Inhibitory effects of neurotransmitters and steroids on human CYP2A6

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Abbreviations: CYP, P450, Cytochrome P450.
Abstract

Human CYP2A6 catalyzes the metabolism of nicotine, cotinine, and coumarin as well as some pharmaceutical drugs. CYP2A6 is highly expressed in liver, and also in brain and steroid related tissues. In this study, we investigated the inhibitory effects of neurotransmitters and steroid hormones on CYP2A6 activity. We found that coumarin 7-hydroxylation and cotinine 3’-hydroxylation by recombinant CYP2A6 expressed in baculovirus-infected insect cells were competitively inhibited by tryptamine (both Ki = 0.2 µM), serotonin (Ki = 252 µM and 167 µM), dopamine (Ki = 49 µM and 22 µM), and histamine (Ki = 428 µM and 359 µM). Cotinine formation from nicotine was inhibited by tryptamine (Ki = 0.7 µM, competitive), serotonin (Ki = 272 µM, non-competitive), dopamine, noradrenaline, and adrenaline (Ki = 11 µM, 54 µM, and 81 µM, un-competitive). Estrogens (Ki = 0.6 – 3.8 µM), androgens (Ki = 60 – 149 µM), and corticosterone (Ki = 36 µM) also inhibited cotinine formation but coumarin 7-hydroxylation and cotinine 3’-hydroxylation did not. Nicotine-Δ5(1’)-iminium ion formation from nicotine was not affected by these steroid hormones, indicating that the inhibition of cotinine formation was due to the inhibitory effects on aldehyde oxidase. The nicotine-Δ5(1’)-iminium ion formation was competitively inhibited by tryptamine (Ki = 0.3 µM), serotonin (Ki = 316 µM), dopamine (Ki = 66 µM), and histamine (Ki = 209 µM). Thus, we found that some neurotransmitters inhibit CYP2A6 activity, being related with inter- and intra-individual differences in CYP2A6-dependent metabolism. The inhibitory effects of steroid hormones on aldehyde oxidase may also contribute to interindividual differences in nicotine metabolism.
Introduction

Cytochrome P450 (CYP, P450) enzymes are of great importance and interest because they catalyze the metabolism of both endogenous compounds and xenobiotics. The substrates for the P450s include a variety of clinically used drugs, environmental pollutants, carcinogens, steroid hormones, fatty acids, and bile acids. Studies on the inhibition of P450 are useful to predict drug efficacy, drug interaction, and drug toxicity. Because many endogenous compounds are substrates of P450s, they may inhibit drug metabolism catalyzed by P450s. Previous studies reported that human CYP1A2 (Agúndez et al., 1998), CYP2C9 (Gervasini et al., 2001), CYP2D6 (Martínez et al., 1997), and CYP3A (Martínez et al., 2000) activities were inhibited by neurotransmitters and their precursors. The inhibition would be of particular importance, since these P450s are expressed in brain and are involved in the metabolism of psychoactive drugs or associated with Parkinson’s disease. In addition, it has been reported that androgens activate or inhibit the enzymatic activities catalyzed by CYP3A4 (Nakamura et al., 1998). Since endogenous compounds are always found in vivo, the inhibitory effects of endogenous compounds may contribute to the intraindividual and interindividual differences in P450 activity.

Human CYP2A6, first purified as a coumarin 7-hydroxylase (Yun et al., 1991), catalyzes the metabolism of pharmaceutical agents such as tegafur, losigamone, letrozole and valproic acid. CYP2A6 is a major enzyme responsible for the metabolism of nicotine to cotinine (Nakajima et al., 1996b) and further to trans-3’-hydroxycotinine (Nakajima et al., 1996a). Furthermore, it can metabolically activate aflatoxin B1 and tobacco specific nitrosamines such as 4-methylnitrosoamo-1-(3-pyridyl)-1-butanone and N-nitrosodiethylamine (Nakajima et al., 2002). CYP2A6 is predominantly expressed in liver and also in brain (Miksys and Tyndale, 2004). Although the systemic clearance of nicotine is due to the metabolism in liver, CYP2A6 expressed in human brain contributes to the local metabolism of nicotine (Miksys and Tyndale et al., 2004; Yamanaka et al., 2005).

