Healthy subject study

Mean dopamine D_2 receptor occupancies in the striatum were 74.8±8.0% at 1.5 h, 60.1±5.6% at 8 h, and 31.9±6.4% at 25.5 h after administration of 16 mg of perospirone in healthy subjects (Fig. 2). The mean plasma concentrations of both perospirone and ID-15036 reached a peak at 1 h after administration, then rapidly decreased, and were not detectable at 25.5 h after (Fig. 3a, b). Estimated half-lives of plasma concentrations of perospirone and ID-15036 were 2.2 and 1.9 h, respectively. No subject complained of severe side effects such as extrapyramidal symptoms or sleepiness.

Discussion

Clinical dose of perospirone

A previous study reported that dopamine D_2 receptor occupancy using [^{11}C]raclopride was 44.4% with 8 mg of perospirone at 1 h post-administration (Sekine et al. 2006). PET studies have suggested that more than 70% dopamine D_2 receptor occupancy is necessary for antipsychotic effect and that 80% occupancy causes extrapyramidal symptoms (Farde et al. 1992; Kapur et al. 2000a; Nordstrom et al. 1993). Two patients (numbers 1 and 5) administered perospirone at 16 mg 2.5 h before PET scanning showed over 70% occupancy. On the other hand, one patient (number 4) taking 8 mg did not reach 70% occupancy in spite of a short interval between the last administration and PET scan. In healthy subjects, a peak of about 75% occupancy was also obtained with 16 mg of perospirone. Although some patients could be maintained at less than 70% occupancy, 16 mg of perospirone seems to be the necessary dose for achieving antipsychotic effect. The plasma concentrations of perospirone and ID-15036 inducing 70% occupancy (EC_{70}) were 0.72 and 4.43 ng/ml, respectively. Side effects could not be evaluated in this study because some patients were taking benzodiazepines or anti-Parkinson drugs, and plasma prolactin levels were not measured.

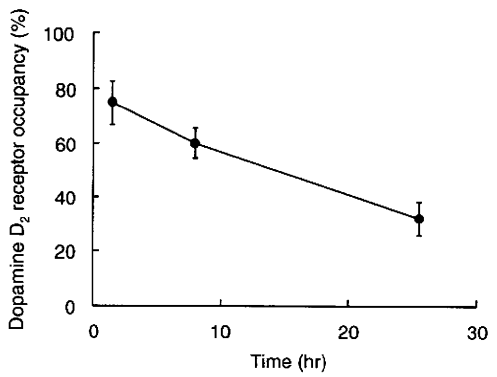

Fig. 2 Time course of mean dopamine D_2 receptor occupancy in healthy subject study. *Bars* represent standard deviation of mean

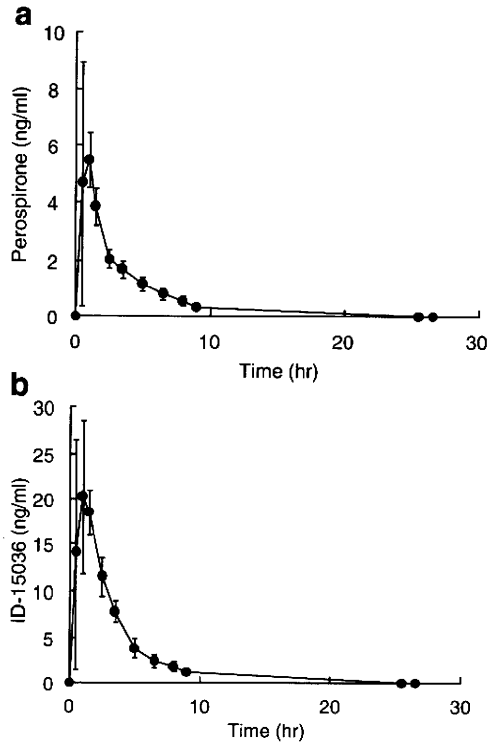

Fig. 3 Time course of mean plasma concentrations of perospirone (**a**) and ID-15036 (**b**) in healthy subjects study. *Bars* represent standard deviation of mean

Pharmacokinetics and contributions to receptor occupancy of perospirone and ID-15036

In healthy subjects, plasma concentrations of perospirone and ID-15036 peaked at 1 h after administration, with the half-lives of plasma concentrations being 2.2 and 1.9 h, respectively. The plasma concentration of ID-15036 was fourfold that of perospirone. These results were in good agreement with the previous study showing that the T_{max} values were 0.8 (perospirone) and 1.1 h (ID-15036), and $T_{1/2}$ was 1.9 h (perospirone; Yasui-Furukori et al. 2004). As ID-15036 has affinity for the dopamine D_2 receptor (K_i= 5.84 nM) and blocks the dopamine D_2 receptor of the in vivo rat brain (Takahashi et al. 1998), both perospirone and ID-15036 contributed to dopamine D_2 receptor occupancy, and the plasma concentrations of both were fitted to the occupancy curve.

Effects of affinity and pharmacokinetics of antipsychotics on time course of receptor occupancy

Dopamine D_2 receptor occupancy was about 75% at 1.5 h after perospirone administration and then showed a rela-

tively rapid decline. After 25.5 h, about 30% occupancy remained, although plasma concentrations of perospirone and ID-15036 were not detectable. The time to reach half of the peak occupancy of 75% was 22 h. The time courses of receptor occupancy and plasma concentration were quite different. In comparison, risperidone and olanzapine showed sustained occupancy; about 80% occupancy 5 or 6 h after administration decreased to only 70% after 24 h (Takano et al. 2004; Tauscher et al. 2002). On the other hand, quetiapine showed transient occupancy; 64% occupancy after 2 h decreased to 0% after 24 h (Kapur et al. 2000b). Some factors such as the time course of plasma concentration of antipsychotics or affinity for dopamine D_2 receptor were considered to affect the time course of receptor occupancy (Kapur and Seeman 2001; Takano et al. 2004). For example, high affinity and long half-life of plasma concentration (e.g., risperidone (K_i=1.1 nM, $T_{1/2}$= 17.8 h) and olanzapine (K_i=5.1 nM, $T_{1/2}$=19.5 h)) expressed sustained occupancy, and low affinity and short half-life of plasma concentration (e.g., quetiapine (K_i= 122 nM, $T_{1/2}$=3.2 h)) expressed transient occupancy (Gefvert et al. 1998; Seeman 2002; Takano et al. 2004; Tauscher et al. 2002). Perospirone has high affinity for dopamine D_2 receptor and a short half-life of plasma concentration (Takahashi et al. 1998; Yasui-Furukori et al. 2004). These features may cause relatively rapid decrease in occupancy, from 75% at 1.5 h of perospirone administration to 32% after 25.5 h, but the occupancy did not completely disappear within a day. In patients taking 32 mg perospirone (number 6), dopamine D_2 receptor occupancy was 65% at 17.5 h after, supporting an intermediate time course between sustained and transient occupancy.

Possibility of new dosing schedule with perospirone

There are several opinions concerning the dosing schedule of antipsychotics. A recent clinical study reported that extended antipsychotic dosing (every second or third day) was effective and decreased side effects for chronic patients with schizophrenia (Remington et al. 2005). An animal study reported that transient antipsychotic medication was more effective for amphetamine-induced behavioral abnormality than continuous one (Samaha et al. 2008). These findings indicate that sustained occupancy might not necessarily be required for antipsychotic therapy of schizophrenia. In prodromal episode-based intervention, antipsychotic drugs were used occasionally, and long antipsychotic-free periods were sometimes inserted. However, some studies reported that intermittent medication increased the relapse rate in schizophrenia (Gaebel et al. 2002; Herz et al. 1991; Schooler et al. 1997). Because perospirone shows an intermediate time course between sustained and transient occupancy, its single administration may become a new dosing schedule choice for an antipsychotic drug. Indeed, the administration of perospirone once a day indicated antipsychotic effects and preventions from relapse for chronic patients with schizophrenia (Kusumi et al. 2008). Four patients in the present study (numbers 1, 4, 5, and 6) taking 16 mg or more at least once a day were maintained for more than 6 months. Further study of relationships between clinical response and receptor occupancy of various dosing schedules in patients with schizophrenia will be needed.

Regional difference of dopamine D_2 receptor occupancy

Regional differences of dopamine D_2 receptor occupancy between the striatum and extrastriatum in some second-generation antipsychotic drugs have been discussed (Arakawa et al. 2008; Ito et al. 2009; Pilowsky et al. 1997; Talvik et al. 2001). In the present study, the mean occupancy of four healthy subject and two patients (number 1 and 5) in a short interval between the administration of 16 mg of perospirone and PET scanning seemed to differ very little (75.1% in the striatum with [^{11}C]raclopride and 77.0% in the temporal cortex with [^{11}C]FLB 457). It is suggested that there were no regional differences of dopamine D_2 receptor occupancy between the striatum and extrastriatum with perospirone despite the subjects, study protocols, and radioligands being different.

Conclusion

Sixteen milligrams of perospirone caused over 70% dopamine D_2 receptor occupancy near its peak level, then becoming about half after 22 h. The time courses of receptor occupancy and plasma concentration were quite different. This single dosage may be sufficient for the treatment of schizophrenia and might be useful as a new dosing schedule choice.

Acknowledgments This study was supported by a consignment expense for the Molecular Imaging Program on "Research Base for PET Diagnosis" from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japanese Government. The sponsors of the study had no role in the study design, collection, analysis, and interpretation of data, in the writing of the report, or in the decision to submit the paper for publication. All authors took part in the study design, collection, analysis, and interpretation of data, in the writing of the report, and in the decision to submit the manuscript for publication. All authors saw and approved the final version of the manuscript. All authors reported no biomedical financial interests or potential conflicts of interest. We thank Mr. Katsuyuki Tanimoto, Mr. Takahiro Shiraishi, and Mr. Toshio Miyamoto for their assistance in performing the PET experiments, and Ms. Yoshiko Fukushima for her help as clinical research coordinator at the National Institute of Radiological Sciences. We thank also Mr. Yoshimasa Inoue for measurement of plasma concentration of perospirone at MP-Technopharma Corporation.

References

Arakawa R, Ito H, Takano A, Takahashi H, Morimoto T, Sassa T, Ohta K, Kato M, Okubo Y, Suhara T (2008) Dose-finding study of paliperidone ER based on striatal and extrastriatal dopamine D2 receptor occupancy in patients with schizophrenia. Psychopharmacology (Berl) 197:229–235

Farde L, Nordstrom AL, Wiesel FA, Pauli S, Halldin C, Sedvall G (1992) Positron emission tomographic analysis of central D1 and D2 dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine. Relation to extrapyramidal side effects. Arch Gen Psychiatry 49:538–544

Gaebel W, Janner M, Frommann N, Pietzcker A, Kopcke W, Linden M, Muller P, Muller-Spahn F, Tegeler J (2002) First vs multiple episode schizophrenia: two-year outcome of intermittent and maintenance medication strategies. Schizophr Res 53:145–159

Gefvert O, Bergstrom M, Langstrom B, Lundberg T, Lindstrom L, Yates R (1998) Time course of central nervous dopamine-D2 and 5-HT2 receptor blockade and plasma drug concentrations after discontinuation of quetiapine (Seroquel) in patients with schizophrenia. Psychopharmacology (Berl) 135:119–126

Herz MI, Glazer WM, Mostert MA, Sheard MA, Szymanski HV, Hafez H, Mirza M, Vana J (1991) Intermittent vs maintenance medication in schizophrenia. Two-year results. Arch Gen Psychiatry 48:333–339

Innis RB, Cunningham VJ, Delforge J, Fujita M, Gjedde A, Gunn RN, Holden J, Houle S, Huang SC, Ichise M, Iida H, Ito H, Kimura Y, Koeppe RA, Knudsen GM, Knuuti J, Lammertsma AA, Laruelle M, Logan J, Maguire RP, Mintun MA, Morris ED, Parsey R, Price JC, Slifstein M, Sossi V, Suhara T, Votaw JR, Wong DF, Carson RE (2007) Consensus nomenclature for in vivo imaging of reversibly binding radioligands. J Cereb Blood Flow Metab 27:1533–1539

Ito H, Arakawa R, Takahashi H, Takano H, Okumura M, Otsuka T, Ikoma Y, Shidahara M, Suhara T (2009) No regional difference in dopamine D2 receptor occupancy by the second-generation antipsychotic drug risperidone in humans: a positron emission tomography study. Int J Neuropsychopharmacol 12:667–675

