Serotonin, cortisol, and stress-related psychopathology

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Chapter 8

Psychopathology in carcinoid patients is only affected by tryptophan levels in 5-HTTLPR s/s homozygotes

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ABSTRACT

The functional triallelic serotonin transporter length polymorphism (5-HTTLPR) has shown to modulate psychopathology during acute transient tryptophan depletion paradigms. We tested the hypothesis that 5-HTTLPR also affects the psychopathological effects of chronic low tryptophan levels. This is done in a unique sample of patients with long-lasting low tryptophan levels due to a metastatic serotonin producing carcinoid tumor. Plasma tryptophan levels, 5-HTTLPR, and the change in psychopathology since the carcinoid diagnosis (using the Symptom Checklist-90; Δ SCL-90) were measured in 41 patients. We developed a MANOVA model including main effects of 5-HTTLPR and plasma tryptophan on the SCL-90 as well as their interaction, while controlling for gender. Borderline significant effects of plasma tryptophan levels (s.e.s.=0.30; P=0.06) and 5-HTTLPR (s.e.s.=0.30; P=0.07) were observed. In addition, an interaction effect was observed on SCL-90 total scores (s.e.s.=0.35; P=0.03) and five of eight sub-scales, being interpersonal sensitivity-mistrust (P=0.003), agoraphobia (P=0.005), depression (P=0.018), cognitive-performance deficits (P=0.021), and somatization (P=0.047). Visualizing the data showed that tryptophan levels affected psychopathology only in subjects carrying two s alleles, suggesting that 5-HTTLPR s/s homozygotes are more vulnerable to lasting tryptophan depletion than s/l or l/l carriers. This effect was most pronounced in the sub-scales interpersonal sensitivity-mistrust and agoraphobia, but in the other sub-domains a similar pattern showed largely similar results, suggesting that low tryptophan is not directly causally related to specific psychiatric symptoms, but merely facilitates the development of psychopathology.

INTRODUCTION

Low cerebral availability of tryptophan leads to a cerebral shortage of serotonin (5-HT) (48) and is associated with psychopathology (116;140;317;328-330). For example, increased irritability has been reported in patients with long-term tryptophan depletion due to carcinoid disease, while an acute transient depletion of plasma tryptophan induced impulsivity and depressed mood ((63;67;328). Depressive symptoms did, however, not precipitate in healthy controls, but predominantly in subjects already at risk for depression, as for instance patients with a personal or family history of major depressive disorder (MDD).

The variability in responsiveness to the low tryptophan condition suggests that individual vulnerability is related to genes contributing to 5-HT functioning. In particular a polymorphism in the promoter region of the serotonin transporter (5-HTTLPR) has been explored in psychopathology (11;97;99;331). Originally, two variants of the 5-HTTLPR, a long (L, functional active) and a short (S, functional less active) version, were distinguished (332). Recently an A>G single nucleotide polymorphism (SNP) was identified within the L allele. The alleles are currently denoted as S, L_G and L_A (105). The S and L_G alleles reduce transcription of the serotonin transporter gene resulting in decreased expression and decreased 5-HT uptake compared with the L_A allel (105;333;334). Although a direct association between 5-HTTLPR and psychiatric disorders is as yet inconclusive (335-337), the 5-HTTLPR has shown to modulate the vulnerability to stress (11;97) and the psychological response during acute tryptophan depletion (ATD) protocols (62;66;338-340).

A unique population to study the association between long-term tryptophan depletion and psychopathology is found in patients with a metastatic carcinoid tumor. Carcinoid is a relatively rare cancer; according to the American Cancer Society, for example, the incidence of carcinoid tumors is about 5,000 cases per year in the United States. Carcinoid tumors are slowly growing neuro-endocrine malignancies, that can produce and secrete various factors, of which 5-HT is the most prominent one (271;273;274). 5-HT is synthesized from its precursor, the essential amino acid L-tryptophan. When metastasized, the production of 5-HT by the carcinoid tumor may increase so dramatically that a major fraction of whole body tryptophan is peripherally converted to 5-HT, leading to low plasma levels of tryptophan and reduced central tryptophan availability (134). 5-HT does not pass the blood brain barrier, hence the cerebral amine is synthesized from tryptophan exclusively in cerebral serotonin containing neurones.

