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The Dopamine Stabilizer (−)-OSU6162 Occupies a Subpopulation of Striatal Dopamine D2/D3 Receptors: An [^{11}C]Raclopride PET Study in Healthy Human Subjects

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(−)-OSU6162 is a dopamine stabilizer that can counteract both hyperdopaminergic and hypodopaminergic states. In this study, D2/D3 receptor occupancy of (−)-OSU6162 in the human brain was investigated using positron emission tomography (PET). Twelve male healthy volunteers underwent [^{11}C]raclopride PET scanning before and 1 h after a single oral dose of (−)-OSU6162 (15–90 mg). Blood samples for determination of (−)-OSU6162 and prolactin plasma levels were collected at T_{max}. Parametric images of [^{11}C]raclopride binding potential relative to nondisplaceable tissue (cerebellar grey matter) uptake (BP_{ND}) at baseline and after (−)-OSU6162 administration were generated using the simplified reference tissue model. MRI-based regions of interest were defined for the striatum, composed of caudate nucleus and putamen, and projected onto the co-registered parametric [^{11}C]raclopride BP_{ND} image. Furthermore, three striatal subregions, ie, anterior dorsal caudate, anterior dorsal putamen, and ventral striatum, were defined manually and additionally analyzed. Plasma concentrations of (−)-OSU6162, ranging from 0.01 to 0.9 μM, showed a linear relationship with prolactin levels, reflecting blockade of pituitary D2 receptors. A concentration-dependent increase in striatal D2/D3 receptor occupancy was observed, reaching a value of about 20% at an (−)-OSU6162 plasma level of 0.2 μM, and which for higher concentrations leveled off to a maximal occupancy of about 40%. Findings were similar in the striatal subregions. The present data corroborate the notion that (−)-OSU6162 binds preferentially to a subpopulation of D2/D3 receptors, possibly predominantly extrasynaptic, and this may form the basis for the dopamine-stabilizing properties of (−)-OSU6162.
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INTRODUCTION

(−)-OSU6162 ((3*S*)-3-[3-(methylsulfonyl)phenyl]-1-propylpiperidine hydrochloride) has been classified as a 'dopamine stabilizer' because of its affinity for dopaminergic D2/D3 receptors in conjunction with its ability to inhibit or stimulate dopamine-related behavior, depending on the baseline motor activity level. At high baseline level (−)-OSU6162 inhibits and at low baseline level it stimulates behavior (Rung *et al*, 2008). *In vitro*, the compound shows moderate (micromolar) affinity for D2/D3 receptors with very low intrinsic activity (Burstein *et al*, 2011). *In vivo*, however, it appears to be an antagonist devoid of intrinsic activity over a wide range of doses (Carlsson *et al*, 2011; Rung *et al*, 2011). Evidence in humans of a dopamine D2 antagonistic action of (−)-OSU6162, at the level of the pituitary, was demonstrated indirectly by a dose-dependent elevation in serum prolactin (Rodriguez *et al*, 2004). Although (−)-OSU6162 also demonstrates affinity for serotonergic 5-HT1 and 5-HT2 receptors, in both cases acting as a partial agonist (Burstein *et al*, 2011; Carlsson *et al*, 2011), and for sigma sites (Sahlholm *et al*, 2013), especially its effects on the dopamine system have been well characterized. For example, the ability of (−)-OSU6162 to counteract amphetamine-induced hyperactivity is lost in dopamine D2 knockout mice (Svensson *et al*, 2009). Activation of habituated rats appears to depend largely on dopaminergic stimulation mediated via preferential antagonism on dopaminergic autoreceptors, possibly in conjunction with an allosteric mechanism (Lahti *et al*, 2007; Rung *et al*, 2008).

Although (−)-OSU6162 receptor occupancy data are available for rats (Natesan *et al*, 2006) and Rhesus monkeys (Ekesbo *et al*, 1999), no human studies have been reported yet. There is an increasing need for human imaging data in view of

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the ongoing accumulation of both preclinical and clinical observations showing the substance's promise for treating a variety of neuropsychiatric disorders such as Huntington's disease, schizophrenia, and mental fatigue following stroke or head trauma (Tedroff *et al*, 1999; Johansson *et al*, 2012; Kloberg *et al*, 2014; Carlsson A, unpublished observation).

The aim of the present study was to investigate D2/D3 receptor occupancy of (−)-OSU6162 in the human brain in order to better understand its dopamine-stabilizing properties.

