Biochemical Pharmacology, Vol. 52, pp. 1925–1929, 1996.

ELSEVIER

ISSN 0006-2952/96/$17.00 + 0.00
PII S0006-2952(96)00665-X

SHORT COMMUNICATION

Strain Differences in Adrenal CYP2D16 Expression in Guinea Pigs

RELATIONSHIP TO XENOBIOTIC METABOLISM

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ABSTRACT. Experiments were done to determine the mechanisms responsible for differences in adrenal microsomal xenobiotic metabolism between Strain 13 and English Short-Hair (ESH) guinea pigs. The rates of adrenal xenobiotic metabolism (bufuralol 1′-hydroxylase, benzo[a]pyrene hydroxylase, benzphetamine *N*-demethylase) were 2–3 times greater in microsomes from the Strain 13 animals. In both strains, xenobiotic-metabolizing activities were far greater in the inner zone (zona reticularis) than in the outer zones (zona fasciculata and zona glomerulosa) of the adrenal cortex. Northern blot analyses of total adrenal RNA with a CYP2D16 cDNA as the probe revealed significantly greater amounts of CYP2D16 mRNA in the Strain 13 guinea pigs. In addition, SDS–PAGE and Western blotting of adrenal microsomes demonstrated higher concentrations of CYP2D16 protein in Strain 13 than in ESH animals. Expression of CYP2D16 was predominantly in the inner zone of the adrenal, coinciding with the major site of xenobiotic metabolism. The results demonstrated higher levels of expression of CYP2D16 in adrenal glands from Strain 13 than from ESH guinea pigs, which may account for the strain differences in adrenal xenobiotic metabolism. Strain 13 guinea pigs should serve as a good experimental model for further studies on the regulation of adrenal CYP2D16. *Copyright © 1996 Elsevier Science Inc.* BIOCHEM PHARMACOL 52;12:1925–1929, 1996.

KEY WORDS. adrenal cortex; xenobiotic metabolism; cytochrome P450; guinea pig; strain differences, CYP2D16

The major function of the adrenal cortex is the synthesis of a variety of steroid hormones. Accordingly, a number of cytochrome P450 isozymes that catalyze steroidogenesis are expressed in the gland [1, 2]. The adrenal cortex is also among the many extrahepatic sites of xenobiotic metabolism [3, 4]. A wide variety of foreign compounds are metabolized by adrenal enzymes, in some cases resulting in the formation of highly reactive metabolites [3, 4]. These metabolites probably contribute to the relatively high incidence of chemical-induced adrenal injuries.

The rates of adrenal xenobiotic metabolism are particularly high in the human fetus and in the guinea pig making the latter a useful model for studies on the enzymes involved. Nonetheless, there is little definitive information available on the adrenal P450 isozymes that catalyze the metabolism of foreign compounds. We recently cloned a cytochrome P450 (CYP2D16) that may account for the high levels of xenobiotic metabolism in the guinea pig adrenal cortex [5, 6]. This microsomal isozyme is highly expressed in the zona reticularis of the cortex, the major region of foreign compound metabolism [6–9]. In addition, the abundance of CYP2D16 in the guinea pig adrenal is highly correlated with the activities of several xenobiotic-metabolizing reactions [6, 9]. Thus, expression of CYP2D16 may be an important determinant of xenobiotic metabolism in the guinea pig adrenal cortex.

Among the factors affecting xenobiotic metabolism within species is the strain of the animal. Strain differences in hepatic xenobiotic metabolism have long been recognized [10, 11]. In addition, we previously reported strain differences in the activities of various adrenal xenobiotic-metabolizing enzymes in guinea pigs [12]. Highly inbred guinea pigs (Strain 13, Strain 2) had far higher levels of activity than did the more common outbred strains such as ESH† guinea pigs. The present studies were carried out to determine the mechanisms responsible for these strain differences in xenobiotic metabolism.

MATERIALS AND METHODS

A full-length guinea pig CYP2D16 cDNA was cloned in our laboratory [6]. Anti-rat CYP2D1 was elicited in rabbits

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Received 6 May 1996; accepted 5 August 1996.

† *Abbreviations:* ESH, English Short-Hair; and BCIP/NBT, 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium.

as previous reported [13, 14]. The random primer DNA labeling kits were obtained from Stratagene (La Jolla, CA). T4 polynucleotide kinase was purchased from New England Biolabs (Beverly, MA). Tri Reagent-RNA/DNA/Protein Isolation Reagent was purchased from the Molecular Research Center, Inc. (Cincinnati, OH). Nitrocellulose and Nytran membranes were from Schleicher & Schuell (Keene, NH). Bufuralol and 1′-hydroxybufuralol were provided by Dr. Bruce Mico (Hoffman–LaRoche, Nutley, NJ). Goat anti-rabbit IgG, protein assay kits, and chemicals for gel electrophoresis were obtained from Bio-Rad (Hercules, CA). Protein and RNA molecular weight standards were purchased from Promega (Madison, WI). All other chemicals were obtained from the Sigma Chemical Co. (St. Louis, MO).

