International Journal of Neuropsychopharmacology (2010), **13**, 951–960. Copyright © CINP 2010
doi:10.1017/S1461145710000374

ARTICLE

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Dopamine $D_{2/3}$ receptor occupancy by quetiapine in striatal and extrastriatal areas

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Abstract

Quetiapine is next to clozapine an antipsychotic agent that exerts hardly any extrapyramidal side-effects at clinical efficacious doses. Some previous receptor occupancy studies reported preferential extrastriatal $D_{2/3}$ receptor ($D_{2/3}$R)-binding properties of second-generation antipsychotics and suggested this as possible reason for improved tolerability. This positron emission tomography (PET) investigation was designed to compare the occupancy of dopamine $D_{2/3}$Rs by quetiapine in striatal and extrastriatal brain regions. Therefore, a cohort of 16 quetiapine-treated psychotic patients underwent an [^{18}F]fallypride (FP) PET scan. Due to the high affinity of FP and its comparatively long half-life, striatal and extrastriatal binding potentials could be determined in one single scan. Receptor occupancy was calculated as percent reduction in binding potential relative to age-matched medication-free patients suffering from schizophrenia. Quetiapine occupied 44 ± 18% in the temporal cortex and 26 ± 17% in the putamen, a difference significant at the level of $p = 0.005$ (Student's t test). Quetiapine showed a mean occupancy of 36 ± 16% and in the thalamus. In the caudate nucleus there was an occupancy of 29 ± 16% ($p = 0.0072$). Individual occupancy levels did not exceed 59% in any of the striatal volumes of interest. The time-interval between scan and last drug ingestion did not influence the relationship between plasma concentration and central $D_{2/3}$R occupancy. Taken together, quetiapine shows preferential extrastriatal binding at $D_{2/3}$Rs; the extent of this difference is comparable to that previously described for clozapine. Both antipsychotics show very low affinity for $D_{2/3}$Rs.

Received 30 October 2009; Reviewed 5 January 2010; Revised 10 February 2010; Accepted 12 March 2010; First published online 15 April 2010

Key words: Antipsychotics, $D_{2/3}$ receptor occupancy, positron emission tomography, preferential extrastriatal binding, quetiapine, schizophrenia.

Introduction

The antipsychotic effect of all clinically used antipsychotics is mediated via their antagonistic (or partial agonistic) effects at cerebral D_2-like dopamine receptors. In recent years, the D_2 receptor (D_2R)-binding characteristics of first- and second-generation antipsychotics (FGA, SGA) have been investigated extensively with positron emission tomography (PET) or single photon emission computed tomography (SPECT; Frankle, 2007). Based on the pioneering investigations of Farde and co-workers (Farde *et al*. 1992; Nordström *et al*. 1993) using [^{11}C]raclopride a threshold of about 60% D_2R occupancy is generally considered a requirement for antipsychotic efficacy. However, it should be noted that important effects of antipsychotic medications may be mediated by extrastriatal dopamine $D_{2/3}$ receptors ($D_{2/3}$Rs), which cannot be assayed in PET studies with raclopride. The less

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abundant extrastriatal sites can be revealed in molecular imaging studies with newer radioligands possessing higher affinity for dopamine $D_{2/3}$Rs. Many molecular imaging investigations using high-affinity ligands have revealed preferential extrastriatal binding for most SGAs (Gründer *et al*. 2006; Kessler *et al*. 2006; Pilowsky *et al*. 1997; Vernaleken *et al*. 2008*b*). Traditionally, the striatum has been used as an index of the extent of $D_{2/3}$R occupancy, although the primary brain region of antipsychotic action is not known. The affinity of individual antipsychotics for dopamine $D_{2/3}$Rs may be the factor that explains the phenomenon of preferential extrastriatal binding by SGAs (Vernaleken *et al*. 2008*a*). Quetiapine shows a comparatively low affinity at $D_{2/3}$Rs (Schotte *et al*. 1996*c*). Accordingly, some molecular imaging studies using radioligands of moderate affinity have shown striatal $D_{2/3}$R occupancy as low as 40% in patients treated with quetiapine (Catafau *et al*. 2008; Gefvert *et al*. 1998; Kapur *et al*. 2000; Kügferle *et al*. 1997; Tauscher *et al*. 2004). These results may explain the lack of severe extrapyramidal side-effects experienced by patients treated with quetiapine, but are not informative about the extent of concomitant blockade at extrastriatal sites, which might well be substantially higher than 40%. Indeed, two previous investigations employing higher affinity ligands revealed relatively higher extrastriatal binding by quetiapine (Kessler *et al*. 2006; Stephenson *et al*. 2000). However, both of those studies suffered from small sample sizes and did not report quetiapine plasma levels. Thus, these results cannot simply be generalized to clinical circumstances. In the present study we have attempted to characterize fully the relative occupancies by quetiapine at striatal and extrastriatal $D_{2/3}$Rs in a group of 16 patients with schizophrenia and related conditions. To this end, we obtained PET recordings with the high-affinity ligand [^{18}F]fallypride (FP), and calculated the occupancy relative to the binding seen in a group of untreated patients. Furthermore, we investigated the relationship between $D_{2/3}$R occupancy and the quetiapine plasma concentrations at the time of PET scanning.