MATERIALS AND METHODS

Subject Selection Procedure

Male subjects, in the age range of 50–60 years, were recruited through newspaper advertisements. All subjects received a screening that included medical history, physical, and neurological examinations, screening laboratory tests, toxicology screening of urine (opiates, cocaine, amphetamines, cannabis, methadone, benzodiazepines, and barbiturates), and brain magnetic resonance imaging (MRI). Exclusion criteria were any history of major psychiatric or neurological illness, use of any centrally active medication, including anti-psychotic or anti-depressant medication, use of any investigational medication within 30 days prior to the start of this study, (history of) alcohol and/or drug abuse (DSM-IV criteria), any sign of cardiovascular disease, and claustrophobia. All subjects were instructed to refrain from alcohol consumption during 24 h prior to testing. Two volunteers were habitual smokers. Subjects were not allowed to smoke or drink grapefruit juice on the day of the positron emission tomography (PET) study.

Prior to inclusion, written informed consent was obtained from all subjects after a complete written and verbal description of the study.

(−)-OSU6162 Clinical Samples, Safety, and Pharmacokinetic Data

(−)-OSU6162 was prepared under GMP conditions at 'Syntagon' (Södertälje, Sweden) and 'Galenica AB' (Malmö, Sweden) as described previously (Sonesson *et al*, 1994). Safety data for (−)-OSU6162 are available from a phase I study using single oral doses of up to 200 mg. Potential adverse effects of (−)-OSU6162 administration are on the central nervous system and the cardiovascular system. Single oral doses of (−)-OSU6162 up to 150 mg appeared safe and well tolerated. (−)-OSU6162 plasma levels were reported to reach peak values (T_{max}) at 1–1.5 h (Rodriguez *et al*, 2004).

Study Design

Twelve male healthy volunteers underwent [^{11}C]raclopride PET scans before and 1 h after a single oral dose of (−)-OSU6162.

Subjects received the following single oral doses of (−)-OSU6162: 15 mg ($n=3$), 30 mg ($n=2$), 45 mg ($n=2$), 60 mg ($n=1$), and 90 mg ($n=3$). A starting dose of 15 mg was chosen based on data from proof-of-concept clinical studies (Johansson *et al*, 2012). Subsequent doses were chosen based on the outcomes of the previous PET scans in the current study. The maximum dose was set to a clinically safe and relevant dose of 90 mg, well below the previously observed maximally tolerated dose of 150 mg (Rodriguez *et al*, 2004). The study had been approved by the Medical Ethics Review Committee of the VU University Medical Center Amsterdam.

[^{11}C]Raclopride Clinical Samples

[^{11}C]raclopride was synthesized according to a slightly modified procedure reported previously (Ehrin *et al*, 1987). It was obtained with a radiochemical purity >98%, a specific activity at the time of injection of 66 ± 26 GBq/μM, and it was manufactured under GMP license at the GMP facility of the department of Radiology and Nuclear Medicine.

Positron Emission Tomography

PET scans were performed using an ECAT EXACT HR+ scanner (Brix *et al*, 1997) (Siemens/CTI, Knoxville, USA), equipped with a neuro-insert to reduce the contribution of outside field of view (FOV) activity. This scanner enables the acquisition of 63 transaxial planes over a 15.5 cm axial FOV, thus allowing the whole brain to be imaged in a single bed position. All subjects received a venous cannula for intravenous infusion of [^{11}C]raclopride, administered using an infusion pump (Med-Rad, Beek, the Netherlands). Patient motion was restricted by the use of a head holder, and throughout the scanning procedure, the position of the head was monitored using laser beams.

First, a 10-min transmission scan was performed in 2D acquisition mode using three retractable rotating line sources. This scan was used to correct the subsequent emission scan for photon attenuation. Next, a 60-min dynamic emission scan in 3D acquisition mode was acquired, starting at the time of [^{11}C]raclopride infusion (369 ± 14 MBq at a rate of 0.8 ml/s, followed by a flush of 42 ml saline at a rate of 2.0 ml/s). Each dynamic emission scan consisted of 21 frames with progressive increase in frame length (6×5, 3×10, 4×60, 2×150, 2×300, 4×600 s). Four hours later, an identical scanning sequence was performed 1 h (ie, at T_{max}) after oral administration of (−)-OSU6162. The total injected amount of [^{11}C]raclopride, averages being 2.31 ± 0.78 μg and 2.16 ± 0.66 μg, respectively, did not differ significantly between PET scans at baseline and after pretreatment with (−)-OSU6162, ($p=0.70$).

