was 4.3 mm in-plane and 4.2 mm full-width at half maximum axially. With a Hanning filter (cutoff frequency, 0.4 cycle/pixel), the reconstructed in-plane resolution was 7.5 mm. Data were acquired in three-dimensional mode. Scatter was corrected using the single scatter simulation technique (Watson et al. 1996). Ten-minute transmission scan using a ^{68}Ge–^{68}Ga line source was performed for attenuation correction. A head fixation device with thermoplastic attachments was used to minimize the subject's head movement during the PET measurements.

PET studies were performed under resting condition (baseline study) and with oral administration of aripiprazole (drug challenge study) on separate days. The average interval between baseline studies and drug challenge studies was 15.1± 17.1 days (7–63 days). In each study, the two dynamic PET scans with [^{11}C]raclopride and [^{11}C]FLB457 were performed sequentially. After the intravenous rapid bolus injection of [^{11}C]raclopride, dynamic PET scanning was performed for 60 min. One hour after the end of [^{11}C]raclopride PET measurement, dynamic PET scanning was performed for 90 min after intravenous rapid bolus injection of [^{11}C]FLB457. The frame sequence consisted of 12 20-s frames, 16 1-min frames, and 10 4-min frames for [^{11}C]raclopride, and 9 20-s frames, 5 1-min frames, 4 2-min frames, 11 4-min frames, and 6 5-min frames for [^{11}C]FLB457. The injected radioactivity was 206–235 and 184–240 MBq in baseline studies, and 211–235 and 217–229 MBq in drug challenge studies for [^{11}C]raclopride and [^{11}C]FLB457, respectively. The specific radioactivity was 131–247 and 138–361 GBq/μmol in baseline studies, and 94–270 and 139–277 GBq/μmol in drug challenge studies for [^{11}C]raclopride and [^{11}C]FLB457, respectively. The injected mass was 0.90–1.64 and 0.66–1.34 nmol in baseline studies, and 0.78–2.51 and 0.79–1.73 nmol in drug challenge studies for [^{11}C]raclopride and [^{11}C]FLB457, respectively. Paired t test revealed no significant differences in injected radioactivity, specific radioactivity, and injected mass between baseline and drug challenge studies for both [^{11}C]raclopride and [^{11}C]FLB457.

In the drug challenge study, 6 mg aripiprazole was orally administered 150 min before the start of PET scan with [^{11}C]raclopride. To estimate the plasma concentrations of aripiprazole, venous blood samplings were performed at the start and end of each PET scan. The plasma concentrations of aripiprazole and its main metabolite, dehydroaripiprazole, were determined by the validated liquid chromatography coupled to mass spectrometry/mass spectrometry method (Sumika Chemical Analysis Service Ltd, Tokyo, Japan).

All MR scans were performed with a 3.0-T MR scanner (General Electric, Milwaukee, WI). Three-dimensional volumetric acquisition of T_1-weighted three-dimensional fast-spoiled gradient-recalled acquisition in the steady state (SPGR) sequence produced gapless series of thin transverse sections (TE 2.848 ms; TR 6.992 ms; prep time 900 ms; flip angle 8°; field of view 260 mm; acquisition matrix 256×256; slice thickness 1 mm; scan time 367 s).

Regions of interest

The MR images were co-registered to each of the summation images of all frames of dynamic PET scans for each subject using PMOD (PMOD Technologies Ltd., Zurich, Switzerland). Regions of interest (ROIs) were drawn on the co-registered MR and PET images of each subject: the cerebellum and striatum (caudate head and putamen) for [^{11}C]raclopride studies; the cerebellum and extrastriatum (midbrain, thalamus, parahippocampal gyrus including amygdala, anterior part of the cingulate cortex, frontal cortex, temporal cortex, and parietal cortex) for [^{11}C]FLB457 studies. Each ROI was drawn over three adjacent sections and data were pooled to obtain the average radioactivity concentration for the whole volume of interest. To obtain the regional time–activity curves, the regional radioactivity was calculated for each frame, corrected for decay, and plotted versus time.

Calculation for dopamine D_2 receptor occupancy

For both PET studies with [^{11}C]raclopride and [^{11}C]FLB57, the binding potential (BP_{ND}) was calculated using the simplified reference tissue model method (Lammertsma et al. 1996; Lammertsma and Hume 1996). With this method, the time–activity curve in the target region is described by that in the reference region with no specific binding, assuming that both regions have the same level of non-displaceable radioligand binding:

$$C_i(t) = R_1 C_r(t) + \{k_2 - R_1 k_2/(1 + \mathrm{BP_{ND}})\} \times C_r(t) \otimes \exp\{-k_2 t/(1 + \mathrm{BP_{ND}})\} \quad (1)$$

where $C_i(t)$ is the radioactivity concentration in the target region, $C_r(t)$ is the radioactivity in the reference region, R_1 is the ratio of K_1/K_1' (K_1, influx rate constant for the target brain region; K_1', influx rate constant for the reference region), k_2 is the efflux rate constant for the target region, and ⊗ denotes the convolution integral. In this analysis, three parameters (BP_{ND}, R_1, and k_2) were estimated by nonlinear least-squares curve fitting using PMOD. The cerebellum was used as reference region. Dopamine D_2 receptor occupancy by aripiprazole was calculated as follows:

$$\text{Occupancy}\,(\%) = 100 \times \left(\mathrm{BP_{ND,\,baseline}} - \mathrm{BP_{ND,\,drug}}\right)/\mathrm{BP_{ND.\,baseline}} \quad (2)$$

where $BP_{ND,\,baseline}$ is BP_{ND} in the baseline study, and $BP_{ND,\,drug}$ is BP_{ND} in the drug challenge study.

The relation between the plasma concentration of antipsychotic drug and dopamine D_2 receptor occupancy can be

expressed as follows (Kapur and Remington 1996; Takano et al. 2004):

$$\text{Occupancy}\,(\%) = 100 \times C/(\text{ED}_{50} + C) \quad (3)$$

where C is the plasma concentration of aripiprazole, and ED_{50} is the plasma concentration required to induce 50% occupancy. ED_{50} values for each of the ROIs were calculated using the Prism software system (GraphPad Software Inc., San Diego, CA, USA) to fit the dose–occupancy data for each region, assuming a range from 0% to 100% occupancy.

Anatomic standardization

The analysis using ROIs does not allow evaluation of data throughout the brain. For visualization of regional differences in dopamine D_2 receptor occupancies, intersubject averaging of occupancy images, which requires transformation of brain images of individual subjects into the standard brain shape and size in three dimensions (anatomical standardization), was performed (Fox et al. 1988). BP_{ND} images of [^{11}C] raclopride and [^{11}C] FLB457 were calculated on a voxel-by-voxel basis by the reference tissue model (Lammertsma et al. 1996; Lammertsma and Hume 1996) with the basis function method (Gunn et al. 1997). Images of dopamine D_2 receptor occupancy were also calculated on a voxel-by-voxel basis. All MR images that were co-registered to PET images were transformed into the standard brain size and shape by linear and nonlinear parameters with SPM5 (Wellcome Trust Center for Neuroimaging, Institute of Neurology, London, UK). The brain templates used in SPM5 for the anatomical standardization were T_1 templates for MR images. All BP_{ND} images were also transformed into the standard brain size and shape using the same parameters as MR images. Thus, brain images of all subjects had the same anatomical format. Average images for BP_{ND} and dopamine D_2 receptor occupancy were calculated on a voxel-by-voxel basis.

Statistical analysis

Statistical analyses were performed with SPSS, version 18.0 (SPSS, Inc., Chicago, IL, USA). Paired t tests were performed to compare (1) dopamine D_2 receptor occupancies between the striatal and extrastriatal regions, and (2) plasma concentrations of aripiprazole between [^{11}C]raclopride and [^{11}C]FLB457 PET studies. In all tests, the two-tailed level of statistical significance was set at α=0.05. Multiple comparisons with Bonferroni corrections were performed to test the regional differences of dopamine D_2 receptor occupancies in the extrastriatal regions.

Results

Striatal and extrastriatal BP_{ND} values and dopamine D_2 receptor occupancies are shown in Tables 1 and 2. Dopamine D_2 receptor occupancies in the striatum were 70.1% and 74.1%, and the corresponding values for the extrastriatal regions ranged from 46.6% to 58.4%. Although direct comparison of dopamine D_2 receptor occupancy values between the striatal and extrastriatal regions should be interpreted with some caution due to the small difference of plasma concentration of aripiprazole during the [^{11}C] raclopride and [^{11}C]FLB457 PET studies as well as systematic errors in occupancy for [^{11}C]FLB457 studies as described in the "Discussion", higher dopamine D_2 receptor occupancies in the extrastriatal regions were not observed. No significant difference in dopamine D_2 receptor occupancy was found among the extrastriatal regions by multiple comparison test with Bonferroni corrections.

Average images of BP_{ND} at the baseline condition and after administration of aripiprazole and dopamine D_2 receptor occupancy for [^{11}C]raclopride and [^{11}C]FLB457 are shown in Figs. 1 and 2, respectively. Intersubject averaging of images allowed visualization of regional differences in dopamine D_2 receptor occupancy throughout the brain. Dopamine D_2 receptor occupancy by aripiprazole was almost uniform among the cerebral cortices.