In addition, CYP2A6 is also expressed in steroid related tissues such as adrenal gland, testis, ovary, and breast (Nakajima et al., 2006b). Recent studies reported that CYP2A6 catalyses the
16β-hydroxylation of estradiol (Lee et al., 2003) and progesterone 6β-hydroxylation (Niwa et al., 1998), although the activities were much lower than those of other CYPs. This background prompted us to investigate the inhibitory effects of neurotransmitters, their precursors and steroid hormones on CYP2A6 activity.

Materials and Methods

Materials

Tryptamine, serotonin, melatonin, tyrosine, dopamine, histamine, corticosterone, testosterone, indole-3-acetic acid, and tryptophol were purchased from Wako Pure Chemical Industries (Osaka, Japan). Tryptophan, L-dopa, noradrenaline, adrenaline, progesterone, 4-androstene-3,17-dione (androstenedione), 5α-androstane-3α,17β-diol (androstanediol), estrone, estradiol, estriol, coumarin, 7-hydroxycoumarin, nicotine, cotinine, and caffeine were from Sigma-Aldrich (St. Louis, MO). Trans-3'-hydroxycotinine was kindly provided by Dr. William S. Caldwell (R. J. Reynolds Tobacco Company, Winston Salem, NC). NADP⁺, glucose-6-phosphate, and glucose-6-phosphate dehydrogenase were from Oriental Yeast (Tokyo, Japan). Pooled human liver microsomes and microsomes from baculovirus-infected insect cells expressing human CYP2A6 with NADPH-cytochrome P450 oxidoreductase and cytochrome b₅ were obtained from BD Gentest (Woburn, MA). All other chemicals and solvents were of the highest grade commercially available.

Enzyme assays

Coumarin 7-hydroxylase activity was determined as described previously (Ohyama et al., 2000). The incubation mixture (200 µl total volume) contained 50 mM Tris-HCl buffer (pH 7.4), the NADPH-generating system (0.5 mM NADP⁺, 5 mM glucose-6-phosphate, 5 mM MgCl₂, 1 U/ml glucose-6-phosphate dehydrogenase), 2 pmol/ml CYP2A6 with coumarin as a substrate. The substrate concentration was 1 µM for the determination of the IC₅₀ values, and ranged from 0.1 to 5 µM for the determination of the Ki values.
Cotinine 3'-hydroxylase activity was determined as described previously (Nakajima et al., 1996a). The incubation mixture (500 µl total volume) contained 50 mM potassium phosphate buffer (pH 7.4), the NADPH-generating system, 3 pmol/ml CYP2A6, with cotinine as a substrate. The substrate concentration was 250 µM for the determination of the IC₅₀ values, and ranged from 100 to 2000 µM for the determination of the Ki values.

Cotinine formation from nicotine was determined as described previously (Nakajima et al., 1996b). The incubation mixture (500 µl total volume) contained 50 mM potassium phosphate buffer (pH 7.4), the NADPH-generating system, 3 pmol/ml CYP2A6, 3 mg/ml human liver cytosol, with nicotine as a substrate. The substrate concentration was 50 µM for the determination of the IC₅₀ values, and ranged from 10 to 200 µM for the determination of the Ki values. Since nicotine is auto-oxidized to cotinine, cotinine contaminants exist in commercially available nicotine to the extent of about 0.15%. Therefore, the content of cotinine in the mixture incubated without microsomal protein was subtracted from that with microsomal protein to correct the activity.

