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Expression and regulation of cytochrome *P*-450I genes (*CYP*1A1 and *CYP*1A2) [1] in the rat alimentary tract

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Cytochrome *P*-450 (P450) enzymes in the mucosa of the alimentary tract may be involved in the activation and/or inactivation of ingested xenobiotics and procarcinogens. Since the multiple P450 enzymes have overlapping substrate specificities and, in some cases, similar antigenic determinants, definitive identification of P450 genes that are expressed in various tissues requires molecular analysis. In this study, a sensitive and specific polymerase chain reaction assay along with hybridization analysis was used to examine the expression of the *CYP*1A gene family in the rat alimentary tract. *CYP*1A1 mRNA was expressed throughout the alimentary tract in untreated rats, as determined by the polymerase chain reaction. However, Northern blot analysis detected CYP1A1 mRNA and enzymatic activity only in small intestine and liver, with greater amounts in intestine. After treatment with β-naphthoflavone, *CYP*1A1 mRNA and enzymatic activity was markedly induced in each alimentary tissue including esophagus, fore-stomach, glandular stomach, small intestine and colon. A single dose of inducer resulted in a rapid rise in *CYP*1A1 mRNA which was shown by nuclear run-on assays to be primarily due to an increase in transcription of the *CYP*1A1 gene. *CYP*1A2 mRNA was detected in significant amounts only in glandular stomach following induction although the polymerase chain reaction assay identified low levels of *CYP*1A2 mRNA in several other tissues. The definitive identification of the *CYP*1A genes that are expressed in alimentary tissue will allow the design of experiments to investigate the importance of these enzymes in the metabolism of carcinogens, and ultimately carcinogenesis, in the gastrointestinal tract. In addition, these data suggest the the aromatic hydrocarbon receptor, which mediates transcriptional induction of multiple genes by xenobiotics, is expressed throught the alimentary tract.

Introduction

The mucosal surfaces of the alimentary tract are continuously exposed to ingested xenobiotics (non-nutritive organic chemicals) including natural compounds synthesized by plants, industrial wastes, pharmaceutical agents and food additives. The metabolic fate of xenobiotics in the alimentary tract, many of which are proven or putative mutagens and carcinogens [2,3], may be one determinant of tissue susceptibility for the development of intestinal cancer. Colon cancer is a common cause of mortality in the United States whereas cancer of the small intestine is rare [4]. Animal studies have substantiated the fundamental difference between the susceptiblity of these two tissues to carcinogenesis [5]. The risk of colon cancer may depend on dietary constituents which may contain either procarcinogens or compounds that modulate the response to carcinogens [6,7].

The majority of carcinogens require activation by cellular enzyme systems before they manifest mutagenic properties [8,9]. Although the metabolic pathways for procarcinogens are complex, enzymes that are members of the cytochrome *P*-450 superfamily [1] are often crucial in either activation or inactivation [8,9]. The CYP1A subfamily [1] plays an integral role in metabolism of two important classes of environmental carcinogens, polycyclic aromatic hydrocarbons [10] and

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Abbreviations: AHH, aromatic hydrocarbon hydroxylase; AMV, avian myoblastosis virus; BNF, β-naphthoflavone; P450, cytochrome *P*-450; DTT, dithiothreitol; ERDE, ethoxyresofurin deethylase; 3-MC, 3 methylcholanthrene; PAH, polycyclic aromatic hydrocarbons; PCR, polymerase chain reaction.

[1] Cytochrome *P*-450 genes in this report are named according to the recent recommendations for the standardization of P450 gene nomenclature [1]. Italicized *CYP* for cytochrome *P*-450 refers to the P450 gene or cDNA and non-italicized CYP refers to the P450 mRNA or protein. *CYP*1A1 and *CYP*1A2 in rat were previously referred to as P450IA1 or P450c and P450IA2 or P450d, respectively.

aryl amines [11]. PAHs are commonly present in the environment as a result of industrial combustion processes [12] and in tobacco products [13]. Several potent carcinogenic aryl amines result from the pyrolysis of amino acids in cooked meats which have been implicated as mutagens [14,15] and can cause colon cancer in rats [16].

The *CYP*1A subfamily of genes appears to contain two members in mouse, rat, rabbit and man, *CYP*1A1 and *CYP*1A2 [1]. Expression of cDNAs in mammalian cells have determined that *CYP*1A1 is primarily responsible for AHH activity and *CYP*1A2 is responsible for activation of aryl amines [17–19]. Environmental and genetic factors alter the expression of these enzymes. Exposure to plant-derived chemicals and other xenobiotics causes an increase in these enzymes in liver and, for *CYP*1A1, in extrahepatic tissues [20,21]. It was recently reported that a human polymorphism of the *CYP*1A1 gene was associated with the development of lung cancers [22]. These findings suggest that genetic factors may be important in the expression of this gene in humans and that *CYP*1A1 may be involved in carcinogenesis in humans.

