

S-ketamine and GABA-A-receptor interaction in humans: an exploratory study with I-123-iomazenil SPECT[†]

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Objective We aimed to probe for regional cerebral effects of S-ketamine on *in vivo* GABA-A-receptor binding in healthy human subjects.

Methods We investigated I-123-iomazenil SPECT before, during and after administration of S-ketamine in a blinded placebo-controlled study design ($n = 12$ in both groups). Analyses of SPECT were performed with voxel-based statistical parametric mapping (SPM), and statistical comparisons were made between the groups. We also assessed biochemical and behavioural changes during S-ketamine infusion.

Results S-ketamine induced positive and negative symptoms measured by the Brief Psychiatric Rating scale (BPRS). It increased the cortisol and prolactin levels. Image analysis revealed significantly decreased I-123-iomazenil binding in bilateral dorsomedial prefrontal cortex during S-ketamine administration when compared to placebo.

Conclusion Our study delivers preliminary evidence for an *in vivo* interaction of S-ketamine with GABA-A-receptors in human dorsomedial prefrontal cortex. Copyright © 2008 John Wiley & Sons, Ltd.

KEY WORDS — S-ketamine; I-123-iomazenil; SPECT; GABA-A; medial prefrontal cortex

INTRODUCTION

Subanaesthetic doses of ketamine and especially its S-enantiomer can elicit psychotic symptoms and cognitive deficits (Northoff *et al.*, 2005; Vollenweider and Geyer, 2001; Vollenweider *et al.*, 2000) as well as changes in cerebral blood flow and cerebral glucose utilisation (Langsjo *et al.*, 2005; Vollenweider *et al.*, 1997) in healthy volunteers. Ketamine is believed to exert its main effects by antagonising the *N*-

methyl-D-aspartate receptor (Gouzoulis-Mayfrank *et al.*, 2006; Passie *et al.*, 2003; Vollenweider and Geyer, 2001). However, based on data derived from animal experiments, it has been suggested that ketamine may additionally influence GABA-A-receptors. It has been demonstrated that GABA-A antagonism can influence the anaesthetic potency of ketamine (Sonner *et al.*, 2003). *In vitro* experiments suggested that ketamine may increase GABA-mediated inhibition and thus act as a GABA-mimetic (Gage and Robertson, 1985; Liske *et al.*, 1990; Little and Atkinson, 1984; Scholfield, 1980).

In vivo experiments in humans showed incongruent results: it was demonstrated that interaction with the benzodiazepine receptor may affect the action of ketamine as benzodiazepines modulate many effects

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induced by ketamine (Restall *et al.*, 1988) and that administration of the benzodiazepine receptor antagonist, flumazenil, increases the effects of ketamine (Restall *et al.*, 1990). On the other hand, Salmi *et al.* (2005) could not demonstrate an interaction of ketamine and flumazenil binding.

We aimed to probe for a possible influence of S-ketamine on GABA-A-receptors *in vivo* in humans on an exploratory basis.

METHODS

Subjects

A total of 24 healthy subjects were recruited from university students and hospital staff. Relying on a structured interview (Spitzer *et al.*, 1990) and clinical examination the following inclusion criteria were applied: (i) no history of any use of benzodiazepines; (ii) no history of any use of glutamatergic substances; (iii) no drug, alcohol and/or substance abuse; (iv) no neurologic or psychiatric diseases of the subject itself or first-degree relatives.

The protocol was approved by the institutional review boards of the University of Magdeburg, Germany. Written consent was obtained from all the subjects.

Study design

Subjects received either S-ketamine ($n = 12$; 6 females, 6 males; 25.42 ± 2.34) or placebo ($n = 12$, 6 females, 6 males; 24.12 ± 2.06 ; age- and sex-matched). The subjects were assigned randomly to both groups and were blind to the substance they received. S-ketamine was administered by an initial 1 min intravenous bolus (0.12 mg/kg) followed by a 1 h intravenous infusion of 0.65 mg/kg/h using an infusion pump. Placebo consisted of saline (0.9% NaCl) administered in an analogous fashion.

