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ORIGINAL ARTICLE

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Decreased prefrontal 5-HT_{2A} receptor binding in subjects at enhanced risk for schizophrenia

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Abstract The brain serotonin-2A receptor (5-HT_{2A}R) has been implicated in both the pathology of schizophrenia and the therapeutic action of atypical antipsychotics. However, little is known about the 5-HT_{2A}R status before the onset of schizophrenia and before the exposure to antipsychotics. We used [^{18}F] altanserin and positron emission tomography (PET) in a pilot study of 6 individuals suspected to be at elevated risk for schizophrenia and seven age-matched controls to test the hypothesis that regional 5-HT_{2A}R binding is altered in the prodromal stages of schizophrenia. Distribution volume ratios (DVRs) as a proxy for 5-HT_{2A}R availability were significantly reduced in prefrontal cortex regions of at-risk subjects, implicating early abnormalities of serotonergic neurotransmission that antecede the onset of schizophrenia.

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Abbreviations DVR: distribution volume ratio · ROI: region of interest · at-risk: subjects with prodromal states for schizophrenia

Introduction

Neurochemical hypotheses of schizophrenia propose a role of the brain 5-HT_{2A}R in both the pathology of the disease and the therapeutic action of atypical antipsychotics (Dean 2003). Transmission of susceptibility for schizophrenia has been linked to allele 2 of the T102C polymorphism of the 5-HT_{2A}R gene (Erdmann et al. 1996; Williams et al. 1996; Abdolmaleky et al. 2004). Substantial evidence indicates a direct role of the prefrontal 5-HT_{2A}R in the cognitive process of working memory (Williams et al. 2002), dysfunction of which is a core deficit in schizophrenia (Fletcher et al. 1998; Cameron et al. 2002). Most research on post-mortem brain tissue converges on locating a decrease of 5-HT_{2A}R binding in the prefrontal cortex of schizophrenic patients (e.g., Arora and Meltzer 1991; Laruelle et al. 1993; but see also Joyce et al. 1993). However, no consensus has evolved on the 5-HT_{2A}R in vivo status in schizophrenia. There are four [^{18}F]setoperone PET reports of either no specific alterations (Trichard et al. 1998; Lewis et al. 1999; Verhoeff et al. 2000) or significant decreases in prefrontal 5-HT_{2A}R binding (Ngan et al. 2000).

The prodromal state is defined as that period preceding the onset of schizophrenia, when there is increasing symptomatic presentation and functional deterioration (McGorry et al. 2000; Phillips et al. 2000; Klosterkotter et al. 2001; Ruhrmann et al. 2003; Hafner et al. 2004). Imaging the brain 5-HT_{2A}R during the prodromal stages provides an opportunity to identify potential abnormalities of serotonergic neurotransmis-

sion associated with the emergence of schizophrenia. In order to quantify the regional distribution of 5-HT_{2A}R binding before the transition to active psychosis and before the exposure to antipsychotics, we used the selective radioligand [^{18}F] altanserin and PET in a pilot study of 6 drug-naïve subjects with prodromal states of schizophrenia and seven age-matched controls. The at-risk subjects did not meet diagnostic criteria for established schizophrenia. However, screening procedures elicited evidence of trait and state symptoms consistent with current research definitions of early and late prodromal stages of schizophrenia (Hafner et al. 2004).

Method

Participants

Study protocols were approved by the ethics committee of the University of Bonn Medical Faculty and the Federal Office for Radiation Protection, Germany. Six at-risk individuals (one female; mean age 25 ± 4.6 years; range 20–30 years) recruited from the local early recognition program and seven healthy control subjects (two females; mean age 23 ± 1.6 years; range 20–25 years) participated after giving written informed consent. None of the subjects had a history of neurological or psychiatric illness, alcohol or substance abuse, or had been exposed to psychotropic drugs.

From the six individuals constituting the at-risk group, two subjects were assigned to late prodromal states and four subjects to early prodromal states (Hafner et al. 2004). Individuals assigned to late prodromal states had experienced brief limited intermittent psychotic symptoms (BLIPS; present for < 1 week; spontaneous remission) and attenuated psychotic symptoms (APS; several times per week; present for > 1 week and < 5 years). BLIPS included hallucinations (positive and negative syndrome scale, PANSS: P3≥4), delusions (PANSS: P1, P5 or P6≥4), and formal thought disorder. APS comprised ideas of reference, odd beliefs or magical thinking, odd thinking or speech, unusual perceptions (PANSS: P2≥4), and paranoid ideation or suspiciousness.

Four at-risk subjects fulfilled research criteria for early prodromal states according to the prospective study by Klosterkotter et al. (2001), who identified symptoms to predict psychosis several years in advance. In this early at-risk sample, one or more of the following predicitve symptoms occurred within the last 3 months, several times a week: Interference, perseveration, pressure, or blocking of thought; disturbance of receptive speech; disability to discriminate between ideas and perception, fantasy and true memories; unstable ideas of reference (subject-centrism); derealisation; acoustic or visual perception disturbances. In three out of these four cases, an additional genetic/obstetric risk plus a reduction in the Global Assessment of Functioning (GAF) Score (DSM IV) of at least 30 points was present.

