Constitutive and Inducible Hepatic Cytochrome P450 Isoforms in Senescent Male and Female Rats and Response to Low-Dose Phenobarbital

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ABSTRACT:

Numerous studies, usually limited to male rodents, have reported an inverse relationship between the age of the animal and the activities of various multi-cytochrome P450-dependent drug-metabolizing enzymes. It has been suggested that the aging-induced decline in hepatic drug-metabolizing capacity is solely a male phenomenon. That is, whereas the levels of male-specific isoforms of P450 decline with senescence, the female-dependent isoforms remain unchanged in females and even increase in male liver. In addition to their baseline activities, induction levels of hepatic monooxygenases have also been reported to decrease with aging. To examine aging- and sex-dependent effects on drug metabolism at a more molecular level, we measured the expression (mRNA, protein, and/or catalytic activity) of a near dozen constitutive and inducible isoforms of P450 in 5- and 23-month-old male and female Sprague-Dawley rats. Moreover, we investigated the induction effects of low concentrations of phenobarbital known to reveal gender differences and the threshold sensitivities of both constitutive and inducible isoforms. With the exception of male-specific CYP2C11 (whose expression declined ~70% in aged male rats), we observed little senescence-associated reduction in either preinduction or induction levels of CYP2B1, CYP2B2, CYP3A1, CYP3A2, CYP2C6, CYP2C7, CYP2C12, and CYP2C13 in either male or female rats. Moreover, the sexually dimorphic expression levels apparent at 5 months of age persisted in the old rats.

People over 65 years of age represent the fastest growing segment of the population and consume at least one-third of all prescription drugs. Stated another way, 82% of people over 65 use prescription medications (Willcox et al., 1994). Moreover, the high prevalence of polypharmacy in the elderly likely contributes to an abnormally high 20 to 25% incidence of adverse drug reactions and toxicities in this age group (Hunt et al., 1992; Willcox et al., 1994). The generally accepted aging-associated decline in drug disposition is a result of senescent changes in drug absorption, distribution, excretion, and/or metabolism (Schmucker and Lonergan, 1987). Direct studies on liver drug metabolism in elderly humans are scant with most evidence of strain, a critical factor (Chengelis, 1988) influencing age-associated changes in drug metabolism.

In this regard, early investigations using laboratory animals had to limit their analyses to nonspecific multi-P4502-dependent drug-metabolizing enzymes. Moreover, the important contribution of sex was often overlooked by using only the “preferred” gender in pharmacological studies, the male rodent, or worse yet, not even recording the sex of the animal. Last, few investigators considered the effect of strain, a critical factor (Chengelis, 1988) influencing age-associated changes in drug metabolism.

One of the earliest studies using rats reported an inverse relationship between age and the metabolism of commonly used model substrates (Kato and Takanaka, 1968). This, as well as additional studies observed an aging related decline in the in vivo and in vitro metabolism of different classes of drugs, subnormal baseline concentrations of various components of the hepatic drug-metabolizing enzyme system, and/or a decrease in the rate and extent of monooxygenase induction by several agents (Birnbaum and Baird, 1978; McMartin et al., 1980; Schmucker and Wang, 1981). However, there have been numerous conflicting reports indicating no senescent-induced changes in both drug-metabolizing enzyme activities or in their response to inducing agents (Adelman, 1971; Kao and Hudson, 1980; Chengelis, 1988).

In the current investigation, we have examined the effects of senescence and sex on the expression of constitutive and inducible isoforms of P450 exposed to the low concentrations of phenobarbital known to reveal gender differences and threshold sensitivities to induction (Shapiro, 1986; Agrawal and Shapiro, 1996).

