28. Nasal Periceptor Processes

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There are myriads of odorous molecules that we perceive and it is remarkable that most of us seem to have very similar odor impressions that originate from a specific stimulus and the sense of smell appears to be robust during much of a lifetime. When perceiving scents, olfactory receptor (OR) proteins are at work to translate chemical information into neuronal signals that are decoded in the olfactory cortex to provide us with an odor image. Proposed in the middle of the last century but only substantiated with intriguing laboratory data during the last decade, there are enzymes expressed at high levels in the olfactory mucosa, and they metabolize xenobiotics including odorants and produce many new chemical species. Examples demonstrate that such perireceptor events can alter the receptor-dependent activation pattern in the olfactory neuroepithelium, which has an impact on the quality and the intensity of odor stimuli. Results that do not seem to fit a model or a hypothesis may make sense if perireceptor events are brought into the equation.

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Our senses provide us with an internal representation of the outside world with a straight impact on our behavior and decision-making processes. While it is generally appreciated that sight and sound are crucial for our quality of life, the important role of the sense of smell is often forgotten and goes far beyond enjoying a subtle splash of a luxury perfume. The perception of fragrances is inevitably linked with joy, well-being, mood, emotions, memories, and both physiological and psychological reactions are responsible for the power of the sense of smell. There is a strong personal flavor to the perception of odors and learning, association, context as well as a genetic predisposition all contribute to a unique individuality for olfaction that is not observed for other senses, such as vision and audition. Evidence for a striking variability for the perception of β-ionone, a floral and woody odorant with a strong freesia character, was based on a large sensory study conducted during a flower show in New York City in 1935 [28.1]. The term *specific anosmia* describes the fact that many people are *odor blind* for specific molecules. The first thorough investigation was published in 1967 by *Amoore* who described anosmia for the sweat odorant isovaleric acid [28.2]. He expanded the studies to other odorants and also discovered that specific anosmia is genetically inherited [28.3, 4]. Today, it is generally known that even for perfumery ingredients, specific anosmia exists for instance for β-ionone, salicylates, musks, and amber odorants. Androstenone is an interesting body odor, that is perceived as unpleasant/urinous/sweaty or pleasant/sweet/floral or odorless, and it has been shown that a genetic variation in one human odorant receptor (OR) is responsible for the difference in odor perception [28.5]. Besides single-nucleotide polymorphisms, receptor gene copy number variation is a possible factor for phenotypic difference in odor perception [28.6]. There is some decrease in the performance of the sense of smell during aging which is generally not dramatic unless the cause is a neurodegenerative disease, such as Alzheimer and Parkinson where an impaired sense of smell is one of the earliest symptoms [28.7]. The decline in the ability to detect and discriminate odors in aged humans is not well understood, and latest investigations suggest

that it is multifactorial, and includes reduced neurogenesis, altered synaptic organization as well as modified odor representation in primary olfactory cortices and beyond [28.8].

Many excellent studies on the sense of smell have been based on sensory behavioral experiments, psychophysical measurements, and electrophysiological and anatomical investigations. A review in National Geographic Magazine in 1986 provides a superb overview on various aspects of olfaction that were known at the time and emphasizes that the mechanisms by which odorous molecules activate neurons that convey respective information to the brain were still to be elucidated [28.9]. An explosion of research in olfaction followed the discovery of the odorant receptor proteins in 1991 by *Buck* and *Axel* [28.10]. Olfactory receptor genes are the largest gene family in the human genome with close to 1000 genes that are scattered across most chromosomes. In humans, the majority of the receptor genes have been mutated to pseudogenes leaving us with close to 400 functional genes [28.5, 11–15]. Interestingly, segregating pseudogenes have been identified, indicating that different people may have a slightly different number of pseudogenes on top of the occurrence of various alleles for each of the functional olfactory receptor genes [28.16]. Investigating and understanding the olfactory gene expression in olfactory sensory neurons, signal transduction, receptor agonist patterns, axonal projections to glomeruli in the olfactory bulb and signal processing in the olfactory cortical areas are fascinating topics that are reviewed and explained in other chapters in this book. There is a chemotopic map preserved on the level of olfactory bulb, the first relay station in the brain [28.17, 18]. Information processing beyond the olfactory bulb toward the olfactory cortical areas is multifaceted and surprisingly, the activation patterns in the piriform cortex, the next processing center for olfactory information get much more complex. The same cortical neuron responds to diverse odorants and it appears that odorants are represented by unique ensembles of active neurons in the piriform cortex. When moving from single odorants to odor mixture, strong suppression and some minor synergistic effects are observed, demonstrating that the representation of complex odors in the piriform cortex is not the integration of individual activation patterns [28.19]. Previously, Buck et al. proposed that cortical neurons might be acting as *coincidence detectors* when receiving inputs that originate from more than one receptor. More recently, it has been elegantly demonstrated that neuronal information from the olfactory bulb is conveyed to multiple cortical centers where odor representation is differently organized. While activated neurons in the piriform cortex show no discernible spatial order, the representation in the cortical amygdala exhibits spatially stereotyped projections from olfactory bulb glomeruli which overlap and allow for the local integration of neuronal signals [28.20]. The authors propose a model, where the representation of activated ensembles of neurons in the piriform cortex is vital for experience and learned olfactory responses, while the odor representations in the cortical amygdala are linked to innate behavioral responses.

The activation of olfactory receptors by odorants at the periphery of the olfactory system is the essential step toward smell perception. Yet there is evidence that perireceptor events also contribute to the shape of the olfactory percept, and this understanding may be important to compare in vitro results derived from receptor screenings and in vivo psychophysical studies, such as the determination of odor detection thresholds, as well as the assignment of odor descriptors to fragrance molecules. It has been speculated for years that enzymes in the respiratory tract and in particular in the olfactory epithelium could have an impact on the perception of odorants. The initial hypothesis that enzymatic activities (or their inhibition) are involved in the nature of the sensation of smell was proposed by the chemist *G.B. Kistiakowsky* from Harvard University more than 60 years ago [28.21]:

> *On the Theory of Odors: I cannot resist the temptation to add on more hypothesis on the nature of the sensation of smell to speculation of others to this subject. Several characteristic traits of this sense can be accounted for without infringing on the basic physical principles if it is attributed to the inhibition of certain enzymes contained in the olfactory organs* [...].

He proposes that sequences of metabolism, on the one hand, and inhibition of enzymes on the other contribute to high impact odors, form the basis of the large collection of molecules having a smell and determines that the complexity of odors also results from the inhibition of various enzymes to different extents. Interestingly, he also proposes that such enzyme inhibition can change the quality of smell, a concept that will be revisited later in this chapter. Around the same time, enzymes were localized in and around the gustatory and olfactory organs of the rabbit, including the olfactory mucosa, and it was suggested that they may be associated in some way with the mechanisms of smell and taste [28.22].

The first evidence of the in-nose metabolism of a volatile compound was provided at the 6th International Symposium of Olfaction and Taste [28.23]. The scientists observed that upon channeling tritium-labeled octane through a frog's nose, some of the labeled mate-

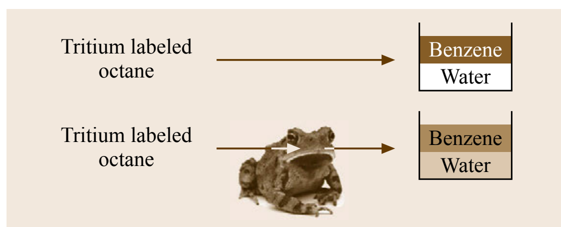

Fig. 28.1 Indication of in-nose oxidative metabolism (after [28.23])

rial became water soluble, and they speculated that the chemical got somehow transformed at the olfactory receptor site (Fig. 28.1). From today's perspective, it can be concluded that the nonwater-soluble alkane was oxidized by cytochrome P450 monooxygenases (CYPs) to produce the water soluble alcohol.

