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The Striatal and Extrastriatal D2/D3 Receptor-Binding Profile of Clozapine in Patients with Schizophrenia

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Positron emission tomography (PET) studies reveal that clozapine at clinically used doses occupies less than 60% of D_2/D_3 dopamine receptors in human striatum. Here, the occupancy of D_2/D_3 dopamine receptors by clozapine in patients with schizophrenia was determined to test the hypothesis that clozapine binds preferentially to extrastriatal dopamine receptors. A total of 15 clozapine-treated inpatients with schizophrenia underwent a [^{18}F]fallypride PET scan. Receptor occupancy was calculated as percent reduction in binding potential relative to unblocked values measured in seven normal volunteers. Mean D_2/D_3 receptor occupancy was statistically significantly higher in cortical (inferior temporal cortex 55%) than in striatal regions (putamen 36%, caudate 43%, $p<0.005$). While the maximum attainable receptor occupancy E_{max} approached 100% both in the striatum and cortex, the plasma concentration at 50% of E_{max} (ED_{50}) was much higher in the putamen (950 ng/ml) than in the inferior temporal cortex (333 ng/ml). Clozapine binds preferentially to cortical D_2/D_3 receptors over a wide range of plasma concentrations. This selectivity is lost at extremely high plasma levels. Occupancy of cortical receptors approaches 60% with plasma clozapine in the range 350–400 ng/ml, which corresponds to the threshold for antipsychotic efficacy of clozapine. Extrastriatal binding of clozapine may be more relevant to its antipsychotic actions than striatal. However, further studies with an intraindividual comparison of untreated *vs* treated state are desirable to confirm this finding.
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INTRODUCTION

Until recently, the occupancy of D_2-like dopamine receptors by antipsychotic drugs was determined in striatal structures only. With positron emission tomography (PET) methodology, Farde *et al* (1992) demonstrated that clinically effective doses of typical neuroleptics occupy D_2-like dopamine receptors in the range between 65 and 90%. The suggestion of a 'therapeutic window' between 60 and 80% striatal D_2 receptor occupancy for sufficient treatment response and a 'ceiling' of around 80% occupancy for extrapyramidal side effects (EPS) was later confirmed by a number of other groups (Kapur *et al*, 2000a). This rule seems to apply also for most of the 'atypical' antipsychotics (Kapur *et al*, 1999), but not for partial agonists (Gründer *et al*, 2003a). Interestingly, the early PET studies with clozapine demonstrated that clozapine occupies striatal D_2 receptors to a significantly lesser extent than other antipsychotics, with the exception of quetiapine (Nordström *et al*, 1995; Kapur *et al*, 2000b, 1999). It was concluded that the low frequency of EPS observed under treatment with clozapine may be a reflection of the comparatively low D_2 receptor occupancy induced by clinical doses of this drug.

With the availability of high-affinity radiotracers belonging to the class of substituted benzamides, it became possible to visualize and quantify extrastriatal dopamine receptors. However, the first studies in drug-treated schizophrenic patients performed with these ligands revealed controversial results with regard to the differential occupancy of striatal *vs* extrastriatal regions, which has been attributed to tracer characteristics and methodological

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pitfalls. Using [^{123}I]epidepride SPECT, Pilowsky *et al* (1997) demonstrated a higher occupancy of temporal cortical than striatal D_2 receptors by clozapine. This group reported the same observation for a number of other atypical antipsychotics (Bigliani *et al*, 2000; Bressan *et al*, 2003). On the other hand, using [^{11}C]FLB-457 PET, the Karolinska group could not detect preferential extrastriatal binding of clozapine (Talvik *et al*, 2001). However, since striatal occupancy is not reliably quantifiable with [^{11}C]FLB-457, they used [^{11}C]raclopride for determination of striatal occupancy. Olsson and Farde (2001) suggested that the finding of preferential mesolimbic binding by clozapine is an artifact resulting from underestimation of striatal D_2 receptor occupancy. This is in line with the observation that striatal D_2 receptor occupancy is markedly underestimated when a high-affinity radiotracer ([^{76}Br]FLB-457) is used, and striatal and extrastriatal D_2 receptor occupancy is determined at the same early time after radiotracer injection (Xiberas *et al*, 2001; Vernaleken *et al*, 2004). On the other hand, it has been argued that cortical binding is underestimated with [^{11}C]FLB-457 (Erlandsson *et al*, 2003). Thus, previous methods and study designs were not completely adequate to resolve the uncertainty in determining the D_2/D_3 receptor occupancy in striatal *vs* extrastriatal regions.

