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ORIGINAL INVESTIGATION

Occupancy of dopamine D_1, D_2 and serotonin$_{2A}$ receptors in schizophrenic patients treated with flupentixol in comparison with risperidone and haloperidol

M. Reimold · C. Solbach · S. Noda · J.-E. Schaefer · M. Bartels · M. Beneke · H.-J. Machulla · R. Bares · T. Glaser · H. Wormstall

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Abstract
Rationale Flupentixol (FLX) has been used as a neuroleptic for nearly 4 decades. In vitro data show comparable affinity to dopamine D_2, D_1 and 5-HT_{2A} receptors and recently, FLX showed to be not inferior to risperidone in schizophrenic patients with predominant negative symptomatology, which was implicated with flupentixol's interaction with 5-HT_{2A} and/or D_1 receptors.
Objectives To assess in vivo receptor occupancy (RO) in patients clinically treated with FLX (n=13, 5.7±1.4 mg/day) in comparison with risperidone (RIS, n=11, 3.6±1.3 mg/day) and haloperidol (HAL, n=11, 8.5±5.5 mg/day).
Materials and methods Each patient underwent two PET scans with 3-*N*-[^{11}C]methylspiperone (target: frontal 5-HT_{2A}), [^{11}C]SCH23390 (striatal D_1) or [^{11}C]raclopride (striatal D_2). RO was calculated as the percentage reduction of specific binding in comparison with healthy controls.
Results D_2-RO under FLX was between 50% and 70%, indicating an ED_{50} of about 0.7 ng/ml serum. 5-HT_{2A} and D_1-RO was 20±10% and 20±5% (mean, SEM). Under HAL, D_1-RO was 14±6% and under RIS not significantly different from zero.
Conclusions We were able to demonstrate a moderate 5-HT_{2A} and D_1 occupancy under clinically relevant doses of flupentixol, albeit lower than expected from in vitro data and clearly below saturation. Therefore, if flupentixol's efficacy on negative symptoms is based on its interaction with 5-HT_{2A} and/or D_1 receptors, it should be highly dependent on serum concentration and thus on dosage and metabolism. However, these data suggest that mechanisms other than D_1 or 5-HT_{2A} antagonism may contribute to flupentixol's efficacy on negative symptoms.

Keywords Positron emission tomography · Receptor occupancy · Dopamine D_2 receptor · Dopamine D_1 receptor · 5-HT_{2A} receptor · Flupentixol · Haloperidol · Risperidone

M. Reimold · R. Bares
Department of Nuclear Medicine/PET Center,
University of Tuebingen,
Tuebingen, Germany

S. Noda · J.-E. Schaefer · M. Bartels · H. Wormstall
Department of Psychiatry and Psychotherapy,
University of Tuebingen,
Tuebingen, Germany

C. Solbach · H.-J. Machulla
Radiopharmacy, University of Tuebingen,
Tuebingen, Germany

M. Beneke · T. Glaser
Bayer Vital GmbH,
Leverkusen, Germany

M. Reimold (✉)
PET Center,
Roentgenweg 15,
72076 Tuebingen, Germany
e-mail: matthias.reimold@uni-tuebingen.de

Introduction

There is a large body of evidence that the neurotransmitter dopamine and serotonin (5-HT) play an important role in schizophrenia and in the action of antipsychotic drugs. Several receptor subtypes for these neurotransmitter seem to be involved herein. It is generally accepted that for antipsychotic activity (with respect to positive symptoms), a blockade of mesolimbic dopaminergic transmission with D_2 receptor antagonists is an essential prerequisite. This

hypothesis is, among numerous other findings, based on the fact that all currently available antipsychotics are more or less potent dopamine D_2 receptor blockers. Positron emission tomography (PET) and single photon emission computed tomography (SPECT) have been used for more than 15 years to assess the in vivo occupancy of various receptor systems under neuroleptic treatment. Receptor occupancy is defined as the percentage of binding sites occupied by medication, assessed by means of percentage reduction of binding potential of a certain receptor system. The first and most freqently investigated receptor system under neuroleptic treatment is the dopamine D_2 receptor (Farde et al. 1986), many studies of the D_1 receptor and the serotonergic system followed. PET and SPECT are especially useful to investigate the relation between occupancy and clinical effects, and can therefore be used to make dose suggestions (Nyberg et al. 1999). Several PET and SPECT studies have come to the conclusion that for antipsychotic efficacy a blockade of 65–70% of dopamine D_2 receptors is necessary and sufficient, whereas a blockade of >80% leads to an increased risk of extrapyramidal symptoms (EPS) (Nordstroem et al. 1993; Nyberg et al. 1995; Kapur et al. 2000a; but also see Gründer et al. 2003). This "therapeutic window" seems to apply for many different neuroleptics. However, for some "atypical" compounds, e.g. clozapine or quetiapine, lower D_2-RO values were observed under clinically effective doses (Kapur et al. 2000b; Tauscher et al. 2004).

It was hypothesized that both superior effectiveness and better tolerability of the prototypical atypical antipsychotic drug clozapine is at least in part due to its additional interaction with dopamine D_1 and 5-HT_{2A} receptors (Farde and Nordstrom 1992; Farde et al. 1992; Meltzer 1992; Lundberg et al. 1996). Consequently, during the past years various so-called atypical or second generation antipsychotics with a combined action on both dopamine D_2 and 5-HT_{2A} receptors were developed for the treatment of schizophrenia. The first of them was risperidone, which leads to a 5-HT_{2A}-RO that is even higher than that of dopamine D_2 receptors (Nyberg et al. 1993, 1996). Similar findings were also reported for most of the other atypical antipsychotics (Seeman 2002; Nyberg et al. 1998). Those data support the hypothesis put forward by Meltzer et al. (1989) that the atypicality of antipsychotic drugs may be related to the ratio of their affinities for 5-HT_{2A} and dopamine D_2 receptors (5-HT_{2A}/D_2 ratio). There is some evidence that the benefit from 5-HT_{2A} receptor antagonism is based on interactions between the serotonergic and the dopaminergic system (Lieberman et al. 1998; Ichikawa et al. 2001) resulting in increased dopaminergic activity. It is interesting to note that the relevance of mesocortical dopaminergic activity in schizophrenic patients for cognitive functions could be demonstrated with PET. Abi-Dargham et al. (2002) showed that increased prefrontal D_1 availability, presumably secondary to deficient dopaminergic function, is a strong predictor of poor performance at the n-back task, a test of working memory. Still, the role of 5-HT_{2A} receptor antagonism for neuroleptic treatment remains controversial and other possible criteria for "atypicality" were described (Kapur and Seeman 2001; Gründer et al. 2003).

