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Immunoblot analysis and immunohistochemical characterization of CYP2A expression in human olfactory mucosa

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Abstract

The aim of the present study was to further characterize the expression of the *CYP2A* genes in human nasal mucosa. Fetal nasal tissues at 12–26 weeks of gestational age and surgical biopsy tissues from various regions of nasal cavity of adult patients were studied to determine whether CYP2A proteins can be detected by immunoblot in adults, whether higher levels of CYP2A proteins are present in adult than in fetal nasal mucosal microsomes, and whether CYP2A13 mRNA is more abundant than CYP2A6 mRNA in fetal nasal mucosa. In adults, immunoblot analysis detected CYP2A proteins in microsomes of the olfactory region from 8 of 10 individuals, but in none of the nasal microsomes of the respiratory region from 47 patients. Quantitative immunoblot analysis confirmed that CYP2A proteins are selectively expressed in the olfactory region in both adult and fetal tissues. Interestingly, the levels of CYP2A proteins in nasal microsomes were generally higher in fetuses than in adults. In the fetus, the level of CYP2A13 mRNA was much higher than that of CYP2A6 mRNA, as has been previously found in adult nasal mucosa. Immunohistochemical studies confirmed that, in the fetus, the CYP2A proteins are expressed in the supporting cells in the olfactory epithelium and in the Bowman's glands in the lamina propria. The prenatal expression of the CYP2A proteins in the olfactory mucosa suggests potential risks of developmental toxicity from maternally derived xenobiotics, since both CYP2A6 and CYP2A13 are known to be efficient in the metabolic activation of tobacco-specific nitrosamines and other respiratory toxicants.

Keywords: Cytochrome P450; CYP2A6; CYP2A13; Olfactory mucosa; Fetus; Human; Nasal mucosa

1. Introduction

The human *CYP2A* gene subfamily has two functional genes, *CYP2A6* and *CYP2A13* [1,2]. Both enzymes are known to be active in the metabolic activation of nasal toxicants and other xenobiotic compounds, such as *N*-nitrosodiethylamine, 2,6-dichlorobenzonitrile, and hexamethylphosphoramide (e.g. [1,2]). CYP2A13 is particularly active in the metabolic activation of NNK, which is a major tobacco-specific procarcinogen, although CYP2A6 is also active in this reaction [2].

Recent studies on tissue samples from human adults [2,3] showed that CYP2A13 mRNA is predominantly expressed in the respiratory tract, with the highest level in the nasal mucosa, followed by the lung and the trachea. On the other hand, CYP2A6 mRNA is predominantly expressed in liver, where its levels are much higher than those of CYP2A13 in the respiratory tract [2]. CYP2A proteins have been detected in adult human nasal mucosa by immunohistochemistry with a polyclonal antibody against rabbit CYP2A10/11 [4].

Both CYP2A6 and CYP2A13 have also been detected in human fetal nasal mucosa by RNA-PCR, but the relative levels of the two mRNAs were not determined [5]. In the same study, CYP2A proteins were detected with a polyclonal antibody to mouse CYP2A5 in nasal and hepatic microsomes prepared from human fetal tissues between GD 91 and 125; however, the CYP2A proteins were expressed in nasal microsomes at much higher levels than in liver microsomes. In addition, the expression of two other CYPs, CYP2B6 and CYP2J2, was also detected in fetal nasal mucosa.

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Abbreviations: CYP or P450, cytochrome P450; OM, olfactory mucosa; GD, gestational day; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; RT, reverse transcription; PCR, polymerase chain reaction; bp, base pair; OMP, olfactory marker protein; NSE, neuron-specific enolase.

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The selective expression of CYP2A13 in the nasal mucosa and the lung implicates a potentially important role for it in tobacco-related xenobiotic toxicity in the respiratory tract. The prenatal expression of CYP2A proteins in the nasal mucosa suggests that this tissue may be at risk for developmental toxicity of maternally derived xenobiotics that are activated by CYP2A6 or CYP2A13. Thus, we conducted additional studies to characterize the developmental expression and cellular distribution of the CYP2A proteins and to compare the levels of CYP2A proteins in prenatal and adult nasal microsomes. In addition, since antibodies that can distinguish between CYP2A6 and CYP2A13 are not yet available, the relative levels of CYP2A6 and CYP2A13 mRNAs in fetal nasal mucosa were determined. Our studies confirm that, in both adult and fetal tissues, CYP2A proteins are selectively expressed in the olfactory region. Furthermore, the level of CYP2A proteins in nasal microsomes was generally higher in fetuses than in adults.

