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Ahr Locus Phenotype in Congenic Mice Influences Hepatic and Pulmonary DNA Adduct Levels of 2-Amino-3-methylimidazo[4,5-*f*]quinoline in the Absence of Cytochrome P450 Induction

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SUMMARY

The potent food mutagen/carcinogen 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) undergoes metabolic *N*-hydroxylation by cytochromes P450, including cytochrome P450 1A2, followed by generation of an unstable ester catalyzed by acetyltransferases; promutagenic DNA adducts result. Genetic polymorphisms in these enzymes have been implicated in human cancer risk related to arylamine exposure. We investigated the effects of *Ahr* locus and acetylator polymorphisms on ^{32}P-postlabeled IQ/DNA adducts in lungs and livers of female C57BL/6 mice congenic for slow acetylation and/or *Ahr*-nonresponsiveness; some groups were pretreated with β-naphthoflavone (βNF), a cytochrome P450 1A inducer. Total adducts in lung were doubled by βNF pretreatment in *Ahr*-responsive mice only and consisted of ≤30% adduct 2 and ≤60% adduct 3. In contrast, in *Ahr*-nonresponsive mice, adducts 2 and 3 were each ≤7% of the total. Livers of noninduced *Ahr*-responsive mice formed 6–18-fold more adducts than those of nonresponsive mice. This striking difference was not due to altered levels of cyp1a-2, as indicated by specific enzyme assays and immunoblotting, and was not accompanied by a comparable increase in the ability of liver preparations to activate IQ to a mutagen in the Ames test. Pretreatment of responsive mice with βNF to induce cyp1a-1 and cyp1a-2 led to a reduction in liver adduct levels. Acetylation phenotype also had a significant effect in *Ahr*-responsive mice, with 3-fold more adducts in slow than in rapid acetylators. These results indicate that in uninduced mice, the normal Ah receptor facilitates formation of IQ/DNA adducts in liver and alters the profile of adducts in lung, via an unknown mechanism, whereas the Ah receptor-dependent enzyme induction reduces adducts in liver, probably due to increased detoxification, but increases them in lung.

IQ belongs to a group of mutagenic and carcinogenic heterocyclic aromatic amines formed in various foods during cooking and possibly involved in cancer risk (1). Metabolic activation of IQ occurs via a two-step process involving, first, CYP450 1A2 (cyp1a-2 in the mouse) -catalyzed *N*-hydroxylation. Studies with recombinant mouse cyp1a-1 and cyp1a-2 showed that the latter, but not the former, catalyzes *N*-hydroxylation of IQ (2). This step is followed by the formation of an unstable *N*-acetoxy ester, a step catalyzed by NATs (3, 4). Although other esterification reactions are implicated in heterocyclic aromatic amine metabolism, the acetylation pathway seems to predominate for IQ (5). It has also been shown that the *in situ* generated *N*-acetoxy-IQ ester is more reactive toward DNA than is *N*-hydroxy-IQ (4). *In vitro* studies with DNA and *N*-hydroxy-IQ have demonstrated that the reactive metabolite of IQ forms a major adduct at the C8 atom of guanine [N^2-(deoxyguanosin-8-yl)-IQ] (4). Detoxification reactions involve ring hydroxylation (at the C5 position) and *N*-demethylation, followed by conjugation (6, 7).

Thus, it seems that the extents of formation of *N*-hydroxy-IQ and of its subsequent ester will be determinants of DNA adduct formation and presumably influence the degree of initiation of carcinogenesis. Both the CYP and NAT enzymes are known to exhibit genetic polymorphisms in ro-

ABBREVIATIONS: IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; NAT, *N*-acetyltransferase; βNF, β-naphthoflavone; RAL, relative adduct labeling; CYP, (human and rat) cytochrome P; cyp, (mouse) cytochrome P; B6, C57BL/6; ANOVA, analysis of variance.

dents and humans. Induction of CYP1A1 and CYP1A2 is regulated by the *Ahr* locus (*AHR* in humans); the molecular genetics of this process are being unraveled for both mice and humans (8, 9). Polymorphisms in human *CYP1A1* have been implicated in lung cancer risk (10). Polymorphism for the *NAT* gene has also been observed in humans (11, and references therein) and other mammals, including Syrian hamsters and mice (12, 13).

The results of several human epidemiological studies have suggested associations between acetylator phenotype and the incidence and/or type of tumors associated with arylamine exposure (14, 15); polymorphisms in both *CYP1A2* and *NAT* were associated with increased frequency of colon cancer (16). This hypothesis has been strengthened by the observation of high IQ/DNA adduct levels in COS-1 cells expressing human CYP1A2 and NAT2 (17) and the recent demonstration of a close link, in human colon cancers, between point mutations in the K-*ras* oncogene and rapid acetylation phenotype (18).