Octane is soluble in the organic solvent (benzene); however, following exposure to the olfactory tissue of a frog, some of the tritium-labeled material is soluble in the aqueous phase, indicating in-nose oxidative metabolism [28.23].

Only a few years later, it was demonstrated that high concentrations of CYPs and other enzymatic activities are located in the nasal cavity of animal species [28.25]. One paper was published showing that the fragrance material heliotropin (piperonal) inhibits rat nasal CYP activity and the author is putting forward the idea that part of the effectiveness of heliotropin as a perfumery ingredient may result from prolonging the half-life and residency time of other odorants in the nasal cavity by inhibiting their enzymatic oxidation and degradation [28.26]. An excellent review by *Alan Dahl* describes animal studies conducted in the 1980s to investigate whether nasal metabolism influences the biological fate and toxicity of inhaled materials as well as to address a potential impact on olfactory physiology [28.24]. Selected substrates and metabolites reported in this review and references cited therein are shown in Fig. 28.2 together with the original classification of olfactory mucosal enzymes.

It is further postulated that olfactory xenobiotic metabolizing enzymes might have an effect on the characteristic odors of compounds, and the author makes suggestions how to direct research efforts to provide data on the role of nasal metabolism in olfaction [28.24]. He lists five specific effects where xenobiotic-metabolizing enzymes may influence odor perception:

1. Conversion of a nonodorant into one or more odorants.
2. Conversion of odorants to nonodorants.
3. Transformation of odorants to other odorants (change in quality).
4. Transformation of lipophilic compounds into more water-soluble ones (change in physicochemical properties and elimination).
5. Inhibition of the metabolizing enzymes (may alter all the previous effects). These possibilities will be discussed in more detail in this chapter.

Another group of proteins which is prone to play a role in perireceptor events are the so-called odorant-binding proteins (OBPs) which are small soluble carrier proteins with binding activity toward volatile compounds [28.27]. These proteins belong to the family of lipocalins which are known to transport small ligands in other body fluids but their role in mammalian ol-

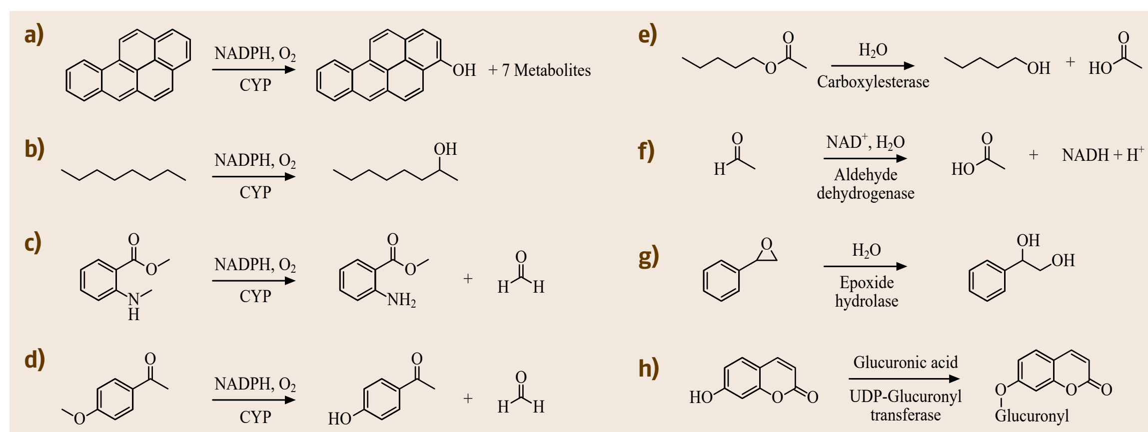

Fig. 28.2a–h Metabolic reactions catalyzed by olfactory mucosal enzymes (after [28.24]): **(a)** hydroxylation of benzo[a]pyrene, **(b)** hydroxylation of octane, **(c)** *N*-demethylation of dimethylanthranilate, **(d)** *O*-demethylation of *p*-methoxyacetophenone, **(e)** hydrolysis of amyl acetate, **(f)** oxidation of acetaldehyde, **(g)** hydrolysis of styrene oxide, **(h)** glucuronidation of 7-hydroxycoumarin (umbelliferone)

faction is still unclear. Proposed functions include the transport of the hydrophobic molecules from the nasal air space across the viscous, hydrophilic mucus layer to the ciliae of olfactory sensory neurons where receptor proteins are located, as well as a role as scavengers for excess odorants and removal of the stimuli. Since the studies on heterologously expressed olfactory receptor genes clearly indicated that they function in the absence of OBPs, their role in vertebrate olfaction is still unanswered. More evidence for the function of lipocalins in chemoreception was found for insects [28.28]. The first identified insect OBP was the pheromone binding protein (PBP) of the silk moth *Antheraea polyphemus* [28.29] shortly before the first mammalian OBP was described. Later, compelling evidence was provided that the *Drosophila* insect pheromone 11-*cis* vaccenyl acetate is requiring a specific OBP for the activation of pheromone-sensitive neurons [28.30] and it has been demonstrated that the detection of the *Drosophila* pheromone is mediated directly by the OBP upon a pheromone-induced conformational change [28.31]. Binding of the pheromone converts an inactive ligand into an activator of pheromone-sensitive neurons, which is in contrast to the general assumption that olfactory neurons are activated by volatile odorants as a result from the direct activation of the membrane-embedded receptor by the volatile stimulus.

It has been nicely demonstrated that OBP/PBP-enabled perireceptor events play an essential role in insect olfaction where a large repertoire of OBP genes are expressed; however, their role in mammalian olfaction is still not clear. Therefore, the upcoming sections are dedicated to review the current understanding on biotransformation reactions that take place in the olfactory mucosa with emphasis on the role of cytochrome P450 enzymes (CYPs) in nasal metabolism, and the potential impact of xenobiotic-metabolizing biochemical reactions for olfaction research.

28.1 Xenobiotic–Metabolizing Enzymes in the Olfactory Epithelium

Metabolism of xenobiotic molecules is primarily assigned to a role of hepatic phase-1 and phase-2 biotransformation enzymes. In phase-1 metabolism, molecules are made reactive and during phase-2 metabolism sugar or peptide moieties are added to make water soluble catabolites and allow excretion via urine. However, there has been strong evidence that xenobiotic metabolism is taking place outside the liver, and various reviews have described that biotransformation enzymes are found in the respiratory tract and in particularly high concentrations in the olfactory mucosa [28.32, 33]. In order to identify the enzyme families that are involved in xenobiotic metabolism, gene expression patterns were compared between human fetal and adult olfactory mucosa and liver specimens, using a combination of gene array analysis and ribonucleic acid polymerase chain reaction (RNA-PCR) [28.34]. A series of biotransformation enzymes which were identified in the nasal tissue are shown in Table 28.1. The family of CYPs is of specific interest, since they are involved in the phase-1 metabolism of very diverse chemical species. About one dozen CYP genes are expressed in the human olfactory mucosa and amongst them, CYP2A13 has been identified to be specifically expressed in the human respiratory tract, predominantly in the olfactory mucosa [28.35], therefore being a primary candidate to explore and test odorants as substrates or enzyme inhibitors.