In the present study we explored the role of tryptophan levels in the development of psychopathology in the presence of specific 5-HTTLPR polymorphisms in patients with a carcinoid tumor. We tested the hypothesis that patients with 5-HTTLPR s/s alleles are more sensitive to decreasing tryptophan levels than the other patients, expressed in more changes in psychopathology (66;99;338)

MATERIALS AND METHODS

Patients and procedure

Eligible for the study were patients who had been diagnosed with a metastatic carcinoid tumor and serotonin overproduction. The carcinoid syndrome was diagnosed by clinical features as well as biochemical measurements, such as urinary 5-hydroxyindolamine acid (5-HIAA) levels and platelet serotonin content (341;342). We included patients who visited the Department of Medical Oncology, University Medical Center Groningen (UMCG) between April 2007 and February 2008. The UMCG is a referral center for carcinoid patients. Most patients visit the center at least every six months. During a regular visit, blood samples were obtained and the patients were asked to fill in a self-report questionnaire at home. The study was approved by the Medical Ethics Committee of the UMCG. All patients gave written informed consent.

Psychiatric assessment

Psychopathology was assessed with the Dutch version of the multidimensional Symptom Checklist-90 (SCL-90). The SCL-90 is a well validated, multidimensional self-report symptom inventory, consisting of 90 items and designed to assess various domains of psychopathology, including agoraphobia, anxiety, depression, somatization, cognitive-performance deficits, interpersonal sensitivity-mistrust, acting-out hostility and sleep difficulties (319;320;343). Since we were particularly interested in the prospective effects of tryptophan depletion on the development of psychopathological symptoms, we asked the patients to fill out the SCL-90 twice: once describing the current situation and once how symptoms had changed since the onset of their disease. Elsewhere we have shown that specifically changes in symptoms were sensitive to tryptophan levels (chapter 7).

Biochemical measurements

Blood samples were obtained during routine lab investigations during regular morning visits to the outpatient clinic, as described elsewhere (279). Plasma tryptophan concentrations were measured by means of High Performance Liquid Chromatography (HPLC) with fluorometric detection (166). Tryptophan reference values range from 40-70 μmol/l (166). Blood for 5-HTTLPR genotyping was collected in a 10 ml EDTA tube and centrifuged at 800g for 10 minutes at 4 °C. The buffy coat was collected and stored at -20 °C until DNA isolation. 5-HTTLPR genotypes were determined using the HTTp2a and HTTp2B primer set to amplify 406 (S) and 450 (L) bp fragments with the polymerase chain reaction (PCR) (105). The L_A, L_G and S alleles were determined by incubation of the PCR product with the restriction enzyme Msp I (New England Biolabs, Westburg, Leusden, The Netherlands) for at least 3 hours at 37 °C. Msp I cuts the GGCC sequence, resulting in fragments of 329, 62, and 59 (L_A), 174, 155, 62 and 59 bp (L_G), and 285, 62 and 59 bp (S) respectively. The resulting restriction fragments were separated using a 2% agarose gel and

visualized using GelStar (SYBR-green; Cambrex Bio Science, Rockland, ME). The triallelic classification was then reclassified into a biallelic model, based on the levels of transporter expression as follows: L_G/S, L_G/L_G, and S/S subjects were classified as s/s; L_A/S and L_A/L_G subject were classified as s/l and L_A/L_A subjects were classified as l/l (62;66;105).

Statistical analysis

All statistical calculations were conducted using SPSS (version 14) and $P \leq 0.05$ was considered significant. Correlations between current tryptophan levels and tryptophan levels one year ago (Δ tryptophan) were calculated with a Pearson Correlation coefficient. The differences between genotype groups on tryptophan, 5-HT and 5-HIAA levels were analyzed using a one-way analysis of variance (ANOVA). Difference in SCL-90 scores between males and females weas tested using an independent samples t-test.Thereafter, we evaluated the univariate effects of the 5-HTTLPR polymorphism and plasma tryptophan on the Δ SCL-90 score using a two-way analysis of variance (ANOVA). Then, we developed a model including both main effects of the 5-HTTLPR polymorphism and plasma tryptophan and their interaction, while controlling for gender. The primary aim was to test whether significant main effects of plasma tryptophan and 5-HTTLPR and a significant interaction effect between the 5-HTTLPR polymorphism and plasma tryptophan concentration was present resulting in changes in psychopathological symptoms assessed with the SCL-90. As the SCL-90 includes eight different sub-domains, we determined whether the interaction effect was stronger on any of these specific sub-domains.

