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INDUCTION OF DRUG-METABOLIZING ENZYMES IN GUNN RAT LIVER

EFFECT OF POLYCYCLIC AROMATIC HYDROCARBONS ON CYTOCHROME P-450 REGULATION

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Abstract—Response of congenitally jaundiced rats (Gunn rats) to administration of polycyclic aromatic hydrocarbons (PAH) was investigated and compared to that of Wistar rats. Unlike Wistar, Gunn males did not exhibit changes in the overall cytochrome P-450 content of hepatic microsomes. The first step in the induction process (i.e. presence of cytosolic receptors for PAH) was found present and functionally similar (number of sites, K_d) to that of Wistar rats from which the Gunn strain is derived. An increase in monooxygenase activities related to P-450c and P-450d isoenzymes specifically induced by PAH was noticed, whereas no effect could be detected on the glucuronidation rate of either 4-nitrophenol, testosterone or estrone. As determined by immunoquantification after Western blotting, the isoenzymatic profile of P-450 from PAH-treated male Gunn rats showed an increase of P-450c and P-450d accompanied by an equivalent decrease in P-4502c (major male-specific isoenzyme). The balance between increase in P-450c and P-450d and decrease in P-4502c may explain the absence of increase in the total P-450 in PAH-treated male Gunn rats. Such a response was not observed in PAH-treated male Wistar rats or in female rats of both strains. In contrast, the response of male Gunn rats to PB treatment was similar to that observed in Wistar rats, i.e. increase in overall cytochrome P-450 content of hepatic microsomes and of specific isoenzyme P-450b/e. A possible regulation of P-450 isoenzyme synthesis by the intracellular haem pool might be involved.

A wide variety of endogenous and exogenous lipophilic compounds are metabolised by a drug-metabolizing system including cytochrome P-450-dependent monooxygenases, epoxide hydrolase and conjugating enzymes like UDP-glucuronosyl transferase (UDP-GT)† and glutathione transferase. These enzymatic systems can be induced by a large number of chemicals, the most classically used are PB and MC.

The mechanism of induction by PB is not fully elucidated but implicates activation of gene transcription for P-450b and e, epoxide hydrolase and glutathione transferase [1, 2]. For MC and other polycyclic aromatic hydrocarbons (PAH), the first step in the induction process is the binding to cytosolic receptors which are further translocated into the nucleus and stimulate gene transcription [3]. The possibility of a common receptor involved in the induction of both cytochrome P-450 and UDP-GT by PAH has been hypothesised in mice but not confirmed [4].

Homozygous Gunn rats (strain derived from Wistar rats) are characterised by their chronic hyperbilirubinemia provoked by the lack of bilirubin UDP-GT activity [5] due to the absence of the enzymatic protein [6]. The glucuronidation of phenols such as 4-nitrophenol is also impaired in this strain of rats whereas activity is normal towards other substrates like testosterone [7, 8]. The response of UDP-GT to inducers constitutes another difference between Gunn and Wistar strains: bilirubin UDP-GT cannot be induced by PB and the response of 4-nitrophenol UDP-GT to PAH administration is very poor in Gunn rats whereas these activities are highly inducible in Wistar rats [9–11]. In Wistar rats, bilirubin is conjugated to UDP-glucuronic acid and then excreted in the bile. In Gunn rats, slow bilirubin elimination passes through the production of hydroxylated metabolites which are directly excreted in the bile without conjugation [12]. The cytochrome P-450 isoenzyme probably implicated in bilirubin hydroxylation has not yet been identified. Few studies have been devoted to the induction of monooxygenase activities by PAH regarding bilirubin catabolism and have been performed only in females while no data are available in male Gunn rats [13].