Liver metabolic enzymes are known to be upregulated as a response to exposure to xenobiotic compounds, including drugs. However, the regulation of xenobiotic-metabolizing enzymes in the olfactory mucosa has been little explored. There are studies indicating an enhanced expression of specific genes following

Table 28.1 Metabolic enzymes that are expressed in the human nasal mucosa

Enzyme	References
Aldehyde dehydrogenase (ALDH6)	[28.34, 36]
Aldehyde dehydrogenase (ALDH7)	[28.34, 36]
Carboxyl esterase (CE)	[28.37, 38]
Cytochrome P450 monooxygenase (CYP1B1)	[28.34]
Cytochrome P450 monooxygenase (CYP2A6)	[28.32, 33]
Cytochrome P450 monooxygenase (CYP2A13)	[28.32, 33, 35]
Cytochrome P450 monooxygenase (CYP2B6)	[28.33]
Cytochrome P450 monooxygenase (CYP2C)	[28.33]
Cytochrome P450 monooxygenase (CYP2E1)	[28.34]
Cytochrome P450 monooxygenase (CYP2F1)	[28.34]
Cytochrome P450 monooxygenase (CYP2J2)	[28.33]
Cytochrome P450 monooxygenase (CYP2S1)	[28.39]
Cytochrome P450 monooxygenase (CYP3A)	[28.33]
Cytochrome P450 monooxygenase (CYP4B1)	[28.34]
Epoxide hydrolase (EH)	[28.36, 40]
Flavin-containing monoxygenase (FMO1)	[28.34]
Glutathion-*S*-transferase (GSTP1)	[28.34, 36, 40, 41]
Nicotinamide adenine dinucleotide phosphate (NADPH)-cytochrome P450 reductase (POR)	[28.32, 36]
Glucuronyl transferase (UGT2A1)	[28.34, 36, 42]

treatment with chemicals [28.43] and a recent publication reports that inducers known to regulate hepatic gene expression also worked in the rat olfactory mucosa and phase-1, phase-2, and transporter genes were up-regulated [28.44].

28.2 Cytochrome P450 Enzymes

CYPs are peripheral membrane proteins anchored to the membrane bilayer of smooth endoplasmic reticulum by their amino-terminal domain. An iron–heme cofactor is present in the catalytic center where the oxygen-dependent monooxygenation of suitable substrates takes place. For a full catalytic cycle, two electrons are required which are supplied via an electron-transfer system from an NADPH-cytochrome P450 reductase (POR) which is also membrane-anchored and in a close proximity to the CYP. NADPH is the ultimate electron donor, and electrons are channeled via two flavin cofactors (flavin adenine dinucleotide (FAD), flavin adenine mononucleotide (FMN)) in POR to the catalytic center of the CYP. The overall reaction is shown in Fig. 28.3 and further described, for instance, in the following reviews [28.45, 46].

A comprehensive overview on classical and particularly uncommon CYP-catalyzed reactions has been published by *Guengerich* [28.47]. This family of enzymes shows low substrate specificity and frequently produces multiple products. The availability of several crystal structures of human CYPs allows rationalizing the fate of substrates and the binding site of inhibitors.

Pharmacological metabolism research and the role of CYPs in chemical toxicology have been the area of strong interest. Various excellent reviews describe recent developments in metabolism studies and safety testing, adverse effects of drugs through biotransformation, and bioactivation of chemicals. Besides identifying metabolites of active pharmaceutical ingredients (APIs) and determining the pharmacogenetics and clearance of drugs, various groups also investigated the role of CYP polymorphisms in the onset and progression of cancer and the role of genetic variability in human CYP genes [28.48–50]. It is remarkable that nasal cytotoxicity and carcinogenic activities are originating from systemically distributed organic chemicals, confirming the metabolic power of the nasal mucosa [28.51]. Several publications conclude that the respiratory tract CYP2A enzymes and particularly CYPA13 play a role in the metabolic activation of nasal toxicants [28.52] and are involved in the bioactivation of tobacco-specific nitrosamines [28.35, 53–57]. Genetic polymorphism of *Cyp2a13* can be linked to lung cancer susceptibility [28.58–64] and CYP enzymes have been mentioned as potential targets for chemoprevention of lung cancer by the use of selective inhibitors (see the following paragraphs). Active site mutations of CYP2A13 influence the orientation and results in altered kinetics for metabolite formation which can be rationalized by docking studies using the CYP2A13 crystal structure [28.65].

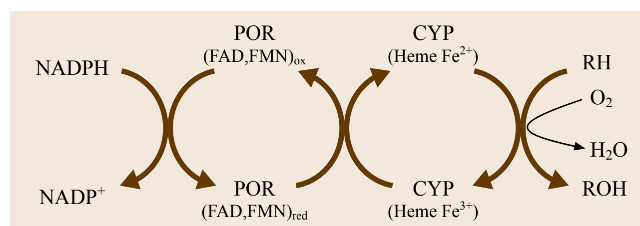

Fig. 28.3 Substrate oxidation by CYP with the concomitant reduction of oxygen to water. Electron transfer from NADPH is taking place via two flavin cofactors (FAD, FMN) which are present in POR. The iron-heme cofactor is the site of chemical oxidation in CYP

28.3 Exploring the Substrate and Inhibitor Range of Olfactory P450 Enzymes

CYP members of family 2 are strongly expressed in nasal tissue, and also known to bind small molecular weight compounds as substrates and inhibitors, including volatile odorants. Studies were conducted with various commercially available CYP sources; however, the respiratory tract-specific CYP2A13 was selected as the primary candidate for further investigations and produced from *Spodoptera frugiperda* cell line 9 (Sf9) insect cells together with the reductase partner POR [28.66, 67]. A library of odorant molecules was used to identify substrates of CYP2A13. For many molecules, a molecular weight increase [M+16] was

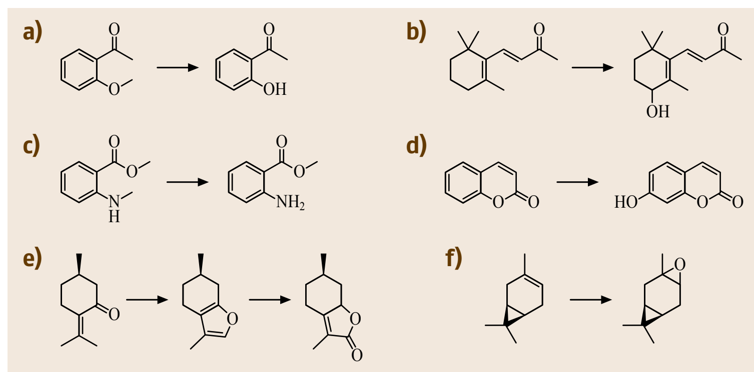

Fig. 28.4 CYP2A13-catalyzed oxidation of odorants: **(a)** demethylation of 2-methoxyacetophenone, **(b)** allylic hydroxylation of β-ionone, **(c)** demethylation of dimethylanthranilate, **(d)** hydroxylation of coumarin, **(e)** oxidation and cyclization of (*R*)-(+)-pulegone to menthofuran and further oxidation to mintlactone, **(f)** epoxidation of delta-3-carene