Nicotine-Δ⁵¹¹-iminium ion formation from nicotine was determined by HPLC. The incubation mixture (200 µl total volume) contained 50 mM Tris-HCl buffer (pH 7.4), the NADPH-generating system, 3 pmol/ml CYP2A6, and nicotine as a substrate. The concentrations of nicotine were the same as described above. The reaction was initiated by the addition of the NADPH-generating system, following a 2 min preincubation at 37°C. After incubation for 10 min, the reaction was terminated by adding 100 µl of ice-cold acetonitrile. After removal of protein by centrifugation at 10,000 rpm for 5 min, a 50 µl portion of the supernatant was injected into a HPLC system. The HPLC analyses were performed using an L-7000 pump (Hitachi, Tokyo, Japan), L-7200 autosampler (Hitachi), and a D-2500 integrator (Hitachi) equipped with a Capcell Pak C₁₈ UG120 (4.6 x 250 mm; 5 µm) column (Shiseido, Tokyo, Japan). The eluent was monitored at 260 nm using an L-7400 UV detector (Hitachi). The mobile phase was 7% acetonitrile containing 0.05% phosphoric acid and 1 mM sodium heptane sulfonate. The retention times of nicotine-Δ⁵¹¹-iminium ion and nicotine were 18 min and 21 min, respectively. Since an authentic standard was not available, the relative...
enzyme activity was determined with the HPLC peak height. This is the first study in which nicotine-Δ⁵'(1')-iminium ion could be detected by HPLC-UV with unlabeled nicotine. It was confirmed that the peak of nicotine-Δ⁵'(1')-iminium ion was decreased to less than half in the presence of cytosol. The nicotine-Δ⁵'(1')-iminium ion formation was verified with LC-MS/MS analysis by the method described previously (Yamanaka et al., 2004) with slight modifications. The column was a Capcell Pak C₁₈ UG120 (4.6 x 250 mm; 5 µm) and the mobile phase was 0.15% acetic acid, and the flow rate was 0.5 ml/min. Nicotine with [M+H]⁺ ion at m/z 163 and product ion at m/z 130 was eluted at 5.0 min. A peak with M⁺ ion at m/z 161 and the product ion at m/z 130, corresponding to nicotine-Δ⁵'(1')-iminium ion (Murphy et al., 2005), was observed at 4.3 min. The 18-min peak in the above ion-pair HPLC analysis was fractionated, and extracted by dichloromethane after alkalinization with sodium hydroxide. The organic fraction was evaporated under a gentle stream of nitrogen and the residue was dissolved in mobile phase. LC-MS/MS analysis of the residue showed a peak at 4.3 min with ions at m/z 161 and 130, demonstrating that it was nicotine-Δ⁵'(1')-iminium ion.

**Inhibition analysis of CYP2A6 enzyme activities**

The structures of the neurotransmitters, their precursors and steroid hormones used in this study are shown in Fig. 1. Tryptophan, tryptamine, serotonin, tyrosine, L-dopa, dopamine, noradrenaline, adrenaline, and histamine were dissolved in distilled water. Melatonin, progesterone, corticosterone, androstenedione, testosterone, androstanediol, estrone, estradiol, and estriol were dissolved in methanol. These endogenous compounds were added to the incubation mixtures described above to investigate their inhibitory effects. The final concentration of methanol in the incubation mixture was 1%. Therefore, the control activity was determined in the presence of 1% methanol for the compounds that were dissolved in methanol. The concentrations of tryptophan, tryptamine, serotonin, dopamine, noradrenaline, adrenaline, and histamine ranged from 1 to 1000 µM, but the concentration of the other substances was limited to 100 µM because of the solubility. The effects of noradrenaline and adrenaline for the cotinine 3’-hydroxylation could not be investigated at 1000 µM, because of
interference with the detection of trans-3’-hydroxycotinine.

**Tryptamine metabolism by CYP2A6**

Indole-3-acetic acid and tryptophol formations from tryptamine was determined according to the methods by Mills et al. (1991) and Yu et al. (2003) with slight modifications. The incubation mixture (200 µl total volume) contained 50 mM Tris-HCl buffer (pH 7.4), the NADPH-generating system, 120 µg/ml human liver microsomes or 20 pmol/ml CYP2A6 with tryptamine as a substrate. The retention times of tryptamine, indole-3-acetic acid, and tryptophol were 11 min, 6.7 min, and 15 min, respectively.

