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## Sertraline and Its Metabolite Desmethylsertraline, but not Bupropion or Its Three Major Metabolites, Have High Affinity for P-Glycoprotein

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### Abstract

The ATP-binding cassette (ABC) transporter protein subfamily B1 line (ABCBI) transporter P-glycoprotein (P-gp) plays an important role in the blood–brain barrier limiting a broad spectrum of substrates from entering the central nervous system. In the present study, the transport activity of P-gp for sertraline, desmethylsertraline, bupropion, and the major metabolites of bupropion, threo-amino alcohol (TB), erythro-amino alcohol (EB), and hydroxy metabolite (HB) was studied using an ATPase assay in expressed human P-gp membranes by measuring concentrations of inorganic  $P_i$  in expressed human P-gp membranes. Verapamil was included as a positive control. The Michaelis–Menten equation was used for characterizing the kinetic data. Sertraline and desmethylsertraline showed high affinity for P-gp. The  $V_{max}/K_m$  values of sertraline ( $1.6 \text{ min}^{-1} \times 10^{-3}$ ) and desmethylsertraline ( $1.4 \text{ min}^{-1} \times 10^{-3}$ ) were comparable with that of verapamil ( $1.7 \text{ min}^{-1} \times 10^{-3}$ ). Bupropion and its three metabolites showed very weak affinity for P-gp, with  $V_{max}/K_m$  values lower than  $0.01 \text{ min}^{-1} \times 10^{-3}$ . The results of the present study indicate that sertraline and desmethylsertraline have high affinity for P-gp, whereas bupropion and its three major metabolites TB, EB, and HB have very weak affinity for P-gp. These findings may help to explain observed drug–drug interactions among antidepressants.

### Keywords

P-glycoprotein; sertraline; desmethylsertraline; bupropion; antidepressant; blood–brain barrier

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The drug transporter protein, P-glycoprotein (P-gp), is a member of the ATP-binding cassette (ABC) superfamily that is widely localized at various human tissues including the apical membranes of the gastrointestinal tract, the biliary canalicular membranes of hepatocytes, the luminal membranes of proximal tubular epithelial cells in the kidney, the testes, the placenta and the luminal membranes of endothelial cells of the blood–brain barrier (BBB).<sup>1,2</sup> As a drug efflux transporter, P-gp plays important role in drug absorption, distribution, and

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excretion.<sup>3-5</sup>) Inhibition and induction of P-gp function can result in significant drug-drug interactions.

The function of P-gp in BBB can significantly limit the brain uptake of its substrate drugs and affect therapeutic outcomes of central nervous system (CNS) acting drugs. By using the ABCB1a/b  $-/-$  knockout mice, P-gp has been shown to significantly limit the brain entry of a wide variety of structurally unrelated drugs.<sup>6-14</sup>) Inhibition of P-gp activity can greatly increase P-gp substrate concentrations in brain and may increase their neurotoxicity.<sup>15-17</sup>) Because of the importance of P-gp in CNS drug disposition, characterization of the binding affinities of CNS drugs for P-gp has clinical implications.

Newer antidepressants, *i.e.* the selective serotonin reuptake inhibitors (SSRIs) and multi-receptor antidepressants, venlafaxine, mirtazapine, bupropion, and nefazodone have advantages over the classical tricyclic antidepressants in lower frequency to cause unwanted side effects and are extensively used worldwide due to established antidepressants efficacy.<sup>18</sup>) However, a substantial number of patients with antidepressant therapy still exhibit treatment resistance despite increasing doses. The reason for this resistance is unknown.

Recently, the transport efficacy of most of the antidepressants by P-gp has been studied by using the ABCB1a/b  $-/-$  mouse or *in vitro* cell culture models<sup>10-12,19</sup>) except for two drugs, sertraline and bupropion. In these reports, most of the studied antidepressants (*e.g.* amitriptyline, nortriptyline, citalopram, and trimipramine) were shown to be substrates of P-glycoprotein<sup>10-12,19</sup>) These results suggest that the variable expression of P-gp among patients may be an important source of variability in treatment response for the antidepressants.

With availability of human P-gp membranes, we have previously used an ATPase assay method to determine the drug stimulated P-gp-ATPase activity and binding affinity of several antipsychotic drugs.<sup>20</sup>) Our results indicated that atypical antipsychotic drugs (AAPs), risperidone, and olanzapine, were effectively transported by P-gp. The findings have been verified by our subsequent *in vivo* *Abcb1a/b* gene knockout mouse experiments,<sup>13,14</sup>) supporting the ATPase assay to provide reliable information of P-gp substrates' binding affinity. In the present report, we studied the binding affinity of sertraline, desmethylsertraline, bupropion and its three major metabolites for P-gp using the ATPase method.