The plasma concentrations of aripiprazole and dehydroaripiprazole in [^{11}C]raclopride PET studies averaged between the start and end of each scanning were 29.4±4.8 and 1.4±0.6 ng/ml (mean ± S.D.), and the corresponding values in [^{11}C]FLB457 PET studies were 25.6±2.2 and 1.7± 0.7 ng/ml (mean ± S.D.), respectively. The plasma concentration of aripiprazole in the [^{11}C]raclopride studies was higher than that in the [^{11}C]FLB457 studies (p<0.01). We generated dose–occupancy curves as described in the "Methods" section for each of the nine regions analyzed, yielding the ED_{50} value for each region. The ED_{50} values were 9.9 ng/ml for the striatum (r=0.40 for curve fitting), 12.2 ng/ml for the putamen (r=0.58), 29.4 ng/ml for the midbrain (r=0.36), 24.4 ng/ml for the parahippocampal gyrus (r=0.15), 18.9 ng/ml for the thalamus (r=0.50), 24.1 ng/ml for the anterior cingulate gyrus (r=0.29),

Table 1 Striatal binding potential (BP_{ND}) values and dopamine D_2 receptor occupancy in [^{11}C] raclopride PET studies

Region	BP_{ND}		Occupancy (%)
	Baseline	Drug challenge	
Caudate head	2.55±0.28	0.66±0.19	74.1±6.7
Putamen	3.52±0.30	1.04±0.18	70.1±6.3

Values are mean ± S.D.

Table 2 Extrastriatal binding potential (BP_{ND}) values and dopamine D_2 receptor occupancy in [^{11}C]FLB457 PET studies

Region	BP_{ND}		Occupancy (%)
	Baseline	Drug challenge	
Midbrain	1.78±0.41	0.94±0.23	46.6±9.9
Thalamus	4.30±0.63	1.81±0.29	57.6±6.7
Parahippocampal gyrus	1.87±0.34	0.89±0.17	51.2±10.7
Anterior cingulate	1.15±0.17	0.53±0.12	51.5±6.6
Frontal cortex	1.06±0.20	0.52±0.12	51.3±9.2
Temporal cortex	1.83±0.29	0.76±0.11	58.4±3.0
Parietal cortex	1.20±0.25	0.53±0.13	55.6±4.8

Values are mean ± S.D.

24.3 ng/ml for the frontal cortex (r=0.25), 18.2 ng/ml for the temporal cortex (r=0.35), and 20.4 ng/ml for the parietal cortex (r=0.21).

Discussion

To test the hypothesis of preferential extrastriatal dopamine D_2 receptor binding by aripiprazole, we performed PET examinations using [^{11}C] raclopride and [^{11}C] FLB457 in healthy subjects. The preferential extrastriatal dopamine D_2 receptor occupancy by aripiprazole was not observed in this study.

PET examinations using [^{11}C]raclopride and [^{11}C]FLB457 in the present study showed higher dopamine D_2 receptor occupancy in the [^{11}C]raclopride studies than in the [^{11}C] FLB457 studies, and therefore lower ED_{50} values were observed in the striatum compared to those in the extrastriatal regions. Two possible sources for these differences should be considered on the basis of our previous simulation study of regional dopamine D_2 receptor occupancies using the identical multiple radioligand method. First, the effects of small but non-negligible specific binding in the cerebellum in [^{11}C] FLB457 PET studies need to be taken into account. We previously showed that non-negligible specific binding in the cerebellum can cause an underestimation of about 8% in dopamine D_2 receptor occupancy measured by [^{11}C]FLB457 PET when BP_{ND} in the cerebellum was 0.3 and assumed occupancy without consideration of specific binding in the cerebellum was 70% (Ito et al. 2001; Ito et al. 2008). This indicated that the occupancies of dopamine D_2 receptors by aripiprazole in the striatum would not be different from those in the extrastriatal regions. Another possible source for the difference in dopamine D_2 receptor occupancy between striatal and extrastriatal regions is related to the time point of the scan and plasma concentrations of aripiprazole. In the present study, [^{11}C]FLB457 PET studies began 2 h after the start of [^{11}C]raclopride PET studies, and therefore the plasma concentration of aripiprazole was slightly lower during [^{11}C]FLB457 studies (25.6±2.2 ng/ml) than during [^{11}C]raclopride PET studies (29.4±4.8 ng/ml). When ED_{50} was considered to be 10 ng/ml, 29.4 and 25.6 ng/ml of the plasma concentrations of aripiprazole were estimated to yield 75% and 72% of dopamine D_2 receptor occupancy, respectively (Eq. 3). This might also partially explain the higher dopamine D_2 receptor occupancies in [^{11}C]raclopride studies than in [^{11}C]FLB457 studies. Taken together, it is concluded that aripiprazole

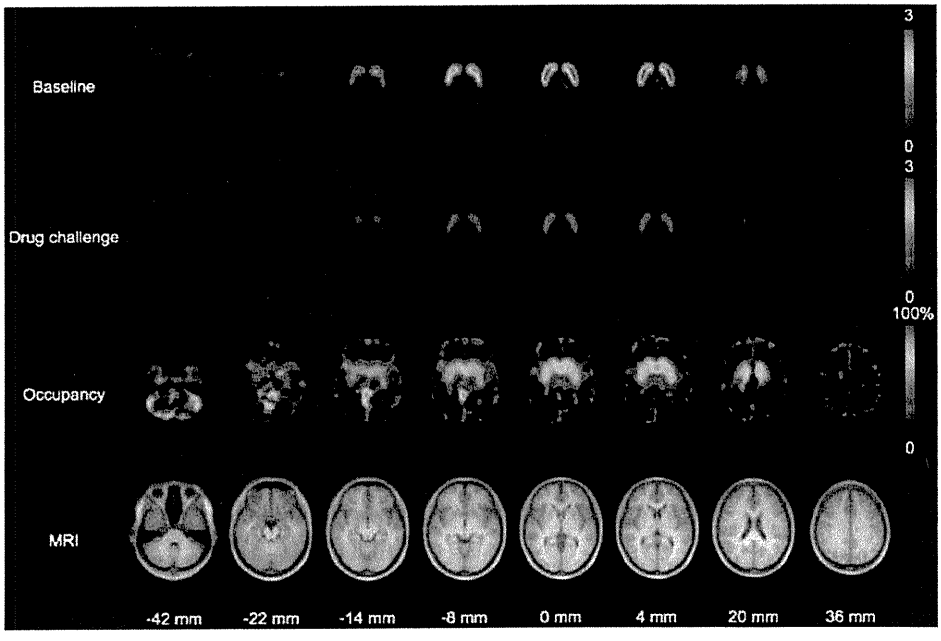

Fig. 1 Average images of binding potential at baseline condition and after administration of aripiprazole, dopamine D_2 receptor occupancy for [^{11}C]raclopride, and T_1-weighted images. In the striatum, no obvious regional differences in dopamine D_2 receptor occupancy were observed

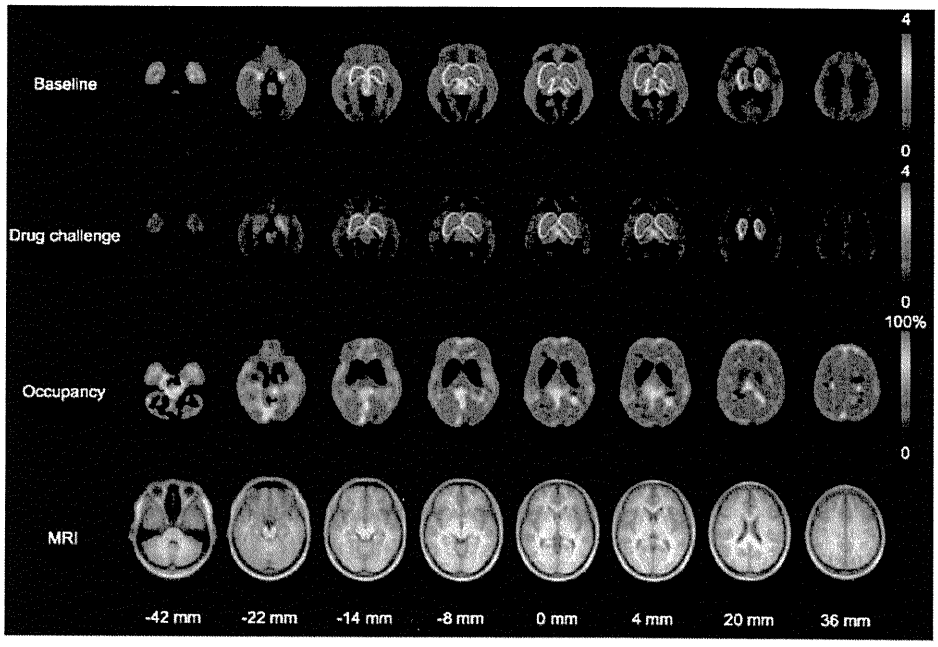

Fig. 2 Average images of binding potential at baseline condition and after administration of aripiprazole, dopamine D_2 receptor occupancy for [^{11}C]FLB457, and T_1-weighted images. Among extrastriatal regions, no obvious regional differences in dopamine D_2 receptor occupancy were observed. Note that dopamine D_2 receptor occupancies in the striatum could not be calculated correctly in [^{11}C]FLB457 studies due to the extremely high BP_{ND} values. Therefore, BP_{ND} values higher than 10.0 were cut off from average images, and this accounts for the striatum appearing to have zero occupancy values

demonstrates similar dopamine D_2 receptor occupancies in the striatal and extrastriatal regions.

