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Venlafaxine ER Blocks the Norepinephrine Transporter in the Brain of Patients with Major Depressive Disorder: a PET Study Using [^{18}F] FMeNER-D$_2$

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Abstract

Background: The in vivo binding of clinical dose of venlafaxine on norepinephrine transporter has been questioned because venlafaxine has higher in vitro affinity to serotonin transporter than that to norepinephrine transporter. Although serotonin transporter occupancy of clinically relevant doses of venlafaxine has been reported, there has been no report of norepinephrine transporter occupancy in the human brain.

Methods: This was an open-label, single center, exploratory positron emission tomography study. Twelve major depressive disorder patients who had responded to venlafaxine extended-release and 9 control subjects were recruited. Each subject participated in one positron emission tomography measurement with [^{18}F]FMeNER-D$_2$. Binding potential in brain was quantified by the area under the curve ratio method with thalamus as target and white matter as reference regions. The difference of binding potential values between control and patient groups divided to 2 dose ranges were evaluated. Norepinephrine transporter occupancy (%) for all the major depressive disorder patients was calculated using mean binding potential of control subjects as baseline. The relationships between dose or plasma concentration of total active moiety and occupancies of norepinephrine transporter were also estimated.

Results: The binding potential of the patient group with 150 to 300 mg/d was significantly lower than that in the control subjects group ($P = .0004 < .05/2$). The norepinephrine transporter occupancy (8–61%) increased in a dose-dependent manner although a clear difference beyond 150 mg/d was not observed.

Conclusions: This study demonstrates that clinically relevant doses of venlafaxine extended-release block the norepinephrine transporter of the major depressive disorder patient's brain. The data support the notion that the antidepressant effect of venlafaxine involves a combination of serotonin transporter and norepinephrine transporter blockades.

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Significance Statement

Based on in vitro data, venlafaxine is classified as a serotonin norepinephrine reuptake inhibitor. However, the in vivo inhibitory effect of clinical doses of venlafaxine on norepinephrine transporter (NET) has been questioned as the in vitro affinity for the serotonin transporter (5-HTT) is 2 orders of magnitude higher than for NET. Thus, we used positron emission tomography to investigate the NET occupancy of clinically relevant doses of venlafaxine extended-release (ER) in the living human brain of patients with major depressive disorder. This study demonstrates for the first time to our knowledge that clinically relevant doses of venlafaxine ER block the NET dose dependently in the human brain in vivo. The data support the notion that the antidepressant effect of venlafaxine involves blockade of both 5-HTT and NET.

Keywords: major depressive disorder, norepinephrine transporter, occupancy, positron emission tomography, venlafaxine ER

Introduction

Major depressive disorder (MDD) represents a major unmet medical need. For example, MDD was the number one cause of years lived with disability in 2015 in the world (World Health Organization, 2017). The development of better treatment options is one way to decrease its impact on global health. It has repeatedly been shown in the living human brain that subjects with MDD in many, but not all cases, show changes in expression levels of markers for the serotonin (5-HT) system, e.g., the serotonin transporter (5-HTT) (Savitz and Drevets, 2013; Gryglewski et al., 2014). In line with this observation, drugs that inhibit the reuptake of 5-HT such as selective serotonin reuptake inhibitors (SSRIs) have repeatedly proven efficacious for the treatment of MDD in many but not all subjects (Jakubovski et al., 2016; Locher et al., 2017; Cipriani et al., 2018). For example, in the STAR*D trial, the remission rate in 2876 MDD patients treated with SSRI was 30% (Trivedi et al., 2006). Another suggested antidepressant target is the norepinephrine transporter (NET). Postmortem data, and recently also in vivo data, suggest changes in NET densities in MDD subjects (Klimek et al., 1997; Moriguchi et al., 2017b). Indeed, it has been reported that dual action of a serotonergic and noradrenergic agent is associated with better clinical outcome in patients with MDD than single-action antidepressants (Nelson et al., 1991, 2004). Also, MDD patients who failed to respond to SSRIs have been shown to improve when switched to serotonin and norepinephrine reuptake inhibitors (SNRIs), which have dual-target sites including 5-HTT and NET (Papakostas et al., 2008; Perahia et al., 2008). On the other hand, a recent report shows no clear difference of the efficacy between SSRI and SNRI (Cipriani et al., 2018).