AHH activity has been identified in the small intestine of rat [23,24], rabbit [25] mouse [26] and man [27], and in the colon of rat [28]. However, few studies have definitively identified the specific P450 protein responsible for the enzymatic activity in the alimentary tract. The importance of this was highlighted by studies that suggested enzymatic activity of rat *CYP*1A enzymes may vary from one tissue to another [23]. Goldstein and Linko [29] identified *CYP*1A1 protein in rat small intestine and Vang et al. [30] were able to induce *CYP*1A1 mRNA in rat colon by treatment with indole-3-carbinol and β-naphthoflavone. However, no systematic study of the expression of *CYP*1A genes has been undertaken in the rat alimentary tract and no information is available on mechanisms of induction.

The goals of this study were to definitively identify *CYP*1A genes expressed in the alimentary tract of rat, to determine if xenobiotics induce these genes, and to investigate the mechanisms of induction. Several chemical inducers of *CYP*1A genes were used in these studies including, β-naphthoflavone a non-carcinogenic inducer which is related to dietary flavones, 3-methylcholanthrene a carcinogenic hydrocarbon and prototypical inducer of *CYP*1A genes, and isosafrole a non-carcinogenic dietary compound that also induces *CYP*1A genes. Each of these classes of compounds are encountered by humans in the environment albeit at lower levels than used for the purposes of this study. Other chemicals were used to assess for the specificity of the responses including phenobarbital and dexamethasone, known inducers of other *CYP* genes. *CYP*1A mRNA was evaluated by a highly sensitive and specific polymerase chain reaction (PCR) assay and by hybridization analysis using probes that discriminate between *CYP*1A1 and *CYP*1A2 mRNAs; induction of mRNA was correlated with enzymatic activity. The mechanism of induction of *CYP*1A gene expression was evaluated by using nuclear run-on assays.

Materials and Methods

Materials

Guanidinium isothiocyanate, cesium chloride, T4 polynucleotide kinase, Klenow enzyme and T4 ligase were obtained from Bethesda Research Laboratories (Gaithersburg, MD). [^{32}P]dCTP (> 3000 Ci/mmol), [^{32}P]ATP (> 5000 Ci/mmol), [^{35}S]dATP (1000 Ci/mmol), Hybond-N nylon membranes an were obtained from Amersham (Arlington Heights, IL). Oligo(dT) cellulose was obtained from Pharamcia. AMV reverse transcriptase, all restriction enzymes, oligo(dT_{15}) and dideoxynucleotides were obtained from Boehringer-Mannheim (Germany). Taq polymerase was purchased from Perkin Elmer-Cetus (Norwalk, CT). Oligonucleotides were synthesized by the University of Michigan Gastrointestinal Peptide Hormone Center's oligonucleotide synthesis facility. NuSieve, SeaKem and SeaPlaque agaroses were obtained from FMC Biochemical (Rockland, ME). *Escherichia coli* RNA was purchased from Calbiochem. All other chemicals were obtained from Sigma (St. Louis, MO).

Animals

Male F344 rats weighing 200–250 g were housed in wire-floored cages and had free access to standard laboratory rat chow and water. The procedures used in these experiments were approved by the Animal Use Committee of the University of Michigan. Animals were treated with one of the following regimens: phenobarbital (80 mg/kg) administered daily for 3 days by intraperitoneal injection; BNF (80 mg/kg) administered daily for 3 days by intraperitoneal injection; 3-MC (20 mg/kg) as a single intraperitoneal dose; dexamethasone (20 mg/kg) as a single intraperitoneal dose; a combination of phenobarbital and dexamethasone; isosafrole (80 mg/kg). Control animals (or 'untreated' rats) were injected with corn oil alone since this was used as a vehicle for administration of each of the inducers except for phenobarbital which was dissolved in 0.15 M NaCl.

RNA extraction and Northern blot analysis

Livers and the entire gastrointestinal tracts were removed from rats that had been anesthetized by diethyl ether inhalation. A portion of each liver was immediately frozen in liquid nitrogen and the gastrointestinal tissue was thoroughly washed with ice-cold 0.15 M NaCl. Esophageal mucosa was obtained by eversion and stripping of the mucosa from the muscu-

lar layer; mucosa from the forestomach, glandular stomach, small intestine and colon was obtained by scraping using a glass slide. RNA was extracted as described by Chomczynski and Sacchi [31]. Polyadenylated RNA was isolated from aliquots of total RNA using using a microbatch method as previously described [32]. Samples of either total or polyadenylated RNA were size separated by electrophoresis in 2.2 M formaldehyde agarose gels. The RNA was transferred to Hybond-N (Amersham) nylon membranes by an electroblotting method and crosslinked to the membranes using ultraviolet radiation. The membranes were prehybridized, hybridized and washed as previously described [33].

DNA probes

A *CYP*1A1 cDNA probe, p210, and a *CYP*1A2 cDNA which were cloned from rat liver were gifts of Dr. J. Fagan (Fairfield, IA [34]). A full-length P450 reductase cDNA, pSP65-OR, that was cloned from rat liver was a gift of Dr. C. Kasper (Madison, WI [35]). Control cDNAs used in nuclear run-on experiments included a human β-actin cDNA, pHFBA-1 and an 18S ribosomal RNA cDNA have been used previously in our laboratory [32]. The cDNAs that were used in Northern blots were isolated from their plasmid vectors and radiolabeled with [α-^{32}P]dCTP using a random-primed oligonucleotide labeling method as previously described [33,36]. Oligonucleotides were 5'-labeled with [^{32}P]ATP using T_4 polynucleotide kinase as previously described [32,36]. Unincorporated nucleotides were removed from the labeled DNA using Sephadex G-50 chromatography (Nick columns, Pharmacia, Uppsala, Sweden).