Aripiprazole is an antipsychotic drug with high affinity for dopamine D_2 receptors (Davies et al. 2006). Past clinical studies have shown that aripiprazole demonstrates clinical efficacy equal to first-generation antipsychotics but with a lower risk of extrapyramidal side effects (Bhattacharjee and El-Sayeh 2008). To explain the clinical properties of aripiprazole, two hypotheses have been proposed. First, it has been assumed that aripiprazole as a partial agonist exhibits an intrinsic activity at dopamine D_2 receptors (Lawler et al. 1999). Therefore, it is theoretically possible that even at 90% receptor occupancy by aripiprazole, extrapyramidal side effects comparable to those expected with first-generation antipsychotics do not occur. Second, preferential extrastriatal dopamine D_2 receptor binding has been proposed as a possible mechanism of second-generation antipsychotics and aripiprazole. However, previous PET studies using [^{18}F]fallypride, where regional differences of dopamine D_2 receptor occupancy by aripiprazole were investigated in patients with schizophrenia, reported inconsistent conclusions. Kegeles et al. (2008) reported higher dopamine D_2 receptor occupancies in the extrastriatal regions compared to the striatum in patients with schizophrenia and schizoaffective disorder treated with aripiprazole. Another PET study using [^{18}F]fallypride was in agreement in showing relatively higher ED_{50} values in the striatum than those in the extrastriatal regions but reported no significant regional difference in dopamine D_2 receptor occupancy across brain regions by aripiprazole in patients with schizophrenia or schizoaffective disorder (Gründer et al. 2008). Since those two PET studies differed in scan durations, subject groups at baseline, and drug challenge studies, it was possible that these methodological differences caused different results. In the present study, preferential extrastriatal dopamine D_2 receptor occupancy was not observed by the multiple radioligands method. Thus, partial agonism at dopamine D_2 receptors is the most likely explanation for the minimal risk of extrapyramidal side effects in the treatment by aripiprazole.

There are several methodological issues to be discussed. While previous studies measured dopamine D_2 receptor occupancies in the striatal and extrastriatal regions using a single high-affinity radioligand, we used [^{11}C] raclopride and [^{11}C] FLB457 for the striatal and extrastriatal regions, respectively. Since dopamine D_2 receptor densities are quite different between the striatal and extrastriatal regions (Hall et al. 1996; Hall et al. 1994), different radioligands with different affinities for dopamine D_2 receptors have been shown to be preferable (Olsson and Farde 2001). It has also been pointed out that insufficient scan durations in PET studies with a single high-affinity radioligand could cause the underestimation of BP_{ND} values especially in regions with high dopamine D_2 receptor densities due to a failure of reaching equilibrium (Ito et al. 2009; Olsson and Farde 2001). Although previous studies indicated that too short data acquisition time in [^{11}C] FLB457 PET studies could cause the underestimation of occupancy in the extrastriatal regions (Erlandsson et al. 2003), the accuracy of the estimation of extrastriatal BP_{ND} and occupancy in [^{11}C]FLB457 studies with a data acquisition time over 60 min has been confirmed (Ito et al. 2001; Olsson and Farde 2001; Sudo et al. 2001). Accuracy of the estimation of striatal BP_{ND} using [^{11}C]raclopride was also confirmed (Ito et al. 1998; Lammertsma et al. 1996). In the present study, we

did not randomize the order of the two PET scans with [^{11}C] raclopride and [^{11}C]FLB457. Since washout from the brain with [^{11}C]raclopride is faster than that with [^{11}C]FLB457, we performed PET scans with [^{11}C]raclopride before those with [^{11}C]FLB457. This could be a potential limitation because the plasma concentration of aripiprazole at the time of two PET scans significantly differed, as mentioned above.

To calculate dopamine D_2 receptor occupancies, we used the subject's own baseline data to determine dopamine D_2 receptor occupancies by aripiprazole. Although previous studies showed little or no difference in dopamine D_2 receptor density in the striatum (Farde et al. 1990) or in the extrastriatal regions (Suhara et al. 2002) between normal subjects and patients with schizophrenia, individual differences in dopamine D_2 receptor density might potentially lead to an error in the estimation of dopamine D_2 receptor occupancy (Farde et al. 1992).

The administered dose of aripiprazole for measuring dopamine D_2 receptor occupancies should also be considered. Aripiprazole treatment has been shown to be well tolerated across a range of previously studied doses (2–30 mg/day) (Bhattacharjee and El-Sayeh 2008). In Japan, the starting dose of aripiprazole was set at 6–12 mg/day, and the recommended daily dose ranging from 6 to 24 mg/day in patients with schizophrenia was confirmed (Ohmori et al. 2006). From an ethical standpoint, a relatively small dose of aripiprazole (6 mg) was administered in the present study, and lower plasma concentrations and dopamine D_2 receptor occupancies were observed compared to the past studies. Since it has been proposed that compounds with a long plasma half-life and/or a high affinity are described by a steep curve in dose–occupancy relationship (Gründer et al. 2008), the single-dose setting and the relatively small dose of aripiprazole in the present study might have been a limitation in the calculation of ED_{50}. Thus, whether aripiprazole has different striatal and extrastriatal occupancies at lower/higher doses remains unclear in the present study. In a future study, administration of lower/higher doses of aripiprazole should be tested in the measurement of dopamine D_2 receptor occupancies to calculate accurate ED_{50} at a wider range of plasma concentrations.

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ARTICLE

THEMATIC SECTION
New Developments in Schizophrenia Research

CINP

Effect of risperidone on high-affinity state of dopamine D_2 receptors: a PET study with agonist ligand [^{11}C](R)-2-CH_3O-N-n-propylnorapomorphine

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Abstract

The increased proportion of the high-affinity state of dopamine $D_{2/3}$ receptors ($D_{2,high}$) is assumed to correlate with dopamine hypersensitivity, implying a relationship with psychotic symptoms observed in psychiatric disorders such as schizophrenia. [^{11}C](R)-2-CH_3O-N-n-propylnorapomorphine ([^{11}C]MNPA), which has an agonistic property to dopamine D_2 receptors (D_2Rs), is expected to bind preferentially to $D_{2,high}$. The occupancy of dopamine D_2Rs by antagonists to receptors has not been investigated using [^{11}C]MNPA. We compared dopamine D_2R occupancies by risperidone, an antagonist to receptors, between [^{11}C]MNPA and [^{11}C]raclopride to confirm whether risperidone occupies $D_{2,high}$ and $D_{2,low}$ at almost identical proportions. PET studies were performed on 11 healthy men under resting condition and following oral administration of a single dose of risperidone (0.5–2.0 mg). Striatal receptor occupancy for each radioligand was calculated. The relationship between dose or plasma concentration of risperidone and dopamine D_2R occupancy was calculated. Striatal dopamine D_2R occupancies measured with [^{11}C]MNPA and [^{11}C]raclopride were 22–65% and 24–69%, respectively. In the striatum, ED_{50} values measured with [^{11}C]MNPA and [^{11}C]raclopride were 0.98 and 1.03 mg, respectively. The striatal ED_{50} values as calculated from plasma concentration were 9.15 ng/ml and 8.01 ng/ml, respectively. Almost identical occupancies and ED_{50} values were observed between the two radioligands, indicating that risperidone bound to $D_{2,high}$ and $D_{2,low}$ at almost identical proportions in a dose-dependent manner.

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Key words: Binding potential, dopamine D_2 receptor, receptor occupancy, risperidone, [^{11}C]MNPA.

Introduction

The dopaminergic neurotransmission system is of central interest in schizophrenia, and dopamine D_2 receptors (D_2Rs) are the main target in the treatment with antipsychotics (Seeman *et al.* 1975). Early *in-vitro* studies reported that dopamine D_2Rs have two interconvertible affinity states for endogenous dopamine, referred to as high-affinity ($D_{2,high}$) and low-affinity ($D_{2,low}$) states (De Lean *et al.* 1982; George *et al.* 1985; Richfield *et al.* 1989; Sibley *et al.* 1982). D_2R antagonists are reported to have equal affinity to both states of receptors, while endogenous dopamine preferentially binds to $D_{2,high}$ (Seneca *et al.* 2006). The increased proportion of $D_{2,high}$ is assumed to correlate with dopamine hypersensitivity, implying a relationship with psychotic symptoms observed in psychiatric disorders such as schizophrenia (Seeman *et al.* 2005).

[^{11}C]raclopride, an antagonist radioligand for dopamine D_2Rs, has been used to measure striatal

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dopamine D_2R binding with positron emission tomography (PET). [^{11}C]raclopride is thought to bind to both $D_{2,high}$ and $D_{2,low}$. Recently, several agonist radioligands for dopamine D_2Rs that are thought to bind preferentially to $D_{2,high}$ were developed, i.e. (−)-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). [^{11}C]MNPA has high selectivity and affinity to dopamine D_2Rs (IC_{50}: 1.02 nM, K_i: 0.17 nM; Gao *et al.* 1990; Neumeyer *et al.* 1990) and is believed to bind preferentially to the high-affinity state of dopamine $D_{2/3}Rs$.

The receptor occupancy for endogenous dopamine and dopamine agonists using [^{11}C]MNPA and [^{11}C]raclopride has been studied (Finnema *et al.* 2009; Seneca *et al.* 2006), but occupancy by dopamine antagonists was not fully investigated using [^{11}C]MNPA (Finnema *et al.* 2005). Most antipsychotic drugs are antagonists to dopamine D_2Rs, and the antipsychotic effects of such drugs have been considered to be mediated by blockade of dopamine D_2Rs. The degree of blockade can be evaluated by the occupancy of dopamine D_2Rs using PET. Because dopamine D_2R antagonists bind to both $D_{2,high}$ and $D_{2,low}$, occupancies of dopamine D_2Rs by antagonist antipsychotic drugs measured with [^{11}C]raclopride and [^{11}C]MNPA are expected to be the same.

In the present study, we measured dopamine D_2R occupancies by administration of a single dose of the antipsychotic risperidone as a dopamine D_2R antagonist using both [^{11}C]raclopride and [^{11}C]MNPA in healthy subjects. The dose–occupancy curves and the ED_{50} values in the putamen and caudate nuclei were compared between [^{11}C]raclopride and [^{11}C]MNPA.

Materials and methods

Subjects

Eleven healthy male volunteers (age range 21–39 yr, mean ± S.D. 27 ± 7.4yr) were recruited and written informed consent was obtained. They were healthy according to T1- and T2-weighted magnetic resonance (MR) imaging (Philips Medical Systems, The Netherlands) and blood (blood cell count, aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, gamma glutamyltransferase, creatine kinase, serum sodium, potassium, chlorine, blood urea nitrogen, serum creatinine, blood sugar) and urine screening tests. None had a history of psychiatric or neurological disorders, and they were free of physical disease. They had no history of current or previous drug abuse. The subjects were assigned to one of three groups according to risperidone dose: 0.5 mg (n=5), 1.0 mg (n=3), 2.0 mg (n=3). This study protocol was approved by the Ethics and Radiation Safety Committees of the National Institute of Radiological Science, Chiba, Japan.

