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REGULAR RESEARCH ARTICLE

Affinity States of Striatal Dopamine D2 Receptors in Antipsychotic-Free Patients with Schizophrenia

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Abstract

Background: Dopamine D2 receptors are reported to have high-affinity ($D2^{High}$) and low-affinity ($D2^{Low}$) states. Although an increased proportion of $D2^{High}$ has been demonstrated in animal models of schizophrenia, few clinical studies have investigated this alteration of $D2^{High}$ in schizophrenia in vivo.

Methods: Eleven patients with schizophrenia, including 10 antipsychotic-naive and 1 antipsychotic-free individuals, and 17 healthy controls were investigated. Psychopathology was assessed by Positive and Negative Syndrome Scale, and a 5-factor model was used. Two radioligands, [^{11}C]raclopride and [^{11}C]MNPA, were employed to quantify total dopamine D2 receptor and $D2^{High}$, respectively, in the striatum by measuring their binding potentials. Binding potential values of [^{11}C]raclopride and [^{11}C]MNPA and the binding potential ratio of [^{11}C]MNPA to [^{11}C]raclopride in the striatal subregions were statistically compared between the 2 diagnostic groups using multivariate analysis of covariance controlling for age, gender, and smoking. Correlations between binding potential and Positive and Negative Syndrome Scale scores were also examined.

Results: Multivariate analysis of covariance demonstrated a significant effect of diagnosis (schizophrenia and control) on the binding potential ratio ($P = .018$), although the effects of diagnosis on binding potential values obtained with either [^{11}C]raclopride or [^{11}C]MNPA were nonsignificant. Posthoc test showed that the binding potential ratio was significantly higher in the putamen of patients ($P = .017$). The Positive and Negative Syndrome Scale "depressed" factor in patients was positively correlated with binding potential values of both ligands in the caudate.

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Significance Statement

It has been documented that dopamine D2 receptors exist in 2 distinct states with high and low affinities for endogenous dopamine. Here, we quantified total and high-affinity-state D2 receptors in the striatal subregions of healthy controls and patients with schizophrenia by PET with a D2-type receptor antagonist, [^{11}C]raclopride, and a D2-type receptor agonist, [^{11}C]MNPA, respectively, and demonstrated a higher proportion of the high-affinity state of D2 receptors in the putamen of the patients. Additionally, the binding of these 2 radioligands in the caudate of the patients was positively correlated with their scores of Positive and Negative Syndrome Scale (PANSS) "depressed" factor. These findings support the utility of PET with a D2-type receptor antagonist and a D2-type receptor agonist in the same individual for investigating a change in the total amount or affinity state of this receptor as a possible molecular basis of symptomatic manifestations in schizophrenia.

Conclusions: The present study indicates the possibilities of: (1) a higher proportion of $D2^{High}$ in the putamen despite unaltered amounts of total dopamine D2 receptors; and (2) associations between depressive symptoms and amounts of caudate dopamine D2 receptors in patients with schizophrenia.

Keywords: PET, [^{11}C]raclopride, [^{11}C]MNPA, striatum, high affinity

Introduction

Dysregulation in dopamine neurotransmission is thought to underlie the pathophysiology of schizophrenia (Meltzer and Stahl, 1976; Davis et al., 1991). This concept has been supported for decades by evidence that the clinical effect of antipsychotics is mediated by dopamine D2 receptor (D2R) blockade (Nord and Farde, 2011).

Previous in vitro studies have suggested that D2R has 2 interconvertible affinity states for endogenous dopamine, referred to as G-protein-coupled high-affinity ($D2^{High}$) and G-protein-uncoupled low-affinity ($D2^{Low}$) states (De Lean et al., 1982; Sibley et al., 1982; George et al., 1985; Richfield et al., 1989). $D2^{High}$ is a functionally active state of D2R (George et al., 1985). Elevations of $D2^{High}$ have been reported in several different animal models of schizophrenia, and it has been suggested that an increased proportion of $D2^{High}$ might play an important role in the pathophysiology of schizophrenia (Seeman et al., 2005; Seeman, 2011).

