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Acute S-ketamine application does not alter cerebral [^{18}F]altanserin binding: a pilot PET study in humans

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Summary Modeling short-term psychotic states with subanaesthetic doses of ketamine provides substantial experimental evidence in support of the glutamate hypothesis of schizophrenia. Ketamine exerts its pharmacological effects both directly via interactions with glutamate receptors and indirectly by stimulating presynaptic release of endogenous serotonin (5-HT). The aim of this feasibility study was to examine whether acute ketamine-induced 5-HT release interferes with the binding of the 5-HT$_{2A}$ receptor (5-HT$_{2A}$R) radioligand [^{18}F]altanserin and positron emission tomography (PET). Two subjects treated with ketamine and one subject treated with placebo underwent [^{18}F]altanserin PET at distribution equilibrium conditions. Robust physiological, psychopathological and cognitive effects were present at ketamine plasma concentrations exceeding 100 μg/l during >70 min. Notwithstanding, we observed stable radioligand binding (changes ±95% CI of −1.0 ± 1.6% and +4.1 ± 1.8% versus −1.2 ± 2.6%) in large cortical regions presenting high basal uptake of both, [^{18}F]altanserin and ketamine. Marginal decreases of 4% of radioligand binding were observed in the frontal lobe, and 8% in a posteriorily specified frontomesial subregion. This finding is not compatible with a specific radioligand displacement from 5-HT$_{2A}$R which should occur proportionally throughout the whole brain. Instead, the spatial pattern of these minor reductions was congruent with ketamine-induced increases in cerebral blood flow observed in a previous study using [^{15}O]butanol PET. This may caused by accelerated clearance of unspecifically bound [^{18}F]altanserin from cerebral tissue with increased perfusion. In conclusion, this study suggests that [^{18}F]altanserin PET is not sensitive to acute neurotransmitter fluctuations under ketamine. Advantageously, the stability of [^{18}F]altanserin PET towards acute influences is a prerequisite for its future use to detect sub-acute and chronic effects of ketamine.

Keywords: Ketamine induced model psychosis, [^{18}F]altanserin, [^{15}O]butanol, PET, 5-HT$_{2A}$-receptor

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Introduction

Ketamine has repeatedly been used in subanaesthetic doses to induce experimental psychosis (Krystal et al. 2005; Malhotra et al. 1996; Newcomer et al. 1999; Aghajanian and Marek 2000; Lahti et al. 2001). Ketamine is an antagonist at the N-methyl-D-aspartate (NMDA) site of glutamate receptors ($K_i = 0.5$ μM), an agonist at dopamine D_2 receptors ($K_i = 0.5$ μM), and has lower affinities to other targets (all K_i in: Kapur and Seeman 2002). In addition ketamine has been suggested to exert indirect effects on the serotonergic system (Aghajanian and Marek 2000). A long-lasting antidepressive effect of single or repeated-dose ketamine has been observed in clinical populations (Berman et al. 2000; Zarate et al. 2006; reviewed by Check 2006) and might involve the serotonergic system. Thus, it is tempting to study ketamine induced indirect plastic changes of serotonergic neurotransmission by in-vivo molecular imaging techniques such as positron emission tomography (PET).

In this context it is important to clarify whether radioligand PET is sensitive to ketamine-induced acute fluctuations of synaptic serotonin levels, since ketamine evokes, via an interneuron-mediated disinhibiting effect, the liberation of many neurotransmitters, particularly serotonin (5-HT), norepinephrine (NE), and dopamine (DA) (Aghajanian and Marek 2000). Thirteen percent (Smith et al. 1998) and 10% (Breier et al. 1998) decreases of the binding potential (BP) of the DA radioligand [^{11}C]raclopride have been reported after ketamine challenge in humans. This PET finding is compatible a 400% increase in DA according to Laruelle (2000).

When it comes to 5-HT, however, in vitro experiments have shown a 46% increase of peak 5-HT liberation in rat brain slices at ketamine concentrations of 100 μM, with an EC_{50} in the range of 50 μM (Tso et al. 2004). In contrast, injection of a 0.5 mg/kg ketamin bolus yields peak concentrations of only 3–12 μM (4 min p.i.) in the human brain, which is the 6.5 fold plasma concentration of 100–425 μg/l (Pfizer 2004; molecular weight of ketamine 237 g/mol). This value represents an upper estimate of brain ketamin levels as in psychiatric research rather lower ketamine doses are infused and not injected as bolus (Zarate et al. 2006). Thus, theoretically only marginal serotonine liberation can be expected under ketamine in vivo.

[^{18}F]Altanserin is one of the most selective 5-HT_{2A}R radioligands for positron emission tomography (PET; Lemaire 1991). The aim of the present study was therefore to examine to what extent acute ketamine-induced release of endogenous 5-HT interferes with [^{18}F]altanserin binding in the human brain.

The stability of [^{18}F]altanserin towards fluctuations in endogenous 5-HT has already been studied using the selective serotonin re-uptake inhibitor (SSRI) citalopram (K_i = 1.66 μM at 5-HT_{2A}R, from Leysen et al. 1982) by Pinborg et al. (2004). These authors detected no change in the [^{18}F]altanserin distribution volume parameter DV'_3 (corresponding to BP_1) although they assumed a 200% increase of 5-HT synaptic levels induced by citalopram. The affinity of ketamine to high-affinity state 5-HT_{2A}Rs ($K_i = 15$ μM; Kapur and Seemann 2002) is negligible at subanaesthetic plasma levels of 0.5 μM in the present study.

Ketamine challenge for less than three hours is considered a model for psychosis at short-term scales (Aghajanian and Marek 1999; Fletcher and Honey 2006), omitting transcriptional changes which typically start three hours after a trigger event. We detected focal decreases in [^{18}F]altanserin binding in individuals at increased risk for schizophrenia (Hurlemann et al. 2005), which are consistent with similar findings in medication-naïve patients with first-episode schizophrenia (Ngan et al. 2000).

