Progress in Neuro-Psychopharmacology & Biological Psychiatry 35 (2011) 118–125

Contents lists available at ScienceDirect

Progress in Neuro-Psychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

Autoantibodies reacting with vasopressin and oxytocin in relation to cortisol secretion in mild and moderate depression

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ARTICLE INFO

Article history:
Received 20 July 2010
Received in revised form 29 September 2010
Accepted 29 September 2010
Available online 13 October 2010

Keywords:
Behavior
Mood disorders
Natural immunity
Neuropeptides
Stress

ABSTRACT

Background: Abnormal vasopressin (VP) and oxytocin (OT) signaling may contribute to the altered activity of the hypothalamo-pituitary-adrenal (HPA) axis in major depression; however, the underlying mechanisms remain uncertain. This study characterized plasma levels and affinities of OT- and VP-reactive autoantibodies (autoAbs) in relation to disease severity and plasma cortisol response to physical exercise in patients with mild and moderate depression and healthy controls.

Methods: Physical exercise was used to elicit plasma cortisol response in 23 male patients with depression and 20 healthy controls and plasma samples were obtained before and after the exercise. Just before the exercise, patients and controls were evaluated by the Montgomery and Åsberg Depression Rating Scale (MADRS) and divided according to depression severity (14 mild and 9 moderate). Plasma levels of total and free VP- and OT-reactive IgG, IgA and IgM autoAbs were measured by ELISA and affinity of IgG and IgM autoAbs were measured by plasmon resonance technique at baseline before the exercise and analyzed with relation to the MADRS and cortisol response. Immunohistochemistry was used to evaluate autoAbs binding to the rat hypothalamus.

Results: Plasma levels of OT- and VP-reactive total IgG autoAbs were lower in patients with moderate depression vs. controls and patients with mild depression. Plasma levels of both OT- and VP-free IgG autoAbs were negatively correlated with MADRS scores. Affinity values of IgG and IgM autoAbs for both OT and VP displayed 100 fold variability among patients or controls but no significant group differences were found. Patients with moderate depression displayed blunted response of cortisol secretion to physical exercise. Baseline levels of VP total IgG and IgM autoAbs correlated negatively and VP-free IgG autoAbs correlated positively with plasma cortisol after physical exercise. Immunostaining of magnocellular hypothalamic neurons of the supraoptic and paraventricular nuclei by plasma IgG was present in 35% of the depression and in 14% of the controls groups, but this staining was not abolished by plasma preabsorption with OT or VP peptides.

Conclusion: These data show that changes of levels but not affinity of OT- and VP-reactive autoAbs can be associated with the altered mood in subjects with moderate depression and that levels of VP-reactive autoAbs are associated with cortisol secretion.

1. Introduction

Major depression is a complex disorder characterized by disturbed mood and behavior as well as by neuroendocrine and immune abnormalities (Irwin and Miller, 2007). Altered activity of the hypothalamo-pituitary-adrenal (HPA) axis is one of the most consistent findings in depression (Pariante and Lightman, 2008; Zunszain et al., 2010). Mild to moderate hyperactivity of the HPA axis resulting in increased plasma cortisol is observed in 30–50% of depressed subjects (Pariante and Lightman, 2008; Rubin et al., 2001). Moreover, non suppression of cortisol secretion in the combined dexamethasone/corticotropin-releasing hormone (CRH) test has approximately 80% sensitivity in major depression, although this

Abbreviations: α-MSH, α-melanocyte-stimulating hormone; ACTH, adrenocorticotropic hormone; autoAbs, autoantibodies; BIA, biospecific interaction analysis; BMI, body mass index; CRH, corticotropin-releasing hormone; CSF, cerebrospinal fluid; DSM, diagnostic and statistical manual of mental disorders; EDI, Eating Disorders Inventory; ELISA, enzyme-linked immunosorbent assay; HPA, hypothalamo-pituitary-adrenal; Ig, immunoglobulin; MADRS, Montgomery and Åsberg Depression Rating Scale; OD, optical density; OC, optic chiasm; OT, oxytocin; PVN, paraventricular nucleus; RT, room temperature; SD, standard deviation; SE, standard error; SON, supraoptic nucleus; TSA, tyramide signal amplification; VP, vasopressin; 3v, third ventricle.

