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Cytochromes P450 NMa, NMb (2G1), and LM4 (1A2) Are Differentially Expressed during Development in Rabbit Olfactory Mucosa and Liver

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SUMMARY

Mammalian olfactory mucosa has a high concentration of cytochrome P450 monooxygenases (P450). The major olfactory P450 isoforms in adult rabbits include P450 NMa, which is found in both olfactory and respiratory mucosa, as well as in liver at a low level, P450 NMb (2G1), which is olfactory specific, and P450 form 4 (1A2), which is found only in liver and olfactory mucosa. In the present study, we have found that the developmental expression of olfactory P450 in rabbits is not coordinated with the ontogenesis of hepatic P450. These three P450 isoforms were detected immunochemically and found to be at a relatively high level in olfactory but not hepatic microsomes in the first 2 weeks after birth. In the liver, NMb is not detectable at any age and NMa is not detectable until the fourth week. P450 1A2 is not detectable until the third week, but its level increases rapidly in the fourth week. These P450 isoforms are also detectable in prenatal olfactory tissue at 2 days before birth, indicating that direct exposure to air is not a prerequisite for their early expression in this tissue and that the early appearance of these enzymes may be controlled by both endogenous and environmental factors. In addition, the developmental expression of 2E1, a minor olfactory P450 isoform, also occurs earlier in olfactory mucosa than in liver, and the same conclusion can be made about the expression of NADPH-P450 reductase, which is detectable in olfactory microsomes but not in hepatic microsomes from prenatal rabbits. Thus, the regulatory mechanisms that control basal prenatal expression in the olfactory tissue may be common for multiple P450 isoforms and perhaps also for other biotransformation enzymes. The tissue-specific early onset of expression of multiple forms of P450 in olfactory tissue suggests that these enzymes may play an important role in the neonatal period, when olfactory ability is vital for the survival of the newborn. The presence of relatively high levels of biotransformation enzymes in the olfactory mucosa may also have important implications for neonatal inhalation toxicology.

The mammalian olfactory mucosa is constantly exposed to numerous foreign compounds in the inhaled air, including odorants, environmental pollutants, allergens, and pheromones, and also to a variety of xenobiotic and endobiotic substances that are present in the rich blood supply to this tissue, such as steroid hormones, dietary constituents, and drugs. The ability to metabolize and dispose of these chemicals is vital for maintenance of the normal function of the olfactory tissue. P450, which is responsible for many of these conversions, has been found in relatively high concentration in the olfactory mucosa of numerous species (1, 2). We have found that in adult rabbits the specific content of P450 in olfactory microsomes is second only to that in hepatic microsomes and the concentration of NADPH-P450 reductase in olfactory microsomes is actually the highest among all tissues examined (3). Several research groups have proposed that the presence of high levels of P450 and other biotransformation enzymes in the olfactory mucosa is important for odorant removal or modification and for protection of the olfactory neuroepithelium and the brain against inhaled toxicants and, in some cases, may unfortunately also lead to a high incidence of inhalation-induced nasal cancer (4-9).

At least nine forms of P450 have been identified in rabbit olfactory microsomes. The three major olfactory isoforms in adult rabbits are NMa, NMb, and form 4 (7). NMa and NMb were discovered in rabbit nasal microsomes several years ago in this laboratory (10), and so far they are the only two P450 isoforms to be purified from the nasal source. Based on the corresponding cDNA sequences we have obtained, NMa is a member of the P450 2A subfamily[1] and NMb belongs to the 2G subfamily (11). The tissue distribution of these isoforms is quite interesting. In adult rabbits, NMb has been detected only

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[1] H.-M. Peng, X. Ding, and M. J. Coon, unpublished observations.

ABBREVIATIONS: P450, cytochrome P450; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TBS, Tris-buffered saline (20 mM Tris-chloride buffer, pH 7.4, containing 0.9% NaCl).

in the olfactory tissue, whereas NMa has been detected in both olfactory and respiratory mucosa, as well as in liver (7). P450 form 4, which has been designated as 1A2, is an abundant isoform in both olfactory mucosa and liver (7, 12) but has not been detected in other extrahepatic tissues. The alcohol-inducible P450 form 3a (2E1) is a minor protein in olfactory microsomes and is apparently the only P450 that is inducible by chemical agents in the olfactory mucosa (13). The other minor isozymes identified in rabbit olfactory microsomes include forms 2 (2B4) (7, 12), 3c (3A6) (10), and 5 (4B1) (12), as well as two isoforms of the 4A subfamily (14).