## MATERIALS AND METHODS

### Materials

Human P-gp membranes (5 mg/ml) prepared from baculovirus-infected insect cells were purchased from Gentest Inc. (Woburn, MA, U.S.A.). Sertraline and desmethylsertraline were obtained from Pfizer (Groton, CT, U.S.A.). Bupropion, and its three major metabolites, the hydroxy metabolite (hydroxy-BUP; 306U), the *erythro*- (17U) and *threo*-metabolites (A494U) were obtained from Burroughs Wellcome Co. (Research Triangle Park, NC, U.S.A.). Other chemicals and reagents were the purest grade available and were obtained from Fisher Scientific Co. (Fairlawn, NJ, U.S.A.) or Sigma Chemical Co. (St. Louis, MO, U.S.A.).

### ATPase Assay

Reactions were carried out in low-binding 96-well plates (Corning Costar, NY, U.S.A.). The reaction mixtures, in a final volume of 60  $\mu$ l, contained 50 mM Tris-Mes buffer (pH 6.8), 40  $\mu$ g P-gp membranes, test drug and 4 mM Mg-ATP. After pre-warming at 37°C for 3 min, the reactions were initiated by the addition of Mg-ATP. Verapamil served as a positive control. For each test incubation, identical incubations containing 100  $\mu$ M *ortho*-vanadate, a specific inhibitor of P-gp, were served as controls for baseline ATPase activity. P-gp-dependent ATPase activity was quantified by determining the increase in  $P_i$  concentration that was subtracted from

the activity generated in the presence of *ortho*-vanadate from the activity generated without *ortho*-vanadate to yield vanadate-sensitive ATPase activity during the energy-dependent P-gp drug transport process. All incubations at each condition (with and without *ortho*-vanadate) were performed in duplicate. An 8 point standard curve of 0—150 nM  $P_i$  was included in duplicate in each plate prior to incubation.

After incubation of the reaction mixtures at 37°C for 40—60 min, 30  $\mu$ l of 10% sodium dodecylsulfate with 0.1% Antifoam A was added to terminate the reaction. Then, to each incubation well, 200  $\mu$ l of 35 mM ammonium molybdate in freshly prepared 15 mM Zinc Acetate: 10% ascorbic acid pH 5.0 in a 1 : 4 proportion was added and incubated at 37°C for 20 min. The  $P_i$  release was measured by a spectrum II microplate reader with winselec T software (Tecan, Austria) at 620 nm using ultraviolet absorption.

### Time-Course and Concentration-Dependent Experiments

For each test compound, the linearity of incubation time-course was tested with 1  $\mu$ M—1 mM of the test compounds at 37°C for 0, 20, 40, and 60 min. The incubation time finally chosen for the study was based on examination of the linearity of the formation rate of  $P_i$  versus the incubation time.

The concentration dependence of the ATPase activity of verapamil, sertraline, desmethylsertraline, bupropion, and its three major metabolites, 306U, A494U, and 17U were assessed at 0, 1, 10, 50, 100, 250, 500, and/or 750 and 1000  $\mu$ M. All incubations were performed in duplicate.

### Data Analysis

The kinetics of the substrate stimulated ATPase activity were analyzed by fitting the data to equations for different enzyme models using an iterative nonlinear regression program (GraphPad Prism 4, San Diego, CA, U.S.A.). The choice of the best-fitted enzyme model was based on the examination of the Michaelis–Menten plots, the residual sum of squares, the standard errors, and Akaike's information criteria. The one enzyme Michaelis–Menten model was found to be the best-fitted enzyme model for all the kinetic data of the tested compounds:

$$V = V_{\max} \cdot S / (K_m + S)$$

where  $K_m$  is the substrate concentration at which the reaction velocity ( $V$ ) is 50% of  $V_{\max}$ , the maximal velocity, and  $S$  is substrate concentration.

## RESULTS

All of the tested compounds stimulated P-gp ATPase in a concentration-dependent manner (Figs. 1A, B). Sertraline and desmethylsertraline showed very strong stimulative effects on P-gp ATPase, with resultant  $V_{\max}/K_m$  values comparable to that of the positive control, verapamil (Table 1). However, bupropion and its three metabolites, HB, TB, and EB only showed weak affinity with P-gp ATPase, with much lower  $V_{\max}/K_m$  values compared with those of verapamil, sertraline and desmethylsertraline (Table 1).

Sertraline and desmethylsertraline showed some degree of inhibitory effects on P-gp ATPase activity at high concentrations (750, 1000  $\mu$ M). Fitting the data to the Michaelis–Menten equation with substrate self inhibition did not yield better fitting than the classical Michaelis–Menten equation. Therefore their kinetic parameters were estimated from the classical Michaelis–Menten equation.