The oligonucleotides used in this study were designed using the rat cDNA sequences as published by Kawajiri et al. [37] for *CYP*1A2 and by Sogawa et al. [38] for *CYP*1A1.

P450IA2:
5' PCR primer: [5'-GGTGGTCTCATCCCTCAGGA-3']
3' PCR primer: [5'-TTTATGAAGATGCAGCACTC-3']
Nested probe: [5'-GGATGTGTATCGGTAGAT-3']
P450IA1:
5' PCR primer: [5'-GTAACCAACCCTAGGATACA-3']
3' PCR primer: [5'-TTCACAAAGACACAGTGTCC-3']
Nested probe: [5'-GGATGAATGTCGTGGAAGGT-3']

Enzymatic amplification and identification of mRNA

Complementary DNA was synthesized from total RNA using oligo(dT_{15}) (1 μg) as a primer and AMV reverse transcriptase (13 U/reaction) in a total reaction volume of 50 μl containing 50 mM Tris-HCl (pH 8.3), 50 mM KCl, 8 mM $MgCl_2$, 10 mM dithiothreitol, and 0.5 mM each of dATP, dCTP, dGTP and dTT. The reaction mixture, without the reverse transcriptase, was incubated at 41°C for 15 min; the reverse transcriptase was then added and the reaction allowed to proceed for 1 h at 41°C. Segments of the *CYP*1A cDNAs were enzymatically amplified using oligonucleotide primers and PCR. 20 μl of the cDNA synthesis reaction mixture was amplified using 1 μM of each oligonucleotide primer (20mers) and 2.5 U of Taq polymerase in a total reaction volume of 100 μl containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 2 mM dithiothreitol, 0.01% gelatin and 200 μM each of dATP, dCTP, dGTP and dTTP. The reaction mixture was overlaid with 100 μl of mineral oil and subjected to 30 cycles of amplification. The samples were cycled as previously described [32] using 55°C as the annealing temperature.

10 μl of the PCR mixture was separated in 3% NuSieve and 1% SeaKem agarose gels containing ethidium bromide (0.5 μ/ml) using a Tris/borate/EDTA buffer. After photography, the DNA was electrophoretically transferred to Hybond N nylon membranes and the DNA was bound to the nylon using ultraviolet radiation crosslinking. The membranes were prehybridized and hybridized with a 5'-labeled oligonucleotide probe ('nested probe') and washed as previously described [32]. The temperature of hybridization was performed at the hypothetical temperature of dissociation of DNA:DNA hybrids (approx. 55°C) for the oligonucleotide to maximize the specificity of hybridization.

Nuclear run-on assay

Nuclear run-on assays were performed as previously described [32]. Mucosal scrapings were washed twice in ice-cold 0.15 M NaCl, 5 mM DTT and resuspended in nuclei lysis buffer (NLB: 10 mM Tris-HCl (pH 7.4), 10 mM NaCl, 5 mM DTT). The suspension was homogenized in a Dounce homogenizer using 10 strokes of the loose pestle, then 10 strokes of the tight pestle and centrifuged at $800 \times g$ and 4°C for 10 min. The resulting pellet was resuspended in sucrose buffer (2.4 M sucrose, 1 mM $CaCl_2$, 5 mM DTT) by vortexing and centrifuged at $50000 \times g$ at 4°C for 1 h. The pellet containing the purified nuclei was resuspended in nuclei storage buffer (NSB: 40% glycerol, 50 mM Tris-HCl (pH 8.3), 0.1 mM EDTA and 5 mM $MgCl_2$), an aliquot counted in a hemacytometer and frozen at −80°C.

Transcription reactions were initiated by adding 60 μl of 5 × run-on buffer (25 mM Tris-HCl (pH 8.0), 12.5 mM $MgCl_2$, 750 mM KCl and 1.25 mM each of ATP, GTP and CTP) and 30 μl of [^{32}P]UTP (> 3000 Ci/mmol, 10 μCi/μl) to 210 μl of NSB containing approx. $5 \cdot 10^7$ nuclei. Following incubation at 30°C for 30 min, the labeled RNA transcripts were isolated as described [32].