Blood samples for determination of plasma levels of (−)-OSU6162 and prolactin were collected just before the start of the second [^{11}C]raclopride scan. Venous whole blood (10 ml) was collected into Vacutainer tubes containing heparin. Specimens were centrifuged for 10 min at 4 °C, after which the plasma layer was transferred to plastic vials and stored at −80 °C.

Magnetic Resonance Imaging

All subjects underwent a structural MRI scan using a 1.5-T Sonata scanner (Siemens Medical Solutions, Erlangen, Germany). The scan protocol included a sagittal T1-weighted 3D MPRAGE (magnetization prepared rapid acquisition gradient echo; slice thickness 1.0 mm, 176 slices; matrix size

256×256; voxel size $1 \times 1 \times 1$ mm; echo time 3.97 ms; repetition time 2700 ms; inversion time 950 ms; flip angle 8°).

Image and Data Analysis

All PET sinograms were corrected for dead time, tissue attenuation using the transmission scan, decay, scatter, and randoms and reconstructed using a standard filtered back projection algorithm with a Hanning filter at a cut-off of 0.5 times the Nyquist frequency. A zoom factor of two and a matrix size of $256 \times 256 \times 63$ were used, resulting in a voxel size of $1.2 \times 1.2 \times 2.4\,mm^3$ and a spatial resolution of approximately 7 mm full-width at half-maximum at the center of the FOV.

Parametric images of [^{11}C]raclopride-binding potential relative to non-displaceable uptake (BP_{ND}) (Innis *et al*, 2007) were generated using a basis function implementation of the 'two-step' simplified reference tissue model with cerebellar grey matter as reference tissue (Wu and Carson, 2002).

MRI images were aligned to corresponding co-registered PET images using a mutual information algorithm (Maes *et al*, 1997).

Segmentation of MRI images and region of interest (ROI) analysis of PET data were performed using PVE lab, a software program that uses a probability map of 35 delineated (grey matter) ROIs (Svarer *et al*, 2005), including caudate nucleus, putamen, and cerebellum. Caudate nucleus and putamen were combined to create a striatal ROI. For an additional subregion analysis, anterior dorsal caudate, anterior dorsal putamen, and ventral striatum were defined manually according to the existing guidelines (Martinez *et al*, 2003; Mawlawi *et al*, 2001).

ROIs were projected onto parametric [^{11}C]raclopride BP_{ND} images at baseline and after treatment with (−)-OSU6162. D2/D3 receptor occupancy Occ (%) was calculated according to:

$$\text{Occ} = 100 \times \left(1 - BP_{ND}^{OSU} / BP_{ND}^{BASELINE}\right) \quad (1)$$

where $BP_{ND}^{BASELINE}$ and BP_{ND}^{OSU} represent BP_{ND} before and after (−)-OSU6162 administration, respectively. For each ROI, Occ was plotted as a function of (−)-OSU6162 plasma levels and fitted to the following binding equation:

$$Occ(\ \%\ ) = \frac{Occ_{\max} * C_p}{\left(C_p + EC50\right)} \quad (2)$$

where $Occ_{\max}$ and C_p represent maximum level of occupancy and plasma level of (−)-OSU6162, respectively. EC_{50} is defined as the required plasma level of (−)-OSU6162 to reach 50% of the maximum occupancy.

Data are presented as mean ± SD, unless otherwise stated.

LC-MS/MS Determination of (−)-OSU6162 Plasma Levels

A 0.25-ml sample of plasma was deproteinized with 0.25 ml of an 11% aqueous trichloroacetic acid solution. After 5 min of ultracentrifugation (20 000 *g*), the supernatant was transferred to a 2.0-ml amber screw cap vial with a 200-µl insert. Plasma concentrations of (−)-OSU6162 were quantitatively determined by LC-MS/MS using an optimized stable isotope dilution method (Yamazaki *et al*, 2009). The injection volume was 2 µl, and [$^2H_7$](−)-OSU6162 (Syntagon, Södertälje, Sweden) was used as an internal standard. Separation of analytes was performed using a Jasco X-Liquid Chromatography (LC) system with a Kinetex $100 \times 2.1\,mm^2$, 1.7 µm column (Phenomenex, Torrance, CA, USA) as stationary phase. A gradient mobile phase of acetonitril and 0.1% ammonium acetate pH 3.95 was used. Mass spectrometry (MS) analysis was performed using a Qtrap5500 (ABSciex, Framingham, MA, USA) with electrospray ionization. Multi-reaction monitoring with transitions *m/z* 282.2–169.1 for (−)-OSU6162 and 289.2–169.2 for [$^2H_7$](−)-OSU6162, respectively, was used. The retention time of (−)-OSU6162 was 3.02 min, and the calibration curve was linear from 0.0029 to 5.0 µM ($r^2 = 1.00$). The limit of quantification was 5 nM, the intra-assay variation was <5%, and the inter-assay variation <6%. The metabolic pathways of OSU6162 have been mapped, and no pharmacologically active metabolites have been identified (Wienkers and Wynalda, 2002).