In the present study, we have examined the ontogenic expression of several P450 isoforms, including NMa, NMb, 1A2, and 2E1, as well as NADPH-P450 reductase, in rabbit liver and olfactory mucosa. Our results indicate that the ontogenic expression of olfactory P450 is not coordinated with that of hepatic P450. The three major olfactory P450 isoforms, NMa, NMb, and 1A2, are expressed at high levels in the olfactory mucosa, but not in the liver, in the first 2 weeks after birth. Furthermore, these cytochromes, as well as P450 2E1 and NADPH-P-450 reductase, are expressed in prenatal rabbits in olfactory but not in hepatic microsomes.

Experimental Procedures

Animals. Late-term pregnant New Zealand White rabbits were allowed free access to rabbit chow and water. Fetuses were removed via caesarean section at approximately day 29 of gestation (the gestational period of the rabbit is 31 days). Newborn rabbits were kept with their mothers. The sex of fetuses and neonates was not determined. Adult male rabbits (4 months of age) were obtained from a local supplier and sacrificed upon arrival. Liver and olfactory mucosa from various age groups were promptly frozen in liquid N_2 and stored at −70° until further processing.

Preparation and analysis of microsomes. Tissues were disrupted with a Brinkman Polytron homogenizer, and microsomes were prepared as previously described (15), except that 0.1 mM phenylmethylsulfonyl fluoride was included in the homogenization buffer and the pyrophosphate washing step was omitted. Protein concentration was determined by the method of Lowry *et al.* (16). The concentration of total P450 in microsomal suspensions was determined according to the procedure of Omura and Sato (17). SDS-PAGE was carried out with use of a discontinuous buffer system (18) in 7.5% polyacrylamide gels. After electrophoresis, proteins were transferred to nitrocellulose sheets (19). The nitrocellulose sheets were treated with 5% nonfat dry milk in TBS containing 0.1% Tween 20 (blocking solution) for 1 hr at room temperature, incubated with an anti-P450 or antireductase antibody in the blocking solution for an additional 1 hr, washed with TBS containing 0.1% Tween 20, and then incubated with an appropriate peroxidase-conjugated anti-IgG antibody at a 1/30,000 dilution in the blocking solution for 1 hr at room temperature. The nitrocellulose sheets were washed again, and the immune complexes were detected with an enhanced chemiluminescence detection kit as described by the manufacturer (Amersham), with the use of Hyperfilm-ECL. Immunoquantitation was performed with a Zeiss soft laser densitometer as previously described (20).

Preparation and analysis of RNA. Total RNA was prepared by the method of Chirgwin *et al.* (21), as previously described. RNA electrophoresis and hybridization were performed as described (22), with the use of ^{32}P-end-labeled oligonucleotides complementary to the P450 2E1 and 2E2 mRNAs (23). The integrity of the RNA samples was confirmed by staining of the blots with methylene blue, to disclose the rRNA subunits, before hybridization.

Antibody production. Monoclonal antibodies to purified rabbit P450 2E1 were prepared as described earlier (3). Sheep antibodies to purified rabbit P450 1A2 were produced by Dr. Dennis R. Koop, now at the Oregon Health Sciences University. Antibodies to rabbit NADPH-P450 reductase were produced in goat (24). The preparation of monospecific sheep anti-NMb has been described previously (7). Chicken antibodies to purified NMa were produced according to a recent immunization protocol described by Gassmann *et al.* (25). The IgY fraction obtained from egg yolk of immunized hens recognized a single band on immunoblots with microsomal proteins from either liver or olfactory mucosa but displayed weak cross-reactivity with purified P450 form 2, which comigrates with purified NMa on SDS-PAGE. Monospecific anti-NMa antibody was subsequently prepared by the method of Thomas *et al.* (26), with an immunosorbent affinity column packed with Affi-gel 10 (Bio-Rad) that was coupled to purified P450 form 2.