Materials and Methods

Animals were housed in the University of Pennsylvania Laboratory Animal Resources facility, under the supervision of certified laboratory animal medicine veterinarians, and were treated according to a research protocol approved by the University’s Institutional Animal Care and Use Committee. At all times, animals were housed on hardwood bedding in plastic cages, with water and commercial rat diet supplied ad libitum. The animal quarters were air conditioned (20–23°C) and had a photoperiod of 12 h of light and 12 h of darkness (lights on at 8:00 AM). After a 2- to 3-week acclimation period in our facilities,
the animals were bred by randomly housing two adult female Sprague-Dawley rats [Crl:CD(SD)BR] with an individual adult male of the same strain. On the day of parturition, all litters were mixed and randomly assigned to the dams at 10 pups/litter, with a sex ratio of 1:1 or close to that as possible. The pups were weaned at 24 days of age. At 5 or 23 months of age, male and female offspring were injected daily, intraperitoneally, with either 1 mg or 10 mg/kg b.wt. of phenobarbital or an equivalent sodium concentration of NaCl diluent, pH 9.1, for a maximum of 6 days. Four to six rats in each treatment group were decapitated 0, 64, and 136 h after the first phenobarbital injection. Livers were quickly removed and perfused with ice-cold saline. Each liver was quickly minced; a portion for mRNA determinations was plunged into liquid nitrogen and subsequently stored at −70°C. The remaining liver mince was used for microsomal preparation.

Total RNA was isolated from ~0.5 g of individual rat liver as described previously (Agrawal and Shapiro, 1996). RNA samples from individual livers were fractionated by electrophoresis under denaturing conditions in 1.2% agarose gels containing 1× (N-morpholino)propanesulfonic acid buffer and 1.28% formaldehyde. The RNA was transferred to GeneScreen nylon membranes (PerkinElmer Life Sciences, Boston, MA) by capillary transfer in 10× standard saline citrate and then fixed to the filters by UV cross-linking. Prehybridizations and hybridizations in Rapid-hyb buffer (Amerham Biosciences Inc., Piscataway, NJ) with 32P-labeled oligonucleotide probes were performed with high-stringency washings. The washed blots were wrapped in clear plastic and exposed to X-ray films with two intensifying screens at −70°C for 1 to 3 days. The nucleotide sequence of oligonucleotide probes for CYP2B1, CYP2B2 (Omicinski et al., 1985), CYP1A1, CYP1A2, CYP2C6, CYP2C7, CYP2C11, CYP2C12, CYP2C13 (Waxman, 1991), CYP3A1 (Genzik et al., 1992), and CYP3A2 (Ram and Waxman, 1991) have been reported.

Evidence that RNA was equally loaded and transferred was obtained by equivalent intensity of ethidium bromide staining of 18S and 28S rRNA bands. Prehybridizations and hybridizations in Rapid-hyb buffer (Amerham Biosciences Inc., Piscataway, NJ) with 32P-labeled oligonucleotide probes were performed with high-stringency washings. The washed blots were wrapped in clear plastic and exposed to X-ray films with two intensifying screens at −70°C for 1 to 3 days. The nucleotide sequence of oligonucleotide probes for CYP2B1, CYP2B2 (Omicinski et al., 1985), CYP1A1, CYP1A2, CYP2C6, CYP2C7, CYP2C11, CYP2C12, CYP2C13 (Waxman, 1991), CYP3A1 (Genzik et al., 1992), and CYP3A2 (Ram and Waxman, 1991) have been reported.

To reduce interanimal variation associated with parallel protocols (Chengelis, 1988) we chose a serial design using littermates euthanized at different ages. This meant, however, that the hepatic mRNA, protein, and enzyme levels for the 5- and 23-month-old rats would be determined about 18 months apart. To evaluate this concern, all the Northern, Western, and radioenzymatic assays for the 23-month-old rats were conducted along with one or two samples from the 5-month-old rats stored at −70°C. Because the variation between assay results for the same 5-month-old rat material was generally ±15% when stored ~1 month and ~18 months, clearly within the treatment group variation, we considered it reasonable to compare results obtained from 5-month-old rats with 23-month-old rats in which assays were conducted under the same conditions ~18 months apart.

Regardless of age, gender or dose of phenobarbital, the extent of induction (mRNA, protein, and catalytic activity) of those isoforms responsive to phenobarbital was invariably greater during the first 3 days of treatment than during the last 3 days of treatment. Because the results obtained after 3 days and after 6 days of phenobarbital administration were only quantitatively different and demonstrated the same trends, data from the former treatment groups are not reported.

CYP1A. Irrespective of age, sex or treatment, CYP1A1 and CYP1A2 mRNAs and CYP1A1/2 protein were undetectable in all samples (data not presented).