We undertook an investigation of the possible roles in cancer risk of polymorphisms in *Ahr* responsiveness and rates of acetylation and of the interactions of these phenotypes by measurement of DNA adducts of IQ in mice congenic for *Ahr* responsiveness versus nonresponsiveness and rapid versus slow acetylation. Results for tissues for which acetylator phenotype has been implicated in cancer risk (i.e., colon, urinary bladder, and kidney) have been given elsewhere (19). In this report, we present the data for mouse liver and lung, both of which are targets for tumor initiation by IQ (20).

Levels of IQ/DNA adducts in lung were low but showed a significant, 2-fold increase after pretreatment with the *Ahr* inducer βNF in *Ahr*-responsive mice. A major contribution of several unidentified adducts occurred in lung but only in the *Ahr*-responsive strains. In liver, adduct levels were reduced 3-fold by βNF pretreatment of both *Ahr*-responsive strains. A surprising and unexpected finding in liver was a large difference in DNA alteration in uninduced mice, with the *Ahr*-responsive strains exhibiting 6-fold (slow acetylators) to 18-fold (rapid acetylators) more IQ/DNA adducts than the *Ahr*-nonresponsive strains.

Materials and Methods

Chemicals. IQ (chemical purity >99.7%) was obtained from the Nara Institute, Ltd. (Osaka, Japan). βNF was obtained from Sigma Chemical Co. (St. Louis, MO). Other reagents used were of analytical grade.

Animal treatment. B6 mice (*Ahr*-responsive, Ahr^{b-1}; rapid acetylator, Nat^{r}) were derived from stock maintained at the University of Michigan. B6 mice congenic for slow acetylation (B6.A, Ahr^{b-1}/Nat^{s}) were produced by introduction of genetic material from strain A mice as described previously (21). B6 mice congenic for *Ahr* nonresponsiveness (B6.D, Ahr^{d}/Nat^{r}) due to substitution of the Ahr^{d} locus from DBA mice were originally obtained from Dr. A. Poland (McArdle Laboratory for Cancer Reseach, University of Wisconsin, Madison, WI) and subsequently produced at the University of Michigan. Both congenic lines were produced by backcrossing for 12 generations. The double-congenic B6.A.D. (Ahr^{d}/Nat^{s}) mice were produced as described previously (22) and were inbred for two generations before carrying out the experiments described here. Mice were housed in stainless steel cages, three to five mice/cage, and were maintained at constant temperature and humidity with a 12-hr light/dark cycle and fed *ad libitum*. All of the mice were housed in the same room and were treated within a 2-week time period.

Groups of four females from each of the above genotypes received a single intraperitoneal injection of IQ (20 mg/kg) in olive oil 48 hr after βNF pretreatment (100 mg/kg). Controls received olive oil instead of βNF. Mice were killed 3 hr after the last treatment. Livers and lungs were frozen immediately in liquid N_2 and stored at −70° until used for DNA isolation, enzyme assays, Western immunoblotting, and mutagenicity testing.

Alkoxyresorufin *O*-dealkylase assays. Ethoxyresorufin, methoxyresorufin, and benzyloxyresorufin dealkylations are specific and sensitive indicators of murine cyp1a-1, cyp1a-2, and cyp2b activity, respectively (23, 24). The dealkylations of all derivatives were measured fluorimetrically in liver homogenates by accumulation of resorufin with an excitation wavelength of 522 nm and an emission wavelength of 586 nm. DT-diaphorase activity was inhibited by the addition of dicumarol. Resorufin standards were assayed with each set of experiments, and activities were demonstrated to be linear over time and as a function of homogenate concentration.

Western immunoblotting. Microsome preparation from two pooled livers, protein determination, gel electrophoresis, and transfer to nitrocellulose were carried out as described previously (25). Briefly, hepatic microsomes diluted in sample buffer were loaded onto 8% minigels (Novex Corp.), and protein separation was carried out with double-strength tank buffer. Blots were probed with monoclonal antibody 1–7-1 to CYP1A1 and CYP1A2 isolated from methylcholanthrene-treated rats (26). The blots were then probed with a secondary antibody (goat anti-mouse IgG) conjugated to alkaline phosphatase, and the bands were visualized with the use of the alkaline phosphatase reaction.

Mutagenic activation. The mutagenesis assay was performed on portions of two livers from oil-treated mice of each phenotype, as described previously (27). Briefly, test compounds dissolved in 0.02 ml of dimethylsulfoxide, *Salmonella typhimurium* strain TA98, 400 μg of protein from a hepatic 9000 × *g* supernatant fraction (S-9), and NADPH in a total volume of 530 μl were preincubated in a 37° water bath. After 20 min, top agar was added, and the mixtures were immediately plated onto Vogel Bonner Medium E plates. Plates were incubated at 37° for 48 hr. The number of histidine-independent mutants was scored with the use of an Artec model 880 automatic colony counter. Plates were run in duplicate, and background colony formation in the presence of the S-9 fraction (22–36 revertants/plate) was subtracted from the results.

