The Induction of Hepatic Cytochrome P450 3A in Rats: Effects of Age (43932)

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Abstract. **Liver microsomal cytochrome P450 3A (CYP3A) concentrations were evaluated by Western blots using specific antisera. Low levels of CYP3A protein were found in untreated animals. Dexamethasone (DX) treatment resulted in a significant induction of CYP3A. The induction was dose and time dependent. Addition of U486 (a specific type II glucocorticoid receptor antagonist), but not spironolactone (a specific type I receptor antagonist) blocked the induction of CYP3A proteins by dexamethasone, suggesting a receptor-mediated mechanism. Concomitant administration of either actinomycin D or cyclohexamide, together with dexamethasone completely abolished the induction of CYP3A protein by dexamethasone, suggesting the requirement of both protein and RNA synthesis. A comparison of the inducibility of CYP3A protein by dexamethasone in rats from different age groups showed that the degree of increase was higher in the younger than in the older groups (e.g., 5 days versus adult). Thus, there is an attenuation in the responsiveness to dexamethasone induction of CYP3A proteins with age. Evaluation of the steady-state levels of CYP3A mRNA by Northern blots showed increases in mRNA following DX treatment in both young and old rats. The final level of CYP3A mRNA reached after DX treatment was higher in the pups than that found in similarly treated older rats. This decrease in responsiveness in older animals appeared to manifest at least in part at the pretranslational level.** [P.S.E.B.M. 1995, Vol 210]

Hepatic microsomal cytochrome P450 is involved in the oxidative metabolism of a variety of drugs and chemicals. Several of the cytochrome P450 activities have been shown to change with age.

CYP3A originally isolated as an unique form in the liver of rats treated with pregnenolone-16a-carbonitrile (PCN) (1) has been shown to exist constitutively (2). This form is highly inducible by glucocorticoids and has striking qualitative and quantitative difference between species in their responses (3). The CYP3A protein is responsible for steroid 6B hydroxylase activity (4) and changes developmentally in rats (4) and rabbits (5).

Most cytochrome P450s exist at low basal levels but increase many fold in response to specific inducers (6). The effect of age on the inducibility of cytochrome P450s has been reported only for CYP1A and 2B. In rabbits, benzo[a]pyrene hydroxylation showed a decrease response to 3-methylcholanthrene (3MC) induction with age (7). In rats, CYP1A mRNA is unresponsive to 3MC before age 22 days (8). Peak response occurs at 46 days. At 62 days the response is reduced. In contrast, an age associated decrease in the inducibility of CYP2B and 1A levels does not occur in rats (9). We have recently shown that there is a blunting in the responsiveness to dexamethasone induction of 6B steroid hydroxylase in older rats (10).

Modulation of the expression of the P450 system may alter the capacity of the individual to metabolize endogenous and exogenous agents such as prostaglandins, steroid hormones, drugs, and carcinogens. Therefore, the influence of age on the capacity of cytochrome P450 expression in response to inducers is an important issue.

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The present study characterized the induction of CYP3A by dexamethasone in rat liver and followed the changes in the inducibility of CYP3A during postnatal development with particular emphasis on the question of whether an age-related difference in the inducibility of CYP3A by dexamethasone exists.

Materials and Methods

Chemicals. Except otherwise stated, all chemicals were from Sigma Chemical Co. (St. Louis, MO). RU 38486 (U486) was a gift from Dr. D. Martini of Roussel Uclaf (Romanville, France). Rabbit anti-rat CYP3A serum was kindly provided by Dr. J. E. Hulla, Pacific Northwest Laboratories (Richland, WA).

Animals. Pregnant Sprague-Dawley rats were housed in individual cages and maintained on a 12:12-hr light:dark cycle. On the expected date of delivery, cages were inspected frequently for birth. The day of birth was regarded as Day 0. Pups were allowed to suckle freely until the time of sacrifice. Rats were sacrificed by decapitation at the age specified. After sacrifice, a portion of liver was removed, snap-freezed, and stored at −75°C for subsequent use. The National Institutes of Health guidelines for the care and use of laboratory animals were followed to ensure that animals were not subjected to pain and discomfort.

