

High striatal occupancy of D₂-like dopamine receptors by amisulpride in the brain of patients with schizophrenia

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Abstract

The 'atypicality' of the antipsychotic drug, amisulpride, has been attributed to preferential extrastriatal binding. Previous investigations of striatal D₂ receptor occupancy by amisulpride revealed conflicting results. The aim of this PET study was to measure the striatal occupancy by amisulpride and to correlate it with the corresponding drug plasma concentrations. Nine amisulpride-treated patients and 12 healthy volunteers serving as controls were studied with PET and [¹⁸F]desmethoxyfallypride. Occupancy values and plasma concentrations were nonlinearly fitted to an E_{max} model. Results showed 43–85% (putamen) and 67–90% (caudate) D₂-like receptor occupancy. Plasma amisulpride concentrations at the time of tracer injection, but not administered doses, were significantly nonlinearly correlated to occupancy levels (putamen: $r_S = 0.88$, $p = 0.0017$; caudate: $r_S = 0.78$, $p = 0.0127$). Calculated E_{max} was similar in both caudate and putamen, but occupancy levels were lower in caudate at lower amisulpride plasma concentrations. Calculated plasma levels to attain 60–80% receptor occupancy ranged from 119 to 474 ng/ml (caudate) and from 241 to 732 ng/ml (putamen). This reveals a broad range of plasma concentrations producing less than 80% striatal receptor occupancy. However, our data show high striatal D₂-like receptor occupancies under rising plasma concentrations. Using the full range of recommended amisulpride dosage, striatal occupancies up to 90% can be measured.

Received 17 September 2003; Reviewed 10 December 2003; Revised 18 February 2004; Accepted 14 March 2004

Key words: Amisulpride, atypical antipsychotic, D₂ receptor occupancy, [¹⁸F]desmethoxyfallypride, positron emission tomography.

Introduction

A consistent property of antipsychotic agents is the antagonism of D₂-like dopamine receptors. In positron emission tomography (PET) studies, classical antipsychotics were found to be effective in doses resulting in at least 60–70% striatal occupancy of D₂-like receptors (Farde et al., 1992; Nordström et al., 1993). Additionally, occupancies of more than approx. 80% are associated with extrapyramidal side-effects (Farde et al., 1992). Atypical agents produce antipsychotic effects with fewer extrapyramidal side-effects. Several mechanisms of atypical action including combined

5-HT₂/D₂ antagonism, selective D₄ antagonism, or rapid dissociation from D₂-like receptor sites are currently being discussed (Kapur and Seeman, 2001; Meltzer et al., 1989; Seeman et al., 1997). Another hypothesis suggests that 'atypicality' of antipsychotics is related to preferential mesolimbic binding. For clozapine, sertindole, olanzapine, quetiapine and amisulpride some investigators reported lower D₂-receptor binding in the striatum compared to extrastriatal regions (Bigliani et al., 2000; Pilowsky et al., 1997; Stephenson et al., 2000; Xiberas et al., 2001). However, the validity of the analytical methods used in these studies is a matter of considerable debate (Olsson and Farde, 2001). Talvik et al. (2001) were not able to find significant spatial differences of D₂-receptor binding in clozapine-treated patients. Analogously, Mukherjee et al. (2001) failed to show preferential extrastriatal binding of clozapine and risperidone in rhesus monkeys.

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The substituted benzamide amisulpride is widely used in Europe. Although acting only on D₂-like dopamine receptors with high selectivity (Schoemaker et al., 1997), this compound can be clearly classified as an 'atypical' antipsychotic (Leucht et al., 2002). The absence of D₄ or 5-HT₂ antagonism makes amisulpride an interesting antipsychotic for investigation with respect to other discussed mechanisms of 'atypicality'. Using PET and the high-affinity ligand [⁷⁶Br]FLB 457, Xiberas et al. (2001) reported a low striatal occupancy in the range of 0–61% compared to a very marked extrastriatal binding in patients treated with amisulpride under sufficient concentrations (occupancy in temporal cortex, 48–94%). This group calculated binding indices 165 min after tracer injection, at which time the radiotracer was expected to be in equilibrium. This approach does not account for regional and occupancy-dependent differences in time to equilibrium. This may result in a miscalculation of occupancy, specifically, an underestimation of striatal occupancy (for detailed discussion see Olsson and Farde, 2001). Interestingly, Martinot et al. (1996) reported earlier a markedly higher striatal binding of up to 76% using PET and a ligand with moderate affinity, [⁷⁶Br]bromolisuride. More recent work from Bressan et al. (2003) found comparatively higher striatal occupancies than Xiberas et al. (2001) but was able to support the hypothesis of preferential extrastriatal binding using the high-affinity SPET ligand [¹²³I]epidepride (striatum, 19–75%; temporal cortex, 68–91%).

