Human Cytochrome P450 2A13 Efficiently Metabolizes Chemicals in Air Pollutants: Naphthalene, Styrene, and Toluene

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Human P450 2A13 is the most efficient enzyme for catalyzing the metabolism of nicotine and metabolic activation of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). It is conceivable that P450 2A13 also metabolizes chemicals in air pollutants because this enzyme is highly expressed in the respiratory tract. In this study, we investigated the possibility that P450 2A13 can metabolize naphthalene, styrene, and toluene, which are included in air pollutants as well as tobacco smoke, although they were known to be metabolized by P450 1A2 or 2E1. We found that P450 2A13 catalyzed 1- and 2-naphthol formations from naphthalene with higher intrinsic clearances (*k*cat/*K*m) (3.1- and 2.2-fold, respectively) than P450 1A2 and also more efficiently catalyzed the styrene 7,8-oxide formation from styrene and the benzylalcohol formation from toluene than P450 2E1. The overlapping substrate specificity of P450 2A13 with P450 2E1 was supported by the finding that P450 2A13 catalyzed chlorzoxazone 6-hydroxylation (8-fold higher value of *k*cat/*K*m) and *p*-nitrophenol 2-hydroxylation (19-fold higher value of *k*cat/*K*m), which are marker activities of P450 2E1. Thus, we found that P450 2A13 metabolizes diverse environmental chemicals and has overlapping substrate specificities of P450 1A2 and 2E1, suggesting that P450 2A13 plays important roles in the local metabolism of environmental chemicals in the respiratory tract related to toxicity or carcinogenicity.

Introduction

The human cytochrome P450 (P450[1]) 2A subfamily comprises three genes: *P450 2A6*, *2A7*, and *2A13* (*1*). Among them, *P450 2A6* and *2A13* encode functional enzymes (*2, 3*). P450 2A13 is the most efficient enzyme in the metabolism of nicotine and metabolic activation of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) with a substrate specificity similar to that of P450 2A6 (*3, 4*) because of the high amino acid identity between them (93.5%) (*1*). P450 2A6 is predominantly expressed in the liver, whereas P450 2A13 is predominantly expressed in the respiratory tract, with the highest level in the nasal mucosa, followed by the lung and trachea (*3, 5, 6*). P450 2A13 also metabolizes aflatoxin B_1 (*7*). Recently, we found that P450 2A13 can metabolize phenacetin, theophylline (*8*), and 4-aminobiphenyl (*9*), although P450 2A6 showed no or negligible activity. Thus, the substrate specificity of P450 2A13 does not necessarily overlap with that of P450 2A6. Since P450 2A13 is mainly expressed in the respiratory tract, it is feasible that P450 2A13 may catalyze the metabolism of environmental chemicals in air pollutants.

In cigarette smoke, more than 4,000 identified constituents are present. Among them, more than 200 constituents are known to be carcinogens or toxicants. Inhaled cigarette smoke deposits chemicals in the bronchial airway passages and lung. Many carcinogens require activation by enzymes such as P450 to elicit the carcinogenicity or toxicity. Naphthalene is a bicyclic aromatic compound included in cigarette smoke and diesel fumes (*10*). It is also used in the manufacturing of naphthylamines, anthranilic and phthalic acids, and synthetic resins (*11, 12*). Naphthalene is mainly converted to 1-naphthol and further to 1,2-naphthoquinone and 1,4-naphthoquinone, the ultimate toxic compounds (Figure 1A). An in vitro study revealed that these naphthoquinones have similar cytotoxicity (*13*). Recently, it has been reported that multiple P450 isoforms are involved in the metabolism of naphthalene and 1-naphthol and that P450 1A2 has the highest activity (*14*). Since the target organs of the toxicity of naphthalene are the lung and bronchus (*15, 16*), and since P450 1A2 is expressed only in the liver, the naphthalene metabolism by the P450 expressed in the respiratory tract should be characterized. Styrene and toluene are widely used as industrial solvents (*17, 18*). High occupational exposure to them results in significant toxicity in the nervous system as well as many organs (*19, 20*). Since they are present in many household products such as aerosols, degreasers, glues, and paints, the general population is likely to be exposed to them. It has been demonstrated that most of the adverse effects of styrene can be attributed to a metabolite, styrene 7,8-oxide (*19*).