Among the classical neuroleptic drugs there are also compounds with combined dopamine D_2 and 5-HT_{2A} receptors activities. One of those is the thioxanthene derivative flupentixol, which has now been marketed as a neuroleptic for nearly 4 decades. The pharmacological receptor profile of flupentixol is characterized by high affinity interactions with dopamine D_1, D_2 and 5-HT_{2A} receptors in vitro (Hyttel et al. 1985; Glaser and Soyka 1998). Corresponding human data were generated only in very few schizophrenic patients by PET measurements (Farde et al. 1992; Nyberg et al. 1996; Lundberg et al. 1996). Based on recent preclinical and clinical studies, a partial atypical profile of flupentixol was proposed (Glaser and Soyka 1998; Kuehn et al. 2000). In line with this profile are the results of a recent double-blind head to head trial with risperidone, which showed flupentixol being not inferior to risperidone in 153 schizophrenic patients with predominant negative symptomatology (Philipp et al. 2002, Philipp et al. 2003). In a recent double-blind study with 28 patients (Gattaz et al. 2004), the efficacy of flupentixol was not significantly different from that of olanzapine, whereas the side effects profile differed in that patients receiving flupentixol experienced more EPS, but less weight gain.

The goal of the present trial was to assess the occupancies of dopamine D_1, D_2 and 5-HT_{2A} receptors in clinically stable schizophrenic patients treated with flupentixol and to compare the occupancy profile of flupentixol with that observed under risperidone and haloperidol under comparable clinical conditions.

Materials and methods

This was a monocenter, randomized, open-label study conducted with patients routinely treated as inpatients or outpatients with one of the three antipsychotic drugs under study. Approvals were obtained from the local Ethics Committee and from the Bundesinstitut für Strahlenschutz (BfS). Subjects gave their written informed consent after a detailed description of the study.

Patients and control subjects

Eligible for the study were patients with the diagnosis of schizophrenia or schizoaffective psychosis according to the

ICD-10 criteria in stable remission, treated in steady state (i.e. et least for 14 days) with flupentixol (4–10 mg/day or a corresponding dose of flupentixol decanoate), haloperidol (5–10 mg/day) or risperidone (2–6 mg/day) with no history of substance abuse and no severe somatic disorder (e.g. stroke, parkinsonism, neurodegenerative disorder). At the time of the PET scan, the patients had to be free of any other psychotropic medication, the required drug-free interval was chosen upon clearance (minimum 3 days, 5 weeks for SSRI, short-term treatment with benzodiazepines and anticholinergic drugs allowed).

A total of 35 patients (Table 1) were enrolled ("intent to treat" population: $n=13$ flupentixol, $n=11$ haloperidol, $n=11$ risperidone), 32 of which underwent two PET scans within 2 weeks, randomly assigned to two of the three tracers [^{11}C]raclopride (RAC; for assessment of striatal D_2 occupancy), 3-*N*-[^{11}C]methylspiperone (NMS; for assessment of frontal 5-HT_{2A} occupancy) and [^{11}C] SCH 23390 (SCH; for assessment of striatal D_1 occupancy). Three patients underwent only one PET scan with one of the abovementioned tracers. In five patients, the urine test at the day of the first PET scan revealed additional psychotropic medication or drugs. Patients that completed both PET scans without a positive drug urine test and without any other protocol violation formed the "per protocol" population. In three of these patients, serum drug concentration was very low (flupentixol: two patients with levels below the detection limit; risperidone: one patient with 0.1 ng/ml), presumably due to non-compliance, and their PET scans were not included in the statistical analysis.

As drug-free controls, 26 age-matched healthy controls (HC) were recruited after completing a health screening questionaire and a urine drug test. Each control subject underwent one PET scan. (RAC: 9, NMS: 9, SCH: 8).

Table 1 Number of patients and PET scans

		Flupentixol	Risperidone	Haloperidol
Total (intent to treat)		13	11	11
Dropouts[a]		2	1	–
Patients[b] with				
Two scans	D_1 and D_2	3	3=2+1[d]	3
	D_1 and 5-HT_{2A}	3	4=3+1[d]	3
	D_2 and 5-HT_{2A}	4=2+2[d]	3	3
One scan	D_1[c]	1		1[d]
	5-HT_{2A}[c]			1

[a] Serum level negligible
[b] All patients except those with negligibe serum level
[c] Consent withdrawn/protocol violation after the first PET scan
[d] Positive drug urine test

Preparation of ^{11}C-ligands

RAC, NMS and SCH were synthesized by alkylation of *S*-(+)-*O*-demethyl-raclopride, spiperone and demethyl-SCH23390 using [^{11}C]methyliodide (Ehrin et al. 1987; Dannals et al. 1986; Halldin et al. 1986). High specific activity [^{11}C]CH3I was prepared using an automated module (MeI Microlab, General Electric Medical Systems, Uppsala, Sweden). Radiolabeling was performed in a PET tracer synthesizer for ^{11}C-methylations from Nuclear Interface (Muenster, Germany). After purification and formulation, the three radiotracers were obtained in radiochemical yields of 25–50% calculated on produced [^{11}C]CH3I. The total synthesis times were 50–60 min from the end of bombardment. The specific activities were in the range of 30–50 GBq/μmol at the end of synthesis. The radiochemical purities of the final formulated product solutions were >95% as determined by radio-HPLC.

Clinical assessments

At the day of each PET scan, patients had to undergo clinical ratings of their psychopathology by means of the positive and negative syndrome scale (PANSS; Kay et al. 1987), EPS (Simpson-Angus scale developed in 1970) and clinical assessment (clinical global impression, CGI). In all patients, a urine drug test was performed at the day of the PET scan.