Adult male guinea pigs weighing approximately 850 g were used in all experiments. ESH guinea pigs were obtained from the Camm Research Institute (Wayne, NJ), and Strain 13 guinea pigs were obtained from Crest Caviary (Prunedale, CA). Animals were killed by CO_2 inhalation, and the adrenal glands were quickly removed and trimmed free of adhering fat and connective tissue. Adrenals were then bisected longitudinally, and the dark-brown inner zone, consisting primarily of zona reticularis, was dissected from the tan outer zone comprised of zona fasciculata and zona glomerulosa [8, 9]. Microsomes were obtained by differential centrifugation as described previously [15].

Polyacrylamide gel electrophoresis of adrenal microsomal proteins was done as described previously [6]. Visualization of protein bands was by Coomassie Blue staining. Western blot analyses with anti-CYP2D1 were done according to Towbin *et al.* [16] using goat anti-rabbit IgG coupled to alkaline phosphatase with BCIP/NBT as substrate for detection. To check protein transfer and to match Coomassie-stained protein bands with immunoreactive proteins, the nitrocellulose membranes were stained with Ponceau S for 10 min, and then rinsed with nanopure water before incubation with primary antibodies. Quantitation of immunopositive bands was done with a Multiscan-R Video Densitometry System (Interactive Technologies International, L.C., St. Petersburg, FL), using the 1-D video densitometry software.

Northern blot analyses were done as described previously [6]. Total RNA was isolated using RNeasy™ total RNA kits from Qiagen Inc. (Chatsworth, CA). RNA preparations (10 μg) were denatured, electrophoresed on a 1% agarose gel containing 2.2 M formaldehyde, and transferred onto a nylon membrane by alkaline transfer [17]. Hybridization with the CYP2D16 cDNA probe was done as described by Ausubel *et al.* [18]. A rat 18S ribosomal RNA oligodeoxyribonucleotide probe, end-labeled with [γ-^{32}P]ATP, was used to assess the amount and integrity of RNA loaded [19]. All Northern blots were visualized by autoradiography following film (Kodak X-OMAT-R) exposures at −70°C. The exposure time was 4 hr for all probes. Quantitation was done by video densitometry, as described above for Western blots, and values were normalized for the amount of 18S loading probe in each sample.

Bufuralol 1′-hydroxylase activity was determined by minor modification of the HPLC method described by Kronbach *et al.* [20], as previously described [6]. Benzo[a]pyrene hydroxylation was determined by the fluorometric method of Nebert and Gelboin [21]. 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 produced using the method of Nash [22]. For all enzyme assays, conditions were established to ensure linearity of product formation with respect to protein concentrations and incubation times. Data are presented as means ± SEM. Statistical analyses of the differences between group means were done with the Newman-Keuls multiple-range test; $P < 0.05$ was considered significant.

RESULTS AND DISCUSSION

In both ESH and Strain 13 guinea pigs, bufuralol 1′-hydroxylase activity, a marker for the CYP2D subfamily [23, 24], was far higher in inner than outer zone microsomal preparations (Table 1). Other xenobiotic-metabolizing activities, including benzphetamine N-demethylation and benzo[a]pyrene hydroxylation (Table 1), were similarly greater in inner than outer zone microsomes. In addition, the rates of all xenobiotic-metabolizing reactions were sig-

TABLE 1. Xenobiotic-metabolizing activities in adrenal inner and outer zone microsomal preparations from ESH and Strain 13 guinea pigs

Enzyme activity	ESH Inner zone	ESH Outer zone	Strain 13 Inner zone	Strain 13 Outer zone
Bufuralol 1′-hydroxylase (pmol/min · mg protein)	813 ± 102	116 ± 12*	2152 ± 119†	243 ± 19*
Benzphetamine *N*-demethylase (nmol/min · mg protein)	373 ± 52	53 ± 8*	886 ± 56†	119 ± 8*
Benzo[a]pyrene hydroxylase (pmol/min · mg protein)	617 ± 94	86 ± 11*	1418 ± 108†	182 ± 14*

Values are means ± SEM of 3–6 animals per group.
* $P < 0.05$ (vs corresponding inner zone value).
† $P < 0.05$ (vs corresponding ESH value).

nificantly greater in inner zone microsomal preparations from Strain 13 guinea pigs than in those from ESH animals (Table 1).

