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Expression and inducibility of CYP1A1, 1A2, 1B1 by β-naphthoflavone and CYP2B22, 3A22, 3A29, 3A46 by rifampicin in the respiratory and olfactory mucosa of pig

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ABSTRACT

The presence and inducibility of specific CYPs (1A1, 1A2, 1B1, 2B22, 3A22, 3A29 and 3A46) and the related transcriptional factors (AhR, CAR, PXR, and HNF4α) were investigated, at activity and/or transcriptional level, in liver, respiratory and olfactory mucosa of control and β-naphthoflavone (βNF)-treated pigs an agonist of AhR, or rifampicin (RIF), an agonist of PXR. Experiments with real-time PCR showed that CYP1A1 mRNA was enhanced by βNF, although at different extent, in liver, respiratory and olfactory tissues, whereas mRNAs of CYP1A2 and 1B1 were increased only in liver. Accordingly, in microsomes of both nasal tissues, the transcriptional activation of CYP1A1 was accompanied by an induction of ethoxyresorufin deethylase activity (a marker of this isoform) but not of methoxyresorufin demethylase activity (a marker of CYP1A2). The rifampicin treatment resulted in a transcriptional activation of CYP2B22 and CYP3As genes in liver but not in respiratory and olfactory mucosa. In parallel, the marker activity of CYP2B (ethoxy 4-(trifluoromethyl)coumarin deethylase) and CYP3As (6β-testosterone hydroxylase and benzyloxyquinoline debenzylase) were induced in liver microsomes but not in the nasal ones. Considering the transcriptional factors, the basal expression of AhR mRNA was found to be as high in liver as in both nasal tissues but not susceptible to induction by βNF. Also PXR mRNA was found, aside liver, well expressed in the nasal tissues, whereas CAR and HNF4α mRNAs were barely detected. In any case, these transcripts appeared to be enhanced by RIF treatment. Our results demonstrated that in the respiratory and olfactory mucosa of pig, although the presence of AhR, only CYP1A1, but not 1A2 and 1B1 resulted to be inducible by βNF. Similarly, it was observed that in these nasal tissues, although the presence of PXR, neither CYP2B22 nor any CYP3A resulted to be inducible by RIF. Thus, the regulation mechanism of CYP1A2, 1B1, 2B22, 3A22, 3A29, and 3A46, in the nasal mucosa involves tissue-enriched transcriptional factors others than AhR, CAR, PXR, and HNF4α, which are fundamental in liver.

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1. Introduction

The cytochrome P450 (CYP) system plays a crucial role in the tissue-selective metabolism and toxicity of a myriad of xenobiotics. Although the liver contains the highest CYP content, also the respiratory and, particularly, the olfactory mucosa of mammals have significant levels of CYP and drug metabolising activity (Thornton-Manning and Dahl, 1997; Ling et al., 2004). Many compounds are known to be potent inducers of individual CYP enzymes in liver and various extrahepatic organs, such as lung, kidney, intestine (Baron and Voigt, 1990; Park et al., 1995), but much less has been documented for the CYP inducibility in nasal tissues. However, this knowledge appears to be important, as inhaled or blood borne xenobiotics may be bioactivated *in situ* to toxicants and as nasal administration of drugs was considered recently to be an interesting route for brain (Minn et al., 2002). For various years, some reports have indicated that nasal CYP isoforms are relatively refractory to induction by classical CYP inducers such as phenobarbital and 3-methylcholanthrene (Thornton-Manning and Dahl, 1997; Ling et al., 2004). Only CYP2E1 and 2J have been demonstrated to be clearly inducible in the nasal tissues, mostly from rat, by various compounds including acetone, pyrazole (Longo and Ingelman-Sundberg, 1993; Xie et al., 2000) and physiopathologic conditions such as starvation (Longo et al.,

Abbreviations: CYP, cytochrome P450; βNF, beta-naphthoflavone; RIF, rifampicin; GAPDH, glyceraldehyde 6-phosphate dehydrogenase; EROD, ethoxyresorufin O-deethylase; MEROD, methoxyresorufin O-demethylase; EFCOD, 7-ethoxy 4-(trifluoromethyl)coumarin deethylase; BQD, benzyloxyquinoline debenzylase.

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Table 1
Primer pairs, annealing temperature and product size for RT-PCR and sequences analysis.