The 5-HTT occupancy has been highlighted as a measure of antidepressant treatment effect (Meyer et al., 2001, 2004; Suhara et al., 2003; Lundberg et al., 2012). But the fact that MDD biology commonly involves changes in NET densities, as well as the additive effect of NET blockade in antidepressant treatment, motivates further examination of NET occupancy in vivo in SNRI treatment. [^{18}F]FMeNER-D$_2$ is a positron emission tomography (PET) radioligand that binds reversibly and selectively to NET (Schou et al., 2004). A method for reliable quantification of [^{18}F]FMeNER-D$_2$ binding to NET in human in vivo using PET has been described (Arakawa et al., 2008). The applicability of [^{18}F]FMeNER-D$_2$ PET in clinical occupancy studies have been shown in several reports (Sekine et al., 2010; Nogami et al., 2013; Nyberg et al., 2013; Takano et al., 2014; Moriguchi et al., 2017a).

Venlafaxine, which was clinically introduced as the first SNRI for the treatment of MDD in 1993, has been widely and globally prescribed and its major prescribed formulation is the extended release (ER) (Thase et al., 2017). The in vitro affinity of venlafaxine has been reported to be higher for 5-HTT than for NET (Ki: 82 nM and 2480 nM, respectively) (Bymaster et al., 2001). Due to the large discrepancy of the affinity between NET and 5-HTT, the in vivo binding of clinical doses of venlafaxine on NET has been questioned, especially in the lower dose range (Koch et al., 2003). In a previous nonhuman primate (NHP) PET study, similar in vivo occupancy between 5-HTT and NET of venlafaxine has been demonstrated (Takano et al., 2013). While 5-HTT occupancy of clinically relevant doses of venlafaxine in the human brain has been reported previously (Meyer et al., 2004; Voineskos et al., 2007; Lundberg et al., 2012), there has been no report of in vivo NET occupancy of venlafaxine in the human brain.

Thus, the aims of this study were to verify that clinically relevant doses of venlafaxine ER occupiy NET in the living human brain, and to identify the relationship between oral dose and plasma concentration of venlafaxine ER and NET occupancy in patients with MDD using PET and [^{18}F]FMeNER-D$_2$.

Methods

Subjects

This was an open-label, single-center, exploratory PET study. Approval was obtained from the Regional Ethical Review Board in Stockholm, Sweden; the Radiation Safety Committee at the Karolinska University Hospital Solna in Stockholm, Sweden; and the Swedish Medical Product Agency (EudraCT 2016-004590-40). Oral and written informed consent was obtained from all participants after thorough oral and written information of the study, and before any study related activity took place.

Twelve MDD patients (age range, 22–65 years; mean ± SD, 37.4 ± 11.7; 6 males, 6 females) who had responded to venlafaxine ER (37.5–300 mg/d) treatment were recruited from Northern Stockholm Psychiatry, Stockholm Health Care Services. The response to the treatment was judged by clinical observations. Nine healthy volunteers (20–62 years; 39.9 ± 14.4 (P = .66 compared with patient group); 3 males, 6 females) were recruited as control subjects through advertisement in local newspapers and social media. Patients were diagnosed with MDD based on the Diagnostic and Statistical manual of Mental Disorders, 4th edition (DSM-IV-TR). The dose of venlafaxine ER had been fixed for more than 2 weeks before PET measurement. The MDD patients did not take any other antidepressants or psychotropic agents or any other medication that might influence 5-HT and NE transmission for at least 4 weeks before PET measurement.

In addition, the MDD patients did not take structured psychotherapy or behavioral therapy for at least 3 months before the PET measurement. Exclusion criteria for patients and controls included past psychiatric (with the exception of MDD in the patients), neurological, or somatic disorders, or alcohol- or drug-related problems. Habitual nicotine use within 3 months prior to the PET examination was also an exclusion criterion. All subjects were healthy according to somatic and psychiatric interview (apart from MDD in the patient group), somatic examination, 12-lead electrocardiography, and blood and urine tests. A pregnancy test was done for female subjects using a urine pregnancy strip test.