## DISCUSSION

Based on the ATP-dependent feature of P-gp, it is generally agreed that drugs stimulating P-gp ATPase activity are transported by P-gp.<sup>21)</sup> The results of the present study indicated that sertraline and desmethylsertraline have very high affinity for P-gp ATPase. The estimated  $V_{\max}/K_m$  values were comparable with that of verapamil, a prototypical substrate of P-gp. In contrast to these drugs, bupropion and its three major metabolites only showed minimal affinity for P-gp. We admit that the ATPase assay method is not a sole method for assessing the substrate specificity for P-gp. A combination of this and cellular transport experiment in cell lines overexpressing P-gp and/or P-gp knockout mouse experiment will help to answer unequivocally if these compounds are substrates of P-gp.

Our results are in good agreement with our recent *in vivo* drug–drug interaction study in CFI mice,<sup>22)</sup> in which the brain concentrations of sertraline in CFI mice 1 h after sertraline administration were significantly increased (about 2.2-fold) by coadministration of risperidone, a potent inhibitor of P-gp.<sup>23)</sup> In addition, sertraline also significantly increased brain concentrations and *AUC* values of risperidone, a substrate of P-gp.<sup>24)</sup> These results suggest that sertraline may not only be a substrate of P-gp, but also be an effective *in vivo* inhibitor of P-gp<sup>22)</sup> and can increase brain entry of other substrates of P-gp. This conclusion is also consistent with an *in vitro* study in which sertraline was reported to be a strong inhibitor of P-gp.<sup>25)</sup>

The involvement of P-gp in the disposition of sertraline and desmethylsertraline is consistent with a recent clinical observation of placental passage of antidepressants<sup>26)</sup> In this study, sertraline and desmethylsertraline exhibited the lowest umbilical cord to maternal serum ratio (0.29) followed by paroxetine (0.54), fluoxetine, (0.64), and citalopram (0.71).<sup>26)</sup> These results are in good agreement with a protective role of P-gp in placenta limiting fetal access of these antidepressants, since several of the antidepressants listed (*i.e.*, paroxetine and citalopram) have been identified in previous studies to be substrates of P-gp.<sup>3,11,12)</sup>

This is the first report for a minimal role of P-gp in disposition of bupropion and its three major metabolites. Our results are in good agreement with our previous pharmacokinetic study in CFI mice, in which risperidone, a substrate and inhibitor of P-gp<sup>22,24,27)</sup> showed negligible effects on plasma and brain concentrations of bupropion and its metabolite HB<sup>22)</sup> The minimal involvement of P-gp in disposition of bupropion suggests that alteration of P-gp activity is not an important source for drug–drug interactions and variable therapeutic response of bupropion. The major mechanism for previous reported alteration of bupropion plasma concentrations by other perpetrator drugs is by changing of the metabolic enzyme activities of bupropion.<sup>28)</sup>

The function of P-gp in disposition of several other antidepressants has been studied using P-gp knockout mouse or *in vitro* cell culture models. These results showed that higher brain concentrations of amitriptyline, paroxetine, venlafaxine, doxepin, citalopram, and trimipramine were found in the P-gp knockout mice than in the wildtype mice.<sup>10,29)</sup> Our present findings along with these findings consistently indicated that P-gp in BBB may be an important factor limiting these antidepressants' brain entry. Therefore alteration of function and expression of P-gp may be responsible for the observed drug–drug interactions and different treatment response of the antidepressants among patients. The high affinity of sertraline for P-gp also suggests that the clinically observed sertraline-related interaction might be caused by a competitive inhibition of P-gp by sertraline in target sites. A good example of such interactions is the sertraline–digoxin interaction. As reported in a population-based assessment of the potential interaction between serotonin-specific reuptake inhibitors and digoxin, an increased risk of digoxin toxicity was observed following initiation of sertraline.<sup>30)</sup> As the

elimination of digoxin solely depends on P-gp-mediated excretion in kidney, an inhibition of P-gp by sertraline is a good explanation of such interaction.

In conclusion, our findings along with previous data consistently indicate that P-gp in BBB may be an important factor limiting brain entry of sertraline and desmethylsertraline, but not bupropion and its three metabolites. P-gp may represent an important target for drug–drug interactions and therapeutic responses associated with sertraline.