PET procedures

All PET data were obtained with a Siemens ECAT Exact HR+ system, which provides 63 sections with an axial field of view of 15.5 cm (Brix *et al.* 1997). The intrinsic spatial resolution was 4.3 mm in-plane and 4.2 mm full-width at half-maximum (FWHM) axially. With a Hanning filter (cut-off frequency 0.4 cycle/pixel), the reconstructed in-plane resolution was 7.5 mm FWHM. Imaging data were acquired in 3D mode. Scatter correction was performed (Watson *et al.* 1996). A thermoplastic head fixation device was used to minimize head movement during PET scanning. A 10-min transmission scan using a ^{68}Ge–^{68}Ge line source was performed for attenuation correction.

PET studies were performed under resting condition (baseline study) and oral administration of risperidone (drug challenge study) on separate days. The average interval between the two studies was 8.3 ± 2.8 d. After intravenous rapid bolus injection of [^{11}C]raclopride, dynamic PET scanning was performed for 60 min. One hour after the end of [^{11}C]raclopride PET measurement, dynamic PET scanning was performed for 90 min after intravenous rapid bolus injection of [^{11}C]MNPA. The frame sequence comprised nine 20-s frames, five 60-s frames, four 120-s frames, eleven 240-s frames and six 300-s frames for [^{11}C]-MNPA, and twelve 20-s frames, sixteen 60-s frames and ten 240-s frames for [^{11}C]raclopride. Injected radioactivities for [^{11}C]MNPA and [^{11}C]raclopride under baseline conditions were 208–248 MBq and 207–241 MBq, respectively, and under drug challenge 214–235 MBq and 182–232 MBq, respectively, for [^{11}C]MNPA and [^{11}C]raclopride. The specific radioactivities for [^{11}C]MNPA and [^{11}C]raclopride under baseline conditions were 148–429 GBq/μmol and 222–736 GBq/μmol, respectively, and under drug challenge 103–411 GBq/μmol and 211–545 GBq/μmol, respectively.

Measurement of plasma concentration of risperidone

In the drug challenge study, 0.5–2 mg risperidone was orally administered 2 h prior to the start of PET scanning with [^{11}C]raclopride. To determine the plasma concentration of risperidone and its active metabolite

(9-hydroxy-risperidone), venous blood samples were drawn at 10–15 min before, and at 1 h, 2 h, 3 h, 4 h, 5.5 and 7 h after its oral administration.

The series of blood samples of each subject were collected in heparinized tubes and centrifuged for 10 min at 3000 rpm. All plasma samples were stored at −20 °C. The plasma concentrations of risperidone and 9-hydroxy-risperidone were determined using the liquid chromatography coupled to mass spectrometry/mass spectrometry (LC–MS/MS) method with a target lower limit of quantification of 0.10 ng/ml (Johnson & Johnson Pharmaceutical Research and Development L. L. C., Belgium). Since risperidone and 9-hydroxy risperidone have similar binding profiles to neuroreceptors (Leysen *et al.* 1994), the sum of the plasma concentrations of risperidone and 9-hydroxy-risperidone was used as the plasma concentration of the antipsychotic drug (Ito *et al.* 2009*a*; Leysen *et al.* 1994).

MR imaging

MR images were acquired with a 1.5-T MR scanner (Philips Medical Systems, The Netherlands). 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).

Data analysis

All data analyses were performed with PMOD 3.0 software (PMOD Technologies, Switzerland). Volumes of interest (VOIs) were defined for the caudate head, putamen, dorsal striatum (caudate head and putamen) and cerebellar cortex. For accurate definition of each VOI, each MR image was co-registered to the corresponding summated PET image. VOIs were drawn manually on each summated PET image with reference to each co-registered MR image. Subsequently, the data of each VOI were applied to each dynamic PET image. In the present study, we applied VOIs obtained from individual PET space according to the previous quantitative study of [^{11}C]MNPA in humans (Otsuka *et al.* 2009). To obtain regional time–activity curves, regional radioactivity was calculated for each frame, corrected for decay, and plotted *vs.* time.

Dopamine D_2R binding was quantified using a three-parameter simplified reference tissue model (Lammertsma & Hume, 1996). The cerebellar cortex was used as reference tissue due to its negligible density of dopamine D_2Rs (Suhara *et al.* 1999). This model allows the estimation of binding potential (BP_{ND}). BP_{ND} was defined as a follows:

$$BP_{ND} = f_{ND} \times B_{max}/K_d, \quad (1)$$

where f_{ND} is the free fraction of radioligand in the non-displaceable tissue compartment, B_{max} the neuroreceptor density, and K_d the dissociation constant of radioligand to receptors (Innis *et al.* 2007). The dopamine D_2R occupancies by risperidone were calculated as follows:

$$\text{occupancy } (\%) = (BP_{base} - BP_{drug})/BP_{base} \times 100, \quad (2)$$

where BP_{base} is the BP_{ND} in the drug-free state, and BP_{drug} the BP_{ND} under administration of risperidone (Takano *et al.* 2004, 2006; Yasuno *et al.* 2001). The relationship between the dose or the plasma concentration of antipsychotic drug and dopamine D_2R occupancy can be expressed as follows:

$$\text{occupancy } (\%) = D/(D + ED_{50}) \times 100, \quad (3)$$

where D is the dose or the sum of the plasma concentrations of risperidone and 9-hydroxy-risperidone (Nyberg *et al.* 1999), and ED_{50} is the plasma concentration required to induce 50% occupancy. The maximal occupancy of D_2Rs was restricted to 100% to reflect the expected maximal occupancy. All the regression analyses were performed using Kaleida Graph 4.01 software (Synergy Software, USA). The regression line was fitted according to equation (3), with calculation of the ED_{50} values and regression coefficients for each radioligand. The mean plasma concentration values at the start and end of PET scanning were used for the calculation of ED_{50} values.

Results

Baseline BP_{ND} values of [^{11}C]MNPA were 0.84 ± 0.1 in the striatum, 0.67 ± 0.1 in the caudate nuclei and 0.93 ± 0.1 in the putamen. Baseline BP_{ND} values of [^{11}C]raclopride were 3.17 ± 0.4 in the striatum, 2.83 ± 0.4 in the caudate nuclei and 3.37 ± 0.4 in the putamen (Table 1).

The dopamine D_2R occupancies using [^{11}C]MNPA ranged from 22% to 66% for the striatum, 34–78% for the caudate nuclei, and 17–67% for the putamen in the three dose groups. Receptor occupancies using [^{11}C]raclopride ranged from 18% to 70% for the striatum, 22–72% for the caudate nuclei, and 17–69% for the putamen.

Significant positive correlations of dopamine D_2R occupancies between [^{11}C]raclopride and [^{11}C]MNPA were observed in the caudate nuclei and putamen (caudate nuclei: $r = 0.65$, $p = 0.04$; putamen: $r = 0.86$, $p = 0.01$).

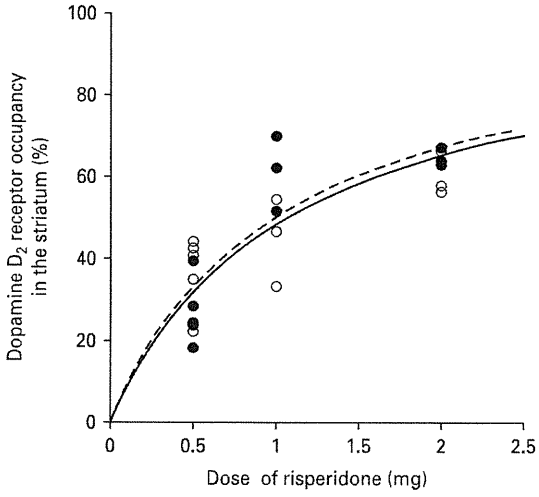

Fig. 1. The relationship between dopamine D_2 receptor occupancy in the striatum and risperidone dose. Open symbols (○) and solid curve (——) indicate receptor occupancy with [^{11}C]MNPA and its fitting curve, and solid symbols (●) and dashed curve (– – –) indicate receptor occupancy with [^{11}C]raclopride and its fitting curve, respectively. ED_{50} in the striatum was 1.08 and 1.00 mg for [^{11}C]MNPA and [^{11}C]raclopride, respectively.

The relationship between dopamine D_2R occupancy in the striatum and risperidone dose are shown in Fig. 1. These relationships are well described by equation (3) (striatum: $r=0.86$ and $r=0.68$; caudate nuclei: $r=0.86$ and $r=0.62$; putamen: $r=0.85$ and $r=0.78$ for [^{11}C]raclopride and [^{11}C]MNPA, respectively). The ED_{50} values calculated from [^{11}C]raclopride and [^{11}C]MNPA, respectively, were 1.00 mg ($r=0.86$) and 1.08 mg ($r=0.68$) in the striatum, 0.88 mg ($r=0.86$) and 0.80 mg ($r=0.62$) in the caudate nuclei, and 1.07 ($r=0.85$) mg and 1.00 mg ($r=0.78$) in the putamen.

The sum of plasma concentrations of risperidone and 9-hydroxy-risperidone during [^{11}C]raclopride and [^{11}C]MNPA studies, averaged between the start and end of each scanning, was 9.37 ± 4.89 ng/ml and 7.74 ± 3.91 ng/ml (mean ± S.D.), respectively. The relationship between dopamine D_2R occupancy in the striatum and the sum of plasma concentrations of risperidone and 9-hydroxy-risperidone are shown in Fig. 2. The ED_{50} values from the plasma concentration–occupancy curves with [^{11}C]raclopride and [^{11}C]MNPA, respectively, were 9.15 ng/ml ($r=0.91$) and 8.32 ng/ml ($r=0.36$) in the striatum, 8.00 ng/ml ($r=0.88$) and 6.12 ng/ml ($r=0.53$) in the caudate nuclei, and 9.75 ng/ml ($r=0.92$) and 7.70 ng/ml ($r=0.74$) in the putamen.

