Acute tryptophan depletion in eating disorders

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Introduction. This work describes the rational bases justifying the use of acute tryptophan depletion technique in eating disorders (ED) and the methods and design used in our studies. Tryptophan depletion technique has been described and used in previous studies safely and makes it possible to evaluate the brain serotonin activity. Therefore it is used in the investigation of hypotheses on serotonergic deficiency in eating disorders. Furthermore, and given the relationship of the dysfunctions of serotonin activity with impulsive symptoms, the technique may be useful in biological differentiation of different subtypes, that is restrictive and bulimic, of ED.

Methods. 57 female patients with DSM-IV eating disorders and 20 female controls were investigated with the tryptophan depletion test. A tryptophan-free amino acid solution was administered orally after a two-day low tryptophan diet to patients and controls. Free plasma tryptophan was measured at two and five hours following administration of the drink. Eating and emotional responses were measured with specific scales for five hours following the depletion. A study of the basic characteristics of the personality and impulsivity traits was also done. Relationship of the response to the test with the different clinical subtypes and with the temperamental and impulsive characteristics of the patients was studied.

Results. The test was effective in considerably reducing plasma tryptophan in five hours from baseline levels (76%) in the global sample. The test was well tolerated and no severe adverse effects were reported. Two patients withdrew from the test due to gastric intolerance.

Conclusions. The tryptophan depletion test could be of value to study involvement of serotonin deficits in the symptomatology and pathophysiology of eating disorders.

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Depleción aguda de triptófano en trastornos de la conducta alimentaria

Introducción. Este trabajo describe las bases racionales que justifican el uso de la técnica de la depleción aguda de triptófano en los trastornos de la conducta alimentaria (TCA), así como la metodología y el diseño empleado en nuestros estudios. La técnica de la depleción de triptófano ha sido descrita y utilizada en estudios previos de manera segura y permite evaluar la función serotoninérgica cerebral. Por ello puede ser de interés en la investigación de las hipótesis sobre la deficiencia serotoninérgica en los trastornos de la conducta alimentaria. Asimismo, y dada la relación de las disfunciones de la actividad de serotonina con los síntomas impulsivos, la técnica puede ser útil en la diferenciación biológica de los distintos subtipos, restrictivo y bulímico, de TCA.

Método. Se estudia la respuesta sintomática de 57 pacientes mujeres diagnosticadas de TCA y de 20 mujeres control a una prueba de depleción de triptófano. Para ello se administra una solución con todos los aminoácidos esenciales a excepción del triptófano precedida por una dieta pobre en triptófano de 2 días de duración. Se miden las cifras de triptófano plasmático a las 2 y a las 5 h tras la administración de la bebida. Se miden las respuestas alimentarias y emocionales mediante escalas específicas a lo largo de las 5 h siguientes a la depleción. Se realiza también un estudio de las características básicas de la personalidad y de los rasgos de impulsividad. Se estudia la relación de la respuesta a la prueba con los distintos subtipos clínicos y con las características temperamentales e impulsivas de las pacientes.

Resultados. La prueba fue efectiva en la reducción del triptófano plasmático, cuyas cifras descendieron significativamente (76%) en la muestra global. La prueba fue bien tolerada y no se produjeron efectos adversos

graves. Dos pacientes abandonaron la prueba por intolerancia gástrica a la bebida.

Conclusiones. La prueba de depleción de triptófano es eficaz en la reducción del triptófano plasmático en los TCA y puede ser válida para el estudio de la función serotoninérgica y de su relación de los síntomas alimentarios e impulsivos de los TCA.

Palabras clave:
Trastornos de la conducta alimentaria. Serotonina. Triptófano. Bulimia. Anorexia. Impulsividad.

INTRODUCTION

Use of the tryptophan depletion technique as a tool to study brain serotonergic systems has increased in the last 15 years. Acute trypotphan depletion (ATD) is a technique that produces and in vivo decrease in serotinin levels. Many studies state that acute trypotphan depletion reduces brain serotonergic function, demonstrating a decrease in the tryptophan and 5 hydroxyindolacetic tryptophan[2] concentrations by positron emission tomography[1]. Brain serotinin synthesis depends on the availability of plasma tryptophan[3]. Rapid decrease of the availability of tryptophan may be produced by substituting a normal diet with an amino acid preparation in which there are no tryptophans. This situation produces a decrease in tryptophan plasma concentrations and inhibits transportation through the brain blood barrier and consequently brain synthesis and release of serotonin decreases[4].

