Brazilian Journal of Medical and Biological Research (2000) 33: 581-587
ISSN 0100-879X

# Effects of tryptophan depletion on anxiety induced by simulated public speaking

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## Abstract

Several lines of evidence point to the participation of serotonin (5HT) in anxiety. Its specific role, however, remains obscure. The objective of the present study was to evaluate the effect of reducing 5HT-neurotransmission through an acute tryptophan depletion on anxiety induced by a simulated public speaking (SPS) test. Two groups of 14-15 subjects were submitted to a 24-h diet with a low or normal content of tryptophan and received an amino acid mixture without (TRY-) or with (TRY+) tryptophan under double-blind conditions. Five hours later they were submitted to the SPS test. The state-trait anxiety inventory (STAI) and the visual analogue mood scale (VAMS) were used to measure subjective anxiety. Both scales showed that SPS induced a significant increase in anxiety. Although no overall difference between groups was found, there was a trend (P = 0.078) to an interaction of group x gender x phases of the SPS, and a separate analysis of each gender showed an increase in anxiety measured by the STAI in females of the TRY- group. The results for the female TRY- group also suggested a greater arousing effect of the SPS test. In conclusion, the tryptophan depletion procedure employed in the present study did not induce a significant general change in subjective anxiety, but tended to induce anxiety in females. This suggests a greater sensitivity of the 5HT system to the effects of the procedure in this gender.

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Research supported by FAPESP and by a joint CAPES-British Council collaborative program. F.S. Guimarães and A.W. Zuardi are recipients of CNPq fellowships.

Received August 20, 1999
Accepted February 14, 2000

**Key words**
- Serotonin
- Anxiety
- Tryptophan depletion
- Public speaking
- Healthy volunteers
- Gender differences

## Introduction

Several lines of evidence point to the participation of serotonin (5HT) in anxiety. Its specific role, however, remains obscure (1,2). Recently, it has been suggested that although the general role of 5HT is to modulate responses to aversive stimuli, different 5HT subsystems have distinct roles. 5HT fibers originating in the dorsal raphe nucleus (DRN) and projecting to 5HT2/5HT3 receptors in the amygdala and frontal cortex would modulate responses to distal danger, facilitating avoidance responses and fear conditioning. On the other hand, activation of 5HT2 or 5HT1A receptors in the dorsal central gray (DCG), a region related to flight/fight reactions to proximal, unconditioned

danger in animals, would be anxiolytic. This dual effect of 5HT might help to explain much of the controversy in the literature (1). At the clinical level these two systems have been related to generalized anxiety and panic disorder, respectively (1,2). Some clinical findings favor this interpretation. For example, ritanserin, a 5HT2 antagonist, is anxiolytic in generalized anxiety but anxiogenic in panic (2-4).

We have recently initiated studies on healthy volunteers to test this theory using two different clinical models: aversive fear conditioning to sounds (AFC) and simulated public speaking (SPS). We hypothesized that the first one would be related to the DRN-amygdala/frontal cortex 5HT system whereas the SPS would be related to the DRN-DCG system. The latter involves an almost universal, probably unconditioned fear, and raises anxiety feelings in healthy subjects independently of their trait anxiety (5). We showed that ritanserin, similar to that found in generalized anxiety and panic disorder patients, has opposite effects in the two models (6), being anxiolytic in the AFC model and anxiogenic in the SPS test.

As a continuation of these studies we have recently completed a study on healthy volunteers with d-fenfluramine, a 5HT neuronal releaser and reuptake blocker. As predicted by the theory (1), the drug was anxiolytic in the SPS model, but tended to be anxiogenic in the AFC model (7). Dietary changes were able to decrease up to 87 and 91% of the plasma concentration of total and free tryptophan (TRY), respectively (8). This amino acid is the precursor of 5HT, and the neurotransmitter synthesis seems to depend on tryptophan availability. This procedure reversed the therapeutic effects of antidepressants in symptom-free depressive patients (8). These results, besides implicating 5HT in the therapeutic effects of antidepressant drugs, also indicate that this is an interesting method for manipulating 5HT neurotransmission in humans.