PET Procedures

Each subject participated in one PET measurement with [^{18}F]FMeNER-D$_2$. For MDD patients, the last administration of venlafaxine ER was approximately 5 hours before radioligand injection. A plastic helmet was used during the PET measurement to minimize head movement. [^{18}F]FMeNER-D$_2$ was prepared as previously reported (Schou et al., 2004). After a bolus injection (<10 seconds) of the radioligand, the emission data were collected from 120 to 180 minutes using a HRRT system (Siemens Molecular Imaging). The injected radioactivity was adjusted by body weight. For an attenuation correction, a 6-minute transmission using a single ^{137}Cs source was also performed. The data were reconstructed using the ordinary Poisson-3D-ordered subset expectation maximization (OP-3D-OSEM) algorithm with 10 iterations and 16 subsets including modeling of the point spread function (Varrone et al., 2009). T1-weighted magnetic resonance imaging (MRI) was also performed for the anatomical reference for PET images using a 1.5T Siemens MAGNETOM Avanto. The protocol was a 3D sagittal magnetization-prepared rapid gradient-echo with the following sequence: repetition time/echo time/inversion time = 1790/3.53/1100 milliseconds, field of view 260 mm, image matrix 256 mm ×208 mm, flip angle 15°, and slice thickness = 1 mm. Before the MRI measurement, compatibility was checked such as absence of metal objects in the body or claustrophobia.

Plasma Concentration of Venlafaxine and O-Desmethylvenlafaxine

For all MDD patients, 3 venous blood samplings were performed before and 120 and 180 minutes after the radioligand injection to measure the plasma concentration of venlafaxine and its main active metabolite, *O*-desmethylvenlafaxine. Samples were analyzed using a validated high performance liquid chromatography-tandem mass-spectrometry method at WuXi Apptec Co. Ltd. (Shanghai, China). In this study, total active moiety as sum of venlafaxine and *O*-desmethylvenlafaxine was chosen as the parameter of interest as reported elsewhere (Hynninen et al., 2008; Owens et al., 2008; Takano et al., 2013). Averaged plasma concentration using a trapezoidal method during 180 minutes, from PET radioligand injection to end of PET measurement, was used for further analysis.

Data Analysis

Anatomical regions of interest (ROIs) were delineated on the reoriented MRI image using the Automated Anatomical Labelling template using Matlab7.5 toolbox (MathWorks Inc.) and SPM5 (Wellcome Trust Centre for Neuroimaging). White matter was defined using SPM5 segmentation. MRI and ROIs were co-registered to summated PET image with the mutual information algorithm using SPM5. Time activity curves were obtained from co-registered ROIs on dynamic PET images. [^{18}F]FMeNER-D$_2$ binding potential (BP_{ND}) in brain was quantified applying the area under the curve (AUC) ratio method with thalamus as target region ($AUC_{thalamus}$ / $AUC_{reference}$ – 1), as previously described (Arakawa et al., 2008; Takano et al., 2008; Moriguchi et al., 2017a). To select the reference region, the difference of standardized uptake value (SUV) of each patient relative to averaged SUV of control subjects was calculated for the 2 previously evaluated reference regions: caudate (Arakawa et al., 2008; Takano et al., 2008) and white matter (Takano et al., 2008; Moriguchi et al., 2017a). Then the SUV difference for all patients was compared between caudate and white matter using a paired *t* test. The reference showing smaller difference was chosen. The difference of BP_{ND} values between control subjects (n = 9) and patients group divided into 2 dose ranges (low dose: 37.5–75 mg/d and high dose: 150–300 mg/d; each n = 6) was evaluated using Wilcoxon rank sum test. Statistical significance was set as $P < .025$ (= .05/2) using Bonferroni correction. To create averaged BP_{ND} images of control and patient groups, spatial normalization by SPM5 was applied to each subject's BP_{ND} image.

NET Occupancy

NET occupancy (%) was calculated as ($BP_{ND:baseline}$ – $BP_{ND:treatment}$) / $BP_{ND:baseline}$ × 100 (Nogami et al., 2013; Takano et al., 2014; Moriguchi et al., 2017a). The mean BP_{ND} of control subjects was used as $BP_{ND:baseline}$ for all the MDD patients. The relationships between dose or plasma concentration of total active moiety and occupancies of NET were modeled by the following equation (Nogami et al., 2013; Takano et al., 2014; Moriguchi et al., 2017a): Occupancy (%) = D / (K_d + D) × O_{max}, where D is the dose of venlafaxine ER or plasma concentration of total active moiety, K_d is the dose of venlafaxine ER or plasma concentration of total active moiety required to induce 50% of O_{max}, and O_{max} is the maximal NET occupancy. Two different analyses about the O_{max} were conducted as (1) O_{max} set to 100% as previously published methods for NET occupancy in human brain (Nogami et al., 2013; Takano et al., 2014; Moriguchi et al., 2017a), and (2) O_{max} was also estimated as well as K_d.