Table 1. The range of dopamine D_2 receptor binding potential values of [^{11}C]MNPA and [^{11}C]raclopride by dose of risperidone

	Baseline	0.5 mg	1.0 mg	2.0 mg
[^{11}C]MNPA				
Caudate head	0.40–0.81	0.32–0.49	0.21–0.33	0.16–0.39
Putamen	0.70–1.22	0.48–0.79	0.45–0.52	0.28–0.44
Dorsal striatum	0.84–0.13	0.44–0.65	0.40–0.40	0.26–0.41
[^{11}C]raclopride				
Caudate head	2.19–3.36	1.65–2.28	0.66–1.22	0.84–1.1
Putamen	2.56–4.11	2.37–2.86	1.00–1.44	1.03–1.44
Dorsal striatum	2.55–3.84	2.15–2.65	0.87–1.37	0.99–1.28

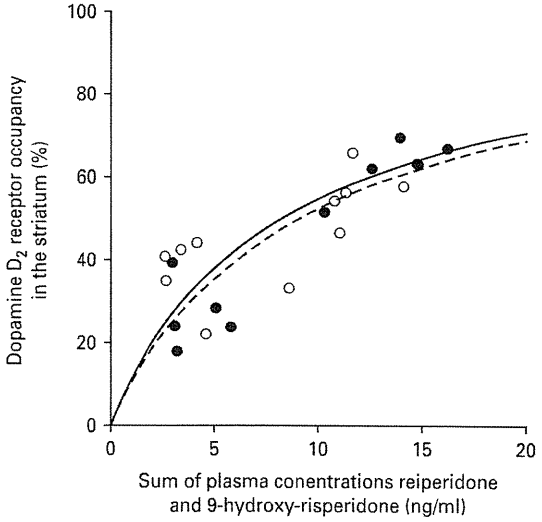

Fig. 2. The relationship between dopamine D_2 receptor occupancy in the striatum and the sum of plasma concentrations of risperidone and 9-hydroxy-risperidone. Open symbols (●) and solid curve (——) indicate receptor occupancy with [^{11}C]MNPA and its fitting curve, and solid symbols (○) and dashed curve (– – –) indicate receptor occupancy with [^{11}C]raclopride and its fitting curve, respectively. ED_{50} in the striatum was 8.32 ng/ml and 9.15 ng/ml for [^{11}C]MNPA and [^{11}C]raclopride, respectively.

Discussion

The present study demonstrated dopamine D_2R occupancies by risperidone measured with both [^{11}C]MNPA and [^{11}C]raclopride, i.e. the effects of risperidone on $D_{2,high}$ and $D_{2,low}$. The dopamine D_2R occupancies and ED_{50} values measured by [^{11}C]raclopride under risperidone administration were in agreement with most previous studies (Kapur *et al.* 1999; Nyberg *et al.* 1999;

Yasuno *et al.* 2001). In addition, the dose–occupancy curves and ED_{50} values with [^{11}C]MNPA were almost identical to those of [^{11}C]raclopride. Therefore, these results indicate that risperidone binds to both $D_{2,high}$ and $D_{2,low}$ at almost identical proportions. This is in accord with the report that risperidone is an antagonist for dopamine D_2Rs (Leysen *et al.* 1994) and has equal binding affinity to both $D_{2,high}$ and $D_{2,low}$ receptors (Seneca *et al.* 2006).

The [^{11}C]MNPA PET studies began 2 h after the start of the [^{11}C]raclopride PET studies. The sums of the plasma concentrations of risperidone and 9-hydroxyrisperidone were thus slightly higher during [^{11}C]-raclopride studies (9.37 ± 4.89 ng/ml) than during [^{11}C]MNPA studies (7.74 ± 3.91 ng/ml), raising the possibility that the occupancy of [^{11}C]MNPA by dose could be underestimated in the present study. However, the ED_{50} value calculated by plasma concentrations showed almost identical values with the ED_{50} value by dose.

With regard to the *in-vivo* proportion of $D_{2,high}$ to $D_{2,low}$ in dopamine D_2Rs, several arguments have been put forward. Seneca *et al.* (2006) demonstrated that the sensitivity of striatal uptake of [^{11}C]MNPA is greater than that of [^{11}C]raclopride in response to amphetamine-induced dopamine release. It was indicated that [^{11}C]MNPA BP_{ND} was ~50% more sensitive to change than [^{11}C]raclopride to amphetamine-induced increase in synaptic dopamine. These authors suggested that 61% of the D_2Rs are configured in the high-affinity state (10% occupied by dopamine at baseline, 23% synaptic and 28% extrasynaptic) (Seneca *et al.* 2006). Narendran *et al.* (2010) also observed greater [^{11}C]NPA BP_{ND} reduction in response to amphetamine-induced dopamine release compared to [^{11}C]raclopride in healthy humans (Narendran *et al.* 2010). Moreover, Seeman (2009) demonstrated in their *ex-vivo* study that the dopamine agonist NPA inhibited [^{3}H]PHNO binding more than [^{3}H]raclopride did in amphetamine-sensitized rodents, indicating *in-vivo* competition of NPA to $D_{2,high}$ (Seeman, 2009). These experimental observations might provide psychopharmacological basis of dopamine D_2R hypersensitivity in patients with schizophrenia, i.e. use of psychostimulants worsens psychotic symptoms in patients with schizophrenia (Curran *et al.* 2004; Lieberman *et al.* 1987).

In contrast, Finnema *et al.* (2009) did not find distinguishable ID_{50} and K_i values of apomorphine for dopamine D_2Rs when measured with [^{11}C]MNPA and [^{11}C]raclopride (Finnema *et al.* 2009). *In-vitro* binding studies demonstrated that apomorphine binds to $D_{2,high}$ with 30- to 60-fold higher affinity compared to $D_{2,low}$ (De Lean *et al.* 1982; Sibley *et al.* 1982), and they suggest that almost all dopamine D_2Rs are in a high-affinity state at *in-vivo* condition. These findings might be the case for dopamine D_2 antagonists, partial as well as full agonists. Peng *et al.* (2010) found no significant differences of dopamine D_2R occupancy with [^{3}H]raclopride, [^{3}H]MNPA and [^{3}H]PHNO by using quinpirole (full agonist), aripiprazole (partial agonist), and halopridol (antagonist) (Peng *et al.* 2010). A similar finding was observed in a previous *ex-vivo* study with [^{11}C]PHNO (McCormick *et al.* 2008). In clinical investigations, there is no clear evidence for the $D_{2,high}$ to $D_{2,low}$ proportion in dopamine D_2Rs. While ~20% differences of dopamine D_2R occupancy between [^{11}C]MNPA and [^{11}C]raclopride were observed in long-term treated patients with schizophrenia by Graff-Guerrero *et al.* (2009*a*), they found no significant differences of [^{11}C]PHNO binding in patients with schizophrenia-spectrum disorder amidst an acute psychotic episode group compared to control group (Graff-Guerrero *et al.* 2009*b*). In any case, further *in-vivo* investigations would be needed to elucidate the plausible ratio of $D_{2,high}$ to $D_{2,low}$. In particular, an *in-vivo* human receptor occupancy PET study using dopamine full agonists and/or partial agonists (e.g. an antipsychotic agent aripiprazole; Mamo *et al.* 2007) might help elucidate the $D_{2,high}$ and $D_{2,low}$ proportions in dopamine D_2Rs.

It is reported that a part of dopamine D_2R agonist radioligands shows higher affinity for dopamine D_3Rs. [^{11}C]PHNO is assumed to have ~50-fold higher selectivity to dopamine D_3Rs than to dopamine D_2Rs (Freedman *et al.* 1994; Narendran *et al.* 2006). This indicates that the observed differences of K_i value between [^{11}C]PHNO and [^{11}C]raclopride could be interpreted as the difference between D_2 and D_3 binding property. Meanwhile, a previous *ex-vivo* study showed that [^{11}C]MNPA has almost identical affinity to dopamine D_2 and D_3 receptors [2.21 nM (D_2) and 2.02 nM (D_3)] (Skinbjerg *et al.* 2009).

The dopamine D_2 antagonist administered could bind to dopamine D_2 autoreceptors as well as to post-synaptic dopamine D_2Rs. Blockade of dopamine D_2 autoreceptors could regulate dopaminergic neurotransmission in the present study. A recent human PET study on acute administration of the dopamine D_2 antagonist (risperidone) showed a stabilizing effect of risperidone on dopamine synthesis (Ito *et al.* 2009*b*). That study raised the possibility that acute administration of dopamine D_2R antagonist affects post-synaptic receptors by regulating the dopaminergic neurotransmission. Further study will be needed to elucidate the effect on dopamine D_2 autoreceptors.

In conclusion, dopamine D_2R occupancies by administration of risperidone, a dopamine D_2R antagonist, were measured in healthy subjects using both [^{11}C]raclopride and [^{11}C]MNPA. Almost identical occupancies were observed with the two radioligands, implying that risperidone binds to $D_{2,high}$ and $D_{2,low}$ at almost identical proportions in a dose-dependent manner. The present results of dose–occupancy curves with both radioligands also suggest that the same therapeutic window could be applicable to $D_{2,high}$ and for dopamine D_2Rs including both $D_{2,high}$ and $D_{2,low}$.

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Statement of Interest

None.

