthesis (1, 2). The steroidogenic P450 isozymes have been extensively investigated, and most have been well characterized with respect to catalytic function and regulatory mechanisms. Many foreign compounds are also metabolized by the adrenal cortex, sometimes resulting in the formation of reactive metabolites and toxicity of the gland (3–5). The rates of adrenal xenobiotic metabolism are particularly high in the human fetal and guinea pig adrenal gland, where activities often exceed those in liver (3, 5, 6–10). However, relatively little is known about the P450 isozymes that catalyze adrenal metabolism of xenobiotics.

Otto *et al.* (11) partially purified a novel P450 from rat adrenal microsomes that appears to be responsible for the activation and adrenal toxicity of 7,12-dimethylbenz[*a*]anthracene in rats (4). Recent cloning experiments have established the identity of this isozyme as *CYP1B1* (12). An apparently different P450 isozyme is associated with the high levels of xenobiotic metabolism in the guinea pig adrenal gland. Black *et al.* (13, 14) demonstrated the presence of a 52-kDa protein in microsomes from the inner zone (zona reticularis) of the guinea pig adrenal cortex that is immunoreactive with polyclonal antibody raised against *CYP1A1/1A2*. The localization of this protein to the inner zone coincides with the site of most xenobiotic metabolism in the cortex (9, 10). In addition, a close correspondence between changes in adrenal xenobiotic metabolism and in concentrations of the 52-kDa protein occurs in response to a number of physiological variables (8, 9, 14–16). These and other observations provide indirect evidence for the involvement of the 52-kDa protein in adrenal xenobiotic metabolism.

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[1] Current affiliation: Albany College of Pharmacy, Union University, Albany, NY 12208.

ABBREVIATIONS: SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; ACTH, adrenocorticotropic hormone; HPLC, high performance liquid chromatography.

We recently cloned and sequenced a P450 isozyme from a guinea pig adrenal cDNA library that is highly homologous with members of the *CYP2D* subfamily (17). This isozyme has been designated *CYP2D16*. Because a variety of drugs and other foreign compounds are metabolized by *CYP2D* isozymes (18–24), we postulated that *CYP2D16* may be of importance in adrenal xenobiotic metabolism (17). We now report on the expression of *CYP2D16* in the different zones of the guinea pig adrenal cortex as well as in other organs. The results suggest that *CYP2D16* probably is the adrenal inner zone microsomal protein described by Black *et al.* (13, 14) and may have a major role in adrenal metabolism of xenobiotics.

Experimental Procedures

Materials. A full-length guinea pig *CYP2D16* cDNA (17) and a partial-length guinea pig *CYP17* cDNA corresponding to base pairs 1–1280 (25) were cloned in our laboratory. A full-length guinea pig *CYP1A1* cDNA was provided by Dr. S. Ohgiya (Government Industrial Development Laboratory-Hokkaido, Agency of Industrial Science and Technology, Sapporo, Japan) (26). Polyclonal antibody to *CYP1A1/1A2* was purchased from Human Biologics (Phoenix, AZ). Anti-rat *CYP2D1*, anti-guinea pig *CYP17*, and anti-bovine *CYP21* antibodies were elicited in rabbits as previously reported (27–29). Goat anti-rabbit IgG and the random primer DNA labeling kits were obtained from Stratagene (La Jolla, CA). T4 polynucleotide kinase was purchased from New England Biolabs (Beverly, MA). Nitrocellulose and Nytran nylon membranes were obtained from Schleicher and Schuell (Keene, NH). Bufuralol and 1′-hydroxybufuralol were provided by Dr. Bruce Mico, (Hoffman-LaRoche, Nutley, NJ). Protein assay kits and chemicals for gel electrophoresis were obtained from Bio-Rad (Hercules, CA). Protein and RNA molecular mass standards were purchased from Promega (Pittsburgh, PA). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Animals and tissue preparations. Adult male English shorthair guinea pigs (800–1000 g) obtained from Camm Research Institute (Wayne, NJ) were used in all experiments. Animals were killed by CO_2 inhalation, and organs were quickly removed and trimmed free of adhering fat and connective tissue. Adrenals were bisected longitudinally, and the dark-brown inner zone, consisting primarily of zona reticularis, was dissected from the tan outer zone, comprising the zona fasciculata and zona glomerulosa (9, 10). Tissues were homogenized, and microsomal preparations were obtained by differential centrifugation as described previously (10). In some experiments, the adrenal inner zone tissue obtained from each animal was divided into two aliquots; one aliquot was used for isolation of RNA, and the other was used for enzyme assays and Western blotting.