Method

This investigation was approved by the local ethics committees in Aachen and Mainz, Germany, and the German radiation safety authorities. All subjects gave written informed consent. They underwent physical and mental-state examinations. Patients suffering from additional neurological or relevant somatic diseases were not included, nor were patients reporting illegal drug consumption in the last 6 months. All PET investigations were performed at the PET Centres of the University of Mainz and RWTH Aachen University, Germany.

Patients

The cohort of 16 patients (11 male, five female, age 22–64 yr, mean ± S.D. 41.4 ± 10.7 yr) had diagnoses of schizophrenia ($n=10$), personality disorder ($n=1$), transient psychosis ($n=2$), schizoaffective disorder ($n=2$) or cyclothymic disorder ($n=1$) according to DSM-IV criteria [Positive and Negative Symptom Scale (PANSS) at day of scanning: 22–74, mean ± S.D. 50.0 ± 15.7]. The 12 patients with (transient) psychosis had a mean duration of psychosis (DUP) of 5.3 yr (S.D. = 6.0 yr). Main inclusion criterion was the clinical need for quetiapine treatment without any other drug with antidopaminergic, anticholinergic, or antiserotoninergic properties. Patients had been treated with stable doses of quetiapine (200–1300 mg/d, mean ± S.D. 753 ± 317) for at least 1 wk. Four patients had no previous antidopaminergic drug treatment history prior to the current episode. Fifteen patients received the immediate release formulation (IR), one patient was treated with 800 mg/d of the extended release quetiapine (ER). Three patients needed concomitant benzodiazepine treatment, two patients received selective norepinephrine/serotonin reuptake inhibitors (SNRI), one a selective serotonin reuptake inhibitor (SSRI) and one patient needed valproic acid. The daily doses of quetiapine were chosen according to clinical needs. Patients under treatment of quetiapine (IR) were assigned to two groups with different time intervals between last quetiapine ingestion and the PET scan (short interval: $\leqslant 10$ h, $n=9$; long interval >10 h, $n=6$).The control group consisted of eight male and two female untreated patients with schizophrenia (20–58 yr; mean ± S.D. 38.7 ± 11.7 yr). Of the eight control subjects, five patients suffered from the paranoid type and three from the undifferentiated type of schizophrenia (DUP 0.25–10 yr, mean ± S.D. 2.7 ± 3.0; PANSS 16–115, mean ± S.D. 82.7 ± 13.1). Treated patients did not significantly differ from unmedicated subjects in age (unpaired two-tailed t test: −2.7 yr, $p=0.55$). The control group was free of any centrally acting medication for at least 6 months prior to the scan; three subjects were drug-naive. All subjects (treated patients and control subjects) had to be free of any relevant somatic complaints and morphological brain lesions. Illegal drug treatment was not allowed for 6 months prior to the PET scan (due to mental health examination). Drug screening was

performed at the beginning of the in-patient stay and 1 d before the scan day. Diagnostics as well as the decision about the ability to participate in the study were performed by the study investigator and the physician with clinical responsibility.

Radiochemistry and data acquisition

The radiosynthesis of FP was a high-yield modification of the method for the synthesis of [^{18}F]desmethoxyfallypride, as described in detail previously (Gründer *et al*. 2003). Emission images were acquired on a Siemens ECAT EXACT 922/47 whole-body PET scanner in 3D-mode (field of view: 16.2 cm, 47 planes; FWHM axial: 4.6 mm, in-plane: 6.0 mm). Data acquisition comprised a series of 39 time-frames (3×20 s, 3×1 min, 3×2 min, 3×3 min, 21×5 min, 2×8 min, 4×10 min), resulting in a total recording period of 180 min. This scan duration was previously found to be sufficient for valid calculation of striatal and extrastriatal binding potentials. An internal inspection of FP scans of 34 medication-free subjects (patients and healthy subjects) revealed that for every subject and every region equilibrium was reached. There was additionally no statistical significant difference in calculated binding potentials when comparing the simplified reference tissue model (SRTM) and the transient equilibrium approach (I. Vernaleken, unpublished data). A 15-min transmission scan using a ^{68}Ge source was performed prior to each study for subsequent attenuation correction. In treated patients 219 ± 26.5 MBq (mean $\pm$ S.D., range 154–252 MBq) FP was injected intravenously as a bolus into a cubital vein over approximately 30 s. In unmedicated patients 221 ± 24.3 MBq (mean $\pm$ S.D., range 176–247 MBq) FP was injected. During the PET scan venous blood samples were taken to measure the quetiapine plasma concentrations at time-points 0, 90, and 180 min after FP injection. The quetiapine concentration in serum was determined in blood by high performance liquid chromatography (HPLC) with column switching as described previously (Sachse *et al*. 2006).