A recent study of Kapitulnik *et al.* [14], reports the high content of cytochrome P-450c isoenzyme (main isoenzyme induced by MC) in non-induced female Gunn rats. This unexpected result prompted us to examine the whole induction pathway from the cytosolic content in PAH-binding proteins to the isoenzyme profile in untreated and PAH-treated animals. Data collected from these experiments demonstrate that the induction pathway is active in male

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† Abbreviations used: MC, 3-methylcholanthrene; TCDD, 2,3,7,8 tetrachlorodibenzo-*p*-dioxin; BP, benzo(*a*)pyrene; PB, phenobarbital; UDP-GT, UDP-glucuronosyl transferase; PAH, polycyclic aromatic hydrocarbons; W, Wistar rats; G, Gunn rats; P-4502c (UT-A; P450IIC11) and P-4502d (UT-I; P450IIC12), major sex-regulated isoenzymes in male and female rats; P-450c (P-450IA1) and P-450d (P-450IA2), main isoenzymes induced by PAH; P-450b (P-450IIB1) and P-450e (P-450IIB2), main isoenzymes induced by PB.

Table 1. Microsomal cytochrome P-450 content and related monooxygenase activities in male and female Gunn rats treated or not by PAH

	Cytochrome P-450 (nmol/mg protein)	λ_{max} (nm)
Male Gunn rats		
Untreated	0.99 ± 0.10	450
MC-treated	0.82 ± 0.13	449.5
	NS	
TCDD-treated	0.86 ± 0.09	449
	NS	
BP-treated	1.07 ± 0.14	448.5
	NS	
Female Gunn rats		
Untreated	0.72 ± 0.03	450
MC-treated	1.15 ± 0.06	449
	$P < 0.001$	

Values are the mean ± SD for 4 animals per group. Animals were treated for 3 or 4 days with either MC (50 mg/kg), TCDD (27 μg/kg) or BP (100 mg/kg). Statistical significance is given in reference to untreated animals. NS: not significant. λ_{max} is given at ±0.5 nm.

Table 2. Concentration (n), dissociation constant (K_d) and effect of different competitors for the binding of 3-methylcholanthrene to male rat hepatic cytosol

	Gunn rats	Wistar rats
	(N = 4)	(N = 3)
n (fmol/mg protein)	315 ± 70	405 ± 28
K_d (nM)	1.28 ± 0.36	1.57 ± 0.23
Competitor IC_{50}		
3-methylcholanthrene	1×10^{-8} M	1.5×10^{-8} M
benzo(a)pyrene	1×10^{-8} M	3×10^{-8} M
5,6 benzoflavone	1.4×10^{-8} M	1×10^{-8} M
Phenobarbital	nc	nc
Bilirubin	nc	nc

For concentration and dissociation constant, values are the mean ± SD for 4 or 3 different animals, each individual determination being performed twice in duplicate. For each determination, specific binding for concentrations of MC from 0.3 to 30 nM was calculated and Scatchard plots drawn. For competition purpose, rat cytosolic proteins (3 mg/ml) were incubated with 6 nM ^{3}H-MC in presence of 10^{-9} to 10^{-6} M, 5,6 benzoflavone MC or benzo(*a*)pyrene, 10^{-9}, 10^{-6} and 10^{-3} M phenobarbital or 10^{-7} to 10^{-5} M bilirubin. IC_{50} was the concentration of competitor required to elicit a 50 percent displacement of specific binding of ^{3}H-MC from rat hepatic cytosol. In these experiments cytosols from different animals were pooled and each determination was performed in duplicate on the pooled fractions. nc (non competitive) indicates that IC_{50} cannot be calculated.

Gunn rats, and that induction of P-450c and P-450d isoenzymes interferes with the expression of non-PAH inducible P-4502c. For comparison, administration of PB to male rats was studied to determine whether the depression of the male-specific P-450 isoenzyme by inducers is a general feature in the Gunn strain.

MATERIALS AND METHODS

Materials. [^{3}H]MC was purchased from Amersham (Arlington Heights, IL). [^{3}H]Acetanilide was synthesised as previously described [15]. Benzphetamine hydrochloride was a gift from the Upjohn Company (Kalamazoo, MI). TCDD was a gift from Dr J. Van Cantfort (Liege, Belgium). Hydroxylapatite, DNA grade, was from Biorad (Richmond, CA). Peroxidase-conjugated swine anti-rabbit immunoglobulins were purchased from DAKO (Copenhagen, Denmark). Other reagents were of the highest purity available.