SDS-PAGE and Western blot analyses. PAGE of adrenal microsomal protein was based on the method of Laemmli (30) with some modifications. Briefly, 10 μg of protein was loaded onto an 8% polyacrylamide gel (14 × 16 cm) made with an acrylamide/bisacrylamide ratio of 19:1. Electrophoresis was done under constant voltage (125 V for stacking gel and 250 V for separating gel) in half-strength Laemmli tank buffer (12.5 mM Tris, 125 mM glycine, 0.1% SDS). The loading buffer contained 50 mM Tris·HCl, pH 6.8, 2% SDS, 10% glycerol, 100 mM dithiothreitol, and 0.2% bromophenol blue. Gels were stained with Coomassie brilliant blue R-250 to visualize protein bands. Western blotting analyses were done according to the method of Towbin *et al.* (31) with goat anti-rabbit IgG coupled to alkaline phosphatase with 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium as substrate for detection. To check for protein transfer and to match Coomassie-stained protein bands with immunoreactive proteins, the nitrocellulose sheets were stained with Poncieux S for 10 min and then rinsed with nanopure water before incubation with primary antibodies.

Amino-terminal amino acid sequencing. After adrenal inner-zone microsomal proteins were separated by SDS-PAGE, they were transferred onto a polyvinylidene difluoride membrane. The membrane was briefly stained with amino black and rinsed thoroughly with nanopure water. The 50-kDa protein band was then cut out for microsequencing. The amino-terminal amino acid sequence was analyzed by automated Edman degradation with a Beckman LF3400 protein sequencer.

Northern blot analyses. Total RNA was isolated by immediate homogenization of tissues in 4 M guanidinium thiocyanate followed by gradient ultracentrifugation in 5.7 M CsCl (32). RNA preparations (4–60 μg for adrenals and 25–60 μg for other organs) were denatured, electrophoresed on a 1% agarose gel containing 2.2 M formaldehyde, and transferred onto a nylon membrane by alkaline transfer (33). Northern blotting was done with a full-length guinea pig *CYP2D16* cDNA as the probe (17). The membrane was then stripped at 90° in 40 mM Tris·HCl, pH 7.8, containing 0.5% SDS and reprobed with a guinea pig *CYP1A1* or *CYP17* cDNA. Hybridization and washing were carried out under conditions described by Ausubel *et al.* (34) with a final wash of 15 min at 60° in 0.2× saline/sodium phosphate/EDTA and 0.1% SDS. A rat 18*S* ribosomal RNA oligodeoxyribonucleotide probe, end labeled with [γ-^{32}P]ATP, was used to assess the amount and integrity of loaded RNA (35, 36). Hybridization with oligodeoxyribonucleotide probes was done according to Clements *et al.* (35) with a final wash of 15 min at 50° in 0.2× saline/sodium phosphate/EDTA (1 × = 150 mM NaCl, 10 mM NaH_2PO_4, and 1 mM EDTA) and 0.1% SDS. All Northern blots were visualized with the use of autoradiography after film (Kodak X-OMAT-R) exposures at −70° for the times indicated.

Enzyme assays. Adrenal microsomal 21-hydroxylase activity was determined from the rate of conversion of 17α-hydroxyprogesterone to 11-deoxycortisol, and 17α-hydroxylase activity was determined as the rate of conversion of progesterone to 17α-hydroxyprogesterone plus 11-deoxycortisol. Incubation conditions and HPLC analyses of metabolites have been previously described in detail (37). Benzo[*a*]pyrene hydroxylation was determined according to the fluorometric method of Nebert and Gelboin (38). Quinine sulfate was calibrated against authentic 3-hydroxybenzo[*a*]pyrene and routinely used as the fluorescence standard. Benzphetamine *N*-demethylation was assayed as the amount of formaldehyde formed according to the method of Nash (39), as previously described (29). Bufuralol-1′-hydroxylase activity was determined according to the HPLC procedure described by Kronbach *et al.* (40) with minor modification. The HPLC effluent was monitored by UV absorbance at 247 nm, and quantification was made by comparison of metabolite peak areas