Plasmids containing the cDNAs of interest were denatured by heating in 0.2 M NaOH and applied to nitrocellulose using a Minifold II slot blot device (Schleicher and Schuell). The following cDNAs were

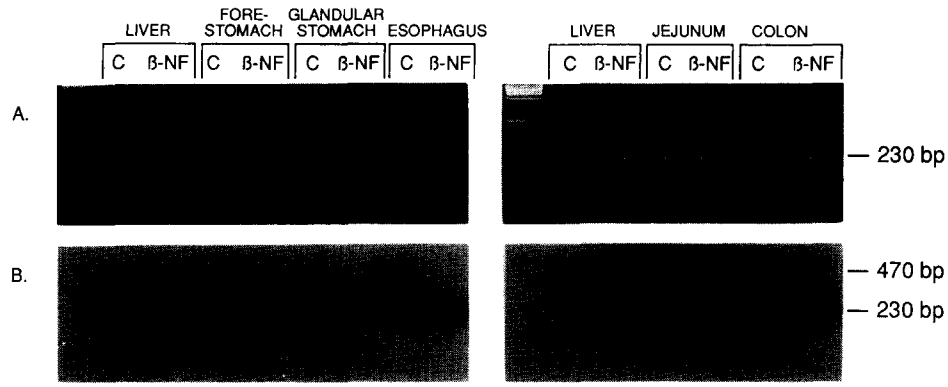

Fig. 1. PCR analysis for expression of CYP1A1 mRNA. RNA was extracted from liver and alimentary tract tissues from control rats and rats treated with BNF. Reverse transcription and PCR was performed as described in Materials and Methods and the products analyzed on a 3% NuSieve, 1% SeaKem agarose gel (A). The DNA was transfered to nylon membrane and hybridized with an end-labeled nested oligonucleotide which is complementary to *CYP*1A1 as described in methods (B). First lane on gel contains size markers. The expected size band for CYP1A1 mRNA is 230 base pairs. The 470 base pair band represents amplification of a small amount of contaminating genomic DNA which includes and intron.

used: pSBF1 (previously cloned in our laboratory [32]), p210 (*CYP*1A1 cDNA probe), pSP450OR (P450 reductase cDNA), pHFBA-1 (human β-actin cDNA), prA (human 18S ribosomal RNA cDNA) and the bacterial vector pGEM which served as a negative control for non-specific binding. The nitrocellulose containing the bound cDNAs was prehybridized at 60°C for 8 h in a solution containing 10 mM Tes (pH 7.4), 0.2% SDS, 10 mM EDTA, 0.3 M NaCl, 1 × Denhardt's solution and 5 μg/ml *E. coli* RNA. This solution was replaced with 2 ml of the same solution containing $1 \cdot 10^7$ cpm/ml of labeled RNA transcripts and hybridization continued for 36 h (cpms were equalized for each sample of nuclei). The membranes were washed in three changes of 0.1 × SSC and 0.1% SDS at the hybridization temperature. Following autoradiography, the blots were quantitated using a Betascope 603 Blot Analyzer. Since there was variation in the number of nuclei used in the reaction, the results of the transcription reaction were normalized for hybridization to the ribosomal RNA cDNA; this correlated with the DNA concentration of the nuclear preparation.

Enzyme assays

The mucosal lining of each organ was scraped with a glass slide and microsomes were isolated as previously described [36]. Protein concentrations in the microsomes were measured by the method of Lowry [39]. The ability of microsomes to dealkylate ethoxyresorufin was used as an enzymatic assay for P450c (*CYP*1A1) and was performed as described by Buke et al. [40]. This assay has been shown to be specific for rat P450c although there is some minimal overlap with P450d (*CYP*1A2) [41]; there is no overlap with six other forms of P450 [41].

Results

Identification of CYP1A mRNA by PCR

The first goal of this study was to definitively identify the *CYP*1A mRNAs that are constitutively ex-

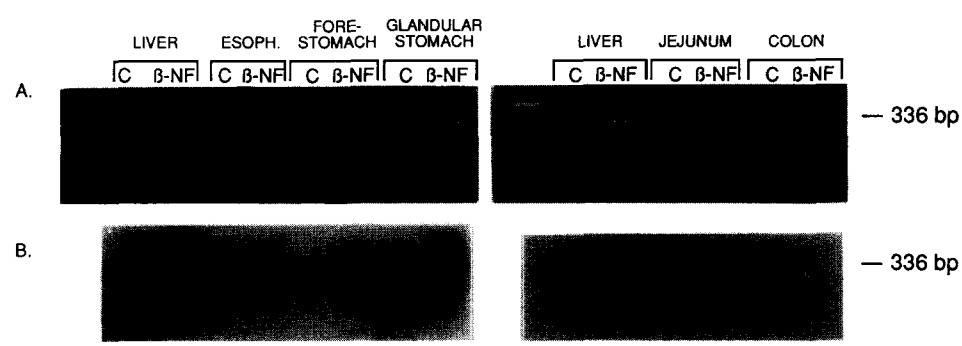

Fig. 2. PCR analysis for expression of CYP1A2 mRNA. RNA was extracted from liver and alimentary tract tissues from control rats and rats treated with BNF. Reverse transcription and PCR was performed as described in Materials and Methods and the products analyzed on a 3% NuSieve, 1% SeaKem agarose gel (A). The DNA was transfered to nylon membrane and hybridized with an end-labeled nested oligonucleotide which is complementary to CYP1A2 as described in methods (B). First lane on gel contains size markers. The expected size band for CYP1A1 mRNA is 336 base pairs. The smaller band noted on the Southern blot represents amplification of single stranded cDNA as previously determined [32].

pressed and inducible in tissues of the rat alimentary tract. Rats were treated with intraperitoneal injections of either corn oil alone or with 80 mg/kg of BNF for 3 consecutive days. RNA was extracted from mucosa of esophagus, fore-stomach, glandular stomach, jejunum and colon as well as from liver. Oligo(dT)-primed cDNA was synthesized from total RNA and used to amplify *CYP*1A1 or *CYP*1A2 sequences. The segment amplified spanned an intron to avoid false positive results that would occur by amplification of genomic DNA which might contaminate the RNA samples.