To pursue the mechanism(s) responsible for the strain differences in adrenal xenobiotic metabolism, studies on the expression of CYP2D16 in adrenals from Strain 13 and ESH guinea pigs were initiated. Recently published studies demonstrated that CYP2D16 levels in the guinea pig adrenal cortex are highly correlated with microsomal xenobiotic-metabolizing activities [6]. As shown in Fig. 1, Northern analyses revealed that CYP2D16 mRNA levels were far greater in adrenal inner than outer zone tissue from both ESH and Strain 13 guinea pigs. Quantitation of the Northern blotting results further indicated that inner zone tissue from Strain 13 animals contained significantly greater amounts of CYP2D16 mRNA than that from ESH guinea pigs (Fig. 2).

Strain and zonal differences in microsomal CYP2D16 protein levels generally paralleled those in CYP2D16 mRNA content. The results of Western blot analyses indicated that CYP2D16 protein concentrations were far greater in inner than outer zone microsomal preparations in both strains (Figs. 1 and 3). In addition, CYP2D16 protein levels were significantly greater in Strain 13 than in ESH guinea pigs (Fig. 3).

The results indicate that higher levels of CYP2D16 expression may account, at least in part, for the higher rates of xenobiotic metabolism in Strain 13 guinea pigs. Both the

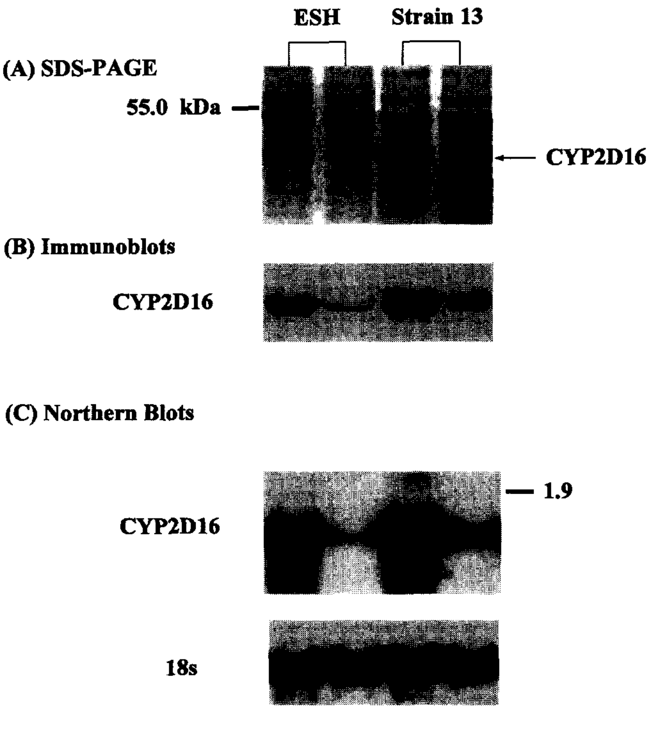

FIG. 1. SDS–PAGE, Western and Northern blot analyses of CYP2D16 in adrenal inner (I) and outer (O) zones of ESH and Strain 13 guinea pigs. Experiments were done as described in the Materials and Methods.

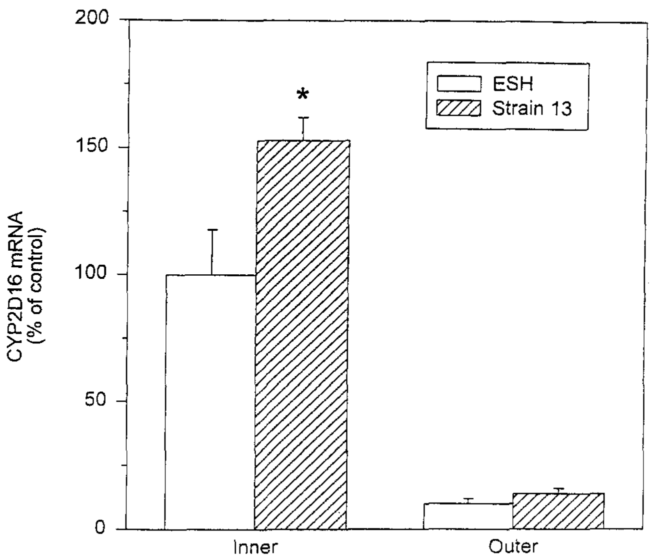

FIG. 2. Quantitative analyses of CYP2D16 mRNA levels in the adrenal inner and outer zones of ESH and Strain 13 guinea pigs. mRNA levels were determined by densitometric analysis of Northern blots as described in Materials and Methods. Values are means ± SEM of three different animals per group and are expressed as a percentage of the ESH inner zone value. Key: (*)$P < 0.05$ (vs corresponding value in ESH group).

zonal localization of CYP2D16 in the adrenal cortex and strain differences in adrenal CYP2D16 content are correlated with xenobiotic-metabolizing activities. To date, CYP2D16 is the only P450 isozyme definitively identified in guinea pig adrenal microsomes that is not primarily ste-

