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SHORT COMMUNICATION

Tryptophan depletion affects the autonomic stress response in generalized social anxiety disorder

J. Frederieke van Veen [a,*], Irene M. van Vliet [a], Roel H. de Rijk [b], Johannes van Pelt [c], Bart Mertens [d], Durk Fekkes [e], Frans G. Zitman [a]

[a] Department of Psychiatry, Leiden University Medical Center, Leiden, The Netherlands
[b] Division of Medical Pharmacology, LACDR/LUMC, Gorlaeus Laboratories, Leiden, The Netherlands
[c] Department of Clinical Chemistry, Leiden University Medical Center, Leiden, The Netherlands
[d] Department of Medical Statistics, Leiden University Medical Center, Leiden, The Netherlands
[e] Departments of Neuroscience and Psychiatry, Erasmus Medical Center, Rotterdam, The Netherlands

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Summary In generalized social anxiety disorder (gSAD), serotonergic dysfunctions are found, as well as abnormalities of the autonomic nervous system (ANS) in basal conditions and of the hypothalamic pituitary adrenal (HPA) axis in response to psychological challenges. These findings raise the question whether these phenomena are interrelated.

Therefore we designed a study in which two groups with nine pair wise age and gender matched gSAD patients (total of 10 men and 8 women), who were successfully treated with a selective serotonin reuptake inhibitor (SSRI), underwent a tryptophan depletion challenge (TD) or a placebo condition. A TD procedure temporarily decreases serotonergic neurotransmission. In order to activate the stress system the TD/placebo challenge was combined with a public speaking task. We assessed ANS responses, as measured with the promising new marker salivary alpha-amylase (sAA), and HPA-axis responses, as measured with salivary cortisol.

The most important result was that the TD group showed a significant larger sAA response to the public speaking task as compared to the placebo group, reflecting hyperresponsivity of the ANS in this group, whereas no differences were seen in cortisol responses. This suggests that in gSAD there is a vulnerability of the ANS more than the HPA-axis.

1. Introduction

Several biological systems behave differently in generalized social anxiety disorder (gSAD). With respect to the serotonergic system higher binding potentials for the serotonin transporter in the thalamus and reduced 5-HT1A receptors levels have been found (see among others Van der Wee et al., 2008). In addition, challenges with various serotonergic

* Corresponding author at: Department of Psychiatry B1-P, Leiden University Medical Center, P.O. Box 9600, 2300 RC Leiden, The Netherlands. Tel.: +31 71 5263785; fax: +31 71 5248156.
E-mail address: j.f.van_veen@lumc.nl (J.F. van Veen).

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agonists and tryptophan depletion induced exaggerated responses (see among others Van Veen et al., 2007). Besides, the favourable effects of selective serotonin reuptake inhibitors (SSRIs) in gSAD suggest serotonergic involvement (Blanco et al., 2003). A reciprocal interaction between the central serotonergic and noradrenergic systems has been proposed (Tassin, 2008), suggesting that SSRIs could also modulate autonomic function.

The autonomic nervous system (ANS) and the hypothalamic pituitary adrenal-axis (HPA-axis) behave also different in gSAD. In basal, non-stressed conditions, diurnal hyperactivation of the ANS was found, as measured with the promising new marker salivary alpha-amylase (sAA) (Van Veen et al., 2008). During psychological stress the increase in systolic blood pressure and heart rate was larger than in normal controls (see among others Gerlach et al., 2003). With respect to the HPA-axis in basal conditions no change was found (Van Veen et al., 2008), but psychological stress induced hyperfunction (see among others Condren et al., 2002).

Taken together, this research leads to the question whether the serotonergic system, ANS and HPA-axis are interrelated in gSAD, and, more specifically, whether manipulation of the serotonergic system with, for example, SSRIs leads to alterations in ANS and HPA-axis function.