[^{11}C]raclopride is a widely used D2R ligand for visualizing this receptor in the brains of living subjects by positron emission tomography (PET). While [^{11}C]raclopride is a D2-type receptor antagonist with similar affinities for $D2^{High}$ and $D2^{Low}$ (Seneca et al., 2006), the D2-type receptor agonists, exemplified by (-)-N-[^{11}C]propyl-norapomorphine ([^{11}C]NPA) (Hwang et al., 2000), [^{11}]C(+)-4-propyl-3,4,4a,5,6,10b-hexahydro-2H-naphtho[1,2-b][1,4]oxazin-9-ol ([^{11}C]PHNO) (Wilson et al., 2005), and [^{11}C](R)-2-CH3O-N-n-propylnorapomorphine ([^{11}C]MNPA) (Finnema et al., 2005), have been shown to have a higher affinity for $D2^{High}$ than $D2^{Low}$ (Sibley et al., 1982; Seneca et al., 2006; Willeit et al., 2007; Shotbolt et al., 2012; Gallezot et al., 2014).

Only a limited number of clinical studies have hitherto investigated $D2^{High}$ availability in patients with schizophrenia in vivo, and whether there are alterations in the ligand binding to $D2^{High}$ or in the proportion of $D2^{High}$ in patients with schizophrenia still remains a question. Previous PET studies using [^{11}C]PHNO have shown that there were no significant changes in its binding potential in patients with schizophrenia compared with healthy controls (Graff-Guerrero et al., 2009; Suridjan et al., 2013). However, [^{11}C]PHNO was reported to have a 50-fold higher affinity for dopamine D3 receptor (D3R) than D2R (Freedman et al., 1994; Narendran et al., 2006), potentially hampering sensitive detection of $D2^{High}$. In contrast to [^{11}C]PHNO, [^{11}C]MNPA has almost identical affinities for D2R and D3R, with dissociation constant (Kd) values of 2.21 nM and 2.02 nM, respectively (Skinbjerg et al., 2009), and thus might be more suitable for assessing $D2^{High}$ (Kodaka et al., 2013). In addition, the binding potential ratio between the 2 ligands, [^{11}C]raclopride and [^{11}C]MNPA, could be a possible index of the proportion of $D2^{High}$ vs total D2Rs, as indicated in a previous study for healthy subjects (Kodaka et al., 2013).

In this study, we aimed to investigate the availability of $D2^{High}$ in antipsychotic-free patients with schizophrenia using PET with both antagonist and agonist ligands.

Methods

Participants

A total of 11 patients with schizophrenia (4 men and 7 women) were recruited from affiliated hospitals or clinics. Seventeen healthy controls (8 men and 9 women) were recruited by the National Institute of Radiological Sciences, Chiba, Japan, for participation in this study.

The patient group was comprised of 10 antipsychotic-naive patients (one had taken benzodiazepines the night before her PET scans) and 1 patient who had been antipsychotic-free for 2 years after 1-year treatment with aripiprazole. All patients fulfilled the diagnostic criteria for schizophrenia according to DSM-IV. None of the patients were comorbid with other neuropsychiatric disorders or had substance abuse. Psychopathology was assessed using the Positive and Negative Syndrome Scale (PANSS) (Kay et al., 1987), and a 5-factor model (Wallwork et al., 2012) was used to calculate PANSS subscale scores (positive, negative, disorganized/concrete, excited, and depressed factors). The healthy control volunteers were recruited by public notices. They had no history of psychiatric disease, neurological injury or disease, severe medical diseases, substance abuse that may affect brain functions, or first-degree relatives suffering from psychotic episodes. All the diagnostic interviews and PANSS ratings were conducted by trained psychiatrists. Table 1 presents the participants' demographic information.

This study was approved by the Radiation Drug Safety Committee and the Institutional Review Board of the National Institute of Radiological Sciences, Japan, and was carried out in accordance with the Code of Ethics of the World Medical Association. After complete description of the study, written informed consent was obtained from all participants. Data were collected between April 2008 and October 2016.