Procedures. Unless otherwise stated, for the induction of CYP3A, rats of both sexes at the ages specified were given daily intraperitoneal (ip) injections of dexamethasone (DX) (30 mg/kg body wt) for 3 days before sacrifice. Age-matched littermate controls were given ip injection of equal volumes of vehicle (dimethylsulfoxide).

Microsomes from frozen livers were prepared by differential centrifugation as described previously (11). Protein concentration was determined by the Bradford method (12) using the BioRad reagent (BioRad Laboratories, Richmond, CA) with BSA as the standard. Western blot for the determination of CYP3A protein was carried out as described previously (11) using an antisera specific for the rat CYP3A (13).

For the quantitation of CYP3A mRNA, a modification of the procedure of Simpson (14) was used to isolate high–molecular weight RNA from fresh liver samples as previously outlined (15). Quantitative changes of CYP3A mRNA were measured by Northern blots using total RNA prepared from rat liver obtained from various age control and dexamethasone treated groups. Hybridization was performed as previously described (15) using cDNA probes containing the gene sequence for CYP3A (plasmid pDEX12, a gift from Dr. P. S. Guzelian from the Department of Medicine, Medical College of Virginia, Richmond, VA).

Statistics. Results are reported as the mean ± SD. Differences between the means of two groups were evaluated by Student's t test. For multigroup comparison, analysis of variance was used. A value of $P \leq 0.05$ was considered significant.

Results

Figure 1 shows the time course of DX induction of CYP3A proteins in 20-day-old pups after a single injection of DX. Increase in CYP3A protein was detected as early as 8 hr after DX administration. Maximal increase was reached at 48 hr. The level remained high at 72 hr.

To see whether DX induced CYP3A expression requires protein and/or RNA synthesis, 10-day-old pups were treated with actinomycin D or cycloheximide 1 hr prior to given DX. Figure 1 (lower panel) shows the outcome of such treatments. Dexamethasone by itself induced CYP3A protein production. Coadministration of actinomycin D or cycloheximide with DX completely abolished the induction of CYP3A protein. Actinomycin D or cycloheximide by itself had no observable effect.

To quantitate the efficacy of DX in the induction of CYP3A protein, pups were given graded increased doses of DX for 3 days and their hepatic microsomal CYP3A proteins were measured (Fig. 2). The degree of induction was dependent on the dose of DX. Induction was observed with DX at a concentration of 1 mg/kg. The degree of inductive effect increased with the dose of DX up to 20–30 mg/kg. Doses higher than 30 mg/kg resulted in extremely sick animals, and the results became very erratic.

Simultaneous treatment of DX with the type II specific glucocorticoid receptor antagonist (U486) at 1:6 M ratio of the hormone (5 mg/kg) to antagonist (30 mg/kg) to pups counteracted the DX effect (Fig. 3). CYP3A protein concentration was significantly re-