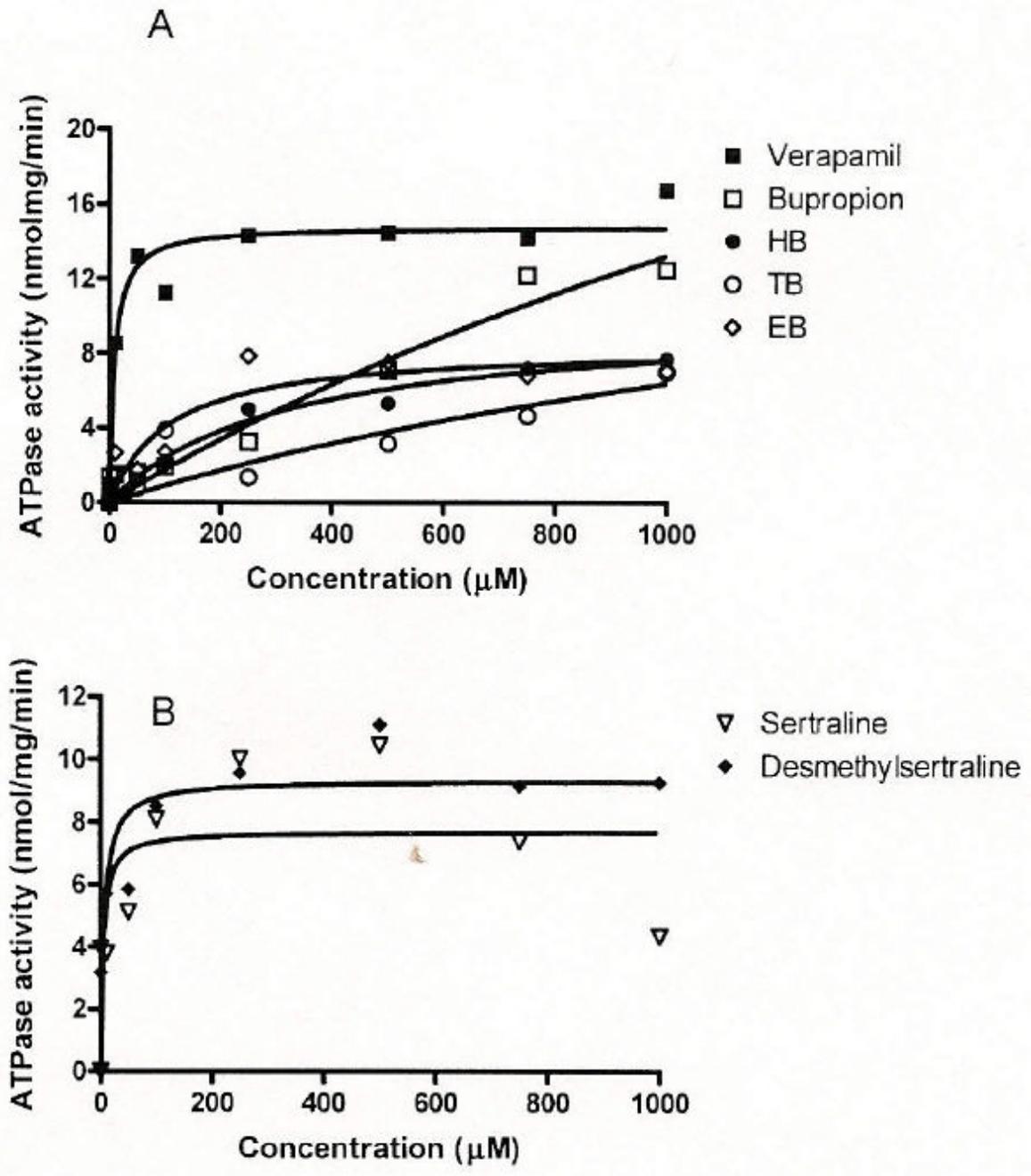
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**Fig. 1.** Concentration-Dependent Simulative Effects of Verapamil, Bupropion and Its Three Metabolites, HB, EB, and TB (A), and Sertraline and Desmethylsertraline (B) on *ortho*-Vanadate-Sensitive ATPase Activity in Expressed P-gp Membranes

**Table 1**

The  $K_m$ ,  $V_{max}$ , and  $Cl_{int}$  ( $V_{max}/K_m$ ) Values of Bupropion and Its Three Major Metabolites and Sertraline and Desmethylsertraline on *ortho*-Vanadate-Sensitive ATPase Activity in Expressed P-gp Membrane<sup>a)</sup>

	$K_m$ ( $\mu M$ )	$V_{max}$ (nmol/mg/min)	$Cl_{int}$ : intrinsic clearance ( $\text{min}^{-1} \times 10^{-3}$ )
Verapamil	8.7	14.8	1.7
Sertraline	4.7	7.7	1.6
Desmethylsertraline	6.5	9.3	1.4
Bupropion	2676	48.6	0.018
HB	318.2	10.0	0.03
TB	2066	19.5	0.01
EB	109.3	8.5	0.08

<sup>a)</sup>Data were mean of duplicate determinations.