The embarrassed brain : towards a neurobiology of generalized socal anxiety disorder

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

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Abstract

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.

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-HT$_{1A}$ receptors levels have been found (see among others (Van Der Wee et al., 2008). In addition, challenges with various serotonergic agonists and tryptophan depletion induced exaggerated responses (see among others (Van Veen et al., 2007). Besides, the favorable 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.

Methods

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, postmenopausal women were included.

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

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, LNAAs, 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 minutes to prepare a 10 minute 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 hours after the public speaking challenge.

Neuroendocrine assessments

<u>TRP, TRP/LNAA and 5-HTP</u>

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).

sAA and cortisol

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

Statistics

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

1. For baseline characteristics, the groups were compared on age, severity of gSAD symptoms after treatment (LSAS score), depressive symptomatology (BDI score), smoking, the use of alcohol and dosage of citalopram.
2. The effects of the TD alone (after the TD and before the public speaking challenge) on sAA_{t1}, $cortisol_{t1}$, $VAS anxiety_{\Delta t1-t0}$, $POMS_{\Delta t1-t0}$, $heart rate_{\Delta t1-t0}$, and $systolic_{\Delta t1-t0}$ and $diastolic_{\Delta t1-t0}$ blood pressure were analyzed.
3. The neuroendocrine data in response to the public speaking test were normalized by dividing all sAA and cortisol values by the first value (t1), which was obtained before the public speaking challenge. The normalized data show the relative increase in sAA and cortisol in response to the public speaking challenge. We normalized the data because of the small sample sizes, large variations between subjects in individual sAA levels, and to take into account the possible influences of the TD/placebo condition on the neuroendocrine data before the start of the public speaking challenge.
4. The TD and placebo group were compared on relative sAA and cortisol levels after the public speaking challenge (t3). Also the $VAS anxiety_{\Delta t3-t1}$, $POMS_{\Delta t3-t1}$, $heart rate_{\Delta t3-t1}$, $systolic_{\Delta t3-t1}$ and $diastolic_{\Delta t3-t1}$ blood pressure at this time point were analyzed.

All analyses were done with SPSS 16.0.

Results

Subjects

The TD and placebo group showed no significant differences on age ($p=0.74$; mean of both groups together 39.1), symptom severity ($p=0.15$; mean LSAS score 28.6), use of alcohol ($p=0.67$; mean 4 units a week), smoking ($p=0.2$; mean 1.1 cigarette a day) and the dosage of citalopram ($p=0.35$; mean 42.4 mg a day). One patient stopped just after the explanation of the public speaking task, because of acute panic. The data that were already collected were included in the analyses. Unfortunately, it appeared that one patient used sertraline (dosage 100 mg a day) instead of citalopram. This patient

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was included in the analyses.

Tryptophan depletion
The TD procedure resulted in a reduction at t1 of total TRP of 90.6%, of the TRP/LNAA ratio of 96.6%, and of 5-HTP of 80.2%. The placebo drink resulted in an increase in total TRP of 84.3%, a decrease of the TRP/LNAA ratio of 46.3%, and an increase of 5-HTP of 12.6%.
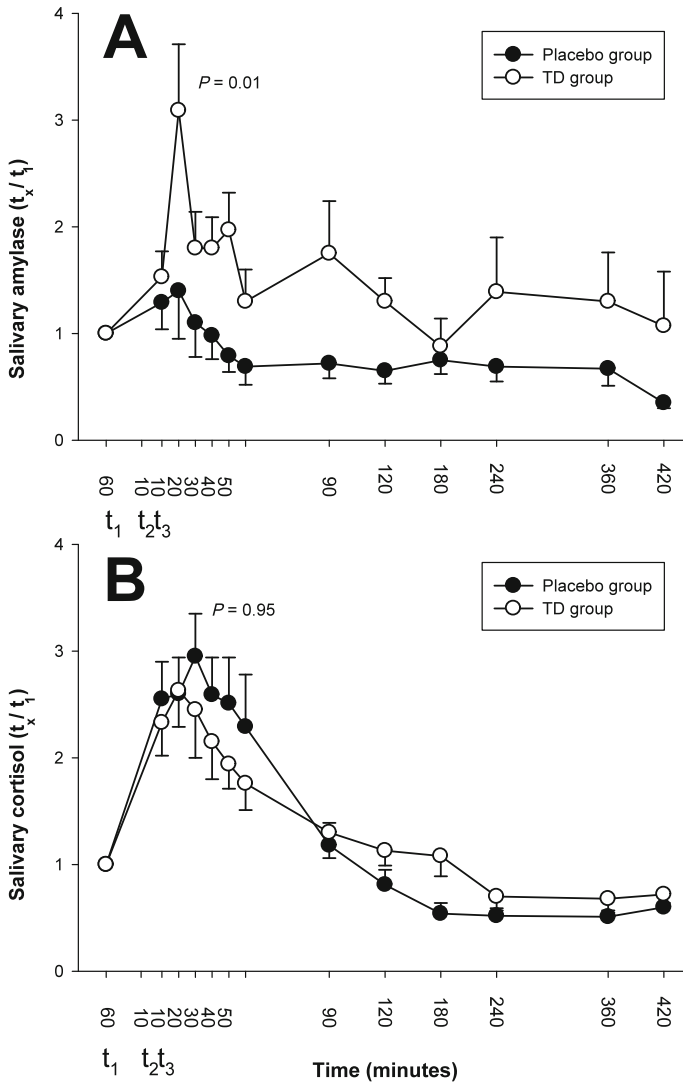
Figure 1. The relative (normalized data) sAA and cortisol responses (± S.E.M.) before and after the public speaking challenge. The p-value is the result of the comparison of the relative increases at t3 between the TD and placebo group by means of a paired-samples *t*-test: t1 is after the TD challenge and before the public speaking challenge; t2 is after the preparation phase and before the public speaking challenge; t3 is after the public speaking challenge.