CYP2B1. Although the 1-mg dose of phenobarbital was clearly less effective than the 10-mg dose, it did produce a doubling in the concentration of CYP2B1 mRNA (P < 0.01) and a smaller but generally significant (P < 0.05) induction in protein and catalytic enzyme activity in all treatment groups (Fig. 1). Due to these limited increases in CYP2B1 expression induced by the lower dose of the barbiturate, it would be injudicious to form any conclusions regarding the influence of sex and/or age other than to note that senescence had no inhibitory effect on CYP2B1 induction by as minimal a dose of phenobarbital as 1 mg/kg b.wt/day.

The inductive responses of CYP2B1 mRNA, protein, and CYP2B1-dependent androstenedione 16β-hydroxylase to the 10-mg dose of phenobarbital were generally in agreement (Fig. 1). As reported previously (Shapiro et al., 1994; Agrawal and Shapiro, 1996), induction of the isoform is sex-dependent; males > females (2:1). Although there were no differences between induction levels of CYP2B1 between the 5- and 23-month-old males, the older females expressed somewhat (P < 0.01) higher levels of the isoform than their younger female littersmates when challenged with 10 mg/kg b.wt/day of the barbiturate (Fig. 1).

CYP2B2. Like CYP2B1, CYP2B2 (sharing a 97% amino acid sequence similarity; Suwa et al., 1985) was responsive to induction by both doses of phenobarbital (10 mg > 1 mg). Although the lower dose of the barbiturate induced an ~2- to 3-fold increase in CYP2B2 mRNA levels in all treatment groups, the 10-mg dose of phenobarbital increased transcript levels ~10-fold above baseline (Fig. 2). In agreement with our previous findings (Agrawal and Shapiro, 1996), there was no sexually dimorphic responsiveness to either dose of phenobarbital at the transcript level. Moreover, there was no statistically consistent effect of age on CYP2B2 mRNA induction by phenobarbital.

The sexual responsiveness of CYP2B2 to phenobarbital seems to be determined post-transcriptionally (Agrawal and Shapiro, 1996) as exemplified by the significantly (P < 0.01) greater accumulation of the protein in male livers exposed to 10 mg of phenobarbital than female livers (2:1). In this regard, only the males exhibited an aging-associated decline in phenobarbital induction levels of CYP2B2 pro-
tein (Fig. 2). Androstenedione 16β-hydroxylase is also a catalytic activity for CYP2B2, but because CYP2B1 is induced by phenobarbital at considerably higher levels than CYP2B2 and the former isoform is a more active 16β-hydroxylase of androstenedione than CYP2B2 (Waxman and Azaroff, 1992), we presented the catalytic enzyme data in Fig. 1 along with CYP2B1.

CYP3A1. Like CYP2B1 and CYP2B2, CYP3A1 (also known as CYP3A23) is an inducible isoform expressed at minimal constitutive levels. Although 10 mg of phenobarbital/kg b.wt. was a more effective inducer than the 1-mg dose, all the animals treated with either dose of the barbiturate exhibited statistically significant \( \text{P} < 0.01 \) increases in CYP3A1 mRNA concentrations (Fig. 3). In contrast to the CYP2B isoforms, it was the females that accumulated the greater concentrations of the CYP3A1 transcript than males \( \text{P} < 0.05 \) when treated with 10 mg of phenobarbital. However, the 23-month-old rats responded to both doses of the barbiturate with similar expression levels of CYP3A1 mRNA as their younger sex-matched littermates.

CYP3A1 protein concentrations could be determined only in female rats because the antibody used to detect the isoform does not discriminate between CYP3A1 and CYP3A2 proteins. CYP3A2 is male-specific, and as is well known, we observed high concentrations of the transcript in males (see below) but no detectable levels in either phenobarbital-induced or-noninduced female liver (data not presented). Understandably then, the antibody could only be used to selectively measure CYP3A1 protein in female liver.

The protein findings for CYP3A1 were in complete agreement with the mRNA data. Both doses of adult-administered phenobarbital induced significant increases in CYP3A1 protein \( \text{P} < 0.01 \); the 10-mg dose, understandably being more effective (Fig. 3). The 23-month-old females were comparably responsive to CYP3A1 protein induction as the 5-month-old females when treated with either dose of the barbiturate.