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Behavioral/Systems/Cognitive

Relation between Presynaptic and Postsynaptic Dopaminergic Functions Measured by Positron Emission Tomography: Implication of Dopaminergic Tone

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Both presynaptic and postsynaptic dopaminergic functions can be estimated by positron emission tomography (PET). While both presynaptic and postsynaptic dopaminergic functions would be regulated by corresponding genes and related to personality traits, the relation between presynaptic and postsynaptic functions in terms of interindividual variation has hardly been investigated. In the present study, both striatal dopamine D_2 receptor binding and endogenous dopamine synthesis rate were measured in the same healthy subjects using PET with [^{11}C]raclopride and L-[β-^{11}C]DOPA, respectively, and these two parameters were compared. Two PET studies with [^{11}C]raclopride and L-[β-^{11}C]DOPA were performed sequentially at rest condition on 14 healthy men. For [^{11}C]raclopride PET, the binding potential was calculated by the reference tissue model method. For L-[β-^{11}C]DOPA PET, the endogenous dopamine synthesis rate was estimated by graphical analysis. A significant negative correlation was observed between the binding potential of dopamine D_2 receptors and endogenous dopamine synthesis rate ($r = -0.66, p < 0.05$). Although the interindividual variation of binding potential of [^{11}C]raclopride would be due to both the interindividual difference in the receptor density and that in the concentration of endogenous dopamine in the synaptic cleft, the negative correlation between parameters for both presynaptic and postsynaptic functions might indicate a compensative relation between the two functions.

Introduction

The central dopaminergic system is of interest in the pathophysiology of schizophrenia and other neuropsychiatric disorders. Both presynaptic and postsynaptic dopaminergic functions can be estimated by positron emission tomography (PET) using several radiotracers. The binding of dopamine receptors representing postsynaptic functions in the striatum can be measured for each of D_1 and D_2 subtypes using [^{11}C]SCH23390 (Halldin et al., 1986; Farde et al., 1987) and [^{11}C]raclopride (Farde et al., 1985; Köhler et al., 1985; Ito et al., 1998), respectively. The relative activity of cerebral aromatic L-amino acid decarboxylase (AADC) representing endogenous dopamine synthesis rate measured by 6-[^{18}F]fluoro-L-DOPA (Gjedde, 1988; Gjedde et al., 1991; Huang et al., 1991) and L-[β-^{11}C]DOPA (Hartvig et al., 1991; Tedroff et al., 1992) can indicate the presynaptic dopaminergic function. Using PET, interindividual variations in both presynaptic and postsynaptic dopaminergic functions in the striatum of normal living human brain were observed (Ito et al., 2008).

It has been reported that the dopamine D_2 receptor density was related to polymorphisms in the dopamine D_2 receptor gene in humans (Jönsson et al., 1999). Genotypes of human monoamine-synthesizing enzymes, e.g., tyrosine hydroxylase (TH) and AADC, were also determined (Nagatsu, 1991), and TH genotypes were reported to participate in the regulation of monoamine turnover in the CNS (Jönsson et al., 1996). It has been reported that dopamine D_2 receptor binding and the endogenous dopamine synthesis rate measured by PET were correlated with personality traits (Farde et al., 1997; Breier et al., 1998; Laakso et al., 2003).

While both presynaptic and postsynaptic dopaminergic functions would be regulated by corresponding genes and related to personality traits, the relation between presynaptic and postsynaptic functions in interindividual variation has hardly been investigated. In the present study, both striatal dopamine D_2 receptor binding and endogenous dopamine synthesis rate were measured in the same healthy subjects using PET with [^{11}C]raclopride and L-[β-^{11}C]DOPA, respectively, and these two parameters were compared.

Materials and Methods

Subjects. The study was approved by the Ethics and Radiation Safety Committees of the National Institute of Radiological Sciences, Chiba, Japan. Fourteen healthy men [20–29 years of age, 23.8 ± 2.9 years

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(mean ± SD)] were recruited and written informed consent was obtained. The subjects were free of somatic, neurological, or psychiatric disorders on the basis of their medical history and magnetic resonance (MR) imaging of the brain. They had no history of current or previous drug abuse.

PET procedures. All PET studies were performed with a Siemens ECAT Exact HR+ system, which provides 63 sections with an axial field of view of 15.5 cm (Brix et al., 1997). The intrinsic spatial resolution was 4.3 mm in-plane and 4.2 mm full-width at half-maximum (FWHM) axially. With a Hanning filter (cutoff frequency: 0.4 cycle/pixel), the reconstructed in-plane resolution was 7.5 mm FWHM. Data were acquired in three-dimensional mode. Scatter was corrected (Watson et al., 1996). A 10 min transmission scan using a ^{68}Ge–^{68}Ga line source was performed for correction of attenuation. A head fixation device with thermoplastic attachments for individual fit minimized head movement during PET measurements. Two PET studies with [^{11}C]raclopride and L-[β-^{11}C]DOPA were performed sequentially at rest condition. After intravenous rapid bolus injection of [^{11}C]raclopride, dynamic PET scanning was performed for 60 min. After 1 h from the end of [^{11}C]raclopride PET measurement, dynamic PET scanning was performed for 89 min after intravenous rapid bolus injection of L-[β-^{11}C]DOPA. In one subject, the L-[β-^{11}C]DOPA PET measurement was performed 5 d after the [^{11}C]raclopride PET measurement. The frame sequence consisted of twelve 20 s frames, sixteen 1 min frames, and ten 4 min frames for [^{11}C]raclopride, and seven 1 min frames, five 2 min frames, four 3 min frames, and twelve 5 min frames for L-[β-^{11}C]DOPA. The radioactivity injected was 194–230 MBq and 342–395 MBq for [^{11}C]raclopride and L-[β-^{11}C]DOPA, respectively. The specific radioactivity was 168–517 GBq/μmol and 26–88 GBq/μmol for [^{11}C]raclopride and L-[β-^{11}C]DOPA, respectively. A venous blood sample was taken at the beginning of L-[β-^{11}C]DOPA PET scanning for measurement of natural neutral amino acid (NAA) concentration in plasma. NAA concentration was measured by HPLC (L-8500 amino acid analyzer system, Hitachi). The amino acids are phenylalanine, tryptophan, leucine, methionine, isoleucine, tyrosine, histidine, valine, and threonine, which are transported via the same carrier at the blood–brain barrier as L-DOPA (Sugaya et al., 2001). A weighted sum of the NAAs, which was the L-DOPA-corresponding concentration of the nine NAAs for the carrier system, was calculated according to our previous study (Ito et al., 2006).

All MR imaging studies were performed with a 1.5 tesla MR scanner (Philips Medical Systems). Three-dimensional 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).

Regions of interest. All MR images were coregistered to the PET images with the statistical parametric mapping (SPM2) system (Friston et al., 1990). Regions of interest (ROIs) were drawn on coregistered MR images and transferred to the PET images. ROIs were defined for the cerebellar cortex, caudate head, putamen, and occipital cortex. Each ROI was drawn in three adjacent sections and data were pooled to obtain the average radioactivity concentration for the whole volume of interest. The radioactivity concentration of the striatum was calculated as the average of values of caudate head and putamen. To obtain regional time–activity curves, regional radioactivity was calculated for each frame, corrected for decay, and plotted versus time. No software correction for head movement during PET measurements was applied to the dynamic PET images.

Calculation of dopamine D_2 receptor binding. For PET studies with [^{11}C]raclopride, the binding potential (BP_{ND}) was calculated by the reference tissue model method (Lammertsma and Hume, 1996; Lammertsma et al., 1996). With this method, the time–activity curve in the brain region is described by that in the reference region with no specific binding, assuming that both regions have the same level of nondisplaceable radioligand binding:

$$C_i(t) = R_1 \cdot C_r(t) + \{k_2 - R_1 \cdot k_2/(1 + BP_{ND})\} \cdot C_r(t) \otimes \exp\{-k_2 \cdot t/(1 + BP_{ND})\},$$

where C_i is the radioactivity concentration in a brain region; C_r is the radioactivity concentration in the reference region; R_1 is the ratio of K_1/K_1' (K_1, influx rate constant for the brain region; K_1', influx rate constant for the reference region); k_2 is the efflux rate constant for the brain region; and 196 denotes the convolution integral. BP_{ND} is defined as $BP_{ND} = f_{ND}B_{avail}/K_D$, where B_{avail} is the receptor density available to bind radiotracer *in vivo* and K_D is the dissociation constant indicating affinity of radiotracer to receptors (Innis et al., 2007). f_{ND} is the free fraction of radiotracer in the compartment of nondisplaceable binding. In this analysis, three parameters (BP_{ND}, R_1, and k_2) were estimated by nonlinear least-squares curve fitting. The cerebellum was used as reference region.

Calculation of dopamine synthesis rate. The uptake rate constant for L-[β-^{11}C]DOPA indicating the dopamine synthesis rate was estimated using graphical analysis (Patlak and Blasberg, 1985; Gjedde, 1988; Ito et al., 2006), which allows for the calculation of the uptake rate constant (k_{ref}) using time–activity data in a reference brain region with no irreversible binding. k_{ref} values can be estimated by using simple linear least-squares fitting as follows:

$$\frac{C_i(t)}{C_i'(t)} = k_{ref} \cdot \frac{\int_0^t C_i'(\tau)d\tau}{C_i'(\tau)} + F \quad t > t^*,$$

where C_i and C_i' are the total radioactivity concentrations in a brain region with and without irreversible binding, respectively, and t^* is the equilibrium time of the compartment for unchanged radiotracer in brain tissue. Plotting $C_i(t)/C_i'(t)$ versus $\int_0^t C_i'(\tau)d\tau/C_i'(t)$, after time t^*, yields a straight line with the slope k_{ref} and intercept F. In the present study, the occipital cortex was used as a reference region with no irreversible binding, because this region is known to have the lowest dopamine concentration (Brown et al., 1979) and lowest AADC activity (Lloyd and Hornykiewicz, 1972). The equilibrium time t^* was set to be 29 min, and data plots of 29–89 min were used for linear least-squares fitting (Ito et al., 2006, 2007).