Results

Developmental changes in the level of total microsomal P450 in liver and olfactory mucosa. As shown in Fig. 1, the microsomal P450 content in adult rabbits was several times higher in liver than in olfactory mucosa. However, such a tissue difference was not observed in the first 2 weeks after birth, and at day 21 the olfactory content was even higher than the hepatic content. Furthermore, whereas the hepatic content reached a maximum at about day 28 after birth, the olfactory level reached a maximum at about day 21. The microsomal P450 contents of adult rabbits were lower than those reported previously (7) because the pyrophosphate washing step was omitted in the present study to accommodate the small samples from neonatal olfactory tissue.

Developmental expression of P450s NMa, NMb, and

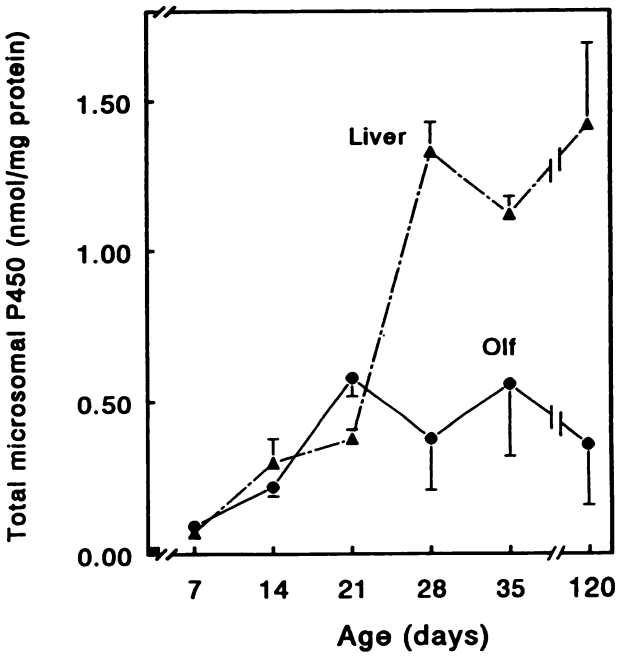

Fig. 1. Developmental change in total microsomal P450 in liver and olfactory mucosa. Microsomes were prepared from pooled tissues from three to six rabbits per age group (for days 7, 14, and 21) or from individual rabbits (for days 28, 35, and 120). The microsomal P450 content in liver (▲) and olfactory mucosa (●) was measured spectrally as described in Experimental Procedures. The data reported are the average of two to five experiments (day 14, $n = 2$; day 21, $n = 3$; day 28, $n = 5$; day 35, $n = 3$; and day 120, $n = 3$) except for day 7 ($n = 1$). The standard deviations are shown in the figure.

1A2 in liver and olfactory mucosa. The level of individual microsomal P450 proteins was determined by immunoblot analysis and is shown in Fig. 2 in arbitrary units, which correspond to the peak intensity from densitometric scans of the immunoblots. The developmental changes in the levels of all three isoforms in olfactory microsomes paralleled the changes in total microsomal P450. The expression of all three reached the adult level by about day 21. In the liver, however, none of the isoforms was detected in the first 2 weeks after birth; NMb was not detected at any age and NMa was not detected until the fourth week. P450 1A2 was not detected until the third week, but its level increased rapidly in the fourth week.

The olfactory expression of NMa, NMb, and 1A2 before and shortly after birth was also examined, as shown in Fig. 3. All three isoforms were detected in prenatal olfactory tissue obtained from the fetus at 2 days before the anticipated birth date, and the levels began to increase at about 24 hr after birth. Thus, direct exposure to air is not a prerequisite for the early expression of these P450 isoforms in the olfactory tissue.

Developmental expression of P450 2E1 and NADPH-P450 reductase in olfactory mucosa. The rabbit *CYP2E* gene subfamily has two known members, 2E1 and 2E2 (23). This laboratory has previously shown that 2E2 mRNA is detectable in liver but not in extrahepatic (including nasal) tissues in adult rabbits (22) and that, in the liver, 2E2 mRNA can be detected immediately after birth but 2E1 mRNA is not detectable until the third week after birth (27). We have found in the present study that, similar to the situation with the three major olfactory P450 isoforms, the developmental expression of 2E1 occured earlier in olfactory mucosa than in liver. Thus, 2E1 mRNA was detected in olfactory but not hepatic tissues at day 12 after birth (Fig. 4, experiment 1). This is in contrast to the profile in adult rabbits, in which the 2E1 mRNA level is at least 5 times lower in olfactory tissue than in liver (22). Immunoblot analysis (Fig. 4, experiment 2) with a monoclonal anti-2E1 antibody, which recognizes both 2E1 and 2E2 proteins (28), indicated that 2E proteins could be detected as early as 2 days before birth in either olfactory or hepatic microsomes. Interestingly, whereas the level of 2E protein in liver (presumably 2E2), which is at a higher level than that in olfactory tissue at 2 days before birth, increased rapidly after birth, the level of 2E protein in olfactory microsomes (presumably 2E1) was not higher at 2 days after birth.