Several ketamine application regimes have been reported to establish a steady state, with ratios of bolus to infusion speed K_{bol} ranging from 0.18 h (Malhotra et al. 1996) and 0.46 h (Krystal et al. 2005) to 2 h (Newcomer et al. 1999; Rowland et al. 2005). For its use in anaesthesia, where an equilibrium state is intended, ketamine is usually applied with a K_{bol} in a 0.5–1.25 h range, according to the summary of product characteristics. We chose a protocol with $K_{bol} = 0.25$ h in order to apply a maximal cumulated dose of ketamine while preventing anaesthesia after peak doses. A higher bolus (higher K_{bol}) induces anaesthesia and adverse peak dose effects which were not intended in the current study. Conversely, with lower infusion speed plasma levels would have been not efficient.

This study is a placebo-controlled, randomized, psychopharmacological trial to investigate the potential effect of ketamine challenge on [^{18}F]altanserin PET. To document that sufficient ketamine concentrations were reached during the experiment, we assessed a number of physiological, psychopathological and cognitive parameters that are known to be sensitive to ketamine challenge (Krystal et al. 2005; Malhotra et al. 1996; Newcomer et al. 1999; Lahti et al. 2001).

Subjects and methods

Subjects

This study was approved by the Ethics Committee of the Medical Faculty of the University of Düsseldorf, Germany, and the German Federal Office for Radiation Protection. Participants were recruited via public advertisements and paid for their participation. Individuals were healthy by physical examination, history, electrocardiography, and laboratory testing. They and their first-degree relative had no personal history of psychiatric illness or substance abuse disorder, no history of extended (>6 months) unemployment, and no major family or occupational disruption in the month before screening. Screening procedures included the *Structured Clinical Interview for* DSM-IV, *Non-patient Edition* (Spitzer et al. 1990) and tests of latent psychosis (Addington 2004). An outside informant was interviewed to confirm information provided by the individual. Participants were instructed to abstain from psychoactive substances for 4 weeks prior to the study. Further exclusion criteria were narrow angle glaucoma or intracranial hypertension. All three subjects were of native German caucasian origin. It was assured that they did not drive for 16 h following ketamine and lived in a stable social environment. They were followed up by two telephone calls 24 h and 7 d after completing the study. Subjects had the possibility to stop the study at any time without financial or other disadvantages. All subjects gave written informed consent. Two males, both 24 years of age and 80 kg body weight, were assigned to ketamine and one male, 23 years, 82 kg, to placebo. Individual structural magnetic resonance imaging (MRI) was performed on a 1.5 T scanner (Sonata, Siemens, Erlangen, Germany) using a 3D T1-weighted magnetization-prepared rapid acquisition gradient echo sequence (MP-RAGE; voxel size $1 \times 1 \times 1\,mm^3$).

Study design

Application schedule of tracer and medication

[^{18}F]Altanserin was infused as a 2 min bolus followed by continuous infusion with a bolus/infusion ratio of $K_{bol} = 2.1$ h. The start of tracer application was referred to as zero. Two subjects received verum in form of a ketamine bolus of 0.05 mg/kg from 120 to 130 min followed by a continuous infusion of 0.2 mg/kg/h ($K_{bol} = 0.25$ h). One subject received placebo composed of saline administered in an analogous manner.

Acquisition of physiological and psychological parameters

Heart rate and blood pressure were monitored at least every 5 min during the study. Serum samples for the determination of ketamine levels were taken at 0 and at 130 min, then every 10 min until 240 min. Ketamine was deter-

mined by high-pressure liquid chromatography electro-spray ionization tandem mass spectrometry (HPLC-ESI-MS^2). Serum samples were spiked with tetradeuterated ketamine (D_4-ketamine) and tetradeuterated norketamine (D_4-norketamine) as internal standards prior to liquid–liquid extraction. Extracts were injected onto a Luna 5u Phenyl-Hexyl 50 × 2 mm column (Phenomenex, Aschaffenburg, Germany) and eluted with isocratic 50 mM acetic acid/acetonitrile 64/36 (v/v). Ketamine was detected on a Quattro-Micro double quadrupole mass spectrometer (Micromass, Darmstadt, Germany) in positive ion mode, at 20 V cone voltage and 23 eV collisional energy (CE) using the transition m/z 238→125 and m/z 242→224 (CE 14 eV) for D_4-ketamine. Norketamine was detected in positive ion mode, at 17 V cone voltage and 11 eV collisional energy using the transitions m/z 224→207 and m/z 228→211 (CE 11 eV) for D_4-norketamine. The calibration for both analytes was linear in the range 5–500 µg/l. The transitions m/z 238→125 (cone 20 V, CE 14 eV) and m/z 224→125 (CE 23 eV) were used for quality control.

Mental status evaluation

The brief psychiatric rating scale (BPRS; Overall and Gorham 1962) was recorded at least twice at baseline (before 0 min and at 115 min) and in 20 min intervals at 125, 145, 165, 185, 205, 225 and 245 min. Cognitive tests were carried out before zero (baseline) and 10 min after the end of tracer and ketamine application (challenge). Cognitive screening included a German language version (Helmstaedter et al. 2001) of the Rey Auditory Verbal Learning Test (AVLT) to assess immediate verbal learning span, new learning, and susceptibility to interference. Lexical and semantic verbal fluency was assessed according to Aschenbrenner et al. (Regensburger Wortflüssigkeitstest, 2000). Forward and backward digit span as well as the letter number span were assessed (Gold et al. 1997).