Kapur S, Seeman P (2001) Does fast dissociation from the dopamine d2 receptor explain the action of atypical antipsychotics? A new hypothesis. Am J Psychiatry 158:360–369

Kapur S, Zipursky R, Jones C, Remington G, Houle S (2000a) Relationship between dopamine D2 occupancy, clinical response, and side effects: a double-blind PET study of first-episode schizophrenia. Am J Psychiatry 157:514–520

Kapur S, Zipursky R, Jones C, Shammi CS, Remington G, Seeman P (2000b) A positron emission tomography study of quetiapine in schizophrenia: a preliminary finding of an antipsychotic effect with only transiently high dopamine D2 receptor occupancy. Arch Gen Psychiatry 57:553–559

Kusumi I, Masui T, Koyama T (2008) Long-term perospirone treatment with a single dose at bedtime in schizophrenia: relevant to intermittent dopamine D2 receptor antagonism. Prog Neuropsychopharmacol Biol Psychiatry 32:520–522

Lammertsma AA, Hume SP (1996) Simplified reference tissue model for PET receptor studies. Neuroimage 4:153–158

Nordstrom AL, Farde L, Wiesel FA, Forslund K, Pauli S, Halldin C, Uppfeldt G (1993) Central D2-dopamine receptor occupancy in relation to antipsychotic drug effects: a double-blind PET study of schizophrenic patients. Biol Psychiatry 33:227–235

Onrust SV, McClellan K (2001) Perospirone. CNS Drugs 15:329–337, discussion 338

Pilowsky LS, Mulligan RS, Acton PD, Ell PJ, Costa DC, Kerwin RW (1997) Limbic selectivity of clozapine. Lancet 350:490–491

Remington G, Seeman P, Shammi C, Mann S, Kapur S (2005) "Extended" antipsychotic dosing: rationale and pilot data. J Clin Psychopharmacol 25:611–613

Samaha AN, Reckless GE, Seeman P, Diwan M, Nobrega JN, Kapur S (2008) Less is more: antipsychotic drug effects are greater with transient rather than continuous delivery. Biol Psychiatry 64:145–152

Schooler NR, Keith SJ, Severe JB, Matthews SM, Bellack AS, Glick ID, Hargreaves WA, Kane JM, Ninan PT, Frances A, Jacobs M, Lieberman JA, Mance R, Simpson GM, Woerner MG (1997) Relapse and rehospitalization during maintenance treatment of schizophrenia. The effects of dose reduction and family treatment. Arch Gen Psychiatry 54:453–463

Seeman P (2002) Atypical antipsychotics: mechanism of action. Can J Psychiatry 47:27–38

Sekine Y, Ouchi Y, Takei N, Yoshikawa E, Okada H, Minabe Y, Nakamura K, Suzuki K, Iwata Y, Tsuchiya KJ, Sugihara G, Mori N (2006) Perospirone is a new generation antipsychotic: evidence from a positron emission tomography study of serotonin 2 and D2 receptor occupancy in the living human brain. J Clin Psychopharmacol 26:531–533

Suhara T, Sudo Y, Okauchi T, Maeda J, Kawabe K, Suzuki K, Okubo Y, Nakashima Y, Ito H, Tanada S, Halldin C, Farde L (1999) Extrastriatal dopamine D2 receptor density and affinity in the human brain measured by 3D PET. Int J Neuropsychopharmacol 2:73–82

Takahashi Y, Kusumi I, Ishikane T, Koyama T (1998) In vivo occupation of dopamine D1, D2 and serotonin2A receptors by novel antipsychotic drug, SM-9018 and its metabolite, in rat brain. J Neural Transm 105:181–191

Takano A, Suhara T, Ikoma Y, Yasuno F, Maeda J, Ichimiya T, Sudo Y, Inoue M, Okubo Y (2004) Estimation of the time-course of dopamine D2 receptor occupancy in living human brain from plasma pharmacokinetics of antipsychotics. Int J Neuropsychopharmacol 7:19–26

Talvik M, Nordstrom AL, Nyberg S, Olsson H, Halldin C, Farde L (2001) No support for regional selectivity in clozapine-treated patients: a PET study with [11C]raclopride and [11C]FLB 457. Am J Psychiatry 158:926–930

Tauscher J, Jones C, Remington G, Zipursky RB, Kapur S (2002) Significant dissociation of brain and plasma kinetics with antipsychotics. Mol Psychiatry 7:317–321

Yasui-Furukori N, Inoue Y, Tateishi T (2003) Determination of a new atypical antipsychotic agent perospirone and its metabolite in human plasma by automated column-switching high-performance liquid chromatography. J Chromatogr B Analyt Technol Biomed Life Sci 789:239–245

Yasui-Furukori N, Furukori H, Nakagami T, Saito M, Inoue Y, Kaneko S, Tateishi T (2004) Steady-state pharmacokinetics of a new antipsychotic agent perospirone and its active metabolite, and its relationship with prolactin response. Ther Drug Monit 26:361–365

Eur Arch Psychiatry Clin Neurosci (2010) 260:345–350
DOI 10.1007/s00406-009-0082-5

ORIGINAL PAPER

Extrastriatal dopamine D_2 receptor occupancy in olanzapine-treated patients with schizophrenia

Ryosuke Arakawa · Hiroshi Ito · Masaki Okumura · Akihiro Takano · Hidehiko Takahashi · Harumasa Takano · Yoshiro Okubo · Tetsuya Suhara

Received: 18 September 2008 / Accepted: 9 October 2009 / Published online: 23 October 2009

Abstract Olanzapine is described as a multi-acting receptor-targeted antipsychotic agent. Although regional differences of dopamine D_2 receptor occupancy, i.e., limbic selectivity, were reported for olanzapine, contradictory results were also reported. We measured dopamine D_2 receptor occupancy of olanzapine in extrastriatal regions in patients with schizophrenia using positron-emission tomography with [^{11}C]FLB457 and the plasma concentrations of olanzapine. Ten patients with schizophrenia taking 5–20 mg/day of olanzapine participated. Dopamine D_2 receptor occupancy in the temporal cortex ranged from 61.1 to 85.8%, and plasma concentration was from 12.7 to 115.4 ng/ml. The ED_{50} value was 3.4 mg/day for dose and 10.5 ng/ml for plasma concentration. The ED_{50} values obtained in this study were quite similar to those previously reported in the striatum. In conclusion, although the subjects and methods were different from previous striatal occupancy studies, these results suggest that limbic occupancy by olanzapine may not be so different from that in the striatum.

Keywords Dopamine D_2 receptor occupancy · Extrastriatum · Olanzapine · Positron-emission tomography · Schizophrenia

R. Arakawa · H. Ito · M. Okumura · A. Takano · H. Takahashi · H. Takano · T. Suhara (✉)
Molecular Neuroimaging Group, Molecular Imaging Center, National Institute of Radiological Sciences, 4-9-1, Anagawa, Inage-ku, Chiba 263-8555, Japan
e-mail: suhara@nirs.go.jp

R. Arakawa · M. Okumura · Y. Okubo
Department of Neuropsychiatry, Nippon Medical School, 1-1-5, Sendagi, Bunkyo-ku, Tokyo, Japan

Introduction

Olanzapine is a second-generation antipsychotic drug that is widely used in the treatment of schizophrenia [7]. Most second-generation antipsychotic drugs, such as clozapine, risperidone, olanzapine and quetiapine, have high affinity for several kinds of neuroreceptors in addition to dopamine D_2 receptors [6]. Olanzapine has high affinity for dopamine D_2 receptors (Ki = 11 nM) as well as for other receptors, i.e., serotonin 5-HT_{2A} (4 nM), 5-HT_{2C} (11 nM), muscarine m_1–m_5 (1.9–25 nM), adrenaline α_1 (19 nM) and histamine H_1 (7 nM) receptors [6]. The pharmacological profile is similar to that of clozapine, described as a multi-acting receptor-targeted antipsychotic agent. The difference in occupancy of dopamine D_2 receptors with clozapine between striatal and extrastriatal regions has been reported as 'limbic selectivity' [23]. This feature was considered one of the reasons for the low risk of extrapyramidal symptoms and a possible effect for negative symptoms [23].

Some animal studies reported greater effects on dopamine D_2 receptors by olanzapine in the extrastriatum than in the striatum [24, 27]. In human studies, higher occupancy in the temporal cortex than in the striatum was also reported for olanzapine [3, 34]. On the other hand, in another human study using olanzapine, no difference in dopamine D_2 receptor occupancies between the striatum and extrastriatum was also reported [16]. In those studies, occupancies in the striatum and extrastriatum were measured from the same data, despite their quite different receptor densities [15].

In the present study, dopamine D_2 receptor occupancy in extrastriatal regions by olanzapine was measured in patients with schizophrenia using positron-emission tomography (PET) with [^{11}C]FLB 457, an optimized

radiotracer for measuring extrastriatal dopamine D_2 receptors [10]. The receptor occupancy in the extrastriatum was compared with that in the striatum by olanzapine previously measured using [^{11}C]raclopride [13].

Methods

Subjects and study protocol

Ten patients, aged 23–47 years (36.2 ± 9.0, mean ± SD), diagnosed with schizophrenia according to DSM-IV criteria, participated in this study (Table 1). After complete explanation of the study, written informed consent was obtained from all patients. Exclusion criteria were current or past substance abuse, organic brain disease or epilepsy. Subjects with severe liver or renal dysfunction, or having undergone electroconvulsive therapy within 90 days prior to this study were also excluded. The patients had been taking fixed dosages of olanzapine for more than 2 weeks before this study. Doses of olanzapine were 5 mg/day in two patients, 7.5 mg/day in two patients, 10 mg/day in three patients, 15 mg/day in one patient and 20 mg/day in two patients. The duration between PET scan and the last administration of olanzapine was between 2 and 20 h. Clinical symptoms were assessed by positive and negative symptom scale (PANSS). This study was approved by the Ethics and Radiation Safety Committee of the National Institute of Radiological Sciences, Chiba, Japan.

PET procedure

A PET scanner system, ECAT EXACT HR+ (CTI-Siemens, Knoxville, TN, USA), was used for all subjects. A head fixation device was used to minimize head movement. Before the dynamic scan, a transmission scan for attenuation correction was performed using a ^{68}Ge–^{68}Ga source. The dynamic PET scan was then performed for 90 min after intravenous bolus injection of 197.0–238.0 MBq (217.5 ± 13.9 MBq, mean ± SD) of [^{11}C]FLB 457. The specific radioactivity of [^{11}C]FLB 457 was 85.8–339.9 MBq/nmol (188.0 ± 79.1 MBq/nmol, mean ± SD); the injected mass of FLB 457 was 0.24–0.90 μg (0.64 ± 0.20 μg, mean ± SD). Venous blood samples were taken before and after PET scanning to measure the plasma concentration of olanzapine. The average values of plasma concentration before and after PET scanning were used. The drug concentration of one patient (No. 8) could not be determined because of a technical error. Magnetic resonance images of the brain were acquired with 1.5 T MRI, Gyroscan NT (Philips Medical Systems, Best, Netherlands). T1-weighted images of 1-mm slices were obtained.

Data analysis

All emission scan data were reconstructed with a Hanning filter. Regions of interest (ROIs) were defined for the temporal cortex as for the extrastriatal region and cerebellar cortex [3, 34]. ROIs were drawn manually on PET images with reference to individual MR images. The values of ROIs for right and left sides were averaged. Binding potential (BP_{ND}) of dopamine D_2 receptor was calculated using a three-parameter simplified reference tissue model [18]. The cerebellum was used as reference tissue because of its negligible density of dopamine D_2 receptors [28].