Although testosterone 6β-hydroxylase is CYP3A1-dependent, other isoforms can express the same enzyme activity (Waxman, 1991), explaining, at least in part, the diminished enzyme levels in senescent females in contrast to apparently unchanged CYP3A1 protein levels. Nevertheless, the greater percentage of increase \( \text{P} < 0.01 \) in hepatic testosterone 6β-hydroxylase in 23-month-old females compared with their younger littermates, both treated with the 10-mg dose of phenobarbital, is in agreement with the mRNA and protein findings.
CYP2C7. In agreement with previous observations summarized elsewhere (Pampori and Shapiro, 1999; Agrawal and Shapiro, 2000), CYP2C7 is a constitutive female-predominant isoform exhibiting at least twice the mRNA and protein levels in females compared with males and comprising \( \frac{1}{4} \) of the total hepatic P450 in female rats and \( \frac{1}{6} \) in male rats (Bandiera and Dworschak, 1992). We observed the expected sexually dimorphic expression and no decline in preinduction levels associated with age (Fig. 4). This gender difference persisted after phenobarbital-induction in spite of the fact that males responded to both doses of the barbiturate with a generally greater percentage of increase \((P < 0.05)\) in transcript and protein levels than females (Fig. 4). Last, hepatic CYP2C7 expression (mRNA and protein) was equally responsive to phenobarbital induction in young adult and old rats.

CYP2C6. Like CYP2C7, CYP2C6 is a constitutive female-predominant isoform in which male rat liver expresses \( \frac{1}{6} \) of the levels found in female liver (Pampori and Shapiro, 1999; Agrawal and Shapiro, 2000). Although we limited our studies to measuring mRNA levels (data not presented), the results were near identical to that observed for CYP2C7. That is, there was no age-associated decline in baseline levels. Moreover, although males exhibited a greater phenobarbital-induced increase in CYP2C6 mRNA, female levels remained higher and age had no effect on induction levels of the transcript.

In contrast to all the other isoforms measured, CYP2C6 expression in all treatment groups plateaued after just three daily injections of phenobarbital. Three additional injections of the barbiturate had no further inductive effect on transcript levels.

CYP2C12. As a constitutive female-specific isoform representing \( \frac{1}{4} \) of the total hepatic P450 in female rats (MacGeoch et al., 1984), we observed no expression of CYP2C12 mRNA or protein in males at either age.\(^4\) Whereas both the 10- and 1-mg dose of phenobarbital induced a similar 20 to 25% elevation in CYP2C12 mRNA, there was no commensurate increase in protein concentrations or CYP2C12-associated testosterone 5α-reductase activities. Moreover, the 5- and 23-month-old females expressed the same preinduction levels of CYP2C12 (mRNA, protein, and enzyme activity) and responded identically to the administered barbiturate (CYP2C12 results not presented).

CYP3A2. In agreement with reports summarized previously (Pampori and Shapiro, 1999; Agrawal and Shapiro, 2000), CYP3A2 is a constitutive male-specific hepatic isoform whose presence was undetectable in any of the female groups (data not presented). Both baseline and phenobarbital induction levels of CYP3A2 mRNA were independent of age (Fig. 5).

As discussed above, the antibody used to measure CYP3A2 protein does not discriminate between the CYP3A2 and CYP3A1 proteins. Nevertheless, we have used the antibody to estimate the levels of CYP3A2 protein because we believe that the protein detected by the antibody in male liver is predominantly CYP3A2. Male liver contains the antibody for CYP3A2, it is not CYP2C12-dependent (Waxman, 1991). Nevertheless, we observed a 4-fold increase in the activity of the reductase in 23-month-old males reducing the male/female ratio of 1:24 in 5-month-old rats to 1:6 at 23 months of age.