Animals. Two-month-old homozygous Gunn rats (CSEAL-CNRS, France) or Wistar rats (IFFA-CREDO, France) were given i.p. either MC (50 mg/kg) daily for 3 days, TCDD (27 μg/kg) 4 days, BP (100 mg/kg) 4 days or PB (80 mg/kg) 4 days. Controls were treated with the vehicle alone. Animals were starved overnight prior to sacrifice and microsomes were prepared as previously described [16]. The cytosol was carefully removed without disturbing the upper lipid layer.

Assays. Protein was determined according to Lowry *et al.* [17] and cytochrome P-450 by the method of Omura and Sato [18]. Aniline hydroxylase [19], benzphetamine-*N*-demethylase measured by the formation of formaldehyde [20], aryl hydrocarbon hydroxylase [21], ethoxycoumarin *O*-deethylase [22], acetanilide 4-hydroxylase [15] and ethoxyresorufin-*O*-deethylase [23] were assayed as described elsewhere. UDP-glucuronosyltransferase (UDP-GT) was assayed with 4-nitrophenol [24], testosterone and estrone [25] using Triton X-100-activated microsomes.

Purified cytochrome P-450 and antibodies. P-450 A, P-450 B_2BNF and P-450 B_2PB (respectively designated in this paper as P-4502c, P-450c and P-450b/e) were prepared from rat liver according to the method of Le Provost *et al.* [26, 27]. P-450d (ISF-G) and P-4502d (UT-I) were kindly provided by F. P. Guengerich. The nomenclature and specificity of antigens and antibodies have been confirmed by double cross reactivity between highly purified antigens and antibodies. It has been reported elsewhere [28, 29]. Concerning anti P-4502c, we observed in Western blot, a single band with adult male liver microsomes comigrating with pure P-4502c; no data were available regarding cross-reactivity with P-450 g and f. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transfer to nitrocellulose were carried out as previously described [30]. Peroxidase was detected with 3 mM 4-chloro-1-naphthol and bands were quantified by densitometric scanning using variable amounts of purified isoenzymes for standardization. In our conditions, the relationship between the amount of pure antigen loaded onto the gel and the intensity of signal is linear in a range of concentrations depending on the antibody titer; consequently, the amount of microsomal proteins loaded is chosen in this range (corresponding to 25 to 250 pmoles pure antigen per mg protein).

MC-binding protein quantitation. The binding of ^{3}H-MC to cytosolic proteins was assayed using the procedure of Gasiewicz and Neal [31]. Supernatants from 105,000 *g* centrifugation were kept up to 3

