Inhibitory and Stimulatory Effects of Selective Serotonin Reuptake Inhibitors on Cytochrome P450 2D6-mediated Dopamine Formation from *p*-Tyramine

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ABSTRACT - PURPOSE: The effects of selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine and paroxetine on dopamine formation from *p*-tyramine, mediated by cytochrome P450 (CYP) 2D6.2 (Arg296Cys, Ser486Thr) and CYP2D6.10 (Pro34Ser, Ser486Thr), were compared with their effects on CYP2D6.1 (wild type)-mediated dopamine formation, to investigate the influence of a *CYP2D6* polymorphism on neuroactive amine metabolism in the brain. METHODS: The Michaelis constants (K_m) and maximal velocity (V_{max}) values of dopamine formation mediated by CYP2D6.1, CYP2D6.2, and CYP2D6.10 (expressed in recombinant *Escherichia coli*), and inhibition constants (K_i) of the SSRIs toward dopamine formation catalyzed by the CYP2D6 variants were estimated. RESULTS: The K_m values for CYP2D6.2 and CYP2D6.10 decreased at lower fluoxetine concentrations, while the V_{max} values for all CYP2D6 variants increased, indicating that fluoxetine stimulated dopamine formation. Conversely, paroxetine competitively inhibited dopamine formation mediated by CYP2D6.1, CYP2D6.2, and CYP2D6.10 with K_i values of 0.47, 1.33, and 31.3 μM, respectively. CONCLUSIONS: These results suggest that the inhibition/stimulation of CYP2D6-mediated dopamine formation by these SSRIs would be affected by *CYP2D6* polymorphisms in the brain.

INTRODUCTION

Cytochrome P450s (P450s or CYPs) catalyze the oxidation of endogenous compounds, including steroid hormones and neuroactive amines, as well as a wide variety of exogenous drugs (1,2). CYP2D6 accounts for only 2–9% of all P450s present in human livers (3,4), but metabolizes approximately 20% of all therapeutic agents (5,6). It is also expressed in the brain (midbrain) (7), howbeit with unknown physiological and pharmacological functions, specifically in the human brain.

CYP2D6 is expressed polymorphically; till date, 137 allelic variants and a series of subvariants of the *CYP2D6* gene have been found, and this number continues to increase (8,9). Interestingly, the *CYP2D6* polymorphism has also been associated with human behavior (10,11). For instance, poor metabolizers of CYP2D6 had a higher frequency of extreme responses compared with extensive metabolizers and scored significantly lower on the Karolinska Psychasthenia scale (12,13). The *CYP2D6*2* allele is present in 27–32% of Caucasian populations, and contains 2 amino acid substitutions; Arg296Cys and Ser486Thr (14). The Michaelis constants (K_m), maximal velocity (V_{max}), and V_{max}/K_m values (intrinsic clearance, CL_{int}) for 62–85% of all CYP2D6.2-mediated metabolic reactions are comparable with those mediated by CYP2D6.1 (wild-type). However K_m values in 31% of CYP2D6.2-mediated reactions are more than 2-fold higher, and 15 and 38% of the V_{max} and V_{max}/K_m values, respectively, are less than one-half (9). The *CYP2D6*10* allele, including *CYP2D6*10A* and *CYP2D6*10B* variants, is widely observed in Japanese (31–38%) (13,14) and Chinese (51%) (15) populations, and contains 2 amino acid substitutions; Pro34Ser and Ser486Thr (16,17). *CYP2D6*36* (formerly *CYP2D6*10C*) has a gene-conversion event at exon 9 derived from CYP2D7 and contains 13 more base substitutions than *CYP2D6*10B* (15). In CYP2D6.10-mediated metabolic reactions, 63% of the K_m values are >2-folds higher, and 74 and 93% of the V_{max} and V_{max}/K_m values, respectively, are less than 1/2 (9).