Determination of drug plasma levels

Venous blood samples were collected directly before injection of the radioligand, centrifuged and frozen. The serum drug concentration in all samples was determined centrally by MDS Pharma Services, Fehraltorf, Switzerland, using standard analytical techniques.

PET data acquisition

In total, 93 PET scans were conducted (67 in patients, cf. Table 1 and 26 in healthy controls). The two PET scans of each patient were conducted at two visits, which were 2–14 days apart from each other.

During the PET scan, the patients head was fixed in an elastic mould with adhesive stripes. Three fiducial markers were attached to the scull to support image realignment. After intravenous bolus injection of 700 MBq RAC (<13.2 μg), NMS (<15.4 μg) or SCH (<10.8 μg), the cerebral radioactivity distribution was measured with a GE Advance PET scanner (GE-Medical Systems, Milwaukee, USA, axial field view of 15 cm) in two-dimension acquisition mode, followed by a transmission scan with 500,000 kilo counts.

Image reconstruction and ROI analysis

We used filtered backprojection (128×128 pixel=30 cm) with a Hanning filter (cutoff 4.6 mm) to reconstruct our images. For realignment and for a coarse anatomical normalization before ROI analysis, we used SPM99 with the standard SPM perfusion template and early summation images (0–5 min p.i.) of each PET-scan with non-linear transformations being disabeled. Time–activity curves were derived from standardized three-dimensional regions of interest (ROI) locally established at the PET Center Tuebingen for the putamen (2×0.7 ml), the frontal cortex (2×2.0 ml, BA10) and the cerebellum (2×10 ml), manually placed on summation images 0–5 min p.i. Due to the coarse spatial pre-normalization, we were able to apply these ROI without modifications to size or shape.

Modelling and calculation of receptor occupancy (RO)

For each type of receptor, we assessed the availability of binding sites with the measure $k_3/k_4 = f_2 \times B_{max}/K_D$, in the literature also denoted V_3" (Laruelle et al. 1994) or binding potential BP_2 with k_3 and k_4 being the transfer rate constants between the first and the second tissue compartment, f_2 the free fraction of the tracer in the first tissue compartment, B_{max} the density of available (i.e. not occupied) binding sites and K_D the equilibrium dissociation constant of the radiotracer. The BP_2 of D_2-like receptors (RAC) was calculated with the simplified reference tissue method (SRTM, Lammertsma and Hume 1996). The BP_2 of 5-HT_{2A} receptors was calculated from BP_2=VR−1 with VR being the distribution volume ratio obtained as slope of the linearization proposed by Logan et al. 1996, assuming a washout from the reference tissue k_2'=0.06 min^{-1}. This washout constant was estimated with the SRTM from inter-individually averaged time–activity curves of the control subjects (cf. Fig. 1). Logan's linearization was also applied to the SCH data, here we used an estimated k_2'=0.14 min^{-1}. The corresponding linearizations of inter-individually averaged data from NMS are shown in Fig. 1 to demonstrate approximately mono-exponential behavior during the chosen regression interval.

The RO was assessed by means of the percentage reduction of specific binding in the striatum (RAC, SCH) and the frontal cortex (NMS). Since no individual baseline PET (i.e. without neuroleptic treatment) was available, the mean BP_2 (age corrected) of the control group was used instead ($RO=100\%-BP_2/BP_{mean}$), thus values of RO below 0% are possible due to baseline variability. For age correction, a mono-exponential function was fitted to BP_2 in healthy controls in a least square sense. Since our control group was age-matched, this age correction had only a small effect on the calculated mean occupancy, however, it allowed for reducing the baseline variance (expressed as $\hat{\sigma}_\varepsilon^2$, see Eq. (2) below) and thus to obtain smaller confidence intervals.

Saturation analysis, statistics

The synaptic concentration of the neuroleptic drug was assumed to be proportional to the serum concentration, which allows determining the plasma concentration equivalent to 50% occupancy (ED_{50}) from a non-linear fit of the saturation curve:

$$RO = RO_{max} \times C_{serum}/(C_{serum} + ED_{50}) \quad (1)$$

with an assumed RO_{max}=100%.

With the given sample sizes, this equation allows to reliably estimate ED_{50} only for higher values of RO, as of D_2 receptors. Therefore, the occupancy of striatal D_1 and frontal 5-HT_{2A} receptors was not compared with serum drug concentration, instead a mean level of occupancy was

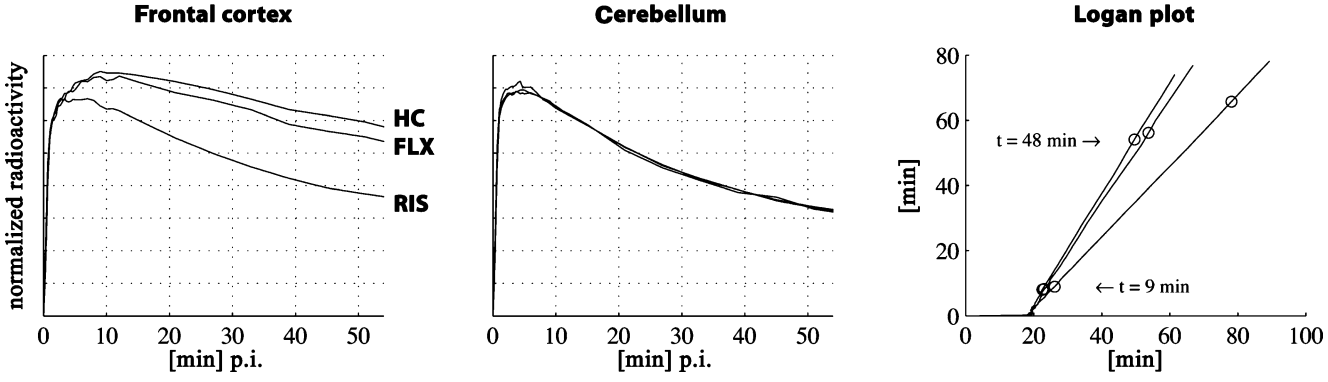

Fig. 1 Frontal NMS binding: time–activity curves and quantification. Mean time–activity curves of NMS in the frontal cortex (*left*) and the cerebellum (*middle*). The Logan plot (*right*) shows straight lines for all groups, data from the haloperidol group is not shown (no 5-HT_{2A} occupancy). NMS is known to bind irreversibly to other receptors (especially D_2) and the linearity of the Logan plot therefore assures that D_2 density in the frontal cortex is small enough to allow for reliable quantification of 5-HT_{2A} binding. The slopes are 1.69 (HC=healthy controls), 1.56 (FLX=flupentixol, n=5) and 1.08 (RIS=risperidone, n=6), which corresponds to a reduction of specific binding by 19% in flupentixol patients