Substituted benzamides with a fluorine-18 label have been developed for broader clinical use, because the fluorine-18 label offers the advantage of a longer half-life compared to the carbon-11 label of, for example, [^{11}C]raclopride. Two of the most promising compounds are [^{18}F]fallypride and [^{18}F]desmethoxyfallypride, with [^{18}F]fallypride having a higher affinity for D_2-like dopamine receptors (IC_{50} 0.6 *vs* 15.0 nM) (Gründer *et al*, 2003b; Mukherjee *et al*, 2001). [^{18}F]fallypride is an ideal tracer for the study of both striatal and extrastriatal receptors in a single PET scan. It has been consistently demonstrated in human studies that a dynamic scan of 180 min duration allows for establishment of a transient equilibrium both in extrastriatal and striatal brain regions (Mukherjee *et al*, 2002; Siessmeier *et al*, 2005). Furthermore, Mukherjee *et al* (2002) have shown that the test–retest variability in all brain regions is below 10%. Thus, the purpose of this [^{18}F]fallypride PET study was to determine the striatal and extrastriatal D_2/D_3 receptor-binding characteristics of the prototypic 'atypical' antipsychotic clozapine in patients with schizophrenia.

PATIENTS AND METHODS

The study was approved by the local ethics committee in Mainz, Germany, and the German radiation safety authorities. In all, 15 patients suffering from schizophrenia and a control group of seven healthy volunteers were included after giving written informed consent. All PET investigations were performed at the PET Center of the University of Mainz, Germany.

Subjects

Healthy comparison subjects. The control group consisted of seven male volunteers (23–41 years; mean $\pm$ SD: 32.0 $\pm$ 6.9). They were free of any relevant somatic complaint, psychiatric diagnosis, and medication. All control subjects received a physical and mental state examination, blood and urine analysis, electroencephalography, electrocardiography, and cerebral magnetic resonance imaging.

Patients. A total of 15 (10 males and five females) patients (25–47 years; mean $\pm$ SD: 36.0 $\pm$ 7.9) were included. The age of the patients did not significantly differ from that of the normals (Mann–Whitney's *U*-test: 35.5; $p = 0.24$). In all, 13 patients were diagnosed with schizophrenia and two with schizoaffective disorder according to DSM-IV. All patients received an ongoing, stable, daily dose of clozapine (100–500 mg/day) according to clinical needs for at least 6 weeks. No patient received any concomitant medication, with the exception of benzodiazepine or zolpidem treatment, respectively, in two patients and lithium treatment in a third. For further patient characteristics, see Table 1.

Radiochemistry

The [^{18}F]fallypride was synthesized as described for [^{18}F]desmethoxyfallypride (Gründer *et al*, 2003b). The tosylated precursor ((S)-*N*-[(1-allyl)-2-pyrrolidinyl)-methyl]-5-(3-toluenesulfonyloxy-propyl)-2,3-dimethoxybenzamide (5 mg, 10 μmol) was dissolved in 1 ml acetonitrile, treated for 5 min at 85°C with potassium carbonate (5 mg, 36 μmol), and subsequently reacted with [^{18}F]fluoride for 20 min at 85°C. [^{18}F]fallypride was isolated using high-performance liquid chromatography (HPLC) and adsorbed on a C18 cartridge, and the product eluted with 1 ml ethanol. The final fraction was diluted with 9 ml of an isotonic sodium chloride solution and sterilized by filtration.

Data Acquisition and Analysis

Images were acquired on a Siemens ECAT EXACT whole-body PET scanner. Data acquisition comprised of a series of 39 time frames (3×20 s, 3×1, 3×2, 3×3, 21×5, 2×8 and 4×10 min) with a total scan duration of 180 min. Following a 15-min transmission scan, a mean of 220 $\pm$ 43 MBq (mean $\pm$ SD) [^{18}F]fallypride was injected intravenously as a bolus. The specific activity at the time of injection was 101 $\pm$ 113 GBq/μmol (mean $\pm$ SD). The injected mass was 2.2 $\pm$ 2.0 μg (mean $\pm$ SD). Specific activities did not significantly differ between normal controls and patients treated with clozapine (controls: 112 $\pm$ 114 GBq/μmol; patients: 96 $\pm$ 116 GBq/μmol). Furthermore, the injected mass was not correlated with the measured binding potentials (BP) in any region, neither in healthy volunteers nor in patients. Thus, it is very unlikely that the radiotracer occupied a significant amount ($>5\%$) of receptors in brain regions with low receptor density.