**Data analysis**

The IC50 values were determined by a nonlinear regression analysis. For the determination of the type of inhibition, the Lineweaver-Burk plot and the kinetic parameters were determined by a nonlinear regression analysis using the computer program Kcat (BioMetallics, Princeton, NJ). All data were analyzed using the average of duplicate determinations. The variances between the duplicate determinations were < 10%.

**Results**

**Effects of endogenous compounds on CYP2A6 activity**

The inhibitory effects of neurotransmitters and their precursors as well as steroid hormones on the CYP2A6 activity were investigated using recombinant CYP2A6 expressed in baculovirus-infected insect cells (Fig. 2). The coumarin 7-hydroxylase activity was strongly inhibited by tryptamine (IC50 = 0.8 µM), and was moderately inhibited by dopamine (IC50 = 117 µM), serotonin (IC50 = 394 µM), and histamine (IC50 = 575 µM) (Fig. 2A, Table 1). Steroid hormones did not show inhibitory effects on the coumarin 7-hydroxylase activity (Fig. 2B). The cotinine 3’-hydroxylase activity was strongly inhibited by tryptamine (IC50 = 1.0 µM), and was moderately inhibited by dopamine (IC50 = 134 µM), serotonin (IC50 = 336 µM),
and histamine (IC50 = 829 µM) (Fig. 2C), but not by the steroid hormones (Fig. 2D). This inhibitory pattern was almost the same as that for the coumarin 7-hydroxylase activity.

The cotinine formation from nicotine was strongly inhibited by tryptamine (IC50 = 4.6 µM), and was moderately inhibited by dopamine (IC50 = 94 µM) and serotonin (IC50 = 592 µM) (Fig. 2E). In addition, noradrenaline (IC50 = 110 µM) and adrenaline (IC50 = 483 µM) also exhibited moderate inhibition. In contrast to the coumarin 7-hydroxylase and cotinine 3'-hydroxylase activities, most steroid hormones inhibited the cotinine formation from nicotine (Fig. 2F). Especially, estriol (IC50 = 5.2 µM), estrone (IC50 = 7.2 µM), and estradiol (IC50 = 8.2 µM) showed prominent inhibition for the cotinine formation from nicotine.

When the cotinine formation from nicotine was measured, cytosol as a source of aldehyde oxidase was usually added in the incubation mixture. To exclude the effects of the aldehyde oxidase activity, we investigated the inhibitory effect of the endogenous compounds on the nicotine-Δ5(1')-iminium ion formation from nicotine in the absence of cytosol (Fig. 3). The nicotine-Δ5(1')-iminium ion formation from nicotine was strongly inhibited by tryptamine (IC50 = 1.0 µM), and was moderately inhibited by dopamine (IC50 = 162 µM), serotonin (IC50 = 701 µM), histamine (IC50 = 722 µM) (Fig. 3A), but not by noradrenaline and adrenaline. In addition, the steroid hormones did not inhibit the nicotine-Δ5(1')-iminium ion formation, in contrast to cotinine formation (Fig. 3B).