Measurement of Plasma Prolactin Levels

Prolactin was measured using an immunometric assay (Centaur, Siemens Diagnostics, Deerfield, IL, USA). The lower limit of quantification was 0.05 U/l (= 2.36 ng/ml), the intra-assay variation was <5%, and the inter-assay variation <6% for the whole concentration range.

RESULTS

Two subjects were excluded from the study, one due to radiosynthesis failure and the other due to an abnormality on MRI. In total, data were obtained for 10 volunteers (age 51–60 years, body mass index 21–34 kg/m^2), with 5 different doses of (−)-OSU6162 corresponding to a range of 0.14–1.25 mg/kg. Because of an experimental error, plasma was not available for one subject. None of the subjects reported any side effects of the drug.

Subject data, administered doses of (−)-OSU6162, plasma concentrations of (−)-OSU6162 and prolactin, and BP_{ND} in the striatum (defined and analyzed using an automated procedure) at baseline and after treatment with (−)-OSU6162, together with occupancy of D2/D3 receptors are shown in Table 1. Plasma concentrations of (−)-OSU6162 ranged from 0.01 to 0.91 µM and plasma prolactin levels from 1.89 to 19.29 ng/ml.

Linear relationships were observed between administered dose (mg/kg) and plasma levels of (−)-OSU6162 (Figure 1a) and between plasma concentrations of (−)-OSU6161 and prolactin (Figure 1b). It should be noted that subject F had likely not swallowed the (−)-OSU6162 tablet, as he showed zero plasma levels, a low prolactin level, and no brain D2/D3 receptor occupancy.

For all subjects, $BP_{ND}^{BASELINE}$ values were in close range for the striatum (2.16–2.67). A decreasing BP_{ND}^{OSU} relative to $BP_{ND}^{BASELINE}$, indicating increasing receptor occupancy, was seen with increasing (−)-OSU6162 plasma levels up to 0.2 µM, at which level D2/D3 receptor occupancy was around 20%. At higher (−)-OSU6162 plasma concentrations, D2/D3 receptor occupancy leveled off.

Table 1 Overview of Subject Data and Findings of the [^{11}C]Raclopride/(−)OSU6162 PET Study in Human Volunteers

Subject code/age	(−)-OSU6162 Dose (mg/kg)	BP_{ND} striatum[a] baseline	BP_{ND} striatum[a] pretreatment	Plasma levels μM	Occupancy striatum %	Prolactin plasma levels ng/ml
A/54 years	15 mg (0.14)	2.41	2.29	0.09	5	3.21
B/57 years	15 mg (0.19)	2.28	2.02	NA	11	NA
C/60 years	15 mg (0.16)	2.45	2.14	0.21	13	6.18
D/51 years[b]	30 mg (0.41)	2.20	1.78	0.16	19	1.89
E/54 years	30 mg (0.36)	2.67	2.05	0.26	23	8.02
F/57 years[b]	45 mg (0.48)	2.26	2.22	0.01	2	2.50
G/57 years	45 mg (0.53)	2.38	1.72	0.19	27	5.71
H/59 years	60 mg (0.74)	2.48	1.83	0.22	26	5.33
I/51 years	90 mg (1.11)	2.22	1.72	0.48	23	6.51
J/52 years	90 mg (1.25)	2.16	1.45	0.91	33	19.29

Abbreviation: NA, not available.
Plasma (−)-OSU6162 and prolactin levels could not be determined for subject B owing to a blood sampling error.
[a]Data from automated analysis.
[b]Smokers, refrained from smoking on day of PET scanning.