DNA adduct study and ^{32}P-postlabeling analysis. DNA was isolated, digested, and used for analysis according to the intensification method as described previously (28, 29). The procedure resolves the [^{32}P]ATP-labeled phosphonucleotide adducts as fingerprints on autoradiograms after chromatography on PEI-cellulose thin layer sheets. For convention, the numbering of adducts is the same as that used previously (28, 29). Adduct 1 is known to be N^2-(deoxyguanosin-8-yl)-IQ; the others adducts have not been identified yet.

Data presentation and statistical analysis. Total adducts, calculated as the sum of the individual adducts detected, and the proportions contributed by each individual adduct were analyzed untransformed with the use of factorial ANOVA. In cases where a particular adduct was observed in some animals but not in others within a treatment group, the RAL at one half of the lowest reported value for that adduct was substituted as a conservative acknowledgment of the fact that the actual adduct level could have been somewhere between zero and the lowest observed value. The data were also analyzed with the use of, for these cases, either zero or one half of a previously determined limit of detection. The results (not shown) were in close agreement with those reported.

We performed a combined ANOVA of all 32 animals followed by separate ANOVAs for the *Ahr*-responsive and *Ahr*-nonresponsive animals to gain a better understanding of possible interactions among the three factors of interest (*Ahr* × *Nat* × βNF). In a number of cases, the ANOVAs were followed by *t* test comparisons among

mean values with use of the pooled residual error from the combined ANOVA. These individual comparisons were those that were built into the design of the study.

Tabled results are expressed as mean ± standard error. Values of $p < 0.05$ were considered to be statistically significant. Values of $p = 0.05$–0.10 are included as possibly indicative of real although weak effects.

Results

Amounts of CYP450s. To confirm the *Ahr*-responsiveness and -nonresponsiveness of the Ahr^{b-1} and Ahr^{d} strains, respectively, and the specific induction of cyp1a-1 and cyp1a-2, we carried out the specific enzyme assays ethoxyresorufin dealkylation (cyp1a-1) and methoxyresorufin dealkylation (cyp1a-2) and benzyloxyresorufin dealkylation (cyp2b) for nonspecific changes. Both cyp1a-1 and cyp1a-2 showed a significant ($p < 0.001$) 20-fold induction by βNF in the *Ahr*-responsive animals (Table 1). As expected, there was no induction of the enzyme activities in the *Ahr*-nonresponsive strains by βNF. Acetylation phenotype did not affect the activities. The benzyloxyresorufin dealkylation activities were similar in all groups.

These findings were further confirmed with respect to enzyme protein with Western blot analysis of hepatic microsomes from each treatment group. Representative blots are shown in Fig. 1. Monoclonal antibody 1–7-1 cross-reacts with both cyp1a-1 and cyp1a-2 which are clearly separated, as illustrated with hepatic microsomes from animals treated with the potent Ah receptor agonist, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (Swiss males, 50 nmol/kg dose, 1 week after treatment) included as positive control animals. Approximate K_D values were 55.5 and 55. The oil-treated animals from all four strains exhibited only cyp1a-2. As expected, βNF pretreatment induced cyp1a-1 proteins only in the *Ahr*-responsive animals. The significance is unknown of some cross-reactive material of lower molecular weight, seen in all samples.

IQ/DNA adduct levels in liver. Three adducts were observed in all strains, as seen on the representative autoradiograms (Fig. 2, A and B). Results are presented in Table 2 and Fig. 3A, with statistical analysis of the data given in Table 3. A striking difference was noted in total adducts in uninduced *Ahr*-responsive mice compared with *Ahr*-nonresponsive animals. The responsive mice had 18-fold (rapid acetylators) or 6-fold (slow acetylators) more adducts than the nonresponsive mice. βNF pretreatment significantly reduced the total adducts (2–3-fold) only in *Ahr*-responsive mice. Even after this reduction, the responsive animals presented significantly more DNA adducts than did the comparable, βNF-pretreated nonresponsive mice.