Assuming allele frequencies of 50% L_A, 10% L_G and 40% S (99;105;344), and an effect of tryptophan and 5-HTTLPR with an medium effect size of 0.4, a sample size of 40 patients would result in 80% power to detect an interaction effect of tryptophan and 5-HTTLPR with the same magnitude (0.4).

In addition, we performed two sensitivity analyses: 1) excluding subjects using antidepressant medication, a history of major depression or using benzodiazepines, and 2) excluding patients for whom the diagnosis of a carcinoid tumor was made >10 years ago.

RESULTS

Of 48 included patients, six patients did not fill in the questionnaires, and in one subject no blood sample could be obtained. Patient characteristics of the resulting 41 patients are presented in table 1. In addition, two patients were known with a psychiatric history of alcohol abuse or major depressive disorder (MDD). Thirty-two patients were treated with the somatostatin analogous octreotide or lanreotide and six patients received interferon-alpha. In addition, four patients used benzodiazepines and two received tricyclic antidepressants (TCAs).

Table 1: Patient characteristics

5-HTTLPR	**s / s**			**s / l**			**l / l**		
N (male/female)	10 (5/5)			19 (10/9)			12 (2/10)		
	mean (SD)			*mean (SD)*			*mean (SD)*		
Age (years)	65 (6)			62 (6)			60 (6)		
Time since diagnosis (years)	4 (3)			7 (5)			6 (4)		
Current plasma tryptophan (µmol/l)	51 (11)			46 (16)			47 (12)		
Δ tryptophan last year (µmol/l)	2 (8)			1 (14)			-2 (13)		
5-HT (nmol/10^9 platelet)	18 (9)			22 (11)			17 (12)		
Urinary 5-HT (µmol/mol kreat)	127 (80)			201 (255)			173 (261)		
Urinary 5-HIAA(mmol/mol kreat)	25 (10)			27 (10)			17 (4)		
SCL-90 score (points)	*median*	*min*	*max*	*median*	*min*	*max*	*median*	*min*	*max*
agoraphobia	**8**	7	23	**8**	7	17	**7**	7	18
anxiety	**18**	10	29	**13**	10	25	**15**	10	18
depression	**25**	16	49	**24**	16	49	**23**	18	40
somatization	**23**	12	43	**17**	12	36	**18**	13	30
cognitive performance deficits	**17**	10	32	**16**	9	33	**16**	9	26
interpersonal sensitivity	**22**	18	41	**23**	18	40	**21**	18	38
acting out hostility	**8**	6	16	**7**	6	10	**7**	6	12
sleep difficulties	**5**	3	13	**5**	3	10	**5**	3	12
Total Score	**146**	97	254	**134**	96	218	**123**	98	180
Δ SCL-90 score (points)	*median*	*min*	*max*	*median*	*min*	*max*	*median*	*min*	*max*
agoraphobia	**0**	-12	8	**0**	-10	6	**0**	-10	5
anxiety	**1**	-12	10	**2**	-10	9	**1**	-16	7
depression	**3**	-15	19	**1**	-14	15	**0**	-19	8
somatization	**5**	-8	13	**2**	-7	11	**1**	-16	7
cognitive performance deficits	**3**	-1	13	**2**	-5	11	**0**	-3	7
interpersonal sensitivity	**1**	-27	12	**1**	-19	7	**0**	-27	5
acting out hostility	**1**	-10	5	**0**	-8	3	**0**	-10	1
sleep difficulties	**2**	-6	6	**0**	-4	3	**0**	-3	3
Total Score	**14**	-97	85	**15**	-82	57	**3**	-112	41

Tryptophan reference values range from 40-70 µmol/l; Δ tryptophan reflects current plasma tryptophan levels minus plasma tryptophan levels one year earlier. The cut-off value for platelet 5-HT is 5.4 nmol/10^9 platelet. Reference values for Urinary 5-HIAA range from 0.8 – 3.8 mmol/mol kreat. Average SCL-90 total scores in the normal population are 108-115 points (men) and 117-129 points (women).