2. Materials and methods

2.1. Immunoblot analysis

Adult and fetal nasal tissues for microsome preparation and immunoblot analysis were obtained at the Qilu Hospital of Shandong University, Jinan, China, with a protocol approved by the Institutional Review Boards of the participating institutions. Patient information, including age, sex, ethnicity, clinical diagnosis and storage condition of biopsy specimens, or cause of death and postmortem time of autopsy samples, is presented in the relevant table and figure legends. Nasal mucosa from the olfactory region (upper septum and superior turbinate) was confirmed to contain olfactory neuron by immunohistochemical detection of OMP. Microsomes were prepared according to Ding and Coon [6]. Protein concentrations were determined by the bicinchoninic acid method (Pierce) with bovine serum albumin as the standard. Nasal microsomal proteins were precipitated by two volumes of 100% ethanol at -20° for 30 min; the precipitates, which were collected by centrifugation at 15,000 *g* in a microcentrifuge for 5 min at 4°, were dissolved directly in a 2× sample dilution buffer, containing 125 mM Tris–Cl buffer, pH 6.8, 2% SDS, 20% glycerol, 10% mercaptoethanol, and 0.002% Bromphenol Blue [7], and were boiled for 5 min before gel electrophoresis. An enhanced chemiluminescence kit from Amersham was used for immunoblot analysis, with a polyclonal antibody to mouse CYP2A5 [8]; this antibody recognizes both CYP2A6 and CYP2A13, but does not recognize a number of other human CYPs on immunoblots, including CYP1A1, 1A2, 1B1, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 3A4, 3A5, and 4A11 [2,9]. Heterologously expressed CYP2A6 [10] was used as a positive control for the identification of immunoreactive bands and as a standard for determination of the amounts of CYP2A proteins in different microsomal samples. Immunoblot quantitation was carried out with the use of an LKB ImageMaster DTS densitometer (Pharmacia).

2.2. Immunohistochemical analysis

CYP2A proteins were detected with a rabbit antiserum to rabbit CYP2A10/11 [6]. OM was identified using polyclonal antibodies to OMP (kindly provided by Dr. Frank Margolis of the University of Maryland at Baltimore) or NSE (BioGenex). Fetal tissues, which were also obtained at Qilu Hospital of Shandong University, Jinan, China, were fixed in 4% paraformaldehyde. Paraffin sections were used for immunohistochemistry, with use of a Super Sensitive Immunostaining Kit from BioGenex, using Antigen Retrieval Citra Solution and 3-amino-9-ethylcarbazole or 3,3′-diaminobenzidine chromogen.

2.3. Quantitative RT-PCR

The relative levels of CYP2A6 and CYP2A13 mRNAs were determined using real-time RT-PCR, with a LightCycler™ (Roche), using conditions essentially as described previously for the determination of mouse CYP2As [11]. Fetal tissues for RNA-PCR analysis were obtained from the Birth Defects Research Laboratory of the University of Washington in Seattle, with postmortem times less than 1 hr. No information was available regarding the medical history of these specimens. Nasal tissues were dissected as described previously [5]. Total RNA was isolated from frozen tissues according to the method of Chomczynski [12], using TRIzol reagent (Gibco BRL). RNA concentrations were determined spectrally. The integrity of the RNA samples was assessed by the A260/A280 ratio and by ethidium bromide staining after electrophoretic analysis on denaturing gels.

RT reactions were performed in a PE9600 PCR machine (Applied Biosystems) with Thermoscript RNase H^- reverse transcriptase (Invitrogen). First-strand cDNAs were synthesized at 50° with use of 2 μg total RNA, 2.5 μM oligo-$(dT)_{16}$ primer, 1 mM each of dNTP, and 5 mM $MgCl_2$ in a total volume of 20 μL. Real-time PCRs were performed according to the instructions in the LightCycler™ FastStart DNA Master SYBR Green I Kit (Roche), using gene-specific PCR primers (CYP2A6: forward primer, 5′-gggccaagatgccctacatg-3′; reverse primer, 5′-aatgtccttaggtgactggga-3; product size, 377 bp; CYP2A13: forward primer, 5′-acctggtgatgaccaccc-3′; reverse primer, 5′-cgtggatcactgcctctg-3; product size, 203 bp). PCR mixtures contained 2 μL of FastStart DNA Master SYBR Green I, 4 mM $MgCl_2$, 0.25 μM each primer, and 2 μL of RT product or serial dilutions of CYP2A6 or CYP2A13 cDNA standard in a total volume of 20 μL. PCR was monitored for 40 cycles with annealing temperature at 62°. At the end of the PCR cycles, melting curve analysis was performed according to LightCycler™