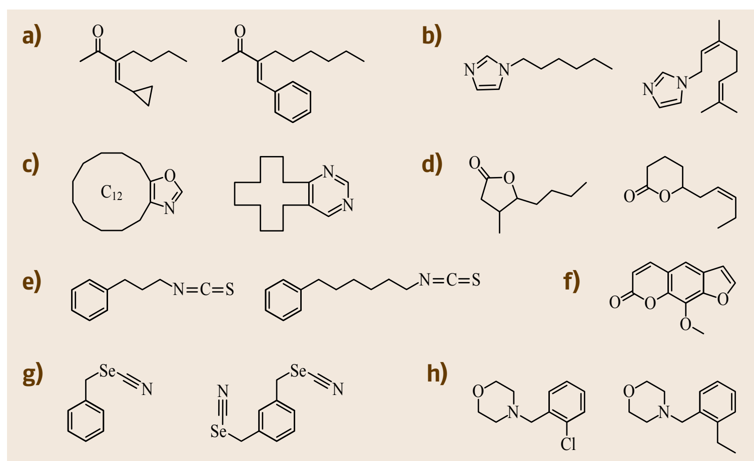

Fig. 28.5 Inhibitors of CYP2A13 exhibiting IC50 values in the low µM range: **(a)** ketone types, **(b)** *N*-heterocycles, **(c)** macrocyclic heterocycles, **(d)** lactones, **(e)** isothiocyanates, **(f)** 8-methoxypsoralen, **(g)** organoselenium types, **(h)** benzylmorpholine types

found indicating a monooxygenation reaction (either hydroxylation or epoxidation). Furthermore, demethylation of methoxy- and *N*-methyl groups was observed. A selection of odorants that are metabolized by CYP2A13 is shown in Fig. 28.4.

Two examples of CYP substrates with available odor intensity and quality data are shown in Table 28.2. In the case of methoxyphenylbutanone (Ketanone), the metabolite is the powerful raspberry ketone, whereas in the case of dimethylanthranilate, the metabolite has a slightly lower threshold and distinct but small differences in the odor description. Depending on the extent of nasal metabolism, one is never smelling the substrate alone, but always a combination of the substrate and the metabolite, which may differ between individuals.

Libraries of diverse chemical classes of small molecular weight compounds were screened for inhibitors of CYP2A13, CYP2A6, and CYP2B6 enzymes [28.68–75]. Chemically diverse inhibitors as

Table 28.2 Odor thresholds and qualities of selected substrate–metabolite pairs

Substrate		Metabolite	
	OTH: 0.05 ng/l Odor description: raspberry, floral, green		OTH: 0.005 ng/l Odor description: raspberry, fruity, sweet
	OTH: 0.59 ng/l Odor description: floral, neroli, sweet, warm		OTH: 0.12 ng/l Odor description: floral, orange blossom, neroli, mandarin

OTH: Odor detection threshold in ng/l air, determined using an olfactometer

well as various substrates were identified, further confirming that the respiratory tract-expressed enzymes CYP2A13 and 2A6 are able to catalyze detoxification as well as metabolic activation reactions of environmental molecules and are also subject to inhibition by xenobiotic compounds. Selected examples of CYP2A13 inhibitors having half minimal inhibitory concentration (IC50) values in the low micromolar range are shown in Fig. 28.5. Different small molecular weight inhibitors have recently been evaluated for their selectivity toward human CYP2A enzymes [28.76–78]. Odors are generally blends of a series of volatile molecules which activate ORs. However, all of them can also be substrates or inhibitors of metabolic enzymes. Odorants which are also CYP inhibitors will reduce the metabolism of other odorants, which can impact the intensity or the quality of odors.

The earliest demonstration of nasal bioactivation took place using insecticides and herbicides. The herbicide 2,6-dichlorobenzonitrile (DCBN) was known to cause tissue-specific toxicity at very low doses in the olfactory mucosa of rodents. Amongst all tested heterologously expressed CYP variants, the 2A subfamily showed strong activity toward DCBN [28.79]. It has recently been shown that the bioactivation of DCBN is also catalyzed by human nasal mucosa microsomes [28.80]. The study was run in parallel using wild type or *Cyp2a5*-null mice (with CYP2A5 being the mouse ortholog of human nasal CYPs 2A13/2A6) demonstrating strong olfactory tissue-specific and CYP-dependent bioactivation of systemically applied DCBN. Metabolites were identified in nasal-wash fluid and these results are particularly interesting, since they demonstrate that products that originate from metabolism in the olfactory sustentacular cells are secreted into the nasal mucus, where metabolites could act as ligands of olfactory receptor proteins, either as agonists or antagonists.

28.4 Evidence for the Role of Biotransformation Enzymes in Olfaction from Animal Studies

It is generally assumed that odorant identity is represented in the chemotopic map by the glomerular activation pattern (see also Chap. 27). *Touhara* et al. reported that there are differences between OR-derived glomerular activation in the olfactory bulb (OB) and response patterns derived from in vitro assays [28.81]. For instance, only modest or no responses were observed for a mouse olfactory receptor protein (mOR-EG) in olfactory glomeruli following exposure of the animal's olfactory system to vanillin, although this odorant was shown to be a potent agonist of mOR-EG in isolated olfactory sensory neurons, as well as in the human embryonic kidney cell line 293 (HEK293) expressing mOR-EG. Most interestingly, it was reported that the nasal olfactory mucus influences the responsiveness to some but not all odorants indicating that some metabolic enzymes appear to be present in the mucus that is surrounding the ciliae where the olfactory receptor proteins are embedded. Later, the same group demonstrated that the enzymatic conversion of odorants in the nasal mucus affects both the olfactory glomerular activation patterns and odor perception in mice [28.82]. It was presented that mucus-secreted enzymes oxidized aldehydes to the corresponding acids and hydrolyzed esters; and that selected inhibitors reduced the metabolism of the odorants. The effect of metabolism taking place at the periphery was shown to influence the pattern of glomerular responses in the olfactory bulb as monitored by calcium imaging. The final study aimed to demonstrate that the enzymatic conversion of odorants in the mucus effects perception. Mice trained to recognize the ester acetyl isoeugenol showed a clear deficit to recognize the target odorant when treated with a carboxylesterase inhibitor, while they behaved no different to the control group when exploring odorants that were not metabolized [28.82]. This study elucidated for the first time that modulated peripheral metabolism in the olfactory epithelium is manifested in the first relay station in the brain, and is influencing the perception and behavior of the animal.

A study in rats further investigated the role of xenobiotic-metabolizing enzymes in the olfactory mucosa including activities of enzymes that are not secreted into the mucus [28.83]. The two CYP substrates coumarin and quinoline, as well as the carboxylesterase substrate isoamyl acetate were investigated. CYPs produced hydroxylated metabolites, while esterase activity resulted in isoamyl alcohol and acetic acid. Electro-olfactogram (EOG) recordings on the olfactory epithelium allowed to determine the activation of olfactory sensory neurons by either substrates or metabolites. When identified metabolites were tested separately in control experiments, the EOG responses were generally lower and weaker amplitudes were recorded, indicating that metabolites are less efficient agonists. In order to determine the functional role of olfactory metabolic enzymes, EOG studies were run in the presence of CYP- or carboxyl esterase-specific inhibitors which in-

hibit the enzymes as demonstrated in in vitro assays. Interestingly, in all cases the recorded EOG signal increased when using specific inhibitors, while in controls where the substrate and the inhibitor were not targeting the same enzymatic activity, no effects were observed [28.83]. This study revealed that peripheral olfactory responses are modulated by enzymes that are located in sustentacular cells.