**Inhibition constant and inhibitory patterns**

The Ki values and inhibition patterns of endogenous compounds exhibiting obvious inhibition of the CYP2A6 enzyme activities were determined (Table 2). The coumarin 7-hydroxylase activity was competitively inhibited by tryptamine (Ki = 0.2 ± 0.1 µM), serotonin (Ki = 252 ± 47 µM), dopamine (Ki = 49 ± 2 µM), and histamine (Ki = 428 ± 152 µM). The cotinine 3'-hydroxylase activity was also competitively inhibited by tryptamine (Ki = 0.2 ± 0.0 µM), serotonin (Ki = 167 ± 14 µM), dopamine (Ki = 22 ± 5 µM), and histamine (Ki = 359 ± 86 µM). These Ki values were significantly correlated between the two activities (r = 0.992, P < 0.005). Fig. 4 shows the typical Lineweaver-Burk plots of cotinine formation.
from nicotine and nicotine-Δ⁵(1⁰)-iminium ion formation from nicotine. First, it was clarified that the Km value of the latter activity (37 µM) was close to that of the former activity (44 µM). The nicotine-Δ⁵(1⁰)-iminium ion formation from nicotine was also competitively inhibited by tryptamine (Ki = 0.3 ± 0.0 µM), serotonin (Ki = 316 ± 48 µM), dopamine (Ki = 66 ± 21 µM), and histamine (Ki = 209 ± 47 µM). In contrast, the cotinine formation from nicotine was non-competitively inhibited by serotonin (Ki = 272 ± 74 µM) and un-competitively by dopamine (Ki = 11 ± 3 µM). Furthermore, the activity was un-competitively inhibited by noradrenaline (Ki = 54 ± 3 µM), adrenaline (Ki = 81 ± 6 µM), corticosterone (Ki = 36 ± 19 µM), testosterone (Ki = 60 ± 10 µM), estrone (Ki = 3.8 ± 0.9 µM), estradiol (Ki = 0.9 ± 0.5 µM), and estriol (Ki = 0.6 ± 0.1 µM).

**Tryptamine metabolism by CYP2A6**

We examined whether CYP2A6 can metabolize tryptamine or not. When pooled human liver microsomes was incubated with tryptamine, two peaks of metabolites, indole-3-acetic acid and tryptophol, were observed. However, recombinant CYP2A6 did not show the formation of these metabolites, even at a high substrate concentration, 1 mM (data not shown).

**Discussion**

Over a decade ago, large interindividual variability was reported for CYP2A6 activity (Shimada et al., 1994). Genetic polymorphisms of CYP2A6 gene can explain the interindividual variability, but our recent study revealed that considerable variability was still observed among homozygotes of the wild CYP2A6 allele (Nakajima et al., 2006a). Three possible reasons for the variability are inferred: 1) the expression level of CYP2A6 varies, possibly owing to differences in transcriptional or posttranscriptional regulation, 2) dietary and/or environmental compounds may affect the enzymatic activity, as we recently reported that dietary isoflavones inhibit nicotine C-oxidation (Nakajima et al., 2006c); 3) endogenous compounds may affect the enzymatic activity. In the present study, to examine the third
possibility, the inhibitory effects of endogenous compounds on CYP2A6 activity were investigated.

In accordance with a previous study using recombinant CYP2A6 expressed in human lymphoblast cells showing that the Ki value of tryptamine for coumarin 7-hydroxylation was 1.7 µM (Zhang et al., 2001), we found that tryptamine is a potent inhibitor of CYP2A6 (Ki = 0.2 – 0.3 µM). The inhibition pattern of tryptamine for CYP2A6 was competitive, but tryptamine was not a substrate of CYP2A6, in accordance with a previous report (Yu et al., 2003). It has been reported that tryptamine inhibits phenacetin O-deethylation catalyzed by CYP1A2 (Ki = 40 µM) (Agúndez et al., 1998), diclofenac 4-hydroxylation catalyzed by CYP2C9 (Ki = 340 µM) (Gervasini et al., 2001), and dextromethorphan O-demethylation catalyzed CYP2D6 (IC₅₀ = 45 µM) (Martínez et al., 1997) in human liver microsomes. Thus, tryptamine exhibited a most potent inhibition toward CYP2A6 among human P450s. In addition to tryptamine, we found that serotonin, dopamine, and histamine also inhibit CYP2A6. The inhibitory effects of serotonin on CYP2A6 activity (Ki = 167 – 316 µM) seem to be weaker than those on CYP1A2 (Ki = 35 µM) (Agúndez et al., 1998), CYP2C9 (Ki = 63.5 µM) (Gervasini et al., 2001), and CYP3A4 (Ki = 26 µM) (Martínez et al., 2000) activities. In contrast, the inhibitory effects of dopamine on CYP2A6 activity (Ki = 22 – 66 µM) seem to be stronger than those on CYP1A2 (Ki = 520 µM) (Agúndez et al., 1998) and CYP2C9 (Ki = 405 µM) (Gervasini et al., 2001) activities. Although adrenaline has been reported to inhibit CYP1A2 (Ki = 175 µM) (Agúndez et al., 1998), CYP2C9 (Ki = 156 µM) (Gervasini et al., 2001), and CYP3A4 (Ki = 42 µM) (Martínez et al., 2000) activities, it did not inhibit CYP2A6. Thus, the inhibitory effects of neurotransmitters depend on human CYP isoforms.