Table 1
Immunoblot detection of CYP2A proteins in adult nasal tissues[a]

Immunoblot detection of CYP2A proteins	Number of individuals examined							
	Olfactory mucosa[b]		Respiratory mucosa[b]			Diseased nasal tissue		
	Superior turbinate	Upper septum	Middle turbinate	Inferior turbinate	Other[c]	Polyps	Tumor	Para-tumor
Detected	6	2	0	0	0	0	1	0
Not detected	1	1	22	15	10	9	5	4
Total	10			47			19	

[a] Biopsy tissues were obtained during surgery and were frozen at −70° within 30 min.
[b] Disease-free.
[c] Including all other types of respiratory mucosa, such as mucosa of the nasal sinus and lateral wall.

Table 2
CYP2A protein level in adult nasal microsomes[a]

Specimens	Age	Sex	Clinical diagnosis	CYP2A protein level (pmol/mg microsomal protein)				
				Olfactory mucosa[b]		Respiratory mucosa[b]		Tumor and peri-tumor tissue
				Superior turbinate	Upper septum	Middle turbinate	Inferior turbinate	
A1	19	M	Nasopharynx fibroangioma	0.1	N/A[c]	N/A	N/A	N/A
A2	25	M	Normal[d]	0.4	0.4	n.d.[e]	n.d.	N/A
A3	30	F	Nasal striated muscle sarcoma	0.5	n.d.	n.d.	n.d.	n.d.
A4	38	F	Nasal sinus cyst	1.1	N/A	n.d.	n.d.	N/A
A5	50	M	Nasal inverted papilloma	N/A	N/A	N/A	N/A	1.1[f]
A6	65	F	Nasal sinusitis and polys	0.6	N/A	N/A	N/A	N/A
A7	67	M	Nasal sinus squamous carcinoma	N/A	0.5	N/A	N/A	N/A

[a] Biopsy tissues were obtained during surgery and were frozen at −70° within 30 min. The autopsy tissue was obtained within 4 hr of death. The level of CYP2A proteins was determined using heterologously expressed CYP2A6 as a standard. The P450 concentration of the CYP2A6 standard in SF9 microsomes was determined spectrally. The anti-CYP2A5 antibody used for immunoblot analysis reacts with CYP2A6 and CYP2A13 equally well.
[b] Disease-free.
[c] N/A, tissue not available.
[d] Autopsy tissue; cause of death: gunshot.
[e] n.d., not detected (less than 0.1 pmol/mg microsomal protein).
[f] The tumor was located in the olfactory region.

kit instructions, to assess the purity of the PCR products. PCR products were also analyzed by electrophoresis on agarose gels, purified using the Qiaquick Gel Extraction Kit (Qiagen), and sequenced to confirm PCR specificity. Negative control reactions (no template) were routinely included to monitor potential contamination of reagents.

The relative levels of β-actin mRNA in various RNA samples were also determined (forward primer, 5′-cctgactgactacctcatg-3′; reverse primer, 5′-tccttctgcatcctgtcggca-3′; product size, 396 bp; annealing temperature, 60°), and the results were used as an internal reference for the actual amounts of RNA added to each RT reaction. For β-actin quantification, a standard curve was constructed with use of a PCR-amplified, purified, β-actin cDNA fragment.

3. Results

3.1. Detection of CYP2A proteins in adult and fetal human nasal mucosa

Although CYP2A-related immunoreactivity was abundantly detected in adult human nasal mucosa in a previous study [4], our initial attempts to detect CYP2A proteins in adult nasal microsomes by immunoblot analysis were unsuccessful (not shown). In the present study, the sensitivity of the immunoblot detection was increased by precipitating microsomal proteins with ethanol prior to electrophoresis. Following the ethanol treatment, CYP2A proteins were detected in adult OM microsomes from 8 of 10 individuals examined, but not in microsomes of nasal respiratory mucosa from 47 individuals (Tables 1 and 2, and Fig. 1, panels A and C). CYP2A proteins were also detected in one nasal tumor, which was located in the olfactory region (Table 2), but they were not detected in diseased nasal tissues from 18 other individuals examined (Table 1). Thus, within the nasal cavity, CYP2A proteins are expressed preferentially in the olfactory region in humans, as has been shown in other mammals [13]. The microsomal CYP2A protein level was quantified with use of the anti-CYP2A5 antibody, which reacts with CYP2A6 and CYP2A13 equally well,[1] and with heterologously expressed CYP2A6 as a standard. The levels ranged from 0.1 to 1.1 pmol/mg microsomal protein among six adult

[1] Ding, unpublished observation.