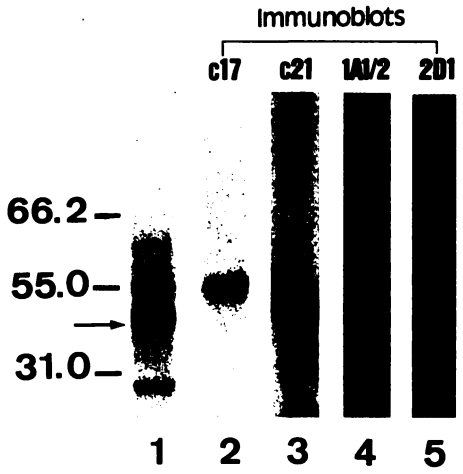

Fig. 1. Electrophoretic and Western blot analyses of adrenal inner-zone microsomes. Analyses were done as described in Experimental Procedures with 10 μg of protein loaded in each lane. Lanes are labeled as follows: *lane 1*, Coomassie-stained gel; *lane 2*, anti-*CYP17* (*c17*) immunoblot; *lane 3*, anti-*CYP21* (*c21*) immunoblot; *lane 4*, anti-*CYP1A1/1A2* (*1A1/2*) immunoblot; and *lane 5*, anti-*CYP2D* (*2D1*) immunoblot. *Arrow*, 50-kDa band.

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2D16P    - GLLTGDALFSVAVAVAIFLLLVDLMHRRQRWAARYPPG
           |||||| |  ||||||||||||||||| ||| |||||
DOG2DP   - MGLLTGDTLGPLAVAVAIFLLLVDLMHRRRRWATRYPPG
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Fig. 2. Amino-terminal amino acid sequences of the guinea pig adrenal microsomal 50-kDa protein (*2D16P*) and of the dog *CYP2D15* (*DOG2DP*). The guinea pig sequence was obtained as described in Experimental Procedures, and the dog sequence is from Genbank. *Vertical lines*, amino acids that are identical in the two sequences.

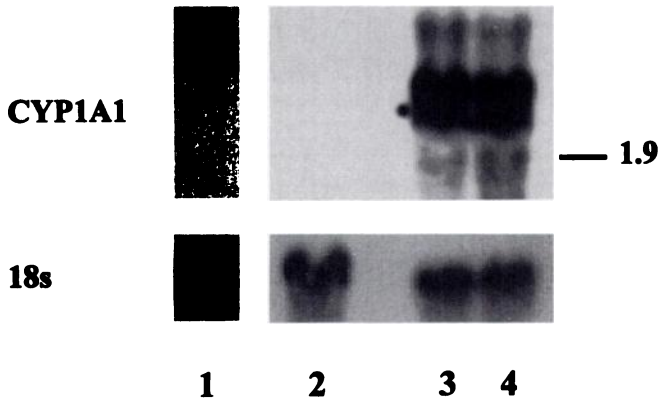

Fig. 3. Northern blot analyses of total RNA (60 μg/lane) from adrenal inner zone (*lanes 1 and 2*) and liver (*lanes 3 and 4*) from two guinea pigs. Preparations were sequentially hybridized with a guinea pig *CYP1A1* cDNA and a rat 18S (*18s*) ribosomal oligodeoxyribonucleotide, as described in Experimental Procedures. Membranes were exposed to the *CYP1A1* and 18S ribosomal probes for 48 and 3 hr, respectively.

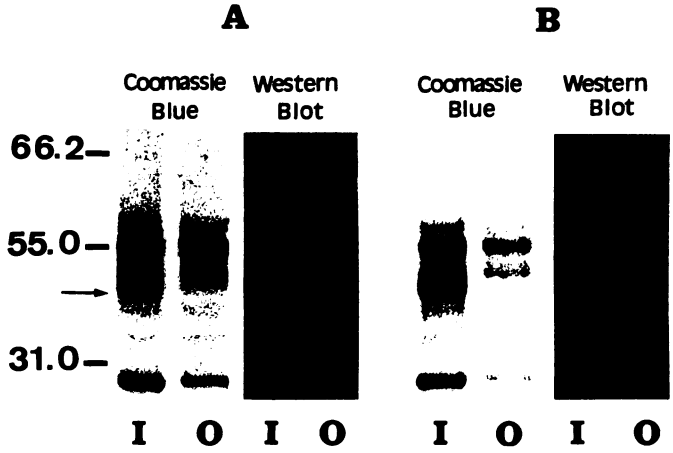

Fig. 4. Electrophoretic and Western blot analyses of adrenal inner (*I*) and outer (*O*) zone microsomes from two different guinea pigs (A and B). Analyses were done as described in Experimental Procedures with 10 μg of protein loaded in each lane; Western blotting was done with anti-*CYP2D1* antibody. *Arrow*, 50-kDa band.