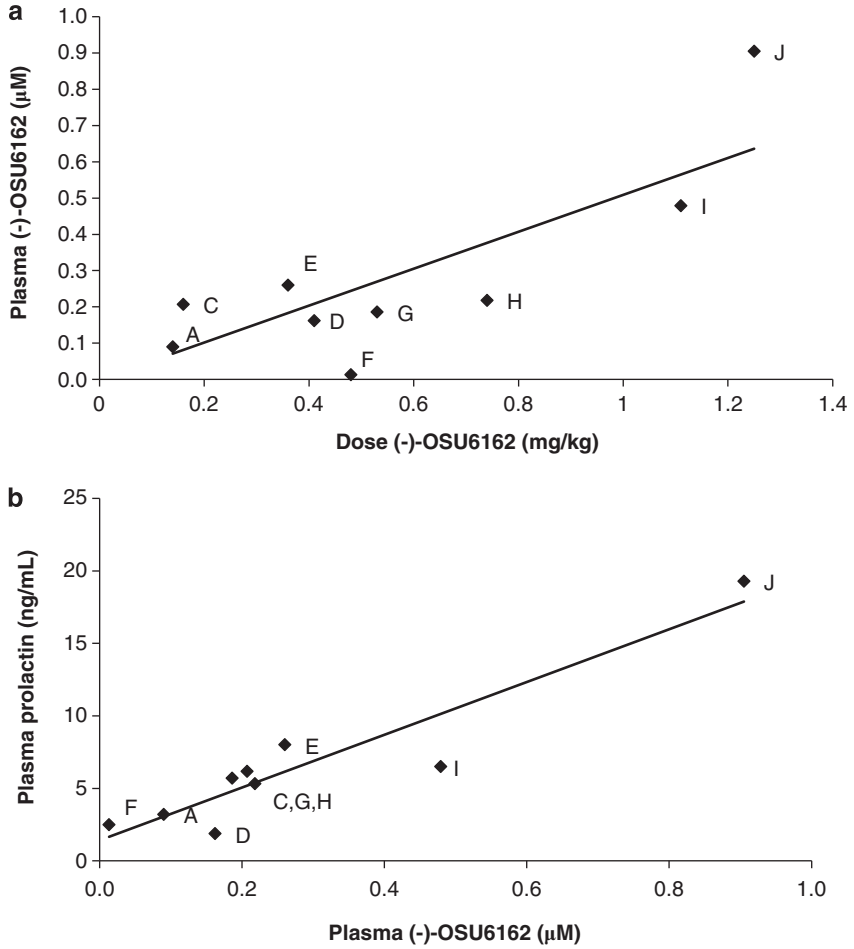

Figure 1 (a) Plasma (−)-OSU6162 levels (μM) as a function of the administered dose of (−)-OSU6162 (mg/kg). Linear correlation coefficient: $R = 0.82$, $n = 9$, $p < 0.01$. (b) Plasma prolactin level as a function of plasma concentration of (−)-OSU6162. Linear correlation coefficient: $R = 0.93$, $n = 9$, $p < 0.001$. Letters indicate individual subjects (see Table 1).

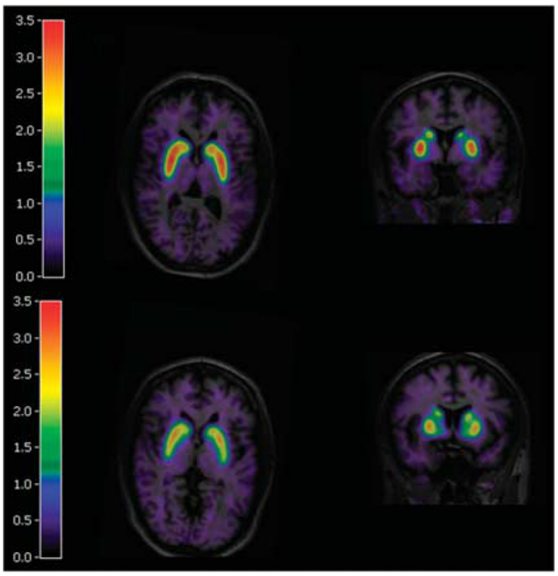

Figure 2 Parametric images of [^{11}C]raclopride BP_{ND} illustrating occupancy of D2/D3 receptors after administration of 90 mg (−)-OSU6162. Top panel: baseline scan, lower panel: scan after treatment with (−)-OSU6162.