The acetylation phenotype also had an impact on total

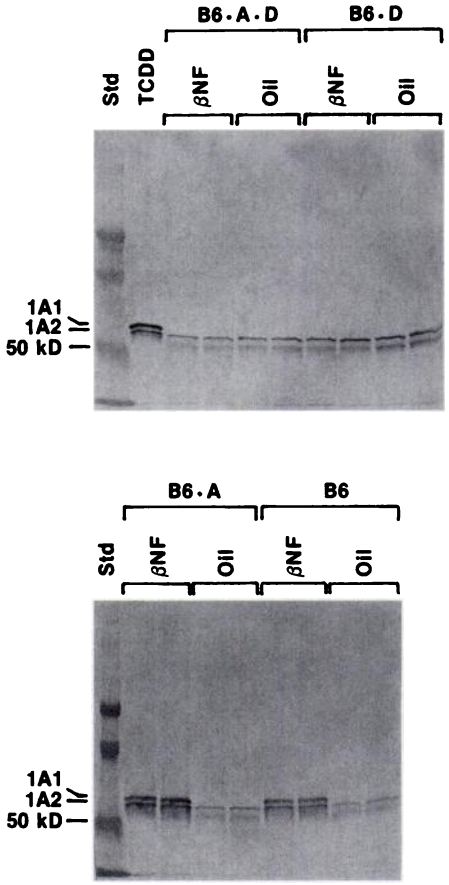

Fig. 1. Western blots of liver microsomal cyp1a proteins pooled from two mice treated with IQ at 20 mg/kg 48 hr after βNF pretreatment (100 mg/kg) in oil intraperitoneally. *Top*, 5.0 μg of microsomal protein from B6.A.D and B6 mice were loaded onto each lane. Liver microsomes (0.5 μg of protein) from 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-treated Swiss males (50 nmol/kg, 1 week after treatment) served as a positive reference for cyp1a-1 and cyp1a-2. *Bottom*, Microsomal proteins from B6.A and B6 mice were loaded at 1 μg of protein per lane for βNF-pretreated livers and at 5 μg of protein per lane for oil-treated livers.

TABLE 1

Basal and induced enzyme activities of hepatic cyp1a-1, cyp1a-2, and cyp2b

Mice (five per group) were treated with IQ (20 mg/kg) 48 hr after pretreatment with oil or βNF (100 mg/kg) and killed 3 hr later. Liver homogenates were assayed for dealkylation of ethoxyresorufin (cyp1a-1), methoxyresorufin (cyp1a-2), and benzyloxyresorufin (cyp2b).

Strain	Treatment	Enzyme activity		
		Ethoxyresorufin	Methoxyresorufin	Benzyloxyresorufin
		nmol/min/g liver		
B6 (Ahr^{b-1}/Nat^{r})	Oil	3.1 ± 1.2	2.0 ± 0.40	5.6 ± 0.14
	βNF	55 ± 4.0	44 ± 3.3	4.3 ± 0.59
B6.A (Ahr^{b-1}/Nat^{s})	Oil	2.3 ± 0.75	2.2 ± 0.10	6.0 ± 0.61
	βNF	45 ± 4.5	44 ± 2.0	7.5 ± 0.39
B6.D (Ahr^{d}/Nat^{r})	Oil	2.6 ± 0.78	2.0 ± 0.60	6.4 ± 0.72
	βNF	2.9 ± 0.84	2.3 ± 0.52	7.8 ± 2.0
B6.A.D (Ahr^{d}/Nat^{s})	Oil	2.5 ± 0.72	1.9 ± 0.39	7.2 ± 0.34
	βNF	2.4 ± 0.29	2.0 ± 0.35	4.8 ± 0.70

Values are mean ± standard deviation.

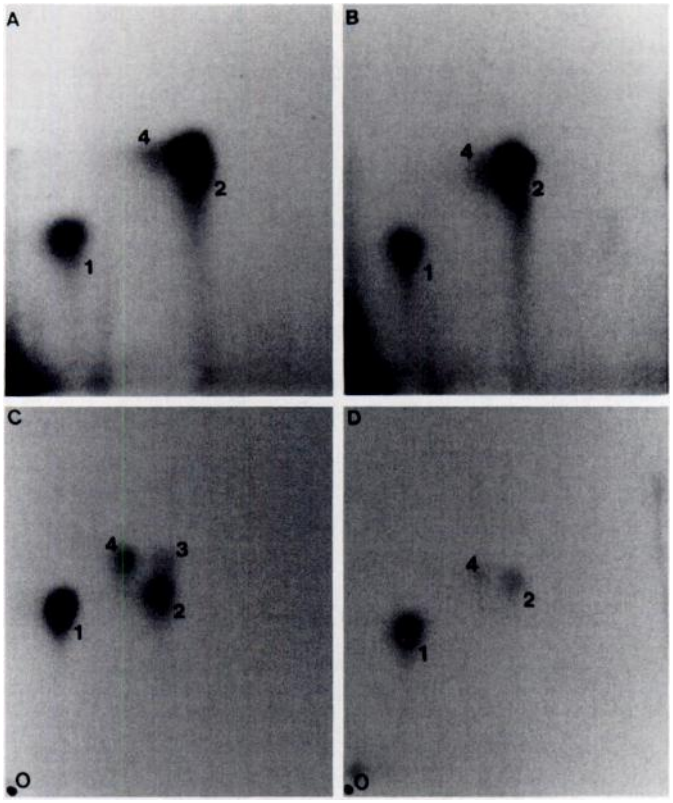

Fig. 2. Autoradiograms of IQ/DNA adducts in control livers from B6 [3-hr exposure (A)] and B6.D [24-hr exposure (B)] congenic mice. Note the similarity in spot intensity with the 8-fold difference in exposure time. Autoradiograms of lung DNA of B6 (C) and B6.A.D (D) congenic mice were exposed for 16 and 17 hr, respectively. *O*, origin.