statistically assessed after excluding a few patients, which had very low level of serum drug concentration, presumably due to non-compliance. The total standard error of the estimated mean occupancy $\sigma_{\overline{RO}}$, which includes the uncertainty from baseline variability, was calculated from:

$$\sigma^2_{RO} = \hat{\sigma}^2_{\overline{RO}} + \left(1 - \overline{RO}/100\%\right)^2 \hat{\sigma}^2_{\varepsilon} \quad (2)$$

with $\hat{\sigma}_{\overline{RO}}$ being the standard error of the mean calculated RO, $\hat{\sigma}^2_{\varepsilon}$ the standard error of the "apparent RO" of the control subjects (i.e. the residual from the age correction expressed in percent) and $\overline{RO}$ the mean receptor occupancy. The 95% confidence intervals were plotted as diamonds ranging from -2σ to $+2\sigma$. For groups with patients having a positive drug urine test, we calculated $\overline{RO}$ twice: once including and once excluding these patients.

For validation, we also calculated the mean receptor occupancy of frontal 5-HT_{2A} and striatal D_1 receptors of each group from inter-individually averaged time–activity curves as described previously (Reimold et al. 2004).

The inference statistical analysis was conducted as an exploratory evaluation because no reasonable sample size calculation could be made beforehand. The analysis was based on a balanced 3×3 split-plot-factorial design (three receptors and three treatments) with block confounding, i.e. subjects were only exposed to two out of three possible treatments. The analysis of the all psychopathological rating scales was based on descriptive statistics using measures of central tendency and spread.

Results

The clinical data of all patients are given in Table 2. The patient groups were widely comparable, however, the duration of neuroleptic treatment was shorter in the risperidone group (mean: 6.4 years) than in the flupentixol (10.3 years) and the haloperidol groups (11.2 years). The mean daily dosages of 5.7 mg flupentixol, 8.51 mg haloperidol and 3.59 mg risperidone corresponded to those usually administered in long-term treatment. Two patients in the flupentixol group and one patient in the risperidone group had a very low or zero drug serum concentration, presumably due to non-compliance, therefore these patients were not used to calculate the mean 5-HT_{2A} and D_1 receptor occupancy. Psychopathology and EPS ratings at study entry by means of the PANSS subscales and the Simpson-Angus scale, respectively, revealed only marginal if any differences in the corresponding mean scores (Table 2). Changes between visit 1 and visit 2 were negligible. According to the CGI scale, 70–80% of patients in each group were mildly to moderately ill at visit 1. Only one (risperidone), two (flupentixol) and three (haloperidol) patients were judged as markedly ill.

Table 2 Patient characteristics

	Flupentixol (n=13)	Risperidone (n=11)	Haloperidol (n=11)
Age [years]	40.4±5.6	39.4±11.2	41.0±9.2
Male/female [n]	5/8	7/4	8/3
Weight [kg]	76.9±18.2	80.8±7.4	76.8±23.7
BMI [kg/m^2]	26.1±5.6	26.6±3.2	26.1±6.5
Diagnosis [n]			
Paranoid schizophrenia	7	6	9
Katatonic schizophrenia	1	–	–
Residual schizophrenia	–	2	1
Schizophrenia simplex	1	–	–
Schizoaffective disorder	1	2	–
Mixed schizoaffective-disorder	3	1	1
Intake of antipsychotics since [years]	10.3±6.3	6.4±7.3	11.2±6.3
Dose/day [mg/day]	5.7±1.4	3.6±1.3	8.5±5.5
Serum concentration: mean (median) [ng/ml]	1.47 (0.97)	10.0 (3.0)	3.8 (1.9)
Range [ng/ml]	0.0–6.0	0.1–77.3	0.6–18.1
Serum variability [%] (mean relative difference between both days)	30	33	13
$PANSS_{tot}$	53.1±8.9	46.8±11.9	50.2±9.8
$PANSS_{pos}$	10.7±2.4	9.7±3.0	11.1±3.3
$PANSS_{neg}$	14.2±3.6	12.8±4.7	13.6±3.6
$PANSS_{glob}$	28.2±5.8	24.3±5.9	25.6±4.6
EPS	0.15±0.18	0.15±0.29	0.24±0.26

Time–activity curves of NMS in the frontal cortex and the reference region are plotted in Fig. 1. Patients with flupentixol treatment exhibited slightly faster washout than the control subjects, the fastest washout was found in patients receiving risperidone treatment. Time–activity curves in the reference tissue showed only negligible group differences (mean time–activity curves being visually identical). The corresponding Logan linearization is shown in Fig. 1 and demonstrates mono-exponential pharmacokinetic behaviour during the chosen regression interval.

D_2 receptor occupancy after flupentixol treatment was below 70% in all patients (Fig. 2), corresponding to an ED_{50} of 0.68 ng/ml (serum concentration). The calculated ED_{50} of risperidone and haloperidol were 2.3 ng/ml and 1.0 ng/ml, respectively. It is interesting to note that the patient with by far the highest risperidone serum concentration (57 ng/ml) presented a considerably lower RO (65%) than predicted from the saturation curve (>95%).