BP were calculated on a voxelwise basis using the Lammertsma Simplified Reference Tissue Model, which is based on a two-tissue compartment model (Gründer *et al*, 2003b; Lammertsma and Hume, 1996). The cerebellum was chosen as a reference region, since it is generally considered dopamine receptor free. We cannot exclude the possibility that the occupancy values in our study are slightly underestimated due to a very small specific binding in the cerebellum (Mukherjee *et al*, 2001). Nevertheless, assuming that [^{11}C]FLB-457 and [^{18}F]fallypride have similar cerebellar

Table 1 Patient Characteristics, Clozapine Plasma Concentrations and Corresponding D_2-Like Dopamine Receptor Occupancies in Several Brain Regions as Determined with [^{18}F]Fallypride PET

					Plasma conc. (ng/ml)		D_2/D_3 receptor occupancy (%)					
Patient no.	Age	Gender	Clozapine dosing scheme (mg)	Interval (h) last dose/PET	Morning[a]	PET[b]	Putamen	Caudate	Inf. temp.	Thalamus	Amygdala	S. nigra
1	28	m	100–0–275–0	8	861	811	55	62	69	63	53	53
2	25	m	0–200–0–0	4	217	816	46	51	65	52	44	56
3	47	m	0–25–0–200	2	362	294	10	19	26	26	3	26
4	40	f	0–0–225–0	23	n.a.	404	34	44	62	50	56	63
5	37	m	0–50–0–250	0.5	378	268	32	38	53	36	47	22
6	34	f	0–200–0–0	4	406	498	40	50	26	47	48	28
7	35	m	0–100–0–0	4	890	1228	42	51	71	42	57	53
8	47	m	0–200–0–0	4	356	694	50	57	80	61	71	60
9	47	m	100–0–0–0	4	694	1079	51	55	74	57	51	56
10	41	f	0–0–300–0	18	376	754	62	66	77	57	62	64
11	33	m	0–0–150–250	18	151	151	23	34	68	30	37	35
12	38	f	0–200–0–0	4	586	849	57	64	72	50	59	52
13	25	m	0–0–250–250	14	599	657	34	41	40	52	50	34
14	42	f	0–0–0–150	16	150	181	0	0	20	5	0	0
15	26	m	0–0–75–100	16	215	246	6	18	27	36	4	23

Clozapine dosing scheme: clozapine was administered at four times: at 0800, 1200, 1800, 2100 h. m = male; f = female; plasma conc. = clozapine plasma concentration.
[a]Determined at 0800 h.
[b]Determined immediately before injection of the radiotracer.
Inf. temp. = inferior temporal cortex; S. nigra = substantia nigra.

binding characteristics, this underestimation should be less than 5% at the occupancy values that were studied (Olsson *et al*, 2004). This is a reasonable assumption given the similar structures of the two radioligands (Halldin *et al*, 1995; Mukherjee *et al*, 1995). Also, it is unlikely that lipophilic metabolites contribute to the occupancy measures reported in this study. Mukherjee *et al* (1995) could not detect specific receptor binding, when they extracted the main lipophilic metabolite of [^{18}F]fallypride from blood plasma of humans and incubated it in rat brain slices containing the striata. For determination of D_2 receptor occupancy, averaged BPs of control subjects were used as the common baseline value, based on the assumption that there is no difference in B_{max} between patients and healthy control subjects when measured with benzamide radiotracers (Farde *et al*, 1987; Hietala *et al*, 1994). BP images were stereotactically normalized for calculation of D_2/D_3 receptor occupancy. First, integrated images (summed images, 3′–19′ p.i.) were calculated and spatially normalized using SPM and a ligand-specific D_2 template (Buchholz *et al*, 2004). Transformation parameters of normalization were then applied to the individual BP image.

Calculation of D_2/D_3 Receptor Occupancy

The individual subject's receptor occupancy was defined as percentage reduction of BP relative to the baseline BP according to the following equation:

$$\text{Occupancy}\,[\%] = \left(1 - \frac{\text{BP}_{\text{Drug}}}{\text{BP}_{\text{Control}}}\right) \times 100$$

A fixed ROI template was defined to evaluate BP values from individual studies on the stereotactically normalized images comprising the following areas: putamen, caudate nucleus, thalamus, inferior temporal cortex, amygdala, and substantia nigra. The inferior temporal cortex (anterior and medial parts) was used as representative of cortical binding, because the D_2/D_3 receptor density in this region is highest compared to all other cortical regions (illustrated in Figure 3). The use of cortical brain regions with even lower D_2/D_3 receptor density such as the prefrontal cortex would have further increased the variability in the binding data (data not shown). A mean $BP_{Control}$ value for each ROI was then calculated by averaging BP values from seven volunteer studies. BP_{Drug} was calculated in an equivalent way for the 15 patient studies.