with those of authentic 1′-hydroxybufuralol. For all enzyme assays, conditions were established to ensure linearity of product formation with respect to protein concentrations and incubation times. Where indicated, enzyme inhibitors (quinidine, quinine) were added to incubation media in small volumes (5–10 μl) of methanol; controls received equal volumes of only the vehicle.

Results

Black *et al.* (13, 14) previously described an adrenal inner-zone microsomal protein that was highly correlated with xenobiotic-metabolizing activity. This protein was reported to be immunoreactive with polyclonal antibody to *CYP1A1/1A2* and had an apparent molecular mass of 52 kDa. In initial attempts to further characterize the protein, we could not consistently resolve it from the *CYP21* with the SDS-PAGE conditions previously described (13, 14). However, when the SDS-PAGE conditions were modified as described in Experimental Procedures, three prominent bands (54, 52, and 50 kDa) were well resolved in the molecular mass range of known cytochromes P450 (Fig. 1). The 54- and 52-kDa bands were immunoreactive with anti-*CYP17* IgG and anti-*CYP21* IgG, respectively, as demonstrated by Western blot analyses (Fig. 1). The 50-kDa protein was immunoreactive with a polyclonal antibody raised against *CYP1A1/1A2* (Fig. 1), but, in addition, the same band was also immunoreactive with anti-rat *CYP2D1* IgG (Fig. 1). The data in Fig. 1 are indicative of results obtained with adrenal microsomes from six to nine animals.

To establish the identity of the 50-kDa protein, amino-terminal amino acid sequencing of the SDS-PAGE 50-kDa band was done after transfer to a polyvinylidene difluoride membrane. The procedure gave an amino acid repetitive yield of 91%, suggesting that only one protein was contained in the 50-kDa band. Sequencing revealed 38 amino acids of the protein (Fig. 2) with high homology for *CYP2D* subfamily members in other species. For example, as shown in Fig. 2, the sequence was 84% homologous with canine *CYP2D15*. More importantly, the amino acid sequence was identical to that deduced from the nucleotide sequence of the *CYP2D16* that was recently cloned from a guinea pig adrenal cDNA library (17). In addition, Northern blot analyses revealed that *CYP1A1*, although constitutively expressed in guinea pig liver (26), is not expressed in the adrenal gland (Fig. 3). Even large amounts of adrenal RNA in two different experiments failed to elicit a detectable signal after hybridization with a guinea pig *CYP1A1* cDNA. These results are consistent with the conclusion that the 50-kDa band corresponds to *CYP2D16* and does not include *CYP1A1*.

Previous studies demonstrated that the guinea pig adrenal inner zone (zona reticularis) has much higher xenobiotic-metabolizing activity than the outer zones (9, 10). To determine whether *CYP2D16* has the same zonal distribution, microsomal preparations from the inner and outer zones were analyzed for *CYP2D16* content by SDS-PAGE and Western blotting. As shown in Fig. 4 for two representative animals (of ~12 that have been studied), the 50-kDa band corresponding to *CYP2D16* is more prominent in the inner than in the outer zone.[2] When Western blotting was done with anti-rat *CYP2D1* IgG, a single band (50 kDa) was identified in the inner zone only (Fig. 4), supporting the conclusion that *CYP2D16* is localized to the zona reticularis of the guinea pig adrenal cortex.

To determine whether the zonal distribution of *CYP2D16* protein is the result of differences in gene expression, Northern blot analyses were done with a full-length *CYP2D16* cDNA as a probe. Hybridization with total RNA isolated from

[2] The apparent zonal differences in the steroidogenic isozymes *CYP17* (54 kDa) and *CYP21* (52 kDa) will be the subject of another report.