When RNA from control and treated tissues was assayed, a DNA fragment of the expected size for CYP1A1 was amplified (Fig. 1). An oligonucleotide complementary to the sequence of CYP1A1 mRNA between the two PCR primers ('nested oligonucleotide') hyhridized to the amplified DNA fragment; this analysis verifies that the correct sequence was amplified and, additionally, increases the sensitivity for detection of amplified product. In a number of samples a 470 bp fragment was identified which corresponds to the expected size of amplified genomic DNA. When the CYP1A2-specific primers were used, a fragment of the expected size (336 bp) was found in the glandular stomach and small intestine of animals treated with BNF (Fig. 2). Small amounts of CYP1A2 mRNA were detected in control and treated esophagus and fore-stomach and in control glandular stomach and jejunum; the colon contained no CYP1A2 mRNA. The second band detected on the Southern blot represents amplification of single-stranded DNA as a result of asymmetric amounts of primers as we and others have previously demonstrated [32]; this band does not represent amplification of CYP1A1 as it did not hybridized to the CYP1A1 probe (data not shown) and is of greater size than expected.

Quantitative analysis of CYP1A mRNA

Northern blot analysis was used to quantitate relative levels of CYP1A1 and CYP1A2 mRNAs. The cDNA probes used for this analysis have been shown to distinguish between the two mRNAs [34]. Rats were treated with three daily intraperitoneal injections of corn oil (control), BNF (80 mg/kg), phenobarbital (80 mg/kg), dexamethasone (300 mg/kg, single dose), or a combination of phenobarbital and dexamethasone. Phenobarbital and dexamethasone were included in this analysis to evaluate for specificity of the response of *CYP*1A1 genes. The CYP1A1 cDNA hybridized to a 2.8 kb band from RNA isolated from control jejunum and from BNF-treated mucosa from each of the tissues (Fig. 3). It should be noted that the intensity of the band in untreated jejunum was greater than that in liver RNA.

When the *CYP*1A2 cDNA was hybridized to a similar blot, only the liver RNA from a BNF treated animal had the expected 2.1 kb band (data not shown). In order to maximize the induction of and detection of CYP1A2 mRNA, rats were treated with intraperitoneal and intragastric doses of isosafrole (80 mg/kg), a potent inducer of *CYP*1A2 in rat liver [42]. This inducer and route of administration was chosen to maximize the possiblity of inducing *CYP*1A2. Northern blots of polyadenylated RNA isolated from these ani-

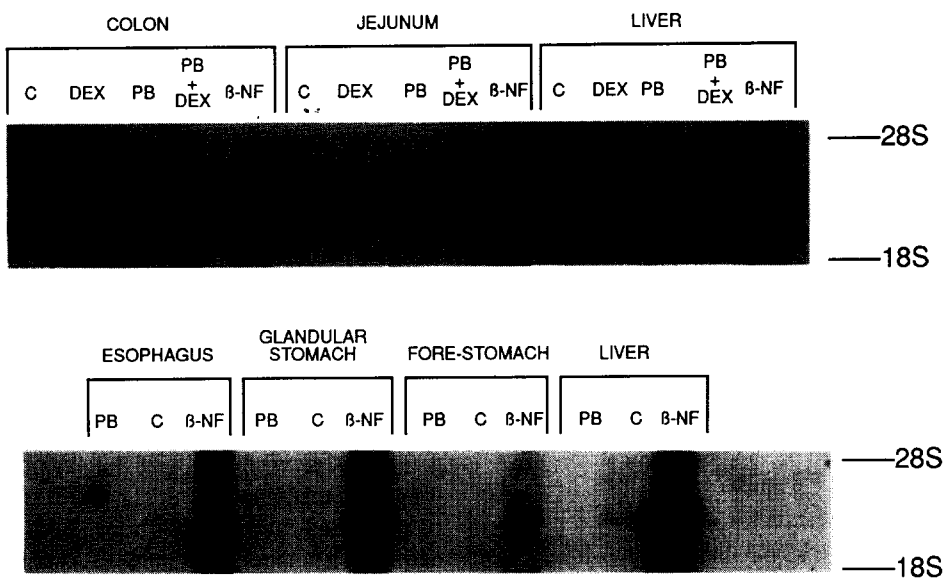

Fig. 3. Northern blot analysis for expression of CYP1A1 mRNA. RNA was extracted from liver and alimentary tract tissues from control rats (C) and rats treated with BNF, dexamethasone (DEX), phenobarbital (PB), or a combination of PB and DEX. 10 μg of RNA was separated in a 1% agarose, 2.2 M formaldehyde gel, transfered to nylon membrane and hybridized with ^{32}P-labeled p210 cDNA as described in Materials and Methods. The upper and lower panels represent different exposure times and, therefore, the intensity of bands are not comparable as indicated by the intensity of the bands in induced liver.

