ELSEVIER

Archives of Medical Research 43 (2012) 375–382

Archives of Medical Research

ORIGINAL ARTICLE

Plasma Amino Acid Levels Discriminate Between Control Subjects and Mildly Depressed Elderly Women

Adriana Mayoral-Mariles,[a] Carmen Cruz-Revilla,[a] Xochitl Vega-Manriquez,[a] Rebeca Aguirre-Hernández,[b] Patricia Severiano-Pérez,[c] Eric Aburto-Arciniega,[a] Araceli Jiménez-Mendoza,[d] and Rosalinda Guevara-Guzmán[a]

[a]*Departamento de Fisiología,* [b]*División de Estudios de Posgrado, Facultad de Medicina,* [c]*Departamento de Alimentos y Biotecnología, Facultad de Química,* [d]*Escuela Nacional de Enfermería y Obstetricia, Universidad Nacional Autónoma de México, México, D.F., Mexico*

Received for publication July 26, 2011; accepted June 20, 2012 (ARCMED-D-11-00365).

Background and Aims. Depression is the most common of all psychiatric disorders and the role of amino acid transmitters in this pathology has been recently studied. We undertook this study to investigate if the plasma levels of L-arginine, L-citrulline, L-glutamic acid, L-glutamine, L-asparagine and other amino acids, the L-citrulline/L-arginine and the L-tyrosine/L-phenylalanine ratios, and the nitrite levels are modified in mildly depressed women and if such changes are related to olfactory dysfunction.

Methods. Plasma samples were obtained from elderly female subjects ($n = 21$) with mild depression and ($n = 48$) controls. Amino acids were analyzed with high-performance liquid chromatography, plasma nitrite levels were measured using the Griess method, and olfactory performance was assessed by the combined testing of odor identification, odor discrimination, odor recognition, and the olfactory threshold.

Results. Compared to controls, depressed patients had a significantly higher concentration of L-arginine and a significantly lower L-citrulline/L-arginine ratio when the effect of other variables is not taken into account. A logistic regression model allowed us to identify two risk factors for mild depression, L-arginine and L-glutamic acid, and two protective factors, L-asparagine and the L-tyrosine/L-phenylalanine ratio. Additionally, a significant increase in nitrite levels in depressed women was found. No significant differences were found between the percentage of depressed and control women that identified the odors.

Conclusions. We identified that the amino acids L-arginine and L-glutamic acid are risk factors for mild depression, whereas L-asparagine and the L-tyrosine/L-phenylalanine ratio are protective factors.

Key Words: Depression, Amino acids, L-glutamic acid, L-arginine, Olfactory functions, Nitric oxide.

Introduction

Depression is the most common of all psychiatric disorders (1). It is a growing social and medical problem affecting millions of individuals worldwide (2); therefore, its study and characterization have become a public health concern. Monoamine neurotransmitters have traditionally been considered to be involved in depression, but the role of amino acid transmitters in this disorder has recently been analyzed (3–5). Amino acid levels have been measured in plasma or serum (6–9), cerebrospinal fluid (10–12), and brain tissue (10,13,14) in subjects with depression. There is evidence that the excitatory glutamatergic system plays a significant role in the neurobiology of depression (3,4), but controversial results have been obtained for the levels of L-glutamic acid. Some authors report an increase

Address reprint requests to: Dr. Rosalinda Guevara-Guzmán, Laboratorio de Análisis Sensorial, Departamento de Fisiología, Facultad de Medicina, Universidad Nacional Autónoma de México, Apartado Postal 70250, Delegación Coyoacán, 04360 México, D.F., Mexico; Phone: (+52) (55) 5623-2396; FAX: (+52) (55) 5623-2155; E-mail: rguevara@servidor.unam.mx

0188-4409/$ - see front matter.
http://dx.doi.org/10.1016/j.arcmed.2012.07.006

(9,14,15), whereas other authors report no changes (6) in this amino acid. A disturbance of the L-arginine-nitric oxide pathway has also been implicated in depression (16–21). Nitric oxide (NO) is synthesized from L-arginine by a family of isoformic enzymes known as nitric oxide synthases (NOSs). In the brain, NO plays an important role in functions such as synaptic plasticity, neuroprotection, neurotoxicity, and behavior (16,18,22), as well as modulating the release of other neurotransmitters and acting as a cellular communicator or acting as a vasodilator regulating blood flow (16,21,23,24).