28.5 Human Sensory Studies

While studies with human beings must be less invasive than the above described studies, several investigations have demonstrated that the respiratory tract-specific metabolism of volatiles is fast and can influence odor perception. The human olfactory mucosa has a very high metabolic activity, and in particular, CYP2A13 acts to oxidize a broad range of substrates and is itself subject to inhibition by small molecular weight compounds.

Two approaches allowed to monitor in vivo formation of metabolites [28.84]. In one case, a mass spectrometer was used to analyze exhaled air in realtime. Saturated headspace of the odorant 2-methoxyacetophenone was inhaled, and the breath exhaled into a glass funnel that was connected to a quadrupole mass spectrometer equipped with an atmospheric pressure chemical ionization (APCI) ion source. Exhaled breath was monitored over several minutes, and the metabolite 2-hydroxyacetophone was already detectable in the first exhalation cycle [28.67]. A second approach enabled better quantification of metabolites, and exhaled breath was captured on a resin, followed by thermal desorption and analysis by gas chromatography-mass spectrometry (GC-MS), where metabolite formation was monitored, for example, for the CYP substrate 2-methoxyacetophenone, or the carboxylesterase substrate styrallyl acetate [28.84] as shown in Fig. 28.6.

Intensity rating is more challenging for panelists than detecting a change in olfactive character. While the former was successfully done for fragrance accords [28.69] the latter demonstration was important to provide evidence that mucosal biotransformation of odorants can impact the olfactory percept. During the substrate screening for CYP2A13 (see above) a metabolite was identified by GC-sniff analysis that had a strong, characteristic raspberry odor, while the substrate is commonly described as woody, fruity with raspberry aspects. The hydroxylated metabolite was isolated, its structure elucidated, and a reference material synthesized to confirm the characteristic raspberry smell of this molecule (Fig. 28.7). In order to determine if indeed that substrate is *woody*, *fruity*, *raspberry*, or if the raspberry note originates from the formation of the metabolite, a volatile odorless inhibitor was selected and used in a sensory experiment. A miniaturized olfactometer was used where the substrate was present in one channel, and the inhibitor in a second one, and a panelist could smell the odorous substrate, the odorless inhibitor, or a combination of the two by switching the pressure control valves. The majority of panelists reported that the raspberry note was reduced or completely eliminated when smelling the inhibitor together with the odor stimulus [28.67, 69]. During the study, some panelists reported that they could only identify a woody smell, while a few individuals described that odor as fruity/raspberry but without any woody facets; a possible explanation is that these panelists are hypo- or hyper-metabolizers of the substrate. This one sensory demonstration further supports the role of biotransformation enzymes as a perireceptor event that contributes to odor perception.

When designing and synthesizing novel odorants, fragrance chemists are building olfactophore models in analogy to pharmacophores, and the question is to what extent metabolism in the olfactory neuroepithelium needs to be considered in such studies to strengthen the model. The above example demonstrates that one needs to know the hydroxylated ketone metabolite to correlate the structure with other odorants that are described as having a raspberry odor. An interesting case is the search for novel green and fruity odorants,

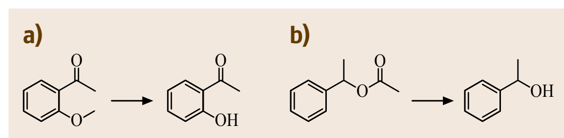

Fig. 28.6a,b Two different odorants were inhaled and exhaled breath was analyzed by real-time mass spectrometer analysis **(a)**, or by trapping on a resin followed by thermal desorption and analysis by GC-MS **(a,b)**

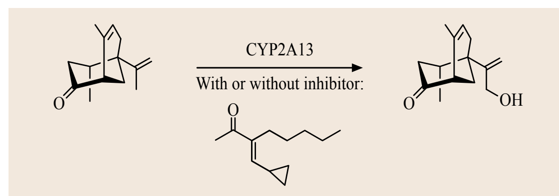

Fig. 28.7 The ketone substrate is olfactively described as woody and raspberry, while the hydroxylated metabolite has a pure raspberry note

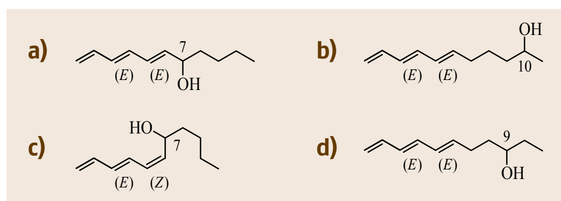

Fig. 28.8a–d Green smelling metabolites of undecatriene, which are produced by CYP2E1. Olfactory descriptions: **(a)** green, floral, metallic, **(b)** undecatriene-like, fatty, green, metallic, **(c)** green, pineapple, **(d)** fatty, oily, green, undecatriene-like, pineapple, fruity, metallic

starting from the signature hydrocarbon odorant 1,3,5-undecatriene which is found in galbanum oil. Series of molecules were synthesized over the years and an olfactophore model was developed that worked well for most molecules, but not for undecatriene that lacks a hydrogen-bonding function, which is present in other prototypic fruity, galbanum-type odorants, and it was speculated that enzymatic oxidation could take place prior to receptor interaction [28.85]. Several CYPs were tested with undecatriene as substrates, and in particular CYP2E1 which is expressed in the human olfactory mucosa produces multiple metabolites, that are described as having a fruity, green, and galbanum smell [28.67, 86] (Fig. 28.8). While the identified metabolites fit the galbanum olfactophore model much better, there is no experimental evidence to date that 1,3,5-undecatriene does not have those odor characteristics.

28.6 Discussion

At first glance, one may assume that deciphering the *olfactive code* is mastered when determining the molecular receptive range of the repertoire of roughly 380 different olfactory receptor proteins that convert the chemical information of odorants into neuronal signals and chemotopic maps in the olfactory bulb. However, recent results clearly demonstrate that the sense of smell is more complex than anticipated and is going to stay a fascinating area of research for many years to come. Latest studies demonstrate that the transformation and coding of neuronal activation patterns in the olfactory cortex is multifaceted and it has been proposed that different cortical areas are involved in learned versus innate behavioral responses providing another scientific approach to investigate emotional components of odor perception [28.20]. Olfactory receptor research has gained much interest in the last two decades, because of their discovery in 1991 and generally because of all the advancements in G-protein coupled receptor (GPCR) research, such as functional, heterologous expression and the growing number of crystal structures that are available for modeling and rationalizing agonist and antagonist interactions in the ligand binding domain of receptor proteins.

The fact that xenobiotic-metabolizing enzymes occur in high concentrations in the olfactory mucosa made scientists speculate on their role in olfaction since the middle of the last century. Recent animal studies demonstrated that inhibiting specific enzymatic activities in the olfactory mucosa changes the olfactory receptor response, the activation pattern in the olfactory bulb and even animal behavior [28.82]. Together with other data reported in this chapter, one can conclude that indeed, the in-nose biotransformation of odorants can modify the quantity (intensity) and the quality of odor stimuli. Since we are normally exposed to complex scents, such biochemistry has little impact on our perception of odors; however, when correlating olfactory receptor response patterns with the hedonics of an odorant this perireceptor event may well play a role and should not be neglected, and ideally one could include a metabolic interface that is mimicking the biotransformation events. When using receptor screening data to predict novel odorants for the fragrance industry, a solid understanding of the structure–activity and the structure–odor relationship is critical, and future activities in cheminformatics and computer modeling will help further valorize the growing number of datasets.