RESULTS

Injected radioactivity was 285 ± 52 (mean ± SD) MBq and 268 ± 53 MBq for MDD patients and control subjects, respectively. Molar radioactivity at the time of injection was 64 ± 27 GBq/μmol and 68 ± 32 GBq/μmol, and injected mass was 1.7 ± 0.7 μg and 1.5 ± 0.6 μg, respectively. There was no statistical difference between MDD patients and control subjects for any of the 3 parameters (Student's *t* test: $P = .47–.76$).

White matter showed a smaller difference than caudate (0.12 vs 0.15; $P = .07$) in SUV of the patient relative to control group. Therefore, white matter was used as reference region in the further analysis. Daily doses of venlafaxine ER varied between 37.5 and 300 mg/d, plasma concentrations of total active moiety between 108 and 947 ng/mL, and NET occupancy in thalamus between 8% and 61% for all MDD patients (Table 1). Averaged BP_{ND} images of controls and patients for 2 dose ranges are shown in Figure 1. BP_{ND} in the thalamus showed an inverse relation to the dose of venlafaxine ER. The BP_{ND} of the 150- to 300-mg/d patient group was significantly lower than that of the control subjects group ($P = .0004$), whereas it was not significant in the patient group with 37.5 to 75 mg/d ($P = .07$) (Figure 2).

Table 1. Age, gender, dose of venlafaxine ER, plasma concentration, and NET occupancy for MDD patients

Subject	Age (years)	Gender	Dose (mg/d)	Plasma concentration (ng/mL)			NET occupancy (%)
				VEN	ODV	Total	
1	37	F	37.5	18	117	135	10
2	65	F	37.5	89	42	131	18
3	22	M	37.5	21	91	112	23
4	41	M	75	38	117	155	8
5	28	M	75	28	94	122	8
6	34	M	75	25	83	108	36
7	47	F	150	163	267	430	54
8	44	F	150	66	268	334	61
9	37	M	187.5	72	272	344	32
10	32	F	225	794	153	947	42
11	23	F	225	231	649	880	51
12	33	M	300	744	72	816	38

ER, extended release; MDD, major depressive disorder; NET, norepinephrine transporter; ODV, O-desmethylvenlafaxine; VEN, venlafaxine.

The relationship between dose of venlafaxine ER and NET occupancy is shown in Figure 3. The relationship between plasma concentration of total active moiety and NET occupancy is shown in Figure 4. The NET occupancy increased in a dose- and plasma concentration-dependent manner, although no obvious escalation was observed in higher range. K_d of dose ($K_{d:dose}$) and plasma concentration ($K_{d:conc}$) was 248 mg/d and 671 ng/mL, respectively, with fixed 100% O_{max}. When fitting O_{max}, $K_{d:dose}$ and $K_{d:conc}$ were 130 mg/d and 246 ng/mL, respectively, and O_{max} of dose ($O_{max:dose}$) and plasma concentration ($O_{max:conc}$) were 71% and 62%, respectively.

Discussion

In this study, we demonstrated that the NET occupancy in MDD patients who had responded to clinically relevant doses (37.5–300 mg/d) of venlafaxine ER was 8% to 61%, increasing in a dose- and plasma concentration-dependent manner. The NET BP_{ND} in patients with MDD compared with controls decreased significantly with higher doses (150 mg/d or more) of venlafaxine ER, whereas no significant differences were detected at lower doses (75 mg/d or less). Several PET studies have demonstrated that 5-HTT occupancy of patients with MDD who were treated with antidepressants was 65% to 80% (Meyer et al., 2001, 2004; Suhara et al., 2003; Lundberg et al., 2012). It has been reported that 5-HTT occupancy of venlafaxine at 75 mg/d already reaches 80% with a plateau for higher doses (Meyer et al., 2004). Therefore, the NET occupancy that we report here at doses 150 mg/d or higher in combination with the 5-HTT occupancy previously shown is one of possible explanations for the clinical efficacy of venlafaxine in the treatment of MDD (Rudolph et al., 1998; Charlier et al., 2002; Linden et al., 2003; Thase et al., 2006). However, there was no clear difference in NET occupancy between the 150 mg/d (n = 2) and higher (187.5–300 mg/d; n = 4) doses in the present study. The question whether the reported higher response rate in doses of 225 or 375 mg/d (Rudolph et al., 1998; Thase et al., 2006) may be explained by even higher NET occupancy than at 150 mg/d should be addressed in a PET study designed and powered for this specific purpose.