PET imaging

Radiosynthesis of [^{18}F]altanserin was performed at the Institute of Nuclear Chemistry, Research Center Jülich, according to Hamacher and Coenen (2006) with a radiochemical yield of ≈30% and a radiochemical purity of >99%. Subjects were injected 226, 224 and 230 MBq [^{18}F]altanserin with mean specific radioactivities of 50, 212, and 90 GBq/ µmol at the time of injection for subjects 1, 2, and 3, respectively. 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 a lead ring insert. A 10 min transmission scan (with three ^{68}Ge/^{68}Ga line sources) was obtained for attenuation correction. The head was fixed using a vacuum cushion. Dynamic emission data were collected in 39 frames of 9 × 30, 3 × 60, 3 × 150, 3 × 300, 21 × 600 s length starting from the start of [^{18}F]altanserin application (zero).

PET data were corrected for randoms, scatters and attenuation, Fourier rebinned into 2D sinograms, reconstructed by filtered backprojection (Shepp filter, 2.5 mm width) with a voxel size of 2 × 2 × 2, 43 mm^3 (63 slices), and decay-corrected. The spatial resolution is given by a full width of half maximum (FWHM) of 5.8 × 5.8 × 6.6 mm^3 at 10 cm from the central axis. Venous blood samples were taken at 1.8, 2, 5, 10 min p.i., than every 10 min until 240 min and additionally at 125 and 135 min. The fraction of radioactive parent compound in plasma was determined by selective liquid-liquid extraction with quantitation of the recovery, followed by thin layer chromatography (Matusch et al. in preparation).

Image analysis

The dynamic volume file in ECAT7 format was converted to analyze format using PMOD version 2.6 (PMOD Group, Zuerich, Switzerland). Frames were realigned to the sum of the first 12 frames using SPM2 (Wellcome Department of Imaging Neuroscience, London, UK) to correct for head movements during the scan. The realigned frames were co-registered to the MR data set orientated to the anterior commissure-posterior commissure line using SPM2. Cerebellar, mediofrontal and temporal regions were delineated on the co-registered MRI at the outset. Radioactivity concentrations were obtained from the dynamic PET using PMOD.

From time-activity curves of a given region ($C_{\mathrm{Region}}(t)$), the reference region cerebellum ($C_{\mathrm{Reference}}(t)$) and plasma parent compound ($C_{\mathrm{PPC}}(t)$) the time dependent distribution volume parameter DV'_3 was calculated according to Eq. (1) (Pinborg et al. 2003),

$$DV'_3(t) = (C_{\mathrm{Region}}(t) - C_{\mathrm{Reference}}(t))/C_{\mathrm{PPC}}(t) \quad (1)$$

with C: radioactivity concentration.

From inspection of the time activity curves the time intervals of optimum equilibrium conditions for all three subjects were set to 90–120 min for baseline and 170–240 min for challenge. Average images across these time intervals were generated using PMOD. From these average images parametric images were generated by voxel-wise linear transformation according to Eq. (2) (Pinborg et al. 2003), with cerebellum as reference region.

$$DV'_3 = (C_{\mathrm{Region}} - C_{\mathrm{Reference}})/C_{\mathrm{PPC}} \quad (2)$$

with C: radioactivity concentration.

Individual difference images were generated according to Eq. (3).

$$\Delta DV'_3 = DV'_{3\ (170-240\,\mathrm{min})} - DV'_{3(90-120\,\mathrm{min})} \quad (3)$$

Average images were corrected for partial volume effects according to the algorithm of Müller-Gärtner (Muller-Gartner et al. 1992) using its voxel-wise application described by Rota Kops (2005). Gray and white matter masks were generated using SPM2 segmentation. Missegmented voxels were manually removed using PMOD. For PVC, gray and white matter masks were thresholded at $p = 0.5$.

Partial volume corrected (PVC) average images were spatially normalized to the Collin single subject T1 brain template using SPM2 and segmented source images (1 mm^3-voxel size).

Partial-volume corrected, spatially normalized parametric images were generated according to Eq. (2), with the cerebellar activity concentration being derived from the PVC image, too. Normalized baseline (90–120) images from subjects 1–3 were averaged, and a verum-minus-placebo difference image (*VP*) was generated according to Eq. (4).

$$VP = \frac{1}{2}(\Delta DV'_{3(\mathrm{Subject1})} + \Delta DV_{3(\mathrm{Subject2})})' - \Delta DV'_{3(\mathrm{Subject3})} \quad (4)$$