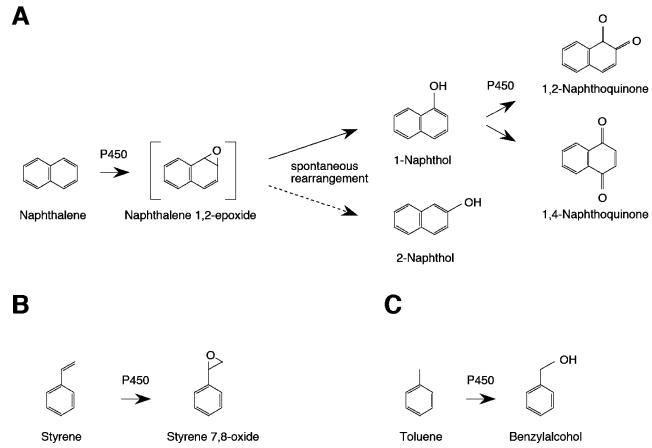

Figure 1. Metabolic pathways of (A) naphthalene, (B) styrene, and (C) toluene by human P450.

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[1] Abbreviations: NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NPR, NADPH-P450 reductase; P450, cytochrome P450.

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The epoxidation of styrene is known to be catalyzed by P450 2E1 and 2B6 to a similar extent (Figure 1B) (*21*). The toxicity of toluene is assumed to depend on the duration and magnitude of exposure as well as its metabolic fate. In vitro studies suggested that methyl-hydroxylation by P450 2E1 is a primary detoxification pathway (Figure 1C) (*22, 23*).

In general, the liver is the principal tissue of the metabolism of xenobiotics, but the lung or bronchus that is directly exposed to tobacco carcinogens or air pollutants would also play an important role in the metabolism of xenobiotics. In this study, we investigated whether P450 2A13 has catalytic ability toward naphthalene, styrene, and toluene.

Experimental Procedures

Chemicals. Naphthalene, 1-naphthol, 2-naphthol, styrene, styrene 7,8-oxide, toluene, benzylalcohol, *p*-nitrophenol, and 1,2-dihydroxy-4-nitrobenzene were from Wako Pure Chemicals (Osaka, Japan). Chlorzoxazone and 6-hydroxychlorzoxazone were from Sigma-Aldrich (St. Louis, MO). $NADP^+$, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase were purchased from Oriental Yeast (Tokyo, Japan). All other chemicals and solvents were of the highest or analytical grade commercially available.

Enzyme Preparations. *Escherichia coli* (*E. coli*) membranes expressing recombinant human P450/NADPH-P450 reductase (NPR) for P450 1A1 (*24*), 1A2 (*24*), 2A6 (*25*), 2A13 (*26*), and 2E1 (*24*) were prepared as described previously (*25*). The P450 content (*27*) and protein concentration (*28*) were determined according to a method described previously. The NADPH-cytochrome *c* reductase activity was determined as described previously (*29, 30*) using $\Delta_{\epsilon 550} = 21.1\ mM^{-1}\ cm^{-1}$, and the content was calculated using the specific activity of 3.0 μmol reduced cytochrome *c*/min/nmol NPR based on purified rabbit NPR preparation (*31*). The molar ratios of NPR to P450 were 3.0 (P450 1A1), 0.8 (P450 1A2), 5.8 (P450 2A6), 2.9 (P450 2A13), and 1.3 (P450 2E1), which were sufficiently high to measure the activities as reported previously (*24*).