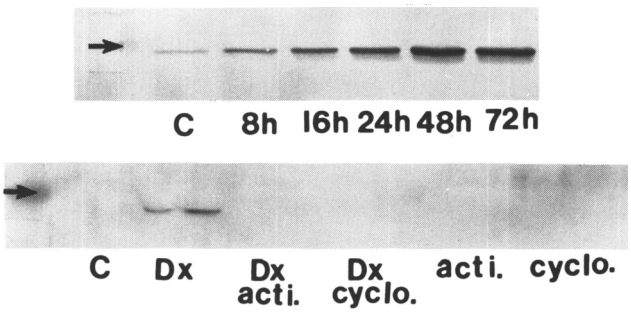

Figure 1. (upper panel) Time course of the response of CYP3A in hepatic microsomes to dexamethasone treatment (30 mg/kg body wt). Dexamethasone was given as a single dose at time zero and liver harvested at 8, 16, 24, 48, and 72 hr after DX administration. (lower panel) Effect of actinomycin D and cycloheximide on CYP3A in hepatic microsome of control and dexamethasone treated rats. C, Control; DX, dexamethasone; DX acti., dexamethasone and actinomycin D; DX cyclo, dexamethasone and cycloheximide; acti., actinomycin only; cyclo., cycloheximide only. DX (30 mg/kg) was given 24 hr before sacrifice. Acti. (1.25 mg/kg) and cyclo. (1.0 mg/kg) were given 1 hr before DX. Arrows indicate position of a molecular weight marker (55.3 kDa).

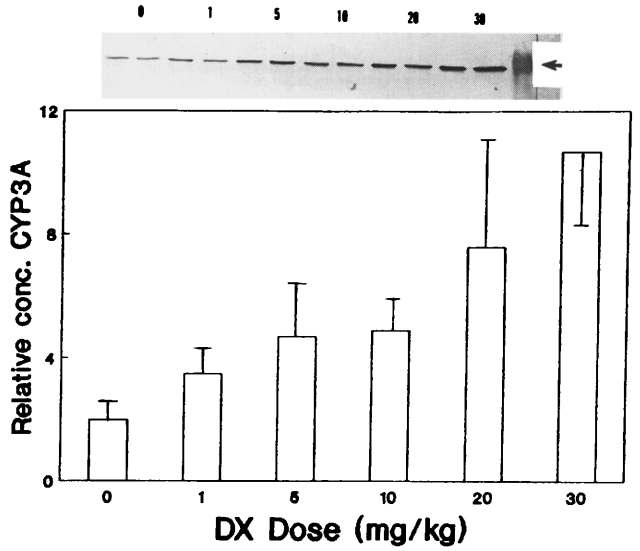

Figure 2. Dose response of CYP3A in hepatic microsome from pups (20 days) receiving no dexamethasone (0) or 1, 5, 10, 20, and 30 mg DX/kg body wt for 3 days before sacrifice. Western blot shows duplicate samples with each sample from a different animal. Arrow indicates the position of a molecular weight marker (55.3 kDa). Values in graph represent Means ± SD of three separate measurements from three separate experiments with two to four animals for each treatment in each experiment. Values from all DX treated groups were significantly different from that of control group with $P \leq 0.05$.