Acute tryptophan depletion is a technique that consists of the administration of an amino acid preparation in which there is no tryptophan and which includes, among others, neutral long chain amino acids (NLCAA) that compete with tryptophan in the brain reuptake. When this preparation is administered in humans, the tryptophan plasma levels decrease substantially (60%-80%) over 5 hours[5]. Reduction of the brain tryptophan levels (and serotonin) is a consequence of decreased reuptake of brain tryptophan.

Different studies suggest that serotonin plays an important role in the pathogenesis of major depression in relationship with the disorders occurring in depressed patients in brain serotinergic activity[6] and the demonstrated effectiveness of antidepressants that selectively increase serotinergic neurotransmission[7]. However, it is not clear if the deficient serotonergic function is responsible for the clinical signs of major depressive disorder. Some studies have tried to evaluate if the decrease in the brain serotonergic activity secondary to depletion of its precursor (trypotphan) can produce a clinically significant relapse in persons vulnerable to suffering this disorder, reaching the conclusion that a rapid decrease in brain serotonergic function could precipitate depressive symptoms[8].

Bulimia Nervosa (BN) is the most frequent eating disorder (ED) among women who have normal weight[11]. BN is characterized by alterations in appetite, binging, purgative behaviors, alterations in body image, dysphoric mood and neuroendocrine disorders[12] and depressive mood, obsessions and comorbid anxiety. The physiopathology is not clear. Some studies suggest that a decrease in serotonin neurotransmission could contribute to the pathogenesis of both bulimia and anorexia nervosa (AN).

Serotonin plays a very important role in the regulation of food intake[9]. Stimulation of post-synaptic 5HT receptors seems to inhibit specifically the consumption of carbohydrates in rats and a more intense stimulation reduces total calorie intake[10]. One hypothesis proposed suggests that binging in bulimia nervosa is precipitating by a reduction in brain serotonergic activity[13]. This fact may be explained by many experimental studies. In the first place, it is well known that reduction of brain serotonergic transmission by drug routes stimulates eating intake[9]. On the other hand, some studies suggest that unrecovered bulimic patients have decreased serotonergic activity seen by a flat response of prolactin after administration of drugs with serotinergic actions[14,15]. In the third place, the drugs involved in serotonin transmission have been shown to be useful in the control of binging in bulimic patients. It is important to state that other antidepressants focalized in noradrenergic activity are also effective in BN, suggesting the probable involvement of other neurotransmission systems.

The effect of acute tryptophan depletion is also of interest in patients at risk of suffering BN. A moderate diet itself may cause a decrease in plasma tryptophan[16] and it is well known that most of the cases of bulimia arise in patients who follow a normal diet. This makes it possible for BN to appear as part of a consequence of the deficits in brain serotonergic function inducted by the diet in persons vulnerable to developing the disorder[17,18]. This could be explained by the fact that release of tryptophan to the brain tissue in bulimic patients is impaiared due to the alterations of the tryptophan/LCAA alterations under baseline conditions or in response to food intake, the synthesis and brain serotonin release decreasing. Post-synaptic changes resulting from the serotonergic action could be precipitated in deviant eating patterns. Since it is known that the role of serotonin in the regulation of eating behavior is predominantly inhibitory, a defect in the serotonergic function could contribute to the appearance of binging in bulimic patients[19]. Some studies support the hypothesis that impulsiveness is one of the cardinal symptoms of bulimia nervosa, it having been seen that low levels of serotonin are associated with impulsiveness and AN, although the relationship between the latter and impulsiveness is less known than the association with BN[20,21]. There is evidence in the literature that involves the serotonergic system in the pathogenesis of AN, the results in these patients being contradictory, so that the relationship between the central serotonergic function and AN is not clear. Thus, several studies have proposed two subtypes of AN: without binging *(a)* and with binging *(b),* maintaining the hypothesis that group *a* would be charac-

terized by low impulsiveness and normal or increased serotonergic activity and group *b* by high impulsiveness and decreased serotonergic activity in relationship to the fact that the changes in serotonin in patients with AN would be associated with impulsive behaviors[22]. On the other hand, it has been demonstrated that low weight anorexic patients have decreased serotonergic activity that is probably related with lack of tryptophan necessary to synthesize serotonin[23].