Cotinine formation from nicotine is frequently used as a specific activity for CYP2A6. However, it consists of two step reactions, CYP2A6-dependent nicotine-Δ⁵₁'-iminium ion formation from nicotine and subsequent cotinine formation from nicotine-Δ⁵₁'-iminium ion that is mainly catalyzed by cytosolic aldehyde oxidase (Brandänge and Lindblom, 1979). Based on the difference in the inhibitory effects on cotinine formation from nicotine and
nicotine-$\Delta^{5(1')}$-iminium ion formation from nicotine, it appeared that some neurotransmitter and steroid hormones could inhibit aldehyde oxidase. These results were supported by a previous study reporting that human cytosolic aldehyde oxidase was inhibited by estradiol, estrone, and ethinyl estradiol (IC$_{50}$ = 0.29 – 0.57 µM), using phthalazine as a substrate (Obach, 2004). Thus, nicotine-$\Delta^{5(1')}$-iminium ion formation from nicotine would be preferable to evaluate CYP2A6 activity rather than cotinine formation from nicotine. However, to predict the \textit{in vivo} situation, the cotinine formation from nicotine in the presence of cytosol should be evaluated.

Cotinine formation from nicotine was inhibited by tryptamine, serotonin, dopamine as well as noradrenaline and adrenaline. Nicotine absorbed from lung by cigarette smoking reaches the brain rapidly. Once nicotine penetrates the central nervous system, it acts as an agonist of nicotinic acetylcholine receptors and evokes dopamine, serotonin, and noradrenaline release (Di Chiara, 2000). It has been reported that monoamine oxidase A and B, metabolizing serotonin, dopamine, and noradrenaline (Johnston, 1968; Youdim and Riederer, 1993), were inhibited in brains of smokers (Fowler et al., 1996a, b). Thus, the inhibition of the nicotine metabolism by the increased neurotransmitters in brain might be associated with nicotine addiction and/or dependence. Previous reports have shown that the serotonin concentration was 6 µM in rat brain homogenate, 4 µM in sheep cerebrospinal fluid, and adrenaline concentration was 0.14 nM in human cerebrospinal fluid (Mousseau, 1993; Ensinger et al., 1992). However, the concentrations in cerebrospinal fluid are not necessarily indicative of the concentrations in brain. There are serious limitations in quantifying of the levels of these substances in brain tissues in human. Furthermore, these transmitter substances are not homogeneously distributed in the brain. The concentrations at the synaptic space, where the neurotransmitters are secreted, must be several orders of magnitude greater. Thus, even though the Ki values in our study are high, an inhibition of CYP2A6 in brain may take place.

Several previous reports suggested the possibility that estrogens might induce CYP2A6. Zeman et al. (2002) reported that the ratio of nicotine/(cotinine + \textit{trans}-3’-hydroxycotinine)
was significantly lower in women compared with men. Similarly, we also found that the ratio of cotinine/nicotine was higher in women than in men (Nakajima et al., 2006a). It has been reported that the clearance of nicotine via the cotinine pathway was higher during pregnancy compared with postpartum (Dempsey et al., 2002) and is accelerated by oral contraceptive use in women (Benowitz et al., 2006). On the other hand, the present study demonstrated that estrogens potently inhibited the cotinine formation from nicotine. Circulating estrogens, which mainly originate from ovarian steroidogenesis in premenopausal women and peripheral aromatization of ovarian and adrenal androgens in postmenopausal women, are eliminated from the body by metabolic conversion in the liver. The major enzymes responsible for the metabolism of estrogens are CYP3A4 and CYP1A2 (Lee et al., 2003). The Km values of CYP3A4 and CYP1A2 for estrogen metabolism have been reported to be 54 – 111 µM and 17 – 28 µM, respectively (Yamazaki et al., 1998). Thus, the extremely low Ki values of estrogens for cotinine formation from nicotine indicated that estrogens would inhibit nicotine metabolism in vivo. The regulation of nicotine metabolism by estrogens might be complicated in vivo, because of the induction of CYP2A6 and inhibition of aldehyde oxidase.