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doi:10.1016/j.pnpbp.2010.09.021

test can be positive in some other psychiatric disorders (Heuser et al., 1994). Hypercortisolemia, however, is not obligatory for major depression which can be accompanied by low plasma cortisol levels as well (Stewart et al., 2005; Vythilingam et al., 2010).

Arginine vasopressin (VP) and oxytocin (OT) are nine amino acid peptide hormones which play critical roles in renal water retention and milk ejection, respectively. Both hormones are also neuromodulators and neurotransmitters acting in the limbic system to regulate memory formation, social behavior, anxiety and stress response (Donaldson and Young, 2008; Veenema et al., 2008). In relation to the HPA axis activity, VP is known to potentiate the effect of CRH on adrenocorticotropic hormone (ACTH) release by the anterior pituitary (Rivier et al., 1984), whereas OT may have either stimulatory or inhibitory effects (Antoni et al., 1983; Legros, 2001; Schlosser et al., 1994). These VP and OT functions suggest their putative involvement in the deregulation of HPA axis activity and in depression. However, data on OT and VP plasma and/or CSF levels in depression showed either an increase, a decrease or no differences vs. healthy controls, while postmortem studies in depressed patients revealed increased hypothalamic expression of both VP and OT in some studies (Bao et al., 2008; Gjerris et al., 1985; Gold et al., 1978; Scott and Dinan, 2002; van Londen et al., 1997). These data suggest that not only increased but also decreased activity of the VP and OT systems may result in altered activity of the HPA axis, and among other effects, may predispose to depression development. However, the underlying causes of pathological changes in the VP and OT signaling remain unknown.

One possible explanation could be related to the altered stability of OT and VP in the circulation as modified by their carrier proteins i.e. molecules which can reversibly bind these peptides. Immunoglobulins, i.e. naturally occurring autoantibodies (autoAbs) may play such a carrier role for peptide hormones. Natural autoAbs are present in the serum of healthy individuals in the absence of deliberate immunization with target antigens (Avrameas, 1991). Detectable levels of autoAbs reactive with OT and VP in healthy subjects have also been demonstrated (Fetissov et al., 2008). Functional relevance of OT and VP autoAbs to the regulation of mood and behavior in healthy subjects was suggested by significant correlations of their plasma levels with the somatization score (Karaiskos et al., 2010) and with some other personality traits as revealed by the Eating Disorders Inventory (EDI-2) test (Fetissov et al., 2005). Moreover, plasma levels of OT and VP autoAbs were altered in subjects with eating disorders (Fetissov et al., 2005) and conduct disorder (Fetissov et al., 2006), both conditions known for abnormal HPA axis activities. These data suggest that autoAbs reacting with OT and VP may also be altered in depression, resulting in altered OT- and VP-mediated signaling and contributing to the deregulation of the HPA axis and cortisol production.

To address this hypothesis, in the present work, we studied plasma levels and affinities of OT- and VP-reactive autoAbs with relation to plasma levels of cortisol in subjects with mild and moderate depression and healthy controls.