Initially, 200 MBq I-123-iomazenil were slowly administered to the subjects in a dimly lit, quiet room. To avoid circadian variability the SPECT scans for all the subjects were obtained at the same time of day (approx. at 1 pm \pm 30 min). SPECT 1 was taken 5–10 min after injection of 200 MBq I-123-iomazenil. It may be considered as an equivalent of rCBF (Bartenstein *et al.*, 1991; Lassen, 1996).

SPECT 2 started 120 min post injection and before administration of either S-ketamine or placebo.

As soon as SPECT 2 was finished, either placebo or S-ketamine was administered. With the beginning of

the infusion of S-ketamine/placebo, SPECT imaging was continued, so that SPECT 3, 4 and 5 could be acquired during continuous infusion of S-ketamine/placebo. SPECT 6 was acquired after infusion of S-ketamine/placebo (Figure 1).

Behavioural measures

Plasma levels of prolactin, cortisol and ketamine were obtained at the following time points: 140 (before), 145, 150, 155, 160, 165, 170, 180, 190, 200, 210 and 220 min.

The brief psychiatric rating scale (BPRS) (Overall and Gorham, 2007) and State-Trait Anxiety Inventory (STAI) (Spielberger, 1980) were obtained before SPECT 1 and after SPECT 6.

The results of the hormonal and the psychometric measures were compared between groups using two-way ANOVA. Post-hoc comparisons were done using t-tests with Bonferroni correction for multiple comparisons.

SPECT imaging

Iomazenil was synthesised and labelled with I-123 at the Paul Scherrer Institute (Villingen, Switzerland) according to the method described by Beer *et al.* (1990). SPECT was performed with a dual head gamma camera (ADAC/Vertex) with a low energy, high-resolution collimator (FWHM: 7.5 mm at 10 cm depth). Images were acquired on a $64 \times 64 \times 16$ matrix size with 64 projections and 40 s per view. Pixel size was $4.33 \times 4.33 \text{ mm}^2$. A filtered back projection was used for reconstruction (analytic Gaussian filter: cut-off = 0.22; order number = 22.0). The images were corrected for attenuation with a uniform linear attenuation correction coefficient of 0.12 cm^{-1} .

Image analysis

Statistical analysis was conducted on the basis of the general linear model as implemented in SPM2 (<http://www.fil.ion.ucl.ac.uk/spm/software/spm2/>) (see Kugaya *et al.*, 2003, Lahorte *et al.*, 2000, Verhoeff *et al.*, 1999 for the application of SPM on SPECT). Images were corrected for motion artefacts, spatially normalised to standard stereotactic space and smoothed with an isotropic 16 mm (FWHM) Gaussian kernel. Additionally, the images were mean-adjusted by proportional scaling.

By applying two-sample t-tests, we calculated comparisons between the two groups. Clusters of activations were reported as significant, when they