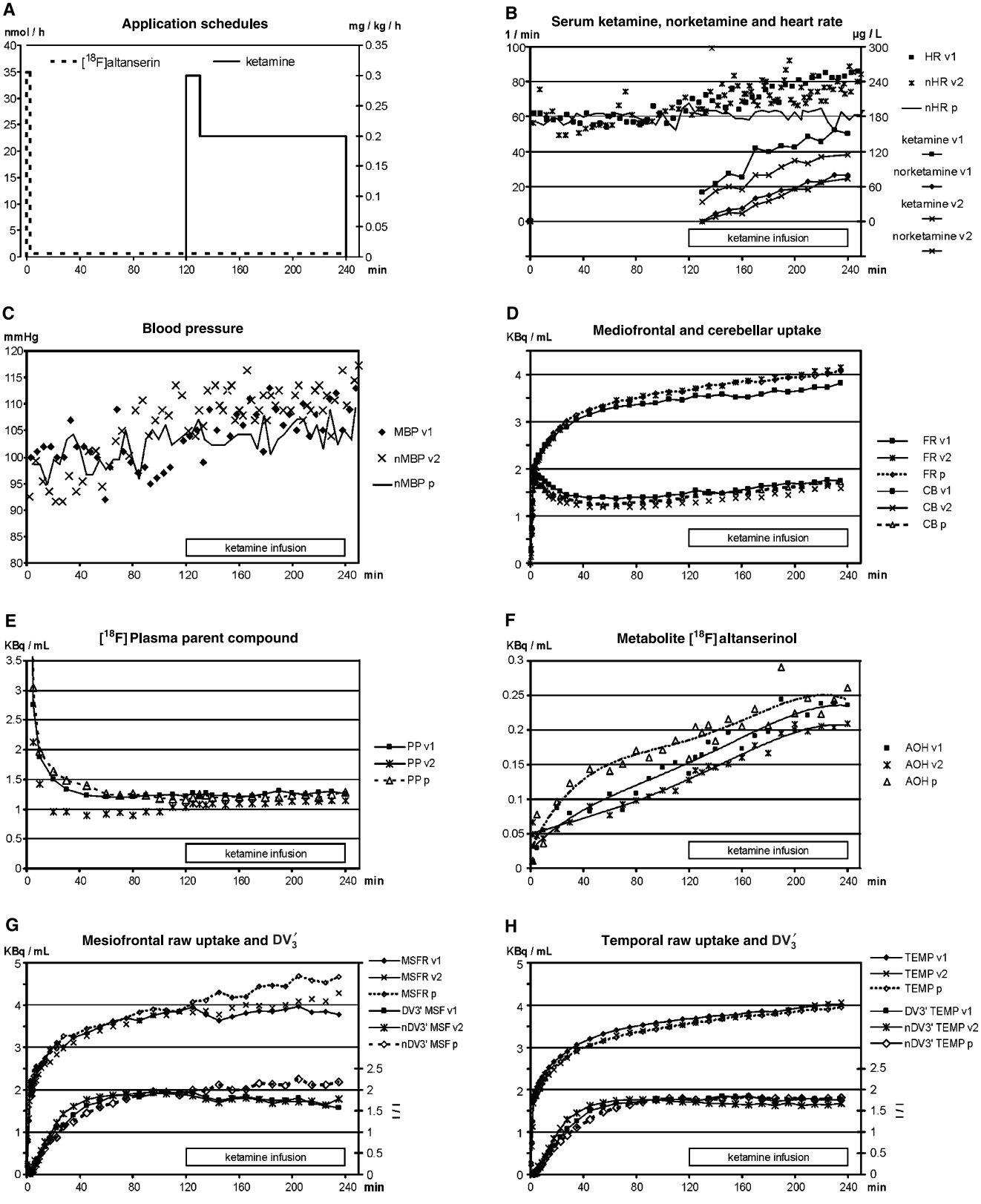
In order to select candidate regions with both, high specific [^{18}F]altanserin uptake and marked difference between baseline and challenge, $VP < -0.3$ and mean $DV'_3 > 1.5$ were chosen as criteria for a posteriori generation of volumes of interest (VOI). A binary 3D data set of the voxels fulfilling these criteria was generated from the VP image. This binary mask, present in the Collin single subject template space was spatially normalized (in "backward" direction) to each individual brain. VOIs from each individual binary mask were generated using PMOD. Time-activity curves of these regions were obtained from the dynamical PET datasets. Confidence intervals for the shift (*shift*) between baseline (*baseline*) and challenge were deduced from each individual $DV'_3(t)$ curves separately by fitting a step function Eq. (5) to the data within the 80–240 min range using GraphPad Prism Plus (GraphPad Software Inc., San Diego, CA).

$$DV'_3(t) = (baseline - shift(t^{30}/(t^{30} + 150^{30})) \quad (5)$$

Results

Ketamine infusion began 120 min after the start of [^{18}F]altanserin infusion (schedule in Fig. 1A) and resulted in continuously increasing plasma levels of ketamine and its metabolite norketamine, reaching peak concentrations

of 157 µg/l in subject 1 and 114 µg/l in subject 2 both at 230 min (Fig. 1B). Assuming a bi-exponential wash-out function, we calculated an optimum bolus/infusion ratio for ketamine with theoretical K_{bol} values of 0.8 and 1.0 h for subject 1 (v1) and 2 (v2), respectively, which corresponds to apparent beta half lives of 32 and 41 min. Heart rate and