\(^4\) Although testosterone 5α-reductase coincidentally reflects the levels of CYP2C12, it is not CYP2C12-dependent (Waxman, 1991). Nevertheless, we observed a 4-fold increase in the activity of the reductase in 23-month-old males reducing the male/female ratio of 1:24 in 5-month-old rats to 1:6 at 23 months of age.
severalfold higher concentrations of CYP3A2 mRNA than CYP3A1 mRNA (A. K. Agrawal, N. A. Pampori, B. H. Shapiro, unpublished data), suggesting that the male liver expresses far more CYP3A2 protein. In addition, male and female liver contained similar levels of CYP3A1 mRNA (Fig. 3) and because the amount of CYP3A1 protein in female liver was only a fraction of the CYP3A2 protein found in male liver, it seems reasonable to conclude that the anti-CYP3A1/2 primarily detected CYP3A2 protein in males. Last, results obtained using the antibody were in agreement with the mRNA findings. That is, baseline concentrations as well as the magnitude of phenobarbital induction of the protein were statistically indistinguishable in the 5- and 23-month-old males (Fig. 5).

In contrast to CYP3A2 mRNA and protein levels, preinduction testosterone 6β-hydroxylase, a CYP3A2-dependent catalytic activity, was reduced ~50% in the senescent males (Fig. 5). As discussed above, this disparity in enzyme activity with mRNA and protein findings may be explained by the fact that other P450s can express 6β-hydroxylase activity (Waxman, 1991). Nevertheless, taking into consideration the lower preinduction enzyme levels in the senescent rats, the percentage of increase in phenobarbital-induced testosterone 6β-hydroxylase in the livers of 23-month-old males were greater than that observed in the younger rats, which is in closer agreement with the mRNA and protein results (Fig. 5).

CYP2C11. CYP2C11 is a constitutive male-specific isoform, and as expected, we were unable to detect the isoform in any female livers. Compared with all the other isoforms examined in this study, CYP2C11 clearly exhibited a dramatic aging associated decline. Preinduction levels of CYP2C11 mRNA, protein, CYP2C11-dependent specific testosterone 2α-hydroxylase as well as testosterone 16α-hydroxylase (the latter not reported) in 23-month-old males was reduced to ~30% of levels found in 5-month-old males (Fig. 6). In spite of the diminished concentration of CYP2C11 mRNA in the

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**Fig. 4.** Expression of hepatic CYP2C7 mRNA and protein in 5- and 23-month-old male and female rats measured after treatment with either 1 mg or 10 mg/kg phenobarbital (PB)/day for 6 consecutive days.

Procedural details are described under Materials and Methods. Values, mean ± standard deviation for four or more rats.

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**Fig. 5.** Expression of hepatic CYP3A2 mRNA, protein, and catalytic activity (testosterone 6β-hydroxylase, T 6β-OH) in 5- and 23-month-old male rats measured after treatment with either 1 mg or 10 mg/kg phenobarbital (PB)/day for six consecutive days.

Procedural details are described under Materials and Methods. Values are mean ± standard deviation for four or more rats. Note different y-axes; one for mRNA and protein, the other for catalytic activity. *, P < 0.01 compared with 5-month-old rats of the same treatment.

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**Fig. 6.** Expression of hepatic CYP2C11 mRNA, protein, and catalytic activity (testosterone 2α-hydroxylase, T 2α-OH) in 5- and 23-month-old male rats measured after treatment with either 1 mg or 10 mg/kg phenobarbital (PB)/day for 6 consecutive days.

Procedural details are described under Materials and Methods. Values are mean ± standard deviation for four or more rats. Note different y-axes; one for mRNA and protein, the other for catalytic activity. *, P < 0.01 compared with 5-month-old rats of the same treatment.
**HEXOBARBITAL HYDROXYLASE**

![Graph](Image)

**FIG. 7.** Expression of hepatic hexobarbital hydroxylase activity in 5- and 23-month-old male and female rats measured after treatment with either 1 mg or 10 mg/kg phenobarbital (PB)/day for 6 consecutive days.

Procedural details are described under Materials and Methods. Values are mean ± standard deviation for four or more rats. *, *P* < 0.01 compared with 5-month-old rats of the same sex and treatment.

617SEX AND PHENOBARBITAL EFFECTS ON P450 EXPRESSION IN OLD RATS

**Discussion**

Based upon the limited number of studies in which both sexes of rats were examined, several laboratories have concluded that aging induces a decline in hepatic drug-metabolizing capacity by “feminizing” the expression of gender-dependent isoforms of CYP (Kamataki et al., 1985; Imaoka et al., 1991; Fujita, 1991). That is, although hepatic content of male-specific P450s declines with age in male rats, female isoforms remain unchanged in females and increase in male liver. Accordingly, only males will experience this decline in drug-metabolizing capacity, while hepatic monooxygenase activities are unaffected by aging in female rats, resulting in the loss of sexually dimorphic enzyme expression.