Table 1. Demographic and Clinical Characteristics of Subjects

	Patient group (n = 11)		Control group (n = 17)		Statistics	
	Mean	SD	Mean	SD	t	P
Age (y)	33.9	6.5	33.8	9.6	0.047	.96
Gender (male/female)	4/7		8/9			.71
Smoker (yes/no)	4/7		4/13			.67
Injected radioactivity of [^{11}C]raclopride (MBq)	224.8	14.4	221.5	15.3	-0.57	.58
Injected radioactivity of [^{11}C]MNPA (MBq)	220.1	17.9	221.2	16.3	-0.17	.87
Specific radioactivity of [^{11}C]raclopride (GBq/mmol)	178.1	87.6	206.2	140.0	-0.65	.52
Specific radioactivity of [^{11}C]MNPA (GBq/mmol)	173.3	99.5	182.5	166.0	-0.17	.87
Age at onset (y)	31.1	5.5				
Duration of illness (y)	3.3	4.3				
PANSS total score	77.6	22.5				
PANSS factor						
Positive	13.5	3.0				
Negative	12.4	5.5				
Disorganized/concrete	7.3	2.6				
Excited	8.5	3.5				
Depressed	8.4	3.0				

PET Procedures

All subjects underwent 2 PET scans on the same day, one with [^{11}C]raclopride and the other with [^{11}C]MNPA, except 1 control subject who took the 2 scans on separate days due to technical trouble. [^{11}C]MNPA-PET preceded [^{11}C]raclopride-PET in 10 patients and 11 controls, and [^{11}C]raclopride-PET was followed by [^{11}C]MNPA-PET in 1 patient and 6 controls. After i.v. rapid bolus injection of [^{11}C]raclopride, a PET scan was performed for 60 minutes. Similarly, after i.v. rapid bolus injection of [^{11}C]MNPA, a PET scan was performed for 90 minutes. The time interval between initiations of the first and second PET scans was 120 minutes or longer. For both PET scans, 3-dimensional dynamic PET data were acquired with a Siemens ECAT Exact HR+ system (CTI/Siemens), which provides 63 sections with an axial field of view of 15.5 cm. PET images were reconstructed with a filtered back-projection method with corrections for attenuation and scatter. The reconstructed in-plane resolution was 7.5 mm full-width at half-maximum. A 10-minute transmission scan using a ^{68}Ge/^{68}Ga line source was performed before each PET scan for attenuation correction. A thermoplastic head fixation device was used to minimize head movement during PET scanning. The dynamic scans consisted of twelve 20-second frames, sixteen 60-second frames, and ten 240-second frames for [^{11}C]raclopride, and nine 20-second frames, five 60-second frames, four 120-second frames, eleven 240-second frames, and six 300-second frames for [^{11}C]MNPA.

MRI Procedures

Because of replacements of MRI machines during this study, 3 types of MRI devices were used depending on the time of participant recruitment. For the initial 4 patients and 7 controls, MR images were acquired with a 1.5-T MR scanner (Intera, Philips Medical Systems). 3D volumetric acquisition of a T1-weighted gradient echo sequence produced a gapless series of thin transverse sections (TE 9.2 ms, TR 21 ms, flip angle 30°, field of view 256 mm, acquisition matrix 256 × 256, slice thickness 1 mm). Then, for the next 6 patients, a 3-T MR scanner (Sigma HDx, General Electric) was used. 3D volumetric acquisition of a T1-weighted 3-dimensional fast spoiled gradient-recalled acquisition in the steady-state sequence produced a gapless series of thin transverse sections (TE 2.8 ms, TR 7.0 ms, flip angle 8°, field of view 260 mm, acquisition matrix 256 × 256, slice thickness 1 mm). Finally, for 1 patient and 10 controls, T1-weighted MR images were obtained with another 3-T MR scanner (MAGNETOM Verio, Siemens). 3D volumetric acquisition of a T1-weighted gradient echo sequence produced a gapless series of thin sagittal sections (TE 1.95 ms, TR 2300 ms, TI 900 ms, flip angle 9°, field of view 250 mm, acquisition matrix 256 × 256, slice thickness 1 mm).

Region of Interest (ROI) Definition

All ROIs used in our analyses were initially defined on a standard anatomic orientation (MNI standard space; Montreal Neurological Institute). Striatum ROIs (caudate, putamen, and ventral striata) were defined using a striatum anatomical atlas (Tziortzi et al., 2011). A cerebellum ROI was defined with a probabilistic cerebellar atlas (Diedrichsen et al., 2009), excluding the vermis.