The sense of smell participates in emotional processes. Olfactory dysfunction has been reported in neuropsychiatric disorders (25). Changes in odor detection or identification have been reported to be present in depression (26–29). Other authors have reported no changes in olfactory function (30–34).

The aim of our study was to determine if changes in the plasma levels of amino acids and nitrites (a metabolite of NO) are associated with mild depression in elderly women and to assess changes in the olfactory function of these patients.

Materials and Methods

Subjects

Female subjects were recruited from nursing homes and from visiting government daycare centers. The subjects essentially preserved a general cognitive function with largely intact functional activities. Written informed consent was obtained from the subjects after explaining all aspects of the study. In accordance with the Declaration of Helsinki, the protocol was approved by the Research and Ethics Committee of the Faculty of Medicine, Universidad Nacional Autónoma de México. All women underwent a standard medical history and a battery of psychological tests. We used the Mini Mental-State Examination (MMSE) to assess global cognitive functioning (35). A cognitive decline was considered when the MMSE score was ≤23. Patients with no cognitive impairment had a MMSE score ≥24 and showed no impairments in the complex activities of daily living. Diagnoses were made with the Beck Depression Inventory (BDI) (cut-off point 10) (36,37). Women with severe depression, after treatment or with any other symptom indicating a somatic disease were excluded from the study. Only those with mild depression were included. Women were also excluded if they consumed alcohol or used tobacco, if they had a history of a brain tumor, epilepsy, stroke, diabetes, or if they needed special diets or medications such as psychotropic or antidepressant drugs. The women reported the absence of any menstruation for at least 4–5 years and were not using any hormonal replacement therapy or taking corticosteroids or had ever taken them in the past. Initially, 127 volunteers accepted to participate in the study but only 69 women fulfilled the inclusion criteria.

This subset of patients was between 60 and 91 years old. Forty eight of the women were not depressed and had no cognitive impairment (controls), and 21 had mild depression but no cognitive impairment.

Sample Collection and Preparation

Qualified nurses from ENEO, the National School of Nursing and Obstetrics, obtained a 5-mL sample of fasting blood from each woman in the morning (0800 to 0900). Blood samples were placed in vacuum tubes containing K_2EDTA (BD Vacutainer, Mexico City). Plasma was then obtained and immediately stored at −70°C for further studies.

Amino Acid Analysis

Plasma levels of 18 amino acids were measured. To carry out high-performance liquid chromatography (HPLC), 200 μL of the sample was mixed with 200 μL of acetonitrile to remove proteins by precipitation. The mixture was refrigerated at 4°C for 10 min and then centrifuged at 14,000 rpm for 7 min. The supernatant was filtered and diluted with Krebs Ringer solution (pH 3). Fifty μL was transferred into microtube vials, placed in amber vials with screw caps, and stored in the refrigerated sampler of the HPLC system at 5°C. The analysis was done with an Agilent 1100 HPLC system (Agilent Technologies, Santa Clara, CA) equipped with a fluorescence detector, a binary pump, an automatic injector with sampler, and a column thermostat. The chromatographic conditions and characteristics were those described in detail by Henderson et al. (38) with some modifications.

Measurement of Plasma Nitrite Levels

Nitrite levels were measured using the Griess method (39). Griess reagents were sulfanilamide, glacial acetic acid, N-(1-napthyl) ethylenediamine, and sodium nitrite (Sigma, St. Louis, MO). The reagent (200 μL) was added directly to an aliquot of the homogenate (200 μL) and incubated under reduced light at 4–8°C for 10 min. Samples were analyzed at 540 nm in a spectrophotometer. The protein level of each sample was determined using the Lowry method (40). Data were calculated as μM of nitrite per milligram of protein.

Olfactory Function Evaluation

Olfactory processes were assessed with the olfactory threshold test and the identification, recognition, and discrimination test (triangular test with two levels of discrimination). Discrimination level 1 indicated whether women were able to identify between two odors that belonged to different groups, e.g., guava (fruit odor) and peppermint (herbal odor). The discrimination level 2