Table 1. The binding potential (BP_{ND}) of [^{11}C]raclopride studies and dopamine synthesis rate k_{ref} of L-[β-^{11}C]DOPA studies

	Caudate head	Putamen	Striatum
BP_{ND}	2.66 ± 0.23	3.40 ± 0.29	3.15 ± 0.26
k_{ref} (min^{-1})	0.0118 ± 0.0019	0.0135 ± 0.0016	0.0129 ± 0.0015

Values are mean ± SD.

Results

The BP_{ND} of the [^{11}C]raclopride studies and dopamine synthesis rate k_{ref} of the L-[β-^{11}C]DOPA studies are shown in Table 1. Weighted sum of the NAAs in plasma was 1262 ± 186 nmol/ml (mean ± SD) for L-[β-^{11}C]DOPA studies. No significant correlation was observed between weighted sum of the NAAs and the dopamine synthesis rate k_{ref} of L-[β-^{11}C]DOPA.

Relations between BP_{ND} and k_{ref} in the striatum are shown in Figure 1. A significant negative correlation was observed between BP_{ND} and k_{ref} ($y = -0.0038x + 0.025$, x: BP_{ND}, y: k_{ref}, $r = -0.66$, $p < 0.05$). A trend of negative correlation was observed between BP_{ND} and k_{ref} in the putamen ($y = -0.0028x + 0.023$, x: BP_{ND}, y: k_{ref}, $r = -0.51$, $p < 0.1$). No significant correlation was observed in the caudate head ($y = -0.0028x + 0.019$, x: BP_{ND}, y: k_{ref}, $r = -0.35$). Typical images of BP_{ND} of subjects with low and high BP_{ND} and corresponding images indicating dopamine synthesis rate are shown in Figure 2.

Discussion

To our knowledge, there are only a few reports concerning the relation between striatal dopamine D_2 receptor binding and endogenous dopamine synthesis ability in the living human brain, and no significant correlation was observed (Heinz et al., 2005; Kienast et al., 2008). Although the coefficient of variation of

BP_{ND} values for [^{11}C]raclopride studies and k_{ref} values for L-[β-^{11}C]DOPA studies were relatively small (8% for BP_{ND} and 12% for k_{ref} in the striatum), a significant negative correlation was observed between the parameters for both presynaptic and postsynaptic functions. One possible reason for this is the competition between [^{11}C]raclopride and endogenous dopamine at dopamine D_2 receptor sites (Reeves et al., 2007). When the endogenous dopamine synthesis rate measured by PET is either small or large, the concentration of endogenous dopamine in the synaptic cleft must be either low or high, and therefore [^{11}C]raclopride binding to dopamine D_2 receptors might become large or small due to competition with the endogenous dopamine, respectively. Recently, the increases in L-[β-^{11}C]DOPA metabolites, [^{11}C]3,4-dihydroxyphenylacetic acid ([^{11}C]DOPAC) and [^{11}C]homovanillic acid ([^{11}C]HVA) in the extracellular space of the rat striatum after intravenous infusion of L-[β-^{11}C]DOPA was reported using microdialysis, indicating that the endogenous dopamine synthesis rate measured by PET can reflect the concentration of endogenous dopamine in the synaptic cleft (Okada et al., 2011). While interindividual variations of BP_{ND} ($f_{ND}B_{avail}/K_D$) of [^{11}C]raclopride were observed in normal human subjects (Ito et al., 2008), it has been reported that interindividual difference in B_{avail} was significant, but not that in K_D, in human [^{11}C]raclopride PET studies (Farde et al., 1995). This indicates that the interindividual variation of BP_{ND} would be mainly due to the interindividual difference in B_{avail} rather than K_D. B_{avail} is the receptor density available to bind radiotracer *in vivo*, and it might be smaller than the receptor density *in vitro* assays due to the competition with endogenous dopamine (Innis et al., 2007). Thus, the interindividual variation of BP_{ND} would be due to both the interindividual difference in the receptor density and that in the concentration of endogenous dopamine in the synaptic cleft.

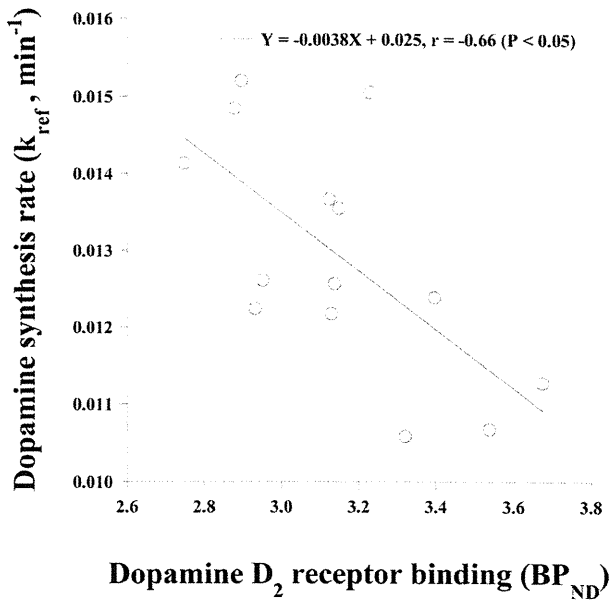

Figure 1. Relation between BP_{ND} of [^{11}C]raclopride studies and k_{ref} of L-[β-^{11}C]DOPA studies in the striatum.

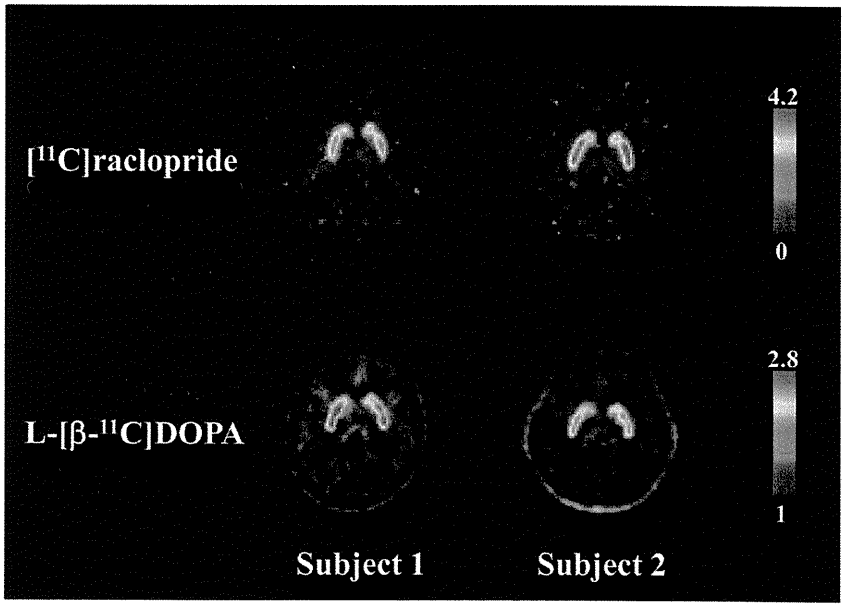

Figure 2. Typical images of BP_{ND} of [^{11}C]raclopride studies for subjects with low and high BP_{ND} (subjects 1 and 2, respectively) and corresponding images indicating dopamine synthesis rate calculated as the ratio of time-integrated radioactivities from 29 to 89 min of L-[β-^{11}C]DOPA studies between brain regions and the occipital cortex (Ito et al., 2007).

Another possible reason for a significant negative correlation between presynaptic and postsynaptic dopaminergic functions might be a compensative relation between the two functions. The mechanism for the regulation of dopamine release from presynapse has been explained by both phasic and tonic dopamine release (Grace, 1991). Phasic dopamine release would be caused by firing of dopaminergic neuron, and tonic dopamine release would set the background level of dopamine receptor stimulation. If the endogenous dopamine synthesis ability at rest condition measured by L-[β-^{11}C]DOPA PET can indicate tonic dopamine release, the background level of dopamine receptor stimulation by tonic dopamine release might be compensatively related to the dopamine D_2 receptor density, indicating that the tone of dopaminergic neurotransmission might not be so different between subjects. In addition, the TaqIA1 allele of dopamine D_2 receptor gene has been reported to be associated with a low density of dopamine D_2 receptors (Jönsson et al., 1999) and with increased striatal activity of AADC in healthy human subjects (Laakso et al., 2005), supporting our present results. They attempted to explain these findings by a lower dopamine D_2 receptor expression leading to decreased autoreceptor function, and therefore increased dopamine synthesis. However, further studies, including animal studies *in vitro* and *in vivo*, will be required to explain the negative correlation between presynaptic and postsynaptic dopaminergic functions in the present study.

Increased striatal dopamine synthesis rate in neuroleptic-naive or -free patients with schizophrenia has been reported using PET with L-[β-^{11}C]DOPA (Lindström et al., 1999; Nozaki et al., 2009) or 6-[^{18}F]fluoro-L-DOPA (Hietala et al., 1995). On the other hand, no significant change in striatal dopamine D_2 receptor density in patients with schizophrenia has been reported using PET with [^{11}C]raclopride (Farde et al., 1990). It might be

valuable to investigate the relation between presynaptic and postsynaptic dopaminergic functions in patients with schizophrenia whether such compensative relation in the striatum was evident or disrupted in patients.

It has been reported that the dopamine D_2 receptor density measured by PET with [^{11}C]raclopride was significantly correlated with a certain personality trait, the detachment score of Karolinska Scales of Personality (Farde et al., 1997; Breier et al., 1998), while no significant correlation was observed between the endogenous dopamine synthesis rate measured by PET with 6-[^{18}F]fluoro-L-DOPA and the detachment score (Laakso et al., 2003). On the other hand, endogenous dopamine synthesis was significantly correlated with anxiety-related personality scales of Karolinska Scales of Personality (Laakso et al., 2003). These findings indicate that dopamine D_2 receptor density and the endogenous dopamine synthesis rate might be related to personality traits independently, although a significant negative correlation was observed between parameters for both presynaptic and postsynaptic functions in the present study. The relations between personality traits and presynaptic or postsynaptic dopaminergic functions should be further investigated in large series of subjects.