Tyramine is exogenously present in fermented foods such as cheese and wine, and endogenously present in the brain, especially in the basal ganglia and limbic system (18).

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Dopamine, a neurotransmitter and precursor of noradrenaline and adrenaline, is formed from the biotransformation of *p*- and *m*-tyramine, and dihydroxyphenylalanine (L-DOPA) in the brain, through CYP2D6-mediated ring-hydroxylation (5,18-20). Initially, we demonstrated that quinidine (a typical human CYP2D6 inhibitor) and quinine (an inhibitor of rat CYP2D subfamily, including rat brain CYP2D4, rather than human CYP2D6) inhibited CYP2D6.10-mediated dopamine formation from *p*-tyramine to a lesser extent compared with those catalyzed by CYP2D6.1 and CYP2D6.2 (21).

We recently observed that imipramine and desipramine (a pharmacologically active *N*-demethylated metabolite derived from imipramine by CYP1A2, CYP2C19, and CYP3A4), older generation tricyclic antidepressants (22-24), competitively or noncompetitively inhibited dopamine formation mediated by CYP2D6.1, CYP2D6.2, and CYP2D6.10, with higher inhibition constants (K_i) for CYP2D6.10 (25). Conversely, V_{max} values for dopamine formation by all CYP2D6 variants gradually increased with increasing concentrations of fluvoxamine, indicating that fluvoxamine stimulated dopamine formation (25). Fluvoxamine is a selective serotonin reuptake inhibitor (SSRI) widely used in the treatment of depression to overcome the toxicity of older generation antidepressants (22,23,26-29). Recently many kinds of SSRIs, including the typical fluoxetine and paroxetine (Fig. 1), have been used (30). Thus, in this study, we investigated how *CYP2D6* polymorphism affects the inhibitory/stimulatory effects of fluoxetine and paroxetine on CYP2D6-mediated formation of endogenous dopamine from *p*-tyramine in the brain.

METHODS

Materials

CYP2D6.1, CYP2D6.2, and CYP2D6.10 co-expressed with NADPH-P450 reductase were all expressed in recombinant *Escherichia coli* (Bactosomes), purchased from Cypex Ltd. (Dundee, UK). *p*-Tyramine, dopamine, fluoxetine hydrochloride, and paroxetine maleate were obtained from Tokyo Chemical Industry (Tokyo, Japan). All other reagents and organic solvents used were of the highest purity commercially available.