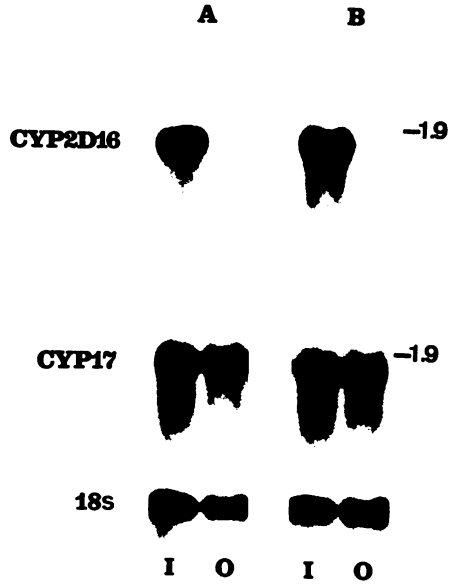

Fig. 5. Northern blot analyses of total RNA (10 μg/lane) from adrenal inner (*I*) and outer (*O*) zones of two different guinea pigs (A and B). The results are representative of ~15 animals studied. Preparations were sequentially hybridized with a *CYP2D16* cDNA for 2 hr, a *CYP17* cDNA for 4 hr, and a rat 18*S* (*18s*) ribosomal oligodeoxyribonucleotide for 3 hr, as described in Experimental Procedures.

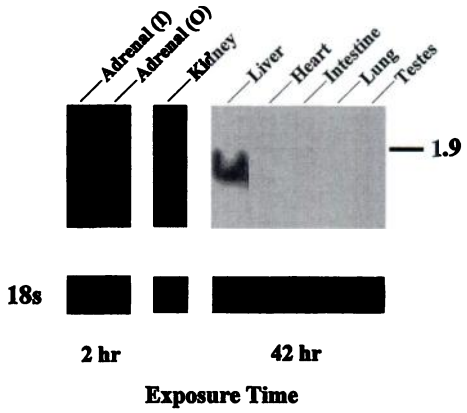

Fig. 6. Northern blot analyses of total RNA (25 μg/lane) from a variety of guinea pig organs. Hybridization was done sequentially with a *CYP2D16* cDNA and rat 18*S* (*18s*) ribosomal oligodeoxyribonucleotide, as described in Experimental Procedures. Lanes are labeled as follows: *lane 1*, adrenal inner zone (*I*); *lane 2*, adrenal outer zone (*O*); *lane 3*, kidney; *lane 4*, liver; *lane 5*, heart; *lane 6*, intestine; *lane 7*, lung; and *lane 8*, testes. With the *CYP2D16* probe, membranes corresponding to lanes 1 and 2 were exposed for 3 hr, and lanes 3–8 were exposed for 42 hr. With the 18*S* probe, all membranes were exposed for 3 hr.

each zone revealed a distinctive 1.7–1.8-kilobase band that was stronger in the inner than in the outer zone (Fig. 5). Rehybridization of the same blot with a *CYP17* cDNA indicated similar levels of expression in the two zones. The results obtained with the rat 18*S* ribosomal oligodeoxyribonucleotide did not reveal any major differences in the amount of RNA loaded from each zone (Fig. 5).

In other species that have been studied, *CYP2D* isozymes are expressed principally in liver, with lower levels found in kidney (20, 21). Accordingly, we determined whether *CYP2D16* mRNA was expressed in extra-adrenal tissues of guinea pigs. Northern blot analyses indicated the presence of *CYP2D16* mRNA in liver and kidney, but none was demonstrable in heart, lung, intestine, or testis (Fig. 6). The concentration of *CYP2D16* mRNA was lower in liver than in the inner zone of the adrenal cortex and was still lower in kidney (Fig. 6). The data in Fig. 6 are representative of the results obtained with three to six RNA preparations from each organ.

One of the substrates that is metabolized almost exclusively by *CYP2D* isozymes is the drug bufuralol (24, 41). To determine whether guinea pig adrenals had catalytic activity characteristic of *CYP2D* subfamily members in other species, microsomal preparations were incubated with bufuralol, and the rates of production of 1′-hydroxybufuralol were monitored by HPLC. As indicated in Fig. 7, bufuralol-1′-hydroxylation was catalyzed by adrenal microsomes and enzyme activity was greater in inner- than in outer-zone microsomes, corresponding to the localization of *CYP2D16*. The ratios of inner- to outer-zone activities for other microsomal xenobiotic-metabolizing reactions, such as benzo[*a*]pyrene hydroxylation and benzphetamine *N*-demethylation, were similar to that for bufuralol-1′-hydroxylation (Fig. 7).