To clarify the discrepancies regarding the striatal binding of amisulpride and to further investigate the relationships between amisulpride doses, plasma concentration and striatal dopamine receptor occupancy, we performed a PET study in patients suffering from schizophrenia and treated with stable amisulpride doses. In contrast to previous studies, scans were carried out close to the expected t_{\max} of 4 h after the last ingestion of amisulpride in order to depict the highest individual extent of receptor occupancy (Le Bricon et al., 1996). The in-vivo D₂-like dopamine receptor occupancy was measured using the radiotracer [¹⁸F]desmethoxyfallypride ([¹⁸F]DMFP), a substituted benzamide that has been previously validated (Gründer et al., 2003; Schreckenberger et al., In Press).

Methods

The study was approved by the local ethics committee in Mainz, Germany, and the German radiation safety authorities. Twelve healthy volunteers and nine patients were included after giving written informed

consent. All PET investigations were performed at the PET Center of the University of Mainz, Germany.

Healthy comparison subjects

The control group consisted of 12 male volunteers (24–60 yr; mean \pm s.d. = 35.4 \pm 15.9 yr). 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

Six male and three female patients (19–53 yr; mean \pm s.d. = 35.9 \pm 12.5 yr) were included. Except for one case, all subjects were in-patients following an acute psychotic exacerbation. The age of the patients did not significantly differ from that of the normals (Mann–Whitney *U* test, 53.5; $p=0.972$). In seven patients schizophrenia, according to the criteria of DSM-IV, was diagnosed. Two of the seven patients suffered from an acute episode of schizoaffective disorder with predominant positive symptoms. The mean duration of illness was 8.4 yr (range 0.5–25 yr). The mean Positive and Negative Syndrome Scale (PANSS) score was 76.0 (range 53–106). All patients received an ongoing, stable, daily dose of amisulpride (200–1200 mg/d) according to clinical needs for at least 2 wk. No patient received any co-medication with antidopaminergic, anticholinergic, or antiserotonergic properties. Two patients needed concomitant benzodiazepine or zolpidem treatment and one patient received lithium co-medication. Patients with addictive, neurological or other relevant somatic diseases were excluded from participation (see also Table 1).

Symptom characteristics were rated using the PANSS (Kay et al., 1987), the Abnormal Involuntary Movement Scale (AIMS; Guy, 1976), the Barnes Akathisia Rating Scale (Barnes, 1989) and the Simpson–Angus Scale (SAS; Simpson and Angus, 1970).

Data acquisition

PET images were acquired on a Siemens ECAT EXACT whole-body PET scanner. The camera has a field-of-view of 16.2 cm in 47 planes with a plane spacing of 3.375 mm and an axial resolution of 5.4 mm FWHM. Scanning was started immediately after a bolus injection of [¹⁸F]DMFP. Data acquisition comprised of a series of 28 time-frames with an overall scan duration of 124 min (4 \times 1, 3 \times 2, 3 \times 3, 15 \times 5,

Table 1. Patient characteristics, amisulpride plasma concentrations and corresponding D₂-like dopamine receptor occupancies in the putamen and caudate nucleus measured by PET with [¹⁸F]DMFP

Patient no.	Age	Gender	PANSS	SAS	Amisulpride dose (mg/d)	Amisulpride plasma concentration (ng/ml)		D ₂ -like dopamine receptor occupancy (%)			
						Morning ^c	PET ^d	Pseudoequilibrium		SRTM	
								Putamen	Caudate	Putamen	Caudate
1	50	M	62	17	400 ^a	74	193	53	70	51	68
2	29	M	84	–	600 ^a	67	194	63	74	62	70
3	30	F	106	12	800 ^a	211	213	38	50	43	67
4	53	F	69	18	200 ^a	71	231	68	76	69	82
5	46	M	75	25	800 ^a	121	283	53	65	65	73
6	44	F	64	14	200 ^a	143	322	69	70	67	68
7	23	M	65	12	1200 ^b	613	771	77	77	80	78
8	29	M	53	11	800 ^a	163	1003	84	90	83	90
9	19	M	106	20	600 ^a	375	1066	82	90	85	88

^a Drug intake once daily in the morning.