The early onset of expression in olfactory tissue was also observed for NADPH-P450 reductase. As shown in Fig. 5, the reductase protein was detected in olfactory but not hepatic microsomes at 2 days before birth, and its level was much higher in olfactory than in hepatic samples at 2 days after birth. In other experiments not shown, we found that the level of reductase protein was higher in olfactory than in liver microsomes at all neonatal time points examined, as well as in adult rabbits.

Discussion

The results from the present study indicate that the developmental expression of olfactory P450 in rabbits is not coordinated with the ontogenesis of hepatic P450. The three major olfactory P450 isoforms, NMa, NMb, and 1A2, are expressed at relatively high levels in the olfactory mucosa, but not in the liver, in the first 2 weeks after birth. The tissue difference in developmental expression is most striking with P450 1A2, which is present in much higher concentrations in hepatic than in olfactory microsomes from adult rabbits (7). The developmental change in hepatic 1A2 expression has been found to be closely related to diet change at weaning (29); however, no

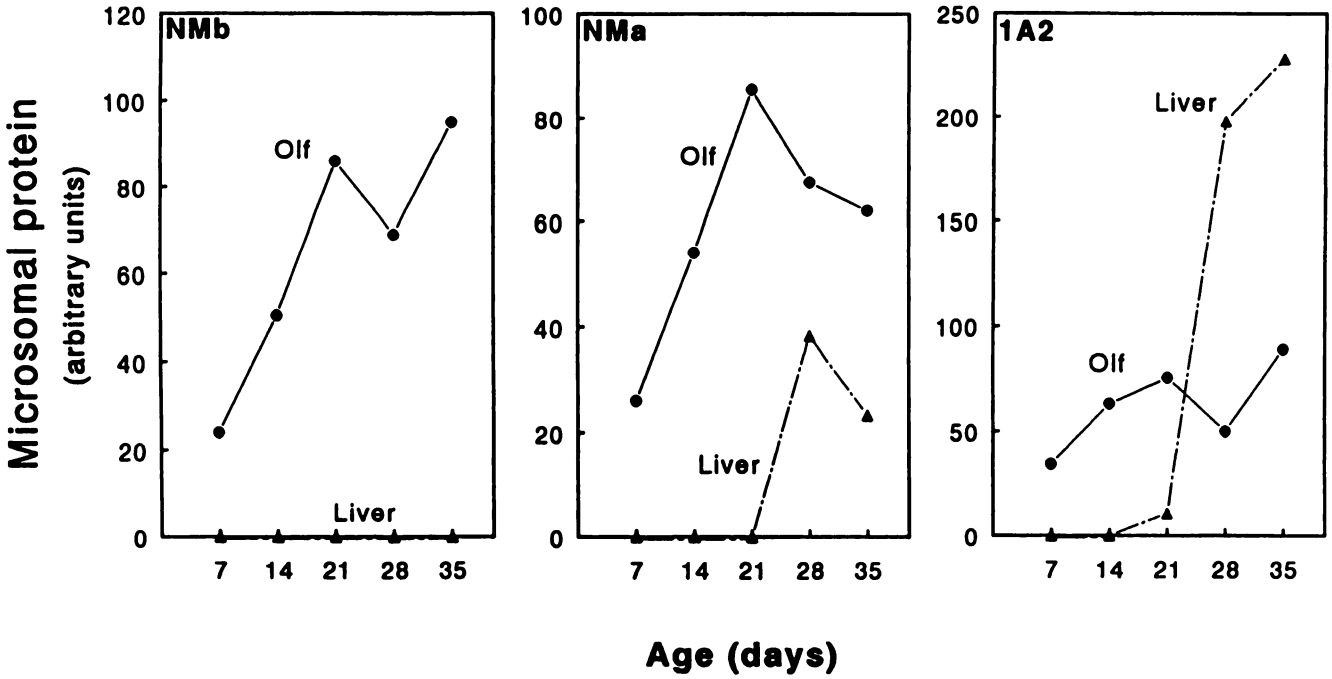

Fig. 2. Developmental expression of P450s NMa, NMb, and 1A2 in liver and olfactory mucosa. Hepatic (▲) and olfactory (●) microsomal samples are the same as described in the legend to Fig. 1. Immunoblot analysis was performed with monospecific chicken anti-NMa IgY (10 μg/ml), monospecific sheep anti-NMb IgG (2 μg/ml), or a sheep antibody that recognizes both 1A1 and 1A2 (2 μg of IgG/ml), as described in Experimental Procedures. The blots were scanned by densitometry and the protein levels were quantified in arbitrary units. Typical results are shown.

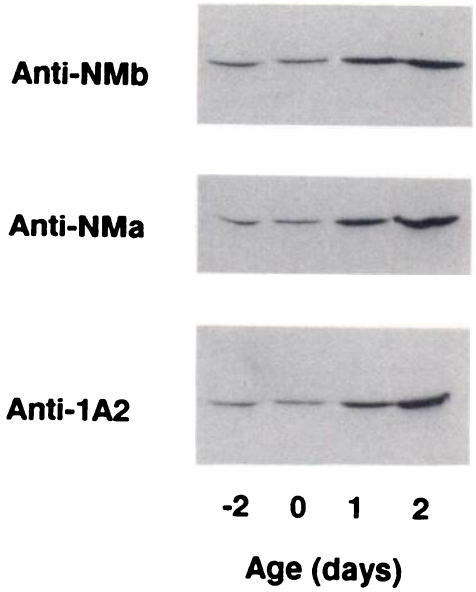

Fig. 3. Perinatal expression of P450s NMa, NMb, and 1A2 in rabbit olfactory mucosa. Microsomal samples (12 μg of protein/lane) were prepared from pooled olfactory tissue from six prenatal or four postnatal rabbits per age group. *Day 0* corresponds to the day of birth and *day −2* corresponds to gestational day 29, which is 2 days before the anticipated birth date. Immunoblot analysis was performed as described in the legend to Fig. 2.