Examples of parametric images of [^{11}C]raclopride BP_{ND}, illustrating inhibition of [^{11}C]raclopride binding after administration of 90 mg (−)-OSU6162, are shown in Figure 2. D2/D3 receptor occupancies in the striatum as functions of (−)-OSU6162 plasma levels are shown in Figure 3, together with the curve of best fit to these data using a single-site binding model (Equation 2). The curve fitting yielded an $Occ_{\max}$ of D2/D3 receptor occupancy of approximately 40% (Table 2).

Because of the finding of leveling off and low maximal D2/D3 receptor occupancies by (−)-OSU6162 in the striatum, three striatal subregions, ie, anterior dorsal caudate, anterior dorsal putamen, and ventral striatum, were defined manually and subjected to a similar analysis as the striatum. Plots of D2/D3 receptor occupancy *vs* (−)-OSU6162 plasma levels for these subregions revealed similar results as for the striatum (graphs not shown). Derived $Occ_{\max}$ and EC_{50}-values are presented in Table 2. $Occ_{\max}$ estimates from all regions, mutually compared using a two-tailed *t*-test, were not significantly different (*p*-value >0.1).

DISCUSSION

This [^{11}C]raclopride PET study aimed to investigate D2/D3 receptor occupancy by the dopamine stabilizer (−)-OSU6162 in the striatum of the human brain. A concentration-dependent increase in D2/D3 receptor occupancy, reaching a value of about 20%, was observed for (−)-OSU6162 plasma levels of up to 0.2 μM; above this concentration, occupancy started to level off. Data were fitted well to a single-site binding curve, suggesting a maximum occupancy level of about 40%.

In contrast to this apparent plateauing of striatal D2/D3 receptor occupancy, plasma prolactin levels increased linearly with (−)-OSU6162 plasma concentrations (Figure 1), which is in agreement with a previous study (Rodriguez *et al*, 2004). It is likely that this reflects an antagonistic action of (−)-OSU6162 on pituitary dopamine D2 receptors, which in the intact system mediate tonic inhibition of prolactin release. The highest prolactin levels observed in this study, however, were still 2–4 times lower than those found in male patients treated with therapeutic doses of amisulpiride (Bressan *et al*, 2004), and they were lower than mean prolactin levels in male patients with schizophrenia under antipsychotic treatment (Sugawara *et al*, 2011).

The present data indicate that striatal D2/D3 receptor occupancy by (−)-OSU6162 reached an asymptotic maximum of about 40% (Table 2). It was of particular interest to compare occupancy in the ventral striatum, which is associated with limbic structures and strongly innervated by the mesolimbic dopamine system, to dorsal striatal regions, which form the sensorimotor-related part of the striatum (Voorn *et al*, 2004; Groenewegen, 2007). [^{11}C]raclopride $BP_{ND}^{BASELINE}$ values in the ventral striatum were lower than in dorsal striatal areas, confirming previous observations (Mawlawi *et al*, 2001; Martinez *et al*, 2003; Alakurtti *et al*, 2013) and suggesting differences in D2/D3 receptor densities between striatal regions. The D2/D3 receptor occupancy by (−)-OSU6162, however, was similar in all the regions investigated. Early PET studies with antipsychotics that are in clinical use for schizophrenia (Farde *et al*, 1992) indicated that the therapeutic window for an antipsychotic effect in relation to D2/D3 receptor occupancy in the striatum lies between 70 and 80%, above which extrapyramidal side effects occur. This finding has subsequently been verified and fine-tuned to 65–80% in a large number of PET studies (for a review, see Pani *et al*, 2007). With antipsychotics displaying partial D2 agonist activity, such as aripiprazole, higher occupancy can be achieved without side effects (Yokoi *et al*, 2002).

With respect to D2/D3 receptor occupancy, the role of the dissociation rate of drugs from the D2 receptor has been investigated previously. It has been claimed that drugs with relatively low D2 receptor-binding affinity and a fast dissociation rate, such as clozapine and quetiapine, exert antipsychotic activity at lower D2 receptor occupancies, ie, 40–50% (Seeman and Tallerico, 1999). Among the so-called 'atypical' antipsychotics, quetiapine has the weakest D2 receptor-binding affinity ($K_i = 0.72$ μM; Leysen, 2002), indicating a six times higher potency than (−)-OSU6162 ($K_i = 4.36$ μM; Kara *et al*, 2010b). It has been reported that (−)-OSU6162 also displays a fast D2 receptor dissociation rate (Dyhring *et al*, 2010). PET studies in schizophrenic patients treated with quetiapine showed striatal D2/D3 receptor occupancy of up to 50%. However, 2–3 h after a single dose, a transiently high occupancy of 58–64% was observed (Kapur *et al*, 2000; Gefvert *et al*, 2001). Recently, a novel compound with fast off rate from the D2 receptor, JNJ-37822681, showed a regular saturation curve of D2/D3 receptor occupancy of up to 70% (Te Beek *et al*, 2012). JNJ-37822681 showed very good uptake in the brain and a moderate affinity for the D2 receptor ($K_i = 0.158$ μM), comparable with clozapine ($K_i = 0.137$ μM; Langlois *et al*, 2012). Hence, neither low (*in vitro*) D2 receptor affinity nor fast dissociation rate seems to be a general cause for low D2 receptor occupancy of a drug.