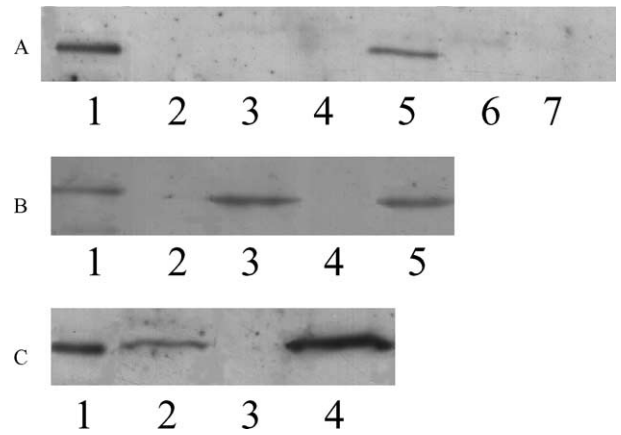

Fig. 1. Immunoblot detection of CYP2A proteins in adult and fetal human nasal mucosa. Nasal mucosa microsomal proteins (30 μg per lane for fetal tissue and about 35 μg per lane for adult tissue) were analyzed on immunoblots with a rabbit antiserum to CYP2A5. Heterologously expressed CYP2A6 in SF9 cell microsomes (about 80 fmol CYP per lane) was used as a standard (lane 1). Panel A: nasal microsomes of normal mucosa from a 38-year-old female with nasal sinus cyst (lane 2, respiratory mucosa from lateral wall of the nasal cavity; lanes 3 and 4, inferior nasal turbinate; lane 5, superior nasal turbinate; lane 6, anterior part of intermediate nasal turbinate; lane 7, maxillary sinus). Panel B: nasal microsomes from a GD 119 male fetus (lane 2, inferior nasal turbinate; lane 4, intermediate nasal turbinate) and nasal microsomes from a GD 185 male fetus (lane 3, upper part of nasal septum; lane 5, superior nasal turbinate). Panel C: nasal microsomes of normal mucosa from 67-year-old male with nasal sinus squamous carcinoma (lane 2, upper part of nasal septum) and nasal microsomes from a GD 182 female fetus (lane 4, upper part of nasal septum). Lane 3 was a blank.

nasal microsomes used for quantitative immunoblot analysis (Table 2). Notably, although all adults (except A2) studied had diseases involving the nasal cavity, only the non-diseased parts of the specimens were used as normal tissues for microsomal preparation.

CYP2A proteins are also expressed in fetal nasal microsomes (Table 3 and Fig. 1, panels B and C). The CYP2A protein content in olfactory microsomes from five fetuses (with gestational ages ranging from 96 to 185 days) ranged from 0.8 to 5.3 pmol/mg microsomal protein, with a trend of developmental increase in expression level (Table 3). CYP2A was detected in both OM and nasal respiratory mucosal microsomes in one fetus (F5, at GD 185), but the levels in olfactory microsomes were much higher. Interestingly, CYP2A protein levels in fetal OM microsomes (Table 3; 0.8–5.3 pmol/mg) were also higher than in adult OM microsomes (Table 2; 0.1–1.1 pmol/mg), as illustrated by a direct comparison in panel C of Fig. 1.

Table 3
CYP2A protein level in prenatal nasal microsomes[a]

Specimens	Gestational age (days)	Sex	CYP2A protein level (pmol/mg microsomal protein)			
			Olfactory mucosa		Respiratory mucosa	
			Superior turbinate	Upper septum	Middle turbinate	Inferior turbinate
F1	96	F	0.8	N/A[b]	n.d.[c]	n.d.
F2	119	M	2.8	2.7	n.d.	n.d.
F3	160	M	5.3	N/A	N/A	N/A
F4	182	F	N/A	3	N/A	N/A
F5	185	M	4.0	4.3	0.7	1.6

[a] Autopsy fetal tissues were obtained at Qilu Hospital of Shandong University, Jinan, China. Causes of death of the fetus were spontaneous abortion (F1 and F2) and premature birth (F3, F4, and F5). Tissues were obtained within 2 hr of death. Immunoblot analysis was performed as described in Table 2.