Enzyme Assays for Naphthalene Hydroxylation and Naphthoquinone Formation from 1-Naphthol. 1- Naphthol and 2-naphthol formation from naphthalene was determined as follows: A typical incubation mixture (0.2 mL total volume) contained an *E. coli* membrane preparation (3 pmol of P450), 100 mM potassium phosphate buffer (pH 7.4), an NADPH-generating system (0.5 mM $NADP^+$, 5 mM glucose-6-phosphate, 5 mM $MgCl_2$, and 1 U/mL glucose-6-phosphate dehydrogenase) and 5–200 μM naphthalene. The reaction was initiated by the addition of the NADPH-generating system after 2-min preincubation at 37 °C. After 5 min of incubation at 37 °C, the reaction was terminated by the addition of 100 μL of cold acetonitrile. After the removal of the protein by centrifugation at 6,500*g* for 5 min, a 20 μL portion of the supernatant was subjected to HPLC. HPLC analyses were performed using an L-7100 pump (Hitachi, Tokyo, Japan), an L-7200 autosampler (Hitachi), and a D-2500 integrator (Hitachi) equipped with a Mightysil RP-18 C18 GP (4.6 × 150 mm; 5 μm) column (Kanto Chemical, Tokyo, Japan). The eluent was monitored at 223 nm with a noise-base clean Uni-3 (Union, Gunma, Japan), which can reduce the noise by integrating the output and increase the signal 3-fold by differentiating the output and 5-fold by further amplification with an internal amplifer, resulting in a maximum 15-fold amplification of the signal. The mobile phase was 30% acetonitrile containing 0.01% phosphoric acid. The flow rate was 1.0 mL/min. The column temperature was 35 °C. The quantification of 1-naphthol and 2-naphthol was performed by comparing the HPLC peak heights with those of authentic standards.

1,2-Naphthoquinone and 1,4-naphthoquinone formation from 1-naphthol was also determined. A typical incubation mixture, reaction condition, and HPLC condition were the same as described above except that 5–200 μM 1-naphthol was added as a substrate, and a 50 μL portion of the supernatant was subjected to HPLC. Once formed, 1,2-naphthoquinone and 1,4-naphthoquinone were effectively reduced by NPR in the membrane of the *E. coli* expression systems. Therefore, instead of the direct estimation with the HPLC peak heights of 1,2-naphthoquinone and 1,4-naphthoquinone, the disappearance of the peak height of the substrate was calculated for the estimation of 1,2-naphthoquinone and 1,4-naphthoquinone formation.

Enzyme Assay for Styrene 7,8-Epoxidation. Styrene 7,8-oxide formation from styrene was determined as follows: a typical incubation mixture (final volume of 0.2 mL) contained an *E. coli* membrane preparation (5 pmol of P450), 100 mM Tris-HCl buffer (pH 7.4), the NADPH-generating system, and 20–1000 μM styrene. The reaction was initiated by the addition of the NADPH-generating system after 2 min of preincubation at 37 °C. After 10 min of incubation at 37 °C, the reaction was terminated by the addition of 10 μL of 60% perchloric acid. After the removal of protein by centrifugation at 9,500*g* for 5 min, a 60 μL portion of the supernatant was subjected to HPLC. The HPLC apparatus was the same as that described above, except for a Capcell Pak C18 UG120 (4.6 × 150 mm, 5 μm) column (Shiseido, Tokyo, Japan). The eluent was monitored at 200 nm with a noise-base clean Uni-3. The mobile phase was 8% acetonitrile containing 0.25% phosphoric acid. The flow rate was 1.0 mL/min. The column temperature was 35 °C. The quantification of styrene 7,8-oxide was performed by comparing the HPLC peak height with that of an authentic standard.

Enzyme Assay for Toluene methyl Hydroxylation. Benzylalcohol formation from toluene was determined as follows: a typical incubation mixture (final volume of 0.2 mL) contained an *E. coli* membrane preparation (5 pmol of P450), 100 mM Tris-HCl buffer (pH 7.4), the NADPH-generating system, and 50–2000 μM toluene. The reaction was initiated by the addition of the NADPH-generating system after 2 min of preincubation at 37 °C. After 5 min of incubation at 37 °C, the reaction was terminated by the addition of 10 μL of 60% perchloric acid. After the removal of protein by centrifugation at 9,500*g* for 5 min, a 60 μL portion of the supernatant was subjected to HPLC. The HPLC apparatus was the same as that described above, except for a Capcell Pak C18 UG120 (4.6 × 250 mm, 5 μm) column. The eluent was monitored at 200 nm with a noise-base clean Uni-3. The mobile phase was 7% acetonitrile containing 0.1% phosphoric acid. The flow rate was 1.0 mL/min. The column temperature was 35 °C. The quantification of benzylalcohol was performed by comparing the HPLC peak height with that of an authentic standard.