The objective of the present study was to use this method to investigate the role of 5HT in anxiety induced by SPS in healthy volunteers.

## Subjects and Methods

### Subjects

Thirty subjects (15 males and 15 females), aged 18 to 35 years, participated in the study as paid volunteers after being submitted to a clinical interview and physical and laboratory examination. They belonged to the Hospital staff and were recruited by advertisement. Exclusion criteria were psychiatric or somatic disease, pregnancy, and current use of medication. Informed consent was obtained from all volunteers and the local Ethical Committee approved the study.

### Dietary procedures

The volunteers were submitted to a previous one-day low TRY (165.11 mg/day) diet that provided an adequate amount of energy (1.363 kcal/day; proteins: 5.3%, lipids: 24.6%, carbohydrates: 70%), supplemented with gelatin capsules containing TRY in the control group and placebo in the TRY depletion group. The diet was planned and administered by the Nutritional Service of the University Hospital of Ribeirão Preto.

On the day of the experiment the subjects drank an amino acid solution (300 ml) with (control group) or without (experimental group) TRY. The solution contained L-alanine (5.5 g), L-arginine (4.9 g), L-cysteine (2.7 g), glycine (3.2 g), L-histidine (3.2 g), L-isoleucine (8.0 g), L-leucine (13.5 g), L-lysine (11.0 g), L-methionine (3.0 g), L-phenylalanine (5.7 g), L-proline (12.2 g), L-serine (6.9 g), L-threonine (6.9 g), L-tyrosine (6.9 g), L-valine (8.9 g), and L-tryptophan (2.3 g). Due to their aversive taste, methionine, cysteine and arginine were administered in gelatin capsules.

### Measurements

Subjective states were evaluated by the following self-rating scales: 1) visual analogue mood scale (VAMS) (9) which consists of 16 analogue items composed of two adjectives with opposite feelings, separated by a 10 cm line on which the subject has to mark the point which best describes his feelings at the time. These items were combined into four factors (anxiety: items - calm-excited, relaxed-tense, tranquil-troubled; physical sedation: items - quick-witted, mentally slow, proficient-incompetent, energetic-lethargic, clear-headed-muzzy, gregarious-withdrawn, well-coordinated-clumsy, strong-feeble; mental sedation: items - alert-drowsy, attentive-dreamy; other feelings and attitudes: items - interested-bored, amicable-antagonistic, happy-sad, contented-discontented) according to a factorial analysis performed on a Brazilian sample (10). 2) State-trait anxiety inventory (STAI) (11), which provides operational measures of intensity of anxiety at a particular moment (STAI-S) and of anxiety as a relatively stable personality trait (STAI-T). Each scale contains 20 items with 4 points. 3) Bodily symptom scale (BSS), a 5-point scale (from "0" indicating no symptoms to "4" indicating extremely marked symptoms) designed to evaluate somatic symptoms (10). Physiological measurements included heart rate and blood pressure.

### Procedure

On the day before the experimental session the subjects came to the laboratory at 7.30 a.m. to receive the diet they would eat during the next 24 h and to perform pretreatment measurements. On the next day they came back at 7.30 a.m. to perform baseline measurements (B) and received the amino acid solutions plus the gelatin capsules with (control) or without tryptophan (experimental group) under double-blind conditions. They were then allowed to leave the laboratory with the instructions not to eat/drink any food but water over the next 5 h. After 5 h they returned to the laboratory for pretest (P) measurements at 13.05 p.m., followed by the instructions about the test, prerecorded by one of us (FSG) on videotape. Subjects were told that they would have 2 min to prepare a 4-min speech about "most anxiety-provoking episodes in their lives", that would be recorded by the video camera and analyzed later. Anticipatory anxiety measurements (A) were made before the subject started speaking in front of the camera while viewing his/her own image on the video screen. The speech was interrupted in the middle so that subjective performance anxiety measurement (S) could be made. The final measurement (F) was made immediately after the end of the speech. At the end of the session a blood sample was collected to measure total plasma tryptophan concentration.