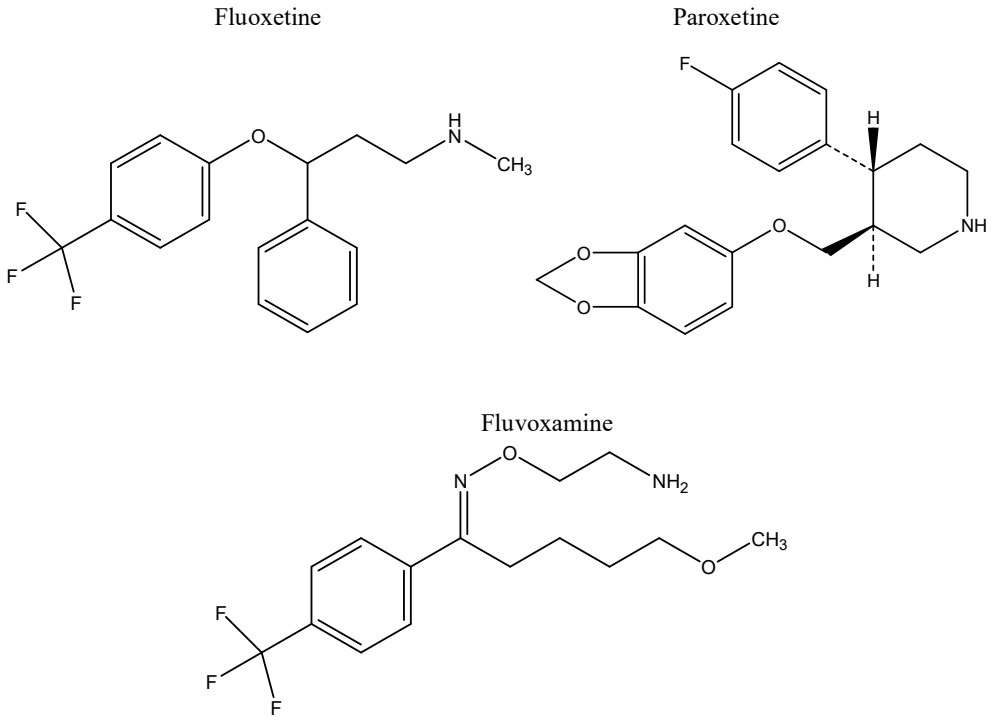

Figure. 1. Chemical structures of fluoxetine, paroxetine, and fluvoxamine.

Determination of dopamine formation

Dopamine formation from *p*-tyramine in the presence or absence of SSRIs was determined as previously described (19,21,31). Briefly, the incubation mixture consisted of 10 nM CYP2D6 variants, 0.05–10 mM *p*-tyramine, 1 mM NADPH, 50 µl of water or 0.5–1000 µM SSRIs dissolved in water, and 100 mM potassium phosphate buffer (pH 7.4) in a final volume of 500 µl. After pre-incubation at 37°C for 3 min, the reaction was activated by adding NADPH, and the mixture was incubated at 37°C for 10 min. Dopamine concentrations in the mixtures were measured using high performance liquid chromatography (HPLC). Tosoh (Tokyo, Japan) TSK-gel ODS-120T (5 µm, 4.6×250 mm) analytical columns were used, and the fluorescence intensities were determined at an excitation wavelength of 280 nm and an emission wavelength of 340 nm (19,21,31). The linearity of the reaction with P450 concentrations and incubation times were confirmed for CYP2D6.1 and its variants in preliminary experiments. Unless stated otherwise, the inhibitory/stimulatory effects of SSRIs on CYP2D6.1- and CYP2D6.2-mediated dopamine formation were investigated at 0.1 mM substrate concentration, and 1 mM substrate concentration was used for CYP2D6.10-mediated reactions. These substrate concentrations were close to or $<K_m$ values previously reported (21,31).

DATA ANALYSES

All data were analyzed using the means of duplicate or triplicate reactions, and K_m, V_{max}, and K_i values and the standard deviations (S.D.) as indices of the precision of the calculated parameters were calculated from Michaelis-Menten kinetics using nonlinear least squares regression by means of MULTI (32). Statistical significance was determined by Student's *t*-test, and the significance level was set at $p<0.05$. JMP 5 software (SAS Institute Inc, Cary, NC, USA) was used for the statistical analysis.

RESULTS

Firstly, we investigated the effect of fluoxetine and paroxetine at 0.1, 0.1, and 1 mM substrate concentrations for CYP2D6.1, CYP2D6.2, and CYP2D6.10, respectively (Fig. 2). Fluoxetine at 1 µM stimulated dopamine formation catalyzed by CYP2D6.2 and CYP2D6.10, but not CYP2D6.1. Conversely, paroxetine at 1 µM and 10 µM inhibited CYP2D6.1- and CYP2D6.2-mediated dopamine formation by 49-68% and 74-89%, respectively, while CYP2D6.10 was inhibited by 42%, only at 100 µM (Fig. 2).