Receptor occupancy of antipsychotic drug is expressed as follows: Occupancy (%) = $(BP_{base} - BP_{drug})/BP_{base} \times 100$, where BP_{base} is BP_{ND} in the drug-free state and BP_{drug} is BP_{ND} after administration of the drug. In this study,

Table 1 Patient characteristics, plasma concentration of olanzapine, and dopamine D_2 receptor occupancy

No.	Age (years)	Sex	Duration of illness (years)	PANSS	Dose (mg/day)	Duration of fixed dose (months)	Other medication	Plasma concentration (ng/ml)	Receptor occupancy (%)
1	41	M	6.5	50	5	14	–	20.1	61.6
2	45	M	8	38	5	25	BZ	12.7	72.2
3	30	F	12	49	7.5	13	–	25.9	65.6
4	45	F	4	95	7.5	0.5	BZ, AP	25.5	76.9
5	23	M	0.8	50	10	7	–	48.5	69.7
6	41	M	17	44	10	30	BZ	21.8	61.1
7	47	M	27	92	10	3	–	44.5	81.8
8	32	M	17	75	15	16	BZ	ND	67.9
9	23	M	5	101	20	0.5	BZ	61.0	79.5
10	37	F	11	92	20	3	BZ	115.4	85.8

BZ benzodiazepine, *AP* anti-parkinsonian drug, *ND* not determined

mean BP_{ND} of age-matched ten normal male subjects (age range 21–49; 36.2 ± 9.1 years, mean ± SD) measured by the same procedure as for the patients was used as BP_{base} because of the lack of individual baseline BP_{ND}.

The relationship between receptor occupancy and dose (or plasma concentration) of antipsychotic drug can be expressed as follows:

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

where D is the dose of olanzapine and ED_{50} is the dose required to induce 50% occupancy [1, 13, 31]. In this study, maximum occupancy was fixed at 100%, the same as previous occupancy studies with olanzapine [13].

Measurement of plasma concentration of olanzapine

Plasma concentrations of olanzapine were determined using a validated high-performance liquid chromatography (HPLC) method (JCL Bioassay Corporation., Hyogo, Japan).

Statistical analysis

Correlations between dopamine D_2 receptor occupancy in the temporal cortex and daily dose, plasma concentration, age, duration of illness and PANSS (total or sub scores) were assessed using Pearson's correlation coefficient.

Results

Dopamine D_2 receptor occupancy in the temporal cortex ranged from 61.1 to 85.8% (Table 1). Plasma concentration of olanzapine ranged from 12.7 to 115.4 ng/ml. ED_{50} was 3.4 mg/day for the daily dose (Fig. 1) and 10.5 ng/ml for the plasma concentration (Fig. 2). The PANSS score ranged from 38 to 101. Average PANSS scores of all patients were 68.6 ± 24.7.

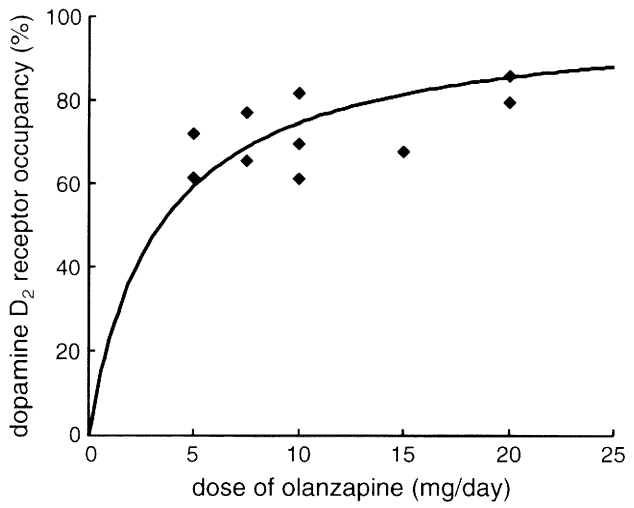

Fig. 1 Relationship between dopamine D_2 receptor occupancy and daily dose of olanzapine

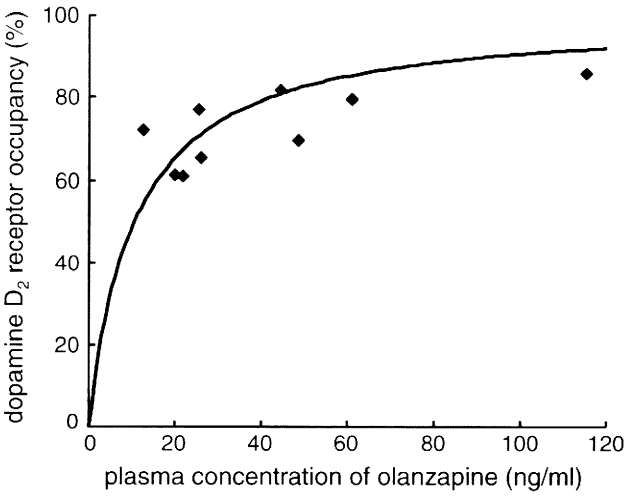

Fig. 2 Relationship between dopamine D_2 receptor occupancy and plasma concentration of olanzapine

A positive correlation was observed between dopamine D_2 receptor occupancy in the temporal cortex and plasma concentration ($r = 0.72$, $P = 0.029$), but not daily dose within this dose range ($r = 0.57$, $P = 0.082$). A positive correlation was also observed with total PANSS scores ($r = 0.80$, $P = 0.0054$), positive scores ($r = 0.78$, $P = 0.0074$), negative scores ($r = 0.68$, $P = 0.032$), and general scores ($r = 0.78$, $P = 0.0072$). No correlations were observed between dopamine D_2 receptor occupancy in the temporal cortex and age ($P = 0.85$) or duration of illness ($P = 0.81$).

Discussion

Although the measured occupancy value was above 50% with 5–20 mg/day of olanzapine, the calculated ED_{50} value from the present result in the temporal cortex was 3.4 mg/day for the daily dose and 10.5 ng/ml for the plasma concentration. The previously reported ED_{50} of olanzapine in the striatum was 4.5 mg/day for the daily dose and 10.3 ng/ml for the plasma concentration [13]. ED_{50} of plasma concentration in the extrastriatum of the present study was similar to that reported in the striatum, meaning that there was no noteworthy regional difference in dopamine D_2 receptor occupancy by olanzapine between the striatum and extrastriatum. Based on 70–80% of dopamine D_2 receptor occupancy [8, 14, 20], the optimal daily dose of olanzapine would be about 8–14 mg/day. This estimated dose was in fairly good agreement with the current clinical dose (5–20 mg/day in Japan).

According to electrophysiological measurement, the effect of olanzapine was reported preferentially in the ventral tegmental area (A10) [27], and olanzapine was reported to increase c-fos expression to a greater degree in the nucleus accumbens than in the dorsolateral striatum [24]. These findings suggested that olanzapine had preferentially different regional effects for extrastriatal regions. The concept of 'limbic selectivity', i.e., differences in dopamine D_2 receptor occupancy between the striatum and extrastriatum, has been discussed. Although there are several reports about 'limbic selectivity' of second-generation antipsychotics, such as clozapine [9, 17, 23, 34], risperidone [5, 34], quetipaine [17, 26] and amisulpride [4, 34], it has also been reported that there is no limbic selectivity with second-generation antipsychotics such as clozapine [32] and risperidone (and paliperidone) [1, 11, 35].

Contradictory results of limbic selectivity have also been reported for olanzapine. Two studies showed higher occupancy in the temporal cortex than in the striatum using [^{123}I]epidepride SPECT (82.8 ± 4.2% in the temporal cortex and 41.3 ± 17.9% in the striatum) [3] and [^{76}Br]FLB 457 PET (83.6 ± 10.5% in the temporal cortex and 45.1 ± 20.9% in the striatum) [34]. On the other hand, no significant difference in occupancy between the temporal cortex (67.5 ± 7.1%) and striatum (70.9 ± 6.9%) was reported using [^{18}F]fallypride PET [16]. Regional differences in occupancies were calculated from the area under the time-activity curve ratio in [^{123}I]epidepride SPECT and [^{76}Br]FLB 457 PET [3, 34]. A previous study reported that the ratio method underestimated striatal occupancy using high-affinity radioligand such as [^{123}I]epidepride or [^{11}C]FLB 457 (probably also [^{76}Br]FLB 457) because radioligand bindings did not reach equilibrium due to the high density of dopamine D_2 receptors in the striatum [21]. In addition, because none of the studies concerning regional difference of occupancy by olanzapine presented plasma concentrations [3, 16, 34], ED_{50} of the extrastriatum could not be compared with the present study.

Differences of occupancy or EC_{50} values in the same brain region (e.g. striatum) were reported using different radioligands ("Discussion" in [19]). As commented above, this difference may be caused using different affinity radioligands at high-density receptor regions [21]. In this study, the dopamine D_2 receptor bindings in the temporal cortex were measured using [^{11}C]FLB 457 because the dopamine D_2 receptor density of the temporal cortex is very low compared with that of the striatum (B_{max} = 0.4 and 16.6 pmol/g tissue, respectively) [15]. Recently, the absence of regional difference between striatal and extrastriatal occupancy of risperidone was reported using [^{11}C]raclopride and [^{11}C]FLB 457 by precise methods [11]. These results suggest that optimal radioligands are necessary for different brain regions with different receptor densities.

The significant positive correlation between temporal dopamine D_2 receptor occupancy and PANSS suggests that higher doses tend to be used for severe symptoms of schizophrenia. However, as this was an open study and the number of patients was limited, further studies (such as randomized controlled trials) are needed.

In the present study, the mean BP_{ND} value of age-matched healthy subjects was used as value of the drug-free state. Although previous studies showed no difference in BP_{ND} values of the temporal cortex between normal subjects and patients with schizophrenia [29, 33] or between the sexes [12], individual differences in BP_{ND} values may lead to potential error in the estimation of dopamine D_2 receptor occupancy [8]. Moreover, there is a possibility of upregulation of dopamine D_2 receptor by neuroleptic treatment [25]. When BP_{base} changes by ±15%, the estimated occupancy ranges from 41 to 57% for an assumed occupancy of 50%. The effect of displaceable binding of [^{11}C]FLB 457 in the cerebellum may also lead to an underestimation of receptor occupancy [2, 22].

Although the time point of the scan following the last drug administration was different among the scans, plasma concentration was measured and the reported time-course of occupancy of olanzapine fitted well with the occupancy simulated by plasma concentration [30].

In conclusion, dopamine D_2 receptor occupancy ranged from 61.1 to 85.8% in the temporal cortex of patients with schizophrenia taking 5–20 mg/day of olanzapine. The ED_{50} values were 3.4 mg/day for dose and 10.5 ng/ml for plasma concentration of olanzapine, in fairly good agreement with the reported values in the striatum using [^{11}C]raclopride. Although the subjects and methods were different from previous striatal occupancy studies, these results suggest that limbic occupancy by olanzapine may not be so different from that in the striatum.

Acknowledgments This study was supported by a consignment expense for the Molecular Imaging Program on "Research Base for PET Diagnosis" from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japanese Government. We thank Mr. Katsuyuki Tanimoto, Mr. Takahiro Shiraishi, Mr. Akira Ando, and Mr. Toshio Miyamoto for their assistance in performing the PET experiments, and Ms. Yoshiko Fukushima for her help as clinical research coordinator at the National Institute of Radiological Sciences. We thank also Dr. Amane Tateno, Dr. Mizuho Sekine, and Dr. Hajime Fukuta for their clinical support.

Conflict of interest statement All authors reported no conflict of interest.