In conclusion, we found that some neurotransmitters and steroid hormones inhibit human CYP2A6 activity. It is known that there are large inter- and intra-individual variations in the level of these endogenous compounds (Curtin et al., 1996). The inhibitory effects of the endogenous substances might be one of the factors influencing the variability of CYP2A6 activity.

Acknowledgements

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References


Brandänge S and Lindblom L (1979) The enzyme “aldehyde oxidase” is an iminium oxidase. Reaction with nicotine $\Delta^{1'(5')}$ iminium ion. *Biochem Biophys Res Commun* 91: 991-996.


Johnston JP (1968) Some observations upon a new inhibitor of monoamine oxidase in brain.


Yamanaka H, Nakajima M, Fukami T, Sakai H, Nakamura A, Katoh M, Takamiya M, Aoki Y, and Yokoi T (2005) CYP2A6 and CYP2B6 are involved in nornicotine formation from nicotine in humans: interindividual differences in these contributions. Drug Metab
Dispos 33: 1811-1818.


Footnotes

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Figure legends

Figure 1. Structure of neurotransmitters and their precursors as well as steroid hormones used in the present study.

Figure 2. Inhibitory effects of neurotransmitters, their precursors (A, C, E) and steroid hormones (B, D, F) on human CYP2A6 activity. Coumarin 7-hydroxylase activity (A, B), cotinine 3’-hydroxylase activity (C, D), and cotinine formation from nicotine (E, F) by recombinant CYP2A6 expressed in baculovirus-infected insect cells were determined at the substrate concentrations of 1 µM, 250 µM, and 50 µM, respectively. The control activity was 7.7 pmol/min/pmol CYP for the coumarin 7-hydroxylation, 0.55 pmol/min/pmol CYP for the cotinine 3’-hydroxylation, and 10.3 pmol/min/pmol CYP for the cotinine formation from nicotine.

Figure 3. Inhibitory effects of neurotransmitters, their precursors (A) and steroid hormones (B) on nicotine-Δ^5(1')-iminium ion formation from nicotine at the substrate concentrations of 50 µM.

Figure 4. Lineweaver-Burk plots of cotinine formation (A, C, E) and nicotine-Δ^5(1')-iminium ion formation (B, D, F) from nicotine by the recombinant CYP2A6 in the presence of tryptamine (A, B), serotonin (C, D), and dopamine (E, F). The inhibitory patterns of tryptamine (A), serotonin (C), and dopamine (E) for the cotinine formative activity were the competitive, non-competitive, and un-competitive types, respectively. Tryptamine (B), serotonin (D), and dopamine (F) competitively inhibit the nicotine-Δ^5(1')-iminium ion formative activity.
Table 1. The IC₅₀ values of endogenous compounds for CYP2A6 enzyme activities.

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<th>Inhibitor</th>
<th>Coumarin 7-hydroxylation</th>
<th>Cotinine 3’-hydroxylation</th>
<th>Cotinine formation from nicotine</th>
<th>Nicotine-Δ²⁻(1')-iminium ion formation from nicotine</th>
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Table 2. The Ki values and inhibition types of endogenous compounds for the CYP2A6 enzyme activities.