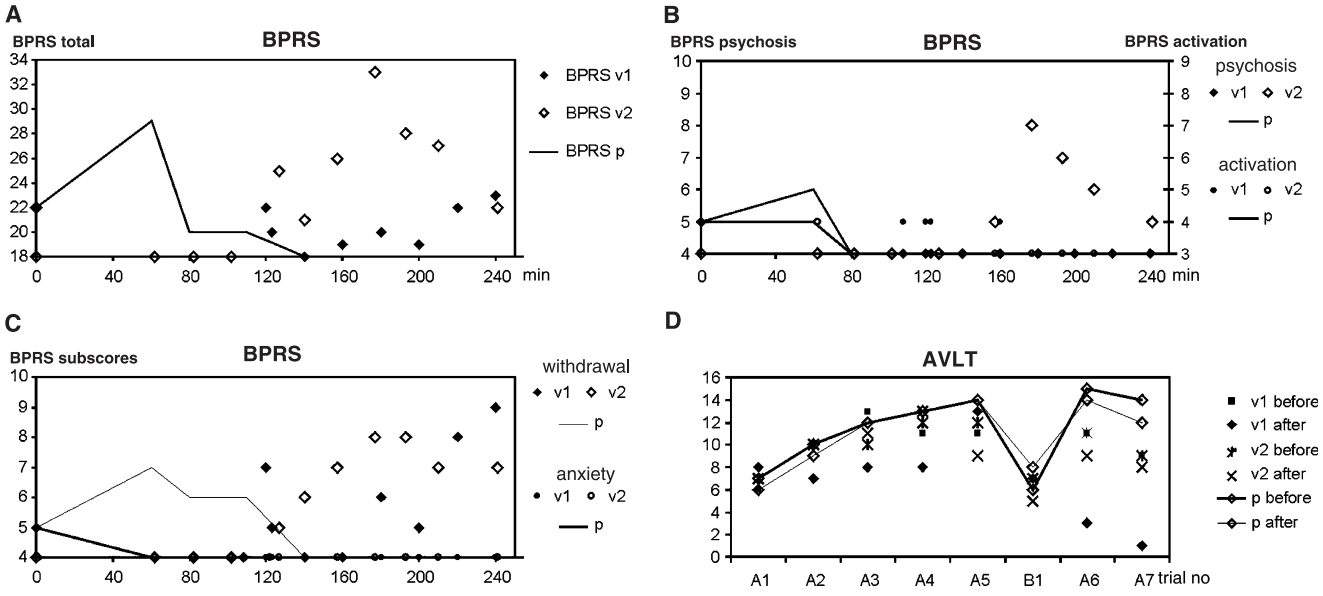


Fig. 2. Psychopathological and cognitive response to ketamine challenge. **A–C** Psychometric tests at baseline (20–10 preceding infusion start) and after ketamine challenge (250–260 min). **A** Total brief psychiatric rating scale (BPRS) scores. **B** BPRS subscores for psychosis and activation. **C** BPRS subscores for withdrawal and anxiety. **D** Auditory verb learning test (AVLT) performance (A1–A5, immediate recall of list A, repeated 5 times; B1, immediate recall of list B; A6 and A7, delayed recall of list A). *v1* Subject 1 on verum; *v2* subject 2 on verum; *p* subject on placebo

blood pressure increased during ketamine infusion and were unchanged during placebo infusion (Fig. 1B and C).

Both, subjects 1 and 2 experienced psychotic symptoms in form of derealisation and optical hallucinations following ketamine infusion. Mood changes included an euphoric mood state in subject 1 and a dysphoric mood state in subject 2. Ketamine-induced changes in mental state increased in quality and quantity during the ketamine infusion, as documented by BPRS changes (Fig. 2A). BPRS subscores (psychosis, activation, withdrawal, anxiety) are given in Fig. 2B and C. Figure 3 illustrates the spontaneous comments of subjects. The verbal fluency decreased to a comparable degree in all subjects. The cognitive performance as measured with the AVLT (Fig. 2D) and digit span tests (Table 1) decreased after ketamine infusion, while remaining stable after placebo infusion.

Equilibrium of [18F]altanserin distribution was reached before start of ketamine infusion with slopes of $DV_3'(t)$ (60–120 min) of $-0.5\%/\text{h}$, $-5.0\%/\text{h}$, $3.0\%/\text{h}$ and slopes of the plasma parent compound radioactivity concentration (C_{PPC}) of $2.3\%/\text{h}$, $14.3\%/\text{h}$, $-6.7\%/\text{h}$ in subjects 1, 2 and 3, respectively. Time-activity curves of cerebral regions were almost identical for all three subjects (mean coefficient of variation $co = 2.4\%$) from 0 to 120 min. After

Table 1. *Neuropsychological performance before and after ketamine challenge. Raw scores are given which correspond to the number of correct items obtained in the first minute of testing*

Test	Ketamine				Placebo	
	Subject 1		Subject 2		Subject 3	
	BL	CH	BL	CH	BL	CH
Word fluency*						
Semantic	33	22	30	27	27	20
Lexicalic	26	23	27	22	18	11
Category shift*						
Semantic	27	21	20	20	19	16
Lexicalic	22	14	21	23	22	24
Digit span						
Forward	14	12	14	12	13	15
Backward	12	10	11	12	13	14
Letter number	10	7	11	9	9	9

* Regensburger word fluency task.
BL Baseline; *CH* challenge

Fig. 1. Kinetic data illustrating [18F]altanserin binding and distribution as well as the course of and the response to ketamine challenge. **A** Application schedule of radioligand and ketamine. **B** Plasma levels of ketamine and its metabolite norketamine (absolute) and heart rates (HR) normalized to subject 1 at baseline (0–110 min). **C** Mean blood pressure (MBP) normalized to subject 1 at baseline (0–110 min). **D** Tissue time-activity curves of a priori defined mediofrontal and cerebellar regions of interest. **E** Plasma radioactivity concentration corrected for parent compound ([18F]altanserin). **F** Radioactivity concentration of the brain penetrating primary metabolite [18F]altanserinol. **G** Tissue radioactivity and distribution volume ratio DV_3' of the a posteriorily defined region with the highest decrease (mesiofrontal). **H** Tissue radioactivity and distribution volume ratio DV_3' of the a priori defined temporal region. *v1* Subject 1 on verum; *v2* subject 2 on verum; *p* subject on placebo; *HR* heart rate; *nHR* heart rate normalized to subject 1; *MBP* mean blood pressure; *nMBP* mean blood pressure normalized to subject 1; *PP* radioactivity concentration of plasma parent compound; *AOH* altanserinol; *FR* frontal region; *MSFR* mesiofrontal region; *TEMP* temporal region