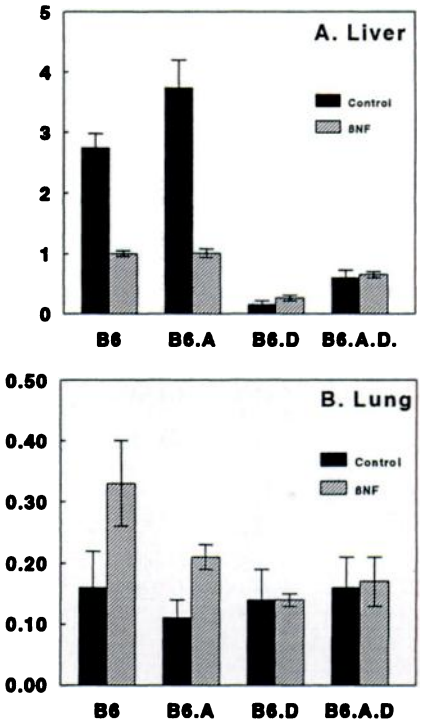

Fig. 3. Summary of the total IQ/DNA RAL × 10^7 in liver (A) and lung (B) of the four congenic strains, control animals, and βNF-pretreated animals.

adducts, to an extent that was most pronounced in the non-responsive B6.D mice, for which slow acetylators had significantly higher (3–4-fold) DNA adducts compared with rapid acetylators.

The predominant adduct 1 formed 50–71% of the total, with adduct 2 forming 18–37% and adduct 4 forming 9–21%. The proportions of the individual adducts varied significantly among groups (ANOVAs in Table 3), with the largest differences being related to *Ahr* genotype. The ratio of adduct 1 to adduct 2 was greater in Ahr^{b-1} than in Ahr^{d} mice. Also, slow acetylators accounted for a higher proportion of adduct 2 than did rapid acetylators, for both *Ahr* genotypes. There was a significant interaction between *Nat* and βNF in the Ahr^{d} group, in that βNF pretreatment decreased the proportion of adduct 1 and increased the proportion of adduct 2 in rapid acetylators but had the opposite effect in slow acetylators. The contribution of adduct 4 did not vary significantly among groups.

TABLE 2

IQ/DNA adducts in livers of congenic mice

Total IQ/DNA adducts are the sum of the individual adducts, and the proportions of individual adducts were calculated. Values are the mean ± standard error of DNA adduct measurements in four animals. Numbers with matched superscripts are significantly different by pairwise *t* tests with pooled error for all groups or for Ah^{b-1} and Ah^{d} groups, respectively. All data analyzed with the use of ANOVA; see Table 3.

Strain	Treatment	Total liver adducts	Proportion of total adducts		
			Adduct 1	Adduct 2	Adduct 4
		RAL × 10^7			
B6 (Ahr^{b-1}/Nat^{a})	Oil	2.74 ± 0.23[a,b]	0.69 ± 0.008	0.19 ± 0.005	0.12 ± 0.006
	βNF	1.00 ± 0.05[a,c]	0.71 ± 0.02	0.18 ± 0.009	0.11 ± 0.007
B6.A (Ahr^{b-1}/Nat^{a})	Oil	3.74 ± 0.45[d,e]	0.65 ± 0.02	0.23 ± 0.02	0.12 ± 0.009
	βNF	1.01 ± 0.07[d]	0.70 ± 0.01	0.19 ± 0.006	0.11 ± 0.009
B6.D (Ahr^{d}/Nat^{a})	Oil	0.15 ± 0.06[b,f]	0.71 ± 0.03	0.20 ± 0.04	0.09 ± 0.02
	βNF	0.26 ± 0.05[c,g]	0.53 ± 0.08	0.26 ± 0.02	0.21 ± 0.09
B6.A.D (Ahr^{d}/Nat^{a})	Oil	0.60 ± 0.12[e,f]	0.50 ± 0.05	0.37 ± 0.06	0.13 ± 0.02
	βNF	0.65 ± 0.05[g]	0.58 ± 0.03	0.28 ± 0.01	0.14 ± 0.04

[a] $p = 0.00043$.
[b] $p < 0.00001$.
[c] $p = 0.017$.
[d] $p < 0.00001$.
[e] $p < 0.00001$.
[f] $p = 0.0013$.
[g] $p = 0.0036$.

TABLE 3

ANOVAs of liver data, all eight groups (top nine entries) and for groups differing at the *Ahr* locus considered separately

Parameter	Total	Proportion of individual adducts		
		Adduct 1	Adduct 2	Adduct 4
Among all groups	0.00001	0.0013	0.0022	NS
Ahr genotype	0.00001	0.00048	0.00075	NS
Nat	0.0022	0.060	0.012	NS
Ahr × *Nat*	NS	NS	NS	NS
βNF	0.00001	NS	NS	NS
Ahr × βNF	0.00001	NS	NS	NS
Nat × βNF	0.063	0.011	0.035	NS
Ahr × *Nat* × βNF	0.096	0.054	NS	NS
Coefficient of variance	30%	12%	25%	57%
Among Ahr^{b-1} groups	0.00001	0.061	0.016	NS
Nat	0.074	0.10	0.034	NS
βNF	0.00001	0.047	0.030	NS
Nat × βNF	0.079	NS	0.085	NS
Coefficient of variance	24%	4.4%	11%	13%
Among Ahr^{d} groups	0.0012	0.066	0.089	NS
Nat	0.00014	NS	0.049	NS
βNF	NS	NS	NS	NS
Nat × βNF	NS	0.029	0.092	NS
Coefficient of variance	37%	18%	30%	72%