The destiny of xenobiotics that reach the olfactory epithelium can be manifold: receptor agonists and antagonists, enzyme substrates and inhibitors, OBP-ligands, precursors of bioactive compounds and allosteric modulators of receptors, enzymes, and other targets in the signal transduction cascade, such as the ion channels that are exposed to the mucus. The CYP2 family, and in particular the CYP2A subfamily of enzymes shows strong activity toward volatile organic molecules, and does not only oxidize odorants, but is also involved in the activation of nasal toxicants and carcinogens, and those enzymes have been proposed as pharmaceutical targets. There is evidence that olfactory biotransformation enzyme concentrations are regulated on the gene transcription level by chemicals acting as inducers of gene expression. This indicates a chance for plasticity and fast adaptation to the environment which impacts the metabolic capacity of this tissue. It is interesting to mention that the olfactory neuroepithelium is constantly regenerating and, in a way, it is remarkable that our sense of smell does not change much as a function of time.

As it invariably happens in scientific research, when answering one question, two more questions arise. Our current understanding on the code of smell advanced significantly over the last two decades and there is still much to learn and to be discovered. Hypotheses, including the ones expressed in this chapter, will be proven incomplete and there is still much incentive to further investigate chemoreception and in particular olfaction.

References

28.1 A.F. Blakeslee: Demonstration of differences between people in the sense of smell, Sci. Mon. **41**, 72–84 (1935)
28.2 J.E. Amoore: Specific anosmia: A clue to the olfactory code, Nature **214**, 1095–1098 (1967)
28.3 J.E. Amoore: Evidence for the chemical olfactory code in man, Ann. NY Acad. Sci. **237**, 137–143 (1974)
28.4 J.E. Amoore: Specific anosmia and the concept of primary odors, Chem. Senses Flavor **2**, 267–281 (1977)
28.5 A. Keller, H. Zhuang, Q. Chi, L.B. Vosshall, H. Matsunami: Genetic variation in a human odorant receptor alters odour perception, Nature **449**, 468–472 (2007)
28.6 Y. Hasin-Brumshtein, D. Lancet, T. Olender: Human olfaction: From genomic variation to phenotypic diversity, Trends Genet. **25**, 178–184 (2009)
28.7 R.L. Doty: Olfaction in Parkinson's disease and related disorders, Neurobiol. Dis. **46**, 527–552 (2012)
28.8 A.S. Mobley, D.J. Rodriguez-Gil, F. Imamura, C.A. Greer: Aging in the olfactory system, Trends Neurosci. **37**(2), 77–84 (2013)
28.9 B. Gibbons: The intimate sense of small, Natl. Geogr. Mag. **170**, 324–361 (1986)
28.10 L. Buck, R. Axel: A novel multigene family may encode odorant receptors: A molecular basis for odor recognition, Cell **65**, 175–187 (1991)
28.11 C.H. Wetzel, M. Oles, C. Wellerdieck, M. Kuczkowiak, G. Gisselmann, H. Hatt: Specificity and sensitivity of a human olfactory receptor functionally expressed in human embryonic kidney 293 cells and Xenopus Laevis oocytes, J. Neurosci. **19**, 7426–7433 (1999)
28.12 M. Spehr, G. Gisselmann, A. Poplawski, J.A. Riffell, C.H. Wetzel, R.K. Zimmer, H. Hatt: Identification of a testicular odorant receptor mediating human sperm chemotaxis, Science **299**, 2054–2058 (2003)
28.13 G. Sanz, C. Schlegel, J.C. Pernollet, L. Briand: Comparison of odorant specificity of two human olfactory receptors from different phylogenetic classes and evidence for antagonism, Chem. Senses **30**, 69–80 (2005)
28.14 K. Schmiedeberg, E. Shirokova, H.P. Weber, B. Schilling, W. Meyerhof, D. Krautwurst: Structural determinants of odorant recognition by the human olfactory receptors OR1A1 and OR1A2, J. Struct Biol. **159**, 400–412 (2007)
28.15 H. Saito, Q. Chi, H. Zhuang, H. Matsunami, J.D. Mainland: Odor coding by a Mammalian receptor repertoire, Sci. Signal **2**, ra9 (2009)
28.16 I. Menashe, O. Man, D. Lancet, Y. Gilad: Different noses for different people, Nat. Genet. **34**, 143–144 (2003)
28.17 P. Mombaerts, F. Wang, C. Dulac, S.K. Chao, A. Nemes, M. Mendelsohn, J. Edmondson, R. Axel: Visualizing an olfactory sensory map, Cell **87**, 675–686 (1996)
28.18 B.A. Johnson, M. Leon: Chemotopic odorant coding in a mammalian olfactory system, J. Comp. Neurol. **503**, 1–34 (2007)
28.19 D.D. Stettler, R. Axel: Representations of odor in the piriform cortex, Neuron **63**, 854–864 (2009)
28.20 D.L. Sosulski, M.L. Bloom, T. Cutforth, R. Axel, S.R. Datta: Distinct representations of olfactory information in different cortical centres, Nature **472**, 213–216 (2011)
28.21 G.B. Kistiakowsky: On the theory of odors, Science **112**, 154–155 (1950)
28.22 A.F. Baradi, G.H. Bourne: Localization of gustatory and olfactory enzymes in the rabbit, and the problems of taste and smell, Nature **168**, 977–979 (1951)
28.23 D.E. Hornung, M.M. Mozell: Preliminary data suggesting alteration of odorant molecules by interaction with receptors, Olfact. Taste, Vol. VI, ed. by J. Le Magnen, P. MacLeod (1977) p. 63
28.24 A.R. Dahl: The effect of cytochrome P450-dependent metabolism and other enzyme activities on olfaction. In: *Molecular Neurobiology of the Olfactory System*, ed. by F.L. Margolis, T.V. Getchell (Plenum, New York 1988) pp. 51–70
28.25 A.R. Dahl, W.M. Hadley, F.F. Hahn, J.M. Benson, R.O. McClellan: Cytochrome P-450-dependent monooxygenases in olfactory epithelium of dogs: Possible role in tumorigenicity, Science **216**, 57–59 (1982)
28.26 A.R. Dahl: The inhibition of rat nasal cytochrome P-450-dependent mono-oxygenase by the essence heliotropin (piperonal), Drug Metab. Dispos. **10**, 553–554 (1982)
28.27 P. Pelosi: The role of perireceptor events in vertebrate olfaction, Cell Mol. Life Sci. **58**, 503–509 (2001)
28.28 R.G. Vogt: Biochemical diversity of odor detection: OBPs, ODEs and SNMPs. In: *Insect Pheromone Biochemistry and Molecular Biology*, ed. by G.J. Blomquist, R.G. Vogt (Elsevier, London 2003) pp. 391–446
28.29 R.G. Vogt, L.M. Riddiford: Pheromone binding and inactivation by moth antennae, Nature **293**, 161–163 (1981)
28.30 P. Xu, R. Atkinson, D.N. Jones, D.P. Smith: Drosophila OBP LUSH is required for activity of pheromone-sensitive neurons, Neuron **45**, 193–200 (2005)
28.31 J.D. Laughlin, T.S. Ha, D.N. Jones, D.P. Smith: Activation of pheromone-sensitive neurons is mediated by conformational activation of pheromone-binding protein, Cell **133**, 1255–1265 (2008)
28.32 X. Ding, A.R. Dahl: Olfactory mucosa: Composition, enzymatic localization and metabolism. In: *Hand-*

book of Olfaction and Gustation, Vol. 2, ed. by R.L. Doty (Marcel Dekker, New York 2003) pp. 51–73