### Statistical analysis

Data from the four factors of the VAMS, together with those from the STAI, heart rate and blood pressure, were analyzed by MANCOVA using the pretreatment measurement as covariant. The analyzed factors were group, gender and phases. The pretreatment measurements of these variables were compared by MANOVA with group and gender as factors. Data from the BSS were analyzed by the Kruskal-Wallis test. The analysis was performed using the SPSS/PC+ statistical package (version 3.1).

## Results

One female subject from the tryptophan depletion group did not follow the prescribed diet and was withdrawn from the study. The groups were comparable in terms of age, gender and STAI-T baseline score (Table 1). At the end of the experiment total plasma tryptophan levels were significantly different between groups ($F = 56.4$; $P<0.001$). No

significant gender effect or interaction was found in this measure.

**Self-rating scales**

*STAI-S*. The SPS test induced a significant increase in anxiety (phase factor, F4,22 = 4.13; P = 0.012). No main group or gender effect was found. However, there was a trend for a group x gender x phase interaction (F4,22 = 2.48; P = 0.074). A separate analysis in each gender showed a significant difference between groups (F1,9 = 10.39; P = 0.008) in females, and analysis of each phase showed that the tryptophan-depleted group presented a higher anxiety state during baseline (B: F1,9 = 7.35; P = 0.02) and speech anxiety (S: F1,9 = 8.93; P = 0.012; Figure 1). No significant difference was found in males.

*VAMS*. Anxiety factor: The SPS test induced a significant increase in anxiety in both groups (phase factor, F4,22 = 7.28; P<0.001; Figure 2). No main group or gender effect was found, but there was also a trend to group x gender x phase interaction (F4,22 = 2.48; P = 0.073). However, a separate analysis in each gender did not detect any difference between groups.

*Mental sedation factor*. There was a significant decrease in mental sedation during the test (phase factor, F4,22 = 3.39; P = 0.027). No main group or gender effect was found, but there was a significant interaction between phases and gender (F4,22 = 5.67; P = 0.003). An analysis of each gender showed an almost significant group x phase interaction (F4,9 = 3.24; P = 0.06) due to an increased arousing effect of tryptophan depletion in females, acceptable as a trend (Figure 3).

*Physical sedation*. There was a significant increase in physical sedation during the experiment (phase factor, F4,22 = 3.8; P = 0.017). Tryptophan depletion decreased physical sedation throughout the session (group factor, F1,24 = 4.99; P = 0.035; Figure 4).

*Other feelings and attitudes*. The tryptophan depletion procedure tended to decrease the scores of this item (group factor, F1,24 = 3.24; P = 0.08; Figure 5). No gender or phase effects were found.

*BSS*. The only significant effects were 1) phase B: greater palpitation in the control group (P = 0.02), and 2) phases A and S: greater urinary urgency in the tryptophan-

Table 1 - Characteristics of the sample.

Data are reported as means ± SD.

| | Group | |
|---|---|---|
| | TRY+ | TRY- |
| Gender | 8 males, 7 females | 7 males, 7 females |
| Age (years) | 27.9 ± 1.3 | 28.1 ± 0.9 |
| STAI-trait | 39.0 ± 1.4 | 39.0 ± 1.7 |
| Plasma total tryptophan levels (mg/dl) | 2.1 ± 0.8 | 0.5 ± 0.2 |

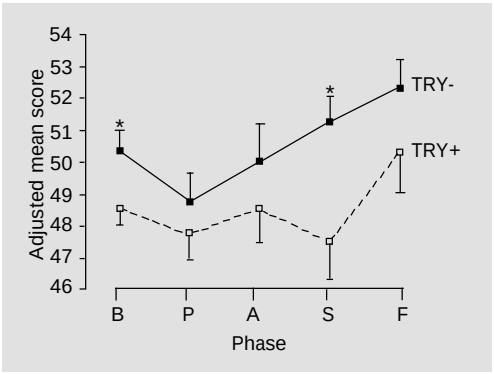


Figure 1 - Effects on females of exposure to a control (TRY+) or tryptophan-free amino acid mixture (TRY-) on subjective state anxiety (STAI) in a simulated public speaking test. In the 24 h that preceded the test the tryptophan-free and control groups were also submitted to a low or normal tryptophan diet, respectively. Symbols indicate the adjusted means (± SD) for the pretreatment measurement of 7 subjects. The phases of the experiment were baseline (B), pre-stress (P), anticipatory anxiety (A), performance anxiety (S), and post-stress (F). Statistical analysis showed a significant phase effect without a group effect (*P<0.05, MANCOVA).