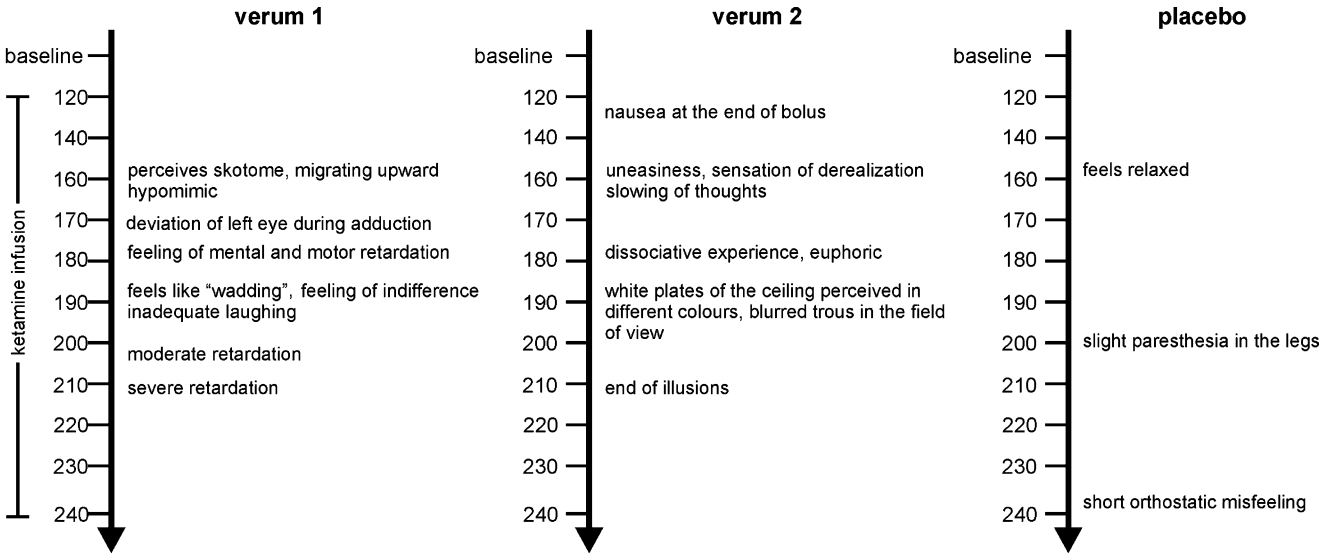


Fig. 3. Self-reported psychopathology during ketamine infusion in temporal order

ketamine application (starting at 120 min) an apparent equilibrium was re-established, with slightly higher [^{18}F]altanserin plasma levels due to interference of ketamine with altanserin metabolization, most probably mediated by competitive P450 isoenzyme blockade by ketamine (Fig. 1E). The concentrations of the primary brain-penetrating metabolite [^{18}F]altanserinol increased almost linearly during the scan with parallel kinetics for all three subjects (Fig. 1F).

Figure 1D illustrates the time-activity curves of a region showing maximum uptake (mediofrontal, corresponding to the gyrus frontalis medialis) and of the cerebellar reference region. Slopes for mediofrontal $DV_3'(t)$ (170–240 min) were $+2.0\%/\text{h}$, $-2.1\%/\text{h}$, $1.0\%/\text{h}$ and slopes for C_{PPC}: $+1.6\%/\text{h}$, $+4.3\%/\text{h}$, $+2.7\%/\text{h}$ in subjects 1, 2 and 3, respectively. The changes between baseline (80–120 min) and challenge (170–240 min) in [^{18}F]altanserin binding potential DV_3' was -1.0% ($\pm 1.6\%$) and $+4.1\%$ ($\pm 1.8\%$) in the two subjects receiving ketamine compared to -1.2% ($\pm 2.6\%$) in the subject receiving placebo in a predefined VOI comprising the lateral temporal lobe. The respective changes in a large mediofrontal region were -3.2% ($\pm 2.4\%$) and -5.1% ($\pm 2.6\%$) versus 0.2% ($\pm 2.0\%$) (raw uptake in Fig. 1D, $DV_3'(t)$ not shown). The maximum decrease of DV_3' was -8.8% ($\pm 4.6\%$) and -8.0% ($\pm 3.9\%$) (verum) versus $+9.7\%$ ($\pm 4.1\%$) (placebo) in a frontomesial region selected a posteriori (Fig. 1G). In a temporal region curves of the raw uptake and DV_3' were almost congruent without change for all three subjects (Fig. 1H).

Individual DV_3' images averaged from 90 to 120 min and from 170 to 240 min are displayed in Fig. 4A and B, difference images in Fig. 4C. There was a remarkable increase of unspecific radioactivity uptake in skull and retinae in the late phase, which was clearly more pronounced in the ketamine subjects as compared to placebo.

A comparison based on spatially normalized difference images (parameter *VP* according to Eq. (4)) of the two subjects on verum ($\text{n}\Delta DV'_{3(\text{Sub1})}$, $\text{n}\Delta DV'_{3(\text{Sub2})}$) and the subject on placebo ($\text{n}\Delta DV'_{3(\text{Sub3})}$) suggested frontopolar, insular, orbitofrontal, occipital and frontomesial regions as candidates for a probable decrease in DV_3' after ketamine application. Analysis of individual time-activity curves obtained from the above mentioned candidate regions showed a slight but clear decrease of $DV_3'(t)$ in the frontomesial region (Fig. 1H).

Discussion

The aim of the present feasibility study was to isolate acute effects of ketamine on [^{18}F]altanserin binding in vivo. Ketamine-induced acute release of endogenous 5-HT from presynaptic sites could theoretically interfere with radioligand binding. We did not observe a global change in [^{18}F]altanserin binding $DV_3'(t)$ although ketamine induced robust physiological, psychopathological, and cognitive effects, which were expected from the literature (e.g., Krystal et al. 2005; Malhotra et al. 1996; Newcomer et al. 1999; Lahti et al. 2001) and served as a proof that effective ketamine doses had been established.

Although there were no global changes in [^{18}F]altanserin binding potentials, we observed a slight decrease of tissue radioactivity and $DV_3'(t)$ in selected regions.

For instance, temporal (Fig. 3H), parietal and frontal regions, displayed high baseline [^{18}F]altanserin binding (DV_3')