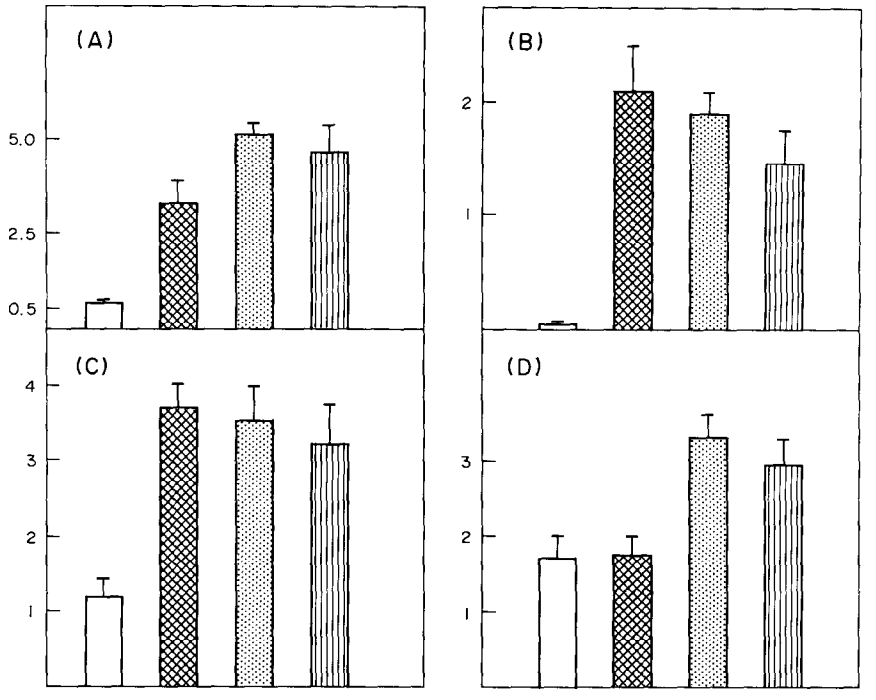

Fig. 1. Monooxygenase activities in male Gunn rat liver microsomes. □ Untreated animals. ▩ MC-treated animals. ▦ TCDD-treated animals. ▥ BP-treated animals. Activities are expressed as nmol substrate hydroxylated/min/mg protein. Results shown are the mean ± SD for four different microsomal preparations. Panel A: Ethoxycoumarin-*O*-deethylase; Panel B: Ethoxyresorufin-*O*-deethylase; Panel C: Benzo(*a*)pyrene hydroxylase; Panel D: Acetanilide 4-hydroxylase.

months at −80° without appreciable loss of the binding capacity. For Scatchard analysis, 400 μl of supernatant (approximately 6 mg protein) were incubated in a final volume of 2 ml of HEDG buffer (25 mM Hepes pH 7.4, 1.5 mM EDTA, 1 mM dithiothreitol, 10% glycerol) with various concentrations of ^{3}H-MC ranging from 0.3 to 30 nM. Incubations were carried out for 1 hr at room temperature. Non-specific binding was estimated in presence of a 100-fold excess of non-radioactive MC or BP dissolved in dioxane. Scatchard analysis were performed from saturation data and allowed to calculate by extrapolation the number of binding sites for MC (n) and the apparent dissociation constant (K_d). To test the effect of competitors on the binding of MC to cytosolic proteins, 6 nM ^{3}H-MC was incubated in presence of 10^{-6} to 10^{-9} M MC, BP or 5,6 benzoflavone, 10^{-3} to 10^{-9} M PB or 10^{-5} to 10^{-7} M bilirubin dissolved in sodium bicarbonate just prior to addition to the reaction mixture.

RESULTS

The effects of PAH administration on the cytochrome P-450 content of hepatic microsomes from Gunn rats are shown in Table 1. No change in the overall P-450 content occurred in males after treatment by either MC, TCDD or BP. This result has been repeatedly obtained in several sets of experiments. However, a shift from 0.5 to 1.5 nm of the wavelength of maximal absorption was observed. In contrast, the microsomal content in cytochrome P-450 was increased by 60% in MC-treated female Gunn rats, comparable to that observed in Wistar rats from both sexes.

In order to further investigate the lack of induction of cytochrome P-450 by PAH in male Gunn rats, we determined the cytosolic content in MC-binding protein. For that purpose we used the procedure of Gasiewicz and Neal with some minor modifications due to the use of MC instead of TCDD as ligand. Even if MC binds to several proteins in the rat cytosol, the procedure including washes with a detergent-containing buffer was convenient and suitable for a screening of the presence of the protein involved in the induction process. In our conditions, the specific binding was clearly saturable by increasing doses of ^{3}H-MC and allowed us to determine the concentration of MC binding protein sites in the rat cytosol (n) and the apparent dissociation constant (K_d) by Scatchard analysis. Results are indicated in Table 2: both the concentration of MC binding sites and the respective K_d were in the same range in male Gunn and Wistar rats. When the effect of competitors upon the MC binding was investigated, the concentration required to displace the radioligand was roughly similar in Gunn and Wistar rats for MC, BP and 5,6 benzoflavone, whereas PB cannot account for more than 10% of displacement (Table 2). In another experiment, bilirubin at concentrations ranging from 10^{-5} to 10^{-7} M, was added to Wistar liver cytosol at a concentration close to that of bilirubin in the Gunn liver: in these conditions, the MC-binding to cytosolic proteins was unaffected.