Meyer et al. have reported the oral dose associated with 50% occupancy (the apparent dose-related affinity, $K_{d:dose}$) of venlafaxine ER in human in vivo for 5-HTT to be 5.8 mg/d (Meyer et al., 2004). $K_{d:dose}$ of venlafaxine ER for NET in the present study was

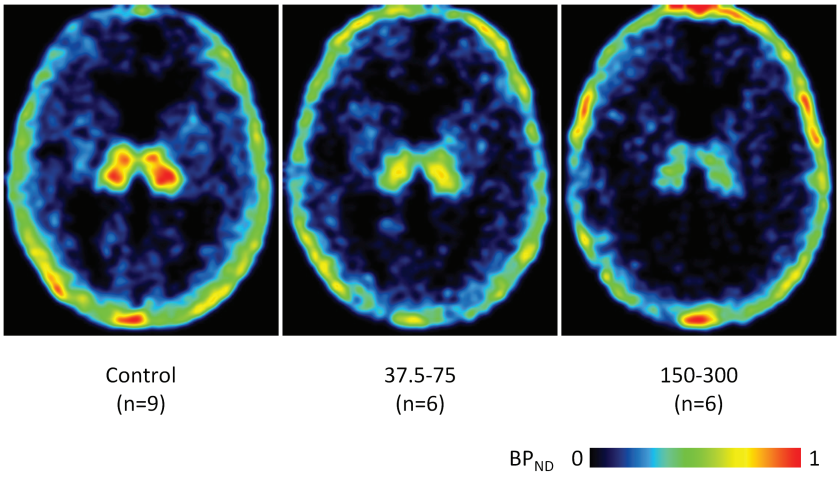

Figure 1. Averaged binding potential (BP_{ND}) images of control subjects and major depressive disorder (MDD) patients for 2 dose ranges.

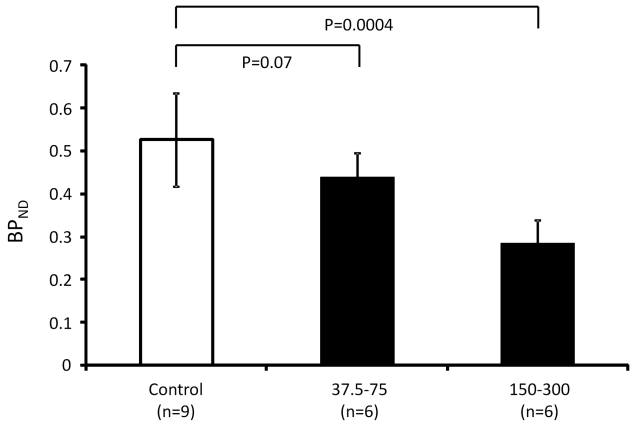

Figure 2. Comparison of binding potential (BP_{ND}) values between control subjects as baseline (n = 9) and major depressive disorder (MDD) patients groups divided into 2 dose ranges (each n = 6). BP_{ND} of patients groups with 150–300 mg/d was significantly lower than that in the control subjects group (P = .0004).