^b Drug intake twice daily (2 × 600 mg) in the morning and evening.

^c Specimen obtained in the morning before drug intake.

^d Specimen obtained at *t*₀ of PET scan (4 h after last drug intake).

3 × 10 min). A 20-min transmission scan using a ⁶⁸Ge source was performed for attenuation correction. PET scans were started approx. 4 h after the last oral ingestion of amisulpride, approximating the *t*_{max} of amisulpride (Le Bricon et al., 1996) [¹⁸F]DMFP was synthesized as previously described (Gründer et al., 2003). A mean of 198 ± 29 MBq (mean ± s.d., range 157–235 MBq) and of 201 ± 41 MBq (mean ± s.d., range 157–308 MBq) [¹⁸F]DMFP was intravenously injected into patients and control subjects respectively. Injections were administered as a bolus into a cubital vein over approx. 30 s. Applied radioactivities did not show any group differences (Mann–Whitney *U* test, 53.0; *p* = 0.943).

The positioning of the head in the scanner was parallel to the cantho-meatal line. A T1-weighted 3D gradient echo MR scan with 1.5 mm slice thickness and 128 slices was acquired. The MR image was re-sliced according to the ac–pc line, which was identified on a mid-sagittal plane. For a volume of interest (VOI)-based analysis, the PET images were realigned for movement correction, co-registered with the re-sliced MRI using the Automatic Image Registration (AIR) algorithm (Woods et al., 1992) and normalized to the space of Talairach and Tournoux (1988) using the MEDx tool. A template of polygonal regions of interest (ROI) for cerebellum, putamen and caudate nucleus was drawn on several planes. Regions for putamen and caudate nucleus were drawn in the slices from *z* = 0 to +12 mm and for the cerebellum,

from *z* = –28 to –24 mm. Corresponding ROIs were grouped across the planes to create VOIs. The volumes of the putamen, caudate nucleus and cerebellum VOIs were 3.12, 1.95 and 4.77 cm³ respectively. For the following analyses, the cerebellum was chosen as a reference region since it is generally considered dopamine-receptor free.

Data analysis

Binding potentials (BPs) of VOIs were calculated using the transient equilibrium method (Farde et al., 1989) and the Lammertsma Simplified Reference Tissue Model (SRTM; Lammertsma and Hume, 1996).

Lammertsma SRTM

A two-tissue compartment model was applied to estimate BPs according to the following equation (Lammertsma and Hume, 1996):

$$C_{RC}(t) = R_1 C_{RF}(t) + \left(k_2 - \frac{R_1 k_2}{1 + BP^*} \right) C_{RF}(t) \otimes \exp\left(\frac{-k_2 t}{1 + BP^*} \right),$$

where *C*_{RC} is the tissue radioligand activity in the receptor-containing region of interest, *C*_{RF} is the tissue radioligand activity of [¹⁸F]DMFP in the reference tissue (cerebellum), *k*₂ is the rate constant for transfer from the free compartment to plasma, BP is the binding potential, *R*₁ is the influx rate constant (*k*₁) ratio for target region vs. reference region, and *t* is time. The parameters *R*₁, *k*₂ and BP (*k*₃/*k*₄) were

estimated with a nonlinear least squares minimization procedure.

Transient equilibrium model (Farde et al., 1989)

Under the assumption that tissue radioligand activity in the non-displaceable compartment is the same in reference and receptor-containing tissues, the tissue radioligand activity bound to receptors $C_3(t)$ equals the difference $C_{RC}(t) - C_{RF}(t)$ (for a definition of terms see above). When $C_3(t)$ is plotted and a curve fitted to these data, at the peak of this curve, $dC_3(t)/dt = 0$ (the point of 'peak equilibrium'), thus, $C_3(t)/C_{RF}(t) = BP = k_3/k_4$.