significant change in olfactory 1A2 expression was found around the time of weaning, at 4–5 weeks after birth, in the present study. In this connection, we have found that olfactory 1A2 is not induced in adult rabbits by inducers of hepatic 1A2, such as 3-methylcholanthrene and isosafrole.[2] It is possible that the expression of olfactory 1A2 and other P450s is activated to the maximum level soon after birth by chemicals in the inhaled air. We have also found that P450 isozymes NMa, NMb, and 1A2, as well as P450 2E1 and NADPH-P450 reductase, are expressed in prenatal rabbits in olfactory but not in hepatic microsomes. Thus, whereas the rapid postnatal increase in olfactory P450 expression may be activated by environmental factors in the inhaled air, the early onset of expression of these enzymes is controlled by endogenous (probably hormonal) factors that may be common for regulating the basal prenatal expression of multiple P450 isozymes and perhaps other biotransformation enzymes.

The tissue-specific expression of the olfactory biotransformation enzymes in the fetal and neonatal period is consistent with the proposed functional importance of these enzymes. Rabbit pups are born with sealed eyes and outer ears, and they depend on olfactory cues, probably pheromones present in the milk, to locate the doe's nipples and to suckle (30). Rabbits living in the wild nurse their pups only once a day for about 3–4 min; therefore, a sensitive and reliable olfactory ability is vital for the survival of the newborn (30). Although direct proof has yet to be obtained, the synchronism of the developmental expression of multiple P450 isozymes in the olfactory mucosa with the ontogenesis of the sense of smell, as well as the known versatility and the abundance of these enzymes in the olfactory tissue, strongly suggests the possible involvement of the olfactory P450s in the odorant and pheromone clearance mechanism

[2] X. Ding, H.-M. Peng, and M. J. Coon, unpublished observations.