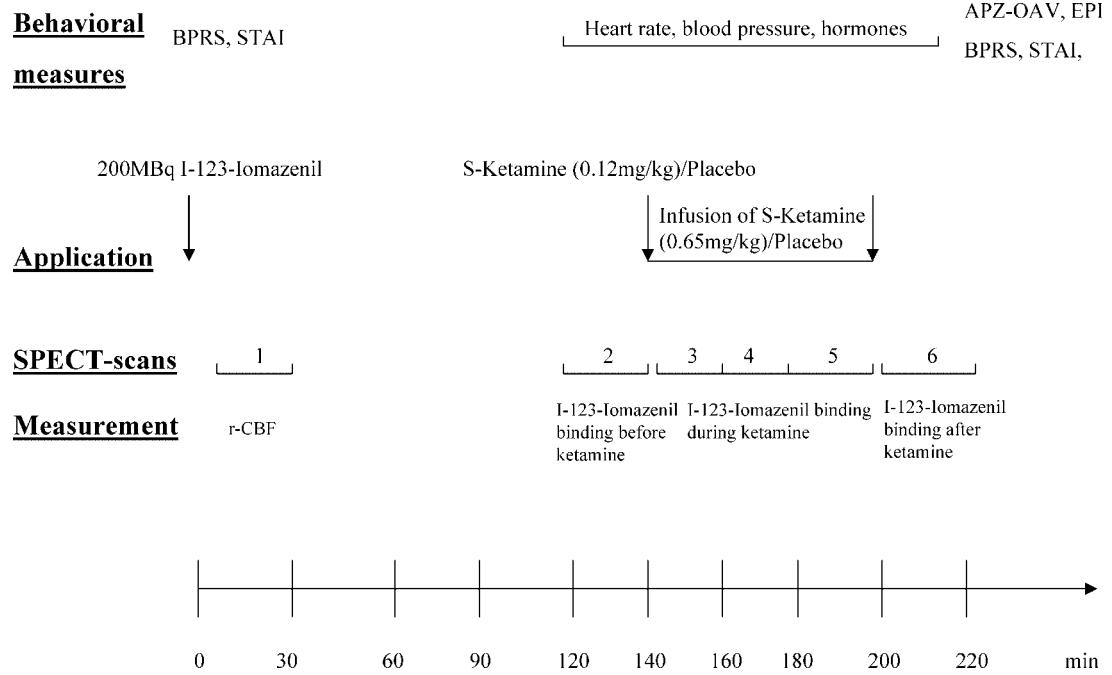


Figure 1. Experimental procedure

surpassed a threshold of $p < 0.05$ on voxel level (FDR-corrected) and comprised 10 or more contiguous voxels.

The following pairwise comparisons (as well as the inverted comparisons) were calculated:

1. SPECT 1 (placebo) > SPECT 1 (S-ketamine)
 2. SPECT 2 (placebo) > SPECT 2 (S-ketamine)
 3. SPECT (placebo) > SPECT(S-ketamine) separately for SPECT 3, 4 and 5
 4. SPECT 6 (placebo) > SPECT 6 (S-ketamine)
- Additionally, we calculated the comparisons:
5. [(SPECT 1/2/6 > SPECT 3/4/5) placebo] > [(SPECT 1/2/6 > SPECT 3/4/5) S-ketamine]
 6. [(SPECT 1/2 > SPECT 3/4/5/6) placebo] > [(SPECT 1/2 > SPECT 3/4/5/6) S-ketamine]

In these comparisons, clusters of activations were reported when they surpassed a threshold of $p < 0.001$ uncorrected.

RESULTS

Behavioural measures

S-ketamine produced positive and negative symptoms as reflected in BPRS: BPRS total score ($p = 0.001$), positive symptoms score ($p = 0.001$), negative symp-

toms score ($p = 0.01$), anxious-depression score ($p = 0.01$), hostility-suspiciousness score ($p = 0.001$) and activation score ($p = 0.01$).

The STAI showed no significant difference between groups.

S-ketamine increased both cortisol ($F = 17.8$; $p = 0.002$) and prolactin ($F = 8.9$; $p = 0.005$) levels over time while such increases could not be observed in the placebo group.

In the ketamine group, the plasma level of S-ketamine ranged from 143.7 to 334.1 ng/ml (mean 202.2 ± 37.5 ng/ml), the level of cortisol ranged from 8.0 to 45.6 ng/ml (mean 19.7 ± 10.3 ng/ml), and the level of prolactin ranged from 8.1 to 41.3 ng/ml (mean 16.2 ± 9.2 ng/ml). In the control group, no significant level of S-ketamine was detected in the plasma, the level of cortisol ranged from 4.4 to 19.7 ng/ml (mean 8.6 ± 4.4 ng/ml), and the level of prolactin ranged from 4.5 to 28.7 ng/ml (mean 9.4 ± 6.6 ng/ml).

SPECT

1. We did not find significant differences in the pre-infusion cerebral blood flow between the groups.
2. We did not find significant differences in the cerebral I-123-lomazenil binding between the groups.

3. The comparison of SPECT 3 (placebo) > SPECT 3 (S-ketamine) revealed significant differences in local cerebral I-123-iomazenil binding with peak voxel MNI-coordinates at $x = 3, y = 66, z = 30$ ($p = 0.014, k = 38$). It is predominately located in the right medial frontal gyrus (BA 10).