Figure 3 shows the individual RO of all patients. Patients treated with flupentixol displayed the highest occupancy of D_1 receptors (19.9%). With the estimated standard error of 5.4% (including baseline uncertainty), this means that the

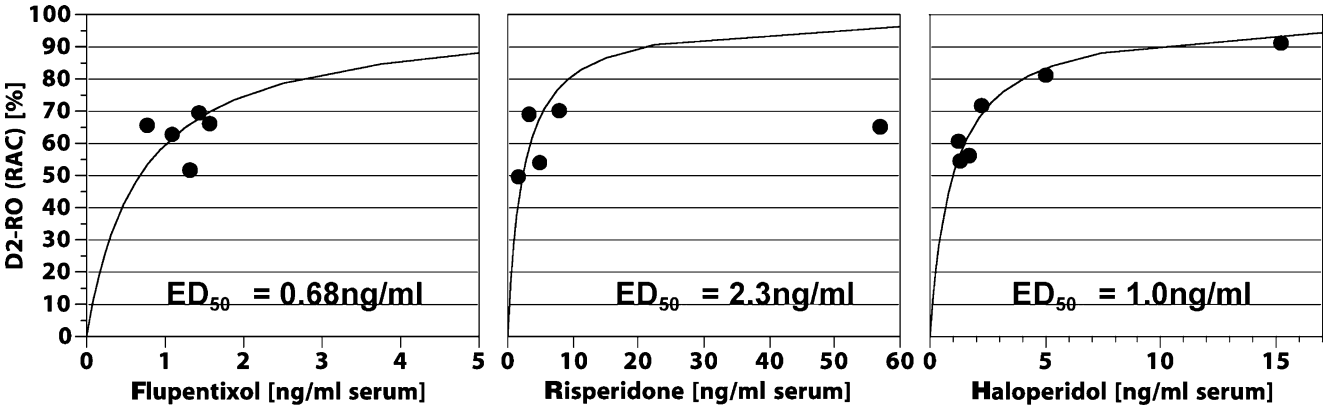

Fig. 2 D_2 receptor occupancy. *Left*: therapeutic doses of flupentixol lead to a D_2 occupancy of 50–70%, the fitted saturation hyperbola corresponds to a ED_{50}=0.68 ng/ml. *Middle* and *right*: corresponding saturation curves for risperidone (not including 9-OH risperidone) and haloperidol

mean D_1-RO is higher than 11% at α=0.05 (unilateral). D_1 occupancy of haloperidol patients was lower (14.0±5.6%; including the patient with a positive drug urine test: 13.0%) and also significantly different from zero. In the risperidone group, we observed no significant D_1 occupancy.

Mean 5-HT_{2A} receptor occupancy under flupentixol treatment was 19.5% (23.6% in females, 13.4% in the two male subjects; including both subjects with a positive drug urine test: 22.9%) with a standard error of 10.0%, which corresponds to a mean RO>3% at α=0.05 (unilateral). Patients of the risperidone group had a much higher RO (87.9±4.3%, including the subject with a positive drug urine test: 88.4%), whereas 5-HT_{2A}-RO under haloperidol was not significantly different from zero (3.8±13.2%). It is interesting to note that the variance of frontal NMS binding was much higher in the haloperidol group (SD 33.3%) than in the controls (11.4%), which might be explained with a higher baseline variability in this group.

The three patients that had been excluded due to likely non-compliance displayed occupancy values close to or even below zero in line with their negligible serum concentrations, except one patient from the flupentixol group with 5-HT_{2A}-RO of 24%.

Discussion

To our knowledge, this is the first study that reports about the in vivo binding profile of flupentixol in a larger number of subjects. As expected from in vitro data, schizophrenic patients clinically treated with flupentixol exhibited reduced D_1 and 5-HT_{2A} receptor availability. The corresponding mean occupancy (20% for both D_1 and 5-HT_{2A}) was significantly lower than that of D_2 receptors (63%). While in a recent clinical trial, it was shown that flupentixol is as efficacious against negative symptoms as risperidone (Philipp et al. 2002) and olanzapin (Gattaz et al. 2004), our data show that 5-HT_{2A} occupancy under flupentixol is much lower than that under risperidone. Thus, this study raises the question whether flupentixol's efficacy against

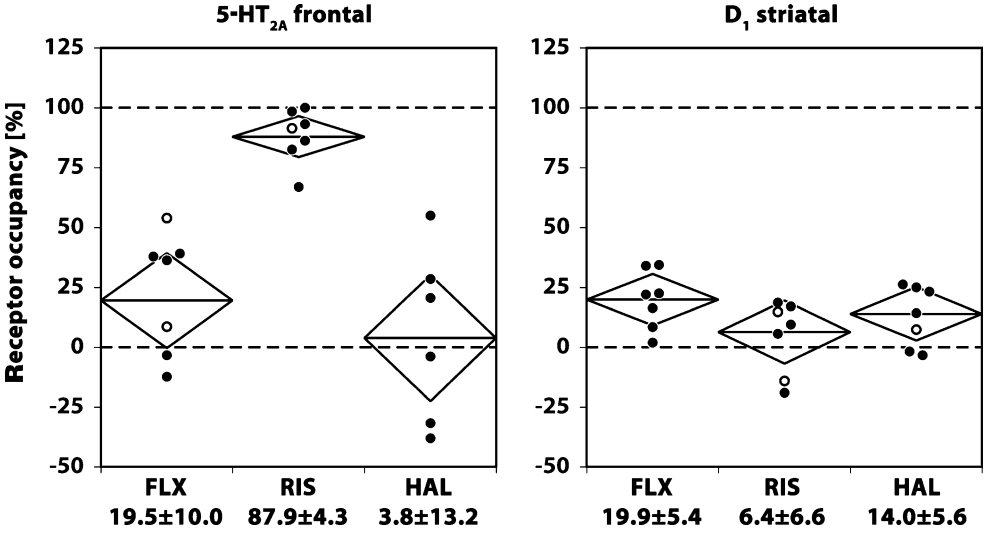

Fig. 3 5-HT_{2A} and D_1 receptor occupancy. Frontal 5-HT_{2A} and striatal dopamine D_1 receptor occupancy of patients treated with flupentixol, risperidone or haloperidol. Numbers reflect the mean receptor occupancy in [%] and the standard error of mean, including the uncertainty from variable baseline conditions. The corresponding 95% confidence interval is shown by *diamonds*. *Open circles* refer to the dropout patients (positive drug urine test) and were not used to calculate the mean occupancy

negative symptoms is based on interactions with other receptor sites than 5-HT_{2A} or if a therapeutically relevant interaction with 5-HT_{2A} receptors does not require high levels of occupation. If the rather low 5-HT_{2A} and/or D_1 occupancy was to mediate the therapeutic efficacy against negative symptoms, one would expect a dependency on dosage and serum levels, as the saturation curve has a higher slope for RO=20% than for occupancy levels near saturation.