Mutagenicity assay. To test whether liver activation of IQ to a genotoxicant might differ among the strains even in the absence of variations in the cyp450s examined (see above), mutagenesis by IQ was tested with *S. typhimurium* strain TA98 (Table 4). Hepatic S9 supernatants from each of two oil-treated mice of all four strains catalyzed mutagenesis. The number of revertants per plate correlated directly with the concentration of IQ used. The liver preparation from the B6 mice was approximately twice as active at the lower IQ doses as those from the other strains, with little difference among the other three strains. Thus, the much greater level of IQ/DNA adduct in *Ahr*-responsive versus -nonresponsive mice did not correlate with activation of IQ to a mutagenic form.

IQ/DNA adducts in lung. Total adducts in lung (Table 5 and Fig. 3B) were overall lower than those in liver. After βNF pretreatment of *Ahr*-responsive mice of either acetylator phenotype, there was a doubling in total adducts (a significant difference by ANOVA). A similar effect was not observed for the nonresponsive mice. There were no other major differences in total adducts.

However, the presence and proportion of the individual adducts in lung were quite strongly influenced by the combination of phenotypes and by βNF pretreatment (Fig. 2, C and D, and Tables 5 and 6). Differences in adducts 1 and 3 were particularly striking. Adduct 1 was the major adduct in all Ahr^{d} mice (Fig. 2D), with much less of adducts 2 and 4 and adduct 3 rarely detected. In contrast, adduct 3 was prominent in Ahr^{b-1} mice, and substantial amounts of adduct 2 occurred, concomitant with a reduction in the percentage of adduct 1 (Table 5). βNF pretreatment of *Ahr*-responsive, slow acetylator mice resulted in a large reduction in the percentage of adduct 1 and a corresponding increase in adduct 3. The net result of these changes was that adduct 1, the major adduct in most tissues, constituting ~90% of the adducts in all of the Ahr^{d} mice, was greatly reduced in proportion in Ahr^{b-1} animals, to as low as 9%, in slow acetylators pretreated with βNF.

TABLE 4

Mutagenesis in *S. typhimurium* strain TA98 catalyzed by liver S-9 fractions from congenic mice

Hepatic supernatant fractions (9000 × *g*) from oil-treated mice, two per group, were incubated with the tester bacteria and the concentrations of IQ indicated. The numbers of mutants were corrected for background (supernatant in the absence of IQ). Values given are the average of duplicate plates for each mouse, which differed by ≤15%.

Mouse strain	μg of IQ			
	0.005	0.02	0.1	0.5
	TA98 revertants/plate			
B6 (Ahr^{b-1}/Nat^{a})	150	256	340	552
	196	354	504	663
B6.A (Ahr^{b-1}/Nat^{a})	76	167	241	487
	59	124	173	451
B6.D (Ahr^{d}/Nat^{a})	87	181	257	464
	52	163	278	495
B6.A.D (Ahr^{d}/Nat^{a})	84	177	270	677
	106	206	273	545

Discussion

The most striking and unexpected finding from this study was the strong dependence of total hepatic IQ/DNA adduct levels on Ah receptor phenotype, with *Ahr*-responsive mice having a ≤18-fold higher level than *Ahr*-nonresponsive mice. This differential was independent of changes in cyp1a-1, cyp1a-2, or cyp2b or in ability to activate IQ to a mutagen in the Ames test. Lack of involvement of P450s of the 1a family was further confirmed by the observation that induction of these enzymes by βNF was associated with a significant (2–4-fold) change in the opposite direction; i.e., there was a decrease in total adducts. Pretreatment of rats with other CYP1A inducers like 3-methylcholanthrene or polychlorinated biphenyls is known to generate more ring hydroxylated IQ, a detoxification product, than in control animals (30, 31) and to reduce IQ/DNA adducts in hepatic and extrahepatic tissues (32).

This remarkable phenomenon suggests that the Ah receptor plays a previously unsuspected direct role in the delivery and/or activation of IQ to enhance the formation of DNA adducts, although a role for another, closely linked gene cannot be ruled out at present. Indications of receptor-mediated effects of arylamines related to tumorigenesis have been noted by others, although not characterized (33). The mechanisms by which the Ah receptor could facilitate IQ/DNA adduction are not known but are of considerable interest because it represents a novel effect of this protein and because of implications for both risk assessment and chemoprevention. IQ and other similar arylamines have been found to have, at best, weak affinity for the Ah receptor in most species (34). It is possible that *N*–OH–IQ has more affinity for the mouse liver Ah receptor than does IQ. Recent findings from a study of a mouse afflicted by targeted disruption of the *Ahr* gene, showing immune system impairment and pathological alterations in liver (35), confirm roles for this protein beyond interception of aromatic xenobiotics. How these functions of the Ah receptor may relate to its novel capacity to increase DNA binding of IQ is unknown.