NAAs are transported by the neutral amino acid carrier system in the blood–brain barrier in a competitive fashion (Oldendorf, 1971; Pardridge, 1977; Ito et al., 1995), and the competitive transport of L-DOPA with NAAs at the blood–brain barrier has been revealed (Ito et al., 2006). We have previously reported a significant negative correlation between the weighted sum of the NAAs and the overall uptake rate constant of L-[β-^{11}C]DOPA calculated using the arterial input function (Ito et al., 2006). The overall uptake rate constant calculated using the arterial input function includes the influx rate constant at the blood–brain barrier, and therefore negatively correlated with the weighted sum of the NAAs due to the competitive transport at the blood–brain barrier. In the present study, no significant correlation was observed between the weighted sum of NAAs in plasma and the dopamine synthesis rate k_{ref} of L-[β-^{11}C]DOPA. Because k_{ref} is calculated using time–activity data in a reference brain region, not in arterial plasma, this parameter does not reflect the influx rate constant at the blood–brain barrier (Ito et al., 2006, 2007). Thus, the dopamine synthesis rate k_{ref} of L-[β-^{11}C]DOPA is independent of the NAA concentration.

In conclusion, a significant negative correlation was observed between parameters for both presynaptic and postsynaptic dopaminergic functions in the striatum of normal human subjects. Although the interindividual variation of BP_{ND} would be due to both the interindividual difference in the receptor density and that in the concentration of endogenous dopamine in the synaptic cleft, this relation might indicate a compensative relation between the two functions. Further studies to elucidate the interindividual variation in dopaminergic neurotransmission tone of neuropsychiatric disorders will be required.

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Serotonergic Neurotransmission in the Living Human Brain: A Positron Emission Tomography Study Using [^{11}C]DASB and [^{11}C]WAY100635 in Young Healthy Men

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ABSTRACT The central serotonergic (5-HT) system is closely involved in regulating various mental functions such as mood and emotion. In this system, the serotonin transporter (5-HTT) and the 5-HT_{1A} receptor play important roles in the pathophysiology and treatment of mood and anxiety disorders. However, only a few integrated databases have considered the intraindividual relationship between pre- and postsynaptic serotonergic transmission. In the present study, we constructed a database of 5-HTT and 5-HT_{1A} receptors using positron emission tomography (PET) with [^{11}C]DASB and [^{11}C]WAY100635, respectively. Seventeen healthy young men participated in this study. After anatomic standardization of original images, BP_{ND} was calculated on a voxel-by-voxel basis using reference tissue methods. The highest binding to 5-HTT was observed in the dorsal raphe nucleus, striatum, and thalamus; moderate binding, in the insula and cingulate cortex; and very low binding, in the cerebral neocortex. In contrast, the highest binding to 5-HT_{1A} receptors was seen in the hippocampal regions, insula, neocortical regions, and dorsal raphe nucleus, and very low binding was found in the thalamus and basal ganglia. These distribution patterns were in agreement with those reported in human postmortem studies and previous PET investigations. In addition, exploratory analysis indicated significant negative correlations between the BP_{ND} values with both radiotracers in certain regions of the brain, such as the cingulate, insula, and frontal, temporal and parietal cortices (Pearson's correlation, $P < 0.05$). These databases facilitate the understanding of the regional distribution of serotonergic neurotransmission function in the living human brain and the pathophysiology of various neuropsychiatric disorders. **Synapse 65:624–633, 2011.**

INTRODUCTION

The central serotonergic (5-hydroxytryptamine, 5-HT) system is one of the major neurotransmitters in the brain and is intricately involved in the regulation of various mental functions such as emotion and cognition. Therefore, dysregulation of this system has been implicated in a variety of neuropsychiatric conditions including mood and anxiety disorders, schizophrenia, etc. (Cooper et al., 2002; Nestler et al., 2008).

To date, seven distinct families (5-HT_1-5-HT_7) and at least 16 subtypes of 5-HT receptors and a serotonin transporter (5-HTT) have been identified (Hoyer et al., 2002; Kitson, 2007). Each subtype has distinct functions, and their distributions in the brain are heterogeneous. Many attempts have been made to develop suita-

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ble radiotracers to visualize and quantify central serotonergic transmission in the living human brain by using PET and single photon emission computed tomography (SPECT) (Brust et al., 2006; Kumar and Mann, 2007; Meyer, 2007; Moresco et al., 2006). In the serotonergic system, only 5-HTT, 5-HT_{1A}, 5-HT_{2A}, and 5-HT_4 receptors and 5-HT synthesis have been visualized in living human brain because of the limited availability of radioligands for humans, although a few have been under development (Diksic and Young, 2001; Lundquist et al., 2006; Marner et al., 2009; Moresco et al., 2006).

The 5-HTT is responsible for the reuptake of 5-HT from the synaptic cleft and the modulation of its extracellular concentration. Therefore, 5-HTT is the primary target molecule of selective serotonin reuptake inhibitors (SSRIs), the most commonly used antidepressants today (Brust et al., 2006; Meyer, 2007). In contrast, the 5-HT_{1A} receptor is localized as a somatodendritic autoreceptor in the dorsal raphe nuclei and as a postsynaptic receptor in the cortical and limbic serotonin terminal fields throughout the brain. The 5-HT_{1A} autoreceptor in the dorsal raphe controls the firing of general 5-HT transmission. In addition, it is the target of 5-HT_{1A} agonists such as buspirone and tandospirone, which have anxiolytic properties. Therefore, both serotonergic functions have been reported to be closely involved in various neuropsychiatric conditions including mood and anxiety disorders, schizophrenia, etc. [see reviews, e.g., (Brust et al., 2006; Drevets et al., 2007; Kumar and Mann, 2007; Savitz et al., 2009; Stockmeier, 2003)].

Recently, we created a normal database for the dopaminergic neurotransmission system by studying healthy volunteers and using five different PET radiotracers, although different cohorts were used for each tracer (Ito et al., 2008). We discussed anatomic localization of receptors and transporters compared with the results of human postmortem studies using autoradiography. In contrast, there has been little integrated database information regarding normal serotonergic transmission, particularly using multiple radioligands to examine both pre- and postsynaptic serotonergic function in the same individuals (Lundberg et al., 2007). In the present study, we conducted two PET scans for each subject using [^{11}C]DASB and [^{11}C]WAY100635 for 5-HTT and 5-HT_{1A} receptor imaging, respectively. We generated parametric images with anatomic standardization of 5-HTT and 5-HT_{1A} binding and subsequently examined the neuroanatomical localization for inter- and intrasubject comparisons.

MATERIALS AND METHODS

Subjects

This study was approved by the Ethics and Radiation Safety Committee of the National Institute of Radiological Sciences, Chiba, Japan. Seventeen healthy men aged 24.4 ± 5.9 (mean ± standard deviation, (SD); range 20–40) years were recruited. All subjects gave their informed written consent before participating in the study. Based on their medical history and an unstructured examination by psychiatrists, the subjects were found to be free of somatic, neurological, and psychiatric disorders. All participants were nonsmokers. The participants had no history of current or previous drug abuse and had not taken any drugs within 2 weeks before the PET studies. The duration between the two PET scans was 7.2 ± 8.7 (range, 0–28) days.

All participants underwent magnetic resonance imaging (MRI) of the brain with a 1.5T MR scanner (Philips Medical Systems, Best, The Netherlands). Three-dimensional volumetric acquisition of a T1-weighted gradient echo sequence produced a gapless series of thin transverse sections (TE: 9.2 msec; TR: 21 msec; flip angle: 30°; field of view: 256 mm; acquisition matrix: 256 × 256; slice thickness: 1 mm). The MRI results revealed no apparent structural abnormalities.

PET procedure

All PET studies were performed with a Siemens ECAT Exact HR+ system (CTI-Siemens, Knoxville, TN), which provides 63 sections with an axial field of view of 15.5 cm. The intrinsic spatial resolution was 4.3 mm in-plane and 4.2 mm full-width at half-maximum (FWHM) axially. With a Hanning filter (cutoff frequency: 0.4 cycle/pixel), the reconstructed in-plane resolution was 7.5 mm FWHM. Data were acquired in three-dimensional mode for [^{11}C]WAY100635 and in two-dimensional mode for [^{11}C]DASB, since [^{11}C]DASB is substantially trapped at first pass through human lungs because of the high expression of 5-HTT on the pulmonary membrane, leading to excessive random counts from three-dimensional head recordings (Suhara et al., 1998). Scatter was corrected for the three-dimensional mode (Watson et al., 1996). A head-fixation device with thermoplastic attachments (Fixster Instruments, Stockholm, Sweden) for individual fit minimized head movement during PET measurements. A 10-min transmission scan using a ^{68}Ge-^{68}Ga line source was performed in order to correct for attenuation. After an i.v. bolus injection of [^{11}C]WAY100635 or [^{11}C]DASB, data were acquired for 90 min in a consecutive series of time frames. The frame sequence consisted of nine 20-s frames, five 1-min frames, four 2-min frames, eleven 4-min frames, and six 5-min frames for [^{11}C]DASB; and twelve 20-s frames, sixteen 1-min frames, ten 4-min frames, and five 6-min frames for [^{11}C]WAY100635. Injected radioactivity was 749.7 ± 37.4 MBq and 224.8 ± 9.1 MBq for [^{11}C]DASB and [^{11}C]WAY100635, respectively. Specific radioactivity was 281.7 ± 92.7 GBq/μmol and