SPECT 4 (placebo) > SPECT 4 (S-ketamine) showed significant differences in the left medial frontal gyrus (BA 8) with peak voxel MNI-coordinates at $x = -6, y = 45, z = 45$ ($p = 0.019, k = 374$). The region extends to the superior frontal gyrus (BA 10).

SPECT 5 (placebo) > SPECT 5 (S-ketamine) demonstrated a significant difference in the left superior frontal gyrus (BA 9) with peak voxel MNI-coordinates at $x = -6, y = 51, z = 36$ ($p = 0.001, k = 500$). Additionally, a difference was located in the left postcentral gyrus (BA 3) with peak voxel MNI-coordinates at $x = -18, y = -39, z = 65$ ($p = 0.021, k = 59$).

The inverted comparisons SPECT (S-ketamine) > SPECT (placebo) for SPECT 3, 4 and 5 revealed no significant differences.

4. We did not find significant differences in the cerebral I-123-iomazenil binding between the groups (see also Figure 2). However at an uncorrected threshold we found a difference at left superior frontal gyrus (BA 9) with peak voxel MNI-coordinates at $x = -3, y = 54, z = 33$ ($p < 0.001, k = 324$), right superior frontal gyrus (BA 9) with peak voxel MNI-coordinates at $x = 18, y = 36, z = 42$ ($p < 0.001, k = 24$), left medial frontal gyrus (BA 6) with peak voxel MNI-coordinates at $x = -3, y = -30, z = 69$ ($p < 0.001, k = 37$), and left middle frontal gyrus (BA 46) with peak voxel MNI-coordinates at $x = -36, y = 27, z = 24$ ($p < 0.001, k = 16$).
5. This comparison did not show significant differences.
6. This comparison revealed a difference in the right medial frontal gyrus (BA 9) with peak voxel MNI-coordinates at $x = 3, y = 55, z = 36$ ($p < 0.001, k = 13$).

DISCUSSION

Our study delivers first evidence that S-ketamine modulates *in vivo* GABA-A-receptor binding in human dorsomedial prefrontal cortex. There are several possible mechanisms, which are, among others, that ketamine interacts with the GABA receptor by allosteric modulation, or that ketamine modulates endogenous release of GABA. It has been demonstrated