For determination of D₂ receptor occupancy averaged BPs of control subjects were used as the common baseline value, assuming that there is no difference in B_{max} between patients and healthy control subjects when measured with benzamide radiotracers of moderate affinity (Farde et al., 1987; Hietala et al., 1994). The individual subject's receptor occupancy was defined as the percentage reduction of BP relative to the baseline BP according to the following equation:

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

Amisulpride plasma concentration

Amisulpride was administered daily as a single oral dose; with the exception of one case (2×600 mg/d). Blood samples were collected immediately before ingestion of the amisulpride morning dose and again immediately before (t_0) as well as 30, 60, 90 and 120 min after [¹⁸F]DMFP bolus injection during the PET scan. Blood samples were centrifuged and the serum stored frozen at -20 °C until assayed for amisulpride. Drug concentrations were determined in plasma by a slightly modified chromatographic method with online solid phase clean-up and subsequent isocratic high performance liquid chromatography (HPLC) with spectrophotometric quantification at 254 nm as previously described for fluvoxamine (Sachse et al., 2003). Plasma was injected on a clean-up column (10×4.0 mm i.d.) filled with silica CN material ($20 \mu\text{m}$) (MZ-Analysentechnik, Mainz, Germany) and cleaned by an eluent consisting of 8% (v/v) acetonitrile in deionized water. Chromatographic separation was performed on an analytical column filled with LiChrospher CN ($5 \mu\text{m}$ particle size, MZ-Analysentechnik) using 50% acetonitrile and 50% K_2HPO_4 (0.008 M), adjusted to pH 6.4 by H_3PO_4 at a flow rate of 1.5 ml/min. The limit of quantification was approx. $10 \mu\text{g/l}$. Inter-assay reproducibility and

inaccuracy ranged between 2.8 and 11.3% and between 0.6 and 9.1% respectively.

Pharmacokinetic and statistical analysis

Plasma concentrations (morning plasma levels, t_0 and AUC) and per cent binding data were fitted to a simple one-site ligand-binding model by nonlinear regression analysis using Sigma Plot, Version 8.0 (SPSS Inc., Chicago, IL, USA), to the following equation:

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

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_{ami} is the plasma concentration of amisulpride. From the fitted concentration/occupancy curves the plasma concentrations that resulted in striatal D₂-like dopamine receptor occupancies of 60% or 80% (C_{Occ60} , C_{Occ80} respectively) were calculated. These values were chosen, because they are believed to represent crucial thresholds for anti-psychotic efficacy and extrapyramidal side-effects respectively (Farde et al., 1992; Nordström et al., 1993). Values calculated from our data were compared with the respective values that were calculated from the fitted curve derived from data for the temporal cortex published by Xiberas et al. (2001).

Statistical evaluations of group differences were made by two-sided Mann-Whitney U tests. Linear correlations between the SRTM and transient equilibrium method, between daily dose and plasma levels and between side-effects and receptor binding were calculated using Spearman's rank order correlation. The level of significance was set at $\alpha = 0.05$.

Results

Both analytical methods (SRTM and transient equilibrium method) revealed similar results for BPs. For the patients studied, the mean BP in the putamen for SRTM (range 0.34–1.33, mean \pm s.d. = 0.77 ± 0.33) correlated significantly ($r_s = 0.917$, $p = 0.001$) with the BP for the transient equilibrium method (range 0.41–1.42, mean \pm s.d. = 0.79 ± 0.34). BPs of the caudate nucleus ranged from 0.17 to 0.54 (mean \pm s.d. = 0.39 ± 0.14) for the SRTM and from 0.18 to 0.86 (mean \pm s.d. = 0.45 ± 0.21) for the transient equilibrium method (correlation of methods: $r_s = 0.88$, $p = 0.002$). Average BPs in control subjects were 2.35 ± 0.34 for putamen and 1.63 ± 0.36 for caudate nucleus (transient equilibrium method: 2.27 ± 0.39 and 1.71 ± 0.36 respectively).

