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Disgust affects TNF-α, IL-6 and noradrenalin levels in patients with obsessive—compulsive disorder

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Summary Neurobiological research of obsessive—compulsive disorder (OCD) has rarely taken in account the context dependent evocation of obsessive—compulsive symptoms. To bypass this obstacle, this study investigated neurobiological parameters during a standardized disgust provocation paradigm in patients with OCD and healthy controls. Ten OCD patients and 10 healthy controls were exposed to 9 disgust related items using a standardized provocation paradigm. Catecholamines and cortisol in plasma and lipopolysaccharide (LPS) stimulated levels of TNF-α and IL-6 by peripheral leucocytes were assessed along with severity of obsessive—compulsive symptoms, disgust, and anxiety levels using Visual Analogue Scales prior, during and after a provocation paradigm. Noradrenalin levels increased, while LPS stimulated TNF-α and IL-6 by peripheral leucocytes decreased during exposure to disgust related objects in OCD patients but not in healthy controls. Cortisol levels were not affected by exposure neither in patients nor in controls, but overall cortisol levels of OCD patients were increased compared to controls. In conclusion, our data suggests that symptom provocation in OCD patients with contamination fear is accompanied by alterations in the immune and neuroendocrine systems but does not affect cortisol levels.

1. Introduction

obsessive—compulsive disorder (OCD) is a common psychiatric disorder with a prevalence of up to 3% (Jenike, 2001). Some theories on the pathophysiology of OCD implicate the immune system. This stems primarily from the observation that children occasionally express obsessive—compulsive symptoms following a bacterial throat infection (Swedo

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et al., 1998), implying that psychiatric symptoms could be triggered by an activation of the immune response.

Consequently various studies have reported immunological abnormalities in adult OCD patients compared with healthy controls (Brambilla et al., 1997; Marazziti et al., 1999; Ravindran et al., 1999). The most robust finding is a change in levels of tumor necrosis factor α (TNF-α). One study found decreased levels after lipopolysaccharide (LPS) stimulation of peripheral leucocytes whereas two studies have reported decreased plasma levels of TNF-α (Brambilla et al., 1997; Monteleone et al., 1998; Denys et al., 2004). One recent study reported increased plasma levels of TNF-α as well as IL-6 in OCD patients (Konuk et al., 2007).

The search for biological correlates for OCD is complicated by the fact that OC symptoms are predominantly present when patients are confronted with aversive situations. This study used a standardized disgust provocation paradigm to measure physiological parameters associated with OCD and stress, such as cortisol and catecholamine levels in plasma as well as ex vivo LPS induced TNF-α and IL-6.

2. Methods

2.1. Subjects

Ten OCD patients (9 female, average age 34.6 years, standard deviation (SD) 9.0) suffering from contamination fear with a minimal Y-BOCS score of 17 and 10 healthy controls (8 female, average age 32.5 years, SD 7.9) participated in the study that had been approved by the Medical Ethics committee of the University Medical Center of Utrecht (UMCU). All subjects provided written informed consent. Diagnoses were made by a psychiatrist and ascertained with the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) (First et al., 1996; Dutch translation and adaptation van Groenestijn et al., 1999). Patients with a concomitant DSM axis I or axis II diagnosis were excluded. The Mini International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998) was used to ensure healthy controls were free of psychiatric diagnoses. All subjects received a general medical health questionnaire and passed a neurological and physical examination. Subjects suffering from inflammatory diseases, endocrine or metabolic disorders, epilepsy or patients who suffered a stroke in the past year were excluded. The average Y-BOCS score in patients was 30.2 (SD 5.4) (14.0 for obsessions (SD 3.1), 16.2 for compulsions (SD 2.8)) while the mean duration of illness was 21 years. Drugs affecting the immune and endocrine system were not allowed, except for SRIs. Five patients were free of psychotropic drugs, 3 patients used paroxetine 60 mg/d, 1 used venlafaxine 150 mg/d and 1 patient used citalopram 60 mg/d.

2.2. Provocation paradigm

The disgust paradigm exposed the subjects to a box containing nine different aversive items that people might encounter in normal life. The items consisted of (artificial) faeces, (artificial) blood in a syringe, garbage, a dirty dust cloth, a vial containing saliva of an unknown subject, a vial with a toxicity label containing a household product, hair on a shower drain, a dirty band-aid and a Petri dish suggesting the presence of a bacteria culture. All items were put in sealed plastic bags. Subjects were not aware of the artificial nature of some of the items, but were informed of this after completion of the test. Beforehand people were asked to rate the nine items, on paper, from least to most aversive. Subsequently, subjects opened each box starting with the item they rated least aversive. They were asked to take out the bag that contained the item and describe it thoroughly. Subjects were then asked to take the item out of the bag if possible. Subjects were exposed to the box for a maximum of 15 min. They were instructed that they could stop at any time. A clinician was always present to monitor the situation.

2.3. Procedure

Subjects arrived at the hospital at 0900 h (timepoint (T) = 0) and were given an indwelling catheter in the forearm after which they rested for 60 min. At 1000 h subjects were exposed to a box containing different disgust provoking stimuli (for exact description and methodology of this box see below). Twenty minutes prior to exposure (T = 1) as well as directly before and after exposure (T = 2 and T = 3) and 30 min (T = 4), 60 min (T = 5) and 120 min (T = 6) after the start of the provocation blood samples were taken. At timepoints T = 0 through T = 6 Visual Analogue Scales (VAS), measuring obsessive—compulsive symptoms, anxiety, agitation, disgust and mood, were filled out. On these scales subjects could score between 0 (no symptoms) and 100 (most symptoms ever experienced). During the test, blood pressure and heart rate were monitored.

2.4. Assessment of neurobiological factors

At T = 2 and T = 6 blood samples were drawn for assessment of immune parameters. For cytokine assessment heparinized diluted blood (100 μl, 1:10 in RPMI-1640 with antibiotics) was stimulated with lipopolysaccharide (100 μl final concentration 2 ng/ml; *E. coli*, 0127:B8, Sigma, St. Louis, MO) and after 24 h supernatants were collected for determination of IL-6 and TNF-α. These levels were determined by ELISA kits (CLB, Amsterdam) that were used according to the manufacturer's instructions. Leukocyte differential counts were performed in the Utrecht University Medical Center hematology laboratory using the CELL-DYN sapphire hematology analyzer (Abbott Diagnostics, Santa Clara, CA, USA).

At T = 1—5 samples were drawn for noradrenaline and adrenaline assessment. These plasma levels were measured using high-performance liquid chromatography (HPLC) with chemical detection after solid phase extraction over aluminum oxide (ClinRep).

In addition at T = 1—6 venous blood was stored in EDTA vials on ice. After the test plasma was collected that was directly stored at −80 °C. Plasma cortisol was measured using an electrochemiluminescence immunoassay on the Modular E170 (Roche Diagnostics GmbH, D-68298 Mannheim, Germany). The lower limit of detection was 3 nmol/l and intra-assay variation was <2%. All samples were measured in one batch. (normative values: 0700—1000 h; 170—540 nmol/l)