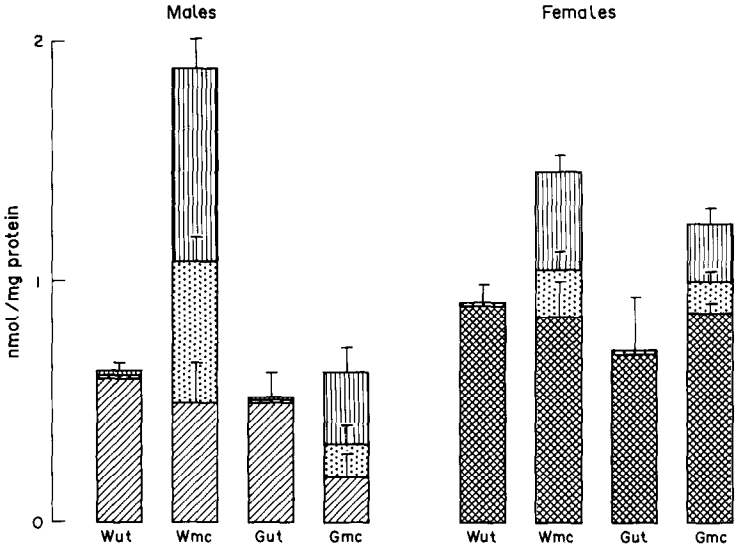

Fig. 2. Cytochrome P-450 isoenzyme concentrations in Gunn and Wistar rat liver microsomes. ▨ P-4502c. ▩ P-450c. ▥ P-450d. ▩ P-4502d. Results shown are the mean of duplicate determinations performed on two different microsomal preparations and expressed as nmol/mg protein. Hepatic microsomal contents in cytochrome P-450 spectrally determined, were respectively in nmol/mg protein: male untreated Wistar (Wut): 0.90 ± 0.03; male MC-treated Wistar (Wmc): 1.85 ± 0.05; female untreated Wistar (Wut): 0.70 ± 0.03; female MC-treated Wistar (Wmc): 1.15 ± 0.05. Gunn rat values are given in Table 1.

Moreover, when Gunn rat cytosol was surcharged *in vitro* with exogenous bilirubin, less than five percent displacement of MC from binding protein could be noticed. Thus, the presence of MC-binding protein and the lack of effect of bilirubin on the binding to this protein in the male Gunn liver was clearly demonstrated.

So, monooxygenase activities have been explored in relation to their dependence on different P-450 isoenzymes. Ethoxycoumarin-*O*-deethylase [32], ethoxyresorufin-*O*-deethylase [23], benzo(a)pyrene hydroxylase [33] and acetanilide-4-hydroxylase [34] are known to be highly induced by PAHs treatment in Wistar rats. All of them were increased in male Gunn rats treated by MC, TCDD or BP (Fig. 1). Furthermore, the extent of induction was in the same range in Wistar and Gunn rats (except for the effect of MC upon acetanilide hydroxylation) suggesting that the whole process is functional whatever the inducer used.

For comparison, UDP-glucuronosyltransferase (a phase II enzyme) has been examined: as previously reported by other groups [9, 10] no increase in activity towards 4-nitrophenolestrone and testosterone was noticed in male Gunn rats whatever the PAH used (data not shown).

The apparent discrepancy between the inefficiency of inducers to elevate the overall cytochrome P-450 content and the presence of a functional induction process for some monooxygenase activities prompted us to quantitate apoproteins by immunoblotting. Results are shown in Figs 2 and 3. In female rats the rise in P-450c and P-450d paralleled the augmentation in total P-450, the content in cytochrome P-4502d (female-specific isoenzyme) remaining constant. This was also true in male Wistar rats where the male-specific P-4502c content was not modified, whereas in male Gunn rats, the increase in P-450c and P-450d was accompanied by a concomitant and equivalent diminution of P-4502c. As a result the total P-450c + P-450d + P-4502c is about the same in male Gunn rats either untreated or MC-induced. This is confirmed by the significant reduction of benzphetamine demethylase activity by PAH: from 4.52 ± 0.09 nmol/min/mg protein in untreated animals to 3.28 ± 0.07, 3.48 ± 0.06 and 2.20 ± 0.09 in MC-, TCDD- and BP-treated males. This activity which is mainly catalysed by P-4502c isoenzyme in untreated rats was reduced in PAH-treated rats reflecting the decreased contribution of P-4502c to the overall P-450 content.

In Fig. 3B, the reactivity of anti P-4502c with rat liver microsomes is shown: only a single band is recognized in MC-treated male liver irrespective of strain, whereas no cross-reaction occurred in untreated samples. Moreover, the respective percentage of P-4502c, P-450c and P-450d are roughly similar in both strains although the total immunochemically determined amount of P-450 isoenzymes was tripled in Wistar vs Gunn males.