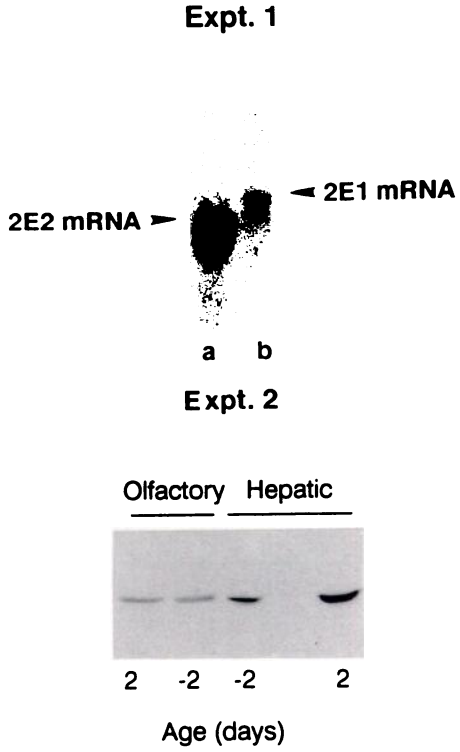

Fig. 4. Developmental expression of P450 2E in liver and olfactory mucosa. Experiment 1, blot hybridization of hepatic and olfactory RNA from 12-day-old rabbits. Total RNA from liver (10 μg) (*lane a*) or olfactory mucosa (20 μg) (*lane b*) was fractionated by agarose gel electrophoresis and hybridized to a radiolabeled oligonucleotide that recognizes mRNA transcribed from *CYP2E1* and *CYP2E2*. The *upper band* (*lane b*) corresponds to 2E1 mRNA; the *lower band* (*lane a*) corresponds to 2E2 mRNA. Experiment 2, immunoblot analysis of P450 2E proteins in liver and olfactory microsomal samples from prenatal (*day −2*) and postnatal (*day 2*) rabbits. Microsomal proteins (12 μg/lane) were analyzed with a monoclonal anti-2E1 antibody (10 μg of IgG/ml) that recognizes both 2E1 and 2E2 proteins.

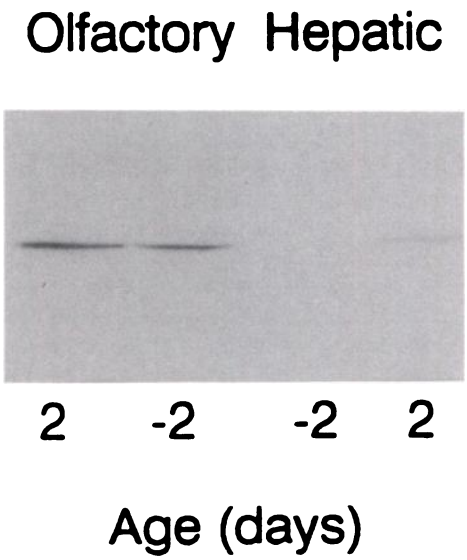

Fig. 5. Perinatal expression of NADPH-P450 reductase in liver and olfactory mucosa. Immunoblot analysis was performed with goat anti-reductase IgG (10 μg/ml). Microsomal samples (12 μg of protein/lane) were the same as described in the legend to Fig. 4.

(2, 31). Nef *et al.* (8) have recently reported the detection of 2G1 mRNA in the rat olfactory neuroepithelium between days 2 and 7 after birth and have suggested a relationship between the temporal gene activation of P450 2G1 and the postnatal increase in the sensitivity of olfactory response to odorants.

The presence of relatively high levels of P450 in the olfactory mucosa of the newborn may also have important implications for neonatal inhalation toxicology. The nasal tissues are exposed to the highest levels of airborne xenobiotics of any body tissue, and the inhalation exposure begins upon birth. Paradoxically, whereas biotransformation is crucial for the disposition of these foreign compounds and for the maintenance of the normal function of the tissue, some of the absorbed compounds may become activated by P450-dependent metabolism and cause pathological changes in the olfactory epithelium. In this regard, P450 NMa, one of the predominant isozymes in nasal microsomes, is highly active in a reconstituted system toward many nasal procarcinogens and toxicants, including hexamethylphosphoramide, phenacetin, *N*-nitrosodiethylamine (10), (*S*)-nicotine (32), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (33), and aflatoxin B1 (34); with each substrate, the consequence of biotransformation seems to be primarily activation rather than detoxification. Some of the activated xenobiotics may enter the brain through the axonal transport system (35) and exert toxic or carcinogenic effects in the central nervous system. Furthermore, the structural and functional damage to the olfactory epithelium resulting from P450-catalyzed bioactivation of inhaled foreign compounds may subject the olfactory receptor neurons and the adjacent brain tissues in the olfactory region to increased risk of invasion by viruses, bacteria, and foreign particles. These hazardous foreign materials are capable of penetrating the olfactory neuroepithelium and entering the brain if not disposed of by the mucocilliary clearance mechanism or the immune surveillance in the nasal cavity; their presence in the brain has been linked to the pathogenesis of diseases of the central nervous system, such as Alzheimer's disease (36, 37). Thus, the early expression of olfactory P450 may result in increased susceptibility to inhalation toxicity in several respects in neonatal mammalian species.