Acetylation phenotype also had an impact on total adducts in liver DNA, most clearly in mice lacking the functional Ah receptor, in that slow acetylators formed more adducts than

TABLE 5

IQDNA adducts in lungs of congenic mice

Total IQDNA adducts and proportions of individual adducts to total were determined. Values are mean ± standard error of DNA adduct measurements in four animals. Numbers with matched superscripts are significantly different by pairwise *t* test with overall pooled error. All data were analyzed with the use of ANOVA; see Table 6.

Strain	Treatment group	Total adducts	Proportion of total adducts			
			Adduct 1	Adduct 2	Adduct 3	Adduct 4
		RAL × 10^7				
B6 (Ahr^{b-1}/Nat^{r})	Oil	0.16 ± 0.06[a]	0.63 ± 0.11[b]	0.13 ± 0.06	0.23 ± 0.14	0.02 ± 0.01
	βNF	0.33 ± 0.07[a,c]	0.48 ± 0.09	0.15 ± 0.04[f]	0.34 ± 0.07[g]	0.03 ± 0.02
B6.A (Ahr^{b-1}/Nat^{a})	Oil	0.11 ± 0.03	0.64 ± 0.10[d,e]	0.22 ± 0.09	0.11 ± 0.06[h]	0.03 ± 0.01
	βNF	0.21 ± 0.02	0.09 ± 0.01[e]	0.30 ± 0.06[f]	0.60 ± 0.06[g,h]	0.01 ± 0.001
B6.D (Ahr^{d}/Nat^{r})	Oil	0.14 ± 0.05	0.89 ± 0.02[b,d]	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01
	βNF	0.14 ± 0.01[c]	0.91 ± 0.01	0.03 ± 0.01	0.02 ± 0.002	0.03 ± 0.02
B6.A.D (Ahr^{d}/Nat^{a})	Oil	0.16 ± 0.05	0.87 ± 0.05	0.05 ± 0.03	0.02 ± 0.01	0.05 ± 0.03
	βNF	0.17 ± 0.04	0.85 ± 0.03	0.07 ± 0.02	0.02 ± 0.01	0.06 ± 0.02

[a] $p = 0.01$.
[b] $p = 0.0074$.
[c] $p = 0.0052$.
[d] $p = 0.012$.
[e] $p < 0.0001$.
[f] $p = 0.033$.
[g] $p < 0.0001$.
[h] $p = 0.0064$.

TABLE 6

ANOVAs of lung data, all eight groups considered together, and for groups differing at the *Ahr* locus considered separately

Parameter	Total adducts	Proportion of individual adducts			
		Adduct 1	Adduct 2	Adduct 3	Adduct 4
Among all groups	0.051	<0.0001	0.0030	<0.0001	NS
Ahr genotype	NS	<0.0001	<0.0001	<0.0001	0.032
Nat	NS	0.019	0.031	NS	NS
Ahr × *Nat*	0.10	NS	NS	NS	NS
βNF	0.033	0.0007	NS	0.0030	NS
Ahr × βNF	0.043	0.0008	NS	0.0019	NS
Nat × βNF	NS	0.021	NS	0.035	NS
Ahr × *Nat* × βNF	NS	0.063	NS	0.046	NS
Coefficient of variance	51%	19%	74%	72%	89%
Among Ahr^{b-1} groups	0.033	0.0019	NS	0.012	NS
Nat	NS	0.051	0.080	NS	NS
βNF	0.012	0.0013	NS	0.0052	NS
Nat × βNF	NS	0.035	NS	0.050	0.093
Coefficient of variance	47%	37%	62%	55%	93%
Among Ahr^{d} groups	NS	NS	NS	NS	NS
Nat	NS	NS	NS	NS	NS
βNF	NS	NS	NS	NS	NS
Nat × βNF	NS	NS	NS	NS	NS
Coefficient of variance	56%	8%	72%	60%	82%

did rapid acetylators. This difference may be ascribed to the polymorphism in *Nat2* because *Nat1* and *Nat3* are not polymorphic (13)[1]. These results, combined with the protective effect of βNF pretreatment, indicate that the level of metabolizing enzymes for IQ in liver correlates positively with protection. Although both rate of enzymatic activation to the DNA-damaging species and rate of detoxification increase, the latter seems to predominate in determination of the final toxic outcome.

Adduct levels were low in lung, which is consistent with a much lower ability of mouse and rat lung microsomes than of liver microsomes to catalyze activation of IQ to a mutagen (36). Lung lacks CYP1a-2; a role of CYP2A3 was postulated (36). One might expect that βNF pretreatment would reduce total IQ/DNA adducts in lung as a result of increased detoxification of IQ in liver, as was seen for the kidneys and colons of these mice (19) as well as in liver itself (see above), and of induction in lung of cyp1a-1, which may carry out detoxifying ring hydroxylations (see above). However, βNF had no such effect. In fact, it caused a significant doubling in total lung DNA adducts in *Ahr*-responsive mice. This effect is consistent with the finding that lung microsomes from βNF-pretreated animals showed an approximate doubling in ability to activate IQ to a mutagen (36).