Comparisons between the present findings in humans and animal data obtained previously are difficult due

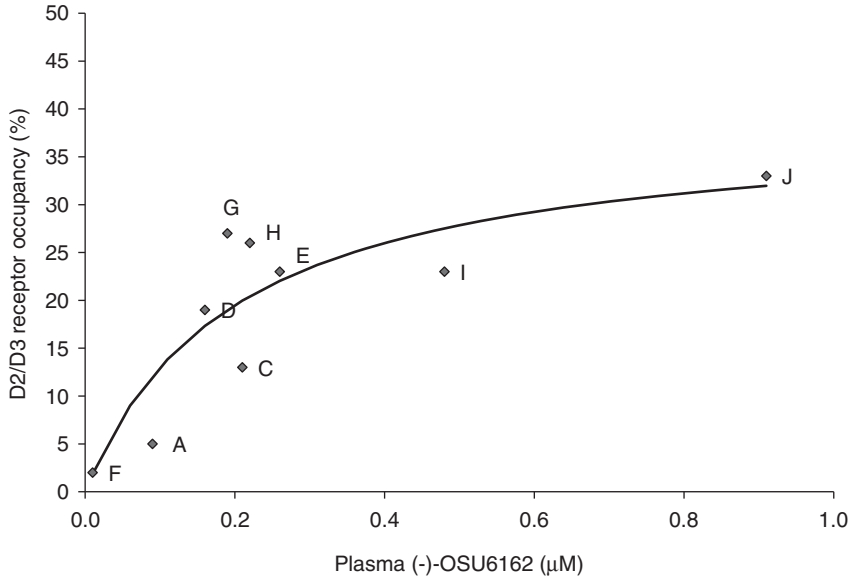

Figure 3 D2/D3 receptor occupancy in the striatum as a function of plasma concentration of (−)-OSU6162. Letters indicate individual subjects (see Table 1).

Table 2 Estimates of Maximal D2/D3 Receptor Occupancy (Occ_{max}) and EC_{50}-Values of (−)-OSU6162 in Human Striatal Regions

Brain region	[^{11}C]raclopride ($BP_{ND}^{BASELINE}$)	Occ_{max} ± SD (% D2/D3 R occupancy)	EC_{50} ± SD (plasma level μM)
Striatum[a]	2.35 ± 0.16	39 ± 10	0.20 ± 0.12
Anterior dorsal caudate[b]	2.14 ± 0.12	51 ± 24	0.34 ± 0.38
Anterior dorsal putamen[b]	2.51 ± 0.22	40 ± 13	0.25 ± 0.19
Ventral striatum[b]	1.67 ± 0.25	41 ± 10	0.14 ± 0.10

All values are mean ± SD, $n = 9$.
[a]Automated segmentation.
[b]Manually defined regions.

to differences in dosage and route of administration. A previous PET study, investigating D2/D3 receptor occupancy by (−)-OSU6162 in rhesus monkeys, revealed an occupancy of up to 75% (Ekesbo *et al*, 1999). In that study, (−)-OSU6162 was administered to anesthetized animals by continuous IV infusion of up to 3 mg/kg/h. In a rat study (Natesan *et al*, 2006), increasing subcutaneous doses of (−)-OSU6162 of up to 120 mg/kg resulted in a D2/D3 receptor occupancy plateau of about 90%. Therefore, it appears that D2/D3 receptor occupancy in humans, in a clinically relevant dosage range, as far as can be judged by the data available, is lower than in non-human vertebrates, where much higher dose ranges were applied.