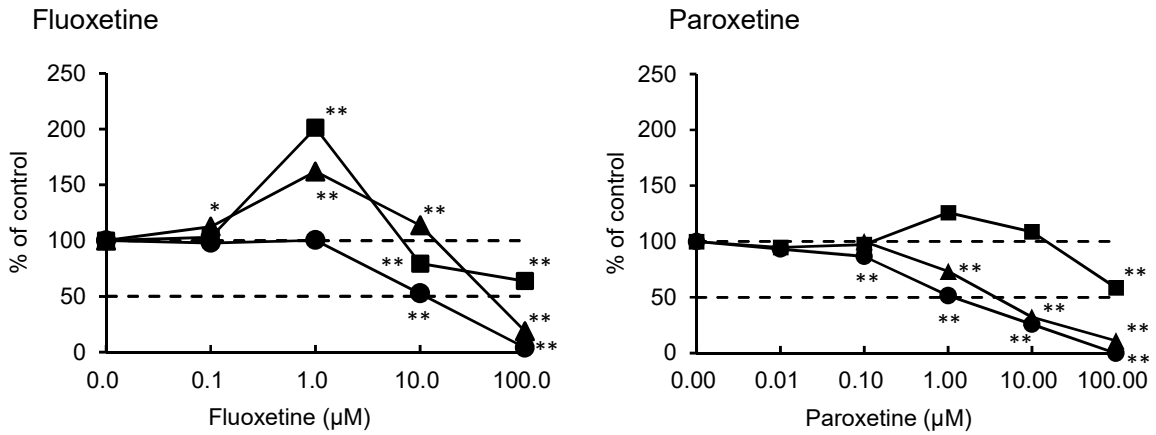

Fig. 2. Effects of fluoxetine and paroxetine on dopamine formation from *p*-tyramine mediated by CYP2D6.1, CYP2D6.2, and CYP2D6.10.

●: CYP2D6.1, ▲: CYP2D6.2, ■: CYP2D6.10. Substrate concentrations for CYP2D6.1 and CYP2D6.2 were 0.1 mM, and 1 mM substrate concentration was used for reactions mediated by CYP2D6.10. Values are means of duplicate or triplicate determinations. * $p<0.05$ and ** $p<0.01$ *vs* control.

Dopamine formation mediated by CYP2D6 and its variants in the presence of various concentrations of fluoxetine was compared (Table 1). The K_m value for CYP2D6.1 increased with increasing fluoxetine, while K_m values for CYP2D6.2 and CYP2D6.10 decreased at concentrations below 1 and 5 μM, respectively and then increased with increasing fluoxetine in CYP2D6.2. Although the V_{max} values for CYP2D6.1 and CYP2D6.2 gradually increased with increasing fluoxetine, the V_{max} value for CYP2D6.10 increased at only 5 μM. Thus, V_{max} and V_{max}/K_m values for all CYP2D6 variants except the V_{max}/K_m value for CYP2D6.1, increased to a maximum of 1.4-1.8 times and 2.3-3.4 times, respectively.

Paroxetine competitively inhibited dopamine formation mediated by CYP2D6.1, CYP2D6.2, and CYP2D6.10 with K_i values of 0.47, 1.33, and 31.3 μM, respectively (Fig. 3); the estimated K_i values of paroxetine against CYP2D6.2 and CYP2D6.10 were 3 and 67 times, higher than that against CYP2D6.1, respectively.

Table 1. Effect of fluoxetine on kinetic parameters of dopamine formation

CYP2D6	Fluoxetine (μM)	K_m (mM)		V_{max} (nmol/min/nmol P450)		V_{max}/K_m (μL/min/nmol P450)	
CYP2D6.1	0	0.14 ± 0.02	(100)	7.1 ± 0.3	(100)	50.2	(100)
	1	0.16 ± 0.03	(114)	9.8 ± 0.6	(137)	60.4	(120)
	4	0.35 ± 0.02	(245)	13.4 ± 0.3	(189)	38.6	(77)
	10	1.38 ± 0.12	(976)	17.3 ± 0.8	(244)	12.5	(25)
CYP2D6.2	0	0.17 ± 0.02	(100)	7.2 ± 0.3	(100)	41.9	(100)
	0.1	0.10 ± 0.02	(57)	6.7 ± 0.3	(92)	68.1	(162)
	1	0.10 ± 0.02	(59)	9.6 ± 0.6	(133)	94.6	(225)
	10	0.29 ± 0.06	(165)	13.1 ± 0.9	(180)	45.8	(109)
CYP2D6.10	0	1.18 ± 0.28	(100)	6.8 ± 0.6	(100)	5.7	(100)
	0.2	1.02 ± 0.38	(86)	6.9 ± 0.9	(102)	6.8	(119)
	1	0.41 ± 0.09	(35)	6.6 ± 0.4	(98)	16.1	(282)
	5	0.47 ± 0.06	(40)	9.2 ± 0.3	(135)	19.6	(342)