SCL-90 scores

The average score on the SCL-90 was somewhat higher for females (146, SD 40) than for males (129, SD 33), although this was not significant (t -1.469, P = 0.15, df 40). These scores are between the 65th and 80th percentile compared to the normal population, but lower than the average scores of psychiatric patients in outpatient clinics (manual SCL-90, Dutch (320)). SCL-90 scores since the onset of the disease (Δ SCL-90 scores) worsened on average 5 points (95% CI: -6 to 17 points) (table 1). For 30 patients (73%), the total number of symptoms increased during their disease, while for 11 patients (27%) it did not increase or even decrease. The mean increase in SCL-90 score for those reporting an increase in symptoms was 15 (95% CI: 12-27). When excluding somatic symptoms, a highly similar pattern emerges suggesting that the increase in symptoms is not due to somatic symptoms per se; a mean increase of 4 points (95% CI: -6 to 13) with 29 patients (71%) reporting an increase.

5-HTTLPR, plasma tryptophan & 5-HT metabolism

Descriptives are displayed in table 1. 5-HTTLPR was in Hardy-Weinburg equilibrium (Chi squared 0.10; P=0.75). In most patients (N=25) were plasma tryptophan levels were in the reference range (40-70 μmol/l). Plasma tryptophan levels were below the reference values in 14 patients and in two patients the plasma tryptophan levels were higher than 70 μmol/l. Plasma tryptophan levels were fairly stable across time. Current tryptophan levels highly correlated with tryptophan levels of one year earlier (Δ tryptophan; Pearson Correlation 0.525; P=0.001). On average, current tryptophan levels were only 0.5 μmol/l lower than one year before (SD 12 μmol/l), this difference was not statistically significant (t -0.262, df 36, P=0.75). There was no significant effect of 5-HTTLPR on tryptophan ($F_{2,39}$=0.731, P=0.731), 5-HT ($F_{2,39}$=0,571, P=0.570) or 5-HIAA levels ($F_{2,39}$=1.398, P=0.260).

Main effects of 5-HTTLPR & plasma tryptophan on Δ SCL-90

Using two-way analysis of variance (ANOVA), neither 5-HTTLPR (P=0.11), nor plasma tryptophan levels (P=0.77) had significant main effects on Δ SCL-90 scores, although the effect size of 5-HTTLPR was moderate (Table 2)(322).

Interaction effect of tryptophan and 5-HTTLPR on Δ SCL-90

Applying the model including 5-HTTLPR, plasma tryptophan, interaction between tryptophan and 5-HTTLPR, while controlling for gender, uncovered the following effects (table 2): borderline significant main effects of both TRP and 5-HTTLPR, a significant gender effect, and a significant interaction effect of 5-HTTLPR and plasma tryptophan. Using a conservative estimate, this model as a whole explained more than 24% of the variance of Δ SCL-90 (R^2 = 0.318; Adjusted R^2= 0.242). The effect size of the interaction effect of 0.35 indicates a medium effect (322), which is rather close to what we anticipated; the post hoc study power for detecting this effect was 76%.

Table 2: Interaction model

	Non-adjusted model			Adjusted model		
	F(1,41)	P	Effect size	F(1,41)	P	Effect size
TRP	0.088	0.768	0.04	3.644	0.064	0.30
5-HTTLPR	2.723	0.107	0.26	3.577	0.067	0.30
TRP*5-HTTLPR	-	-	-	5.202	0.029	0.35
Sex	-	-	-	5.259	0.028	0.36

Effect of serotonin transporter length polymorphism (5-HTTLPR) and plasma tryptophan levels (TRP) on change in total SCL-90 scores. Adjusted model: R Squared = 0.318 (Adjusted R Squared =0 .242)

In Figure 1, the interaction between 5-HTTLPR and plasma tryptophan on Δ SCL-90 is visualised, using tertile scores of plasma tryptophan, confirming the observation that tryptophan levels only affect Δ SCL-90 in the presence of the s/s allele of the 5-HTTLPR polymorphism.