Although there may be significance to the fact that the above-cited reports used 2-year-old Wistar and Fischer-344 rats, whereas we used 2-year-old Sprague-Dawley rats (Chengelis, 1988), our results are only somewhat in agreement. As regards multi-P450-dependent drug-metabolizing enzymes, we did limit our investigation to a single monooxygenase, albeit a prototypical gender-dependent form. And although we did find a dramatic decrease in baseline hexobarbital hydroxylase activity in 23-month-old male rats, we also observed, in agreement with a previous report (Bitar and Shapiro, 1987), a similar percentage of decline in the concentration of the monooxygenase in senescent females, thus maintaining the sexually dimorphic expression of the enzyme.

Our observation of an ~70% decrease in CYP2C11 mRNA, protein, and catalytic activities in untreated 23-month-old male rats agrees favorably with previous reports of a near 100% decline in protein and catalytic levels of the isoform (Kamataki et al., 1985; Fujita, 1991; Imaoka et al., 1991). Because CYP2C11 is the dominant isoform in the male rat comprising ~50% of the animals’ total hepatic P450 (Morgan et al., 1985), it is not surprising that its dramatic decline in the senescent male rat would result in a similar decrease in the activities of the many male-dependent drug-metabolizing enzymes to which it contributes. The cause for this profound aging-dependent decline in CYP2C11 expression is likely due to an age-induced change in the masculine episodic growth hormone profile, the sole regulator of CYP2C11 expression (Pampori et al., 1991; Waxman, 1991). More specifically, CYP2C11 expression is dependent upon a minimum growth hormone devoid interpulse period in the circulating masculine growth hormone profile that is normally characterized by large (~250 ng/ml) hormone pulses every 3.5 to 4 h interrupted by growth hormone-devoid interpulses of 2.5 to 3 h (Shapiro et al., 1995). As male rats age, the interpulse may be compromised by a reduced duration and/or by the secretion of low concentrations of growth hormone (Dhir et al., 2002), either of which could severely depress CYP2C11 expression (Agrawal and Shapiro, 2001).

Except for CYP2C11 suppression, we found no other evidence of “feminization” of P450 isoforms in the 23-month-old male rat. Ex-

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5 This uncoupling of transcription from translation may be explained by the induction of an aberrant, untranslatable species of CYP2C11 mRNA characterized by the retention of its terminal intron (Pampori and Shapiro, 2000).

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7 This uncoupling of transcription from translation may be explained by the induction of an aberrant, untranslatable species of CYP2C11 mRNA characterized by the retention of its terminal intron (Pampori and Shapiro, 2000).
pression of CYP3A2, another male-specific isoform, was reported to disappear in 104-week-old males (Imaoka et al., 1991), whereas we observed no aging-induced change in the isoform. Like CYP2C11, CYP3A2 expression can be suppressed by changes in the masculine growth hormone profile characterized by shortened interpulse durations and/or elevated baselines during this period. However, CYP3A2 expression is considerably less responsive to these profile changes requiring much shorter interpulse durations and significantly greater hormone levels during the interpulse (changes unlikely to occur during aging; Xu and Sonntag, 1996) than CYP2C11 to initiate inhibition of expression (Pampori and Shapiro, 1999).

Aging-induced feminization of hepatic P450 isoforms has been described to entail not just the suppression of male-specific isoforms, but also the concurrent elevation of female-dependent P450s in senescent males (Kamataki et al., 1985; Imaoka et al., 1991). In particular, CYP2C12, which is never detected in young males, was expressed in 2-year-old males at levels approaching that observed in both young and old females (Kamataki et al., 1985; Imaoka et al., 1991). In contrast, aging did not induce detectable levels of CYP2C12 mRNA or protein (although there was some increase in non-CYP2C12-dependent testosterone 5α-reductase) in our study. Moreover, masculine levels of female-predominant CYP2C7 and CYP2C6 were similarly unaffected in senescent males. We did find, however, in agreement with a previous report (Imaoka et al., 1991), a small but significant (P < 0.01) elevation in female-predominant CYP2A1-dependent testosterone 7α-hydroxylase in 23-month-old males (data not presented). In general, we would have to conclude that the only consistent senescent-induced change observed in constitutive P450 isoforms was a profound decline in CYP2C11 expression in male rats.