28.33 X. Ding, L.S. Kaminsky: Human extrahepatic cytochromes P450: Function in xenobiotic metabolism and tissue-selective chemical toxicity in the respiratory and gastrointestinal tracts, Annu. Rev. Pharmacol. Toxicol. **43**, 149–173 (2003)

28.34 X. Zhang, Q.Y. Zhang, D. Liu, T. Su, Y. Weng, G. Ling, Y. Chen, J. Gu, B. Schilling, X. Ding: Expression of cytochrome p450 and other biotransformation genes in fetal and adult human nasal mucosa, Drug. Metab. Dispos. **33**, 1423–1428 (2005)

28.35 T. Su, Z. Bao, Q.Y. Zhang, T.J. Smith, J.Y. Hong, X. Ding: Human cytochrome P450 CYP2A13: Predominant expression in the respiratory tract and its high efficiency metabolic activation of a tobacco-specific carcinogen, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, Cancer Res. **60**, 5074–5079 (2000)

28.36 P.G. Gervasi, V. Longo, F. Naldi, G. Panattoni, F. Ursino: Xenobiotic-metabolizing enzymes in human respiratory nasal mucosa, Biochem. Pharmacol. **41**, 177–184 (1991)

28.37 J.L. Lewis, K.J. Nikula, R. Novak, A.R. Dahl: Comparative localization of carboxylesterase in F344 rat, beagle dog, and human nasal tissue, Anat. Rec. **239**, 55–64 (1994)

28.38 M.S. Bogdanffy, M.L. Taylor, D.R. Plowchalk: Metabolism of vinyl acetate by human nasal tissues using a mini vapor uptake technique, Toxicologist **15**, 6 (1995)

28.39 S.T. Saarikoski, H.A. Wikman, G. Smith, C.H. Wolff, K. Husgafvel-Pursiainen: Localization of cytochrome P450 CYP2S1 expression in human tissues by in situ hybridization and immunohistochemistry, J. Histochem. Cytochem. **53**, 549–556 (2005)

28.40 T. Green, R. Lee, A. Toghill, S. Meadowcroft, V. Lund, J. Foster: The toxicity of styrene to the nasal epithelium of mice and rats: Studies on the mode of action and relevance to humans, Chem. Biol. Interact. **137**, 185–202 (2001)

28.41 N.S. Krishna, T.V. Getchell, N. Dhooper, Y.C. Awasthi, M.L. Getchell: Age- and gender-related trends in the expression of glutathione S-transferases in human nasal mucosa, Ann. Otol. Rhinol. Laryngol. **104**, 812–822 (1995)

28.42 G. Jedlitschky, A.J. Cassidy, M. Sales, N. Pratt, B. Burchell: Cloning and characterization of a novel human olfactory UDP-glucuronosyltransferase, Biochem. J. **340**, 837–843 (1999)

28.43 X.X. Ding, M.J. Coon: Induction of cytochrome P-450 isozyme 3a (P-450IIE1) in rabbit olfactory mucosa by ethanol and acetone, Drug Metab. Dispos. **18**, 742–745 (1990)

28.44 N. Thiebaud, M. Sigoillot, J. Chevalier, Y. Artur, J.M. Heydel, A.M. Le Bon: Effects of typical inducers on olfactory xenobiotic-metabolizing enzyme, transporter, and transcription factor expression in rats, Drug Metab. Dispos. **38**, 1865–1875 (2010)

28.45 B. Meunier, S.P. de Visser, S. Shaik: Mechanism of oxidation reactions catalyzed by cytochrome p450 enzymes, Chem. Rev. **104**, 3947–3980 (2004)

28.46 F.P. Guengerich: Mechanisms of cytochrome P450 substrate oxidation: MiniReview, J. Biochem. Mol. Toxicol. **21**, 163–168 (2007)

28.47 F.P. Guengerich: Common and uncommon cytochrome P450 reactions related to metabolism and chemical toxicity, Chem. Res. Toxicol. **14**, 611–650 (2001)

28.48 F.P. Guengerich: Cytochrome p450 and chemical toxicology, Chem. Res. Toxicol. **21**, 70–83 (2008)

28.49 M. Ingelman-Sundberg: Pharmacogenetics of cytochrome P450 and its applications in drug therapy: The past, present and future, Trends Pharmacol. Sci. **25**, 193–200 (2004)

28.50 U.M. Zanger, M. Schwab: Cytochrome P450 enzymes in drug metabolism: Regulation of gene expression, enzyme activities, and impact of genetic variation, Pharmacol. Ther. **138**, 103–141 (2013)

28.51 A.M. Jeffrey, M.J. Iatropoulos, G.M. Williams: Nasal cytotoxic and carcinogenic activities of systemically distributed organic chemicals, Toxicol. Pathol. **34**, 827–852 (2006)

28.52 C. Liu, X. Zhuo, F.J. Gonzalez, X. Ding: Baculovirus-mediated expression and characterization of rat CYP2A3 and human CYP2a6: role in metabolic activation of nasal toxicants, Mol. Pharmacol. **50**, 781–788 (1996)

28.53 S.S. Hecht, J.B. Hochalter, P.W. Villalta, S.E. Murphy: 2′-Hydroxylation of nicotine by cytochrome P450 2A6 and human liver microsomes: Formation of a lung carcinogen precursor, Proc. Natl. Acad. Sci. USA **97**, 12493–12497 (2000)

28.54 V. Megaraj, X. Zhou, F. Xie, Z. Liu, W. Yang, X. Ding: Role of CYP2A13 in the bioactivation and lung tumorigenicity of the tobacco-specific lung procarcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone: In vivo studies using a CYP2A13-humanized mouse model, Carcinogenesis **35**, 131–137 (2014)

28.55 Y. Weng, C. Fang, R.J. Turesky, M. Behr, L.S. Kaminsky, X. Ding: Determination of the role of target tissue metabolism in lung carcinogenesis using conditional cytochrome P450 reductase-null mice, Cancer Res. **67**, 7825–7832 (2007)

28.56 S.S. Hecht: Progress and challenges in selected areas of tobacco carcinogenesis, Chem. Res. Toxicol. **21**, 160–171 (2008)

28.57 H. Wang, W. Tan, B. Hao, X. Miao, G. Zhou, F. He, D. Lin: Substantial reduction in risk of lung adenocarcinoma associated with genetic polymorphism in CYP2A13, the most active cytochrome P450 for the metabolic activation of tobacco-specific carcinogen NNK, Cancer Res. **63**, 8057–8061 (2003)

28.58 X. Zhang, T. Su, Q.Y. Zhang, J. Gu, M. Caggana, H. Li, X. Ding: Genetic polymorphisms of the human CYP2A13 gene: Identification of single-nucleotide polymorphisms and functional characterization of an Arg257Cys variant, J. Pharmacol. Exp. Ther. **302**, 416–423 (2002)