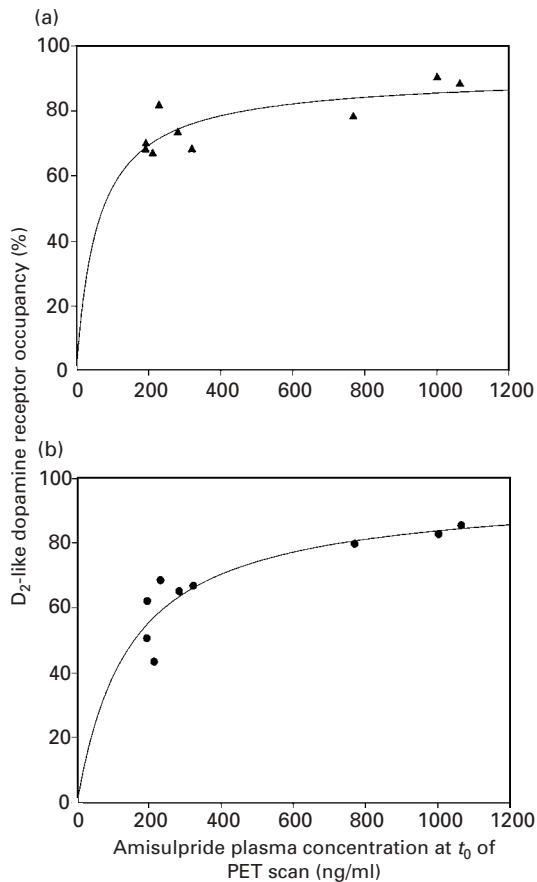


Figure 1. Relationship between amisulpride plasma concentration at t_0 of PET scan and D₂-like dopamine receptor occupancy in (a) caudate nucleus and (b) putamen. [Nonlinear curve fitting was performed following the assumption of one site ligand binding as described in the text. Drop lines characterize the concentrations at Occ₆₀ (temporal cortex) or Occ₈₀ (putamen) which are believed to be thresholds for antipsychotic action and extrapyramidal side-effects respectively (see also Table 2).]

The calculated striatal D₂-like dopamine receptor occupancy 4 h after the last ingestion of amisulpride ranged from 43 to 85% in the putamen using SRTM and 38–84% using the transient equilibrium method. The corresponding occupancy in the caudate nucleus was 67–90% and 50–90% respectively (Table 1).

Receptor occupancy did not correlate with morning plasma levels ($r_s = 0.452$, $p > 0.1$) or daily amisulpride doses ($r_s = 0.095$, $p > 0.1$). Amisulpride morning plasma levels showed a positive linear trend to daily doses ($r_s = 0.598$, $p = 0.089$), but failed to correlate with plasma concentrations during the PET scan ($r_s = 0.299$, $p = 0.434$).

Statistically significant nonlinear correlations were found between D₂-like dopamine receptor occupancy

Table 2. Fitted pharmacokinetic parameters of D₂-like dopamine receptor occupancy in the putamen and caudate nucleus compared to data of the temporal cortex taken from Xiberas et al. (2001)

Region	E_{max}^a	EC_{50}^b	Occ ₆₀ ^c	Occ ₈₀ ^d
Putamen	95.7	143.6	241.3	731.6
Caudate nucleus	90.1	59.9	119.3	474.0
Temporal cortex (according to Xiberas et al., 2001)	90.5	24.1	47.4	183.7

^a E_{max} is the maximum attainable receptor occupancy (%).

^b EC_{50} is the plasma concentration (ng/ml) predicted to provide 50% occupancy of E_{max} .

^c Occ₆₀ is defined as plasma concentration (ng/ml) predicted to provide 60% occupancy of all D₂-like receptors.

^d Occ₈₀ is defined as plasma concentration (ng/ml) predicted to provide 80% occupancy of all D₂-like receptors.

(SRTM) in the putamen or caudate nucleus, and amisulpride plasma concentration before tracer injection. Under the assumption of a nonlinear relationship between amisulpride plasma concentration and striatal receptor occupancy (see Methods section), we found highly significant correlations both in the putamen ($r_s = 0.88$, $p = 0.0017$; Figure 1b) and caudate nucleus ($r_s = 0.78$, $p = 0.0127$; Figure 1a). Additionally, tests for linear correlations yielded significant results for putamen (two-tailed Pearson correlation: $r_s = 0.85$, $p = 0.04$) and caudate ($r_s = 0.84$, $p = 0.05$).

The maximal attainable D₂ receptor occupancy E_{max} in the putamen (96%) and caudate (90%) was very similar to the E_{max} value in the temporal cortex (91%) calculated from the data reported by Xiberas et al. (2001). However, there were marked differences in ED_{50} , C_{Occ60} , and C_{Occ80} (see Table 2 for details). While 60% of D₂ receptors in the putamen were occupied at amisulpride plasma levels of approx. 240 ng/ml, plasma concentrations of less than 47 ng/ml (<20%) were needed for 60% occupancy of temporal cortical D₂ receptors based on the data published by Xiberas et al. (2001) (for details see also Table 2).

No linear correlation could be found between D₂-like dopamine receptor occupancy and side-effects as assessed by the SAS rating scale.