Image and data analysis

Images were reconstructed with filtered back-projection using a Ramp filter and a Hanning filter (filter width 4 mm). After motion correction the whole dynamic scan was spatially normalized into MNI coordinates using the MEDx software (v. 3.43; Medical Numerics, USA). Templates of polygonal volumes of interest (VOI) were applied to the spatially normalized dynamic recordings, for extraction of time–activity curves for cerebellum, caudate nucleus, putamen, thalamus, amygdalae, and inferior temporal cortex (inferior temporal gyrus; ITG). This VOI template has been chosen in several previous FP-PET investigations (Gründer *et al*. 2008; Vernaleken *et al*. 2008*b*) and focuses on regions providing sufficient $D_{2/3}$R availability for FP binding and a stable statistics. The binding potentials (BP_{ND}) of these VOIs were calculated using the Lammertsma Simplified Reference Tissue Model, with the cerebellum serving as the non-binding reference region (Lammertsma & Hume, 1996). The individual subject's receptor occupancy was defined as the percentage reduction of BP_{ND} relative to the corresponding averaged BP of the unmedicated control group according to the following equation

$$\text{Occupancy (\%)} = \left(1 - \frac{BP_{ND}(\text{drug})}{BP_{ND}(\text{control})}\right) \times 100.$$

Statistical analyses

Statistical analyses were performed using commercial SPSS software (v. 15.0; SPSS Inc., USA). Unpaired *t* tests were used to compare binding values between the treatment groups. Plasma concentrations and dopamine $D_{2/3}$R occupancy values were analysed by Spearman rank-order correlations, and additionally fitted by nonlinear regression analysis using SigmaPlot (v. 10.0; Systat, USA) to the following equation:

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

where E_{max} reflects the maximum attainable receptor occupancy and C_{Quet} is the mean plasma concentration of quetiapine during the PET scan. EC_{50} is the plasma concentration predicted to provide 50% of the maximum attainable dopamine $D_{2/3}$R occupancy. In all analyses, the two-tailed level of statistical significance was set at $\alpha = 0.05$. For better comparison of dopamine $D_{2/3}$R occupancies after long and short intervals between the last dose and the PET scan, we found a linearization procedure to be appropriate for applying linear statistical models. To this end, we performed a linearization of the above equation in analogy to the Hanes–Woolf transformation:

$$\frac{[C_{Quet}]}{\text{Occupancy (\%)}} = \frac{EC_{50}}{E_{max}} + \frac{[C_{Quet}]}{E_{max}}.$$

After linear regression, the corresponding individual standardized residuals (Pearson residuals) were calculated. Pearson residuals of the short- and long-duration groups were compared by the use of non-parametric tests (Mann–Whitney test).

In order to evaluate whether 180 min of data acquisition is sufficient to reach equilibrium in high-binding regions, we performed nonlinear fits on the time–activity curves (TACs) using the three-parameter Hörl function [$fx = A*(B\hat{}X)*X\hat{}C$] for the putamen TACs and a modified Hörl function [$fx = \exp(A + B/X + C*\ln(X))$] for the cerebellum TACs. In every case of medication-free patients, equilibrium occurred within 180 min of the PET scan (mean ± S.D.: 110.9 ± 21.5 min). Additionally to the SRTM, we calculated putamen BP_{ND} values according to the transient equilibrium method (Farde *et al*. 1989). There was no statistically significant difference between the SRTM and the equilibrium-based results [putamen: ΔBP_{ND} 0.40 (1.8 ± 2.95%), $p = 0.68$, $n = 10$, two-sided paired t test].

In order to estimate the effect of changing quetiapine plasma levels during the PET scan we performed a nonlinear fit to depict the time-course of quetiapine concentrations based on the analyses of the three blood samples during the scan (see below). For one striatal region (i.e. putamen) and one extrastriatal region (i.e. thalamus) we could thus estimate the quetiapine plasma level at the time-point of the individual equilibrium in these VOIs. Similar to the previously mentioned nonlinear fit of concentration/occupancy relationships (based on C_0), we calculated these fits based on the estimated concentration at equilibrium. Using these corrected equations we finally estimated the expected $D_{2/3}R$ occupancies at the individual C_0 concentration.