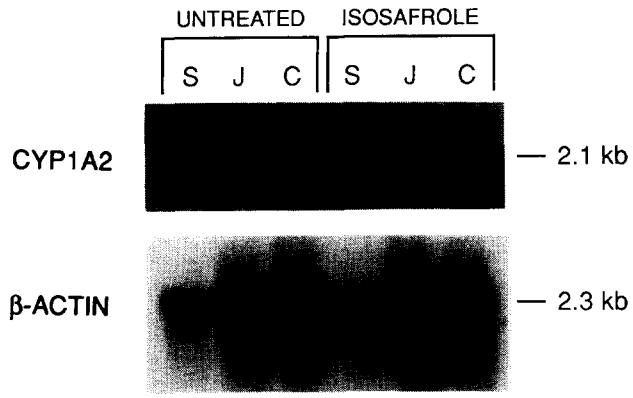

Fig. 4. Northern blot analysis for expression of CYP1A2 mRNA. Polyadenylated RNA was extracted from stomach (S), jejunum (J) and colon (C) from untreated rats and rats treated with isosafrole. 10 μg of polyadenylated RNA was separated in a 1% agarose, 2.2 M formaldehyde gel, transfered to nylon membrane and hybridized with ^{32}P-labeled p72 cDNA as described in Materials and Methods. After autoradiography, the blot was stripped of the probe and rehybridized with ^{32}P-labeled β-actin cDNA. Sizes of the bands were calculated from size markers run on the same gel.

mals demonstrated that CYP1A2 mRNA was induced in glandular somach, but not jejunum or colon (Fig. 4). Rehybridization of the same blot with β-actin cDNA showed that, although there were tissue-specific differences in the β-actin mRNA levels, similar amounts of RNA were present for each tissue (Fig. 4).

Induction of enzyme activity

Ethoxyresorufin deethylase (ERDE) activity has been shown to be relatively specific for CYP1A1 [40,41]. Therefore, ERDE activity was assayed in micosomes isolated from untreated and BNF-treated animals (Fig. 5). ERDE activity was greatest in jejunum of control rats with a low level in control liver and no detectable activity in the remainder of the alimentary tract. Following treatment with BNF, ERDE activity was increased in each alimentary tract tissue (Fig. 5). Therefore, the pattern of constitutive and induced enzymatic activity was similar to the pattern of CYP1A1 mRNA expression.

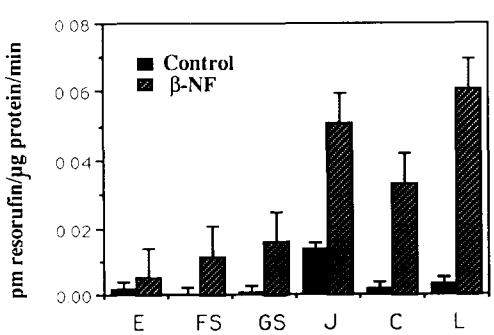

Fig. 5. Ethoxyresorufin deethylase activity in liver and alimentary tract microsomes. Microsomes were isolated from esophagus (E), fore-stomach (FS), glandular stomach (GS), jejunum (J), colon (C) and liver (L) from control and BNF-treated animals. ERDE activity was determined in duplicate as described in Materials and Methods. $n = 3$ separate animals.

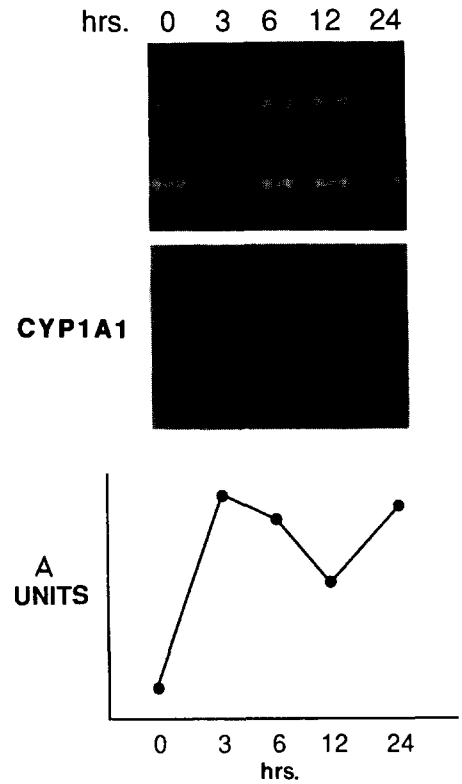

Fig. 6. Time course of induction of CYP1A1 mRNA in small intestinal mucosa. RNA was isolated from small intestinal mucosa of untreated rats and 3, 6, 12 and 24 h following a single intraperitoneal injection of 3-methylcholanthrene (20 mg/mg body weight). Northern blot analysis was performed as described in methods using the p210 cDNA as a probe. Top panel shows ethidium-bromide stained gel and lower panel shows audoradiograph after hybridization. Complete transfer of the RNA to the nylon membrane was confirmed by UV illumination of the membrane after transfer and restaining of the gel. The lower graph shows densitometric analysis of CYP1A1 mRNA normalized for the density of ribosomal RNA as determined from the ethidium bromide stained gel.