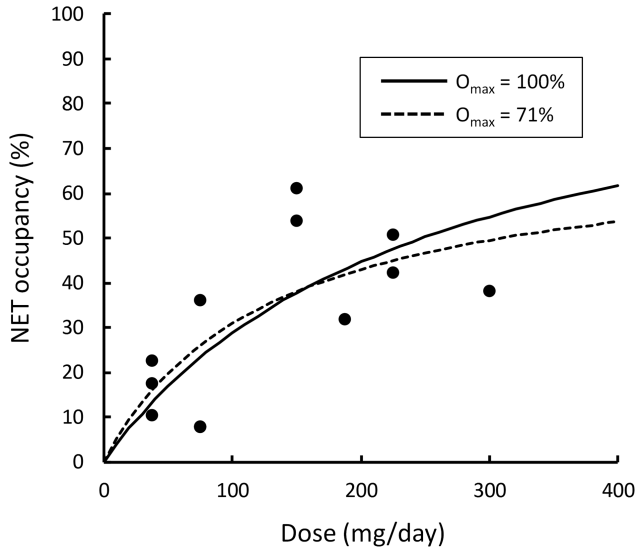

Figure 3. Relationship between dose of venlafaxine extended release (ER) and norepinephrine transporter (NET) occupancy for major depressive disorder (MDD) patients. Solid line: dose of venlafaxine ER required to induce 50% of NET occupancy ($K_{d:dose}$) was 248 mg/d. R = 0.68. Dashed line: $K_{d:dose}$ was 130 mg/d and maximal NET occupancy by dose ($O_{max:dose}$) was 71%. R = 0.71.

20 to 40 times higher (130 or 248 mg/d with or without fitting O_{max}). This ratio is in the same order of magnitude as the ratio of in vitro affinity for 5-HTT (82 nM) and NET (2480 nM) (Bymaster et al., 2001). The plasma concentration of venlafaxine associated with 50% occupancy (the apparent plasma concentration, $K_{d:conc}$) in human in vivo for 5-HTT has been reported to be 3.4 ng/mL (parent venlafaxine only) (Meyer et al., 2004). In the present study, $K_{d:conc}$ for NET was 246 and 671 ng/mL (total active moiety as sum of venlafaxine and *O*-desmethylvenlafaxine), and 34 or 245 ng/mL (parent venlafaxine only; data not shown). In a previous PET study in NHP, it was demonstrated that the ratio of $K_{d:conc}$ for 5-HTT (14.5 ng/mL) and NET (26.1 ng/mL) was 1.8 (Takano et al., 2013). Another study using a human ex vivo serum assay reported the ratio of K_i for 5-HTT (85 ng/mL) and NET (325 ng/mL) was 3.8 (Owens et al., 2008). Although there are numerical

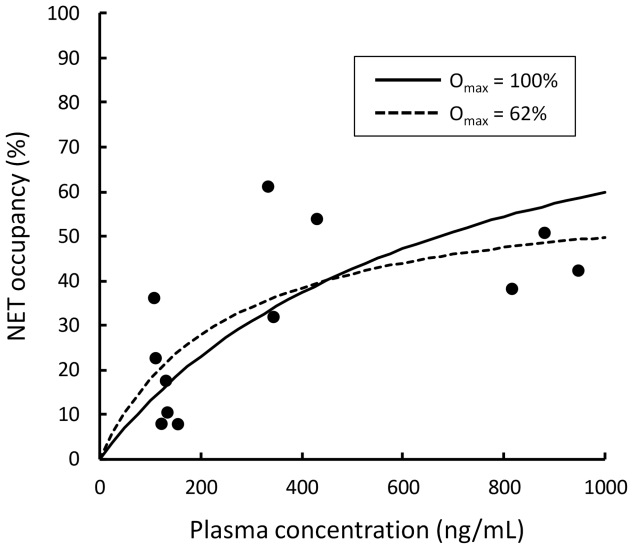

Figure 4. Relationship between plasma concentration of total active moiety of venlafaxine and norepinephrine transporter (NET) occupancy for major depressive disorder (MDD) patients. Plasma concentration of total active moiety was defined as sum of venlafaxine and its main active metabolite O-desmethylvenlafaxine. Solid line: plasma concentration of total active moiety required to induce 50% of NET occupancy ($K_{d:conc}$) was 671 ng/mL. R = 0.61. Dashed line: $K_{d:conc}$ was 246 ng/mL and maximal NET occupancy by concentration ($O_{max:conc}$) was 62%. R = 0.71.

differences between methods, it has consistently been shown that venlafaxine has higher affinity for 5-HTT than NET.