Other Enzyme Assays. Chlorzoxazone 6-hydroxylation was determined as described previously (*32*). *p*-Nitrophenol 2-hydroxylation was also determined as described previously (*33*), except that 100 mM potassium phosphate buffer (pH 7.4) was used.

Statistical Analysis. Statistical analyses of the kinetic parameters were performed by two-tailed Student's *t*-test. A value of $P < 0.05$ was considered statistically significant.

Results

Naphthalene Hydroxylation and Naphthoquinone Formation from 1-Naphthol. The catalytic activities of P450 2A13 for 1-naphthol and 2-naphthol formation from naphthalene were measured by comparing them with those of P450 1A1, 1A2, and 2A6. Naphthalene was predominantly converted to 1-naphthol rather than 2-naphthol, in agreement with a previous report (*14*). As shown in Figure 2A and B, P450 2A13 showed the highest activities for these formations. The activities catalyzed by P450 2A6 were similar to those by P450 1A2. P450 1A1 showed the lowest 1-naphthol formation and no 2-naphthol formation. The kinetic parameters of these reactions are summarized in Table 1. For the 1-naphthol formation, the *K*m value of P450 2A13 was similar to those of P450 1A2 and P450 2A6, and the value of P450 1A1 was prominently high. The *V*max value of P450 2A13 was the highest of all four isoforms. Thus, P450 2A13 showed the highest intrinsic clearance. For the 2-naphthol formation, the *K*m value of P450 2A13 was similar

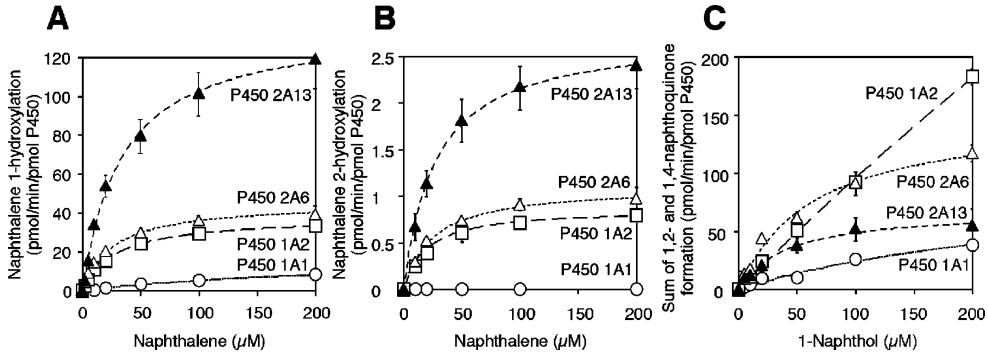

Figure 2. Kinetic analyses of (A) 1-naphthol and (B) 2-naphthol formation from naphthalene, and (C) the total formation of 1,2-naphthoquinone and 1,4-naphthoquinone from 1-naphthol catalyzed by recombinant P450 1A1, 1A2, 2A6, and 2A13 expressed in *E. coli*. The kinetic parameters were estimated from the fitted curve using the computer program KaleidaGraph designed for nonlinear regression analysis. Each data point represents the mean of triplicate determinations.

Table 1. Kinetic Parameters for 1-Naphthol and 2-Naphthol Formation from Naphthalene[a]

	*K*m (μM)	*k*cat (min^{-1})	*k*cat/*K*m (min^{-1} μM^{-1})
1-naphthol formation from naphthalene			
P450 1A1	244 ± 45^d	17 ± 1^d	0.07 ± 0.01^d
P450 1A2	29 ± 1	39 ± 1	1.34 ± 0.02
P450 2A6	23 ± 4^c	43 ± 5	1.90 ± 0.16^d
P450 2A13	36 ± 2^c	143 ± 15^d	4.03 ± 0.58^d
2-naphthol formation from naphthalene			
P450 1A1	ND[b]	ND	ND
P450 1A2	19.1 ± 2.5	0.9 ± 0.0	0.05 ± 0.01
P450 2A6	26.5 ± 5.2	1.2 ± 0.1^c	0.05 ± 0.01
P450 2A13	28.2 ± 2.4^c	2.9 ± 0.4^d	0.10 ± 0.00^d