Route of administration of inducer

The aforementioned experiments were performed using intraperitoneal injections of the inducers since systemic adminstration was felt to be the most consistent method of administration. To determine if similar results were obtained by intragastric administration, rats were given 3-MC (20 mg/kg) by either intraperitoneal or intragastric injection and microsomes isolated from jejunal mucosa 16 h later. 3-MC was used in these experiments since this inducer yieled similar induction in the entire alimentary tract as did BNF. In control animals the level of resorufin deethylase activity was 0.109 ± 0.53 pmol resorufin produced/μg protein per min. This activity increased in animals treated with both intraperitoneal and intragastric injections of 3-MC

to 0.516 ± 0.179 and 0.814 ± 0.098 pmol resorufin produced/μg protein per min, respectively. The greater induction by the intragastric route may be due to a higher dose of inducer reaching the intestinal mucosa. This demonstrates that both routes of administration induce enzymatic activity; CYP1Al mRNA was similarly increased by both routes (data not shown).

Mechanism of induction of mRNA in small intestine

Northern blot analysis suggested that the major level of regulation for *CYP*1A1 induction was alteration in the steady-state level of CYP1A1 mRNA. Therefore, further experiments were performed to determine if induction of gene transcription was responsible for the observed increase in CYP1A1 mRNA. Following a single dose of 3-MC there was a prompt increase in CYP1A1 mRNA by 3 h and levels remained relatively stable for 24 h (Fig. 6); BNF gave a similar pattern of induction as did intragastric administration (data not shown). Therefore, transcriptional assays were performed 6 h following injection. Nuclear run-on assays were performed using nuclei isolated from jejunal mucosa from rats treated with corn oil (controls), 3-MC (20 mg/kg) and isosafrole (80 mg/kg). Fig. 7 demonstrates that there was approx. 6-fold induction of *CYP*1A1 gene transcription following 3-MC treatment which was roughly equivalent to the increase in CYP1A1 mRNA (Fig. 6). These data indicate that, at least in part, the induction of CYP1A1 mRNA is due to an increase in gene transcription.

Discussion

CYP1A1 mRNA and enzymatic activity were present in the small intestinal mucosa of untreated rats, at levels greater than those in liver. Expression of CYP1Al in untreated jejunal mucosa may represent constitutive expression or the inductive effect of low levels of chemical inducers in the rat chow. The PCR assay also indicated that CYP1A1 mRNA was constitutively expressed in the other gastrointestinal tissues studied. Because of the great sensitivity of PCR, constitutive levels of CYP1A1 mRNA detected by PCR in tissues other than liver and small bowel represent very low levels of expression. The lack of detectable mRNA on northern analysis or enzymatic activity in these tissues suggests that the low levels of mRNA may be of little biological significance; however, expression of CYP1A1 in a small subset of cells could potentially be of functional importance. After treatment with BNF, CYP1A1 mRNA and ERDE activity were markedly induced throughout the gastrointestinal tract and in liver. The

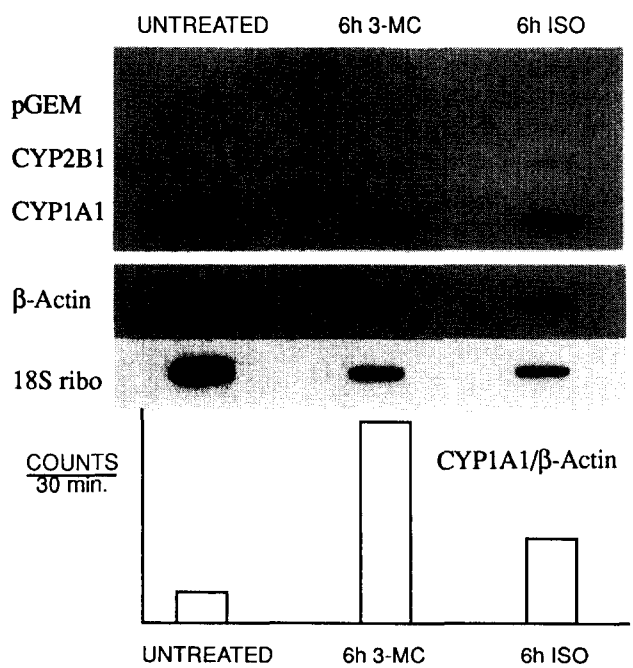

Fig. 7. Nuclear run-on assays on small intestinal nuclei. Nuclei were isolated from small intestinal mucosa of untreated rats and rats treated with 3-methylcholanthrene (3-MC) and isosafrole (ISO). Nuclear run on assays were performed as described in methods and transcripts hybridized to pGEM plasmid as a negative control, CYP2B1 cDNA, CYP1A1 cDNA-p210 and β-actin cDNA. Counts over 30 min were measured using a Betascope counter. In the graph, counts for CYP1A1 were normalized for counts for β-actin. The relative intensities of the actin and 18S ribosomal bands were comparable. Furthermore, as discussed in Materials and Methods, the intensity of the ribosomal and actin bands correlated with the amount of DNA (and hence nuclei) that were used in the individual reactions.

combined approach of PCR and Northern analysis provided complementary data. Northern analysis assessed the relative levels of mRNA and indicated the size of the mRNA. The PCR provided greater sensitivity and, by hybridization with internal oligonucleotides, confirmed the identitiy of the amplified mRNAs. This was useful since hybridization of the Northern blots with cDNAs could potentially detect other mRNAs with similar sequences.