The degree of D2/D3 receptor occupancy in human striatal brain regions, as compared with that of the antipsychotic drugs mentioned above, illustrates a unique feature of (−)-OSU6162 that may help to elucidate its dopamine-stabilizing properties. Various explanations can be proposed for the partial occupancy of D2/D3 receptors *in vivo*. D2 receptors have been reported to function as asymmetrical dimers (Han *et al*, 2009; Kara *et al*, 2010a). It is possible that either (−)-OSU6162 has a preference for certain dimers or that raclopride and (−)-OSU6162 bind to allosteric sites, which are only partially mutually exclusive. These hypotheses, however, are hard to investigate and prove experimentally.

A possible explanation for the different results in human and non-human species could be that we are dealing with a biphasic binding curve. This could theoretically arise from a reduction in specific receptor labeling by [^{11}C]raclopride caused by both unlabeled (−)-OSU6162 and increased extracellular dopamine. Treatment with (−)-OSU6162, because of the blockade of D2/D3 autoreceptors, will stimulate the activity of dopaminergic neurones and thus cause an increase in dopamine release. Because of the preferential blockade of dopaminergic autoreceptors (Carlsson *et al*, 2004), the contribution of endogenous dopamine following low doses of the drug would be high, and this should lead to a relatively steep initial rise of the binding curve. However, when the dose-dependent binding to the autoreceptors is reaching saturation the rise will slow down. With further increasing dosage, the relative contribution of (−)-OSU6162 to the displacement of [^{11}C]raclopride will increase, and the binding curve would then again assume a steeper course. To demonstrate this further increase in humans would, however, require doses higher than those used in the present study, and such high doses may not be well tolerated.

According to another hypothesis, which would not be incompatible with the previous one, (−)-OSU6162 will preferentially bind to extrasynaptic D2/D3 receptors (Carlsson and Carlsson, 2006). The D2 autoreceptors belong to the extrasynaptic subpopulation of dopaminergic receptors, and it is reasonable to assume that the blocking action of (−)-OSU6162 extends to all extrasynaptic D2/D3 receptors. Hence, it might be concluded from the present data that the extrasynaptic subpopulation of the D2/D3 receptor represents <40% of the total D2/D3 receptor population in human striatal regions. The presumed selectivity of (−)-OSU6162 for extrasynaptic D2/D3 receptors seems to provide a plausible explanation for some unique aspects of its pharmacological profile. Behavioral stimulation of individuals (animals or humans) displaying a low psychomotor activity could be due to a blocking action on autoreceptors, which at low doses may prevail over a blocking action on heteroreceptors (Rung *et al,* 2008, Carlsson *et al,* 2011, Johansson *et al,* 2012). With increasing dose of (−)-OSU6162, however, its action on extrasynaptic heteroreceptors will increase and thus counterbalance its effect on autoreceptors. Ultimately, the effect on the heteroreceptors will prevail, especially under conditions of high behavioral baseline levels, thus causing reduced psychomotor activity. Lack of binding to synaptic heteroreceptors could explain both the virtually complete absence of extrapyramidal side effects and the lack of a number of non-motor side effects. The buffering effect of (−)-OSU6162 on brain dopaminergic transmission, ie, its ability to mitigate both deficient and excessive dopamine activity, may at least in part explain why this compound has shown promise in conditions as disparate as Huntington's disease, schizophrenia, Parkinson's disease, and mental fatigue following brain trauma and stroke (Tedroff *et al*, 1999; Johansson *et al*, 2012; Kloberg *et al,* 2014; Carlsson A, unpublished observations).

The interpretation of the binding data given above, although plausible, should still be considered tentative, given the limited number of subjects. For example, although data fit in well with a single-site binding curve, the conclusion that this curve is leveling off with a maximum around 40% may be questioned, especially in view of the animal data showing a much higher binding capacity of (−)-OSU6162 to dopamine D2/D3 receptors. A limitation of the study is the small number of data points between plasma levels of 0.4–0.9 μM, which could have caused an apparent leveling off. In spite of our study design with dosages well spread out between subjects, the measured plasma levels showed some clustering leading to limited data points between 0.4–0.9 μM. This was probably due to some differences in pharmacokinetics between subjects along with the fact that plasma levels could not be obtained for three subjects (two subjects were excluded from the study, and for one subject, plasma levels could not be measured due to an experimental error). Furthermore, the lack of data points beyond a dose of 90 mg could also cause the apparent leveling off. The perceived species difference could therefore also arise from the fact that the dosage of the compound to humans has to be limited for tolerability reasons. Future studies are needed to clarify these issues.

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