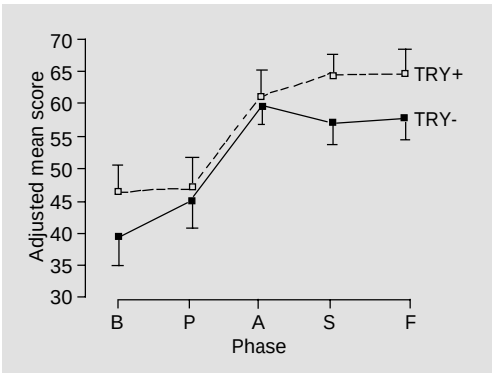


Figure 2 - Effects on healthy subjects of exposure to a control (TRY+) or tryptophan-free amino acid mixture (TRY-) on subjective anxiety (VAMS) in a simulated public speaking test. Symbols indicate the adjusted means (± SD) for the pretreatment measurement of 14-15 subjects. Phases are identified in the legend to Figure 1.

depleted group (P = 0.005 and 0.048, respectively).

**Physiological measurements**

The test induced a significant increase in heart rate and systolic and diastolic blood pressure in both groups (phase factor, P<0.001). No group difference was found.

## Discussion

Confirming previous results, the SPS test induced an increase in subjective feelings of anxiety and in their accompanying physiological phenomena (7,10,12). The tryptophan depletion procedure produced a significant decrease in tryptophan levels. Studies on both laboratory animals and humans have suggested that this effect is accompanied by a decrease in central 5HT neurotransmission (13-15) and that this procedure is able to induce mood changes in 5HT reuptake inhibitor-treated depressive patients (14,16). It also seems to exacerbate panic and aggression. However, results for other psychiatric disorders are not very consistent (16).

In the present study the tryptophan depletion procedure failed to cause a significant change in the subjective anxiety induced by the SPS test. Reports on the effects of tryptophan depletion or amino acid loading on healthy subjects are conflicting. An increase in anxiety induced by $CO_2$ (17) or yohimbine (18), and changes in sleep (19) and learning and memory (20) have been reported. However, tryptophan depletion fails to induce mood changes either alone or in combination with administration of alpha-methyl-para-tyrosine (21), or to modify the panic symptoms induced by cholecystokinin-tetrapeptide (22). Moreover, two recent reports also showed negative results in similar public speaking challenge tests (23,24). Previous studies, however, have shown increased anxiety in the SPS test after treatment with the 5HT receptor antagonists metergoline or ritanserin (6,25). It is possible, therefore, that the degree of impairment of 5HT-mediated neurotransmission obtained by the tryptophan depletion procedure in the present study was not sufficient to induce changes in the SPS model.

Nevertheless, there were gender interactions and analysis of each gender showed that females from the low tryptophan group presented a significant, although small, in-

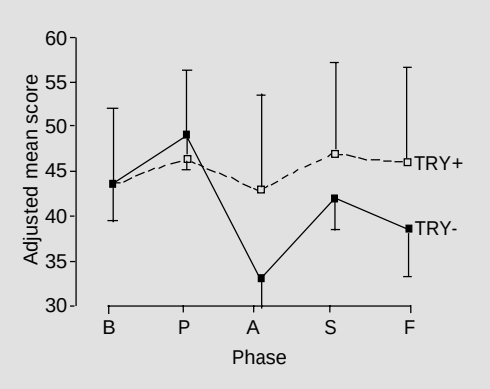


Figure 3 - Effects on females of exposure to a control (TRY+) or tryptophan-free amino acid mixture (TRY-) on subjective mental sedation (VAMS) in a simulated public speaking test. Points represent the adjusted means (± SD) for the pretreatment measurement of 7 subjects. Statistical analysis showed a barely significant group x phase interaction (P = 0.06, MANCOVA). Phases are identified in the legend to Figure 1.