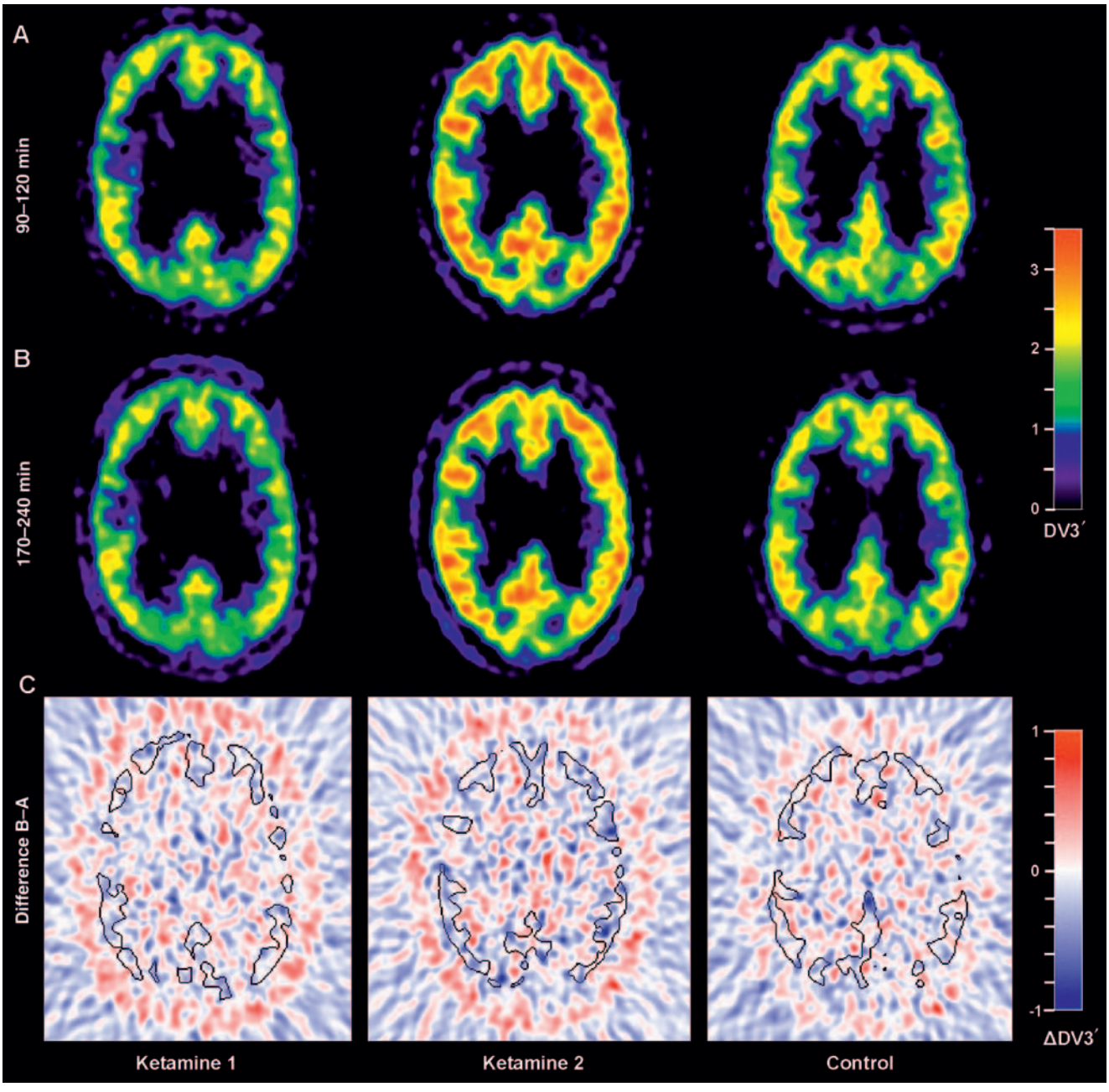


Fig. 4. Individual parametric images of the distribution volume parameter DV_3' at baseline 90–120 min (**A**), during ketamine challenge 170–240 min (**B**) and the difference of B-A (**C**). Isocontur lines indicating regions with $DV_3' > 2.5$ from row A are superimposed on (**C**) in black. Images from subjects 1 and 2 on ketamine are displayed in the left and in the middle column respectively, while images from subject 3 (control) are shown in the right column with identical scaling. Unspecific uptake in the scalp is increased in both subjects who received ketamine

and are known to bind ketamine at a high and comparable level (PET-studies using [^{11}C]ketamine; Hartvig et al. 1995; Kumlien et al. 1999). However, in contrast to frontal regions no decreases of $DV_3'(t)$ were observed in temporoparietal regions during ketamine challenge. This speaks against displacement effects which should occur proportionally in all regions.

Conversely, the spatial pattern of the decreases in [^{18}F]altanserin binding is in accordance with ketamine-induced increases of cerebral perfusion (rCBF) measured by [^{15}O]butanol and PET. We verified this spatial congruence by transforming data from a previous study conducted at our institution to the same reference space. The analogue study-design comprised baseline and challenge scans after

bolus infusion of 0.1 mg/kg and 0.38 mg/kg ketamine (in comparison with a cumulated dose of 0.42 mg/kg in the present study) of two subjects and one control subject. A dose-dependent increase of rCBF in the frontoparietal cortex with maximum increases in the anterior cingulum (about 25 and 70%, respectively) and mesiofrontal (about 16 and 55% respectively) regions was observed (Herzog et al. 2005). Långsjö and coworkers (2003) reported similar changes in cerebral perfusion measured by [^{15}O]water PET and also in cerebral blood volume measured by [^{15}O]carbon monoxide under ketamine at three different concentrations. The plasma level of 132 ± 19 µg/l reported for the group which received a medium dose of ketamine is comparable to our study. At this dose the highest increase in rCBF of 19% occurred in the anterior cingulum whereas the relative change of blood volume did not exceed ±3%.

Hence, we assume that a perfusion effect is responsible for the slight and restricted decrease in [^{18}F]altanserin. According to general theoretical assumptions, changes in perfusion should not alter radiotracer binding at tracer equilibrium. Rather than a distribution equilibrium, there might be a pseudo-equilibrium with balanced reciprocal dynamic processes. In cerebral tissue a remaining quantity of tracer after high first pass capillary extraction of the bolus is continuously washed out, while a cumulative amount of this very lipophilic tracer may be trapped in deep compartments of unspecific binding. Moreover, several antidromic effects could occur in higher perfused regions during a bolus/infusion study. On the one hand, continuously accumulating brain penetrating radioactive metabolites (cf. Fig. 3F) increase the raw radioactivity uptake. On the other hand, the increase in perfusion achieved here may exceed the range within which the distribution equilibrium of parent compound is stable. As ketamine concentrations continuously increase during the experiment, there is a high occupation of plasma protein binding sites by ketamine in arterial plasma, which during the venous passage becomes available for the clearance of [^{18}F]altanserin from tissue. Furthermore, ketamine may induce a capillary leak. The latter effects could explain the observed decrease (maximum ≈10%) of apparent DV'_3 values in defined regions.