Results

There was no statistically significant difference of injected activity between the groups (ΔMBq = 2.1, $p = 0.88$, unpaired two-sided t test). BP_{ND} values in the control group ranged from 0.54 to 1.01 (mean ± S.D.: 0.79 ± 0.16) in ITG, from 1.70 to 3.29 (mean ± S.D.: 2.25 ± 0.51) in the thalamus, from 15.7 to 25.1 (mean ± S.D.: 21.2 ± 3.3) in the CN, and from 16.9 to 26.8 (mean ± S.D.: 23.2 ± 4.1) in the putamen. In CN only, the negative correlation between age and BP_{ND} reached statistical significance ($r = -0.690$, $p = 0.027$); in the putamen this correlation was on a trend level ($r = -0.600$, $p = 0.093$).

Mean quetiapine-induced $D_{2/3}R$ occupancies (not corrected for age effects) ranged from 25 ± 20% (mean ± S.D.) in the putamen to 44 ± 19% in the ITG. In the CN, $D_{2/3}R$ occupancy was 29 ± 18% and in the thalamus 36 ± 19% (see Table 1 for details). Occupancy values of the patient subgroup suffering from (transient) psychosis ($n = 12$; mean quetiapine concentration 246 ng/ml) did not show any significant differences to the non-psychotic subgroup ($n = 4$; mean quetiapine concentration 273 ng/ml) in all regions; differences did not exceed +3.4% (amygdalae) and were on average +0.32% (S.D. ±1.93; p in all VOIs > 0.45).

Occupancies in all VOIs (except amygdala *vs.* ITG and amygdala *vs.* thalamus) differed statistically significantly from each other, with ITG *vs.* putamen, ITG *vs.* CN and thalamus *vs.* putamen surviving a Bonferroni correction ($\Delta_{ITG-Put} = +20.8\%$, $p < 0.0001$; $\Delta_{ITG-CN} = +16.5\%$, $p = 0.0008$; $\Delta_{ITG-Thal} = +8.9\%$, $p = 0.0020$; $\Delta_{Thal-Put} = +11.9\%$, $p = 0.0003$; $\Delta_{Thal-CN} = +7.6\%$, $p = 0.0032$; $\Delta_{CN-Put} = +4.3\%$, $p = 0.0032$; two-tailed paired t test) (see Fig. 1). The age-corrected $D_{2/3}R$ occupancy values correlated with intra-scan quetiapine plasma levels significantly in the CN and at trend-level in the putamen (CN: $r = 0.50$, $p = 0.048$; putamen: $r = 0.453$, $p = 0.078$; two-sided Spearman rank-order correlation), but not in other brain regions. The nonlinear regressions for estimating Michaelis–Menten parameters yielded significant fits for E_{max}, but did not furnish valid fits for EC_{50} values in any brain region (see Table 2).

For estimating the influence of decreasing quetiapine concentrations during the scan duration, time-corrected (see above) and uncorrected analyses have been compared. As expected, uncorrected occupancies were lower than the estimated and corrected data in the putamen (Δ_{Occ}: −1.32 ± 0.78, $p < 0.0001$; two-sided paired t test) due to decreasing quetiapine plasma levels during the PET scan and the late time-point of equilibrium [medicated patients (mean ± S.D.): 94.9 ± 32.1 min]. Quetiapine plasma levels decreased on average by 19.8% until equilibrium was reached. Due to the early time-point of equilibrium this correction did not change the occupancy estimations in the extrastriatal region (i.e. thalamus) substantially.

The interval between last drug ingestion and start of the PET scan was without any significant effect on the magnitude of BP_{ND} in any brain region: the long- and short-interval group did not differ in comparison of standardized residuals (Pearson residuals) after Hanes–Woolf linearization, either in the CN (median short group: −0.45; long group: −0.28; $p = 0.328$, two-sided Mann–Whitney test), or in the putamen (median short group: −0.37; long group: −0.01; $p = 0.279$, two-sided Mann–Whitney test).

Discussion

While the $D_{2/3}R$ occupancy of quetiapine was the focus of several previous PET and SPECT investigations (e.g. Catafau *et al*. 2008; Gefvert *et al*. 1998; Hagberg *et al*. 1998; Kapur *et al*. 2000; Kessler *et al*.