Gene	Forward (5′–3′)	Reverse (5′–3′)	Annealing temperature (°C)	Product size (bp)
CYP1A1	GCCCGACCTCTACAGCTT	TGGGATGGTGAAGGGGA	49	578
CYP1A2	GACATGACAAGCGCCCTATT	CTGGTAACTTCATTTGATGG	54	710
CYP1B1	CCTTCGCCTCYTTCCGTGT	GGATGGAGATGAAGAGAAA	53	1104
AhR	CATGCTTTGGTCTTTTCTGC	TTCCCTTTCTTTTTCTGTCC	60	1036

Table 2
Primer pairs and TaqMan probes for real-time PCR.

Gene	Forward (5′–3′)	Reverse (5′–3′)	Probe (5′-3′)	Product size (bp)
CYP1A1	GGATGGGCTGAGGACTATGTG	TCCTGGCGCCTGAATTTG	AAGAGGAGCAGAGAGCAGAGGGAGTGC	69
CYP1A2	CTGGAGCTCTTCCGACATACCT	GCGTCGTGTCCCTTGTTGT	CTTCGTTCCCTTCACCATCCCTCACA	69
CYP1B1	CCATGCGCTTCTCTAGCTTTG	AGCCTAAGACCGAGGCATTG	ACCCGTCACCATTCCCCATGCC	72
AhR	AGCTGCACTGGGCGTTAAA	GCCACTCGCTTCATCAATTCT	CCTTCACAGTGTCCAGACTCTGGAC	67
GAPDH	TTCCACCCACGGCAAGTT	GGCCTTTCCATTGATGACAAG	CACGGCACAGTCAAGGCGGAGA	69

2000), whereas contrasting results have been reported for CYP1A1, 1A2 and 2B (Gillner et al., 1987; Voigt et al., 1993; Wardlaw et al., 1998; Genter et al., 2002). Concerning the inducibility of nasal CYP3As by classical agonists (i.e. dexamethasone, rifampicin) of PXR and CAR, to our knowledge, no reports have been published.

In the present study, we examined the constitutive presence and inducibility of various CYP and receptor genes in the respiratory and olfactory mucosa of control and treated pigs, a species recently proposed as an useful model for human (Swindle and Smith, 1998; Skaanild, 2006). In particular, it was examined at transcriptional and activity levels: (a) CYP1A1, 1A2, 1B1 and AHR in the control pigs and pigs treated with β-naphthoflavone (βNF), a strong AHR ligand, (b) CYP3A22, 3A29, 3A46 and CYP2B22 along with the PXR, CAR and HNF4α in the control pigs and pigs treated with rifampicin (RIF), a potent ligand of porcine PXR (Moore et al., 2002).

2. Materials and methods

2.1. Chemicals

Ethoxyresorufin, methoxyresorufin, 7-ethoxy 4-(trifluoromethyl)coumarin, benzyloxyquinoline, rifampicin were all supplied by Sigma (St. Louis, USA). Testosterone and its metabolites were obtained as previously reported (Longo et al., 1991). Rabbit polyclonal antibodies raised against rat CYP1A1, rat CYP2B1 and rat CYP3A2 were purchased from Gentest (Woburn, USA). All chemicals and solvents were of analytical grade and were obtained from common commercial sources.

2.2. Animal treatment and preparation of microsomes

To perform this study we used nine male castrated Large white × Landrace hybrid pigs (25–30 kg body weight, aged about 2 months), supplied by a commercial farm. The animals were acclimated for 10 days in floored indoor pens, with free access to drinking water and food and on a 12 h light/dark cycle. After the adaptation period, three pigs were treated i.p. with β-naphthoflavone (30 mg/kg b. wt./day in corn oil) for 4 days; other three pigs were treated i.p. with 40 mg/kg RIF in 40 mM NaOH solution for 4 days, and the other three animals were used as controls. Pigs were sacrificed by bleeding 24 h after the administration of the last dose of treatment. Throughout the study all the animals were under clinical observation. Surgical procedures and experimental protocols were approved by the Animal Care Committee of Bologna University. Liver, respiratory and olfactory mucosa were excised, frozen in liquid nitrogen and stored at −80 °C. Microsomes from hepatic and nasal tissues were prepared according to Longo et al. (1991). Protein content was determined by the method of Lowry et al. (1951), using bovine serum albumin as standard.