In this regard, we can see that a marked decline in the levels of just a single isoform, albeit the predominant CYP2C11, is sufficient to profoundly reduce drug-metabolizing capacity (e.g., hexobarbital hydroxylase) in the senescent rat. Accordingly, the comparable aging-associated decline in the predominant human isoform CYP3A4/5 (Hunt et al., 1992) could similarly explain any decline in drug metabolism observed in the elderly. Moreover, most aging studies in humans examine drug “disposition” (Schmucker and Lonergan, 1987), which is effected by changes in drug absorption, distribution, binding, and excretion as well as biotransformation. Changes in any or all of these factors could contribute to the high incidence of adverse drug reactions and toxicities reported in older people (Hunt et al., 1992; Wilcox et al., 1994).

Earlier studies restricted to male rats and administering large doses of phenobarbital (60–100 mg/kg b wt/day) reported either a decline (Kao and Hudson, 1980; Schmucker and Wang, 1981) or no aging-related change (Abraham et al., 1985) in the induction of several multi-P450-dependent drug-metabolizing enzymes. In spite of an ~50% decrease in preinduction levels of hexobarbital hydroxylase in old rats of both sexes, we found that administration of the 10-mg as well as 1-mg dose of phenobarbital induced a percentage of increase in the hepatic monoxygenase of 23-month-old male and female rats that was equal to or exceeded the response observed in the 5-month-old rats. It is clear from our findings that the induction of hexobarbital hydroxylase by phenobarbital in both age groups could not be a result of a commensurate induction of CYP2C11 or CYP2C12, the most heavily expressed hepatic isoforms in untreated male and female rats, respectively. Rather, expression of CYP2B1 and CYP2B2 (~4:1, respectively), the prototypical and most phenobarbital responsive isoforms (Waxman and Azaroff, 1992) as well as major contributors to hexobarbital hydroxylase activity (Ryan and Levin, 1990) were generally induced to the same degree in the 5- and 23-month-old rats. Previous results examining phenobarbital inducibility of CYP2B1 and CYP2B2 in aged male rats are conflicting, reporting a significant decline in transcript levels (Horbach et al., 1990; Van Beezooijen et al., 1994) but no change in the protein concentration (Horbach et al., 1992) of senescent male rats.

In our hands, the nonconstitutive, phenobarbital responsive CYP3A1, like CYP2B1 and CYP2B2, exhibited no aging-associated decline in induction. Moreover, several constitutive isoforms in young male rats have been shown to be inducible by phenobarbital, albeit at considerably lower levels than that reported for the CYP2B isoforms (Waxman and Azaroff, 1992). In this regard, we report no aging- or sex-associated difference in phenobarbital induction levels of female-predominant CYP2C6 and CYP2C7 and male-specific CYP3A2, all contributors, to various degrees, to the activities of different drug-metabolizing enzymes (Ryan and Levin, 1990).

In summary, with the exception of CYP2C11, we found no consequential senescence associated reduction in the pre- and/or postphenobarbital induction of CYP2B1, CYP2B2, CYP3A1, CYP2C7, CYP2C6, CYP2C12, CYP3A2, and CYP2C13 in either male or female rats. Moreover, when determined, mRNA, protein, and catalytic enzyme activities for each isoform (with the possible exception of multi-P450-dependent testosterone 6β-hydroxylase) were in agreement. Why we did not observe the same degree of P450 feminization as others (Kamataki et al., 1985; Fujita, 1991; Imaoka et al., 1991) is not clear, but may be due, in part, to our use of Sprague-Dawley rats of both sexes, administration of suboptimal induction doses of phenobarbital, and 5-month-old adults for baseline values in contrast to the use of Fischer-344 and Wistar rats, 6- to 10-fold higher doses of phenobarbital, and baseline values derived from much younger 1- to 3-month-old rats.

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SEX AND PHENOBARBITAL EFFECTS ON P450 EXPRESSION IN OLD RATS

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