Table 1. Individual data of the patients receiving stable doses of quetiapine (age-corrected values). The long- and short-interval groups were defined by an interval between last ingestion of quetiapine and the PET scan of >10 h and $\leqslant 10$ h, respectively

No.	Gender	Age (yr)	Quetiapine daily dose (mg)	Interval: last dose – PET		C_{Quet} (ng/ml)	$D_{2/3}R$ occupancy (%)				
				Long (h)	Short (h)		ITG	Thalamus	Amygdala	Caudate nucleus	Putamen
1	F	46	600	–	10	36	58	49	58	43	46
2	M	42	600	–	10	58	38	27	48	10	9
3	F	28	800	–	3.50	171	71	60	56	35	39
4	M	39	900	–	6.50	187	29	43	61	41	42
5	F	42	1200	–	9	444	47	47	28	33	31
6	M	44	900	–	4	473	20	24	0	19	10
7	M	45	600	–	4.75	488	42	31	50	41	31
8	F	41	1300	–	3.75	621	71	57	80	59	56
9	M	22	450	–	1.75	831	53	61	55	50	43
10	F	48	500	20.75	–	13	53	41	66	35	28
11	M	52	1200	24	–	15	60	32	2	6	3
12	M	23	900	21.50	–	24	3	0	0	1	0
13	M	64	200	16	–	40	35	23	66	21	28
14	M	41	800	16.25	–	58	37	24	63	17	14
15	M	50	300	17	–	99	40	27	41	20	13
16	M	35	800 (ER)	4.75		814	53	29	0	26	18
Average	–	41	753	19	6	273	44	36	42	29	26
Standard	–	10.7	317.0	4.3	3.1	293.0	18.0	16.3	28	16.3	16.7

ITG, Inferior temporal gyrus.
Subject: 16 received quetiapine in an extended release (ER) formulation and was not attributed to any of the last-dose interval groups for statistical analysis. C_{Qeut} refers to the quetiapine concentrations at the beginning of the PET scan.

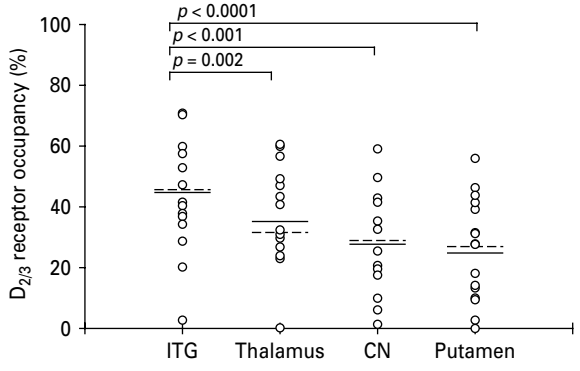

Fig. 1. Individual age-corrected $D_{2/3}R$ occupancy values. Scatter plot of individual age-corrected $D_{2/3}R$ occupancy values in the inferior temporal gyrus (ITG), the thalamus, the caudate nucleus (CN) and the putamen. Occupancies have been calculated in comparison to an age-matched cohort of 10 medication-free patients suffering from schizophrenia. The 16 patients received quetiapine daily doses ranging from 200 to 1300 mg/d. The average occupancies are represented by a solid line, the median by a dashed line, respectively. Group differences were tested according to a two-sided paired *t* test.

Table 2. Results of rank-order correlations (Spearman rank-order correlation, two-sided) and nonlinear regression data (according to the Michaelis–Menten equation) between the intra-scan quetiapine plasma level (C_{Quet}) and the age-corrected $D_{2/3}R$ occupancies in patients under stable quetiapine treatment ($n=16$)

		Putamen	Caudate nucleus	Thalamus	ITG
Spearman	*R*	0.45	0.50	0.34	0.10
	p	0.078	0.048	0.193	0.707
Nonlinear fit	*R*	0.44	0.52	0.35	0.01
E_{max} (%)		34.5	40.1	42.9	44.4
	p	0.0003	<0.0001	<0.0001	<0.0001
EC_{50}		29.7	37.1	15.6	0.3
(ng/ml)	*p*	0.319	0.198	0.283	0.958

ITG, Inferior temporal gyrus.
Due to the high variance, nonlinear fitting approaches did not reveal any suitable and significant fitting result (E_{max}, maximum attainable receptor occupancy; EC_{50}, plasma concentration predicted to provide 50% of the maximum attainable dopamine $D_{2/3}R$ occupancy).

2006; Kügferle *et al*. 1997; Stephenson *et al*. 2000; Tauscher *et al*. 2004), this is the first molecular imaging study to measure the striatal and extrastriatal receptor binding of quetiapine and its relationship to the corresponding drug plasma concentrations in a patient group of adequate size ($n=16$).

Striatal vs. extrastriatal binding

The major finding of this study is a profound difference between striatal and the extrastriatal $D_{2/3}R$ occupancies obtained under stable quetiapine treatment. Specifically, the extent of quetiapine occupancy was about 20% lower (relative to drug-free receptor availability) in both the putamen and the CN than in the inferior temporal lobe (ITG). $D_{2/3}R$ occupancy in the thalamus was intermediate compared to that in the striatal and cortical regions. The type I error probability of $<1\%$ suggests that these results are of high statistical significance.

Our results are thus in line with the two previous observations with high-affinity PET or SPECT ligands, in which small groups ($n=7$ and 6, respectively) of quetiapine-treated patients were included (Kessler *et al*. 2006; Stephenson *et al*. 2000). In addition, our finding of less pronounced differential occupancy between thalamus and striatum is in accord with the report of Kessler *et al*. (2006).