2. Subjects and methods

2.1. Study subjects

Plasma samples were obtained from both patients with depression and healthy controls previously studied for the effect of physical exercise on cortisol response (Kiive et al., 2004). The study was approved by the University of Tartu Ethic Committee for Studies on Humans. All subjects gave written informed consent for study participation. Twenty patients were recruited at the in- and three patients at the outpatients services of the Clinic of Psychiatry, Tartu University. Patients with major depression, all males (age, mean ± S.D., 43.5 ± 1.8 years) fulfilled strict criteria for major depressive disorder according to DSM-IV (DSM-IV, 1994). Twenty male healthy participants (age, mean ± S.D., 42.8 ± 3.0 years) were also recruited and interviewed by a psychiatrist to exclude depression or other psychiatric disorders. Immediately before the first blood sampling, all study subjects completed the Montgomery–Åsberg Depression Rating Scale (MADRS) (Montgomery and Åsberg, 1979) and measures for body mass index (BMI) were taken. Patients were divided into two groups according to MADRS scores: mild depression (MADRS scores: 8 to 19, n = 14) and moderate depression (MADRS scores: 20 to 31, n = 9) (Muller et al., 2003). Exclusion criteria were significant psychiatric comorbidity, organic mental disorder, mental retardation, bipolar disorder, anxiety disorders if primary and/or predominant, alcohol abuse or dependence during the last 12 months.

Five consecutive venous blood samples were taken between 0900 and 1030 h after overnight fast. Blood was collected 10 min before starting 2 min cardiopulmonary exercise (baseline), immediately after the exercise, and another three times with a 30 min interval. The subjects underwent a bicycle cardiopulmonary exercise testing using stepwise increasing workload by 25 W per 2 min (Ergometry System, Siemens AG, Munich, Germany). Plasma was separated by centrifugation and frozen at −80 °C until analyzed. Plasma levels of OT and VP autoAbs were assayed at baseline and compared with known values of plasma cortisol from each of the five samples.

2.2. Serum levels of VP- and OT-reactive autoantibodies

Plasma levels of autoAbs (IgG, IgM and IgA) reacting with VP and OT were measured using enzyme-linked immunosorbent assay (ELISA) technique. The VP or OT peptides (Bachem AG, Bubendorf, Switzerland) were coated on Maxisorp plates (Nunc, Rochester, NY, USA) using 100 µl and a concentration of 2 µg/ml in 100 mM $NaHCO_3$ buffer, pH 9.6 for 24 h at 4 °C. Plates were washed (5 min 3×) in phosphate-buffered saline (PBS) with 0.05% Tween 20 (Sigma, Saint Louis, MO, USA), pH 7.4, and then incubated overnight at 4 °C with 100 µl of human plasma diluted 1:200 in PBS to determine free autoAbs levels or diluted 1:200 in dissociating buffer (3 M NaCl, 1.5 M glycine, pH 8.9) to determine total autoAbs levels. The optimal dilutions of plasma (1:200) were determined by dilution curves (1:50, 1:100, 1:200, 1:400 and 1:800). The plates were washed (3×) and incubated with 100 µl of alkaline phosphatase-conjugated goat anti-human IgG, IgM or IgA (1:2000) (Sigma) for 3 h at room temperature (RT). Following washing (3×), 100 µl of p-nitrophenyl phosphate solution (Sigma) was added as alkaline phosphatase substrate. After 40 min of incubation at RT, the reaction was stopped by adding 3 N NaOH. The optical density (OD) was determined at 405 nm using a microplate reader Metertech 960, (Metertech Inc., Taipei, Taiwan). Blank OD values resulting from the reading of plates without addition of human plasma were subtracted from the sample OD values. Each determination was done in duplicate. The variation between duplicate values was less than 5%.

2.3. Plasma immunoglobulin concentration

Concentrations of plasma total IgG, IgM and IgA were assayed by incubation with appropriate anti-human IgG, IgM and IgA antibodies (Siemens) and nephelometry (Behring, Marburg, Germany) followed by comparison with known concentration standards.

2.4. Affinity assay of VP- and OT-reactive autoAbs

Total IgG was purified from 0.5 ml of baseline plasma samples using protein G agarose (Sigma) according to manufacturer's instructions. The IgG containing eluates were lyophilized and diluted to 3 mg/ml in water. For the total IgM extraction, the effluents were dialyzed against distilled water and the precipitate was diluted in PBS.

Affinity of IgG and IgM autoAbs for VP and OT was assayed by a biospecific interaction analysis (BIA) based on the surface plasmon