These ROIs were transformed into individual MR spaces with transformation matrices calculated with the "normalize" function of statistical parametric mapping software (SPM12; Wellcome Department of Imaging Neuroscience) and were thresholded using FreeSurfer's automated anatomical segmentation (Fischl et al., 2002) to exclude neighboring white matter, ventricles, and non-brain regions. Time-activity curves for each ROI were extracted by applying these ROIs to individual dynamic PET images based on the transformation parameters for the coregistration of MR images to the PET spaces using PMOD software ver. 3.7 (PMOD Technologies Ltd).

Quantification of Total D2R and D2^{High} Availabilities in the Striatum

Availabilities of striatal total D2R and D2^{High} were quantified as binding potentials relative to the nondisplaceable tissue (BP_{ND}) of [^{11}C]raclopride and [^{11}C]MNPA, respectively. [^{11}C]raclopride BP_{ND} and [^{11}C]MNPA BP_{ND} were calculated for each ROI using a 3-parameter simplified reference tissue model (Lammertsma and Hume, 1996) and cerebellar cortex as reference tissue,

which has negligible density of D2R (Suhara et al., 1999). BP_{ND} was defined as follows:

$$BP_{ND} = f_{ND} \bullet B_{avail} / Kd \quad (1)$$

where f_{ND} is the free fraction of radioligand in the nondisplaceable tissue compartment and B_{avail} indicates the neuroreceptor density. All kinetic analyses were performed using PMOD software ver. 3.7.

Statistical Analysis

Statistical analyses were conducted with SPSS 23.0 (SPSS Inc). First, MANCOVA was applied to examine group differences in: (1) BP_{ND} of [^{11}C]raclopride; (2) BP_{ND} of [^{11}C]MNPA; and (3) the BP_{ND} ratio (ratio of [^{11}C]MNPA BP_{ND} to [^{11}C]raclopride BP_{ND}) in 3 striatal ROIs (caudate, putamen, and ventral striata), separately. A fixed factor was diagnosis (patients = 1, controls = 0). Nuisance covariates were defined as age, gender, and smoking state (smoker = 1, nonsmoker = 0) to control for their potential effects on D2Rs (Wong et al., 1997; Okita et al., 2016). Statistical significance threshold was defined as $P < .05$ (2-tailed).

Second, correlational analyses were performed between the BP_{ND} values mentioned above and each of the PANSS factor scores (positive, negative, disorganized/concrete, excited, and depressed factors) in patients. The statistical threshold was set at $P < .05$ with Bonferroni correction for the 3 striatal subregions (caudate, putamen, and ventral striata), as in previous studies (Talvik et al., 2006; Okita et al., 2016). Because of the small sample size and exploratory nature of this study, corrections were not applied for multiple comparisons of the 5 PANSS factor scores and the 3 binding parameters ([^{11}C]raclopride BP_{ND}, [^{11}C]MNPA BP_{ND}, and their ratio). In case significant correlations were found, partial correlation analyses were also performed, controlling for age, gender, smoking state, or duration of illness. Statistical significance threshold was defined as $P < .05$ (2-tailed).

Results

Demographic Data

Demographic data are shown in Table 1. Patients and controls did not significantly differ in terms of age, gender, or proportion of smokers. Injected dose and specific radioactivity of [^{11}C]raclopride and [^{11}C]MNPA were not significantly different between the 2 groups.

Group Comparisons of Radioligand Binding

Results of group comparisons of BP_{ND} values are shown in Table 2 and Figure 1. MANCOVA demonstrated a significant main effect of diagnosis on the BP_{ND} ratio ($F = 4.16$, $P = .018$), although the effects of diagnosis on BP_{ND} values obtained with either [^{11}C]raclopride or [^{11}C]MNPA were nonsignificant. Posthoc analysis by univariate test revealed a significant effect of diagnosis on the putaminal BP_{ND} ratio ($F = 6.64$, $P = .017$), indicating that the BP_{ND} ratio in the putamen of patients was significantly higher.