To determine whether or not the repression of P-450 expression by PAH is specific of this class of inducers, we have investigated the effects of PB on the P-450 isoenzyme content and on dependent monooxygenase activities. As shown in Table 3, the overall P-450 content, as well as the aniline hydroxylase activity, where markedly enhanced in PB-treated rats. The increase in the total P-450 content is due to a considerable augmentation in P-450b/e concentration, whereas the male-specific P-4502c isoenzyme level is unchanged. Resulting from this rise in isoenzyme P-450b/e, the benzphetamine

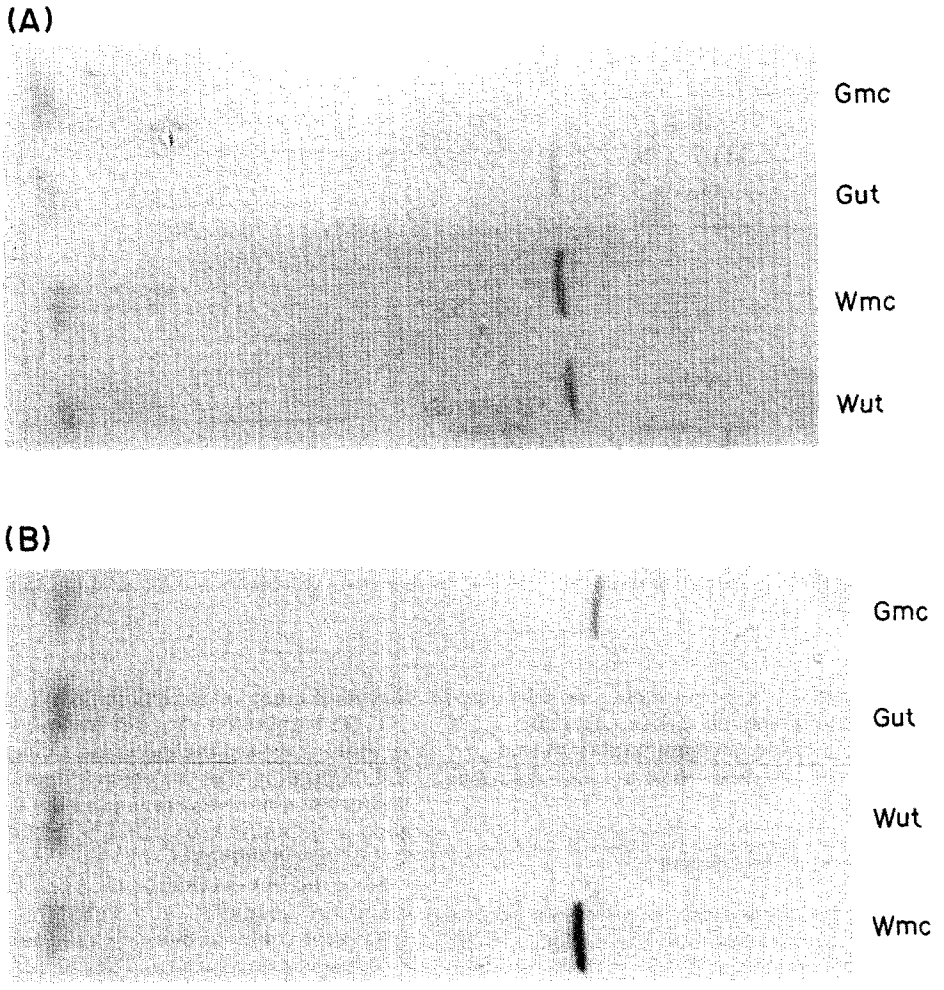

Fig. 3. Western blots of Gunn and Wistar liver microsomes. Wut: untreated Wistar; Wmc: MC-treated Wistar; Gut: untreated Gunn; Gmc: MC-treated Gunn; 10 μg proteins were loaded per lane. Blots were incubated with anti P-4502c diluted 1/100 (A) or with anti P-450c diluted 1/250 (B).