PET imaging

Radiosynthesis of [^{18}F]altanserin was performed at the Institute of Nuclear Chemistry, Research Center Jülich, according to Lemaire et al. (1991), followed by high performance liquid chromatography (HPLC) purification (Hamacher and Hamkens 1995) with a radiochemical yield of ≈30% and a radiochemical purity of > 99%. At the time of injection, the mean specific radioactivity was 85 GBq/μmol (range 27–138 GBq/μmol). PET measurements were performed in 3D mode on a Siemens ECAT EXACT HR + scanner (Siemens-CTI, Knoxville, TN, USA). Scatter from outside the field of view was reduced by inserting a lead ring into the scanner gantry. A 10-min transmission scan (^{68}Ge/^{68}Ga) was obtained for attenuation correction. A slow bolus (20 s) of 240.5 ± 24.8 MBq (6.49 ± 0.66 mCi) of [^{18}F] altanserin (injected quantity, at-risk subjects: 4.4 ± 2.5 nmol; controls: 4.1 ± 2.9 nmol) in 10 ml saline was injected intravenously. Dynamic data were collected in 24 frames over a 60-min period starting with injection. PET data were corrected for randoms, scatters, and attenuation, rebinned into 2D sinograms (FORE), reconstructed by filtered backprojection (Shepp filter, filter width = 2.5 mm) with a voxel size of 2×2×2.43 mm^3 (63 slices), and decay-corrected.

Image analysis

High-resolution structural magnetic resonance imaging (MRI) was performed on a Siemens Magnetom VISION 1.5-T scanner using a 3D T1-weighted magnetization-prepared rapid-acquisition gradient-echo sequence. Individual MRI data sets were oriented according to the anterior commissure—posterior commissure line using a 3D image registration software (MPITool, ATV Co., Germany). Subsequently, a summed PET image (5–60 min) was co-registered to the realigned MRI, and co-registration parameters were applied to each PET frame. Volumes of interest (VOIs) were defined on the MR images and subsequently, superimposed onto all PET frames with a dedicated software (PMOD, V2.4, PMOD Group, Switzerland) to generate time-activity curves (TACs).

Due to a priori predictions of reduced 5-HT_{2A}R availability in established schizophrenia (Dean 2003), the following three target VOIs were selected: orbitofrontal cortex (OFC), prefrontal cortex without OFC (PFC), and anterior cingulate gyrus (ACC). In addition, the striatum (STR) and the occipital cortex (OCC) were included. The occipital cortex served as a cortical control region, where 5-HT_{2A}R availability is thought to be less affected in schizophrenia. The cerebellum served as a *reference* region based on the assumption that cerebellar [^{18}F]altanserin uptake mostly reflects free and non-specifically bound ligand (Pazos et al. 1987; Pinborg et al. 2003).

For kinetic analyses, Logan's non-invasive graphical analysis (GA) was applied (Logan et al. 1996).

Distribution volume ratio (DVR) given by the slope of the linear part of the GA plot was used as an outcome parameter reflecting 5-HT_{2A}R availability. DVR is directly related to the maximum number of receptors available for ligand binding (Bmax′): DVR = $1 + f_2 \times B\text{max}'/\text{KD}$ (f_2, tissue fraction of free ligand; KD, dissociation constant of the receptor-ligand complex) (Abi-Dargham et al. 1994; Laruelle et al. 1994). Statistical group comparison was performed by repeated measures ANOVA followed by independent samples t-tests to determine the source of significance. Statistical significance was accepted if the likelihood p for a chance effect was below .05.

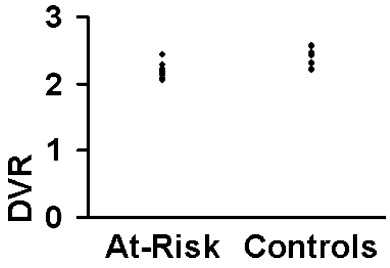

Fig. 1 Individual DVR values. Subjects at enhanced risk for schizophrenia (*left*) showed a lower [^{18}F] altanserin binding in orbitofrontal and prefrontal cortex ROIs than controls (*right*).

Results

At-risk individuals and control volunteers did not differ with regard to age (25 ± 4.6 years vs. 23 ± 1.8 years, $T = -1.279$, df = 6.31, $p = 0.227$ two-tailed two-sample t-test). A group (at-risk sample, control sample) × volume of interest (OFC, PFC, ACC, STR, and OCC) 2×5 ANOVA revealed a significant main effect of group ($F = 5.298$, df = 11, $p = 0.042$). As illustrated in Fig. 1, post hoc two-tailed two-sample t-tests indicated that at-risk individuals displayed a significantly lower 5-HT_{2A}R binding in OFC (DVR 2.13 ± 0.04 vs. 2.42 ± 0.13; $T = 5.419$, df = 11, $p < 0.0001$) and PFC (DVR 2.21 ± 0.08 vs. 2.40 ± 0.13; $T = 3.153$, df = 11, $p = 0.009$) than controls. No significant differences between at-risk subjects and controls were found in the other examined regions including ACC (DVR 2.29 ± 0.15 vs. 2.43 ± 0.18; $T = 1.624$, df = 11, $p = 0.133$), STR (DVR 1.37 ± 0.08 vs. 1.32 ± 0.09; $T = -0.987$; d.f = 11, $p = 0.345$), and OCC (DVR 2.62 ± 0.14 vs. 2.59 ± 0.19; $T = 0.362$, df = 11, $p = 0.725$). The regional DVR values (mean ± SD) are listed in Table 1.

