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ORIGINAL PAPER

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SPECT imaging of serotonin transporter binding in patients with generalized anxiety disorder

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Abstract The purpose of this study was to characterize the binding properties of serotonin transporter (5-HTT) in the brain of the patients with generalized anxiety disorder (GAD) in comparison to healthy subjects using single photon emission computer tomography (SPECT) with the radioligand [^{123}I]nor-β-CIT. The subjects were 7 patients with GAD and 7 matched healthy volunteers. The regions of interest (ROI) were the midbrain and the thalamus. The comparison of the volumes of distribution did not show significant differences between the patients and controls in the binding of nor-β-CIT to 5-HTT in the ROI. Binding of 5-HTT in the midbrain of patients was significantly and negatively correlated with their anxiety levels measured by the visual analogue scale immediately before the first scan ($r = -0.79$, $p = 0.035$). This study failed to demonstrate an altered functional activity of 5-HTT in patients with GAD when compared with controls.

Key words generalized anxiety disorder · serotonin transporter · SPECT · nor-β-CIT

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Introduction

Generalized anxiety disorder (GAD) is a common and chronic psychiatric disease characterized by excessive and uncontrollable worry and anxiety over everyday life matters and the symptoms of autonomic hyperarousal and tension. Epidemiological studies have found that patients with GAD commonly have comorbid psychiatric disorders, most often major depression and panic disorder (PD) (Wittchen et al. 1994). High comorbidity and poor specificity of GAD symptoms have raised doubts about the validity of GAD as an independent diagnosis. Thus, GAD is sometimes considered to be a residual condition and may be diagnosed only in the absence of other anxiety or mood disorder. However, a large body of evidence from the treatment, neuroimaging and genetic studies suggest that GAD, PD and depression have different underlying neurobiology (Wu et al. 1991; Bell and Nutt 1998; Scherrer et al. 2000). Clinical efficacy of the selective serotonin (5-HT) reuptake inhibitors in the treatment of GAD (Rocca et al. 1997) and their mechanism of action indicate involvement of the brain 5-HT system and 5-HT transporter (5-HTT) in the neurobiology of this disorder. Moreover, several genetic studies have demonstrated associations between certain polymorphisms in the 5-HTT gene and anxiety traits as well as GAD (Lesch et al. 1996; Ohara et al. 1999). In addition, the short allele of the 5-HTT gene linked polymorphic region (5-HTTLPR) has been associated with a greater activation of the amygdala in response to fearful face stimuli in healthy subjects (Hariri et al. 2002). On the other hand the studies *in vitro* have provided controversial data showing a normal or decreased platelet 5-HTT binding in patients with GAD (Hernandez et al. 2002; Iny et al. 1994). To our knowledge, so far there have been no brain imaging studies on the function of 5-HTT in GAD. A recent study showed that patients with depression have a reduced midbrain 5-HTT binding (Malison et al. 1998). In our recent SPECT study in PD we detected a significantly lower 5-HTT binding

in the midbrain of patients with current PD in comparison to healthy subjects that was normalized in remitted PD patients (Maron et al. *in press*). It seemed therefore of interest to carry out an imaging study of 5-HTT function in patients with GAD in order to understand whether the role of 5-HTT in GAD differs from PD and depression. Thus, the purpose of the current study was to investigate the brain 5-HTT binding potential in patients with GAD in comparison to healthy subjects using the SPECT methodology.

Methods

Subjects

In total 7 (4 male and 3 female) patients with GAD participated in this study. The 7 healthy controls with no personal or family history of psychiatric disorders were matched by sex, age and smoking status. The mean age (standard deviation) in the two groups was similar: 39.3 (12.3) and 37.1 (12.1) years, respectively. The patients were recruited from the out-patient services at the Clinic of Psychiatry of Tartu University Clinics and by newspaper advertisement in Tartu, Estonia. The control subjects were recruited by newspaper advertisement. The Human Studies Ethics Committee of the University of Tartu approved the study protocol and all study subjects gave written informed consent prior to participation. All subjects were right-handed, in good physical health, and female subjects were not pregnant. The participants were asked to abstain from alcohol and benzodiazepines for at least 2 weeks, which was verified by questioning and medical records. None of the subjects had current or lifetime alcohol dependence or abuse. None of the subjects had received any antidepressant or other medication known to affect the 5-HTT binding for at least 6 months before the study.