The mechanism of induction of CYP1A1 mRNA was further investigated using nuclear run-on studies. This assay provides a relative measure of the number of inititated primary transcripts at the time of isolation of the nuclei. The increased labeling of transcripts after a single dose of 3-MC suggests that an increase in transcription of the gene is responsible for a major portion of the increased CYP1A1 mRNA levels. Therefore, it appears that the mechanism for induction of the *CYP*1A1 gene in intestine is similar to that in liver. In hepatocytes, inducing agents bind to a cytosolic receptor protein, the Ah receptor, which is translocated to the nucleus and binds to DNA elements in the 5′-flanking region of the *CYP*1A1 gene to enhance transcription [43,44]. Ah receptor-like binding activity has also been identified in small intestinal mucosa [45]. The presence of the Ah receptor in intestinal tissues may have implications beyond those of xenobiotic metabolism since it is responsible for induction of a battery of genes [46] and may play a role in the control of cellular growth and differentiation [47].

CYP1A2 mRNA was identified in several tissues of the gastrointestinal tract by PCR analysis. On Northern blots CYP1A2 mRNA was detectable only in the glandular stomach after induction with isosafrole, a potent inducer of *CYP*1A2 in liver. A sufficiently sensitive and specific enzymatic assay is not available for CYP1A2 to determine if this mRNA is translated into active enzyme. Although levels of CYP1A2 mRNA were low in stomach it is possible that this expression represents significant levels for metabolism in a select population of cells located within the gastric mucosa. If CYP1A2 is expressed only in a minority of these cells, localized levels of CYP1A2 might be high enough to activate or metabolize ingested carcinogens in those specific cells. This is a potentially important issue for gastric carcinogenesis since a number of ingested procarcinogens, notably aryl amines, require activation by CYP1A2 before they are mutagenic. It is of interest that recent data suggested that an increased risk of stomach cancer was associated with cigarette smoking [48] which can induce *CYP*1A2 in humans [21]. Previous studies in rat [29,49,50] or human [50] have not identified *CYP*1A2 in extrahepatic tissues; however, this study represents the first evaluation for expression in stomach. In contrast, P_3450 mRNA, the mouse *CYP*1A2 ortholog, was expressed and inducible in multiple mouse tissues including liver, spleen, lung, kidney, small intestine and colon [26]. Therefore, there are important species differences in the extrahepatic expression of the *CYP*1A2 gene.

The observed pattern of expression of CYP1A1 mRNA and enzymatic activity allows speculation as to the significance of these findings for the resistence of the small intestine to carcinogenesis. The fact that *CYP*1A1 is expressed constitutively only in the small intestine raises the question of whether this enzyme provides a protective mechanism for the mucosa. Pretreatment with inducers of CYP1A1 has been shown to provide a protective effect for subsequent administration of carcinogens in other tissues [51,52]. Although CYP1A1 itself may be responsible for this effect, enzymes that are co-regulated with CYP1A1 may be more important. CYP1A1 may metabolize procarcinogens to inactive compounds which are not mutagenic. This might occur with colonic carcinogens such as 2-amino-3,4-dimethylimidazo[4,5-f]quinoline which may be inactivated by CYP1A1 through ring hydroxylation as are other aromatic amines [8]. Although inactivation of procarcinogens is plausible, it does not explain how the small bowel protects itself from ingested compounds that are known to be activated by CYP1A1 such as polycyclic aromatic hydrocarbons. One possible explaination for this paradox might be the distribution of CYP1A1 in the small intestinal mucosa. The epithelial lining of the small intestine is continually renewed by division of a stem-cell population located in intestinal crypts, migration of daughter cells along the villus and, finally extrusion of senescent cells into the intestinal lumen. As the cells migrate from the crypt zone onto the villus, they cease cell to proliferate and acquire differentiated functions. We previously demonstrated that CYP2B1 mRNA was only expressed in the differentiated villus cells [36] and others have shown that CYP1A1 protein [53] or enzymatic activity [54] is expressed in the same cells. Additional data from our laboratory showed that CYP1A1 mRNA was expressed in villus cells constitutively, but was inducible in crypt cells after intraperitoneal injection of 3-MC [55]. It is conceivable that procarcinogens activated in the nonproliferating villus cells would bind to DNA in these cells and, without further division, mutations in DNA would not occur. Since the villus cells are destined for extrusion into the intestinal lumen, binding of mutagens to the DNA in villus cells can be viewed as a detoxification process. This possibility requires further investigation.

In conclusion, we have identified members of the CYP1A family that are expressed constitutively and are inducible along the entire length of the rat gastrointestinal tract. Further investigation of *CYP*1A1 expression in the gastrointestinal tract and *CYP*1A2 expression in stomach will be directed towards the role of these

enzymes in metabolism of ingested xenobiotics and carcinogens as well as the possible role of Ah receptor in cellular proliferation and differentiation.

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