While a D_1 and 5-HT_{2A} occupancy lower than that of D_2 receptors is in agreement with in vitro data and with PET results reported for a small number of subjects (D_1 vs D_2: Farde et al. 1992, two subjects; 5-HT_{2A} vs D_2: Nyberg et al. 1996, one subject), we expected the absolute level of D_1 and 5-HT_{2A} occupancy to be higher. In vitro data (Glaser and Soyka 1998) suggested Ki values for D_1 and 5-HT_{2A} receptors to be in the same order of magnitude as those for D_2-like receptors (D_1, 3.8±3.1 nM; 5-HT_{2A}, 4.3±1.2 nM; D_{2S}, 3.6±1.2 nM; D_3, 2.5±0.1 nM). Assuming the intra-individual non-specific pharmacokinetic behavior of a psychotropic drug to be uniform across the investigated brain regions and transmitter systems, Ki-ratios are frequently used equivalent to ED_{50}-ratios to characterize the pharmacological profile of antipsychotics. With the given Ki-ratios (5-HT_{2A} over D_2=1.2; D_1 over D_2=1.06) and a D_2-ED_{50} of 0.68 ng/ml (cf. Fig. 2), Eq. (1) yields for our patients a predicted occupancy of 65% for 5-HT_{2A} and 66% for D_1 receptors, which is more than threefold higher than measured. This discrepancy is one example that emphasizes the need of in vivo PET imaging to confirm in vitro data. But, although the assessment of receptor occupancy with PET is well-established, it is not without methodological uncertainties, which may have lead to an underestimation of D_1 and 5-HT_{2A} occupancy in the present study. Specific binding of the radioligand to another type of binding sites may result in a false low apparent occupancy if these binding sites are occupied at a lower level. This applies to different receptor subtypes (e.g. 5-HT_{2C} receptors to which NMS also binds) and to different cellular locations of the same type of receptor. The latter was discussed to be the reason why NMS and SCH are apparently not being displaced by the endogenous ligand or exhibit even paradoxical behaviour (for review, see Laruelle 2000). In general, "specific" binding that is not blocked by medication leads to an underestimation of receptor occupancy. Usually it is corrected for by defining a maximum "occupancy" RO_{max} below 100% (Eq. (1). Using MDL 100907, a highly selective 5-HT_{2A} antagonist, Andree et al. (1998) report about a maximum reduction of specific binding of NMS by 77%, so one might want to correct the observed RO by a factor of 100/77 to obtain the "true" 5-HT_{2A} occupancy under flupentixol (26% instead of 20%). Little was published that would allow to draw reliable conclusions about the maximum reduction of specific binding of SCH achievable in vivo with a *selective* D_1 antagonist. To our knowledge, the maximum reduction observed in patients was in the range of 50–60% (e.g. apparent occupancy under clozapine=55±15%, Tauscher et al. 2004), therefore the "true" D_1 occupancy under flupentixol may well be above the observed 20%. Another possible source of error comes from estimating the individual baseline binding potential from healthy controls, e.g. a hypothetical compensatory upregulation, due to either diagnosis or treatment (Huang et al. 1997; Schröder et al. 1998), would lead to an underestimation of occupancy. This is usually ignored because several SPECT/PET studies failed to detect significant baseline differences, in spite of altered dopaminergic transmission (Abi-Dargham et al. 1998). No baseline differences were found with NMS and SCH either (Okubo et al. 2000; Karlsson et al. 2002), however, one has to keep in mind that such a baseline shift may induce a rather small error for high levels of occcupancy, but not for lower levels (at 80% true occupancy, a hypothetical upregulation by 25% would result in an apparent occupancy of 75%; at 40% true occupancy, the same baseline shift would result in 25% apparent occupancy). Furthermore, the fact that no significant baseline differences were observed after a certain drug-free interval does not generally exclude an altered baseline status under neuroleptic treatment, especially since different neuroleptics may interact differently with the receptor (Weiner et al. 2001; Gründer et al. 2003). The PET ligands SCH and NMS were used in the literature with reference tissue methods without experimental validation against full kinetic modelling (including arterial plasma samples, metabolite correction and plasma protein binding), however, we are confident that the chosen methods of quantification do not introduce further relevant uncertainties to the calculated occupancy values. The chosen beginning of the regression interval to obtain the slope of the Logan plot had only negligible impact on the calculated binding potential (data not shown). A noise-dependent bias, a particular drawback from Logan's method, and the bias that might result from a variability of non-specific binding in the reference region was ruled out by demonstrating that between-subject averaging (Reimold et al. 2004) had only negligible impact on the calculated mean binding potential (difference <2% in all groups, cf. Fig. 1).

Our data from patients under treatment with risperidone are in good agreement with other PET studies, which also showed a 5-HT_{2A} occupancy higher than that of D_2 receptors (Nyberg et al. 1999; Kapur et al. 1999). It is interesting to note that we have not observed a significant occupancy of D_1 receptors under risperidone, which is in agreement with in vitro data, but differs from Tauscher et al. (2004) who previously reported a 25% reduction of

specific binding of SCH under 4.3 mg risperidone per day (which is only little more than the 3.6 mg per day in our patients).

In the haloperidol group, D_2 occupancy was closely related to the serum concentration. In two patients, the occcupancy was higher than the threshold suggested to be critical for EPS. The observed D_1 occupancy (14%, significantly different from zero) is in agreement with in vitro data, as was the negligible 5-HT_{2A} occupancy. To our knowledge, this is the first study that reports about the D_1 occupancy under haloperidol in a larger number of subjects.

The focus of this study was to characterize the receptor profile of flupentixol in a typical clinical setting that was comparable to that for the two reference neuroleptics. A limitation of such a design is that estimation of the ED_{50} may be slightly biased because the dosage of the neuroleptic is not randomized.