In order to illustrate regional differences in clozapine occupancy in an observer-independent manner, a parametric mean occupancy image was generated (Figure 3) based on the above-mentioned occupancy equation. To decrease noise, voxels with a ratio $BP_{Drug}/BP_{Control}$ exceeding a threshold <0.01 or >100, were excluded from calculation (resulting in exclusion of less than 5% of all voxels, mainly located in areas without relevant specific binding: cerebellum and occipital cortex). The occupancy value of these voxels was set to 0. Finally, the generated parametric image was smoothed with a Gaussian filter (filter width: 8 mm).

Clozapine Pharmacokinetic Data

Clozapine was usually administered in divided doses. In most cases, the largest portion of the daily dose was

administered in the evening. In order to induce very high clozapine plasma levels at the time of the PET scan, the total daily dose was administered 3 h (mean time from administration to t_{max}) before tracer injection in some patients. Dosing details for individual patients in relation to tracer injection are given in Table 1. Blood samples were collected at 0800 h (immediately before ingestion of clozapine, when a morning dose was administered) and again immediately before [^{18}F]fallypride bolus injection. The PET scans were started between 1200 and 1700 h. Clozapine and its demethylated metabolite were determined in serum by a previously published method using HPLC with column switching and quantified by ultraviolet (UV) spectroscopy at 254 nm (Weigmann *et al*, 2001). There was linear correlation between drug concentration and UV signal from 100 to at least 1000 ng/ml. The limits of quantification were below 53 ng/ml for clozapine and 51 ng/ml for *N*-desmethylclozapine. At therapeutic concentrations of clozapine, the intra- and inter-assay reproducibility of quality control samples was below 5%.

Statistical Analyses

Means and standard deviations were calculated for plasma concentrations and occupancy values. Unpaired *t*-tests were used to compare D_2/D_3 BP values for the healthy volunteers and the clozapine-treated patients. A general linear model for repeated measures with within-subjects factor at four levels was applied to compare D_2/D_3 receptor occupancy in four regions evaluated: putamen, caudate nucleus, thalamus, and inferior temporal cortex. Spearman rank correlations were calculated for relationships between clozapine doses and plasma concentrations and brain D_2/D_3 receptor occupancy values. Plasma concentrations (trough plasma levels in the morning and at time of injection) and D_2/D_3 receptor occupancy values were fit to a one-site ligand-binding model by nonlinear regression analysis using Sigma Plot, Version 8.0, to the following equation:

$$\text{Occupancy}\,[\%] = \frac{E_{max} \times [C_{Clz}]}{EC_{50} + [C_{Clz}]}$$

where E_{max} is the maximum attainable receptor occupancy, EC_{50} is the plasma concentration predicted to provide 50% of the maximum attainable receptor occupancy and C_{Clz} is the plasma concentration of clozapine. In all analyses, the two-tailed level of statistical significance was set at $\alpha = 0.05$.

RESULTS

Clozapine-treated patients had statistically significantly lower mean D_2/D_3 receptor-BP values than healthy volunteers in the putamen (13.6 ± 4.2, mean ± SD, *vs* 21.1 ± 3.7, respectively; $T = 4.1$, df = 20, $p = 0.001$), caudate nucleus (11.7 ± 4.0 *vs* 20.5 ± 3.8; $T = 4.9$, df = 20, $p < 0.001$), temporal cortex (0.35 ± 0.17 *vs* 0.78 ± 0.29; $T = 4.5$, df = 20, $p < 0.001$), thalamus (1.13 ± 0.32 *vs* 2.03 ± 0.53; $T = 5.0$, df = 20, $p < 0.001$), amygdala (0.73 ± 0.31 *vs* 1.25 ± 0.32; $T = 3.6$, df = 20, $p = 0.002$), and substantia nigra (0.92 ± 0.35 *vs* 1.55 ± 0.36; $T = 3.9$, df = 20, $p = 0.001$).

Multivariate tests revealed significant differences in the D_2/D_3 receptor occupancy observed in the four regions examined ($F = 30.0$, df = 3.0, $p < 0.001$). *Post hoc* contrasts showed that D_2/D_3 receptor occupancy was significantly lower in striatal regions than in the inferior temporal cortex (Table 2, Figures 1–3). The mean D_2/D_3 receptor occupancy in striatal regions was statistically significantly lower than in the inferior temporal cortex (putamen: $F = 33.3$, df = 1, $p < 0.001$; caudate: $F = 12.7$, df = 1, $p = 0.003$). Occupancy in the caudate nucleus was significantly higher than in the putamen ($F = 78.7$, df = 1, $p < 0.001$). Similarly, the D_2/D_3 receptor occupancy in the thalamus was between those obtained in the putamen ($F = 11.4$, df = 1, $p = 0.004$) and the inferior temporal cortex ($F = 7.3$, df = 1, $p = 0.017$). D_2/D_3 receptor occupancy values for the other brain regions are given in Table 2.