Because age distribution was reasonably matched between the 2 groups both numerically and statistically (Table 1), we repeated the analysis excluding age from nuisance covariates to confirm our results on the BP_{ND} ratio. This MANCOVA also showed a significant main effect of diagnosis on the BP_{ND} ratio ($F = 3.68$, $P = .028$). Univariate posthoc analysis revealed a significant effect of diagnosis on the putaminal BP_{ND} ratio ($F = 5.83$, $P = .024$), indicating that the BP_{ND} ratio, compared with that of controls, was significantly higher in the putamen of patients.

Correlation Analyses

Significant positive correlations were found between PANSS depressed factor and BP_{ND} of [^{11}C]raclopride (Pearson's $r = 0.773$, $P = .005$) and of [^{11}C]MNPA ($r = 0.701$, $P = .016$) in the caudate (Figure 2). No other significant correlation was found. Partial correlations of PANSS depressed factor with the BP_{ND} of both [^{11}C]raclopride and [^{11}C]MNPA in the caudate were significant after controlling for age, gender, smoking state, or duration of illness.

Discussion

The binding of [^{11}C]raclopride and [^{11}C]MNPA did not significantly differ between patients and controls. This is consistent with most of the previous D2/3 receptor studies using different radioligands, in which no clear differences were shown between the 2 diagnostic groups (Talvik et al., 2006; Graff-Guerrero et al., 2009; Howes et al., 2012; Brunelin et al., 2013; Suridjan et al., 2013). On the other hand, the current study revealed that the

Table 2. Comparisons of BP_{ND} of [^{11}C]raclopride, BP_{ND} of [^{11}C]MNPA, and BP_{ND} Ratio of [^{11}C]MNPA to [^{11}C]raclopride between the Patient and Control Groups

	Patient group (n = 11)		Control group (n = 17)		Multivariate tests		Between-subject effects (posthoc)	
	Mean	SD	Mean	SD	F	P	F	P
BP_{ND} of [^{11}C]raclopride					1.50	.24		
Caudate	2.21	0.34	2.30	0.35				
Putamen	2.89	0.36	3.04	0.31				
Ventral striatum	1.98	0.36	2.12	0.22				
BP_{ND} of [^{11}C]MNPA					2.48	.09		
Caudate	0.45	0.10	0.47	0.11				
Putamen	0.76	0.06	0.75	0.07				
Ventral striatum	0.49	0.09	0.54	0.07				
BP_{ND} ratio					4.16	.018[a]		
Caudate	0.203	0.020	0.202	0.032			0.11	.74
Putamen	0.265	0.022	0.249	0.020			6.64	.017[a]
Ventral striatum	0.256	0.054	0.258	0.031			0.00	.98

[a]$P < .05$.

BP_{ND} ratio of [^{11}C]MNPA to [^{11}C]raclopride in the putamen was significantly higher in patients with schizophrenia.