In contrast to the absence of an effect of the *Ahr* and *Nat* genotypes *per se* on total adducts in lung and the relatively modest effect of βNF pretreatment, the profile of lung DNA adducts was markedly affected by both *Ahr* genotype and βNF treatment. The spectrum of changes was completely different from that seen in liver. Interpretation of this remarkable finding is hampered by the fact that of the several IQ/DNA adducts detected in this study, only adduct 1 has been identified. It has been suggested that others are reflective of incomplete digestion (37), but, if so, the occurrence of

[1] L. Rogers, unpublished observations.

TABLE 7

Summary of major findings

Includes kidney, bladder, and colon data from Ref. 19. Only changes occurring at the $p < 0.01$ level or better are noted.

Organ	Range[b]	Total adducts: Correlation[a] *Ahr*	Total adducts: Correlation[a] *Nat*	Total adducts: Correlation[a] βNF	Total adducts: Interaction	Adduct proportions
Liver	0.3–3.7	↑ Ahr^{b-1}	↑ Nat^{a}	↓ Ahr^{b-1}	*Ahr* × βNF	Adduct 1 ↑ in Ahr^{b-1}
Kidney	0.9–1.8			↓ Ahr^{b-1}		Trace amount of adduct 3
						Adduct 1 ↓, adduct 4/adduct 5 ↑ in Ahr^{d}/Nat^{a} (βNF)
Colon	0.4–1.1		↑ Ahr^{d}/Nat^{r}	↓ Ahr^{b-1}	*Ahr* × *Nat*	Adduct 1 ↑ in Ahr^{d}
Bladder	0.04–0.06			↑ Ahr^{b-1}/Nat^{r}	βNF × *Nat*	Adduct 2 ↑ in Ahr^{b-1} (βNF); Nat^{a} (βNF)
Lung	0.11–0.33			↑ Ahr^{b-1}		Adduct 2, adduct 3 ↑, adduct 1 ↓ in Ahr^{b-1}; adduct 3 ↑, adduct 1 ↓, βNF, in Ahr^{b-1}/Nat^{a}

[a] Correlations across all groups as a function of these phenotypes.
[b] Total adduct RAL × 10^{7} in oil-treated animals.

this artifact is strongly, specifically, and reproducibly dependent on the genotype, the tissue, and the treatment of the mice.

The major findings from our analyses of the adducts in the five organs of these mice, given in this and a preceding report (19), are summarized in Table 7. The relative levels of adducts were similar to those reported by Zu and Schut (38), with liver > colon ≫ lung, and by Hall *et al.* (39), with liver > kidney > colon ≫ lung. The complexity of the effects of the two phenotypes in combination with βNF pretreatment is apparent. The simplest model for the toxicodynamics of IQ activation is that the first step, *N*-hydroxylation, takes place in liver, the only organ expressing significant levels of cyp1a-2, and the resultant proximate carcinogen *N*–OH–IQ is transported by blood or bile to downstream targets, where activation is completed by NAT. NAT is present in all tissues studied here, with the polymorphic mouse NAT highest (as measured by activity toward *p*-aminobenzoic acid and 2-aminofluorene) in liver and colon, and lower by a factor of 2 in lung, kidney, and bladder (40). Induction of cyp1a-2 in livers of *Ahr*-responsive mice would increase the rate of primary activation of IQ but also the rate of detoxification through this pathway. In addition, detoxifying ring hydroxylation due to induced cyp1a-1 would increase after βNF treatment.

The collected data do not integrate neatly into this model. An apparent general finding was that for organs with relatively high basal levels of adducts (e.g., liver, kidney, and colon), βNF pretreatment reduced adducts in responsive animals, whereas for bladder and lung, with low basal adducts, βNF increased these levels but only in *Ahr*-responsive mice. Otherwise, each organ presented its own particular set of prominent correlations. The discovery that rodent lung microsomes have a βNF-inducible capacity to activate IQ to a mutagen, inhibitable by an antibody to CYP450 2A (36), together with our finding of increased IQ/DNA adducts in lung after βNF pretreatment of *Ahr*-responsive mice, raises the question of whether activation in target organs by CYP450s other than 1A2 plays a more important role than events in liver (e.g., formation of the proximate carcinogen and/or detoxification).

Overall, our data show that *Ahr* and *Nat* phenotypes and exposure to an Ah receptor agonist can significantly alter the level of IQ/DNA adducts in all exposed organs, in an organ-specific way, with puzzling quantitative and qualitative contributions of the Ah receptor that are not yet understood. It seems likely that simplistic paradigms of IQ metabolism versus risk may fall short because of the complexities of organismal and local tissue metabolism and other important but mysterious factors. Although DNA adducts do not equate to cancer risk and congenic mice may not adequately model humans, these congenic mice may continue to be useful in unraveling this complexity to enhance our understanding of risk factors and to aid in the development of prevention strategies.

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