[b] N/A, tissue not available.

[c] n.d., not detected (less than 0.1 pmol/mg microsomal protein).

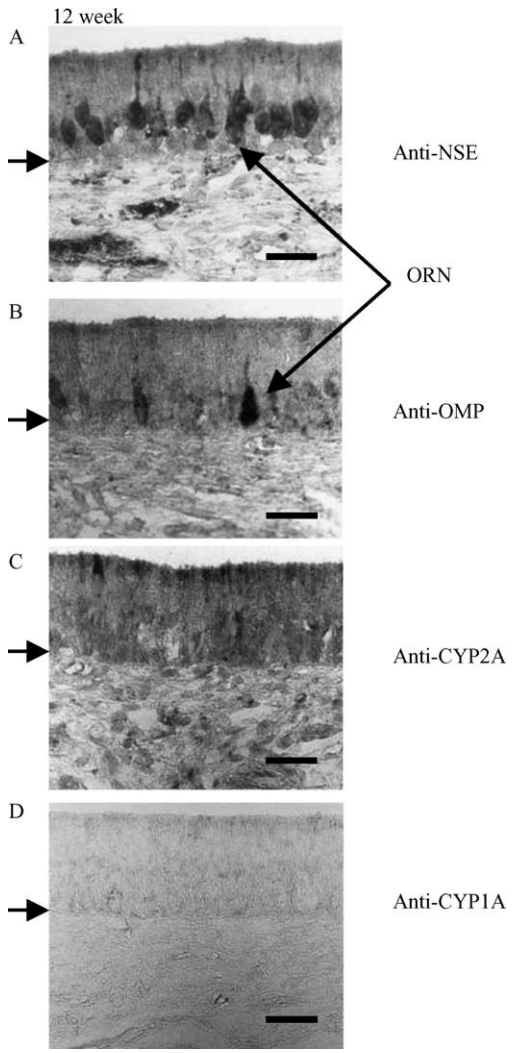

Fig. 2. Immunohistochemical detection of CYP2A proteins in the OM of a 12-week-old fetus. Tissues from a 12-week-old fetus (male; cause of death, extrauterine pregnancy) were fixed in 4% paraformaldehyde. Paraffin sections (5 μm) were used. Immunoreactive proteins were detected with a Super Sensitive Immunostaining Kit from BioGenex, using Antigen Retrieval Citra Solution and 3-amino-9-ethylcarbazole chromogen. OM was identified using antibodies to NSE (panel A; ready-to-use super sensitive antibody from BioGenex; 30 min at room temperature) and OMP (panel B; goat anti-OMP antiserum, 1:2000 dilution; room temperature for 60 min). Panel C, rabbit anti-2A10/11serum (1:1000 dilution, 4° overnight); panel D, monoclonal anti-rat CYP1A1/2 (Oxford Biomedical Research; 1:500 dilution, 4° overnight). ORN, olfactory receptor neuron. Short arrows indicate position of basement membrane. Bar, 20 μm.

3.2. Cellular distribution of CYP2A proteins in human fetal olfactory mucosa

A rabbit antiserum to rabbit CYP2A10/11 was used for localization of the human CYP2A proteins; the same antibody had been used previously for immunohistochemical analysis of CYP2A expression in adult human nasal mucosa [4]. The results from a 12-week-old male fetus and a 26-week-old female fetus are shown in Figs. 2 and 3, respectively. At 12 weeks of gestational age, NSE-positive cells were numerous (Fig. 2, panel A), but OMP-positive cells were only found occasionally (Fig. 2, panel B), which confirms the immature development of the OM at this age. No staining was observed in negative control experiments, in which the primary antibody was replaced by species-appropriate negative control serum (data not shown). Low levels of CYP2A immunoreactivity were observed at the apical region of supporting cells in the epithelium and immature Bowman's glands in the lamina propria (Fig. 2, panel C). No signal was detected on sections incubated with an anti-CYP1A1/2 (Fig. 2, panel D), which served as additional negative controls.