Discussion

Amisulpride, like other substituted benzamides, acts as a selective antagonist at D₂/D₃ dopamine receptors (K_i values, 2.8 and 3.2 nM respectively). This

antipsychotic shows negligible affinities to other receptors including 5-HT₂ and D₄ receptors (Schoemaker et al., 1997). However, amisulpride clearly shows an 'atypical' clinical profile (Leucht et al., 2002) with a lower incidence of extrapyramidal side-effects compared to conventional antipsychotics (Delcker et al., 1990; Wetzel et al., 1998) and favourable effects on negative symptoms (Boyer et al., 1995). The prominent role of substituted benzamides for the understanding of 'atypicality' of antipsychotic drugs has been recognized by Gründer and Benkert (2002) and Kapur and Remington (2001). Both the preferential binding to dopamine autoreceptors at low doses and the preferential binding to mesolimbic dopamine receptors have been proposed to explain amisulpride's atypical properties (Ferrault et al., 1997; Schoemaker et al., 1997).

Xiberas et al. (2001), using the high-affinity ligand [⁷⁶Br]FLB 457, detected high D₂/D₃ dopamine receptor occupancies in the temporal cortex (48–94%) and the thalamus (31–90%) compared to markedly lower, and in three cases negligible occupancies, in the striatum (0–62%) of patients undergoing treatment with amisulpride (with plasma concentrations above 27 ng/ml). These occupancy levels were calculated using binding indices determined at fixed time-points during the scan. Using the high-affinity SPET ligand [¹²³I]epidepride and dynamic measurements with a subsequent reference tissue model analysis, Bressan et al. (2003) were able to support this finding of preferential extrastriatal binding but found comparatively higher striatal occupancies (temporal cortex 68–91%; striatum 19–75%). Using [⁷⁶Br]bromolisuride, a radiotracer with lower affinity, Martinot et al. (1996) found striatal occupancies ranging from 10 to 76%. In this investigation specific ligand binding was estimated by binding indices at fixed time-points. Furthermore, no corresponding amisulpride concentrations were available.

In our study, we measured occupancy levels using the radioligand [¹⁸F]DMFP in patients undergoing antipsychotic treatment with amisulpride. [¹⁸F]DMFP is a D₂/D₃ selective substituted benzamide with a moderate affinity ($K_i=15$ nM). We have previously shown that striatal D₂-like dopamine receptors can be reliably quantified with [¹⁸F]DMFP using various invasive and non-invasive methods (Gründer et al., 2003). In the present study, we found markedly higher striatal D₂/D₃ receptor occupancies (putamen 43–85%, caudate nucleus 67–90%, using SRTM) than the reported values from Xiberas et al. (2001) and Bressan et al. (2003). The images in Figure 2 exemplify the marked reduction of striatal [¹⁸F]DMFP uptake in a

typical patient treated with 800 mg/d amisulpride. In seven patients amisulpride treatment led to more than 60% D₂-like receptor occupancy in the putamen. In the caudate nucleus all patients were above the 60% level (Figure 1, Table 1). Two patients exceeded the 80% receptor occupancy level in the putamen and three patients in the caudate nucleus.

There are remarkable differences between our study protocol compared to previous investigations. In the three studies mentioned above, only a minority of the patients received a medication of more than 400 mg/d amisulpride, whereas our patient sample consisted of in-patients with prominent positive symptoms, requiring doses of amisulpride usually higher than 400 mg/d. Furthermore, we performed the PET scans close to the reported t_{max} of amisulpride. As consequences of both factors, the amisulpride plasma concentrations obtained in our sample were comparably higher (see also Table 1).

Nevertheless, the pronounced discrepancies in observed striatal D₂-like receptor occupancies between Xiberas et al. (2001) and the present study do not depend only on lower plasma concentrations. Taking advantage of the long half-life of [⁷⁶Br]FLB 457 (16.1 h), Xiberas et al. (2001) measured brain radioactivity statically at a fixed time post-injection, i.e. at 165 min. This time was chosen from animal studies, because it corresponds to the time of transient equilibrium in extrastriatal regions. However, a transient equilibrium in striatum of rhesus monkeys is observed markedly later (Delforge et al., 1999; Loc'h et al., 1996). Calculating binding ratios at fixed time-points in regions with considerably different concentrations of receptor sites and under varying states of blockade using high-affinity ligands appears to be highly unreliable. Under these conditions, the time-point at which the binding ratio is calculated can be very distant from the time at which equilibrium occurs. This problem may be most crucial in the unblocked striatum, where the activity curve of specifically bound [⁷⁶Br]FLB 457 is continuously rising for a remarkable time-interval after the time-point of measurement. The most likely explanation for the considerably lower striatal D₂ receptor occupancies (at comparable amisulpride plasma levels) reported by Xiberas et al. (2001) may, therefore, be an underestimation of [⁷⁶Br]FLB 457 binding in the unblocked striatum, whereas possible miscalculations of receptor occupancies in extrastriatal regions with lower receptor density might be less pronounced. Interestingly, Martinot et al. (1996) measured comparatively high striatal occupancies of up to 76% under an antipsychotic dose regimen. As mentioned above, they also calculated binding ratios at fixed time-points but