Linkage between receptor affinity and preferential extrastriatal binding

The phenomenon of preferential extrastriatal binding has never, to our knowledge, been demonstrated in molecular imaging studies for FGAs, but is frequently reported in studies of SGAs. Treatment with amisulpride, risperidone, olanzapine, and ziprasidone all revealed differences between cortical and striatal dopamine $D_{2/3}$ occupancy of about 10% (Vernaleken *et al*. 2004, 2008*b*; Xiberas *et al*. 2001). The phenomenon of preferential extrastriatal binding of SGAs holds especially true at moderate plasma concentrations, but is less apparent at high plasma concentrations (Vernaleken *et al*. 2004, 2008*a*, *b*). The prototypic atypical antipsychotic clozapine showed an occupancy of 20% (Gründer *et al*. 2006).

Uniquely for SGAs, the partial dopamine D_2 agonist aripiprazole showed little or no preferential extrastriatal binding in PET studies with FP (Gründer *et al*. 2008; Kegeles *et al*. 2008). Aripiprazole and FGAs share a comparatively high affinity at D_2Rs, in displacement studies against human dopamine D_{2L}Rs labelled *in vitro* with [^{3}H]spiperone, i.e. IC_{50}s of 0.16 nM for haloperidol and 0.7 nM for aripiprazole (Burris *et al*. 2002). In similar binding paradigms, the SGAs with moderately preferential extrastriatal binding had approximately 10-fold affinities at dopamine D_2Rs, i.e. IC_{50}s of 5.6 nM for risperidone, 4.8 nM for 9-OH-risperidone, 25 nM for amisulpride, and 4.6 nM for ziprasidone) (Castelli *et al*. 2001; Schotte *et al*. 1996*b*). Kapur & Seeman (2000) calculated K_i values (using [^{3}H]spiperone) for quetiapine and clozapine as well as for haloperidol and risperidone. Quetiapine and clozapine in contrast to haloperidol and risperidone showed remarkably low affinities at the dopamine D_{2L}R, i.e. 82 nM for clozapine, 155 nM for quetiapine; 1.6 nM for risperidone, and 0.7 nM for haloperidol. Taken together, the present results strengthen our recently stated hypothesis that the phenomenon of preferential extrastriatal binding of antipsychotics is a function of their $D_{2/3}R$ affinities and the extent of competition with endogenous dopamine (Gründer *et al*. 2006; Vernaleken *et al*. 2008*a*). In this scenario, local differences in distribution, concentration and regulation, of receptors, and the prevailing occupancy by extracellular dopamine at the receptors, account for regionally different occupancy levels. In support of this conjecture, Schotte *et al*. (1996*a*) reported regionally different binding behaviour of risperidone at D_3Rs with or without reserpine pretreatment in rats and noted the influence of competition from endogenous dopamine on the results. Indeed, extracellular dopamine concentrations have previously been claimed to be higher in striatal regions (Brown *et al*. 1979; Devoto & Flore, 2006).

Methodological issues

The observation of preferential extrastriatal binding has been dismissed by some authors as a methodological artifact. Olsson & Farde (2001) stated that BP_{ND} values might be overestimated in receptor-rich regions if determined with short half-life high-affinity ligands. Thus, research centres using the short half-life [^{11}C]FLB457 prefer a dual tracer approach using raclopride for the estimation of striatal $D_{2/3}R$ occupancies. In fact, the half-life of FP should be long enough to reach equilibrium in striatal regions (see below). Nevertheless, some dual-tracer investigations – in contrast to some FP investigations – failed to show a pronounced preferential extrastriatal binding of SGAs (Farde *et al*. 1997; Ito *et al*. 2009; Talvik *et al*. 2001). Although some of these negative reports suffered from very low group sizes, the influence of high tracer affinities still requires discussion. Therefore, the absolute $D_{2/3}R$ occupancies measured in striatum under antipsychotic treatment need to be compared between

FP and raclopride studies: raclopride-PET investigation resulted in 47% (Mamo *et al*. 2008; 800 mg/d quetiapine) and 41% striatal $D_{2/3}R$ occupancy (Gefvert *et al*. 2001; 750 mg/d quetiapine). In our short-interval group we found 32.5% (817 mg/d quetiapine). The striatal occupancy of clozapine measured against high-affinity ligands was about 40% (Gründer *et al*. 2006; Kessler *et al*. 2006); in contrast, Tauscher *et al*. (2004) found $D_{2/3}R$ occupancies by quetiapine to be 61% using raclopride-PET. Thus, the affinity of the radioligand may be a relevant factor in the receptor-occupancy estimates. This might help to explain the isolated observation of absent striatal $D_{2/3}R$ occupancy by quetiapine with the high-affinity ligand *N*-[^{11}C]methylspiperone, compared to 51% by raclopride-PET (Hagberg *et al*. 1998).