Conclusion

With respect to D_2 occupancy, our data indicate an ED_{50} of flupentixol of about 0.7 ng/ml serum. The occupancy profile of flupentixol with respect to 5-HT_{2A} and dopamine D_1 receptors differs from both risperidone and haloperidol. In schizophrenic patients clinically treated with stable doses of flupentixol, the average specific binding of NMS and SCH to frontal 5-HT_{2A} and striatal D_1 receptors was reduced by 20% corresponding to a moderate D_1 and 5-HT_{2A} occupancy below the level of D_2 occupancy and clearly below saturation. Flupentixol's affinity to dopamine D_1 receptors was not high enough to allow for a clear delineation from haloperidol with respect to D_1 blockade, but among the investigated compounds, the highest dopamine D_1 occupancy was indeed observed under flupentixol. In summary, we were able to demonstrate a moderate 5-HT_{2A} and D_1 occupancy under clinical treatment with flupentixol. However, 5-HT_{2A} occupancy under flupentixol lies clearly below that of atypical compounds, suggesting that other mechanisms than D_1 and 5-HT_{2A} antagonism might contribute to flupentixol's efficacy on negative symptoms. We assume the serotonergic effects of flupentixol and effects from D_1 blockade to highly depend on serum level and thus on dosage and metabolism.

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References

Abi-Dargham A, Gil R, Krystal J, Baldwin RM, Seibyl JP, Bowers M, van Dyck CH, Charney DS, Innis RB, Laruelle M (1998) Increased striatal dopamine transmission in schizophrenia: confirmation in a second cohort. Am J Psychiatry 155:761–767

Abi-Dargham A, Mawlawi O, Lombardo I, Gil R, Martinez D, Huang Y, Hwang DR, Keilp J, Kochan L, Van Heertum R, Gorman JM, Laruelle M (2002) Prefrontal dopamine D1 receptors and working memory in schizophrenia. J Neurosci 22(9):3708–3719

Andree B, Nyberg S, Ito H, Ginovart N, Brunner F, Jaquet F, Halldin C, Farde L (1998) Positron emission tomographic analysis of dose-dependent MDL 100,907 binding to 5-hydroxytryptamine-2A receptors in the human brain. J Clin Psychopharmacol 18 (4):317–323

Dannals RF, Ravert HT, Wilson AA, Wagner HN Jr (1986) An improved synthesis of (3-*N*-[^{11}C]methyl)spiperone. Int J Rad Appl Instrum, A Appl Radiat Isot 37:433–434

Ehrin E, Gawell L, Högberg T, de Paulis S, Ström P (1987) Synthesis of [methoxy-^{3}H]- and [methoxy-^{11}C]-labelled raclopride. Specific dopamine-D2 receptor ligands. J Label Compd Radiopharm 24:931–940

Farde L, Nordstrom AL (1992) PET analysis indicates atypical central dopamine receptor occupancy in clozapine-treated patients. Br J Psychiatry Suppl (17):30–33

Farde L, Hall H, Ehrin E, Sedvall G (1986) Quantitative analysis of D_2 dopamine receptor binding in the living human brain by PET. Science 231(4735):258–261

Farde L, Nordstrom AL, Wiesel FA, Pauli S, Halldin C, Sedvall G (1992) Positron emission tomographic analysis of central D_1 and D_2 dopamine receptor occupancy in patients treated with classical neuroleptics and clozapine. Relation to extrapyramidal side effects. Arch Gen Psychiatry 49(7):538–544

Gattaz WF, Diehl A, Geuppert MS, Hubrich P, Schmitt A, Linde I, Maras A, Dittmann RW (2004) Olanzapine versus flupenthixol in the treatment of inpatients with schizophrenia: a randomized double-blind trial. Pharmacopsychiatry 37:279–285

Glaser T, Soyka M (1998) Flupentixol—typisches oder atypisches Wirkspektrum? Steinkopff, Darmstadt

Gründer G, Carlsson A, Wong DF (2003) Mechanism of new antipsychotic medications: occupancy is not just antagonism. Arch Gen Psychiatry 60(10):974–977

Halldin C, Stone-Elander S, Farde L, Ehrin E, Fasth KJ, Långström B, Sedvall G (1986) Preparation of ^{11}C-labelled SCH 23390 for the in vivo study of dopamine D-1 receptors using positron emission tomography. Int J Rad Appl Instrum, A Appl Radiat Isot 37:1039–1043

Huang N, Ase AR, Hebert C, van Gelder NM, Reader TA (1997) Effects of chronic neuroleptic treatments on dopamine D_1 and D_2 receptors: homogenate binding and autoradiographic studies. Neurochem Int 30(3):277–290

Hyttel J, Larsen JJ, Christensen AV, Arnt J (1985) Receptor-binding profiles of neuroleptics. Psychopharmacology Suppl 2:9–18

Ichikawa J, Ishii H, Bonaccorso S, Fowler WL, O'Laughlin IA, Meltzer HY (2001) 5-HT_{2A} and D_2 receptor blockade increases cortical DA release via 5-HT_{1A} receptor activation: a possible mechanism of atypical antipsychotic-induced cortical dopamine release. J Neurochem 76:1521–1531

Kapur S, Seeman P (2001) Does fast dissociation from the dopamine D_2 receptor explain the action of atypical antipsychotics?: a new hypothesis. Am J Psychiatry 158:360–369

Kapur S, Zipursky RB, Remington G (1999) Clinical and theoretical implications of 5-HT_2 and D_2 receptor occupancy of clozapine, risperidone, and olanzapine in schizophrenia. Am J Psychiatry 156:286–293

Kapur S, Zipursky R, Jones C, Remington G, Houle S (2000a) Relationship between dopamine D_2 occupancy, clinical response, and side effects: a double-blind PET study of first-episode schizophrenia. Am J Psychiatry 157:514–520

Kapur S, Zipursky R, Jones C, Shammi CS, Remington G, Seeman P (2000b) A positron emission tomography study of quetiapine in schizophrenia: a preliminary finding of an antipsychotic effect with only transiently high dopamine D_2 receptor occupancy. Arch Gen Psychiatry 57(6):553–559