Study objectives

The main objective is to demonstrate the existence of serotonergic dysfunction in ED patients (bulimia and anorexia nervosa) versus a healthy control group by response to tryptophan depletion.

The following are found among the secondary objectives:

- Investigate if the symptomatic response to tryptophan depletion can discriminate between patients with bulimia nervosa and those with restrictive anorexia given the relationship of serotonergic dysfunctions with impulsive symptoms.
- Obtain preliminary data that justify studies on the importance of an adequate tryptophan rich diet in the treatment and prevention of disorders in which there is an impulse control disorder.
- Identify the existence of a possible biological heterogeneity within ED based on other temperamental variables.
- In general, have preliminary data that make it possible to design a study on a larger scale aimed at validating the usefulness of the tryptophan depletion test in the detection of bulimic patients susceptible to drug treatment.

METHODS

A total of 57 women diagnosed according to DSM-IV criteria of eating behavior disorders on evaluation (22 restrictive anorexia, 30 purgative anorexia and 27 bulimia nervosa) who came consecutively as outpatients to the outpatient clinical of the hospital, were enrolled. We used a sample of 20 healthy volunteers having the same age and weight characteristics as control group. The control group was enrolled among the female staff of the hospital and student population, balancing the samples by age and educational levels. TRP depletion test (diet + amino acid solution), TRP plasma depletions and application of measurement instruments of psychopathological variables were conduced in the same way in the healthy control group.

The patients did not take any medication within 15 days prior to the study (5 weeks in the case of fluoxetine). If the patients were taking any medication, a progressive washout was conducted, allowing for the consumption of 1 mg of lorazepam at night if benzodiazepines had been taken previously. Those patients with severe desnutrition, organic diseases, current history of substance abuse, mood disorders and background of bipolar disorder or schizophrenia were excluded. The patients were informed on the objectives and characteristics of the project and were asked to give written consent on their participation in the study.

Table 1 Sample characteristics

Age (years)	24.2; SD: 6.4 (18-34)
BMI	17.1; SD: 2,8 (16.5-21)
rAN	14 (23%)
c-pAB	19 (32%)
BN	24 (45%)
Disorder time (year)	5.9; SD: 2.7 (range: 1.5-11.5)
University studies	21 (36%)
Secondary studies	36 (64%)

BMI: body mass index; rAN: restrictive anorexia nervosa; cpAN: compulsive-purgative anorexia nervosa; BN: bulina nervosa; SD: standard deviation.

Tryptophan depletion test

To achieve transitory tryptophan depletion (TRP), the patients followed a low tryptophan diet for two days (less than 150 mg/day) followed by the intake of an amino acid solution without tryptophan the next morning to be able to decrease the tryptophan levels more and acutely. Administration of the amino acid solution was necessary since the central serotonergic system could initiate compensatory mechanisms that would avoid a flare-up of the symptoms if the depletion was done progressively.

A TRP poor diet (less than 160 mg/day) was elaborated by the Ramón y Cajal hospital nutrition service. The diet had no risk for the patients, it being calibrated with 30 grams of olive oil, which is sufficient to assure the necessary supply of essential fatty acids. We also supplied 1,073 calories, 19 g of protein, 48 g of lipids and 149 g of carbohydrates, 158.6 mg of TRP that were distributed as follows (the amounts correspond to the weights of the uncooked food):

- Breakfast: coffee with whole milk (100 g), sugar (10 g), apple (200 g) and orange (200 g).
- Lunch: sautéed green beans (100 g), white rice (30 g) with tomato sauce (25 g), apple (200 g), Jell-O flavors (1/2 packet = 40 g).
- Snack: Jell-O flavors (1/2 packet = 40 g).

— Dinner: salad: lettuce (100 g), tomato (75 g), vinegar, oil, orange (200 g), coffee with milk (100 g), sugar (10 g), granola type cookies (40 g = 4 cookies).

The patients followed the diet at home and were told not to eat any foods that were not prescribed. After 2 days on the diet, they were given a dietary drink with all the amino acids (alanine, arginine, cisterne, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, valine Y tyrosine) except tryptophan (Xlys, Xtry maxamaid), which was marketed by SHS. Administration was done at 9 a.m., while fasting, in the hospital and under medical control. The patients reinitiated a normal diet after completing the clinical and biological studies which were finished at 2 p.m. on the same day.