In line with this reasoning are the findings of Elfving et al. (2003) who conducted an occupation study in rats which were kept under continuous ketamine/xylazine anaesthesia for 5 h and received a single bolus injection of [^{18}F]altanserin at 4 h. A frontal-to-cerebellum ratio of 6.1 (±0.64) was observed (concordant with 6.0 ± 0.59 for the ketamin analogue tiletamine mixed with zolazepam) versus 5.5 (±0.69) for placebo. This may reflect a higher bolus first pass tissue extraction of the radioligand in higher perfused regions. In zones with higher perfusion following challenge opposite effects will be observed depending on the experimental conditions. An increase of radioligand binding in repeated occupation studies corresponds a decrease in displacement experiments.

In this context it is of particular interest, that previous [^{18}F]altanserin studies using citalopram in 3 naïve and 4 pindolol-pretreated subjects to increase 5-HT levels (Pinborg et al. 2004) also failed to demonstrate any endogenous displacement.

Altanserin is a very lipophilic ligand: $\log P = 3.2$ (octanole/water). The reported in vivo rate constants for [^{18}F]altanserin dissociation from 5-HT$_{2A}$R is $k_4 = 0.041\ \text{min}^{-1}$ (Biver et al. 1994), while in vitro rate constants are $k_{off} = 0.062\ \text{min}^{-1}$ for [^{18}F]altanserin (Kristiansen et al. 2005) and $k_{off} = 0.110\ \text{min}^{-1}$ for [^{3}H]ketanserin (Rashid et al. 2002). Ketanserin is the thioketone analogue of altanserin with a similar receptor affinity profile (K_i at 5-HT$_{2A}$R of altanserin 0.13 nM, of ketanserin 0.63 nM, Leysen 1984; Lemaire et al. 1991). Autoradiographic studies have shown competition of 5-HT with [^{18}F]altanserin binding at K_i values ranging from 900 to 3400 nM (Kristiansen et al. 2005) and with [^{3}H]ketanserin binding at K_i values ranging from 60 nM (Bonhaus et al. 1995) to 600 nM (Boess and Martin 1994). Baseline 5-HT concentrations in brain tissue in vivo are in the range of only 5 nM (Hume et al. 2001). In brain slices ketamine at a concentration of 23.8 mg/l, the 200 fold of plasma concentrations reached in the present study, provoked only a 46% increase in 5-HT concentrations measured with 5-HT selective microelectrodes (Tso et al. 2004). Even under conditions as present during [^{11}C]raclopride scanning, this would lead to only a 1% decrement in radioligand binding potential (Laruelle 2000).

For comparison, the dynamical range for dopamine concentrations in the synaptic cleft encompasses up to 3 or 4 orders of magnitude (Laruelle 2000), and K_i for DA relative to raclopride on D$_2$ is 2–75 nM for the high affinity state, (Kapur and Seemann 2002), and 1 µM (Kapur and Seemann 2002) or 24.8 µM (Stormann et al. 1990) for the low affinity state. Under basal conditions intrasynaptical DA levels have been reported to vary between 6 and 200 nM (mean 100 nM, Endres et al. 1997). Thus, DA levels, but not 5-HT levels, can reach the range of K_i of the respective radioligands.

Dopamine D$_2$ receptors are equally distributed across high-affinity and low-affinity states (Laruelle 2000). The high-affinity state of 5-HT$_{2A}$R was only occupied by 4% of receptors when expressed on culture cells (Song et al. 2005). Only this fraction would be susceptible to endogenous displacement. Atypical for an antagonist, ketanserin discriminates a high-affinity state at $K_i = 3$ nM from a low-

affinity state at $K_i = 790$ nM (measured by displacement of the agonist [^{125}I]DOI, Lopez-Gimenez et al. 2001) which is not influenced by 100 μM guanosine 5′-[β,γ-imido]triphosphate (Gpp(NH)p). Also agonist-induced internalization of high-affinity state 5-HT$_{2A}$R has been reported (Bhatnagar et al. 2001). In the case of maintained altanserin binding to 5-HT$_{2A}$R this would lead to intracellular trapping of the radioligand, a process blunting other effects leading to decreased receptor availability.

Hirani et al. (2003) detected no change in the uptake of the 5-HT$_{2A}$-ligand [^{11}C]MDL100907 40 min after 10 mg/kg fenfluramine which induces a strong liberation of 5-HT compared to control in rats. These findings illustrate that no 5-HT$_{2A}$ radioligand is currently available for PET studies which is sensitive to endogenous displacement.

Conclusion

Acute ketamine as administered in the present protocol provoked no specific displacement of [^{18}F]altanserin from 5-HT$_{2A}$R. Instead, we observed a slight focal decrease in radioligand binding compatible with an increase in perfusion seen in a previous [^{15}O]butanol study. The marked physiological, psychopathological, and cognitive reaction of subjects confirmed effective tissue ketamine levels. Here we provide evidence that acute effects of ketamine on [^{18}F]altanserin PET are highly unlikely. This is an important prerequisite to measure chronic plastic effects of ketamine on 5-HT$_{2A}$R by this technique. Based on our findings we suggest a washout time of 6 h following a typical dose 0.5 mg/kg ketamin to avoid any significant interference with [^{18}F]altanserin binding by fluctuations of cerebral perfusion during the measurement.

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