28.59 C. Cauffiez, J.M. Lo-Guidice, S. Quaranta, D. Allorge, D. Chevalier, S. Cenee, R. Hamdan, M. Lhermitte, J.J. Lafitte, C. Libersa, J.F. Colombel, I. Stucker, F. Broly: Genetic polymorphism of the human cy-

tochrome CYP2A13 in a French population: Implication in lung cancer susceptibility, Biochem. Biophys. Res. Commun. **317**, 662–669 (2004)
28.60 K.E. Schlicht, N. Michno, B.D. Smith, E.E. Scott, S.E. Murphy: Functional characterization of CYP2A13 polymorphisms, Xenobiotica **37**, 1439–1449 (2007)
28.61 X. Zhang, J. D'Agostino, H. Wu, Q.Y. Zhang, L. von Weymarn, S.E. Murphy, X. Ding: CYP2A13: Variable expression and role in human lung microsomal metabolic activation of the tobacco-specific carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, J. Pharmacol. Exp. Ther. **323**, 570–578 (2007)
28.62 J. D'Agostino, X. Zhang, H. Wu, G. Ling, S. Wang, Q.Y. Zhang, F. Liu, X. Ding: Characterization of CYP2A13*2, a variant cytochrome P450 allele previously found to be associated with decreased incidences of lung adenocarcinoma in smokers, Drug Metab. Dispos. **36**, 2316–2323 (2008)
28.63 K.E. Schlicht, J.Z. Berg, S.E. Murphy: Effect of CYP2A13 active site mutation N297A on metabolism of coumarin and tobacco-specific nitrosamines, Drug Metab. Dispos. **37**, 665–671 (2009)
28.64 M.N. Timofeeva, S. Kropp, W. Sauter, L. Beckmann, A. Rosenberger, T. Illig, B. Jager, K. Mittelstrass, H. Dienemann, H. Bartsch, H. Bickeboller, J.C. Chang-Claude, A. Risch, H.E. Wichmann: CYP450 polymorphisms as risk factors for early-onset lung cancer: Gender-specific differences, Carcinogenesis **30**, 1161–1169 (2009)
28.65 B.D. Smith, J.L. Sanders, P.R. Porubsky, G.H. Lushington, C.D. Stout, E.E. Scott: Structure of the human lung cytochrome P450 2A13, J. Biol. Chem. **282**, 17306–17313 (2007)
28.66 B. Schilling: Metabolic method to identify compounds, Patent WO 2006/7751 A3 (2006)
28.67 B. Schilling, E. Locher, T. Granier: Smell perception – The role of perireceptor events, Wartburg Symp. Flavour Chem. Biol., ed. by T. Hofmann, W. Meyerhof, P. Schieberle (2010) pp. 55–62
28.68 A. Chougnet, W.D. Woggon, E. Locher, B. Schilling: Synthesis and in vitro activity of heterocyclic inhibitors of CYP2A6 and CYP2A13, two cytochrome P450 enzymes present in the respiratory tract, ChemBioChem **10**, 1562–1567 (2009)
28.69 B. Schilling, T. Granier, G. Fráter, A. Hanhart: Organic compounds, Patent WO 2008/116 338 (2008)
28.70 B. Schilling, W.D. Woggon, A. Chougnet, T. Granier, G. Fráter, A. Hanhart: Organic compounds, Patent WO 2008/116 339 (2008)
28.71 B. Schilling, T. Granier: Organic compounds, Patent 2010/37 244 (2010)
28.72 L.B. von Weymarn, J.A. Chun, G.A. Knudsen, P.F. Hollenberg: Effects of eleven isothiocyanates on P450 2A6- and 2A13-catalyzed coumarin 7-hydroxylation, Chem. Res. Toxicol. **20**, 1252–1259 (2007)
28.73 L.B. von Weymarn, Q.Y. Zhang, X. Ding, P.F. Hollenberg: Effects of 8-methoxypsoralen on cytochrome P450 2A13, Carcinogenesis **26**, 621–629 (2005)
28.74 T. Shimada, D. Kim, N. Murayama, K. Tanaka, S. Takenaka, L.D. Nagy, L.M. Folkman, M.K. Foroozesh, M. Komori, H. Yamazaki, F.P. Guengerich: Binding of Diverse Environmental Chemicals with Human Cytochromes P450 2A13, 2A6, and 1B1 and Enzyme Inhibition, Chem. Res. Toxicol. **26**(4), 517–528 (2013)
28.75 T. Shimada, N. Murayama, K. Tanaka, S. Takenaka, F.P. Guengerich, H. Yamazaki, M. Komori: Spectral modification and catalytic inhibition of human cytochromes P450 1A1, 1A2, 1B1, 2A6, and 2A13 by four chemopreventive organoselenium compounds, Chem. Res. Toxicol. **24**, 1327–1337 (2011)
28.76 E.S. Stephens, A.A. Walsh, E.E. Scott: Evaluation of inhibition selectivity for human cytochrome P450 2A enzymes, Drug Metab. Dispos. **40**, 1797–1802 (2012)
28.77 N.M. DeVore, K.M. Meneely, A.G. Bart, E.S. Stephens, K.P. Battaile, E.E. Scott: Structural comparison of cytochromes P450 2A6, 2A13, and 2E1 with pilocarpine, FEBS Journal **279**, 1621–1631 (2012)
28.78 L.C. Blake, A. Roy, D. Neul, F.J. Schoenen, J. Aube, E.E. Scott: Benzylmorpholine analogs as selective inhibitors of lung cytochrome P450 2A13 for the chemoprevention of lung cancer in tobacco users, Pharm. Res. **30**, 2290–2302 (2013)
28.79 X. Ding, D.C. Spink, J.K. Bhama, J.J. Sheng, A.D. Vaz, M.J. Coon: Metabolic activation of 2,6-dichlorobenzonitrile, an olfactory-specific toxicant, by rat, rabbit, and human cytochromes P450, Mol. Pharmacol. **49**, 1113–1121 (1996)
28.80 F. Xie, J. D'Agostino, X. Zhou, X. Ding: Bioactivation of the nasal toxicant 2,6-dichlorobenzonitrile: An assessment of metabolic activity in human nasal mucosa and identification of indicators of exposure and potential toxicity, Chem. Res. Toxicol. **26**, 388–398 (2013)
28.81 Y. Oka, S. Katada, M. Omura, M. Suwa, Y. Yoshihara, K. Touhara: Odorant receptor map in the mouse olfactory bulb: In vivo sensitivity and specificity of receptor-defined glomeruli, Neuron **52**, 857–869 (2006)
28.82 A. Nagashima, K. Touhara: Enzymatic conversion of odorants in nasal mucus affects olfactory glomerular activation patterns and odor perception, J. Neurosci. **30**, 16391–16398 (2010)
28.83 N. Thiebaud, S. Veloso Da Silva, I. Jakob, G. Sicard, J. Chevalier, F. Menetrier, O. Berdeaux, Y. Artur, J.M. Heydel, A.M. Le Bon: Odorant metabolism catalyzed by olfactory mucosal enzymes influences peripheral olfactory responses in rats, PLoS One **8**, e59547 (2013)
28.84 B. Schilling: Method to identify or evaluate compounds useful in the field of fragrances and aromas, Patent WO 2006/7752 A1 (2006)
28.85 P. Kraft, J.A. Bajgrowicz, C. Denis, G. Frater: Odds and trends: Recent developments in the chemistry of odorants, Angew. Chem. Int. Ed. Engl. **39**, 2980–3010 (2000)
28.86 T. Granier, B. Schilling: Organic compounds, Patent WO 2009/9 916 (2009)