References

1. Arakawa R, Ito H, Takano A, Takahashi H, Morimoto T, Sassa T, Ohta K, Kato M, Okubo Y, Suhara T (2008) Dose-finding study of paliperidone ER based on striatal and extrastriatal dopamine D2 receptor occupancy in patients with schizophrenia. Psychopharmacology (Berl) 197:229–235
2. Asselin MC, Montgomery AJ, Grasby PM, Hume SP (2007) Quantification of PET studies with the very high-affinity dopamine D2/D3 receptor ligand [11C]FLB 457: re-evaluation of the validity of using a cerebellar reference region. J Cereb Blood Flow Metab 27:378–392
3. Bigliani V, Mulligan RS, Acton PD, Ohlsen RI, Pike VW, Ell PJ, Gacinovic S, Kerwin RW, Pilowsky LS (2000) Striatal and temporal cortical D2/D3 receptor occupancy by olanzapine and sertindole in vivo: a [123I]epidepride single photon emission tomography (SPET) study. Psychopharmacology (Berl) 150:132–140
4. Bressan RA, Erlandsson K, Jones HM, Mulligan R, Flanagan RJ, Ell PJ, Pilowsky LS (2003) Is regionally selective D2/D3 dopamine occupancy sufficient for atypical antipsychotic effect? An in vivo quantitative [123I]epidepride SPET study of amisulpride-treated patients. Am J Psychiatry 160:1413–1420
5. Bressan RA, Erlandsson K, Jones HM, Mulligan RS, Ell PJ, Pilowsky LS (2003) Optimizing limbic selective D2/D3 receptor occupancy by risperidone: a [123I]-epidepride SPET study. J Clin Psychopharmacol 23:5–14
6. Bymaster FP, Calligaro DO, Falcone JF, Marsh RD, Moore NA, Tye NC, Seeman P, Wong DT (1996) Radioreceptor binding profile of the atypical antipsychotic olanzapine. Neuropsychopharmacology 14:87–96
7. Duggan L, Fenton M, Rathbone J, Dardennes R, El-Dosoky A, Indran S (2005) Olanzapine for schizophrenia. Cochrane Database Syst Rev CD001359
8. Farde L, Nordstrom AL, Wiesel FA, Pauli S, Halldin C, Sedvall G (1992) Positron emission tomographic analysis of central D1 and D2 dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine: relation to extrapyramidal side effects. Arch Gen Psychiatry 49:538–544
9. Grunder G, Landvogt C, Vernaleken I, Buchholz HG, Ondracek J, Siessmeier T, Hartter S, Schreckenberger M, Stoeter P, Hiemke C, Rosch F, Wong DF, Bartenstein P (2006) The striatal and extrastriatal D2/D3 receptor-binding profile of clozapine in patients with schizophrenia. Neuropsychopharmacology 31:1027–1035
10. Ito H, Sudo Y, Suhara T, Okubo Y, Halldin C, Farde L (2001) Error analysis for quantification of [^{11}C]FLB 457 binding to extrastriatal D2 dopamine receptors in the human brain. Neuroimage 13:531–539
11. Ito H, Arakawa R, Takahashi H, Takano H, Okumura M, Otsuka T, Ikoma Y, Shidahara M, Suhara T (2009) No regional difference in dopamine D2 receptor occupancy by the second-generation antipsychotic drug risperidone in humans: a positron emission tomography study. Int J Neuropsychopharmacol 12:667–675
12. Kaasinen V, Nagren K, Hietala J, Farde L, Rinne JO (2001) Sex differences in extrastriatal dopamine d2-like receptors in the human brain. Am J Psychiatry 158:308–311
13. Kapur S, Zipursky RB, Remington G, Jones C, DaSilva J, Wilson AA, Houle S (1998) 5-HT2 and D2 receptor occupancy of olanzapine in schizophrenia: a PET investigation. Am J Psychiatry 155:921–928
14. Kapur S, Zipursky R, Jones C, Remington G, Houle S (2000) Relationship between dopamine D2 occupancy, clinical response, and side effects: a double-blind PET study of first-episode schizophrenia. Am J Psychiatry 157:514–520
15. Kessler RM, Whetsell WO, Ansari MS, Votaw JR, de Paulis T, Clanton JA, Schmidt DE, Mason NS, Manning RG (1993) Identification of extrastriatal dopamine D2 receptors in post mortem human brain with [125I]epidepride. Brain Res 609:237–243
16. Kessler RM, Ansari MS, Riccardi P, Li R, Jayathilake K, Dawant B, Meltzer HY (2005) Occupancy of striatal and extrastriatal dopamine D2/D3 receptors by olanzapine and haloperidol. Neuropsychopharmacology 30:2283–2289
17. Kessler RM, Ansari MS, Riccardi P, Li R, Jayathilake K, Dawant B, Meltzer HY (2006) Occupancy of striatal and extrastriatal dopamine D2 receptors by clozapine and quetiapine. Neuropsychopharmacology 31:1991–2001
18. Lammertsma AA, Hume SP (1996) Simplified reference tissue model for PET receptor studies. Neuroimage 4:153–158
19. Meisenzahl EM, Schmitt G, Grunder G, Dresel S, Frodl T, la Fougere C, Scheuerecker J, Schwarz M, Boerner R, Stauss J, Hahn K, Moller HJ (2008) Striatal D2/D3 receptor occupancy, clinical response and side effects with amisulpride: an iodine-123-iodobenzamide SPET study. Pharmacopsychiatry 41:169–175
20. Nordstrom AL, Farde L, Wiesel FA, Forslund K, Pauli S, Halldin C, Uppfeldt G (1993) Central D2-dopamine receptor occupancy in relation to antipsychotic drug effects: a double-blind PET study of schizophrenic patients. Biol Psychiatry 33:227–235
21. Olsson H, Farde L (2001) Potentials and pitfalls using high affinity radioligands in PET and SPET determinations on regional drug induced D2 receptor occupancy–a simulation study based on experimental data. Neuroimage 14:936–945
22. Olsson H, Halldin C, Farde L (2004) Differentiation of extrastriatal dopamine D2 receptor density and affinity in the human brain using PET. Neuroimage 22:794–803
23. Pilowsky LS, Mulligan RS, Acton PD, Ell PJ, Costa DC, Kerwin RW (1997) Limbic selectivity of clozapine. Lancet 350:490–491
24. Robertson GS, Fibiger HC (1996) Effects of olanzapine on regional C-Fos expression in rat forebrain. Neuropsychopharmacology 14:105–110
25. Schroder J, Silvestri S, Bubeck B, Karr M, Demisch S, Scherrer S, Geider FJ, Sauer H (1998) D2 dopamine receptor up-regulation, treatment response, neurological soft signs, and extrapyramidal side effects in schizophrenia: a follow-up study with 123I-iodobenzamide single photon emission computed tomography in the drug-naive state and after neuroleptic treatment. Biol Psychiatry 43:660–665
26. Stephenson CM, Bigliani V, Jones HM, Mulligan RS, Acton PD, Visvikis D, Ell PJ, Kerwin RW, Pilowsky LS (2000) Striatal and extra-striatal D2/D3 dopamine receptor occupancy by quetiapine in vivo. [^{123}I]-epidepride single photon emission tomography(SPET) study. Br J Psychiatry 177:408–415
27. Stockton ME, Rasmussen K (1996) Electrophysiological effects of olanzapine, a novel atypical antipsychotic, on A9 and A10 dopamine neurons. Neuropsychopharmacology 14:97–105
28. Suhara T, Sudo Y, Okauchi T, Maeda J, Kawabe K, Suzuki K, Okubo Y, Nakashima Y, Ito H, Tanada S, Halldin C, Farde L (1999) Extrastriatal dopamine D2 receptor density and affinity in the human brain measured by 3D PET. Int J Neuropsychopharmcol 2:73–82
29. Suhara T, Okubo Y, Yasuno F, Sudo Y, Inoue M, Ichimiya T, Nakashima Y, Nakayama K, Tanada S, Suzuki K, Halldin C, Farde L (2002) Decreased dopamine D2 receptor binding in the anterior cingulate cortex in schizophrenia. Arch Gen Psychiatry 59:25–30
30. Takano A, Suhara T, Ikoma Y, Yasuno F, Maeda J, Ichimiya T, Sudo Y, Inoue M, Okubo Y (2004) Estimation of the time-course of dopamine D2 receptor occupancy in living human brain from plasma pharmacokinetics of antipsychotics. Int J Neuropsychopharmacol 7:19–26

31. Takano A, Suhara T, Yasuno F, Suzuki K, Takahashi H, Morimoto T, Lee YJ, Kusuhara H, Sugiyama Y, Okubo Y (2006) The antipsychotic sultopride is overdosed–a PET study of drug-induced receptor occupancy in comparison with sulpiride. Int J Neuropsychopharmacol 9:539–545
32. Talvik M, Nordstrom AL, Nyberg S, Olsson H, Halldin C, Farde L (2001) No support for regional selectivity in clozapine-treated patients: a PET study with [^{11}C]raclopride and [^{11}C]FLB 457. Am J Psychiatry 158:926–930
33. Talvik M, Nordstrom AL, Olsson H, Halldin C, Farde L (2003) Decreased thalamic D2/D3 receptor binding in drug-naive patients with schizophrenia: a PET study with [11C]FLB 457. Int J Neuropsychopharmacol 6:361–370
34. Xiberas X, Martinot JL, Mallet L, Artiges E, Loc HC, Maziere B, Paillere-Martinot ML (2001) Extrastriatal and striatal D2 dopamine receptor blockade with haloperidol or new antipsychotic drugs in patients with schizophrenia. Br J Psychiatry 179:503–508
35. Yasuno F, Suhara T, Okubo Y, Sudo Y, Inoue M, Ichimiya T, Tanada S (2001) Dose relationship of limbic-cortical D2-dopamine receptor occupancy with risperidone. Psychopharmacology (Berl) 154:112–114

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

HARUMASA TAKANO,[1]* HIROSHI ITO,[1] HIDEHIKO TAKAHASHI,[1] RYOSUKE ARAKAWA,[1] MASAKI OKUMURA,[1] FUMITOSHI KODAKA,[1] TATSUI OTSUKA,[1] MOTOICHIRO KATO,[2] AND TETSUYA SUHARA[1]

[1]*Department of Molecular Neuroimaging, Molecular Imaging Center, National Institute of Radiological Sciences, Chiba 263-8555, Japan*

[2]*Department of Neuropsychiatry, Keio University School of Medicine, Shinjuku-ku, Tokyo 160-8582, Japan*

KEY WORDS normal database; serotonin; serotonin transporter; serotonin 1A receptor; positron emission tomography

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-

Contract grant sponsors: Grant-in-Aid for Molecular Imaging Program from the Ministry of Education, Culture, Sports and Technology (MEXT), Japanese Government and a Grant-in-Aid for Scientific Research (C) from the Japan Society for the Promotion of Science; Contract grant number: 20591382

*Correspondence to: Harumasa Takano, Molecular Neuroimaging Group, Molecular Imaging Center, National Institute of Radiological Sciences, 4-9-1 Anagawa, Inage-ku, Chiba, 263-8555, Japan. E-mail: hrtakano@nirs.go.jp

Received 27 September 2010; Accepted 10 November 2010

DOI 10.1002/syn.20883

Published online 23 November 2010 in Wiley Online Library (wileyonlinelibrary.com).

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

153.9 ± 61.8 GBq/μmol at the time of injection for [^{11}C]DASB and [^{11}C]WAY100635, respectively.

Preprocessing

A PET summation image of all frames and dynamic images were coregistered to each individual MR image by using PMOD (PMOD Technologies, Zurich, Switzerland). The individual MR image was spatially normalized to the Montreal Neurological Institute (MNI) stereotaxic brain, and subsequently, the transformation parameters were applied to the coregistered PET dynamic images. Thus, the PET and MR images of all subjects were anatomically standardized to the MNI template. Volumes of interest (VOIs) were manually delineated by a rater (H.T.) in the cerebellar cortex, dorsal raphe nuclei, thalamus, and striatum for [^{11}C]DASB, and in the cerebellar cortex and hippocampal area for [^{11}C]WAY100635, on the averaged and standardized PET summation and MR images by using the PMOD fusion tool. Circular VOIs with a diameter of 8 mm in the transaxial planes were set for the dorsal raphe nucleus from $z = -38$ to $z = -30$ in the coordinate of the MNI template. To obtain regional time-activity curves, regional radioactivity was calculated for each time frame, corrected for decay and plotted versus time.

Calculation of binding potentials and generation of parametric images for both tracers

For the PET study with [^{11}C]WAY100635, the binding potential (BP) for nondisplaceable radioligand in tissue (BP_{ND}) (Innis et al., 2007) was calculated using a reference tissue model on a voxel-by-voxel basis (Lammertsma and Hume, 1996).

$$C_T(t) = R_1 C_R(t) + \left(k_2 - \frac{R_1 k_2}{1 + BP_{ND}}\right) C_R(t) \otimes \exp\left(\frac{-k_2 t}{1 + BP_{ND}}\right),$$

where $C_T(t)$ is the radioactivity concentration in a brain region, and $C_R(t)$ 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 $\otimes$ denotes the convolution integral. In this analysis, three parameters (BP_{ND}, R_1, and k_2) were estimated by the basis function method (Gunn et al., 1997, 1998). The cerebellum was used as reference region because a postmortem study indicated that the cerebellum cortex is almost devoid of 5-HT_{1A} receptors (Varnas et al., 2004).