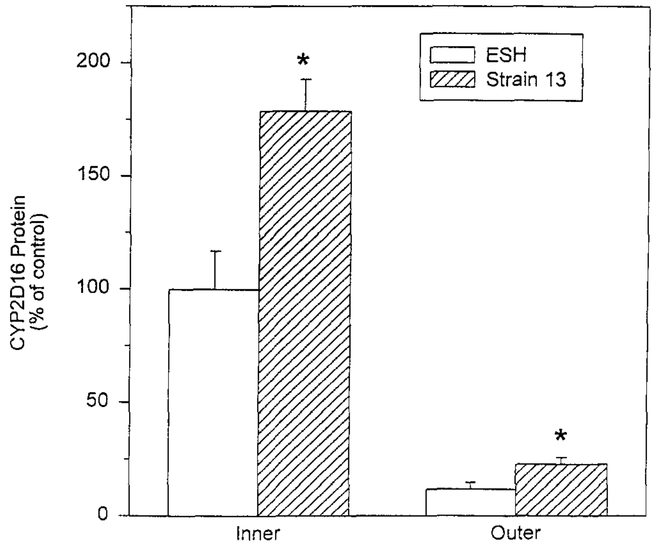

FIG. 3. Quantitative analyses of CYP2D16 protein concentrations in the adrenal inner and outer zones of ESH and Strain 13 guinea pigs. Protein levels were determined by densitometric analysis of Western blots as described in Materials and Methods. Values are means ± SEM of three different animals per group and are expressed as a percentage of the ESH inner zone value. Key: (*)$P < 0.05$ (vs corresponding value in ESH group).

roidogenic in function. Black [25] found that several other adrenal microsomal proteins were immunoreactive with antibodies to CYP2B, CYP2C, or CYP3A subfamily members, but none has yet to be isolated or cloned. In addition, the immunoreactivity noted by Black did not parallel xenobiotic metabolism with respect to either intraadrenal localization or hormonal responsiveness [25]. By contrast, adrenal CYP2D16 levels coincide with xenobiotic-metabolizing activities under a wide variety of experimental conditions [5, 6]. Thus, CYP2D16 is the P450 isozyme most closely linked to xenobiotic metabolism in the guinea pig adrenal cortex.

Among the xenobiotics metabolized by guinea pig adrenal microsomes, bufuralol is a CYP2D-selective substrate and has been used as a marker for catalytic function of CYP2D subfamily members [23, 24]. Since the amino acid sequence of CYP2D16 is approximately 70% homologous with those of other CYP2D isozymes [5], it seems likely that the bufuralol 1′-hydroxylase activity in guinea pig adrenal microsomes is catalyzed by CYP2D16. In addition, adrenal bufuralol metabolism is diminished substantially by the CYP2D-selective inhibitors quinidine and quinine [6].

Demethylation reactions are also catalyzed by guinea pig adrenal microsomes and are among the enzymatic activities of CYP2D isozymes in other species [26, 27]. Although benzphetamine *N*-demethylase activity is more commonly associated with P450 isozymes of the 2B, 2C, and 3A subfamilies [24] some 2D isozymes have also been found to have activity [28, 29]. Sequence relatedness within the CYP2 family probably accounts for some overlap of enzyme activities. Benzo[a]pyrene hydroxylase activity is also high in guinea pig adrenals, but this reaction is characteristically catalyzed by CYP1A1/2 and not CYP2D isozymes. However, we recently reported that CYP1A1 is not expressed in the guinea pig adrenal cortex and that adrenal benzo[a]pyrene metabolism is at least partly blocked by quinidine and quinine [6]. Thus, CYP2D16 may have a role in the adrenal metabolism of a wide variety of xenobiotics. Heterologous expression studies are now in progress to definitively establish the catalytic capabilities of this isozyme.

The mechanism(s) responsible for the high level of CYP2D16 expression in adrenals from Strain 13 guinea pigs remains to be determined. The strain differences in adrenal CYP2D16 mRNA content suggest that transcriptional differences or differences in mRNA half-life are involved. The high level of expression in the adrenal cortex relative to other organs makes CYP2D16 unique among the CYP2D subfamily members but little is known about the factors involved. In ESH guinea pigs there is considerable variability in the level of expression of adrenal CYP2D16 [6]. By contrast, our experience with the inbred Strain 13 animals indicates that expression is not only at high levels but is very consistent from animal to animal. Thus, Strain 13 guinea pigs should serve as an excellent model for further investigations on the regulation of adrenal CYP2D16, particularly for *in vivo* studies in which experimental variability often limits the interpretation of results.

These investigations were supported by USPHS Research Grant AG11987 awarded by the National Institute on Aging.

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