Table 1 Relative increases of sAA and cortisol (t3) and Δ_{t3-t1} mood and anxiety scores in response to a TD directly after a public speaking challenge.

	t-Value	Mean difference (TD – placebo)	95% CI (lower-upper)	*p*-value
sAA$_{t3}$	-3.29	-1.69	-2.91 to -0.48	0.013*
cortisol$_{t3}$	-0.06	-0.03	-1.28 to 1.22	0.95
Δ_{t3-t1} **VAS-anxiety**	-0.78	-7.75	-31.35 to 15.85	0.46
Δ_{t3-t1} **POMS**	0.16	-0.63	-8.89 to 10.14	0.88
Δ_{t3-t1} **heart rate**	-0.77	-5.88	-24.03 to 12.28	0.47
Δ_{t3-t1} **systolic BP**	-0.54	-4.75	-25.43 to 15.93	0.60
Δ_{t3-t1} **diastolic BP**	-2.04	-6.00	-12.95 to 0.95	0.08

Paired-samples t-test analyses; for both groups n=8. * means that the p-value is statistically significant. sAA = salivary alpha-amylase; TD = tryptophan depletion; VAS-anxiety = anxiety levels measured with a visual analogue scale; POMS = total scores of the Profile of Mood States; BP = blood pressure; t1 = 1500h, just before the preparation phase; t3 = 1610h, just after the public speaking challenge.

Neuroendocrine assessments
The effects of the TD/placebo condition alone on sAA and cortisol (t1) revealed no statistical differences ($p = 0.124$, $p = 0.394$). However, significant increases in the relative sAA reponses to the public speaking task (t3) were seen in the TD group compared to placebo ($p=0.013$; bonferroni corrected *p*-value = 0.026). No significant differences were seen in relative cortisol increases ($p=0.95$). For details see Figure 1 and Table 1.

Behavioural and physiological assessments
No statistical significant differences were found in heart rate, blood pressure, and anxiety and mood assessments between the TD and the placebo condition.

Discussion

The most important finding of this study is that in successfully SSRI-treated gSAD patients sAA responses to a social stress test were significantly higher in the TD condition as compared to placebo. No differences were found in salivary cortisol, as well as anxiety, mood, blood pressure and heart rate responses. The TD condition without public speaking did not differ from the placebo condition in sAA, cortisol, anxiety, depressive symptoms, blood pressure and heart rate.

The higher sAA response to stress in the TD group was not accompanied by higher responses of the more traditional markers of autonomic functioning: heart rate and blood pressure. Although these contradictory results may be due to the small number of subjects, they are also compatible with a modest effect on heart rate and blood pressure. All in all, sAA might be a more sensitive and stable marker for autonomic functioning than heart rate and blood pressure, as it is less influenced by confounding factors like posture and exercise (Nater et al., 2007). The

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TD procedure may have decreased the effects of public speaking on sAA concentrations as sAA contains the amino-acid TRP which is depleted. Nevertheless, the TD group showed significantly larger sAA increases as compared to the placebo group.

We did not find an effect of TD on anxiety in response to the public speaking challenge. This finding is in agreement with Argyropoulos et al. (2004). However, Argyropoulos et al. did find effects of TD on anxiety using an autobiographical script. Such a script may be more specific for generalized social anxiety than public speaking is.

To the best of our knowledge, this is the first study to describe the effects of TD on sAA and also the first TD study in gSAD with the assessment of neuroendocrine parameters. The TD procedure induced a profound decrease of TRP and the TRP/LNAA ratio in the TD group. However, in the control group the TRP/LNAA ratio was also decreased with 46.3%, which may be related to the lack of effect seen on the behavioural measures. Strength of the study is that we only included patients with pure gSAD and without medical disorders to rule out the possibility of confounding effects of comorbid disorders on the neuroendocrine data.

In conclusion, the present study shows that in successfully SSRI-treated gSAD patients the stress of public speaking during TD induces higher sAA responses compared to the placebo condition, but has no effect on the HPA-axis. This suggests that in gSAD there is a vulnerability of the ANS more than the HPA-axis, which seems to be mediated by the serotonergic system.

Because of the small number of subjects this study needs replication in a larger group. Besides, future investigations should be directed at the specificity of these findings for gSAD, and of the TD effects in gSAD patients who were treated with cognitive behavioural therapy or noradrenaline reuptake inhibitors. Implications for the future are that, given the involvement of the ANS in gSAD, treatment studies should be directed at the ANS and HPA-axis rather than the HPA-axis alone.

Acknowledgements

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