For the PET study with [^{11}C]DASB, BP_{ND} was calculated by using a multi-linear reference tissue model 2 (MRTM2) (Ichise et al., 2003),

$$C_T(T) = -\frac{V}{V'b}\left(\int_0^T C_R(t)dt + \frac{1}{k_2'} C_R(t)\right) + \frac{1}{b}\int_0^T C_T(t)dt$$

where $C_T(T)$ is the radioactivity concentration in a brain region, and $C_R(t)$ is the radioactivity concentration in the reference region. V and V' are the corresponding total distribution volumes (i.e., $V = K_1/k_2$, $V' = K_1'/k_2'$; k_2', the efflux rate constant from the reference tissue to plasma). b is the intercept term, which becomes constant for $T > t^*$. The k_2' value was estimated by the MRTM method (Ichise et al., 2002) and, to minimize the variability of k_2' estimation, a weighted mean k_2' value according to the VOI size over the raphe nucleus, thalamus, and striatum was calculated with the cerebellum as reference region, because postmortem studies indicated that the cerebellum cortex is almost devoid of 5-HTT (Kish et al., 2005; Varnas et al., 2004). BP_{ND} is then calculated from the two regression coefficients as,

$$BP_{ND} = -(\gamma_1/\gamma_2 + 1),$$

where $\gamma_1 = -V/(V'b)$ and $\gamma_2 = 1/b$, respectively.

Image data analysis

VOIs were placed on the standardized BP_{ND} images for the bilateral putamen; caudate nucleus; globus pallidus; thalamus and its subregions (pulvinar, mediodorsal, and anteriodorsal); hippocampal regions including the parahippocampus, uncus, and hippocampus; anterior and posterior cingulate; and the lateral temporal cortex, basal side as well as the convex side of the frontal cortex, parietal cortex, and occipital cortex (Fig. 1, Table I). The same VOI set was applied for both [^{11}C]WAY100635 and [^{11}C]DASB in all subjects.

Statistical analysis

Data are presented as mean ± SD. Pearson's correlation coefficient was calculated for the evaluation of correlation. Correction for multiple comparisons was not performed during the analysis because of the large number of correlations performed, and these results were considered exploratory. Laterality of the hemispheres was tested by performing a paired t-test for each region. SPSS package version 16 (SPSS, Chicago, IL) was used for statistical analysis.

RESULTS

For both radiotracers, parametric BP_{ND} images with anatomic standardization were obtained from 17 participants. The averaged images for all subjects are presented in Figures 2 and 3; mean and SD values of BP_{ND} are shown in Table II and Figure 4.

Distribution patterns of the 5-HTT and 5-HT_{1A} receptors in the human brain were measured with radioligands [^{11}C]DASB and [^{11}C]WAY100635, respectively, and they were observed to be quite different. The highest binding to 5-HTT was observed in the dorsal raphe nuclei, thalamus, and striatum. Moderate binding was observed in the insula, hippocampal area, and the anterior and posterior cingulate; very

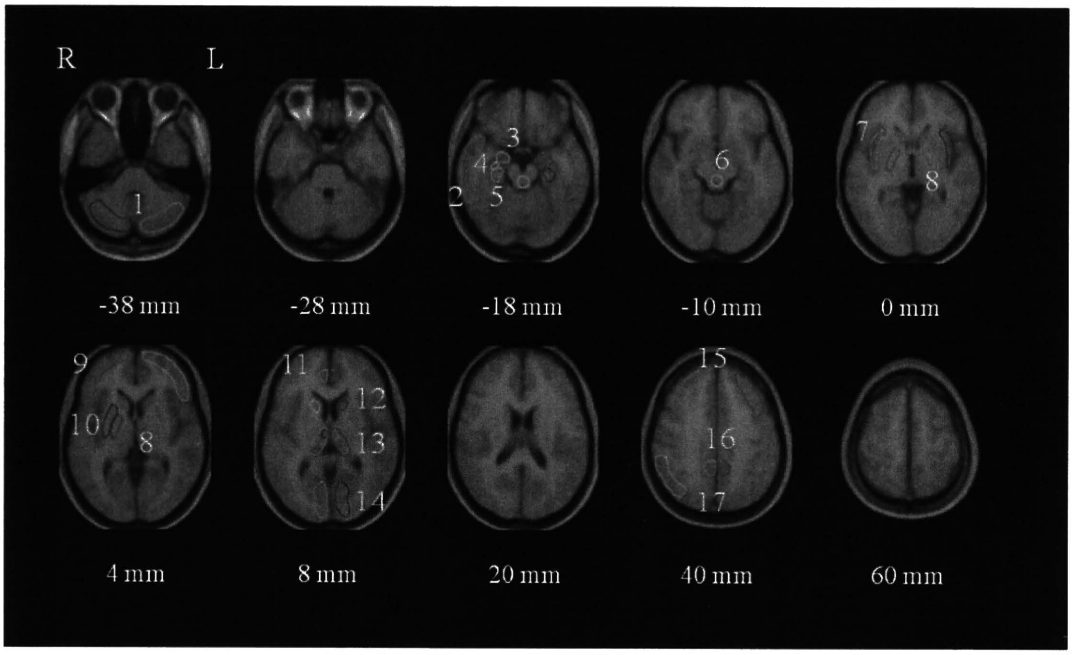

Fig. 1. VOIs drawn on the anatomically standardized MR images averaged for all subjects. Z = −38, −28, −18, −10, 0, 4, 8, 20, 40, 60 mm parallel to the anterior commissure and posterior commissure (AC-PC) line in the MNI template brain. L denotes left side of the brain; R denotes right side; 1, cerebellar cortex; 2, lateral temporal cortex; 3, uncus (amygdala); 4, hippocampus; 5, parahippocampal gyrus; 6, dorsal raphe nucleus; 7, insula; 8, globus pallidus; 9, base of frontal cortex; 10, putamen; 11, anterior cingulate; 12, head of caudate nucleus; 13, thalamus; 14, occipital cortex; 15, frontal convexity; 16, posterior cingulate; 17, parietal cortex.

TABLE I. Representative MNI coordinates and volumes in VOIs drawn on the MNI template image

	Right				Left				
Region	X	Y	Z	Volume (cm^3)	X	Y	Z	Volume (cm^3)	Total volume (cm^3)
Cerebellum	25	−70	−38	4.20	−31	−69	−38	4.30	8.50
Dorsal raphe nucleus	0	−31	−14						1.13
Striatum				1.69				1.99	3.68
Putamen	26	−1	4	1.34	−28	−2	4	1.54	2.88
Caudate head	14	14	8	0.35	−15	12	8	0.45	0.80
Globus Pallidus	20	−8	0	0.89	−18	−6	0	0.95	1.84
Thalamus	10	−23	8	1.67	−12	−22	8	1.60	3.27
Hippocampal complex				1.62				1.73	3.34
Uncus	21	−7	−18	0.70	−27	−24	−18	0.80	1.50
Hippocampus	29	−16	−18	0.19	−28	−13	−18	0.22	0.41
Parahippocampus	26	−24	−18	0.70	−27	−24	−18	0.80	1.50
Insula	38	4	−2	1.74	−38	3	−2	1.46	3.20
Anterior cingulate	5	43	8	0.53	−7	43	8	0.58	1.10
Posterior cingulate	6	−47	40	0.91	−9	−47	40	0.72	1.63
Base side of frontal cortex	34	45	4	4.58	−36	45	4	4.66	9.24
Frontal convexity	31	29	40	3.23	−35	24	40	3.25	6.51
Lateral temporal	53	−17	−18	5.58	−55	−17	−18	5.26	10.84
Occipital cuneus	13	−81	8	2.47	−9	−81	8	2.54	5.02
Parietal	49	−56	40	3.17	−51	−57	40	3.30	6.46

low binding was observed in the neocortical regions. In contrast, the highest binding to 5-HT_{1A} receptors was observed in the medial temporal regions including the hippocampus, uncus (amygdala), parahippocampus, and insula. Then, slightly lower binding was observed in the cingulate cortex and other cortical regions in descending order, with very low binding being seen in the basal ganglia and thalamus. As shown in Figure 4, when we divide brain regions into 5-HTT transporter rich regions and 5-HT_{1A} receptor rich regions, the relationships between mean values of BP_{ND} in 5-HTT and 5-HT_{1A} receptors were quite different. No linear correlation was found in 5-HTT transporter-rich regions between the mean values of BP_{ND} in 5-HTT and 5-HT_{1A} receptors, while a positive linear correlation, except the occipital cortex (r = 0.862, P = 0.006), was found between them in the 5-HT_{1A} receptor-rich regions.

There was no significant difference between the two sides of the brain with either of the radiotracers,

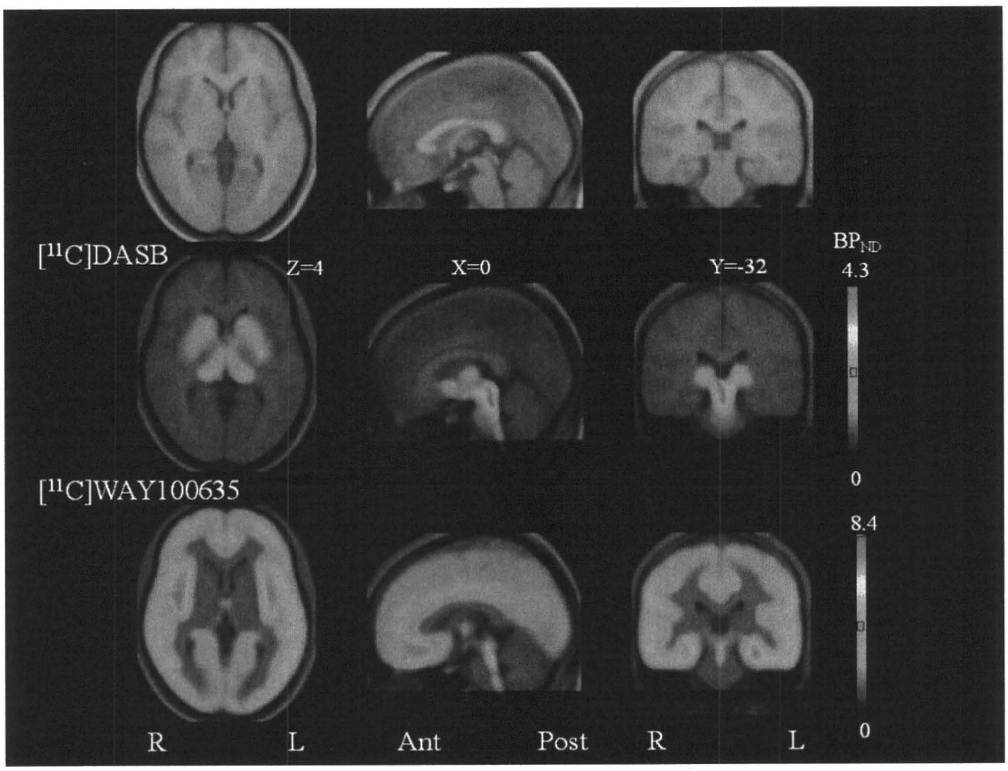

Fig. 2. Averaged images of MRI, [^{11}C]DASB image fused with MRI, and [^{11}C]WAY100635 image fused with MRI for all subjects. In left to right columns, transaxial, saggital, and coronal planes of the brain are displayed. X = 0 mm, Y = −32 mm, and Z = 4 mm indicate the coordinates of the three planes in the MNI template brain. R represents right; L, left. Ant indicates anterior; Post, posterior.