Values in parentheses show the percentage of control. Values are means ± S.D. of the data set using a nonlinear kinetic analysis from mean values obtained in duplicate or triplicate at each substrate/inhibitor concentration.

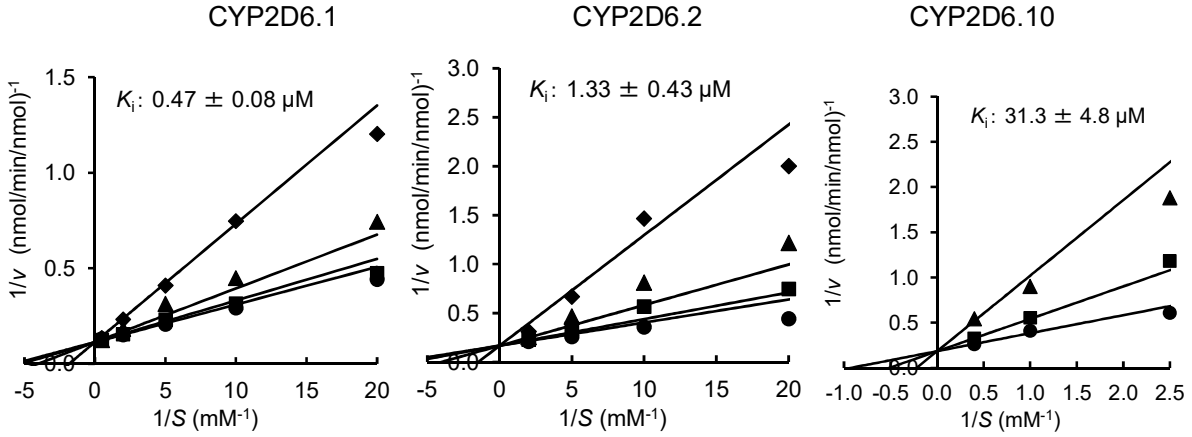

Figure. 3. Inhibitory effects of paroxetine on dopamine formation from *p*-tyramine mediated by CYP2D6.1, CYP2D6.2, and CYP2D6.10.

Paroxetine concentrations for CYP2D6.1, ●: 0 μM, ■: 0.05 μM, ▲: 0.2 μM, ◆: 1 μM, for CYP2D6.2, ●: 0 μM, ■: 0.2 μM, ▲: 1 μM, ◆: 5 μM, for CYP2D6.10, ●: 0 μM, ■: 25 μM, ▲: 100 μM. K_i values are means ± S.D. of the data set using a nonlinear kinetic analysis from mean values obtained in duplicate or triplicate at each substrate/inhibitor concentration.