Table 3. Microsomal cytochrome P-450 content and related monooxygenase activities in male Gunn rats

	Untreated (N = 4)	PB-treated (N = 3)
Cytochrome P-450* (nmol/mg protein)	0.99 ± 0.10	2.04 ± 0.15 P < 0.001
Aniline hydroxylase (nmol/min/mg protein)	0.70 ± 0.12	1.84 ± 0.30 P < 0.001
Benzphetamine demethylase (nmol/min/mg protein)	4.52 ± 0.09	19.8 ± 2.4 P < 0.001
Isoenzymes† (nmol/mg protein)		
P-4502c	0.5	0.4
P-450c	n.d.	n.d.
P-450d	0.05	n.d.
P-450b/e	0.05	1.8

* Spectrally determined.
† Immunochemically determined.
Statistical significance is given in reference to untreated animals. n.d.: not detectable.

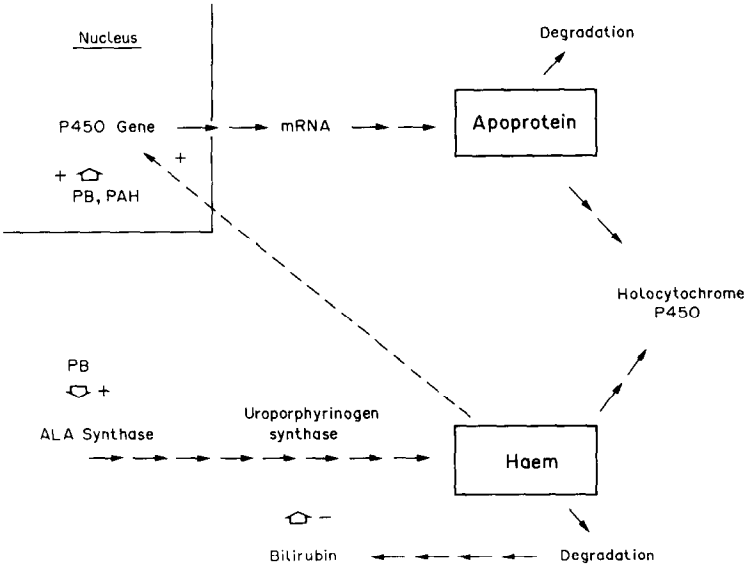

Fig. 4. Possible regulation of cytochrome P-450 synthesis by xenobiotics and bilirubin in Gunn rats.

demethylase activity is 5-fold higher in PB-treated than in untreated animals. Thus, data are qualitatively and quantitatively comparable to that previously reported in male Wistar rats, regarding induction by PB.

DISCUSSION

In rat liver, MC binds avidly to a cytosolic protein and is further translocated into the nucleus [35–38] for stimulating cytochrome P-450 synthesis through an activation of the genome. The values observed in Gunn rats clearly indicate the presence of a MC-binding protein exhibiting the same characteristics as the receptor from Wistar rat liver. In order to explain the inefficiency of MC to increase the overall content in P-450 in male Gunn rat liver, a possible inhibitory effect of bilirubin on the binding of MC to cytosolic protein can be hypothesised. However, neither the addition of bilirubin to Wistar rat cytosol, nor an overload of Gunn rat cytosol by exogenous bilirubin modified the MC binding to cytosolic proteins. So, in Gunn as well as in Wistar liver, the first step of the induction process is present.

It was next of interest to test further the gene expression potentially stimulated by PAH. Molecules known to have different inductive potencies have been tested in order to overcome any trouble linked to a poor translocation into the nucleus or/and activation of the genome. Activities dependent on P-450c and P-450d (ethoxyresorufin deethylase, benzopyrene hydroxylase, ethoxycoumarin deethylase) were dramatically increased by a pretreatment with these inducers whereas the effect on acetanilide-4-hydroxylase activity was not so clear-cut. This definitively indicates that the induction process is present and functional in male Gunn rat as well as in Wistar rat.

The lack of induction of UDP-GT activities in PAH-treated Gunn rats addresses the question about the mechanism of induction. In the case of UDP-GT, the basal activity for 4-NP is lower in Gunn than in Wistar rats and the induction capacity by PAH is lost, indicating that a defect (either in the coding and/or the non-coding region) in the 4-NP UDP-GT gene is responsible for the low activity and/or the loss of inducibility [11].