The mean clozapine plasma concentration at the time of injection of the radiotracer was 595 ng/ml (SD = 335 ng/ml,

Table 2 D_2/D_3 Receptor Occupancy (Means ± SD), E_{max}, and EC_{50} Values (Means ± SEM) in Selected Brain Regions in 15 Patients with Schizophrenia and Schizoaffective Disorder Receiving Therapeutic Doses of Clozapine

	D_2/D_3 occupancy (%)	E_{max} (%)	EC_{50} (ng/ml)
Putamen	36 ± 5	103 ± 44	950 ± 720
Caudate nucleus	43 ± 5	94 ± 25	582 ± 337
Inf. temp. cortex	55 ± 6	93 ± 24	333 ± 235
Thalamus	44 ± 4	74 ± 12	324 ± 151
Amygdala	43 ± 6	96 ± 39	624 ± 531
Substantia nigra	42 ± 5	89 ± 28	572 ± 392

E_{max} is the maximum attainable receptor occupancy and EC_{50} is the plasma concentration predicted to provide 50% of the maximum attainable receptor occupancy.

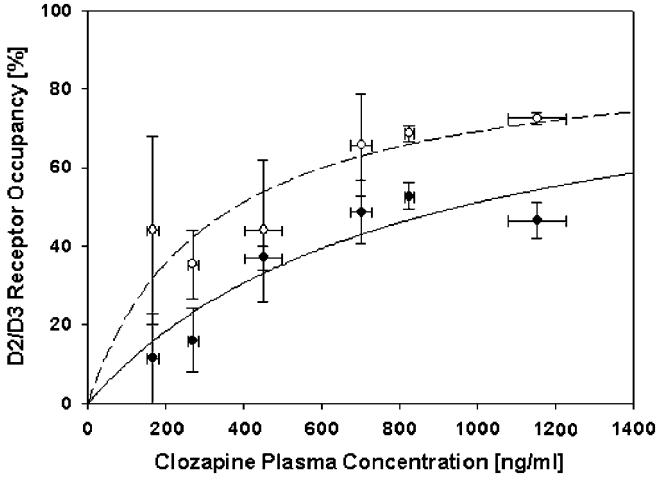

Figure 1 Relationship between clozapine plasma levels (*x*-axis) and dopamine D_2/D_3 receptor occupancy in the putamen (*y*-axis, filled circles, solid line) and in the inferior temporal cortex (open circles, dashed line) in 15 patients with schizophrenia and schizoaffective disorder receiving therapeutic doses of clozapine. For illustrative purposes, plasma concentrations were averaged stepwise (0–199, 200–399, 400–599 ng/ml, etc) and, together with their corresponding occupancy values, presented as means ± SEM. However, statistical values presented in the text are based on individual values.

range 151–1228 ng/ml). Clozapine plasma concentrations determined in the morning were correlated with plasma levels at time of the PET scan on a trend level ($r_s = 0.58$, $p = 0.060$). The daily clozapine dose was neither significantly correlated with the clozapine plasma concentration ($p = 0.099$) nor with D_2/D_3 receptor occupancy in any brain region ($p > 0.1$).

When clozapine plasma concentrations and occupancy values were related to each other according to the law of mass action (for details, see Patients and methods section, paragraph Statistical analyses), plasma concentrations were significantly positively correlated with D_2/D_3 receptor occupancy values for all regions evaluated. Positive correlations were found between clozapine plasma concentrations and occupancy values in the putamen ($r = 0.82$, $df = 1$, $p = 0.0002$; Figure 1), the caudate nucleus ($r = 0.81$, $df = 1$, $p = 0.0003$), the inferior temporal cortex ($r = 0.58$, $df = 1$, $p = 0.023$; Figure 1), the thalamus ($r = 0.80$, $df = 1$, $p = 0.0004$), the amygdala ($r = 0.70$, $df = 1$, $p = 0.004$), and the substantia nigra ($r = 0.75$, $df = 1$, $p = 0.001$). Figure 1 shows the relationship between clozapine plasma concentration and receptor occupancy in the putamen and the inferior temporal cortex. While the occupancy of temporal cortical D_2/D_3 dopamine receptors by clozapine is higher over a wide range of plasma concentrations, occupancy values in the putamen approach the cortical values at very high plasma levels, which are usually not achieved in clinical practice. This is further supported by the calculated E_{max} and EC_{50} values, which are given in Table 2. The maximum attainable receptor occupancy was close to complete saturation in all brain regions, with the exception of the thalamus (Table 2). However, the plasma concentrations leading to half-maximal D_2/D_3 receptor occupancy were markedly different (Figure 1; Table 2). Interestingly, with an E_{max} value of just 74% (SE = 12%), the thalamus was the only brain region where D_2/D_3 receptors were theoretically not saturable.