Assessment

The Mini International Neuropsychiatric Interview M. I. N. I. 5.0.0. (Sheehan et al. 1998) was used to confirm the DSM-IV diagnosis of GAD and exclude other psychiatric morbidity. According to the M. I. N. I. none of the patients had a lifetime diagnosis of panic disorder, major depression, bipolar disorder, psychotic disorder or a history of suicidal attempt. On the study day the patients were assessed for the symptoms of anxiety with the Hamilton Anxiety Scale (HAMA) ranging from 0 to 42, and for symptoms of depression with the Montgomery-Åsberg Depression Rating Scale (MADRS) ranging from 0 to 60. Prior to the first scan the patients rated their anxiety on a Visual Analogue Scale (VAS), ranging from 0–100 mm (most relaxed to most anxious).

Imaging procedure and data processing

The imaging procedures were carried out at the Department of Radiology of the Tartu University Clinics in Tartu, Estonia. Iodine-123 labeled 2 β-carbomethoxy-3β-(4-iodophenyl)nortropane or [^{123}I]nor-β-CIT was used in this study as a radioligand (Kuikka et al. 2001). Nor-β-CIT is an analogue of previously used β-CIT with a high affinity to both 5-HTT and dopamine transporter (DAT), and a higher affinity to 5-HTT in comparison to β-CIT, also with well characterized kinetics (Bergström et al. 1997; Hiltunen et al. 1998). A single dose of 185 MBq of [^{123}I]nor-β-CIT (supplied by MAP Medical Oy, Tikkakoski, Finland) was diluted in a volume of 10 ml physiological saline and slowly injected into the right antecubital vein in a dark and quiet imaging room. Whole head serial scans (5 min, 6 hours, and 24 hours after injection of tracer) were performed on a two-head General Electric 2000 gamma camera with high-resolution collimators. The radius of rotation was 14 cm. Scan was acquired for an angular step of 3° over 180°/camera head. Total scanning time was 30 min.

The SPECT scans were decay-corrected and reconstructed with Butterworth-filtered back projection in a 128x128 matrix with a pixel size of 3x3 mm, and were attenuation-corrected with a Chang's algorithm (Kuikka et al. 1993). The imaging resolution was 13–15 mm.

The SPECT slices were summarized to the total slice thickness of 6 mm and realigned along with the lines of Talairach coordinates using a semiautomatic brain quantification program of the HERMES software (Nuclear Diagnostics AB, Stockholm, Sweden). Regions of interest (ROI) were the midbrain, the thalamus, the temporal cortex, and the cerebellum as a reference region. The ROIs were manually positioned with the help of the laboratory's own 5-HTT based control template.

The specific binding of nor-β-CIT to 5-HTT in a given region was calculated using a reference region model. The main assumptions of this model are that the distribution volume of nonspecifically bound ligand is the same for both target and reference tissues, and that the delivery of tracer from arterial blood is the same in both regions (Acton et al. 1999). We applied a graphical method (Logan et al. 1990) to estimate the specific binding (= distribution volume ratio, V_D) from the slope of the time-activity curves (Fig. 1). The analyses were done blindly to the diagnostic status of the subjects.

To test the reproducibility of our ROI and analyzing method, the whole data sets of index subjects were re-analyzed within 3 months interval by the same analyzer. The intraclass correlation coefficient (ICC) was used. The ICC was 0.67 for the midbrain, 0.63 for the thalamus and less than 0.60 for the temporal lobes, respectively. The agreement between the two analyses was relatively good for the midbrain and the thalamus, and poor for the temporal lobes. We considered that the values of the temporal lobes could not be further used for comparison purposes between the groups.

Statistical analysis

The data were analyzed using software package STATISTICA 5.1 (StatSoft, Inc. 1997). The results are presented as mean values and standard deviations. One-way analysis of variance (ANOVA) was used to compare the binding data and clinical characteristics between the groups. The level of significance was set at $p < 0.05$ with a two-tailed test. Correlations were estimated with Pearson product-moment correlation analysis.

Results

The mean scores on the HAMA and MADRS for the patients were respectively 25.0 (5.5) and 12.3 (3.4). On the VAS the patients were somewhat more anxious immediately before the first scan than the control subjects, but this difference was not statistically significant (18.3 [16.4] vs. 6.7 [10.6]; $F_{1.12} = 2.46$ $p = 0.14$). The analysis of the first SPECT scan (5 min) demonstrated a significantly higher perfusion in the midbrain in patients than in healthy subjects (81.4 [10.2] vs. 66.7 [11.0]; $F_{1.12} = 6.76$ $p = 0.02$). In addition, the patients had significantly higher perfusion in the cerebellum (73.7 [7.9] vs. 58.3 [10.1]; $F_{1.12} = 10.05$ $p = 0.008$).