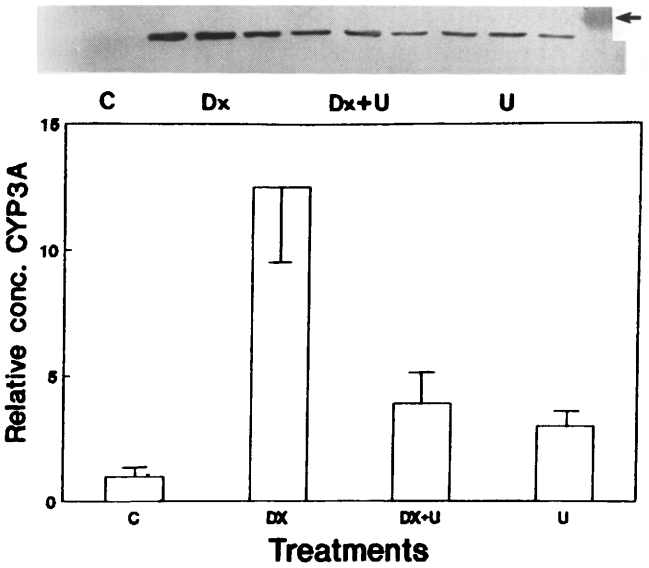

Figure 3. Effect of Type II glucocorticoid receptor antagonist (U486) on dexamethasone induction of hepatic microsomal CYP3A in 10-day-old pups. Dexamethasone (5 mg/kg) was given with or without antagonist (30 mg/kg) 24 hr before sacrifice. C, control; Dx, dexamethasone; Dx + U, dexamethasone and U486; U, U486 alone. Lanes in Western blot shown are in triplicate with each lane representing microsomal fraction from a different animal. Arrow indicates the position of a molecular weight marker (55.3 kDa). Values in graph represent Means ± SD of three separate measurements from three separate experiments with two to four animals for each treatment in each experiment. Values from all treated groups were significantly different from that of control group with $P \leq 0.05$. Value from DX + U group was significantly different from DX group with $P \leq 0.05$.

duced compared with DX-treated animals without antagonist. U486 by itself caused some induction of CYP3A. When spironolactone, a type I specific glucocorticoid receptor antagonist, was used at the same 1:6 *M* concentration of hormone:antagonist, no inhibitory effect on the DX induction of CYP3A was seen (Fig. 4). Interestingly, spironolactone by itself at the concentration used was a good inducer of CYP3A proteins.

To see if the inducibility of CYP3A protein changes during development, rats at selected ages (4, 10, 15, and 20 days, and young [4 months] and old [18 months or older] adults were given dexamethasone (30 mg/kg body wt, daily injection for 3 days) and CYP3A protein concentrations were evaluated (Fig. 5). In control rats, CYP3A protein concentration was low in suckling animals (4–15 days). An increase was seen in weaning rats (20 days) and adults (4 and 18 months). In 4-month-old animals, a lower concentration of CYP3A was found in female compared with male rats. Following dexamethasone treatment, 4-day-old rats showed an increase of CYP3A protein to around 40 times that of untreated control rats in both sexes. As the rats got older, the increase of CYP3A protein after dexamethasone treatment fell until it reached around 20- to 25-fold in older rats (≥18 months old).

Figure 6 shows a comparison of the behavior of CYP3A mRNA in response to dexamethasone in 5-day and older (>18-month-old) rats. In both male and female rats, at 5 days of age, dexamethasone caused an increase in CYP3A mRNA over the corresponding controls. In adult rats, dexamethasone treatment resulted also in increased CYP3A mRNA, but there appeared to be a marked decrease in responsiveness at the mRNA level compared with that in the 5-day-olds. No difference in the induction of CYP3A mRNA in female and males was detected at these two ages.

Discussion

Age-dependent changes in drug metabolism have been documented in both animals and humans (16, 17). Hepatic cytochrome P450s are important enzymes involved in the phase 1 reaction in detoxification. Many cytochrome P450s are inducible and exist in relatively low basal levels. There is, however, considerable variation in the induction of individual cytochrome P450 depending on the animal species. Further, the degree of responsiveness of the system might change with age.

In the present study, we measured the CYP3A protein targeted for the metabolism of steroids in rats at different ages. Our results indicated that CYP3A is highly inducible by dexamethasone. Induction was relatively rapid in that a significant accumulation of CYP3A protein was detected 8 hr after DX administration. It was a time-dependent process: a higher level