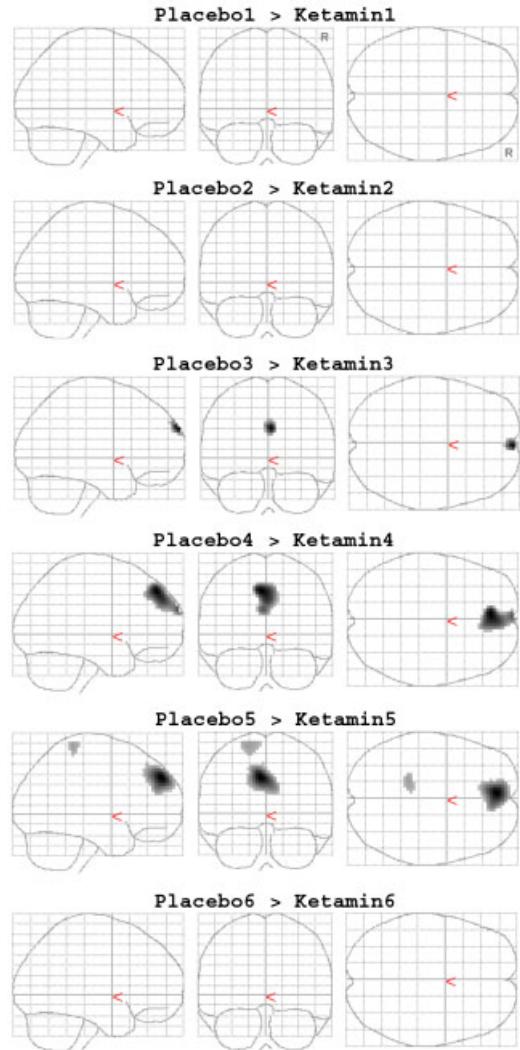


Figure 2. The contrast SPECT (Placebo) > SPECT (S-ketamine) was calculated for the images SPECT 1 (representing cerebral blood flow), SPECT 2 (representing I-123-iomazenil binding before S-ketamine infusion), SPECT 3, 4 and 5 (all representing I-123-iomazenil binding during S-ketamine infusion) as well as SPECT 6 (representing I-123-iomazenil binding after S-ketamine infusion). Only Clusters of activations were shown that surpassed a threshold of $p < 0.05$ on voxel level (FDR-corrected) and comprised 10 or more contiguous voxels. The MNI-coordinates of the peak voxels are reported. Third row: significant difference in local cerebral I-123-iomazenil binding with predominant location in the right medial frontal gyrus (BA 10; $x = 3, y = 66, z = 30$ ($p = 0.014, k = 38$)). Fourth row: significant differences in the left medial frontal gyrus (BA 8; $x = -6, y = 45, z = 45$ ($p = 0.019, k = 374$)). The region extends to the superior frontal gyrus (BA 10). Fifth row: significant difference in the left superior frontal gyrus (BA 9; $x = -6, y = 51, z = 36$ ($p = 0.001, k = 500$))). Additionally, a difference was located in the left postcentral gyrus (BA 3; $x = -18, y = -39, z = 65$ ($p = 0.021, k = 59$))).

that GABA-A antagonism/agonism decreases/increases the effects of ketamine in animals (Irifune *et al.*, 1998, 2000; Sonner *et al.*, 2003). Moreover, by using voltage clamping in recombinant GABA-A-receptors it has been shown that ketamine significantly enhanced GABA-induced currents at concentrations being clinically relevant (Lin *et al.*, 1992). Furthermore, intracellular recordings in native neuronal receptors in olfactory cortex and hippocampus showed similar results (Gage and Robertson, 1985; Scholfield, 1980). Thus, the effects of ketamine may be partly due to an enhancement of GABAergic function.

Our findings are consistent with an allosteric modulation of the GABA receptor inducing an enhanced GABAergic neurotransmission and a reduced capacity for benzodiazepine binding causing decreased binding of iomazenil.

In contrast, applying ketamine for total anaesthesia GABA-A-receptor agonists seem to mask and GABA-A-receptor antagonists seem to unmask some of the side effects induced by ketamine (Restall *et al.*, 1988) hinting at additional interaction of ketamine and GABA-A-receptors that did not become evident in our study.

We found significant differences between the groups in the BPRS, but not in the STAI indicating that S-ketamine produced positive and negative symptoms without increasing anxiety. This is in line with the findings of Lipp *et al.* (1995).

In contrast, in a study by Salmi *et al.* (2005) ketamine did not affect C-11flumazenil binding to GABA-A-receptor in the brain. Unlike in our study they applied a racemic ketamine infusion. It has been shown that the effects of the S-enantiomer of ketamine are different compared with the R-enantiomer and with racemic ketamine infusion leading to differences in cerebral blood flow, metabolic rate of oxygen and cerebral glucose metabolism (Langsjo *et al.*, 2005; Vollenweider *et al.*, 1997).

In a pilot study, Matusch *et al.* (2007) could not demonstrate interaction of S-ketamine with F-18altanserin binding. However, due to their lower dosage of ketamine and a small number of subjects (ketamine = 2, placebo = 1) it cannot be excluded that a bigger sample size may lead to different results.

One limitation of our study is that we did not perform tracer kinetics modelling. Therefore we are not able to exclude the alternative explanation that our results are due to changes in the brain washout or plasma clearance of I-123-iomazenil. However, such an explanation appears unlikely, since it is difficult to explain our observation of localised changes mainly in the dorsomedial prefrontal cortex.

Comparison 5 did not show significant differences. This is probably due to the influence of Scan 6 showing a difference between both groups. Accordingly, comparison 6 reveals a difference between S-ketamine and placebo fitting in with the results of the pairwise comparisons.

In psychiatric research, ketamine serves to produce a model psychosis (Vollenweider *et al.*, 1997) and may also induce antidepressive effects (Zarate *et al.*, 2006). Our results deliver preliminary evidence for modulatory effects of S-ketamine on GABA-A-receptor, thereby contributing to a deeper understanding of possible mechanisms underlying these effects.

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