As demonstrated by the distribution volume ratios there were no significant differences in the [^{123}I]nor-β-CIT binding to 5-HTT in the midbrain or in the thalamus between the patients and controls (Table 1). The female patients had numerically lower 5-HTT binding in the midbrain raphe region in comparison to healthy female controls (0.63 [0.10] vs. 0.71 [0.10]; $F_{1.4} = 1.12$ $p = 0.35$), while the male patients had numerically higher 5-HTT binding in the midbrain raphe region than healthy males (0.73 [0.10] vs. 0.61 [0.10]; $F_{1.4} = 2.79$

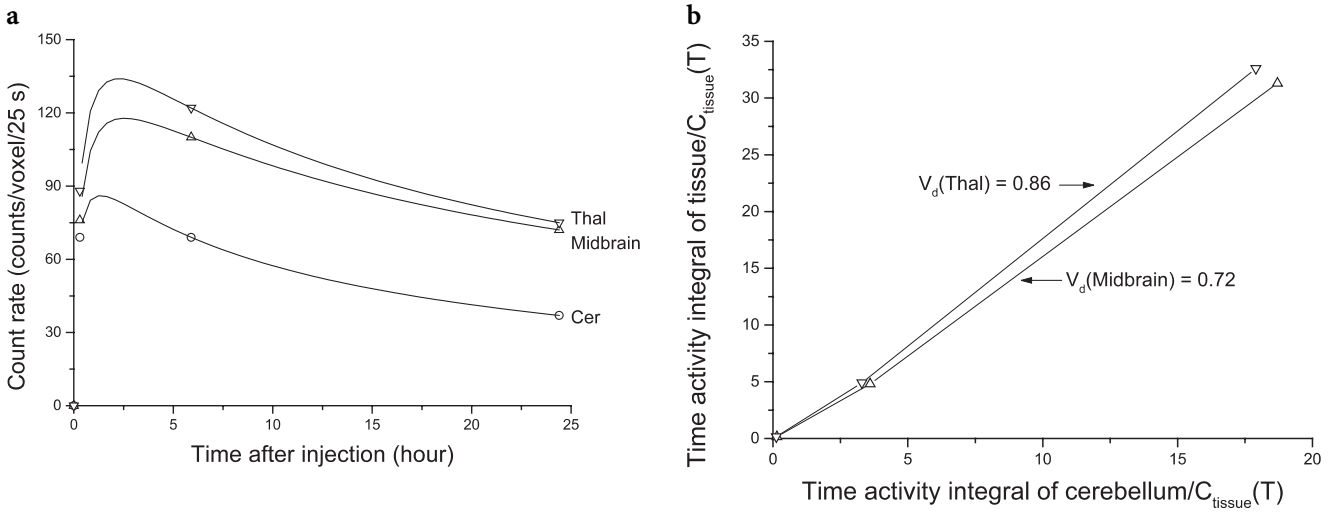

Fig. 1 **a** Time activity curves after injection of 185 MBq of [^{123}I]nor-β-CIT in a patient with GAD. The curves were smoothed with a log-normal function (*Thal* thalamus; *Cer* cerebellum). **b** A graphical reference tissue model was applied to the time activity curves in **a**. The 5-HTT specific binding is given by the distribution volume ratio (V_D) which is the slope of the plot – 1

Table 1 5-HTT binding characteristics assessed by [^{123}I]nor-β-CIT SPECT

Brain region	Patients (n = 7) Mean (SD)	Controls (n = 7) Mean (SD)	Patients vs. Controls ANOVA
Midbrain	0.68 (0.11)	0.65 (0.11)	F = 0.34, df = 1.12, p = 0.57
Thalamus	0.96 (0.12)	0.90 (0.22)	F = 0.32, df = 1.12, p = 0.58

p = 0.15). However these differences did not reach the level of statistical significance.

The correlation analysis in the patients group showed a significant and negative association between VAS score of anxiety immediately before the first scan and midbrain 5-HTT binding (r = –0.79, p = 0.035) (Fig. 2). This correlation was not present in the controls or in the total group or with thalamic 5-HTT binding. There was no correlation between HAMA scores and midbrain or thalamic 5-HTT binding characteristics in the patients.