Another possible methodological objection might claim that the scan duration of 180 min is insufficient to reach equilibrium in receptor-rich regions especially under unblocked conditions. This could bias the results towards lower control BP_{ND} values and lower occupancy rates. We therefore analysed the time-point of equilibrium for the putamen TAC of the control group. None of the datasets suggested an equilibrium state after 180-min scan duration. BP_{ND} values calculated by SRTM and the transient equilibrium method showed only 1.8% difference and were highly correlated.

Finally, the fast kinetics of quetiapine might bias the results. All correlations between plasma levels and occupancy data are principally based on the quetiapine concentration at the time-point of tracer injection. However, quetiapine concentrations are decreasing over the scan interval. In receptor-rich regions FP equilibrium is reached generally during later parts of the PET scan. Thus, changes in quetiapine concentrations might influence our results preferentially in striatal regions. In an attempt to simulate this effect, we calculated the underestimation of putamen occupancies to be 1.3% on average, whereas there were no relevant differences in the thalamus as an example of extrastriatal regions. Despite all restrictions and unproven assumptions of such simulations, we conclude that the effect of preferential extrastriatal binding is not solely caused by the fast kinetics of quetiapine.

Locus of antipsychotic action

The ongoing debate about differences in absolute $D_{2/3}R$ occupancy data striatum and extrastriatal regions is pertinent to understanding the locus of action of antipsychotic medications. The early PET investigations with low-affinity ligands suggested a minimum of 60% D_2R binding to be sufficient for antipsychotic effects, whereas a drug binding of more than 80% severely increases the risk of extrapyramidal side-effects (Farde *et al*. 1992; Nordström *et al*. 1993). However, these observations were obtained mainly for FGAs, and were limited to the striatum. With the advent of high-affinity radioligands, there arose reports of preferential extrastriatal binding, which begs the question whether blockade at striatal or cortical regions is responsible for antipsychotic efficacy. Agid *et al*. (2007) reported significant correlations between striatal $D_{2/3}R$ occupancy values and the clinical benefit under treatment with risperidone and olanzapine, SGAs of moderate $D_{2/3}R$ affinity, but saw no such relationship in extrastriatal regions. As we have previously noted (Vernaleken *et al*. 2008*a*), competitive binding investigations of clozapine, the medication with the highest clinical efficacy, clearly indicate preferential extrastriatal binding, with striatal receptor occupancy much lower than the 60% proposed to be necessary for antipsychotic action. However, with clozapine plasma concentrations in the clinically useful range, occupancy was greater than 60% in the temporal cortex. The present result with quetiapine similarly calls into question the striatum as the critical locus of antipsychotic action. With only 30% $D_{2/3}R$ occupancy in striatum at the time of maximal plasma concentration (t_{max}), only a weak pharmacodynamic effect is to be expected. As with clozapine, the extrastriatal $D_{2/3}R$ occupancy is about 20% higher that in striatum. Nevertheless, the mean receptor binding at t_{max} was still comparatively low (50%) in the ITG. Using the same methodology, patients receiving clinically sufficient daily doses of clozapine reached on average 55% occupancy in the ITG (Gründer *et al*. 2006). In the present study, the individual analysis of occupancy data shows that 7/10 patients who received PET scans shortly after the last dosing failed to exceed the putative 60% occupancy threshold, even in the cortex. This is noteworthy, as the mean dosage of this patient group (815 mg/d) was practically equal to the FDA-approved maximum daily dose (800 mg/d). This finding calls into question the claim that a 60% threshold must be valid for all antipsychotic medications, irrespective of their receptor selectivity profile. However, in the ITG the competition between ligand and antipsychotic drug has to be accounted for, as discussed for the striatum.

Plasma level vs. $D_{2/3}R$ occupancy relationship

Another important finding of the present study is the absence of any linear, rank-order or nonlinear