So far, 5-HTT and NET occupancy in the human brain has been reported for 2 other SNRIs: milnacipran and duloxetine. Nogami et al. demonstrated 33% to 62% 5-HTT occupancy and 25% to 50% NET occupancy in MDD patients after administration of 25 to 200 mg/d milnacipran (Nogami et al., 2013). The $K_{d:dose}$ was estimated to 122.5 mg for 5-HTT and 149.9 mg for NET. This relatively equivalent in vivo occupancy of 5-HTT and NET is in line with the relation of the in vitro affinity of milnacipran (K_i = 8.44/22 nM for 5-HTT/NET) (Vaishnavi et al., 2004). In addition, both 5-HTT and NET occupancy have been examined in control subjects after single oral doses of duloxetine. The 5-HTT occupancy after 5 to 60 mg of duloxetine was 44% to 82% and $K_{d:dose}$ was estimated to 7.9 mg (Takano et al., 2006). The NET occupancy after 20 to 60 mg of duloxetine was 30% to 40% and $K_{d:dose}$ was 76.8 mg (Moriguchi et al., 2017a). The difference between $K_{d:dose}$ values of duloxetine for 5-HTT and NET corresponds to the difference in the reported in vitro affinity (K_i = 0.8/7.5 nM for 5-HTT/NET) (Bymaster et al., 2001). Our data suggest that NET occupancy from clinical doses of venlafaxine may be higher than those from milnacipran and duloxetine in human brain, although a direct comparison is difficult because of the differences in study design and target subjects.

Previous PET studies using reference tissue models to quantify [^{18}F]FMeNER-D_2 binding have applied either caudate (Arakawa et al., 2008; Takano et al., 2008) or white matter (Takano et al., 2008; Moriguchi et al., 2017a) as reference region. In the present analysis, we used white matter as reference region. Theoretically, the caudate is suitable reference for quantification of [^{18}F]FMeNER-D_2 because of its negligible density of NET (Arakawa et al., 2008; Takano et al., 2008). However, this region is smaller than white matter, and its radioligand uptake is likely to be affected by radioligand signal in surrounding tissues such as

ventricle, resulting in an unstable estimation of NET binding in target regions. The smaller difference in SUV between groups for white matter compared with caudate indicates that white matter reference should produce more valid occupancy estimates in this sample.

In this study, O_{max} was set to 100% as in previously published reports of NET occupancy in human brain (Nogami et al., 2013; Takano et al., 2014; Moriguchi et al., 2017a), and fitted as well as K_d. When fitting O_{max}, it was estimated <100% (71% and 62% for dose and plasma concentration, respectively). One possible reason is that relatively low occupancy (up to 60%) was obtained in this study. However, almost fully blocked NET was observed in previous NHP PET studies (Takano et al., 2009, 2013; Gallezot et al., 2011). We reported both results because there might not be strict reason to exclude any of the methods.

There are several limitations in this study. First, we used the average BP_{ND} of control subjects as an estimation of baseline BP_{ND} for all MDD patients. Recently, around 30% higher NET availabilities in unmedicated patients with MDD compared with control subjects was reported using [^{18}F]FMeNER-D_2 (19 patients vs 19 controls, P = .007) (Moriguchi et al., 2017b). Our patients were responders to the antidepressant treatment, and it is not clear whether an increased NET binding in MDD is a state or trait phenomenon. However, if the baseline NET binding is underestimated due to the study design, we may have underestimated the NET occupancy systematically in all the examined patients, so that in the present data set, where occupancy was calculated to between 8% and 61%, the true occupancy may have been between 29% and 70%.

Second, up- or downregulation of NET proteins by chronic administration of antidepressants has been reported in rodent brain using the autoradiography method (Bauer and Tejani-Butt, 1992; Hébert et al., 2001; Weinshenker et al., 2002; Benmansour et al., 2004). Although it is unclear if this phenomenon exists in MDD patients exposed to clinical doses of venlafaxine ER, we cannot rule out that this may have affected our estimation of NET occupancy.

Third, the relationship between the clinical efficacy and NET occupancy by venlafaxine ER could not be investigated with the present protocol, as this included one PET examination in MDD patients who had responded to venlafaxine treatment. To determine the lower limit of NET occupancy needed for clinical effect, a prospective study in unmedicated MDD patients would be needed.

Conclusions

This study demonstrates for the first time to our knowledge that clinically relevant doses of venlafaxine ER block the NET in the living human brain of MDD patients. Additionally, it is shown that the NET occupancy of venlafaxine varies in a dose- and plasma concentration-dependent manner. The present results are in line with the notion that venlafaxine ER exerts its clinical effect via blockade of both 5-HTT and NET.

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Statement of Interest

YA and YH are the employees of Pfizer Japan Inc. Other authors declare that they have no conflict of interest.

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