[a] Data are the mean ± SD of three independent experiments. [b] ND, not detected. [c] $P < 0.05$. [d] $P < 0.005$ compared with P450 1A2.

to that of P450 2A6, but higher than that of P450 1A2. The *V*max value of P450 2A13 was higher than those of P450 1A2 and 2A6. Thus, P450 2A13 showed the highest intrinsic clearance.

The total metabolism of 1-naphthol to 1,2-naphthoquinone plus 1,4-naphthoquinone was determined (Figure 2C), and therefore, the kinetic parameters were not calculated. When the total formation of 1,2-naphthoquinone and 1,4-naphthoquinone by the four P450 isoforms was compared, P450 2A6 showed the highest activity at low substrate concentrations (5–20 μM), but P450 1A2 showed the highest activity at the high substrate concentration (200 μM). At the substrate concentrations of 5–50 μM, P450 2A13 showed activity similar to that of P450 1A2. P450 1A1 showed the lowest activity. Collectively, these results suggested that P450 2A13 has the highest catalytic activity for naphthalene and subsequent 1-naphthol metabolisms.

Styrene 7,8-Epoxidation. The catalytic activity of P450 2A13 for styrene 7,8-oxide formation from styrene was measured by comparing it with those of P450 2E1 and 2A6. As shown in Figure 3A, the activities catalyzed by the three P450s were increased in a substrate concentration-dependent manner. The maximum substrate concentration (1 mM) was not sufficiently high to determine the *K*m values because the solubility of styrene limited the substrate concentration that could be achieved in the incubation mixture. Therefore, the *CL*int values were calculated with the initial slope of the plots of velocity versus substrate concentration. The values in P450 2A13, 2A6, and 2E1 were 46.8, 17.2, and 18.5 min^{-1} mM^{-1}, respectively. These results suggest that P450 2A13 has the highest catalytic activity for styrene 7,8-oxide formation from styrene.

Toluene Methyl Hydroxylation. The catalytic activity of P450 2A13 for benzylalcohol formation from toluene was measured by comparing it with those of P450 2E1 and 2A6. As shown in Figure 3B, the activities catalyzed by the three P450s were increased in a substrate concentration-dependent manner. The maximum substrate concentration (2 mM) was not sufficiently high to determine the *K*m values because the solubility of toluene limited the substrate concentration that could be achieved in the incubation mixture. Thus, accurate kinetic parameters could not be obtained. The *CL*int values calculated with the initial slope of the plots of velocity versus substrate concentration in P450 2A13, 2A6, and 2E1 were 13.3, 2.6, and 5.7 min^{-1} mM^{-1}, respectively. These results suggest that P450 2A13 has the highest catalytic activity for benzylalcohol formation from toluene.

Chlorzoxazone 6-Hydroxylation and *p*-Nitrophenol 2-Hydroxylation. The results of our study prompted us to investigate the possibility that the substrate specificity of P450 2A13 might overlap with that of P450 2E1. The chlorzoxazone 6-hydroxylation and *p*-nitrophenol 2-hydroxylation, which are marker activities of P450 2E1, were measured using recombinant P450 2A13, 2A6, and 2E1. Interestingly, P450 2A13 showed significantly higher catalytic activities for both chlorzoxazone 6-hydroxylation and *p*-nitrophenol 2-hydroxylation than P450 2E1 (Figure 4A and B). As shown in Table 2, the *K*m value for chlorzoxazone 6-hydroxylation by P450 2A13 was approximately one-eighth of that by P450 2E1, and their *V*max values were similar, resulting in 8-fold higher intrinsic clearance than that by P450 2E1. P450 2A6 also catalyzed the chlorzoxazone 6-hydroxylation, but the intrinsic clearance was one-fifth of that by P450 2E1. P450 2A13 catalyzed *p*-nitrophenol 2-hydroxylation with significantly higher (19-fold) intrinsic clearances than that by P450 2E1 (Figure 4B and Table 2) and with a lower *K*m value and higher *V*max value than those of P450 2E1. P450 2A6 also catalyzed the *p*-nitrophenol 2-hydroxylation with clearance similar to that by P450 2E1. These results suggest that P450 2A13 can catalyze the metabolism of P450 2E1 substrates.