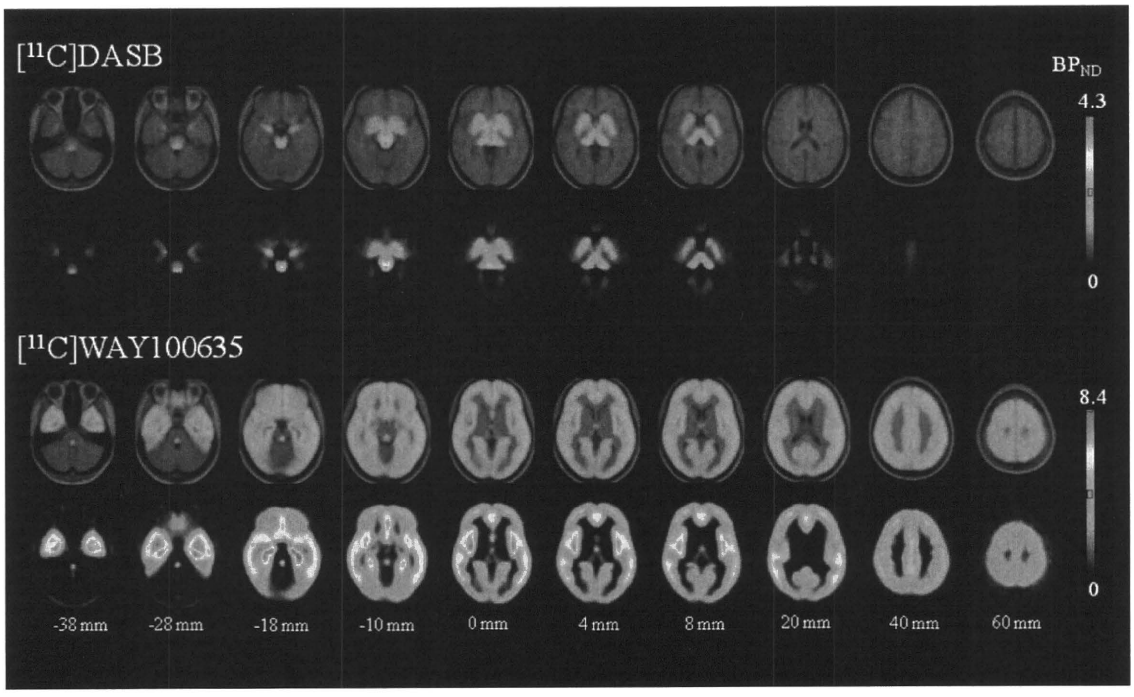

Fig. 3. Averaged BP_{ND} images with anatomical standardization of both radiotracers (axial planes). From the top row to the bottom: fused [^{11}C]WAY100635 and MRI, only [^{11}C]WAY100635, fused [^{11}C]DASB and MRI, only [^{11}C]DASB. In left to right columns: from ventral to dorsal planes of transverse images. L denotes left side of the brain; R denotes right side. Color bar indicates the value of BP_{ND}.

TABLE II. BPND values for each VOI

Region	[^{11}C]DASB			[^{11}C]WAY100635				
	Total	Right	Left	Total	Right	Left	r	P
Dorsal raphe nucleus	3.10 ± 0.97			2.17 ± 0.54			−0.191	0.464
Striatum	1.42 ± 0.28	1.40 ± 0.27	1.43 ± 0.30	1.42 ± 0.39	1.23 ± 0.38	1.58 ± 0.53	−0.333	0.192
Putamen	1.46 ± 0.29	1.45 ± 0.28	1.47 ± 0.30	1.72 ± 0.47	1.48 ± 0.48	1.94 ± 0.66	−0.368	0.146
Caudate head	1.25 ± 0.30	1.20 ± 0.28	1.29 ± 0.35	0.32 ± 0.23	0.29 ± 0.24	0.34 ± 0.25	0.208	0.423
Globus Pallidus	1.15 ± 0.36	1.14 ± 0.48	1.15 ± 0.31	0.35 ± 0.20	0.30 ± 0.22	0.40 ± 0.24	−0.446	0.073
Thalamus	1.88 ± 0.48	1.89 ± 0.53	1.87 ± 0.44	0.95 ± 0.27	1.03 ± 0.33	0.87 ± 0.26	−0.210	0.420
Anterior nucleus	1.93 ± 0.48	2.01 ± 0.57	1.84 ± 0.41	1.38 ± 0.41	1.37 ± 0.60	1.38 ± 0.34	−0.407	0.105
Dorsomedial nucleus	2.01 ± 0.51	2.08 ± 0.55	1.93 ± 0.50	1.23 ± 0.45	1.30 ± 0.55	1.16 ± 0.51	−0.328	0.199
Pulvinar	2.18 ± 0.73	2.03 ± 0.71	2.31 ± 0.79	0.89 ± 0.41	1.02 ± 0.43	0.77 ± 0.51	−0.079	0.762
Hippocampal complex	0.83 ± 0.18	0.83 ± 0.18	0.82 ± 0.19	6.30 ± 0.97	6.27 ± 1.03	6.33 ± 0.95	−0.306	0.232
Uncus	1.20 ± 0.27	1.18 ± 0.26	1.23 ± 0.29	5.78 ± 1.05	5.90 ± 1.10	5.66 ± 1.06	−0.268	0.297
Hippocampus	0.61 ± 0.18	0.58 ± 0.22	0.65 ± 0.17	7.01 ± 1.34	7.34 ± 1.55	6.71 ± 1.36	−0.226	0.383
Parahippocampus	0.52 ± 0.12	0.54 ± 0.13	0.51 ± 0.13	6.61 ± 1.03	6.36 ± 1.10	6.82 ± 1.11	−0.322	0.208
Insula	0.70 ± 0.17	0.75 ± 0.19	0.65 ± 0.15	6.65 ± 1.13	6.64 ± 1.14	6.67 ± 1.20	*−0.506	0.038
Anterior cingulate	0.45 ± 0.09	0.42 ± 0.11	0.47 ± 0.10	5.39 ± 0.83	5.46 ± 0.93	5.33 ± 0.90	*−0.539	0.026
Posterior cingulate	0.35 ± 0.07	0.37 ± 0.07	0.33 ± 0.10	4.76 ± 0.69	4.91 ± 0.73	4.57 ± 0.81	*−0.484	0.049
Base side of frontal cortex	0.21 ± 0.07	0.22 ± 0.08	0.20 ± 0.06	4.45 ± 0.76	4.54 ± 0.80	4.36 ± 0.75	*−0.539	0.026
Frontal convexity	0.20 ± 0.06	0.20 ± 0.06	0.19 ± 0.06	4.55 ± 0.82	4.53 ± 0.78	4.56 ± 0.87	−0.464	0.061
Lateral temporal	0.25 ± 0.05	0.26 ± 0.06	0.23 ± 0.05	5.73 ± 0.86	5.70 ± 0.86	5.76 ± 0.87	*−0.603	0.010
Occipital cuneus	0.39 ± 0.10	0.38 ± 0.11	0.40 ± 0.11	2.59 ± 0.51	2.55 ± 0.56	2.62 ± 0.51	−0.433	0.083
Parietal	0.12 ± 0.04	0.13 ± 0.04	0.12 ± 0.04	4.54 ± 0.71	4.61 ± 0.73	4.46 ± 0.74	*−0.718	0.001

r indicates Pearson's correlation coefficients between total BPND of [^{11}C]DASB and that of [^{11}C]WAY100635 for each VOI.
*Indicates $P < 0.05$.

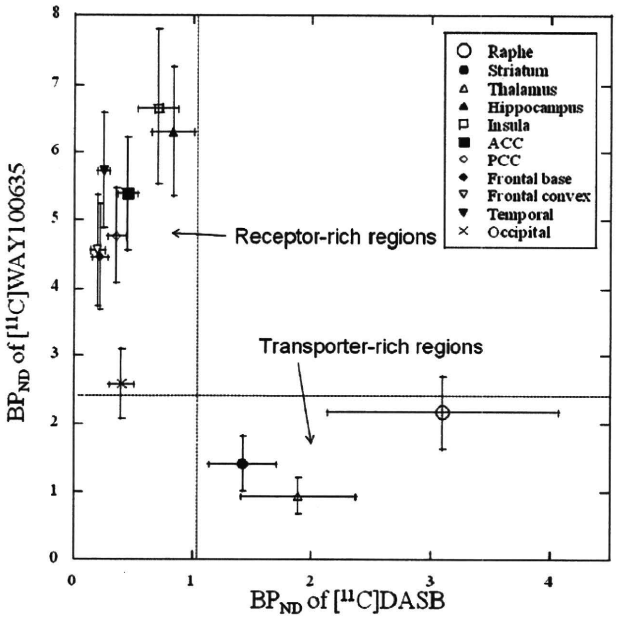

Fig. 4. Relationships between mean BP_{ND} values of [^{11}C]DASB and those of [^{11}C]WAY100635. The error bars indicate standard deviation.

indicating no apparent laterality in the distribution of 5-HTT and 5-HT_{1A} (paired t-test, data not shown). Moreover, no significant correlation between age and the binding of 5-HTT and 5-HT_{1A} was found in any of the brain regions within the age range (20–40 yr) of our sample (Pearson's correlation coefficient, $P > 0.05$, data not shown). In addition, with regard to the intraindividual relationship between the binding of both [^{11}C]DASB and [^{11}C]WAY100635 in the same region of the brain, significant negative correlations were observed in the anterior and posterior cingulate, insula, basal side of the frontal cortex, lateral temporal cortex, and parietal cortex (Table II and Fig. 5), whereas no significant positive correlation was observed.

DISCUSSION

We constructed a database of 5-HTT and 5-HT_{1A} serotonergic function by using parametric images generated by the anatomic standardization of the brains of 17 healthy young men. Anatomical standardization enables visualization of the entire neuroanatomy along the same coordinates with the help of multiple radiotracers that permit inter- and intrasubject comparisons. In the current study, the distributions of the two serotonergic markers differed. In addition, an exploratory study revealed significant inverse linear correlations between BP_{ND} of presynaptic 5-HTT and that of postsynaptic 5-HT_{1A} receptor binding in certain areas of the brain, such as the frontal, temporal, and parietal cortices; insula; and anterior and posterior cingulate.

Overall, the distribution patterns of 5-HTT and 5-HT_{1A} binding in the human brain were in accordance with those observed in previous human PET and postmortem studies (Brust et al., 2006; Drevets et al., 2007; Hall et al., 1997; Hoyer et al., 1986; Ito et al., 1999; Kish et al., 2005; Laruelle et al., 1988; Pazos et al., 1987; Rabiner et al., 2002; Savitz et al., 2009; Stockmeier, 2003; Varnas et al., 2004).

Localization of 5-HTT and 5-HT_{1A} in respective regions of the brain

In general, distributions in 5-HTT and 5-HT_{1A} receptors are quite different, and can be categorized into two groups, 5-HTT-rich regions and 5-HT_{1A} receptor-rich regions (Fig. 4). In 5-HT_{1A} receptor-rich regions such as the cerebral cortex and limbic