When a mean D_2/D_3 receptor occupancy map is generated on a voxel basis, the preferential extrastriatal binding of clozapine in the clinically used dose range becomes even

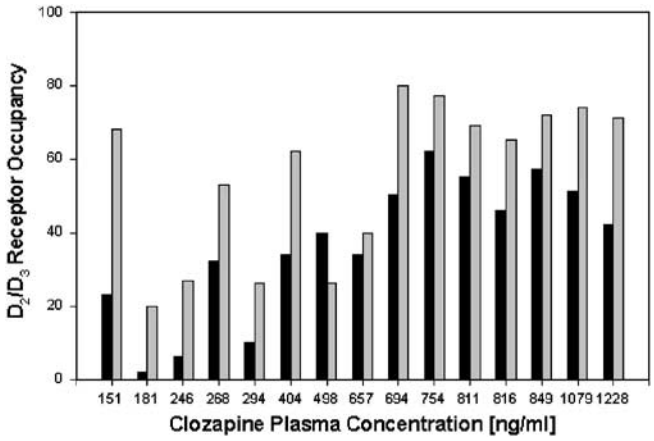

Figure 2 Individual occupancy values in the putamen (black bars) and in the inferior temporal cortex (gray bars) over a wide range of clozapine plasma concentrations determined in 15 patients with schizophrenia and schizoaffective disorder receiving therapeutic doses of clozapine. In 14 out of 15 patients, temporal cortical occupancy was higher than the occupancy in putamen. The statistical analysis is given in the Results section.

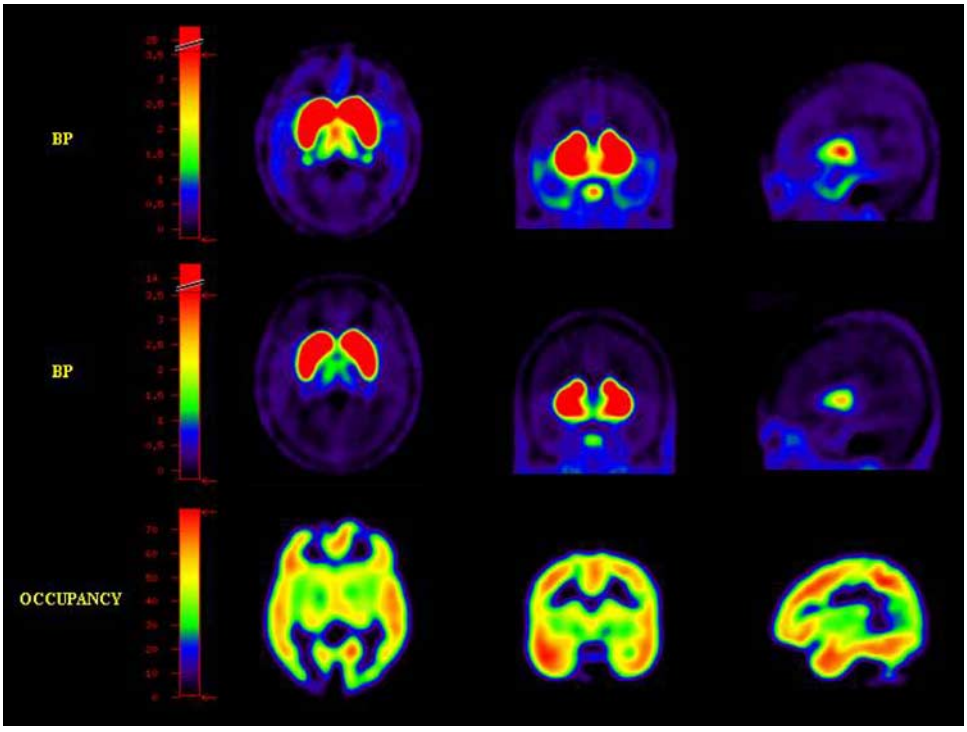

Figure 3 Parametric mean BP images in normal volunteers ($n = 7$, top row) and in clozapine-treated patients ($n = 15$, middle row). The bottom row illustrates the D_2/D_3 receptor occupancy in each single voxel in the patient group, clearly demonstrating the significantly higher binding in cortical regions compared to the striatum.

more apparent (Figure 3), revealing the significantly higher binding in cortical regions compared to the striatum.