Discussion

In the present study, we found that P450 2A13 is catalytically active toward chemicals in air pollutants. P450 2A13 showed significantly higher intrinsic clearances for naphthalene hydroxylation than P450 1A2. Recently, we reported that P450 2A13 is catalytically active toward substrates of P450 1A2, such as aminobiphenyl (*9*), theophylline, and phenacetin (*8*). Especially, it was noteworthy that the catalytic activity of P450 2A13 for phenacetin *O*-de-ethylation was apparently higher than that of P450 1A2. On the basis of the present results, naphthalene

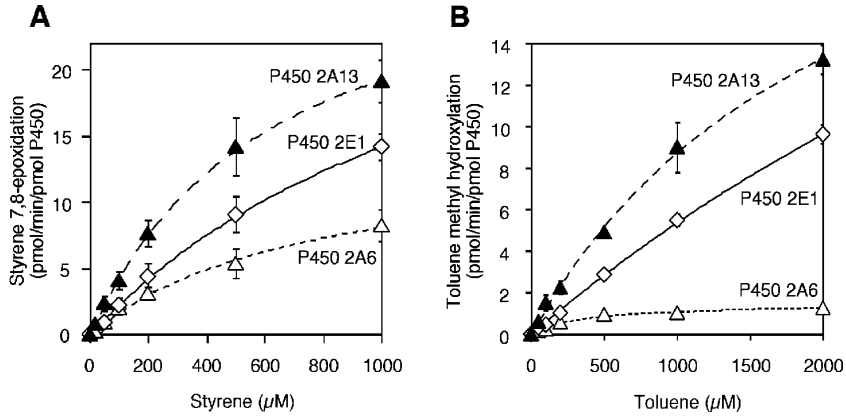

Figure 3. Kinetic analyses of (A) styrene 7,8-oxide formation from styrene and (B) benzylalcohol formation from toluene catalyzed by recombinant P450 2A6, 2A13, and 2E1 expressed in *E. coli*. The styrene and toluene concentrations ranged from 20 to 1000 μM and from 50 to 2000 μM, respectively. Each data point represents the mean of triplicate determinations.

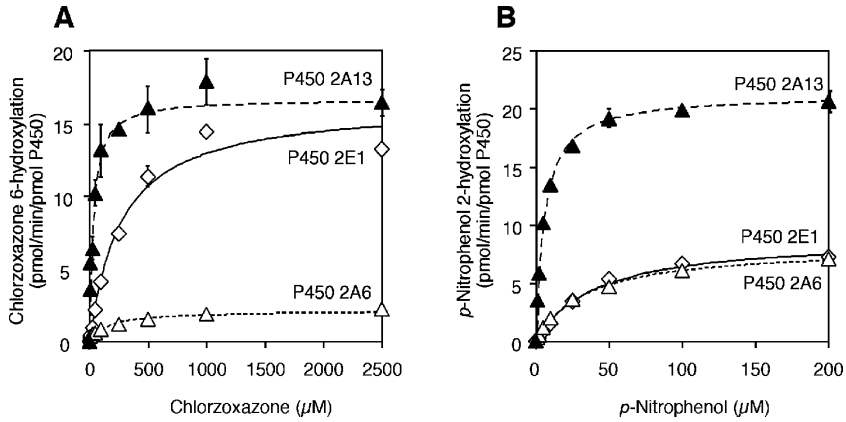

Figure 4. Kinetic analyses of (A) chlorzoxazone 6-hydroxylation and (B) *p*-nitrophenol 2-hydroxylation catalyzed by recombinant P450 2A6, 2A13, and 2E1 expressed in *E. coli*. The kinetic parameters were estimated from the fitted curve using the computer program KaleidaGraph designed for nonlinear regression analysis. Each data point represents the mean of triplicate determinations.