Applying the same model to the eight Δ SCL-90 sub-scales separately, resulted in largely comparable findings with significant interaction terms for interpersonal sensitivity-mistrust ($F_{1,41}$=10.497, P=0.003), agoraphobia ($F_{1,41}$=8.831, P=0.005), depression ($F_{1,41}$=6.136, P=0.018), cognitive-performance deficits ($F_{1,41}$=5.766, P=0.021), and somatization ($F_{1,41}$=4.225, P=0.047), but not for anxiety ($F_{1,41}$=2.597, P=0.116), hostility ($F_{1,41}$=2.656, P=0.112), sleep difficulties ($F_{1,41}$=0.160, P=0.692), although even in these sub-domains a similar pattern is observed, in which tryptophan levels are only associated with Δ SCL-90 in the presence of the s/s allele of the 5-HTTLPR polymorphism.

Sensitivity analyses

When excluding patients with a psychiatric history and/or using TCAs or benzodiazepines (N = 6), we still found in the remaining 35 patients a significant interaction of plasma tryptophan and 5-HTTLPR on overall Δ SCL-90 scores ($F_{1,35}$=4.212 en P=0.049, effect size: 0.36). Similarly, excluding patients for whom the diagnosis of a carcinoid tumor was made >10 years ago (N=4), did not alter our findings as the interaction term remained significant and highly comparable in magnitude: ($F_{1,37}$=4.157, P=0.05, effect size 0.34).

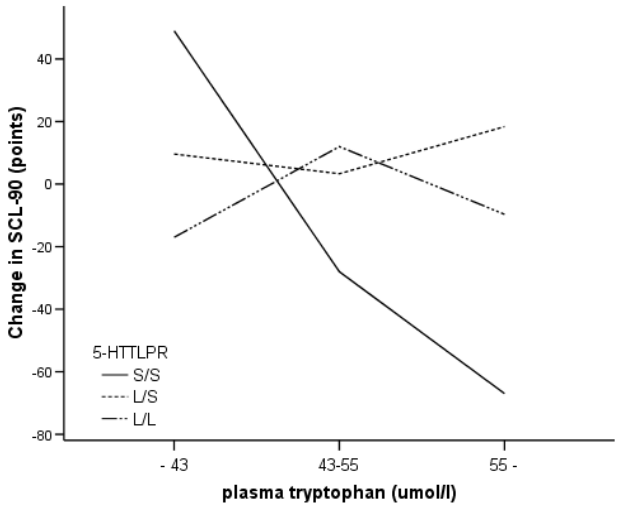

Figure 1: A visualization of the interaction between triallelic serotonin transporter length polymorphism (5-HTTLPR) and plasma tryptophan levels on Δ SCL-90 total score (P=0.03), using tertile scores of tryptophan confirms that tryptophan levels only affect SCL-90 in the presence of the s/s allele of the 5-HTTLPR polymorphism. Applying the same model to the SCL-90 sub-scales separately resulted in highly significant interaction terms for interpersonal sensitivity-mistrust and agoraphobia, and weaker, but still significant interaction terms in the sub-domains of depression, cognitive-performance deficits, and somatisation.

DISCUSSION

This preliminary study confirms and extends previous observations that chronic low plasma tryptophan levels and 5-HTTLPR may precipitate psychopathology (115;116). Overall, the patients in the present study scored above average on the SCL-90. Tryptophan levels were only associated with changes in the SCL-90 scores (Δ SCL-90) in the presence of the s/s allele of the 5-HTTLPR polymorphism. Trends towards significance were observed in all sub-domains largely replicating the same pattern, but most pronounced in the SCL-90 subscales Δ interpersonal sensitivity-mistrust and Δ agoraphobia. These results suggest 1) that low tryptophan is not directly causally related to specific psychiatric symptoms, but may merely facilitate the development of psychopathological symptoms. This is consistent with previous studies, in which chronic low tryptophan is associated with a wide variety of symptoms (115;116;133;140;317;330). 2) This development is facilitated specifically in subjects with an s/s genotype. The highly significant effects are particularly convincing considering the relatively small number of s/s homozygotes.