DISCUSSION

In this study, we could demonstrate that clozapine occupies a significantly larger proportion of cortical than striatal D_2/D_3 receptors over a wide range of plasma levels. Thus, our results are consistent with the finding initially reported by Pilowsky *et al* (1997). However, this group possibly found a serendipitous finding, because the methodology was not optimal (Olsson and Farde, 2001; Talvik *et al*, 2001). Since we performed dynamic PET scanning and, in addition, could relate the occupancy values to clozapine plasma levels, more conclusions can be drawn from our study.

Firstly, while both Nordström and Kapur in their [^{11}C]raclopride PET studies determined an E_{max} of approximately 70% in the striatum, we calculated a maximum attainable receptor occupancy of 94 and 103% in the caudate nucleus and the putamen, respectively (Kapur *et al*, 1999; Nordström *et al*, 1995). Theoretically however, these values are reached at plasma concentrations that are never obtained under clinical conditions in humans (approximately 3300 ng/ml). Our findings confirm earlier observations from monkey PET studies that very high intravenous doses are able to occupy striatal D_2 receptors almost completely (Mukherjee *et al*, 2001; Suhara *et al*, 2002a). At such high doses, the differential occupancy of cortical *vs* striatal D_2/D_3 receptors is lost. The reason why both Nordström *et al* (1995) and Kapur *et al* (1999) could not determine E_{max} values above 70% could be due to the fact that in their studies a number of patients had high D_2 occupancies at relatively low plasma concentrations. Consequently, the curve fits obtained from these data were driven by those few patients.

On the other hand, the half-maximal D_2/D_3 receptor occupancy in the temporal cortex was determined to be slightly above 300 ng/ml in our study. A threshold plasma level for antipsychotic efficacy of clozapine of 350–400 ng/ml is now well established (Perry *et al*, 1991; Kronig *et al*, 1995), although a few studies found somewhat higher thresholds (Potkin *et al*, 1994). Thus, our results suggest that clozapine's antipsychotic efficacy could be related to a D_2/D_3 receptor occupancy in the temporal cortex of about 60%. Such an occupancy has been initially proposed as the threshold occupancy value for striatal D_2-like dopamine receptors, when conventional antipsychotics were studied (Kapur *et al*, 2000a; Farde *et al*, 1992). Low-affinity drugs such as clozapine or, more recently, quetiapine, seemed to be exceptions from the classical rule (Farde *et al*, 1992; Kapur *et al*, 2000b). This observation led Nordström *et al* (1995) to state 'that a high D_2 receptor occupancy is not an absolute prerequisite for antipsychotic effect'. This might indeed be true for the occupancy of striatal D_2 receptors. However, our results suggest that antagonism of cortical D_2 receptors seems to be the more general principle for antipsychotic efficacy. The validity of this principle can be easily tested by applying it to other low-affinity antipsychotics, especially quetiapine, where striatal D_2 binding is low (Kapur *et al*, 2000b; Gefvert *et al*, 2001). While there is evidence for a direct relationship between disrupted patterns of D_2 dopamine receptors in the temporal cortex in schizophrenia and certain aspects of its psychopathology (Goldsmith *et al*, 1997), there are numerous actions of antipsychotic drugs on cortical molecular targets that are mediated by antagonism of D_2-like dopamine receptors. These actions include downregulation of D_1-like dopamine receptors and modification of NMDA receptor expression, among many others (Lidow *et al*, 1997; Riva *et al*, 1997). We could not detect statistically significant relationships between binding to D_2/D_3 receptors in any brain region and improvement in psychopathology. Larger sample sizes and different study designs would be needed to find such associations. Interestingly, even some of the patients with relatively low cortical receptor occupancy (below 40%) were at least partial responders to clozapine treatment. The exact (genetic?) mechanisms that translate receptor occupancy into response in individual patients remain to be elucidated.

Our results are in line with those obtained by Kessler *et al* (2002), who determined higher extrastriatal D_2 binding in a small sample ($n = 5$) of patients treated with clozapine, but not in patients treated with olanzapine or risperidone. However, since plasma levels were not reported by these investigators, the results are somewhat inconclusive. In the same study, it was reported that clozapine occupies significantly less D_2 receptors in the substantia nigra than any other drug that was evaluated. These authors suggest that the reduced binding to D_2 autoreceptors could explain clozapine's unique clinical properties. We could not confirm this finding in our larger patient sample. Since the substantia nigra is a very small structure, determination of receptor occupancy in this brain region is extremely sensitive to partial volume effects. Therefore, we believe that a partial volume correction of the acquired radioactivity is necessary for determination of dopamine receptor occupancy in the substantia nigra to give a definite answer to the question whether clozapine exerts a reduced binding to dopamine autoreceptors.