2.3. Enzyme assay

Ethoxyresorufin O-deethylase and methoxyresorufin O-demethylase activities were determined by measuring the production of resorufin, as previously reported (Lubet et al., 1985; Tsyrlov et al., 1993), using a model LS 45 PerkinElmer spectrofluorimeter. The dealkylation activities of benzyloxyquinoline and 7-ethoxy 4-(trifluoromethyl)coumarin were measured by monitoring fluorimetrically the formation of the corresponding hydroxyl products as previously reported (Stresser et al., 2000; Ekins et al., 1997). Testosterone hydroxylase activity was determined by using a Waters 1525 HPLC apparatus equipped with a Supelco LC-18 column (250 mm × 4.6 mm). The testosterone metabolites were resolved using an isocratic elution with methanol + tetrahydrofuran (THF) 7.5% and H_2O + THF 7.5% (27:73, v/v) for 8 min following by a linear gradient reaching a 42:58 (v/v) for 12 min and a final period of isocratic elution for a further 10 min, substantially in agreement with Amato et al. (1996).

2.4. Immunoblot analysis

Microsomal proteins from liver (5 μg), respiratory and olfactory tissues (40 μg) were separated according to Laemmli (1970) on SDS-7.5% (w/v) polyacrylamide gel and then transferred electrophoretically onto nitrocellulose membranes following the method of Towbin et al. (1979). Immunodetection was performed with polyclonal antibodies anti-rat CYP1A1 (dilution 1:4000), anti-rat CYP2B1 (dilution 1:1000) and anti-rat CYP3A2 (dilution 1:1500). Immunoreactive proteins were visualized with a chemiluminescence reaction kit (Amersham) and bands were electronically scanned and quantified by a Scion Image program.

2.5. Isolation of total RNA and cDNA synthesis

Total RNA was isolated from frozen swine liver, nasal respiratory and olfactory mucosa (about 100 mg of tissue) by RNeasy Midi Kit (Qiagen), following the supplied protocol. Genomic DNA elimination and reverse transcription of total RNA were performed using QuantiTech Reverse Transcription Kit (Qiagen). RNA was quantified using NanoDrop (Celbio); its purity and integrity were evaluated by checking ratio of the absorbance at 260–280 nm and assessing the sharpness of 18S and 28S ribosomal RNA bands on ethidium bromide gel.

2.6. RT-PCR and sequence analysis

As the swine cDNA sequences for CYP1B1 and AhR were not available in GenBank, we performed RT-PCR experiments to obtain their partial coding sequences, using primer pairs (Table 1) designed by OLIGO 4.0 program on the basis of multialignment of ortholog sequences known in other species. Two μl of cDNA was added to a PCR Master Mix (GoTaq Green Master Mix, Promega) for the amplification reaction (35 cycles) performed using for each transcript 400 nM of forward–reverse primers and the annealing temperature indicated in Table 1. Primer pairs specific for the CYP2B22, 3A22, 3A29, 3A46, CAR, PXR and HNR-4α and PCR conditions have been previously described (Nannelli et al., 2008). The DNA fragments were separated on ethidium bromide-stained 1% agarose gel and visualized by transillumination with ultra-violet light. PCR products were purified by the Wizard SV Gel and PCR Clean-Up system (Promega), as indicated by the manufacturer, then they were sequenced by automated fluorescent cycle sequencing (CRIBI, PD, Italy). The partial sequences of 1B1, and AhR are available on request.

Table 3
CYP marker activities in hepatic, respiratory and olfactory microsomes from control and βNF-treated pigs.

Activity	Liver		Respiratory		Olfactory	
	Control	βNF	Control	βNF	Control	βNF
EROD	8 ± 3	187 ± 24*	6.9 ± 3	18 ± 5*	19 ± 7	48 ± 15*
MEROD	3.2 ± 1	58 ± 11*	2.2 ± 0.8	4.8 ± 1.7	11 ± 5	24 ± 9
CoH	49 ± 25	62 ± 28	67 ± 21	52 ± 17	148 ± 38	199 ± 54
AnH	136 ± 42	164 ± 63	28 ± 12	37 ± 16	253 ± 35	312 ± 89

Values are the means ± S.D. of three individual pigs and are expressed as pmol/(min mg protein).

* Significantly different from control by Student's t test, $p < 0.05$.

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