Much greater staining intensity was seen in the 26-week-old fetus, with CYP2A immunoreactivity in mature Bowman's glands in the lamina propria and at the apical region and foot processes of supporting cells in the epithelium, which is typical for supporting cell staining (Fig. 3, top panel). No signal was seen in the negative control section (Fig. 3, bottom panel), indicating staining specificity. These results are consistent with the trend of developmental increase in CYP2A protein expression that was revealed by immunoblot analysis, and indicate that OM CYP2A proteins are expressed in non-neuronal cells, as reported previously for CYP2A expression in adult human OM [4]. The age-related increase in immunohistochemical staining intensity was confirmed in several other samples, and was apparently not related to gender differences (data not shown).

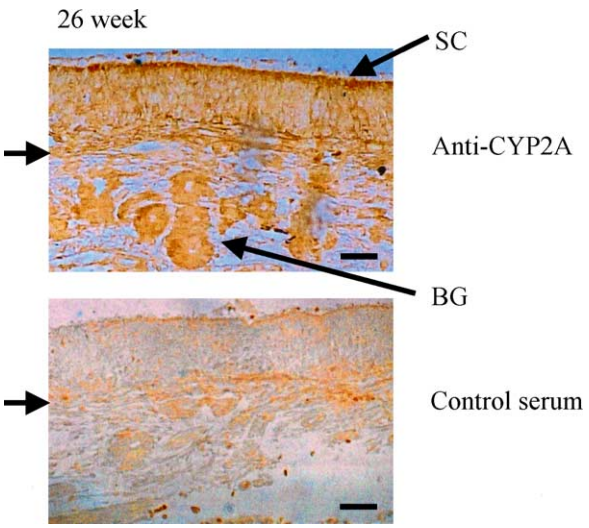

Fig. 3. Immunohistochemical localization of CYP2A proteins in the OM of a 26-week-old fetus. The results shown were obtained with adjacent sections from a 26-week-old female fetus (F4 in Table 3). Immunohistochemical procedures were the same as described in Fig. 2, except that 3,3′-diaminobenzidine was used as a chromogen. Top panel, rabbit anti-2A10/11serum (1:1000 dilution, 4° overnight); bottom panel, control rabbit serum (1:1000 dilution, 4° overnight). BG, Bowman's glands; SC, (apical region of) supporting cells. Short arrows indicate position of basement membrane. Bar, 20 μm.

Table 4
Levels of CYP2A6 and CYP2A13 mRNAs in fetal nasal mucosa[a]

Specimens[b]	Relative mRNA level (arbitrary units)[c]		2A13/2A6 ratio
	CYP2A6	CYP2A13	
#15777	3.3×10^4	5.9×10^5	18
#16005	9.4×10^3	3.9×10^5	41

[a] CYP2A6 and CYP2A13 mRNAs were quantitated using real-time RT-PCR, with a LightCycler, using conditions essentially as described recently [11], but with gene-specific PCR primers, and with CYP2A6 and CYP2A13 cDNAs as standards. RNA concentrations were determined by UV spectroscopy, and were confirmed by relative β-actin mRNA levels in the same tissues, which were also determined by real-time, quantitative RT-PCR. Samples were analyzed in duplicate. For all three genes, the amounts of PCR products were within the linear range of the standard curves. Values obtained from duplicate reactions were consistent, and showed differences less than 5% of the mean. For the standard curves, coefficients from linear regression were always greater than 0.95.

[b] Both fetuses were male and at GD 91; one was Caucasian (#15777) and one was African-American (#16005).

[c] Equivalent to copy number of individual cDNA template used, normalized by the levels of β-actin cDNA in each sample.

3.3. Determination of relative abundance of CYP2A6 and CYP2A13 mRNAs in fetal olfactory mucosa

Quantitative RNA-PCR was performed for a determination of the relative levels of CYP2A6 and CYP2A13 mRNAs in fetal OM. The results obtained with the nasal mucosa from two fetuses (both male and at GD 91) are shown in Table 4. In both RNA samples, the level of CYP2A13 was much higher than that of CYP2A6, with the individual ratios of the two transcripts being 18 and 41. Thus, CYP2A13 mRNA is the main CYP2A transcript in fetal nasal mucosa, as it is in adult nasal mucosa and lung [2].