Since the lack of effect on the overall P-450 content cannot be attributed to a defect in the induction process, it could be due to a rearrangement of the P-450 isoenzyme pattern. The sum of the immunochemically determined concentrations of the individual P-450s was greater than the spectrally determined levels. This fact has been repeatedly reported by us and other groups [31, 39]. Only male Gunn rats exhibit an important diminution of the male-specific P-4502c in such way that the increase of P-450c and P-450d is balanced by an equivalent decrease in the concentration of P-4502c. In male Wistar, as well as in Gunn and Wistar female rats, the elevation in P-450c and P-450d content does not influence the level of P-4502c or P-4502d.

Previously, it has been reported that a partial and transient decline in P-4502c content accompanied the induction by various xenobiotics in adult Sprague Dawley male rats (but not in females) [40]. However, this diminution in P-4502c is balanced by an important rise in inducible P-450s, the total P-450 content exceeding in induced animals the value observed in untreated animals. We did not observe this decline in our "control" (male Wistar) after MC treatment. These data have been repeatedly obtained using several groups of male rats and is probably not an artifact. To date, no valuable explanation can be given for that discrepancy, but difference in strain.

Cytochrome P-450c and P-450d isoenzymes are barely detectable in adult rat liver microsomes and rise after pretreatment with PAH [41]. Recently, Kapitulnik *et al.* [14] reported a high level of P-450c in untreated Gunn rats and its possible involvement

in bilirubin metabolism. We have not found such a level in untreated Gunn rat liver. Moreover the pattern of proteins visualised in Western blot and probed with anti P-450c antibody was quite different from that reported by Kapitulnik *et al.* We observed one unique band at the same apparent molecular weight as the reference P-450c and this band appeared mainly in microsomes from MC-treated rats. It was just at the limit of detection in microsomes from untreated Gunn and Wistar rats of both sexes (i.e. 25 pmoles/mg protein). Unlike Kapitulnik data, no contaminant cross-reaction occurred between anti-P-450c and Gunn liver microsomes. The reasons for that discrepancy remain unexplained but might be relative to either the antibody or the microsomal preparation (age, sex or local strain of homozygous Gunn rats).

In contrast, PB stimulated to a comparable extent the expression of P-450b/e in both strains without modification of the content in the male-specific P-4502c. So, only male Gunn rats exhibit an altered response in P-450 gene expression to PAH administration. This suggests the interference of bilirubin in the regulation of P-450 biosynthesis by PAH (see Fig. 4).

A possible regulation of P-450 gene transcription through the intracellular haem pool has been hypothesised [42]. In this model, a low haem concentration can repress the expression of P-450 genes, whereas the addition of exogenous hemin restored the P-450 gene transcription to its normal level [43]. In male Gunn rats, the high level in circulating bilirubin can modulate haem biosynthesis since bilirubin has been shown to partially inhibit uroporphyrinogen I synthase [44]. Thus, the total haem synthesis might be reduced in male Gunn rats, the amount of haem available for incorporation into apocytochrome P-450 remaining sufficient to maintain a normal level of holocytochrome P-450 (detected by its CO spectrum) and the normal expression of P-450 genes. However, the haem pool content might be limiting in case of induction by xenobiotics: with MC which stimulates only P-450c and P-450d gene transcription without increasing haem synthesis, the amount of haem could not be sufficient to be incorporated into all apoproteins synthesised. Apoprotein without haem is then degraded at a higher rate than the holoprotein [42] and thus cannot be immunochemically detected. Furthermore, the balance between isoenzymes remains nearly identical to that of Wistar rats. So, haem is randomly distributed among all species, excluding a direct relationship between haem biosynthesis and the regulation of a peculiar P-450 species.

PB is involved in the induction process at two levels: first it activated the transcription of P-450b and P-450e genes and secondly increased the activity of ALA synthase [45]. Thus, in PB-treated male Gunn rats, the flow passing through the first steps of haem biosynthesis is augmented and will overcome the partial inhibition of uroporphyrinogen I synthase by bilirubin. The result is an elevated haem availability, allowing its incorporation into all the newly synthesised P-450 apoproteins and consequently a rise in total cytochrome P-450 holoprotein. This does not account for the sex difference observed in Gunn rats which remains unclear and requires more information to be elucidated.

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