Previous studies have verified the perfect tolerability to the diet and amino acid rich solution without TRP and the absence of side effects described during and after its administration.

Measurement of plasma tryptophan levels

Measurements of total and free plasma TRP were conducted with HPLC (high efficacy liquid chromatography) with consumable material (Buffers supplied by BECKMAN) for the measurement of amino acids.

Blood samples for measurement of TRP were obtained before beginning the low TRP diet and five hours after administration of the amino acid solution without TRP to verify transitory depletion of TRP.

Clinical and personality studies

All the patients and control group were administered analogic symptoms scales specifically designed for the purpose before the initiation of the diet, at one hour, at three hours and at five hours of the administration of the amino acid solution. It included symptoms related with emotions (sadness, anxiety, irritability, rage, fear) or food circle symptoms (appetite, anxiety to eat, sensation of empty stomach, fullness, nausea, thoughts related with weight or figure, distortion of body image). The SCL-90, Hamilton scale for depression, Hamilton scale for anxiety and different impulsiveness specific scales were also administered.

Furthermore, before beginning the diet, the patients were administered a personality study with Cloninger's Temperament and Character Inventory (TCI) and Eysenck's Personality Questionnaire (EPQ).

Statistical analysis

Initially, a descriptive statistics of the sociodemographic variables of the sample was performed. Comparisons in the symptomatic response between ED diagnosed patients and controls and between the different clinical ED subgroups were made with the analysis of variance (ANOVA) of the different quantified variables and then subsequent multiple paired comparisons were performed with those variables in which the ANOVA was significant.

Multiple bivariant correlation tests with subsequent Bonferronni correction were used for the analysis of the correlations between the symptomatic response and the baseline temperamental and impulsive characteristic. The analysis was done for the global group and within each clinical subgroup of the sample.

Analysis of the modifications in free and total TRP plasma levels was performed by comparing TRP plasma levels before beginning the depletion test with the levels obtained at 3 hours of performing the test by paired tests (Student's *t* test). Decrease of plasma TRP achieved correlated with the symptomatic variables measured.

Statistical calculations were done with the SPSS statistical program.

RESULTS

Reduction of the plasma free tryptophan values and protein bound tryptophan was 25% at two hours of administration and 72% at five hours in comparison with baseline values of the global sample.

The test was well tolerated in general, although 26 patients (35%) had nausea when they tried to drink the drink, due to the unpleasant flavor and also to the volume required (200 cc). Two patients had to stop the test due to this and were withdrawn from the study. No patient had clinically reportable adverse events.

DISCUSSION

The analysis of this first part of the study makes it possible to verify that the tryptophan depletion test has been effective in the subjects studied. This means that plasma tryptophan values have been considerably reduced. In this sense, the results agree with those of the previous studies on tryptophan depletion in other mental disorders[3,8].

Use of depletion as a measure of serotonergic activity has a double explanation in the eating disorders. In the first place, as any other affective or impulsive type disorder where it is suspected that there may be a serotonin dysfunction, the test makes it possible to know the rela-

tionship of this neurotransmitter with the disorder symptoms. On the other hand, as tryptophan is an element of the diets, the test may supply data in relationship to the pathogenic or healthy properties of tryptophan in them and its implication in phenomena such as binging, appetite or fullness[19,20].

On the contrary to challenge tests based on serotonin receptor agonists or antagonists, such as fenfluramine, clomipramine, buspirone or cyproheptadine, the use of the tryptophan depletion test produces a global reduction of brain serotonergic activity. More specific and partial elements of the system may be modified in the receptor tests, which produce different clinical-biological modifications among them[25,26]). Tryptophan depletion alone does not make it possible to discriminate the parts of the serotonergic system that can be distorted (pre- or post-synaptic receptors, different subtypes of receptors, etc.) but does make it possible to know the vulnerability of the system to a sudden reduction of the serotonin concentration and the relationship of the clinical phenomena with the global activity of the serotonergic system[27]. Clinical (differentiation of clinical subtypes, classification of symptoms) and psychobiological (primary presynaptic serotonergic deficits, post-synaptic activation deficits) conclusions can be drawn from this[24,28].

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