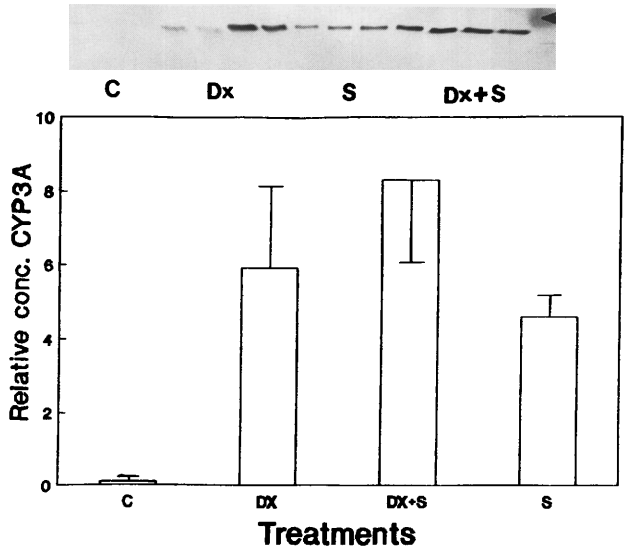

Figure 4. Effect of Type I glucocorticoid receptor antagonist (spironolactone) on dexamethasone induction of hepatic microsomal CYP3A in 10-day-old pups. Dexamethasone (5 mg/kg) was given with or without antagonist (30 mg/kg) 24 hr before sacrifice. C, control; Dx, dexamethasone; Dx + S, dexamethasone and spironolactone, S, spironolactone alone. Lanes in Western blot shown are in triplicate with each lane representing microsomal fraction from a different animal. Arrow indicates the position of a molecular weight marker (55.3 kDa). Values in graph represent means ± SD of three separate measurements from three separate experiments with two to four animals for each treatment in each experiment. Values from all treated groups were significantly different from that of control group with $P \leq 0.05$. No significant difference was found between DX and DX + S groups with $P > 0.05$.

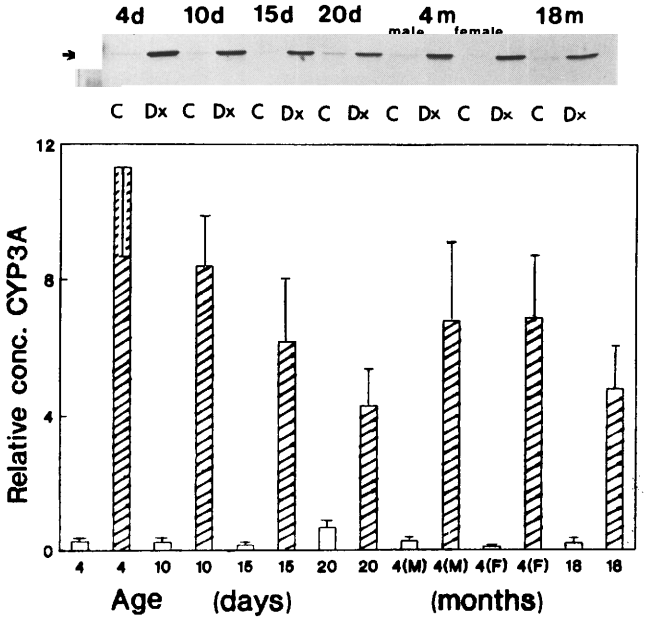

Figure 5. Age-dependent responses of CYP3A in hepatic microsomes from control (C in Western blot and open bars in graph) and dexamethasone-treated (Dx in Western blot and hatched bars in graph) rats at various ages. Dexamethasone (30 mg/kg body wt) was given for 3 days prior to sacrifice at the age stated. Values in graph represent means ± SD of three separate measurements from three separate experiments with three to six (depending on age) animals for each treatment in each experiment. At 4 months of age, values for male (M) and female (F) animals were presented separately. Only those from males were shown for the other ages.

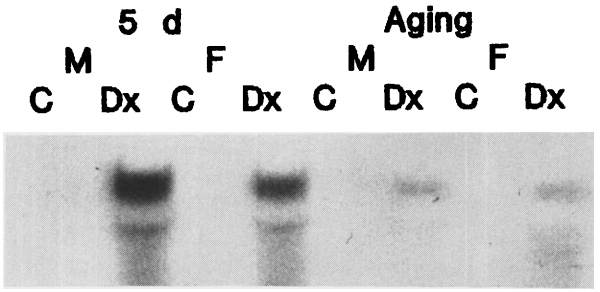

Figure 6. Northern blot showing CYP3A and mRNA from male (M) and female (F) young pups (5 day) and old rats (>18 months) with (Dx) and without (C) treatment of dexamethasone (30 mg/kg, for 3 days prior to sacrifice). RNA levels were comparable between samples as determined from the intensity of ethidium bromide staining of 28s and 18s ribosomal RNAs.

was reached at 48 hr and lasted at least till 72 hr after DX treatment. The induction process was dependent on DX dose and required a relatively high dose (minimal dose of 1 mg/kg) of the inducer to have a significant effect.