Table 2. Kinetic Parameters for Chlorzoxazone 6-Hydroxylation and *p*-Nitrophenol 2-Hydroxylation[a]

	*K*m (μM)	*k*cat (min^{-1})	*k*cat/*K*m (min^{-1} μM^{-1})
chlorzoxazone 6-hydroxylation			
P450 2E1	274 ± 18	16.2 ± 0.3	0.06 ± 0.00
P450 2A6	161 ± 19[c]	2.0 ± 0.2[c]	0.01 ± 0.00[c]
P450 2A13	36 ± 4[c]	16.9 ± 1.3	0.47 ± 0.02[c]
p-nitrophenol 2-hydroxylation			
P450 2E1	39.6 ± 0.2	9.1 ± 0.1	0.20 ± 0.00
P450 2A6	30.9 ± 1.9[c]	8.4 ± 0.3[b]	0.27 ± 0.06[b]
P450 2A13	5.5 ± 0.1[c]	20.4 ± 0.6[c]	3.73 ± 0.06[c]

[a] Data are the mean ± SD of three independent experiments. [b] $P < 0.05$. [c] $P < 0.005$ compared with P450 2E1.

can be added to the P450 2A13 substrates with overlapping substrate specificity with P450 1A2. P450 2A6 also catalyzed naphthalene hydroxylation with similar or higher intrinsic clearance than P450 1A2. A previous study reported that 1-naphthol and 2-naphthol formation from naphthalene in human liver microsomes was inhibited by antibodies toward p450 2a5, mouse orthologue of P450 2A6, to 50–60% and 30–40% of control, respectively (*34*). In addition, Asikainen et al. reported that coumarin 7-hydroxylation in human liver microsomes, a marker activity of P450 2A6, was inhibited by naphthalene in a competitive manner ($K_i = 1.2 – 5.6$ μM) (*34*). Supporting the study, we directly demonstrated that P450 2A6 catalyzes naphthalene hydroxylation.

We found that P450 2A13 showed higher activity for styrene 7,8-oxide formation from styrene than P450 2E1. Styrene 7,8-oxide, which is classified in group 2A (probably carcinogenic to humans) (*18*), is a major reactive metabolite of styrene, and its genotoxicity has been demonstrated by several in vitro test systems (*35, 36*). P450 2A13 also showed higher activity for benzylalcohol formation from toluene, a major detoxification pathway. Toluene is known to be mainly metabolized by P450 2E1. These results suggest that P450 2A13 is active in the metabolism of the P450 2E1 substrates, supported by the fact that P450 2A13 can catalyze chlorzoxazone 6-hydroxylation and *p*-nitrophenol 2-hydroxylation. The bicistronic P450/NPR *E. coli* membranes used in the present study do not include cytochrome b_5. Since it has been reported that the P450 2E1 activity was enhanced by cytochrome b_5 (*24*), we performed a preliminary study to compare the activities of P450 2E1 and 2A13 in the presence of cytochrome b_5. When cytochrome b_5 expressed in *E. coli* was added with equal molar concentration to P450, chlorzoxazone 6-hydroxymation (30 μM substrate concentration) and *p*-nitrophenol 2-hydroxylation (5 μM substrate concentration) catalyzed by P450 2E1 were increased by approximately 2-fold, but these were still considerably (one forth) lower than the activities catalyzed by P450 2A13 in the absence of cytochrome b_5 (Figure S1, Supporting Information). The effects of cytochrome b_5 on styrene 7,8-oxide formation from styrene and benzylalcohol formation from toluene by P450 2E1 and 2A13 were trivial (Figure S1, Supporting Information). Thus, our findings that P450 2A13 showed higher activities toward styrene, toluene, chlorzoxazone, and *p*-nitrophenol were not reversed in the presence of cytochrome b_5. It is generally accepted that P450 2E1 metabolizes a variety of toxins and carcinogens included in air pollutants, such as benzene, xylene,

acrylonitrile, and ethylcarbamate (urethane) (*37*). Therefore, it would be interesting to investigate whether P450 2A13 can catalyze the metabolism of such chemicals in the future.