In the course of investigating adrenal xenobiotic metabolism in numerous outbred (English short-hair) guinea pigs over a period of several years, we noted that in some animals adrenal inner-zone activities were unusually low. Because of the polymorphic nature of hepatic *CYP2D6* expression in humans, the possibility was investigated of genetic variability in *CYP2D16* expression in guinea pig adrenals. As illustrated in Fig. 8, among 12 randomly selected mature male guinea pigs, four animals had far lower bufuralol-1′-hydroxylase and benzo[*a*]pyrene hydroxylase activities in adrenal inner zone microsomes than did the other eight. The rates of adrenal bufuralol and benzo[*a*]pyrene metabolism were highly correlated in those animals ($r = 0.82$; $p < 0.05$). Both reactions are also inhibited by the *CYP2D*-selective inhibitors quinidine (Table 1) and quinine (not shown), raising the possibility of catalysis by the same P450 isozyme. In contrast, microsomal 17α-hydroxylase and 21-hydroxylase activities were similar in the high and low xenobiotic metabolizers (not

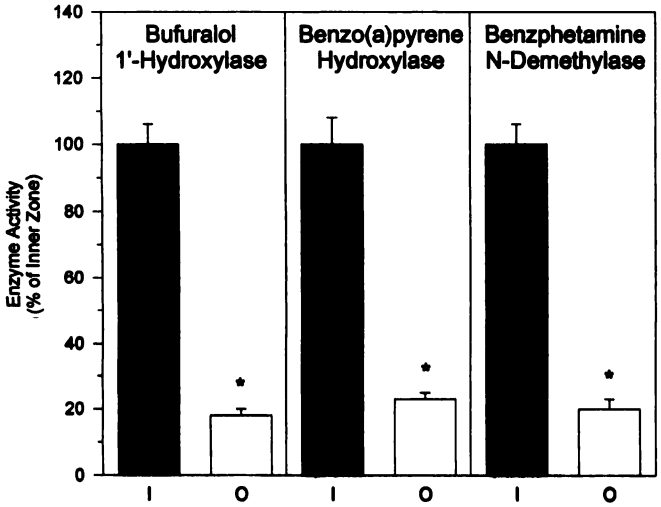

Fig. 7. Bufuralol-1′-hydroxylase, benzo[*a*]pyrene hydroxylase, and benzphetamine *N*-demethylase activities in adrenal inner and outer zone microsomes. Values are mean ± standard error of six different preparations and are expressed as percentage of the corresponding inner-zone value. *, $p < 0.05$ (versus inner zone).

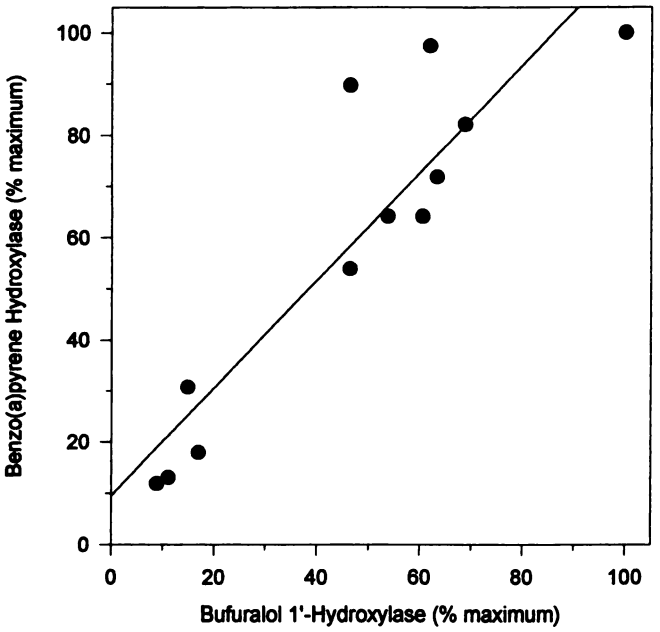

Fig. 8. Relationship between bufuralol 1′-hydroxylase and benzo-[a]pyrene hydroxylase activities in adrenal inner-zone microsomal preparations from 12 randomly chosen English short-hair guinea pigs. ●, Data for one animal. Values are expressed as a percentage of the maximal activity in the group. The correlation coefficient for the line is 0.82 ($p < 0.05$).