The induction of CYP3A protein by DX required both protein and RNA synthesis since it was blocked by both actinomycin D and cycloheximide. In relation to this, Burger *et al.* (18) had reported that actinomycin D blocked while cycloheximide potentiated the induction of CYP3A mRNA by steroid. Our results indicated that for final expression of CYP3A, protein synthesis is still required. Mechanistically, the induction process was mediated by the type II glucocorticoid receptor since the induction was effectively inhibited by U486 (a type II specific glucocorticoid receptor antagonist) (19, 20) but not by spironolactone (a type I glucocorticoid receptor specific antagonist) (21). Interestingly, spironolactone at the concentration used (30 mg/kg) was also an effective inducer of CYP3A in our study. Similar induction of CYP3A ($P450_{PCN}$) by spironolactone has been reported in cultured rat hepatocytes (22) and *in vivo* (23). When U486 was given at similar concentration as spironolactone, some increase in CYP3A protein was observed. The same concentration of U486 however, attenuated the induction of CYP3A by DX. Inhibition of DX-induced cytochrome P450 by U486 has been reported by Chasserot-Golaz and Beck (24, 25).

Our results showed that CYP3A protein concentrations had a unique developmental profile paralleled that of steroid 6B hydroxylase activity reported previously (10, 26). CYP3A protein showed a low concentration in the suckling stage reaching a higher level in weaning animals followed by a sustaining high level in the male adults. In females, there was, however, a significant decrease in the CYP3A level in adult life. Similar sex differences in testosterone 6B hydroxylase

activity have also been reported during development of the white-footed mouse (27) and Sprague-Dawley rats (28, 29).

The present study of dexamethasone induction of CYP3A protein in rats of different age groups provides some information that may be important physiologically. The older adults (≥18 months) had a lesser degree of responsiveness (as measured by the ratio of induced/basal level of CYP3A protein) than did the very young rats (5 days). These results are in agreement with our previous observation on the behavior of steroid 6B hydroxylase activities in older rats (10) and further confirm that the CYP3A protein constitutes the majority if not all of steroid 6B hydroxylase activities.

Quantitation of CYP3A mRNA levels in control and DX induced rats at the younger and older stages confirmed the CYP3A protein measurement. There was an apparent marked decrease in responsiveness to DX in the older animals. These results show that induction occurs in the pretranslational level and confirm those from our actinomycin D studies. The decrease in inducibility in the older animal was controlled only in part at the pretranslational level since the decrease in mRNA levels was much greater than the decrease in CYP3A protein when old animals were compared with young animals. This nonparallel change in protein and mRNA concentrations suggests that another level of regulation may also be occurring to account for the differences. Our results are, however, in agreement with the observation that old rats show a lower rate of CYP3A transcription than do young rats in response to dexamethasone (30).

Mechanistically, the attenuation in the responsiveness to dexamethasone in older rats might be related to the observed decrease in glucocorticoid receptors in older animals (31–33). On the other hand, other steps involved in the induction cascade events such as the availability and activity of modulator(s) of glucocorticoid action, or nuclear factors that control the transcription of the CYP3A gene, might also limit their expression. A comparison of glucocorticoid receptor binding parameters has to be carried out to delineate among the actual mechanisms involved. Nevertheless, the decrease in responsiveness of CYP3A to DX induction might have important physiological implications and needs to be examined in more detail and also in a broader scope to pinpoint the precise mechanism(s) that are responsible for the developmental changes observed.

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