Discussion

To our knowledge, this is the first PET study exploring the 5-HT_{2A}R status in individuals at increased risk for schizophrenia. As study groups did not differ with regard to age and DVR values of the cortical control region, neither age-related decline in 5-HT_{2A}R binding (Adams et al. 2004) nor differential cerebellar uptake of [^{18}F]altanserin are plausible confounds. In the light of whole-genome linkage scans associating the 5-HT_{2A}R gene with susceptibility for schizophrenia (Erdmann et al. 1996; Williams et al. 1996; Abdolmaleky et al. 2004), the presence of prefrontal decreases of 5-HT_{2A}R availability in the prodromal stages of schizophrenia may reflect early disturbances of serotonergic neurotransmission. Such dysregulation might arise from interactions with other neurotransmitter receptors. For instance, interconnected imbalances of the dopamine and glutamate system in schizophrenia (Laruelle et al. 2003) may affect the serotonin system and *vice versa*.

Our findings complement evidence of prefrontal 5-HT_{2A}R reductions in antipsychotic-naïve patients at onset of schizophrenia (Ngan et al. 2000), but conflict with reports of no significant abnormalities of 5-HT_{2A}R binding in the established illness (Trichard et al. 1998; Lewis et al. 1999; Verhoeff et al. 2000). The observed discrepancies are unlikely to result from different techniques of image analysis, as Verhoeff et al. (2000) used a voxel-based approach that replicated the negative outcome of a previous region of interest analysis of identical PET data (Lewis et al., 1999). However, it cannot be ruled out that PET findings of unchanged 5-HT_{2A}R availability in schizophrenia are confounded by the

Table 1 Regional 5-HT_{2A}R binding

Volume of interest	At-risk subjects ($n = 6$)		Controls ($n = 7$)				
	DVR		DVR				
	Mean	SD	Mean	SD	T	df	Exact Sig.
OFC	2.13	0.04	2.42	0.13	5.419	11	< 0.0001
PFC	2.21	0.08	2.40	0.13	3.153	11	0.009
ACC	2.29	0.15	2.43	0.18	1.624	11	0.133
STR	1.37	0.08	1.32	0.09	0.987	11	0.345
OCC	2.62	0.14	2.59	0.19	0.362	11	0.725

Post hoc two-tailed two-sample t-tests indicated that subjects with prodromal states of schizophrenia showed a significantly lower [^{18}F] altanserin binding in both orbitofrontal cortex (*OFC*) and prefrontal cortex (*PFC*) without OFC, where a decrease of 5-HT_{2A}R availability has been postulated a priori. *Abbreviations*: *5-HT_{2A}R* serotonin-2A receptor, *DVR* distribution volume ratio, *SD* standard deviation, *df* degrees of freedom, *Sig.* Significance, *ACC* anterior cingulate gyrus, *STR* striatum, *OCC* occipital cortex

heterogeneity of schizophrenic psychoses or sustained effects of pharmacotherapy, given the fact that existing studies (Trichard et al. 1998; Lewis et al. 1999; Verhoeff et al. 2000) investigated samples of combined drug-naïve and drug-free patients.

The failure of the 5-HT_{2A}R antagonist MDL 100 907 as an effective treatment for schizophrenia (de Paulis, 2001) indicates that 5-HT_{2A}R dysregulation is not central to the clinical phenomenology of schizophrenia. However, the 5-HT_{2A}R decreases in prefrontal cortex regions observed in our study may be involved in precepitating some of the affective and cognitive symptoms associated with the prodromal state of schizophrenia (McGorry et al. 2000; Phillips et al. 2000; Klosterkotter et al. 2001; Ruhrmann et al. 2003; Hafner et al. 2004).

Prefrontal reductions of 5-HT_{2A}R, as detected in our at-risk sample are not specific to schizophrenia, but are implicated in both psychotic and mood spectrum disorders (Lopez-Figueroa et al. 2004). Biver et al. (1997) demonstrated significant prefrontal 5-HT_{2A}R decreases using [^{18}F] altanserin PET in major depression, while Ngan et al. (2000) reported equivalent findings in first-episode schizophrenia. Consistent with an overlap of psychopathology and functional deficits at early stages of both disorders (Hafner et al. 2004), schizophrenic and affective psychoses might share a common serotonergic dysfunction in prefrontal cortex. In conclusion, the present data hold promise that [^{18}F] altanserin PET might serve as a sensitive indicator for populations at enhanced risk for schizophrenia. Further studies of larger at-risk samples are needed to test the hypotheses generated by our preliminary findings.

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