TABLE 1

Effects of quinidine on adrenal microsomal bufuralol-1′-hydroxylase and benzo[a]pyrene hydroxylase activities

Quinidine concentration	Bufuralol-1′-hydroxylase		Benzo[a]pyrene hydroxylase	
μM	pmol/min × mg protein	% control	pmol/min × mg protein	% control
0	927 ± 89	100	769 ± 82	100
5	844 ± 76	91	731 ± 62	95
10	763 ± 81	83	622 ± 57	81
25	473 ± 53	51	569 ± 51	74
100	241 ± 29	26	420 ± 53	54

Values are mean ± standard error of three to six experiments.

shown), indicating that adrenal xenobiotic and steroid metabolism are independently regulated. In the animals with low xenobiotic-metabolizing activities, adrenal *CYP2D16* protein concentrations were correspondingly low. As illustrated in Fig. 9 for four representative animals, microsomal *CYP2D16* content is considerably greater in the high (*lanes 1, 3, and 4*) than the low (*lane 2*) metabolizers. Similar results were obtained with the other high and low metabolizers illustrated in Fig. 8, indicating a close relationship between adrenal *CYP2D16* content and xenobiotic metabolism.

Discussion

Black *et al.* (13, 14) demonstrated that the presence of a 52-kDa microsomal protein is highly correlated with xenobiotic-metabolizing activities in the guinea pig adrenal cortex. Our attempts to isolate and further characterize this protein were initially impeded by its similar mobility to that of *CYP21* under standard SDS-PAGE conditions. However, modification of the gel and buffer compositions as described in Experimental Procedures resulted in the clear resolution of two distinct bands: a 52-kDa band that is immunoreactive

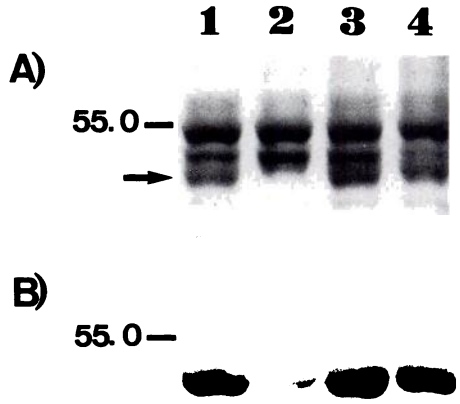

Fig. 9. SDS-PAGE (A) and Western blotting analyses (B) of adrenal inner-zone microsomes from four of the guinea pigs indicated in Fig. 8. Western blotting was done with anti-*CYP2D1* antibody, as described in Experimental Procedures.

with antibody to *CYP21* and a 50-kDa band that is immunoreactive with anti-*CYP1A1/1A2* antibody. We do not know why the modified SDS-PAGE conditions cause an apparent reversal of the band positions described by Black *et al.* (13, 14), but this method gives consistently good separation of the two proteins. Amino-terminal analysis of the 50-kDa band revealed an amino acid sequence identical to that deduced from our recently cloned *CYP2D16* cDNA (17). Immunoreactivity with anti-*CYP2D1* antibody further suggested that the 50-kDa band was *CYP2D16*. The latter conclusion was reinforced by the results of Northern analyses indicating that *CYP1A1* is not expressed in the guinea pig adrenal cortex. Overall, these observations indicate that the SDS-PAGE 50-kDa band consists of *CYP2D16* and suggest that this isozyme may cross-react with *CYP1A1/1A2* antisera. The latter possibility is now being investigated. Thus, it seems likely that *CYP2D16* is the "52-kDa" protein described by Black *et al.* (13, 14).

In other species, *CYP2D* isozymes are expressed principally in the liver and kidney (42–44). However, in the guinea pig, expression of *CYP2D16* mRNA in the inner zone of the adrenal cortex exceeds that in the other organs we investigated. *CYP2D16* mRNA was demonstrable in guinea pig liver and kidney, but at low levels compared with that in adrenal. *CYP2D16* protein, like *CYP2D16* mRNA, seems to be expressed at much higher levels in the inner than in the outer zones of the adrenal cortex. Differences in the levels of expression of steroidogenic P450 isozymes among the three anatomic zones of the adrenal cortex have long been known to exist (1, 2). Nevertheless, the mechanisms responsible for the unique enzymatic profile within each zone have yet to be fully resolved. The relatively selective expression of *CYP2D16* in the inner zone of the guinea pig adrenal cortex seems to provide another example of this functional zonation. It remains to be determined whether even the apparent small amount of *CYP2D16* in the outer zones of the cortex is the result of contamination by inner zone tissue. As noted by Martin and Black (45), it is impossible to totally eliminate the inner-zone from adrenal outer-zone preparation by microdissection. Immunohistochemical approaches are being used in an attempt to unequivocally establish the intra-adrenal distribution of *CYP2D16*.