In previous studies on animal models of schizophrenia, the proportion of $D2^{High}$ in the striatum was markedly increased (Seeman et al., 2005; Seeman, 2011), while D2R density in this area was not significantly changed. Several different animal models of schizophrenia have been reported, such as amphetamine-sensitized rats, mice deficient in types 2 and 3 metabotropic glutamate receptors, trace amine 1 receptor knockout mice, and rats with a neonatal hippocampus lesion. Interestingly, dopamine super-sensitivity (Lieberman et al., 1987) and marked increase in $D2^{High}$ without significant increase in total D2Rs have been observed in most of these animal models (Bhardwaj et al., 2003; Seeman et al., 2005; Wolinsky et al., 2007; Seeman, 2009; Seeman et al., 2009). These findings imply that there may be a variety of mechanisms that can lead to increases in $D2^{High}$. As such, it appears that $D2^{High}$ could be one of the common mechanisms of schizophrenia. Although molecular processes triggering this change are not clear, one explanation might be that the constant oscillation between the high- and low-affinity states of D2R is altered in schizophrenia, and the conversion rate of $D2^{High}$ to $D2^{Low}$ may be slower than a normal condition, resulting in an increase in the proportion of $D2^{High}$ (Seeman, 2013). The extent of the group difference in the BP_{ND} ratio observed in our study was not as prominent as the increase in the proportion of $D2^{High}$ shown in animal models of psychosis. This might be due to the difference between in vitro tissue assays and in vivo PET imaging (Graff-Guerrero et al., 2009), and it is likely that a low-grade but long-lasting abnormality of $D2^{High}$ over decades may lead to the onset of schizophrenia. There is also a possibility that the contribution of $D2^{High}$ alterations vs other neurochemical factors to the etiology of schizophrenia might be variable among individuals in consideration of the heterogeneity of neuroreceptor statuses in subjects with this disease. The current work indicated a higher proportion of $D2^{High}$ in the putamen of patients with schizophrenia, while it is yet to be elucidated how neurochemical and functional abnormalities in this striatal subregion contribute to the onset of the disease. MRI studies have documented involvements of putaminal volume changes in the evolution of a clinical high risk status for psychosis (Bin Hong et al., 2015) and worsening of clinical outcomes in schizophrenia (Mitelman et al., 2009). Hence, mechanistic links between these morphological alterations and a dysregulated affinity state of D2R in the putamen may need to be investigated in subjects at clinical high risk and with schizophrenia by longitudinal PET and MRI assays.

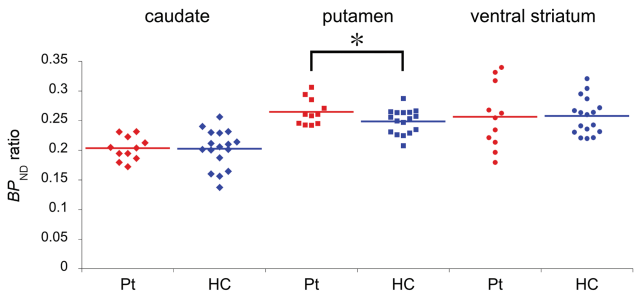

Figure 1. A group comparison of binding potential ratio (BP_{ND} ratio) ([^{11}C](R)-2-CH3O-N-n-propylnorapomorphine ([^{11}C]MNPA) to [^{11}C]raclopride) in striatal subregions. A significant diagnostic effect was found on the BP_{ND} ratio ($P = .018$; MANCOVA controlled for age, gender, smoking). Univariate posthoc analysis showed that the BP_{ND} ratio in the putamen was significantly higher in patients ($P = .017$). HC, healthy control group; Pt, patient group.

For the D2R/D3R selectivity of radioligands, unlike [^{11}C]PHNO, both [^{11}C]raclopride and [^{11}C]MNPA have similar affinities to D2R and D3R according to an in vitro study (Skinbjerg et al., 2009). Because D2Rs are predominant and only low levels of D3R have been detected in the caudate and putamen by previous binding assays or postmortem studies (Murray et al., 1994; Seeman et al., 2006), and [^{11}C]MNPA has a higher affinity for $D2^{High}$ than $D2^{Low}$ (Seneca et al., 2006), the BP_{ND} ratio of [^{11}C]MNPA to [^{11}C]raclopride in these regions could reflect the proportion of $D2^{High}$ relative to total D2Rs with minimal influence of the D3R status.

PANSS depressed factor was positively correlated with both BP_{ND} of [^{11}C]raclopride and [^{11}C]MNPA in the caudate. A previous PET study with [^{11}C]raclopride has reported its elevated binding in the caudate and putamen in medication-free patients with depression compared with healthy subjects (Meyer et al., 2006), suggesting the possible involvement of dopaminergic neurotransmission in depression and depressed subjects. Our results might indicate specific associations between depressive symptoms in schizophrenia and D2R availability in the caudate, in a manner independent of its affinity states. Since BP_{ND} values of both antagonistic [^{11}C]raclopride and agonistic [^{11}C]MNPA were correlated with the depression score, densities of D2Rs rather