Recently, Smith et al. (*38*) determined the structure of P450 2A13 by X-ray crystallography and compared it with the structure of P450 2A6. They reported that the overall P450 2A13 and 2A6 structures are very similar. Like 2A6, the P450 2A13 active site is small, planar, and highly hydrophobic with a cluster of phenylalanine residues for $\pi-\pi$ interactions with aromatic ligands. Sansen et al. (*39*) reported that the active site of P450 1A2 is also relatively small and narrow throughout its extent. The X-ray crystallography of P450 2E1 has not yet accomplished, but it is easily assumed that the P450 2E1 active site is relatively small because its substrate molecules are typically small (molecular weight <100). Smith et al. (*38*) reported that the differences in residues 117, 208, 300, and 301 (for definition of the plane of ligands), and residues 365 and/or 366 (for determination of the ligand binding and catalysis) in the active site may be accountable for the difference of substrate specificity between P450 2A13 and 2A6. We cannot compare the residues at comparable sites in P450 1A2 and 2E1 since the overall structures of P450 1A2 and 2E1 are likely to show considerable changes from those of P450 2A6 and 2A13. However, the relative size and planarity of the P450 1A2 active site compared to that of P450 2A13 have some bearing on overlapping substrate specificity.

Naphthalene, styrene and toluene are included in environmental air as hazardous solvents in many industrial workshops and homes. Because they are easily absorbed by inhalation, their local metabolism in the respiratory tract may be as important as that in the liver. P450 2A13 is predominantly expressed in the respiratory tract such as in the lung and trachea (*3, 5*). Recently, the expression of P450 2A13 protein in the epithelia of the bronchus and lung was shown by immunohistochemistry using a P450 2A13-specific antibody (*40*). Therefore, P450 2A13 could play important roles in the metabolic activation or detoxification of numerous procarcinogens and toxicants in air pollutants or tobacco constituents. Since P450 1A1, 2A6, and 2E1 are expressed in the human lung (*41*), they may also contribute to their metabolism. If we can quantitatively compare these expression levels in the human lung, it would be possible to determine which P450 isoform makes the major contribution.

For the *P450 2A13* gene, genetic polymorphisms (http://www.cypalleles.ki.se/cyp2a13.htm) are known. The *P450 2A13*2* allele leading to a substitution of Arg to Cys at the 257 position has been reported to decrease enzyme activities for NNK α-hydroxylation, hexamethylphosphoramide *N*-demethylation, *N*,*N*-dimethylaniline *N*-demethylation, 2′-methoxyacetophenone *O*-demethylation, and *N*-nitrosomethylphenylamine *N*-demethylation (*42*). The *P450 2A13*3* allele causing a frame-shift and *P450 2A13*7* allele having a stop codon at exon 2 (*43*) are predicted to produce the protein lacking enzymatic activity. Wang et al. reported that the *P450 2A13*4* allele leading to a substitution of Arg to Glu at the 101 position caused the lack of protein expression and enzyme activity (*44*). It has been reported that a single nucleotide polymorphism of 7520C > G in the 3′-untranslated region was related to decreased expression in the lung (*45*). The reported association between *P450 2A13* genetic polymorphisms and lung cancer risk is of particular interest (*46, 47*) because P450 2A13 catalyzes the metabolic activation of tobacco-specific carcinogens such as NNK. The genetic polymorphisms of *P450 2A13* might be responsible for the interindividual variability in the carcinogenicity or toxicity of naphthalene, styrene, and toluene.

In conclusion, we found that P450 2A13 can metabolize naphthalene, styrene, and toluene that are included in air pollutants and tobacco smoke. This knowledge increases our understanding of the toxicological significance of P450 2A13 expressed in the respiratory tract.

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Supporting Information Available: Effects of Cytochrome b_5 on chlorzoxazone 6-hydroxylation, *p*-nitrophenol 2-hydroxylation, styrene 7,8-epoxidation, and toluene methyl hydroxylation catalyzed by P450 2A6, 2A13, and 2E1 (Figure S1). This material is available free of charge via the Internet at http://pubs.acs.org.

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