correlation between quetiapine plasma level and $D_{2/3}R$ occupancy in the ITG. Even in the striatum, nonlinear fits of the occupancy equation were weak, and failed to provide significant estimations of EC_{50}. Only non-parametric rank-order correlations led to statistically significant results. This is noteworthy as the corresponding analyses in our previous investigations of amisulpride, ziprasidone and aripiprazole yielded stable and statistical significant nonlinear estimates of E_{max} and EC_{50} (Gründer *et al*. 2008; Vernaleken *et al*. 2004, 2008*b*). Interestingly, the reported relationship between clozapine binding and plasma drug level was much looser than for the latter drugs (Gründer *et al*. 2006). In a [^{123}I]IBZM-SPECT investigation, Catafau *et al*. (2008) presented the relationship between plasma drug concentration and occupancy at $D_{2/3}Rs$ for risperidone, olanzapine, clozapine and quetiapine. Whereas risperidone and olanzapine showed moderate to good fits according to the same receptor saturation model we used, only weak relationships were found for clozapine and quetiapine. It is difficult to explain this observation. Active metabolites may have an influence on the present $D_{2/3}R$ occupancies. In particular, *N*-desalkylquetiapine shows a broad range of receptor and transporter interaction. It has been discussed whether *N*-desalkylquetiapine's norepinephrine transporter antagonism and partial 5-$HT_{1A}R$ agonism are responsible for antidepressant action; moreover, a moderate D_2 antagonistic effect is known (Jensen *et al*. 2008). Unfortunately, we have been unable to measure this metabolite. According to a pharmacokinetic study (Winter *et al*. 2008) expected plasma concentrations of *N*-desalkylquetiapine under steady-state conditions attain about 25% of those of the mother compound 2 h after drug intake, and metabolite concentrations are about 1.8-fold higher than those of quetiapine at the end of the dosing interval (trough concentrations). Consideration of this compound would probably result in better correlations between active moiety concentrations and receptor occupancies.

Blood–brain dissociation

The present study design was additionally intended to probe for dissociation between the time-course of quetiapine plasma levels and that of $D_{2/3}R$ occupancies. Such a dissociation was formerly described by Tauscher *et al.* (2002) for the cases of raclopride-PET against olanzapine and risperidone plasma levels. To explain their findings, Tauscher *et al*. (2002) suggested that very slow dissociation of the bound drug from the receptors could results in disparate plasma/tissue drug concentrations. Around the same time, Kapur *et al*. (2000) showed that quetiapine binding in the brain declines rapidly after the last ingestion, and suggested that the combination of fast dissociation and short plasma half-life were sufficient to account for the finding. In fact, we found no influence of the delay between last ingestion of quetiapine and the time-point of PET scanning on the concentration/occupancy relationship. This was proven by the use of Hanes–Woolf linearization because this approach is comparatively independent from differences of plasma concentrations in the long- and short-interval grous. No dissociation between brain and plasma levels for quetiapine could be detected. However, it is noteworthy that a similar investigation in respect of ziprasidone binding also failed to find blood–brain dissociations although the kinetics of ziprasidone are much slower (Vernaleken *et al*. 2008*b*).

In summary, we conducted the first investigation of occupancy by quetiapine at striatal and extrastriatal binding sites in a large group of patients, in whom plasma medication levels were measured during the PET session. Similarly to clozapine, quetiapine showed particularly high $D_{2/3}Rs$ in cerebral cortex relative to that in striatum, which may possibly arise due to its very low affinity. Quetiapine also shares with clozapine an inexplicably rather loose plasma concentration/$D_{2/3}R$ occupancy relationship. Finally, we found that cerebral $D_{2/3}R$ binding very rapidly accommodates to changing plasma quetiapine concentrations.

Acknowledgements

The authors thank Sabine Höhnemann and Markus Piel for performing the syntheses of [^{18}F]fallypride. This work was funded by the German Research Council (DFG, grant KFO 112/10).

Statement of Interest

Dr Vernaleken has served as consultant for Bristol–Myers Squibb (New York, NY), Eli Lilly (Indianapolis, IN), and GlaxoSmithKline (London, UK). Dr Gründer has served as a consultant for AstraZeneca (London, UK), Bristol–Myers Squibb (New York, NY), Johnson & Johnson (Beerse, Belgium), Lundbeck (Copenhagen, Denmark), Otsuka (Rockville, MD), and Pfizer (New York, NY). He has served on the speakers' bureaux of AstraZeneca, Bristol–Myers Squibb, Eli Lilly (Indianapolis, IN), Janssen Cilag, Otsuka, Pfizer, Servier (Paris, France), and Wyeth (Madison, NJ). He has received grant support from Alkermes

(Cambridge, MA), Bristol–Myers Squibb, Eli Lilly, Johnson & Johnson, and Pfizer. Dr Schaefer has served as a consultant for GE Healthcare Buchler (München, Germany), Siemens Medical Solutions (Erlangen, Germany), Philips (Aachen, Germany), and Bristol–Myers Squibb (München, Germany). He has received grant support from Mediso Medical Imaging Systems (Budapest, Hungary). Dr Hiemke has served as a consultant for Servier (Paris, France) and Pfizer (New York, NY). He has served on the speakers' bureaux of Bristol–Myers Squibb, Janssen Cilag, Otsuka, Pfizer, AstraZeneca, Sanofi-Aventis (Berlin, Germany), Lundbeck (Copenhagen, Denmark), Servier, and Eli Lilly (Indianapolis, IN). He has received grant support from Sanofi and Pfizer.

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