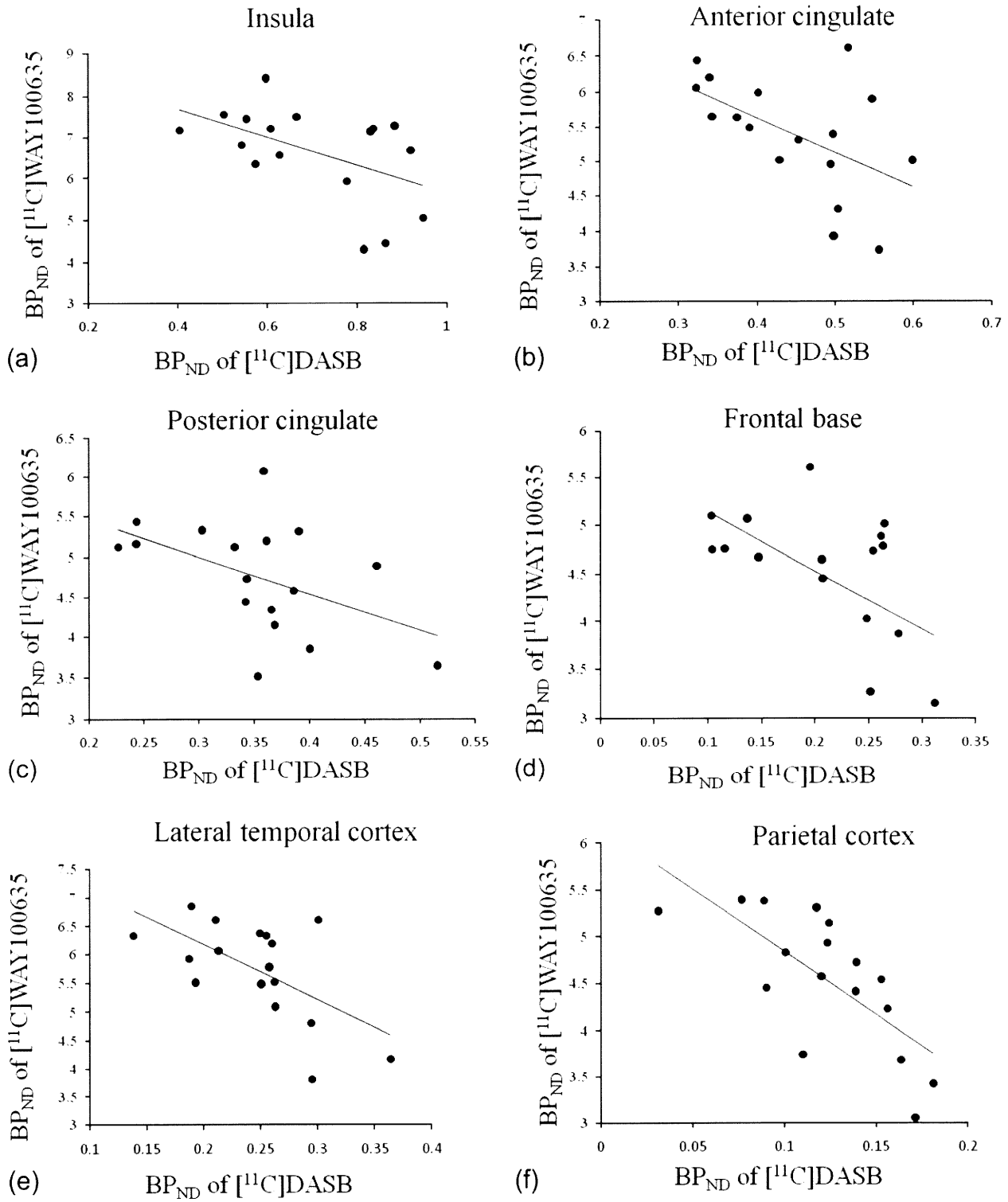

Fig. 5. Areas showing significant correlations of BP_{ND} values between [^{11}C]DASB and [^{11}C]WAY100635 in intrasubject comparisons. (a) insula, (b) anterior cingulate, (c) posterior cingulate, (d) base side of frontal cortex, (e) lateral temporal cortex, (f) parietal cortex.

regions, they tend to positively correlate with each other except the occipital cortex. This finding could reflect innervation of serotonergic fibers in those regions.

5-HTT-rich region

Brain stem. High binding to 5-HTT was observed in large areas of the midbrain, and it continuously

extended to the thalamus; this finding indicated rich distribution in these regions. In contrast, moderate binding to 5-HT_{1A} receptors was observed only in the dorsal and medial raphe, with this distribution indicating that 5-HT_{1A} receptor exists only in these regions of the midbrain (the sagittal section, middle column in the bottom row in Figure 2; the transverse sections, the first to fourth columns in the second row from the bottom; further, refer to the volume of interest 6 in Figure 1, which represents the raphe nuclei). These distribution patterns of 5-HTT and 5-HT_{1A} were consistent with those observed in previous human postmortem autoradiography studies (Hall et al., 1997; Varnas et al., 2004). In the present study, however, 5-HT_{1A} binding was not very high as compared with that in other areas (Table II). For example, the mean BP_{ND} value in the dorsal raphe was approximately one-third of that in the hippocampus, which exhibited the highest value of all regions examined. Further, in a detailed human autoradiography study with [^{3}H]WAY100635, binding to the dorsal raphe was highest (140 pmol/g tissue), followed by the hippocampus (123 pmol/g tissue). However, this discrepancy may be a result of the limited spatial resolution of the PET scanner, resulting in excessive spillover from the raphe and other small structures.

Subcortical regions. In the striatum (putamen and caudate) and globus pallidus, relatively high levels of binding to 5-HTT were observed. In contrast, 5-HT_{1A} receptor binding was very low or absent in the caudate nucleus and globus pallidus, while a low level of binding was found in the putamen. However, postmortem autoradiography studies showed very low levels of binding in the putamen, similar to those in the caudate and globus pallidus (Hall et al., 1997; Varnas et al., 2004); the high BP_{ND} values in the putamen may be attributed to spillover from the insula, which exhibits a very high level of 5-HT_{1A} binding.

The level of 5-HTT binding in the thalamus was the second highest among all brain regions, after the dorsal raphe nucleus; further, there was no marked difference in 5-HTT distribution among the subregions of the thalamus. These findings were similar to those of a previous human postmortem study (Varnas et al., 2004). On the other hand, the level of binding to the 5-HT_{1A} receptor was very low in the thalamus, although a little binding was observed in its medial parts regions (Table II and Figs. 2–4); this finding was similar to those of previous autoradiography studies showing much less or no binding in these regions (Hall et al., 1997; Varnas et al., 2004).

5-HT_{1A} receptor-rich region

Cerebral neocortex. Relatively high binding to 5-HT_{1A} and low binding to 5-HTT were observed in the neocortical regions. The 5-HT_{1A} receptor was widely distributed in the cerebral cortex but sparsely in the occipital cortex. The distribution of 5-HTT was very low in the neocortical regions as compared with other brain regions, but was found to be homogeneous among the cortical regions (Figs. 2–4, Table II). These findings were consistent with those of previous postmortem autoradiography studies (Hall et al., 1997; Laruelle et al., 1988; Varnas et al., 2004).

Limbic regions. In the hippocampal regions that include the parahippocampus, hippocampus, and uncus (amygdala), highest binding to 5-HT_{1A} and moderate binding to 5-HTT were observed (Figs. 2–4, Table II). Postmortem autoradiography studies in humans revealed that the highest binding to 5-HT_{1A} was observed particularly over the CA1 field in the hippocampus (Hall et al., 1997; Varnas et al., 2004). In contrast, binding to 5-HTT was higher in the uncus as compared to other hippocampal regions, a finding in agreement with a previous postmortem study (Varnas et al., 2004).

Within the cingulate cortex, both 5-HTT and 5-HT_{1A} binding can be described in descending order as follows: ventral (subcallosal) cingulate > anterior cingulate > posterior cingulate (Table II and Figs 2 and 3). In particular, the ventral cingulate is thought to be involved in the regulation of emotions and has been repeatedly reported to be associated with depression (Drevets et al., 2008; Seminowicz et al., 2004).

Very high 5-HT_{1A} binding and intermediate 5-HTT binding were observed in the insula. These distributions are in accordance with those found in previous human postmortem autoradiography studies (Varnas et al., 2004), although BP_{ND} values in the insula can be affected by spillover from the striatum, which exhibits high levels of 5-HTT binding.

Our findings together suggest that the limbic regions, including the hippocampal area, cingulate cortex, and insula, are relatively rich in serotonergic innervation. Thus, serotonergic transmission in these regions might play a pivotal role in modulating emotion and cognition.

Intraindividual relationship between the binding of both [^{11}C]DASB and [^{11}C]WAY100635 in the same region of the brain

With respect to the intraindividual relationship between regional 5-HTT and 5-HT_{1A} distribution, we found significant negative linear correlations between the binding of [^{11}C]DASB and [^{11}C]WAY100635 in the insula; anterior and posterior cingulate; and lateral temporal, frontal base, and parietal cortices. This result suggests that serotonergic transmission might be modulated by a cooperative relationship between 5-HTT and 5-HT_{1A}.

There have been only a few reports on pre- and postsynaptic serotonergic functions in individuals. In a study of 12 men, Lundberg et al. (2007) showed a positive linear correlation between 5-HTT and 5-HT_{1A} binding in the raphe nuclei and hippocampal complex using [^{11}C]MADAM and [^{11}C]WAY100635, respectively; however, a positive linear correlation was not observed in the frontal cortex. Another study that examined gender differences in binding using [^{11}C]MADAM and [^{11}C]WAY100635 also reported an interrelationship between 5-HTT and 5-HT_{1A} receptors (Jovanovic et al., 2008). They found a significant positive correlation between BP_{ND} of 5-HTT and 5-HT_{1A} receptors in the hippocampus of eight women, whereas no significant correlation was observed in seven men. The discrepancy between their results and ours could be attributed to the use of different 5-HTT radiotracers, sample size, and subjects' background. In particular, the age range of subjects in the study by Lundberg et al. was wider than ours, and different degrees of atrophy can cause positive correlation in BP_{ND} between 5-HTT and 5-HT_{1A} receptor. This is because older subjects are likely to have more brain atrophy, because of which both BP_{ND} values are lower than those of young subjects. Our results suggest that subjects exhibiting higher 5-HTT binding are likely to have less 5-HT_{1A} receptor binding and vice versa. One possible interpretation of this inter-subject difference is that individuals with higher 5-HT synthesis and release show a decrease in the 5-HT_{1A} receptor function to dampen the transmission at the postsynaptic site and an increase in 5-HTT function in order to reuptake more 5-HT at the presynaptic site, whereas individuals with lower 5-HT synthesis and release show an increase in the 5-HT_{1A} receptor function and a decrease in 5-HTT function to reuptake less 5-HT; that is, pre- and postsynaptic 5-HT functions are modulated cooperatively to compensate the overall 5-HT transmission. However, these studies were done under resting condition, and therefore challenge study designs using drugs or stress may help to better understand the relationship between pre- and postsynaptic functions.

Limitation

There are several limitations to the current study. First, the two PET studies were not always performed on the same day. Good reproducibility for both ligands (Kim et al., 2006; Rabiner et al., 2002), however, has been demonstrated, and it has been shown that the endogenous 5-HT level has no direct effect on binding (Rabiner et al., 2002; Talbot et al., 2005); hence, it is unlikely that the endogenous level affected the results. Second, the sample size was small, especially for a correlation study between pre- and postsynaptic functions, and our study should necessarily be regarded as a preliminary one. Third, we focused only on 5-HTT and 5-HT_{1A} receptors because they reportedly play pivotal roles in serotonergic functions. However, there are more than 10 receptor systems in the brain that affect serotonergic transmission. In addition, monoamine oxidase as well as 5-HTT are known to control the 5-HT concentration in the synapse (Nestler et al., 2008). Finally, the current study includes only young men, and we should expand it to women and individuals of a wider range of ages. Such a database will be helpful even for clinical studies on depression and anxiety disorders, since these disorders are known to show different prevalence and clinical features depending on gender and age (Fernandez et al., 1995; Gorman, 2006; Pigott, 1999). The physiological differences in the 5-HT system based on gender and age might explain the characteristics of depression and anxiety disorders.

In summary, we constructed a normal database to elucidate regional distributions of 5-HT_{1A} and 5-HTT binding. The neuroanatomy of the 5-HT_{1A} and 5-HTT serotonergic systems was discussed mostly by comparing our findings with those of previous postmortem studies. Furthermore, we explored the linear negative correlation between pre- and postsynaptic functions in certain parts of the brain. The results obtained indicate the involvement of a cooperative or complementary process in serotonergic transmission. Further studies are required to elucidate the modulation of 5-HT transmission in neuropsychiatric disorders and to clarify the various serotonergic systems involving pre- and postsynaptic functions in different regions of the brain.

ACKNOWLEDGMENTS

The authors thank Mr. Katuyuki Tanimoto, Mr. Takahiro Shiraishi, and Mr. Akira Ando for their assistance in performing PET examinations at the National Institute of Radiological Sciences. The authors are also grateful to Ms. Yoshiko Fukushima for the help as a clinical research coordinator.

REFERENCES

Brust P, Hesse S, Muller U, Szabo Z. 2006. Neuroimaging of the serotonin transporter. Curr Psychiatry Rev 2:111–149.
Cooper JR, Bloom FE, Roth RH. 2002. The biochemical basis of neuropharmacology, 8th ed. New York, USA: Oxford University Press.
Diksic M, Young SN. 2001. Study of the brain serotonergic system with labeled alpha-methyl-L-tryptophan. J Neurochem 78:1185–1200.
Drevets WC, Savitz J, Trimble M. 2008. The subgenual anterior cingulate cortex in mood disorders. CNS Spectr 13:663–681.
Drevets WC, Thase ME, Moses-Kolko EL, Price J, Frank E, Kupfer DJ, Mathis C. 2007. Serotonin-1A receptor imaging in recurrent depression: replication and literature review. Nucl Med Biol 34:865–877.
Fernandez F, Levy JK, Lachar BL, Small GW. 1995. The management of depression and anxiety in the elderly. J Clin Psychiatry 56(Suppl 2):20–29.
Gorman JM. 2006. Gender differences in depression and response to psychotropic medication. Gend Med 3:93–109.