The increased sensitivity of s/s homozygotes to tryptophan levels might be explained by the effects of 5-HTTLPR on 5-HT synthesis. The rate of synthesis of cerebral 5-HT depends on tryptophan availability (47-49), re-uptake of 5-HT through the 5-HTT (chapter 4), and auto-

inhibition via 5-HT_{1A} and 5-HT_{1B} receptors (51). In the case of s/s homozygotes, reuptake of 5-HT is decreased (105), possibly leading to increased extracellular 5-HT, which results in increased auto-receptor mediated inhibition of 5-HT synthesis. Accordingly, 5-HT synthesis is relatively more dependent on tryptophan availability. Consequently, at low tryptophan levels, the intracellular stores of serotonin may become depleted to some extent in the s/s homozygotes and this could also impair the neurotransmission of serotonin. Conversely, in times when tryptophan is abundant, s/s homozygotes may have an additional advantage, because of higher extracellular serotonin levels that synergisytically support the efficacy of 5-HT neurotransmission.

The carcinoid patients were selected based on elevated serotonin production. In these patients, the tumor produces high levels of 5-HT draining on the tryptophan pool going far beyond the normal variability of plasma tryptophan levels (134;166;284). Using this sample of patients, we are the first to evaluate the effect of the 5-HTTLPR in combination with long-lasting tryptophan depletion. The results of the present study help to clarify previous results. For example, during ATD studies in individuals with a history of MDD, most deterioration in mood is observed in l/l homozygotes. The effect of ATD in individuals without a history of MDD is smaller and more pronounced changes in anxiety, impulsivity and motivation rather than mood are observed which are associated with an s/s genotype (for review: (345)). These results fit well in the hypothesis suggesting a general behavior modulating function of the brain serotonin system (55). Also in our previous studies in carcinoid patients and in animal experiments, decreased tryptophan availability resulted in a higher sensitivity to stress observed in both physiology and behavior (chapter 2,chapter 6).

A relatively unexpected observation was that s/s homozygotes with relatively normal plasma tryptophan levels tended to report less increase in psychopathology than s/l or l/l subjects. Most studies only focus on the negative influences of the environment on the development of psychopathology. Interestingly, two studies that included positive environmental factors suggest that individuals with the 5-HTTLP s/s phenotype also have more benefits from positive events and social support (346;347). This suggests that the s/s genotype is not just a "vulnerability" gene, but renders its carriers more sensitive and reactive to life circumstances in general (345). This would also explain the lack of a direct association between 5-HTTLPR polymorphism and psychopathology in an unselected population (337).

The study has a few limitations. Because carcinoid tumors are rare, the study sample was relatively small. However, according to our a priori power calculations, a sample of 41 patients had sufficient power to detect the predicted interaction effect. Moreover, in a post-hoc power analysis using the actual prevalence and effect size found in our sample, we found that in this specific sample we had 76% power to detect this interaction effect. In other words, the relatively small sample size appears to be balanced with the relatively large interaction effect that we anticipated. Still, a consequence of the relatively small sample was that we were not able to distinguish between males and females, while in previous studies is

shown that males and females may react different to tryptophan depletion (66).

Second, unlike studies exploring the effects of predictable tryptophan depletion for example during interferon-treatment (116;317), we had to rely on retrospective assessments of subjects on changes in psychopathological symptoms. This may have been biased by memory limitations. Although this may have resulted in more error variance, we do not expect this to have had a major impact on the estimation of the interaction effect per se. Also, blood for tryptophan measurements was sampled under non-fasting conditions, which may result in larger variability. Still, such possible variability did not hinder to observe some highly significant relationships. Moreover, dietary intake does not influence plasma levels of tryptophan in the extent that is seen in carcinoid patients (47;283).

In conclusion, the present study suggests that patients with an s/s alleles are more sensitive to plasma tryptophan levels than patients with either s/l or l/l alleles. This effect was not specifically related to either depression or any other SCL-90 subscale. These results are found in a relatively small sample, so the study should be replicated in a larger, preferably prospective, sample.

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