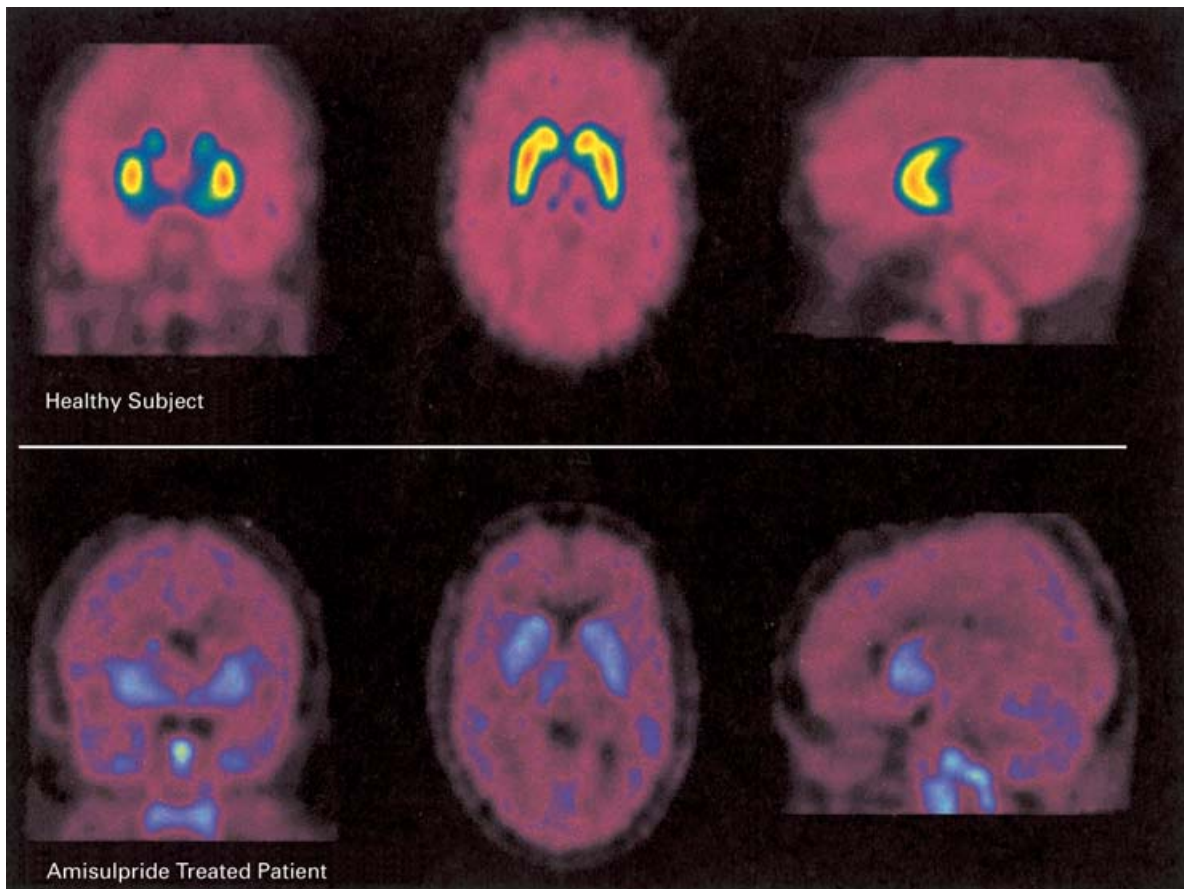


Figure 2. Averaged image over all time-frames of a healthy volunteer compared to the patient with highest extent of D_2 -receptor blockade (patient no. 8). Images are shown in coronal, horizontal and paramedian sagittal orientation.

used a ligand with lower affinity. In addition to receptor density the ligand affinity also influences at what time equilibrium occurs. Both high available receptor density and high ligand affinity lead to a later time-point of equilibrium conditions. This may result in determination of binding ratios before the equilibrium appears. Performing a simulation study, Olsson and Farde (2001) calculated considerable under-estimations of occupancy results under such pre-equilibrium conditions. The use of [^{76}Br]bromolisuride with moderate affinity enabled Martinot et al. (1996) to determine striatal binding ratios nearby the equilibrium. Hence, their striatal occupancy data can be assumed to be more reliable than those measured by Xiberas et al. (2001) using a high-affinity ligand.