In this paper we report about the effects of a tryptophan depletion (TD) challenge compared to placebo on ANS and HPA-axis responses to public speaking stress in SSRI-treated gSAD patients. Acute TD is a procedure that temporarily decreases serotonergic neurotransmission, decreasing the efficacy of SSRIs (Hood et al., 2005). The stress system was activated by means of a public speaking challenge. Anticipatory anxiety and learning were avoided by dividing the gSAD patients in two groups, instead of using a crossover design. The neuroendocrine parameters sAA, as a marker of the ANS, and cortisol, as a marker of the HPA-axis, were measured. Based on our findings in basal conditions (Van Veen et al., 2008) we expected to find the ANS to be more sensitive to stress than the HPA-axis.

2. Methods

2.1. Subjects

Eighteen patients with gSAD (10 men, 8 women), pair wise matched on age and gender, were randomly assigned to two conditions, TD and placebo. They were responders to 20 weeks of treatment with citalopram 20—60 mg a day. No life-time psychiatric comorbidity (confirmed with the MINI Plus 5.0.0) (see also Van Veen et al., 2008) or clinically significant medical disorders, such as endocrinological disorders, were allowed. Before the test day the Liebowitz social anxiety scale (see also Van Veen et al., 2008) and the Beck depression inventory (BDI) were used to measure symptom severity (Beck and Steer, 1987).

In case of heavy smoking, abuse of alcohol, or use of drugs of abuse subjects were excluded. Use of psychotropic medication (including beta-blocking agents) had to be stopped at least 14 days before the trial. Women were tested during the follicular phase of the menstrual cycle and women using oral contraceptives in the stop week. Perimenopausal women were excluded and postmenopausal women were included.

2.2. Procedures

The protocol was approved by the Medical Ethical Committee of the Leiden University Medical Center. The study was carried out in accordance with the Declaration of Helsinki. All procedures were conducted with the adequate understanding and written informed consent of the subjects.

At the test day an indwelling catheter was placed in an antecubital vein. At 1000 h the TD amino-acid mixture or placebo was ingested. At 1525 h patients received instructions for the public speaking task. At 1600 h the public speaking task started, and ended at 1610 h. The test day ended at 1700 h.

For the TD patients fasted overnight and were kept on a low protein diet during the test day until the following morning. The TD amino-acid drinks we used were the standard 100 mg drink (see Hood et al., 2005). During the test day, LNAA's, total TRP and 5-hydroxy-trypthophan (5-HTP) were assessed. The public speaking test was based on the principles of the Trier social stress test (Kirschbaum et al., 1993), but slightly modified. Patients were given 15 min to prepare a 10 min speech on a subject of choice. It was suggested (but not the case) that they were judged by an audience behind the one-way mirror-wall and that the whole session would be video-taped.

During the test day, plasma was obtained, behavioural measurements such as the visual analogue scale (VAS) anxiety and a short version of the profile of mood states (POMS) (Wald and Mellenbergh, 1990), and physiological assessments, such as heart rate and tension (Dinamap® Pro 100), were done at baseline (t0), after the TD (t1), after the preparation for the public speaking challenge (t2), and after the public speaking challenge (t3). Saliva for cortisol and sAA measurements was first collected after the TD and thereafter at 12 time points until 7 h after the public speaking challenge.

2.3. Neuroendocrine assessments

2.3.1. TRP, TRP/LNAA and 5-HTP

Plasma total TRP, the LNAAs phenylalanine, tyrosine, TRP, isoleucine, leucine and valine, and 5-HTP were assessed to evaluate the efficacy of the TD procedure. For the amino acids, quantitative amino-acid analysis was performed by high-performance liquid chromatography as described elsewhere (Fekkes et al., 1995). The ratio total TRP/LNAA was calculated as 100 times the concentration of TRP divided by the summed concentrations of the other LNAAs. For the 5-HTP assay, see Gijsman et al., 2002. The lower limits of detection and quantification were 0.5 and 1.7 ng/mL, respectively. The coefficients of variability for precision and reliability were 2.6% and 7.9%, respectively (Gijsman et al., 2002).

2.3.2. sAA and cortisol

The determination of sAA and cortisol was described in our previous study (Van Veen et al., 2008).

2.4. Statistics

Since the subjects were pair wise matched, the TD and placebo group were compared with paired-samples *t*-tests.