Gunn RN, Lammertsma AA, Hume SP, Cunningham VJ. 1997. Parametric imaging of ligand-receptor binding in PET using a simplified reference region model. Neuroimage 6:279–287.

Gunn RN, Sargent PA, Bench CJ, Rabiner EA, Osman S, Pike VW, Hume SP, Grasby PM, Lammertsma AA. 1998. Tracer kinetic modeling of the 5-HT1A receptor ligand [carbonyl-^{11}C]WAY-100635 for PET. Neuroimage 8:426–440.

Hall H, Lundkvist C, Halldin C, Farde L, Pike VW, McCarron JA, Fletcher A, Cliffe IA, Barf T, Wikstrom H, Sedvall G. 1997. Autoradiographic localization of 5-HT$_{1A}$ receptors in the post-mortem human brain using [^{3}H]WAY-100635 and [^{11}C]way-100635. Brain Res 745:96–108.

Hoyer D, Hannon JP, Martin GR. 2002. Molecular, pharmacological and functional diversity of 5-HT receptors. Pharmacol Biochem Behav 71:533–554.

Hoyer D, Pazos A, Probst A, Palacios JM. 1986. Serotonin receptors in the human brain. I. Characterization and autoradiographic localization of 5-HT$_{1A}$ recognition sites. Apparent absence of 5-HT$_{1B}$ recognition sites. Brain Res 376:85–96.

Ichise M, Liow JS, Lu JQ, Takano A, Model K, Toyama H, Suhara T, Suzuki K, Innis RB, Carson RE. 2003. Linearized reference tissue parametric imaging methods: application to [^{11}C]DASB positron emission tomography studies of the serotonin transporter in human brain. J Cereb Blood Flow Metab 23:1096–1112.

Ichise M, Toyama H, Innis RB, Carson RE. 2002. Strategies to improve neuroreceptor parameter estimation by linear regression analysis. J Cereb Blood Flow Metab 22:1271–1281.

Innis RB, Cunningham VJ, Delforge J, Fujita M, Gjedde A, Gunn RN, Holden J, Houle S, Huang SC, Ichise M, Iida H, Ito H, Kimura Y, Koeppe RA, Knudsen GM, Knuuti J, Lammertsma AA, Laruelle M, Logan J, Maguire RP, Mintun MA, Morris ED, Parsey R, Price JC, Slifstein M, Sossi V, Suhara T, Votaw JR, Wong DF, Carson RE. 2007. Consensus nomenclature for in vivo imaging of reversibly binding radioligands. J Cereb Blood Flow Metab 27:1533–1539.

Ito H, Halldin C, Farde L. 1999. Localization of 5-HT$_{1A}$ receptors in the living human brain using [carbonyl-^{11}C]WAY-100635: PET with anatomic standardization technique. J Nucl Med 40:102–109.

Ito H, Takahashi H, Arakawa R, Takano H, Suhara T. 2008. Normal database of dopaminergic neurotransmission system in human brain measured by positron emission tomography. Neuroimage 39:555–565.

Jovanovic H, Lundberg J, Karlsson P, Cerin A, Saijo T, Varrone A, Halldin C, Nordstrrom A-L. 2008. Sex differences in the serotonin 1A receptor and serotonin transporter binding in the human brain measured by PET. Neuroimage 39:1408–1419.

Kim JS, Ichise M, Sangare J, Innis RB. 2006. PET imaging of serotonin transporters with [^{11}C]DASB: test-retest reproducibility using a multilinear reference tissue parametric imaging method. J Nucl Med 47:208–214.

Kish SJ, Furukawa Y, Chang LJ, Tong J, Ginovart N, Wilson A, Houle S, Meyer JH. 2005. Regional distribution of serotonin transporter protein in postmortem human brain: is the cerebellum a SERT-free brain region? Nucl Med Biol 32:123–128.

Kitson SL. 2007. 5-hydroxytryptamine (5-HT) receptor ligands. Curr Pharm Des 13:2621–2637.

Kumar JSD, Mann JJ. 2007. PET tracers for 5-HT(1A) receptors and uses thereof. Drug Discov Today 12:748–756.

Lammertsma AA, Hume SP. 1996. Simplified reference tissue model for PET receptor studies. Neuroimage 4:153–158.

Laruelle M, Vanisberg MA, Maloteaux JM. 1988. Regional and subcellular localization in human brain of [^{3}H]paroxetine binding, a marker of serotonin uptake sites. Biol Psychiatry 24:299–309.

Lundberg J, Borg J, Halldin C, Farde L. 2007. A PET study on regional coexpression of 5-HT$_{1A}$ receptors and 5-HTT in the human brain. Psychopharmacology 195:425–433.

Lundquist P, Blomquist G, Hartvig P, Hagberg GE, Torstenson R, Hammarlund-Udenaes M, Langstrom B. 2006. Validation studies on the 5-hydroxy-L-[beta-^{11}C]-tryptophan/PET method for probing the decarboxylase step in serotonin synthesis. Synapse 59:521–531.

Marner L, Gillings N, Comley RA, Baare WF, Rabiner EA, Wilson AA, Houle S, Hasselbalch SG, Svarer C, Gunn RN, Laruelle M, Knudsen GM. 2009. Kinetic modeling of ^{11}C-SB207145 binding to 5-HT$_{4}$ receptors in the human brain in vivo. J Nucl Med 50:900–908.

Meyer JH. 2007. Imaging the serotonin transporter during major depressive disorder and antidepressant treatment. J Psychiatry Neurosci 32:86–102.

Moresco RM, Matarrese M, Fazio F. 2006. PET and SPET molecular imaging: Focus on serotonin system. Curr Top Med Chem 6:2027–2034.

Nestler E, Hyman S, Malenka R. 2008. Molecular neuropharmacology: A foundation for clinical neuroscience, 2nd ed. New York, USA: McGraw-Hill Professional.

Pazos A, Probst A, Palacios JM. 1987. Serotonin receptors in the human brain. III. Autoradiographic mapping of serotonin-1 receptors. Neuroscience 21:97–122.

Pigott TA. 1999. Gender differences in the epidemiology and treatment of anxiety disorders. J Clin Psychiatry 60(Suppl 18):4–15.

Rabiner EA, Messa C, Sargent PA, Husted-Kjaer K, Montgomery A, Lawrence AD, Bench CJ, Gunn RN, Cowen P, Grasby PM. 2002. A database of [^{11}C]WAY-100635 binding to 5-HT$_{1A}$ receptors in normal male volunteers: Normative data and relationship to methodological, demographic, physiological, and behavioral variables. Neuroimage 15:620–632.

Savitz J, Lucki I, Drevets WC. 2009. 5-HT$_{1A}$ receptor function in major depressive disorder. Prog Neurobiol 88:17–31.

Seminowicz DA, Mayberg HS, McIntosh AR, Goldapple K, Kennedy S, Segal Z, Rafi-Tari S. 2004. Limbic-frontal circuitry in major depression: A path modeling metanalysis. Neuroimage 22:409–418.

Stockmeier CA. 2003. Involvement of serotonin in depression: Evidence from postmortem and imaging studies of serotonin receptors and the serotonin transporter. J Psychiatr Res 37:357–373.

Suhara T, Sudo Y, Yoshida K, Okubo Y, Fukuda H, Obata T, Yoshikawa K, Suzuki K, Sasaki Y. 1998. Lung as reservoir for antidepressants in pharmacokinetic drug interactions. Lancet 351:332–335.

Talbot PS, Frankle WG, Hwang DR, Huang Y, Suckow RF, Slifstein M, Abi-Dargham A, Laruelle M. 2005. Effects of reduced endogenous 5-HT on the in vivo binding of the serotonin transporter radioligand ^{11}C-DASB in healthy humans. Synapse 55:164–175.

Varnas K, Halldin C, Hall H. 2004. Autoradiographic distribution of serotonin transporters and receptor subtypes in human brain. Hum Brain Mapp 22:246–260.

International Journal of Neuropsychopharmacology (2010), **13**, 943–950. Copyright © CINP 2010
doi:10.1017/S1461145710000313

ARTICLE

CINP

Peripheral benzodiazepine receptors in patients with chronic schizophrenia: a PET study with [^{11}C]DAA1106

Akihiro Takano[1], Ryosuke Arakawa[1], Hiroshi Ito[1], Amane Tateno[2], Hidehiko Takahashi[1], Ryohei Matsumoto[1], Yoshiro Okubo[2] and Tetsuya Suhara[1]

[1] *Molecular Neuroimaging Group, Molecular Imaging Center, National Institute of Radiological Sciences, Chiba, Japan*
[2] *Department of Neuropsychiatry, Nippon Medical School, Tokyo, Japan*

Abstract

Inflammatory/immunological process and glial contribution are suggested in the pathophysiology of schizophrenia. We investigated peripheral benzodiazepine receptors in brains of patients with chronic schizophrenia, which were reported to be located on mitochondria of glial cells, using [^{11}C]DAA1106 with positron emission tomography. Fourteen patients and 14 age- and sex-matched normal controls participated in this study. PET data were analysed by two-tissue compartment model with metabolite-corrected plasma input. Clinical symptoms were assessed using the Positive and Negative Syndrome Scale. There was no significant difference between [^{11}C]DAA1106 binding of the cortical regions of normal controls and patients with schizophrenia, whereas the patients showed a positive correlation between cortical [^{11}C]DAA1106 binding and positive symptom scores. There was also a positive correlation between [^{11}C]DAA1106 binding and duration of illness. Although the correlations need to be interpreted very cautiously, involvement of glial reaction process in the pathophysiology of positive symptoms or progressive change of schizophrenia might be suggested.

Received 18 November 2009; Reviewed 14 December 2009; Revised 29 January 2010; Accepted 27 February 2010; First published online 30 March 2010

Key words: Microglia, peripheral benzodiazepine receptor, positive symptoms, schizophrenia.

Introduction

An accumulating body of evidence has suggested that the pathophysiology of schizophrenia could be related to the dysregulation of the inflammatory response system, such as increased levels of *in vivo* IL-1RA, sIL-2R, and IL-6 (Lin *et al.* 1998; Nawa & Takei, 2006; Potvin *et al.* 2008; Zhang *et al.* 2004). Microglia has been regarded as a mediator of neuroinflammation via the release of pro-inflammatory cytokines, nitric oxide (NO) and reactive oxygen species (ROS) in the central nervous system (CNS). Peripheral benzodiazepine receptor (PBR) was reported to reflect neuronal injury and inflammatory lesions in the brain by increased expression of the number of binding sites in glial cells including activated microglia and reactive astrocytes as visualized *in vivo* using PET with [^{11}C]PK11195 (Shah *et al.* 1994). Recent reports demonstrated that [^{11}C]PK11195 binding was increased in patients with acute-onset schizophrenia (van Berckel *et al.* 2008) and in patients with schizophrenia during psychosis (Doorduin *et al.* 2009). However, the affinity (Chaki *et al.* 1999) and permeability of the blood–brain barrier was low for PK11195, reportedly a substrate of efflux transporter P-glycoprotein (Jakubikova *et al.* 2002; Vaalburg *et al.* 2005). Low uptake of [^{11}C]PK11195 in the brain could hamper stable quantitative analysis.

(*N*-5-fluoro-2-phenoxyphenyl)-*N*-(2,5-dimethoxyl-benzyl) acetamide (DAA1106) is a potent and selective ligand for PBR with high affinity (Chaki *et al.* 1999; Okuyama *et al.* 1999). [^{11}C]DAA1106 is accumulated at high levels in the mouse brain (Zhang *et al.* 2003), and the radioactivity of [^{11}C]DAA1106 at 30 min after injection was reported to be four times higher than that of [^{11}C]PK11195 in the monkey brain (Maeda *et al.* 2004). A quantitative analysis method for [^{11}C]DAA1106 binding in the human brain has been well established with the two-tissue compartment model (Ikoma *et al.* 2007). [^{11}C]DAA1106 was

Address for correspondence: T. Suhara, M.D., Ph.D., Molecular Neuroimaging Group, Molecular Imaging Center, National Institute of Radiological Sciences, 4-9-1, Anagawa, Inage-ku, Chiba, 263-8555, Japan.
Tel.: +81-43-206-3251 *Fax*: +81-43-253-0396
Email: suhara@nirs.go.jp