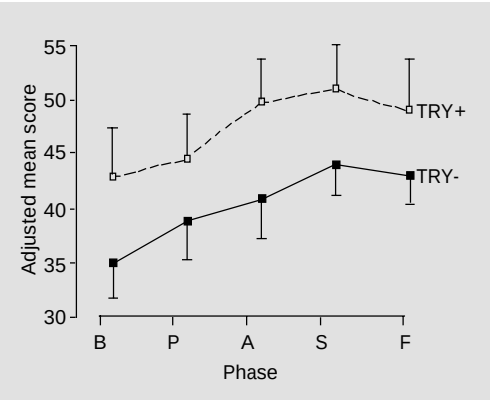


Figure 4 - Effects on healthy subjects of exposure to a control (TRY+) or tryptophan-free amino acid mixture (TRY-) on subjective physical sedation (VAMS) in a simulated public speaking test. Points represent the adjusted means (± SD) for the pretreatment measurement of 14-15 subjects. Statistical analysis showed a significant group difference during the whole test (P<0.05, MANCOVA). Phases are identified in the legend to Figure 1.

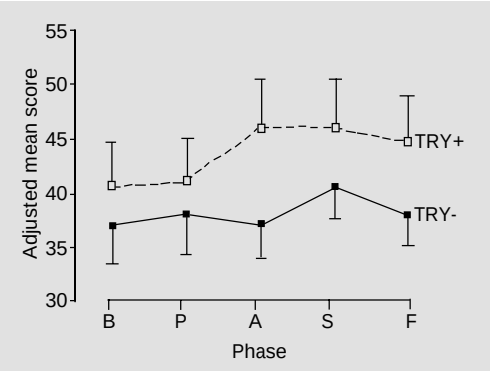


Figure 5 - Effects on healthy subjects of exposure to a control (TRY+) or tryptophan-free amino acid mixture (TRY-) on other feelings and attitudes (VAMS) in a simulated public speaking test. Points represent the adjusted means (± SD) for the pretreatment measurement of 14-15 subjects. Statistical analysis showed a trend to a significant group difference during the whole test (P<0.10, MANCOVA). Phases are identified in the legend to Figure 1.

crease in anxiety during both the baseline and speech anxiety phases, and a decrease in mental sedation. This effect cannot be attributed to a lower plasma tryptophan level in females since no gender difference was found in this measurement. A gender difference in response to tryptophan depletion was recently found, with normal females being more sensitive to mood changes than males (26). It has been shown that the rate of 5HT synthesis is lower in normal females than males (15), a fact which provides a biochemical basis to support the behavioral findings described (26). Therefore, our data may reflect a greater sensitivity of females to the procedure. However, changes in 5HT activity along the menstrual cycle have been reported in the literature (27), especially in the late luteal phase. The lack of control for the menstrual cycle phase could be a limitation of the present study.

Overall group differences were actually found in other factors of the VAMS. Tryptophan depletion decreased physical sedation (items: mentally slow, incompetent, lethargic, muzzy, withdrawn, clumsy, feeble), suggesting an improvement in subjective feelings of cognitive performance competence. Although a general sedative effect of tryptophan administration in the control group could also be involved in this effect, no general change was found in subjective feelings of mental sedation or in items of the BSS related to sedation. It is also interesting to note that a study using objective learning and memory tests showed that tryptophan depletion actually impaired performance (20). Since no such tests were used in the present study, it is not possible to directly compare these contrasting results. In addition to physical sedation, the procedure also tended to change the scores of the factor concerning other feelings and attitudes. This may indicate a decrease in discontentment by the depletion procedure, although this effect was not significant.

In conclusion, although the tryptophan depletion procedure employed in the present study was not able to induce a general change in the subjective anxiety response to simulated public speaking, it may have been anxiogenic in females, perhaps due to a greater sensitivity of the 5HT system of females to the effects of this procedure.

## Acknowledgments

We are indebted to Dr. Osvaldo de Freitas for helpful support in the preparation of the gelatin capsules.

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