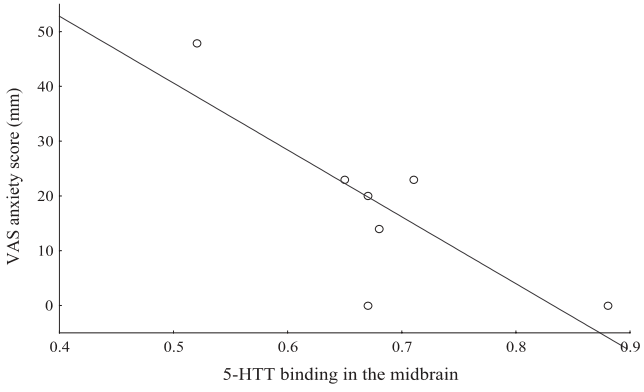

Fig. 2 Scatterplot of 5-HTT binding in the midbrain and VAS in the patients (n = 7)

Discussion

In the current study we did not find a difference in the binding properties of brain 5-HTT between the patients with GAD and healthy subjects. This may indicate that the functional activity of 5-HTT is intact in GAD. However, the study has several limitations to be considered when interpreting these results. The 5-HTT binding properties especially in the midbrain were unusually low in all subjects. This was likely a result of a relatively weak resolution of the SPECT camera used in the study. A small sample size should also be taken into account as a possible reason of negative findings. The power analysis showed that the study was only adequately powered (0.8) to detect a 33 % difference in 5-HTT binding in the thalamus, and a 28 % difference for the midbrain. This suggests that if there was a difference in 5-HTT binding between patients with GAD and healthy controls it was likely to be less than approximately 30 %.

There is some evidence that brain expression of 5-HTT may depend on genetic variability. A recent SPECT study showed that healthy subjects with short alleles of 5-HTTLPR had significantly lower 5-HTT availability in the midbrain as compared to healthy carriers of long alleles with no significant difference between the carriers of short or long alleles in alcoholic subjects (Heinz et al. 2000). We have genotyped the 5-HTTLPR in our sample and found an equal distribution of genotypes in the patients and control subjects (data not presented). Thus, we have excluded possible influence of the 5-HTTLPR polymorphism on our results.

In our previous study we found that the patients with current PD had significantly lower 5-HTT binding in the midbrain, in both temporal lobes as well as in the thalamus in comparison to the controls. The 5-HTT binding in patients with PD in remission was similar to the

healthy subjects in the midbrain and in the temporal lobes, but significantly lower in the thalamus (Maron et al. *in press*). Despite the similar design and identical radiotracer used in these two studies we can not be confident in comparing the results because of the use of different SPECT cameras and dissimilar gender distribution, as most of the subjects in PD study were females. Nevertheless, our data may imply a different functional role of 5-HTT in GAD than in PD. A number of pharmacological studies have also demonstrated different response patterns in patients with GAD and PD to 5-HT-ergic stimulation (Bell and Nutt 1998; Germine et al. 1992; Charney et al. 1987; den Boer and Westenberg 1990). According to the hypothesis of Deakin and Graeff (1991), the 5-HT system in anxiety disorders may have a dual role with an excess activity in GAD and deficit in PD or depression.

The clinical effects of 5-HT_{1A} agonists and 5-HT_2 antagonists suggest the possible involvement of these receptors' subtypes in GAD. There are few available radioligands that are suitable for studying the 5-HT receptors or 5-HT synthesis rate (Staley et al. 1998). The future brain imaging studies with radioligands that specifically bind to 5-HT receptor subtypes could be useful in further characterization of the functional role of the 5-HT system in GAD.

Similarly to our previous study in PD (Maron et al. *in press*) we found that state anxiety immediately before the start of imaging procedure may have influenced the end-results of the 5-HTT binding properties, at least in the midbrain. There was a significant and inverse correlation between VAS scores and midbrain 5-HTT binding in the patients, but not in controls or in the total sample. The patients were also somewhat more anxious before the first scan and had significantly higher perfusion in the studied brain regions than control subjects. The association between the midbrain 5-HTT binding and state anxiety in patients may indicate an increased sensitivity of the midbrain 5-HT system to anxious stimuli.

In conclusion, the results of this study failed to demonstrate a robust alteration in the functional activity of 5-HTT in patients with GAD. However, our study was lacking sufficient power to detect the possible smaller 5-HTT binding differences between the patients and controls. Further imaging studies in larger samples are needed for validation of the current results and more reliable conclusions.

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