DISCUSSION

Human CYP2D6 is expressed in the liver and brain, especially the midbrain (7). Previously we observed that dopamine formation from *p*-tyramine as well as progesterone hydroxylation were affected by *CYP2D6* polymorphism (21,31). In addition, many of the reported kinetic parameters such as K_m, V_{max}, and V_{max}/K_m, of CYP2D6.2 and CYP2D6.10 were different from those of CYP2D6.1 (wild type) (9). Unlike reports on metabolic activities, only a few reports exist on the inhibitory effects of *CYP2D6* polymorphism on the inhibition of CYP2D6-mediated reactions induced by exogenous compounds such as antidepressants. We recently observed that the K_i values of quinidine (a typical strong inhibitor of CYP2D6 *in vitro*, as recommended in the guidance for drug interaction studies by US FDA (33), EMA (34), and Japanese PMDA (35)) and quinine (a potent inhibitor of the rat CYP2D subfamily, including rat brain CYP2D4 (36-39)) against CYP2D6.1 were lower than those against CYP2D6.10. Furthermore, we previously demonstrated that psychotropic drugs such as imipramine, desipramine, fluoxetine, and mazindol, inhibited 21-hydroxylation of progesterone and/or allopregnanolone (a neuroactive steroid), mediated by CYP2D6 and CYP2D4. However, fluoxetine increased the K_m and V_{max} values of CYP2D6-mediated progesterone 21-hydroxylation (40). Imipramine and desipramine inhibited dopamine formation from *p*-tyramine and the K_i values for CYP2D6.10 were higher than those for CYP2D6.1 (25). In this study, paroxetine inhibited dopamine formation with a higher K_i value for CYP2D6.10 compared with that for CYP2D6.1. These results suggested that the inhibition of CYP2D6 by various psychotropic drugs, including these antidepressants, would be affected by *CYP2D6* polymorphism in the brain.

We have reported that steroid hormones such as progesterone and testosterone, stimulate the metabolism of CYP3A4 substrates; however, selection of the enzyme source (recombinant P450s or liver microsomes) can affect enzyme activation (41). More so, some researchers have demonstrated the activation of CYP3A4-mediated metabolic reactions, with no information on the detailed mechanism (40-42). This activation has similarly been demonstrated for other P450s such as CYP1A2, CYP2C8, CYP2C9, CYP2D6, and CYP3A7 (42). We previously observed that fluoxetine increased the K_m and V_{max} values for CYP2D6-mediated progesterone 21-hydroxylation (40), and that fluvoxamine and other SSRIs increased the K_m and V_{max} values for dopamine formation mediated by CYP2D6.1, CYP2D6.2, and CYP2D6.10, but decreased the K_m value for CYP2D6.10 to the level for CYP2D6.1 and CYP2D6.2 (25). Fluoxetine-induced activation of CYP2D6-mediated progesterone 21-hydroxylation was the first finding in relation to CYP2D6 (40,42). In this study, fluoxetine also increased K_m and V_{max} values for dopamine formation mediated by CYP2D6.1 and its variants and decreased the K_m value for CYP2D6.10 to similar levels as those of CYP2D6.1 and CYP2D6.2. These results suggest that the CYP2D6 activation pattern would be affected by *CYP2D6* polymorphism. Apart from selective inhibition of serotonin reuptake, some SSRIs such as fluoxetine and fluvoxamine that are metabolized by CYP2D6, exhibit novel physiological actions *via* the activation of dopamine formation and 21-hydroxylation of neurosteroids, including progesterone and allopregnanolone, mediated by brain CYP2D6 (43). Interestingly, fluoxetine and fluvoxamine but not paroxetine have a trifluoromethylphenoxy group in their chemical structure (Fig. 1), suggesting that this functional moiety may be essential for activation. On the other hand, not only paroxetine but also fluoxetine were demonstrated to inhibit CYP2D6-catalyzed sparteine oxidation in human liver microsomes (44, 45), indicating that the activation/inhibition depends on the substrates. Further investigations like using three-dimensional structural analyses such as molecular docking simulation (46,47), should be conducted to determine the underlying reason.

In conclusion, this study suggests that unlike paroxetine, fluoxetine stimulate CYP2D6-mediated dopamine formation from *p*-tyramine, and *CYP2D6* polymorphism affect the inhibitory/stimulatory potency of SSRIs. However, further clinical studies are required to confirm the therapeutic relevance of our findings.

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