Karlsson P, Farde L, Halldin C, Sedvall G (2002) PET study of D(1) dopamine receptor binding in neuroleptic-naive patients with schizophrenia. Am J Psychiatry 159(5):761–767

Kay SR, Fiszbein A, Opler LA (1987) The positive and negative syndrome scale (PANSS) for schizophrenia. Schizophr Bull 13(2): 261–276

Kuehn KU, Meyer K, Maier W (2000) Flupentixol—ein partiell atypisches Neuroleptikum? Fortschr Neurol Psychiatr 68(Suppl 1):S38–S41

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

Laruelle M (2000) Imaging synaptic neurotransmission with in vivo binding competition techniques: a critical review. J Cereb Blood Flow Metab 20:423–451

Laruelle M, van Dyck C, Abi-Dargham A, Zea-Ponce Y, Zoghbi SS, Charney DS, Baldwin RM, Hoffer PB, Kung HF, Innis RB (1994) Compartmental modeling of iodine-123-iodobenzofuran binding to dopamine D_2 receptors in healthy subjects. J Nucl Med 35:743–754

Lieberman JA, Mailman RB, Duncan G, Sikich L, Chakos M, Nichols DE, Kraus JE (1998) Serotonergic basis of antipsychotic drug effects in schizophrenia. Biol Psychiatry 44(11):1099–1117

Logan J, Fowler JS, Volkow ND, Wang GJ, Ding YS, Alexoff DL (1996) Distribution volume ratios without blood sampling from graphical analysis of PET data. J Cereb Blood Flow Metab 16 (5):834–840

Lundberg T, Lindstrom L, Hartvig P, Reibring L, Agren H et al (1996) Serotonin-2 and dopamine-1 binding components of clozapine in frontal and striatum in the brain visualized by positron emission tomography. Psychiatry Res 67:1–10

Meltzer HY (1992) The importance of serotonin–dopamine interactions in the action of clozapine. Br J Psychiatry Suppl (17):22–29

Meltzer HY, Matsubara S, Lee JC (1989) Classification of typical and atypical antipsychotic drugs on the basis of dopamine D_1, D_2 and serotonin$_2$ pKi values. J Pharmacol Exp Ther 251:238–246

Nordstroem AL, Farde L, Wiesel FA, Forslund K, Pauli S, Halldin C, Uppfeldt G (1993) Central D_2-dopamine receptor occupancy in relation to antipsychotic drug effects: A double-blind PET trial of schizophrenic patients. Biol Psychiatry 33:227–235

Nyberg S, Farde S, Eriksson L, Halldin C, Eriksson B (1993) 5-HT_2 and D_2 dopamine receptor occupancy in the living human brain. A PET study with risperidone. Psychopharmacology (Berl) 110:265–272

Nyberg S, Nordstrom AL, Halldin C, Farde L (1995) Position emission tomography studies on D_2 dopamine receptor occupancy and plasma antipsychotic drug levels in man. Int Clin Psychopharmacol 10(Suppl 3):81–85

Nyberg S, Nakashima Y, Nordstrom AL, Halldin C, Farde L (1996) Positron emission tomography of in-vivo binding characteristics of atypical antipsychotic drugs. Review of D_2 and 5-HT_2 receptor occupancy studies and clinical response. Br J Psychiatry Suppl (29):40–44

Nyberg S, Nilsson U, Okubo Y, Halldin C, Farde L (1998) Implications of brain imaging for the management of schizophrenia. Int Clin Psychopharmacol 13(Suppl 3):S15–S20

Nyberg S, Eriksson B, Oxenstierna G, Halldin C, Farde L (1999) Suggested minimal effective dose of risperidone based on PET-measured D_2 and 5-HT_{2A} receptor occupancy in schizophrenic patients. Am J Psychiatry 156:869–875

Okubo Y, Suhara T, Suzuki K, Kobayashi K, Inoue O, Terasaki O, Someya Y, Sassa T, Sudo Y, Matsushima E, Iyo M, Tateno Y, Toru M (2000) Serotonin 5-HT_2 receptors in schizophrenic patients studied by positron emission tomography. Life Sci 66 (25):2455–2464

Philipp M, Lesch OM, Walter H, Patras L, Kurtz G, Schmauß M, Seemann U, Roth G, Stöhr K, Dose M, Hetzel G, Ruhrmann S, Beneke M, Rappard F, Glaser T (2002) Wirksamkeit von Flupentixol vs Risperidon auf die Negativsymptomatik schizophrener Patienten. Psychopharmakotherapie 9:67–74

Philipp M, Lesch OM, Schmauss M, Dose M, Glaser T (2003) Comparative effectiveness of flupenthixol and risperidone on negative symptoms of schizophrenia. Psychiatr Prax 30(Suppl 2):S94–S96

Reimold M, Mueller-Schauenburg W, Becker GA, Reischl G, Dohmen BM, Bares R (2004) Non-invasive assessment of distribution volume ratios and binding potential: tissue heterogeneity and interindividually averaged time–activity curves. Eur J Nucl Med Mol Imaging 31(4):564–577

Schröder J, Silvestri S, Bubeck B, Karr M, Demisch S, Scherrer S, Geider FJ, Sauer H (1998) D_2 dopamine receptor up-regulation, treatment response, neurological soft signs, and extrapyramidal side effects in schizophrenia: a follow-up study with ^{123}I-iodobenzamide single photon emission computed tomography in the drug-naive state and after neuroleptic treatment. Biol Psychiatry 43(9):660–665

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

Tauscher J, Hussain T, Agid O, Verhoeff NP, Wilson AA, Houle S, Remington G, Zipursky RB, Kapur S (2004) Equivalent occupancy of dopamine D_1 and D_2 receptors with clozapine: differentiation from other atypical antipsychotics. Am J Psychiatry 161(9):1620–1625

Weiner DM, Burstein ES, Nash N, Croston GE, Currier EA, Vanover KE, Harvey SC, Donohue E, Hansen HC, Andersson CM, Spalding TA, Gibson DF, Krebs-Thomson K, Powell SB, Geyer MA, Hacksell U, Brann MR (2001) 5-hydroxytryptamine2A receptor inverse agonists as antipsychotics. J Pharmacol Exp Ther 299(1):268–276