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References

1. Dahl, A. R., and W. M. Hadley. Nasal cavity enzymes involved in xenobiotic metabolism: effects on the toxicity of inhalants. *Crit. Rev. Toxicol.* **21**:345–372 (1991).
2. Ding, X., and M. J. Coon. Extrahepatic microsomal forms: olfactory cytochrome P450. *Handb. Exp. Pharmacol.* (1992).
3. Ding, X., D. R. Koop, B. L. Crump, and M. J. Coon. Immunochemical identification of cytochrome P-450 isozyme 3a (P-450alc) in rabbit nasal and kidney microsomes and evidence for differential induction by alcohol. *Mol. Pharmacol.* **30**:370–378 (1986).
4. Getchell, T. V., and M. L. Getchell. Peripheral mechanisms of olfaction: biochemistry and neurophysiology, in *Neurobiology of Taste and Smell* (T. E. Finger and W. L. Silver, eds.). John Wiley & Sons, New York, 91–123 (1987).
5. Dahl, A. R. The effect of cytochrome P-450-dependent metabolism and other enzyme activities on olfaction, in *Molecular Neurobiology of Olfaction* (F. L. Margolis and T. V. Getchell, eds.). Plenum, New York, 51–70 (1988).
6. Jenner, J., and G. H. Dodd. Xenobiotic metabolism in the nasal epithelia. *Drug Metab. Drug Interact.* **6**:123–148 (1988).
7. Ding, X., and M. J. Coon. Immunochemical characterization of multiple forms of cytochrome P-450 in rabbit nasal microsomes and evidence for tissue-specific expression of P450s NMa and NMb. *Mol. Pharmacol.* **37**:489–496 (1990).
8. Nef, P., T. M. Larabee, K. Kagimoto, and U. A. Meyer. Olfactory-specific cytochrome P-450 (P-450olf1; IIG1): gene structure and developmental regulation. *J. Biol. Chem.* **265**:2903–2907 (1990).
9. Lazard, D., K. Zupko, Y. Poria, P. Nef, J. Lazarovits, S. Horn, M. Khen, and D. Lancet. Odorant signal termination by olfactory UDP-glucuronosyl transferase. *Nature (Lond.)* **349**:790–793 (1991).
10. Ding, X., and M. J. Coon. Purification and characterization of two unique forms of cytochrome P-450 from rabbit nasal microsomes. *Biochemistry* **27**:8330–8337 (1988).
11. Ding, X., T. D. Porter, H.-M. Peng, and M. J. Coon. cDNA and derived amino acid sequence of rabbit nasal cytochrome P450NMb (P450IIG1), a unique isozyme possibly involved in olfaction. *Arch. Biochem. Biophys.* **285**:120–125 (1991).
12. Sabourin, P. J., R. E. Tynes, R. M. Philpot, S. Winquist, and A. R. Dahl. Distribution of microsomal monooxygenases in the rabbit respiratory tract. *Drug Metab. Dispos.* **16**:557–562 (1988).
13. Ding, X., and M. J. Coon. Cytochrome P-450-dependent formation of ethylene from *N*-nitrosoethylamines. *Drug Metab. Dispos.* **16**:265–269 (1988).
14. Laethem, R. M., C. L. Laethem, X. Ding, and D. R. Koop. P450-dependent arachidonic acid metabolism in rabbit olfactory microsomes. *J. Pharmacol. Exp. Ther.* **262**:433–438 (1992).
15. Coon, M. J., T. A. van der Hoeven, S. B. Dahl, and D. A. Haugen. Two forms of liver microsomal cytochrome P-450, P-450LM2 and P-450LM4 (rabbit liver). *Methods Enzymol.* **52**:109–117 (1978).
16. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265–275 (1951).
17. Omura, T., and R. Sato. The carbon monoxide-binding protein of liver microsomes. I. Evidence for its hemoprotein nature. *J. Biol. Chem.* **239**:2370–2378 (1964).
18. Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.)* **227**:680–685 (1970).
19. Towbin, H., T. Staehelin, and J. Gordon. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA* **76**:4350–4354 (1979).