Bressan et al. (2003) in contrast to Xiberas et al. (2001) and Martinot et al. (1996) used the simplified reference tissue model to analyse their data. Their calculated striatal D_2/D_3 receptor occupancies are higher than those reported by Xiberas et al. (2001) but below the occupancies we found in the present

investigation. As discussed previously, differences in dosage and different study protocols led to lower plasma levels in the investigation of Bressan et al. (2003) compared to our study. Nevertheless, there is a range of similar amisulpride plasma concentrations that overlaps between these two studies (approx. 200–400 ng/ml). Focusing on this overlapping range of concentrations, Bressan et al. (2003), as well as our investigation, found a similar extent of amisulpride binding (approx. 40–70%). When considering these results, our data are in accordance with Bressan et al. (2003). Moreover, the present study expands the range of investigated amisulpride concentrations to the entire spectrum that is expected under antipsychotic treatment (from 400 up to 1200 mg/d).

These high amisulpride plasma concentrations result in D_2 -like dopamine receptor binding of up to 90%, which apparently may contradict the hypothesis of lower striatal than extrastriatal binding. Nevertheless, our data indicate that there is a broad range of plasma levels that lead to occupancy levels below

the crucial 80% threshold for the occurrence of EPS. This is illustrated by the concentration/occupancy curves (Figure 1) that can be obtained by a nonlinear correlation between amisulpride concentrations (at time of injection) and striatal D₂ receptor occupancy. Using these concentration/occupancy curves, amisulpride plasma concentrations can be calculated that statistically lead to the EPS threshold of 80% D₂-like receptor occupancy (Occ₈₀) and to the efficacy threshold of 60% (Occ₆₀). The Occ₈₀ is reached later at comparably high plasma levels (putamen 732 ng/ml, caudate nucleus 474 ng/ml), whereas the Occ₆₀ is reached much earlier (putamen 241 ng/ml, caudate nucleus 119 ng/ml). This provides a broad range of amisulpride plasma concentrations that lead to sufficient but well-tolerated D₂-like dopamine receptor occupancies. In the present study, a direct comparison of drug binding in striatal and extrastriatal areas is not possible due to the moderate affinity of [¹⁸F]DMFP. A comparison of our striatal concentration/occupancy curves with the extrastriatal curves of Bressan et al. (2003) and Xiberas et al. (2001) is limited by the use of different radiotracers, methods of analysis and study designs. However, Xiberas et al. (2001) as well as Bressan et al. (2003), independently from the used analytical method, found remarkably high drug binding in extrastriatal regions even at low drug concentrations. Approx. 80% of D₂/D₃ dopamine receptor occupancy in the temporal cortex was found at drug levels of 200 ng/ml. These results demonstrate that, in extrastriatal regions, occupancy levels approximate the E_{max} at much lower amisulpride plasma concentrations than in striatal regions (see also Table 2).

However, methodological limitations that influence the course of the measured concentration/occupancy curves should also be considered. At lower amisulpride concentrations, the receptor occupancies measured in this study also differed between the caudate nucleus and putamen at lower amisulpride concentrations. This might be due to a stronger influence of partial volume effects in the smaller region of the caudate nucleus. The presence of considerable partial volume effects may be reflected by remarkably lower binding potentials in the caudate nucleus compared to the binding potentials in the putamen.

In conclusion, our data support the results of Bressan et al. (2003) regarding moderate striatal amisulpride binding at lower drug concentrations. Moreover, we found that higher drug concentrations result in remarkably high striatal D₂-like occupancies of up to 90%. These results may suggest that a possible preferential extrastriatal binding of amisulpride may occur only at lower plasma levels.

Acknowledgements

The authors thank Markus Piel, Ralf Schirmacher, Sabine Höhnemann and Stephan Maus for providing [¹⁸F]desmethoxyfallypride and Heike Armbrust for assistance in performing the PET studies. We gratefully acknowledge Matthias J. Müller's statistical advice. Part of this work was supported by the Deutsche Forschungsgemeinschaft DFG (Grant Ba 1011/2-1) and by the University of Mainz (MAIFOR).

Statement of Interest

None.

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