4. Discussion

This is the first report in which the expression of CYP2A proteins can be detected by immunoblotting in adult human nasal mucosa. The success was partly due to improvements in the sample preparation procedure, which included ethanol precipitation of microsomal proteins prior to immunoblot analysis. Because of this success, we were able to demonstrate that CYP2A proteins are expressed mainly in the olfactory region in both adult and fetal nasal mucosa. Of particular interest, we found that the level of CYP2A proteins in microsomal preparations of fetal OM is higher than that in adult OM. Furthermore, through the use

of gene-specific quantitative RNA-PCR, we showed that in fetal nasal mucosa, as in adult nasal mucosa, CYP2A13 mRNA is present at much higher levels than is CYP2A6 mRNA. Although mRNA and protein levels are not always directly related, this finding implies that CYP2A13 is the predominant fetal nasal CYP2A enzyme.

The upper respiratory tract is an important target for environmental toxicity. In mammalian animals, the OM has high concentrations of cytochrome P450 and other biotransformation enzymes [13]. Nasal toxicity is a frequent consequence of xenobiotic exposure in experimental animals (for a review, [14]). Human nasal tumors are generally rare, although common in parts of China, but they are most frequently found in smokers and in those occupationally exposed to wood dust, chromate, and other chemicals [15–18]. It has also been reported that children exposed to urban pollution have extensive DNA damage in their nasal epithelium [19] and that three commonly used pesticides, permethrin, *N*,*N*-diethyl-*m*-toluamide (DEET), and diazinon, cause genotoxic effects in primary adult human nasal mucosal cells [20]. However, studies on the gene expression or metabolic activity in human nasal mucosa are very limited, primarily because of the limited tissue availability.

Although most P450s expressed in the liver are also expressed in the nose, the OM is unique in having high levels of CYP2A and CYP2G enzymes in various animal species [13]. The nasal CYP2A enzymes, such as rat CYP2A3 (e.g. [10,21–23]), mouse CYP2A5 (e.g. [8,24–26]), and rabbit CYP2A10/11 (e.g. [6,27–29]), are highly active in the metabolic activation of many xenobiotic compounds, and have been implicated in the tissue-selective toxicity of several compounds in the nasal mucosa (e.g. [9,25,30]). The first evidence for the presence of CYP2A in adult human nasal mucosa was obtained by immunohistochemistry [4]. CYP2A immunoreactivity was localized in sustentacular cells in the olfactory epithelium, and in Bowman's gland acinar cells and vascular endothelial cells in the lamina propria. In the respiratory mucosa, ciliated epithelial cells, as well as serous gland acinar cells and vascular endothelial cells in the lamina propria, were also immunoreactive. In two earlier studies [31,32], human nasal microsomes were found to be active in the metabolic activation of *N*-nitrosodiethylamine and hexamethylphosphoramide, both of which are now known to be substrates for human CYP2A6 and CYP2A13 enzymes (e.g. [2]). The nasal expression of the *CYP2A6* and *CYP2A13* genes has been confirmed by several studies in human adult tissues by RNA-PCR [2,3,33,34] and recently also in human fetuses by RNA-PCR and immunoblot analysis [5].

The presence of a higher level of CYP2A proteins in fetal than in adult OM microsomes is intriguing. Although fetus-selective expression of certain CYP and non-CYP biotransformation enzymes, such as CYP3A7 [35] and flavin-containing monooxygenase 1 [36], has been reported, the expression of the CYP2A proteins may be more influenced by the abundance of neuronal epithelium in the nasal mucosa used for microsomal preparation than by regulatory events associated with postnatal development. It is known that the orderly organization present in neonatal olfactory epithelium becomes increasingly interspersed with non-sensory epithelium in adults [37], which would "dilute" the P450 enzymes in microsomal preparations from adults. There is also a possibility that, although the adult nasal biopsies used in this study were apparently disease-free, their levels of P450 enzymes were affected by the pathological conditions in the nasal cavities of these patients or by the surgical procedures involved.

The toxicological significance of prenatal, OM-selective expression of the CYP2A enzymes remains to be determined. The present finding that CYP2A13 is the predominant CYP2A mRNA in fetal OM, taken in combination with our recent observation that CYP2A13 is the most efficient P450 in the metabolic activation of NNK [2] and the report that NNK can be transplacentally transferred from women who smoke cigarettes to fetuses [38], suggests that *in situ* metabolic activation of NNK in fetal nasal mucosa may occur. Thus, further studies to assess the metabolic capacity of fetal OM toward NNK and other known or potential toxicants are warranted.

Acknowledgments

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