20. Koop, D. R., B. L. Crump, G. D. Nordblom, and M. J. Coon. Immunochemical evidence for induction of the alcohol-oxidizing cytochrome P-450 of rabbit liver microsomes by diverse agents: ethanol, imidazole, trichloroethylene, acetone, pyrazole, and isoniazid. *Proc. Natl. Acad. Sci. USA* **82**:4065–4069 (1985).
21. Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **18**:5294–5299 (1979).
22. Porter, T. D., S. C. Khani, and M. J. Coon. Induction and tissue-specific expression of rabbit cytochrome P450IIE1 and IIE2 genes. *Mol. Pharmacol.* **36**:61–65 (1989).
23. Khani, S. C., T. D. Porter, V. S. Fujita, and M. J. Coon. Organization and differential expression of two highly similar genes in the rabbit alcohol-inducible cytochrome P-450 subfamily. *J. Biol. Chem.* **263**:7170–7175 (1988).
24. French, J. S. NADPH-cytochrome P-450 reductase: properties and interactions with other microsomal components. Ph.D. dissertation, University of Michigan, Ann Arbor, MI (1979).
25. Gassmann, M., P. Thommes, T. Weiser, and U. Hubscher. Efficient production of chicken egg yolk antibodies against a conserved mammalian protein. *FASEB J.* **4**:2528–2532 (1990).
26. Thomas, P. E., D. Korzeniowski, D. Ryan, and W. Levin. Preparation of monospecific antibodies against two forms of rat liver cytochrome P-450 and quantitation of these antigens in microsomes. *Arch. Biochem. Biophys.* **192**:524–532 (1979).
27. Peng, H.-M., T. D. Porter, X. Ding, and M. J. Coon. Differences in the developmental expression of rabbit cytochromes P-450 2E1 and 2E2. *Mol. Pharmacol.* **40**:58–62 (1991).
28. Ding, X., S. J. Pernecky, and M. J. Coon. Purification and characterization of cytochrome P450 2E2 from hepatic microsomes of neonatal rabbits. *Arch. Biochem. Biophys.* **291**:270–276 (1991).
29. Pineau, T., M. Daujat, L. Pichard, F. Girard, J. Angevain, C. Bonfils, and P. Maurel. Developmental expression of rabbit cytochrome P450 CYP1A1, CYP1A2 and CYP3A6 genes. *Eur. J. Biochem.* **197**:145–153 (1991).
30. Hudson, R., and H. Distel. Olfactory guidance of nipple-search behaviour in newborn rabbits, in *Ontogeny of Olfaction* (W. Breipohl, ed.). Springer-Verlag, Berlin, 243–254 (1986).
31. Coon, M. J., X. Ding, S. J. Pernecky, and A. D. N. Vaz. Cytochrome P450: progress and predictions. *FASEB J.* **6**:669–673 (1992).
32. Williams, D. E., X. Ding, and M. J. Coon. Rabbit nasal cytochrome P-450 NMa has high activity as a nicotine oxidase. *Biochem. Biophys. Res. Commun.* **166**:945–952 (1990).
33. Hong, J.-Y., X. Ding, T. Smith, M. J. Coon, and C. S. Yang. Metabolism of

tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) by rabbit nasal microsomes and P450s NMa and NMb. *Proc. Am. Assoc. Cancer Res.* **32**:120 (1991).
34. Putt, D., X. Ding, M. J. Coon, and P. F. Hollenberg. Cytochrome P-450-dependent metabolism of aflatoxin B1 by rabbit and rat nasal mucosa. *FASEB J.* **6**:A1569 (1992).
35. Ghantous, H., L. Dencker, J. Gabrielsson, B. R. G. Danielsson, and K. Bergman. Accumulation and turnover of metabolites of toluene and xylene in nasal mucosa and olfactory bulb in the mouse. *Pharmacol. Toxicol.* **66**:87–92 (1990).
36. Roberts, E. Alzheimer's disease may begin in the nose and may be caused by aluminosilicates. *Neurobiol. Aging* **7**:561–567 (1986).
37. Ferreyra-Moyano, H. The olfactory system and Alzheimer's disease. *Int. J. Neurosci.* **49**:157–197 (1989).

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