The mechanisms responsible for the selectively high ex-

pression of *CYP2D16* in the inner zone of the adrenal cortex remain to be resolved. It is possible that the cells of the inner zone contain some unique transcriptional activating factor or factors for *CYP2D16* that are not found in the outer zones. The low level of *CYP2D16* expression in the outer zones of the adrenal coupled with high expression in the inner zone should facilitate the identification of such factors. Based on the observations of Black *et al.* (13, 14), it seems likely that adrenal *CYP2D16* is down-regulated by ACTH. The nature of adrenal blood flow (46), consisting of a capillary plexus that perfuses the outer regions of the cortex before supplying the inner zone, may create a relative ACTH deficiency in the inner zone and thereby promote *CYP2D16* expression. Because ACTH seems to have opposite effects on the expression of steroidogenic P450s (up-regulation) and of *CYP2D16* (down-regulation), the hormonal environment of adrenal cells may be of major importance in determining the profiles of P450 isozyme expression. Thus, investigations on the regulation of *CYP2D16* expression in the inner zone may simultaneously contribute to a more complete understanding of the mechanisms responsible for the overall functional zonation of the adrenal cortex.

It is not known which of the xenobiotic-metabolizing activities of the guinea pig adrenal cortex are attributable to *CYP2D16*. The high level of adrenal bufuralol-1′-hydroxylase activity, a marker for *CYP2D* isozymes (18, 19), is consistent with *CYP2D16* expression in the gland. Many other enzymatic reactions that have been attributed to *CYP2D* subfamily members (20–24) are also catalyzed by guinea pig adrenal microsomes (3, 7–10). One notable exception is benzo[*a*]pyrene hydroxylase activity, which is high in the guinea pig adrenal but is not characteristic of other *CYP2D* isozymes. However, as illustrated in Fig. 8, benzo[*a*]pyrene hydroxylase and bufuralol-1′-hydroxylase activities are highly correlated in guinea pig adrenal microsomes, suggesting metabolism by similarly regulated isozymes. In addition, adrenal benzo[*a*]pyrene metabolism is at least partially inhibited by the *CYP2D*-selective inhibitors quinidine and quinine. The apparent lack of *CYP1A1* expression in the adrenal (Fig. 3) precludes a role for this isozyme in benzo[*a*]pyrene metabolism. It has been demonstrated that the guinea pig *CYP17* catalyzes benzo[*a*]pyrene hydroxylation (47), but the zonal distribution of this isozyme fails to account for the preponderance of enzyme activity being localized to the inner zone of the adrenal. It will be of interest to determine whether *CYP2D16*, unlike other *CYP2D* isozymes, catalyzes the metabolism of polycyclic aromatic hydrocarbons such as benzo[*a*] pyrene.

Of additional interest is the possible involvement of *CYP2D16* in adrenal steroid metabolism, in particular, cortisol 6β- and 2α-hydroxylase activities. Both reactions are catalyzed by guinea pig adrenal microsomes (48), and prior investigations demonstrated a close relationship with adrenal xenobiotic metabolism (49). Down-regulation of these steroid-inactivating reactions by ACTH could be of physiological relevance because inhibition of intra-adrenal steroid catabolism would contribute to an increase in active hormone secretion. The suppression of steroid inactivation by ACTH in combination with its well-known effects to increase steroid synthesis (1, 2) could serve as complementary mechanisms to optimize hormone output. Studies are under way to evaluate the catalytic capabilities of *CYP2D16* to determine its role in steroid as well as in xenobiotic metabolism by the adrenal.

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Send reprint requests to: Dr. Howard D. Colby, Professor of Pharmacology, Albany College of Pharmacy, Union University, 106 New Scotland Avenue, Albany, NY 12208