In order to obtain occupancy values for a broad range of clozapine plasma levels, we investigated patients on various dosing schedules. However, regardless of the time of the PET scan relative to the last drug administration, the estimated data could be described by a nonlinear fit according to the law of mass action. As long as the occupancy is determined at a range of plasma concentrations, the time of the PET scan relative to the last drug administration is not relevant for illustration of the drug kinetics at the target receptor. The time-course of dopamine D_2 receptor occupancy of an antipsychotic is a function of its plasma pharmacokinetics and *in vivo* affinity (Takano *et al*, 2004). Thus, compounds with a short half-life and/or a low affinity such as clozapine and quetiapine are described by a flat plasma concentration/occupancy curve, whereas compounds with a long half-life and/or a high affinity such as haloperidol are described by a steep curve. A possible explanation for these regionally varying dose occupancy curves might be the following: D_2 receptor occupancies observed under antipsychotics with moderate or low affinity might be influenced by endogenous dopamine (Seeman, 2002). Dopamine concentrations in animal striatum are significantly higher compared to cortical concentrations when measured with tissue homogenate or microdialysis techniques (Brown *et al*, 1979; Pehek, 1999). Furthermore,

there are markedly different kinetics of dopamine release and reuptake across brain regions (Garris and Wightman, 1994). These differences in competition of clozapine against endogenous dopamine could contribute to the above-mentioned regional differences of concentration/occupancy curves. Additionally, upregulation of D_2 but not D_3 receptors under antipsychotic treatment together with different spatial distributions of D_2 and D_3 receptors may contribute to the described observations (Joyce, 2001).

To explain the finding that D_2/D_3 receptor binding was not saturable in the thalamus is more challenging. Several independent groups have reported that D_2 receptor availability is reduced in the thalamus in patients with schizophrenia (Talvik *et al*, 2001; Yasuno *et al*, 2004). Calculation of receptor occupancy for a brain region with reduced receptor numbers from a comparison with normal volunteers should lead to *over*estimation of the occupancy, not *under*estimation. However, if D_2 and D_3 receptors are differentially regulated by clozapine in selected brain regions, or if D_2 and D_3 receptors are differentially regulated in patients with schizophrenia and normal controls, then the estimation of occupancy values in patients from comparison with normative values obtained in healthy subjects might be misleading (Gurevich *et al*, 1997; Joyce, 2001).

The same might of course be true with regard to temporal cortical binding. Tuppurainen *et al* (2003), using epidepride SPECT, have recently reported on significantly reduced D_2 receptor availability in the temporal cortex of seven patients with schizophrenia. If there is indeed a reduced temporal cortical D_2 receptor density in schizophrenia, this would produce spuriously high levels of dopamine D_2/D_3 receptor occupancy in patients with schizophrenia when using normal volunteers as control subjects. However, both Suhara *et al* (2002b) and Talvik *et al* (2003), using PET with [^{11}C]FLB-457, failed to find significant reductions in temporal cortical D_2 receptor availability in larger samples of 11 and nine patients, respectively. Furthermore, with the exception of the thalamus, we demonstrated a higher extrastriatal than striatal occupancy not only in the temporal cortex but also throughout the brain. Thus, it is unlikely that reduced temporal cortical D_2 receptor density in schizophrenia consistently biased our results. However, the low D_2 receptor density in the cortex in conjunction with the use of a normal control group for the baseline condition contributes to the comparatively high variability in our data for these regions. Nevertheless, Mukherjee *et al* (2002) found a test–retest variability of less than 10% both for striatal and temporal cortical regions, supporting the view that D_2 receptors can be reliably quantified with [^{18}F]fallypride even in brain regions with a low D_2 density (Mukherjee *et al*, 2002).

In conclusion, we could demonstrate that clozapine occupies a significantly larger proportion of cortical compared to striatal D_2/D_3 dopamine receptors over a broad range of plasma levels, and that clinically effective plasma concentrations are related to a temporal cortical D_2/D_3 receptor occupancy above approximately 60%. Thus, our study with the largest sample to date suggests that the cerebral cortex rather than the striatum could be the target structure for the evaluation of antipsychotics with PET.

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Dr Gründer has served as a consultant for Bristol-Myers Squibb (New York, NY), Otsuka (Rockville, MD), Pfizer (New York, NY), and Astra Zeneca (London, UK). He has served on the speakers' bureau of Bristol-Myers Squibb, Otsuka, Pfizer, Astra Zeneca, and Eli Lilly (Indianapolis, IN). He has received grant support from Bristol-Myers Squibb, Pfizer, and Sanofi Synthélabo (Paris, France). Dr Wong has served as a consultant for Bristol-Myers Squibb, Lilly and Abbott Labs (Chicago, IL). He has received grant support from Aventis (Strasbourg, France), Janssen (Beerse, Belgium), Eli Lilly, Pfizer, and Sumitomo (Osaka, Japan).

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