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<th>Type of inhibition</th>
<th>Cotinine 3'-hydroxylation Ki (µM)</th>
<th>Type of inhibition</th>
<th>Cotinine formation from nicotine Ki (µM)</th>
<th>Type of inhibition</th>
<th>Nicotine-∆⁵(1')-iminium ion formation from nicotine Ki (µM)</th>
<th>Type of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tryptophan</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Tryptamine</td>
<td>0.2 ± 0.1</td>
<td>Competitive</td>
<td>0.2 ± 0.0</td>
<td>Competitive</td>
<td>0.7 ± 0.1</td>
<td>Competitive</td>
<td>0.3 ± 0.0</td>
<td>Competitive</td>
</tr>
<tr>
<td>Serotonin</td>
<td>252 ± 47</td>
<td>Competitive</td>
<td>167 ± 14</td>
<td>Competitive</td>
<td>272 ± 74</td>
<td>Non-competitive</td>
<td>316 ± 48</td>
<td>Competitive</td>
</tr>
<tr>
<td>Melatonin</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>L-Dopa</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Dopamine</td>
<td>49 ± 2</td>
<td>Competitive</td>
<td>22 ± 5</td>
<td>Competitive</td>
<td>11 ± 3</td>
<td>Un-competitive</td>
<td>66 ± 21</td>
<td>Competitive</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>54 ± 3</td>
<td>Un-competitive</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Adrenaline</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>81 ± 6</td>
<td>Un-competitive</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Histamine</td>
<td>428 ± 152</td>
<td>Competitive</td>
<td>359 ± 86</td>
<td>Competitive</td>
<td>–</td>
<td>–</td>
<td>209 ± 47</td>
<td>Competitive</td>
</tr>
<tr>
<td>Progesterone</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Corticosterone</td>
<td>–</td>
<td>–</td>
<td>36 ± 19</td>
<td>Un-competitive</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>–</td>
<td>–</td>
<td>149 ± 25</td>
<td>Mixed</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Testosterone</td>
<td>–</td>
<td>–</td>
<td>60 ± 10</td>
<td>Un-competitive</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Androstanediol</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>3.8 ± 0.9</td>
<td>Un-competitive</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Estrone</td>
<td>–</td>
<td>–</td>
<td>0.9 ± 0.5</td>
<td>Un-competitive</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Estradiol</td>
<td>–</td>
<td>–</td>
<td>0.6 ± 0.1</td>
<td>Un-competitive</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Data generated by nonlinear regression analysis are expressed as mean ± SE of duplicate determinations.

- : Not applicable.
Fig. 1. Neurotransmitters and their precursors:

- Tryptophan
- Tyrosine
- L-Dopa
- Dopamine
- Serotonin
- Noradrenaline
- Melatonin
- Adrenaline
- Histamine

Steroid hormones:

- Progesterone
- Androstenedione
- Estrone
- Testosterone
- Estradiol
- Corticosterone
- Androstanediol
- Estriol
Fig. 3.

A

Nicotine-Δ5(1)',-iminium ion formation (% of Control)

Concentration (μM)

B

Nicotine-Δ5(1)',-iminium ion formation (% of Control)

Concentration (μM)
Cotinine formation from nicotine

Tryptamine

1/V (pmol/min/pmol P450)⁻¹ vs. 1/ [Nicotine] (μM)⁻¹

- None
- 1 μM
- 2 μM
- 4 μM

Serotonin

1/V (pmol/min/pmol P450)⁻¹ vs. 1/ [Nicotine] (μM)⁻¹

- None
- 100 μM
- 300 μM
- 600 μM

Dopamine

1/V (pmol/min/pmol P450)⁻¹ vs. 1/ [Nicotine] (μM)⁻¹

- None
- 30 μM
- 60 μM
- 100 μM

Nicotine-Δ5'(1')-iminium ion formation from nicotine

Tryptamine

1/height vs. 1/ [Nicotine] (μM)⁻¹

- None
- 0.3 μM
- 0.6 μM
- 1.0 μM

Serotonin

1/height vs. 1/ [Nicotine] (μM)⁻¹

- None
- 200 μM
- 500 μM
- 700 μM

Dopamine

1/height vs. 1/ [Nicotine] (μM)⁻¹

- None
- 50 μM
- 100 μM
- 150 μM