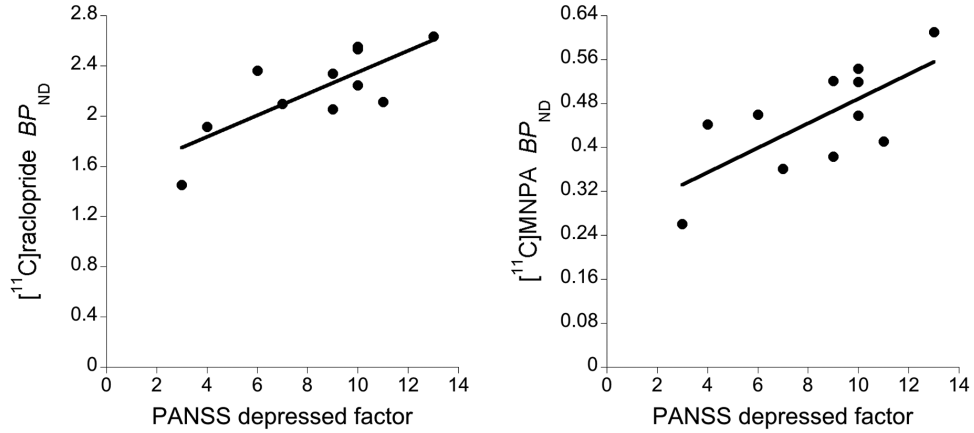

Figure 2. Scatter plots of binding potential (BP_{ND}) values against Positive and Negative Syndrome Scale (PANSS) "depressed" factor and linear regressions in the caudate of patients with schizophrenia. PANSS "depressed" factor was significantly and positively correlated with BP_{ND} of [^{11}C]raclopride (left: Pearson's $r = 0.773$, $P = .005$) and of [^{11}C](R)-2-CH3O-N-n-propylnorapomorphine ([^{11}C]MNPA) (right: $r = 0.701$, $P = .016$). Statistical significance threshold: $P < .05$ for the 3 striatal subregions (Bonferroni correction).

than occupancy of D2R by endogenous dopamine may increase in association with a depressive state. This dopaminergic modulation might not efficiently compensate for the mood change, in light of somewhat limited therapeutic application of dopaminergic stimulants to depressive conditions (Hardy, 2009). Accordingly, further studies with a larger sample size will be needed to better understand the current observations and to confirm them on the basis of more rigorous statistical criteria.

Several technical limitations of the current study should be taken into account. First, the sample size is relatively small, and the demographic compositions of gender and smokers vs non-smokers are not equal between the 2 groups numerically, if not statistically. In addition, a previous PET study in a healthy population reported that central striatal D2R/D3R availability was negatively correlated with recent and lifetime smoking, and also with nicotine dependence (Okita et al., 2016). Another PET study reported a significant gender-by-smoking interaction on D2R/D3R availability in the caudate and putamen (Brown et al., 2012). These findings provide a rationale for the inclusion of gender and smoking as covariates of no interest, but an expanded analysis of patients and controls with a larger sample size and minimal inter-group differences in these factors will be required for more robust proof of the current results. Second, the temporal sequence of PET scans with [^{11}C]raclopride and [^{11}C]MNPA differed among the subjects. However, the occupancy of D2R by these ligands was <1% based on their injected mass doses, and the interval between the initiations of the first and second PET scans was longer than 120 minutes in all subjects. Hence, effects of the sequence of PET scans with the 2 ligands on quantitative data are considered negligible. Third, 3 different MRI devices were used to acquire anatomical information on the brains of the subjects. To minimize a possible confounding effect of MRI qualities on kinetic analyses, we used the standard MNI space to define ROIs, which were eventually transformed into individual PET spaces. Finally, we used only PANSS to evaluate symptoms in patients with schizophrenia. Using multiple scales to assess delusion, hallucination, depression, and other symptoms of schizophrenia would be more desirable.

In conclusion, the present study has provided in vivo clinical evidence that a modulated proportion of $D2^{High}$ is implicated in the molecular etiology of schizophrenia. Relationships between depressive symptoms and D2R levels in schizophrenia patients have also been demonstrated by the consistent observations with 2 radioligands. The combined use of [^{11}C]raclopride and [^{11}C]MNPA validated here could be applied to a PET study on a larger scale to further clarify involvement of the dopaminergic statuses in schizophrenia as a molecular basis of symptomatic manifestations.

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Statement of Interest

None.

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