Psychoneuroendocrinology (2005) **30**, 325–332

ELSEVIER

PNEC

www.elsevier.com/locate/psyneuen

Increased levels of glucocorticoid receptors and enhanced glucocorticoid receptor auto-regulation after hydrocortisone challenge in B-lymphoblastoids from patients with affective disorders

Uwe Henning[a,*], Klaus Krieger[a], Stefan Loeffler[a], Fabio Rivas[b], Guillermo Orozco[b], Manuel G. de Castro[c], Marcella Rietschel[d], Markus M. Noethen[e], Ansgar Klimke[a]

[a]*Neurobiochemical Research Unit, Department of Psychiatry, Heinrich-Heine-University Duesseldorf, Bergische Landstraße 2, D-40629 Duesseldorf, Germany*
[b]*Hospital General Carlos Haya 4, Málaga, Spain*
[c]*Centro de Salud Mental de Velez Málaga, Málaga, Spain*
[d]*Central Institute of Mental Health, J5, D-68159 Mannheim, Germany*
[e]*Department of Medical Genetics, University of Antwerp, B-2610 Antwerp, Belgium*

Received 17 October 2003; received in revised form 24 September 2004; accepted 27 September 2004

KEYWORDS
Blood cells;
Glucocorticoid receptor;
Hydrocortisone;
Affective disorders;
Autoregulation;
Biochemical challenge

Summary The stress response is mediated by a negative feedback effect of glucocorticoids on corticosteroid receptors. Here, we examine the potential contribution of these receptors and their response to a glucocorticoid challenge to dysfunctions of the hypothalamic–pituitary–adrenal axis reported for patients with affective disorders. In a pilot-study, we established B-lymphoblastoid cell lines from patients suffering from affective disorders and healthy subjects and measured the quantity of glucocorticoid receptors at steady state conditions after 12-weeks cell culture. After short-term incubation with 0.1 μM hydrocortisone for 48 h, the decrease of glucocorticoid receptors was also investigated. After 12-weeks cell culture, we found a significantly higher number of cytosolic glucocorticoid receptors in B-lymphoblastoids from patients ($B_{max}=804.9\pm342.5$ fmol/mg protein) compared to those from healthy subjects ($B_{max}=576.9\pm190.3$ fmol/mg protein: $p=0.045$; *t*-test). The increase of the glucocorticoid receptor level in the group of patients could be attributed largely to the higher number of these receptors measured in B-lymphoblastoids of patients suffering from major depressive disorder. The in vitro regulation of glucocorticoid receptors in response to 0.1 μM hydrocortisone for 48 h

* Corresponding author. Tel: +49 211 922 2712.
E-mail address: uwe.henning@lvr.de (U. Henning).

doi:10.1016/j.psyneuen.2004.09.004

resulted in a significantly larger decrease in cultures of B-lymphoblastoids derived from patients (to 32.9±7.5%) than in those from healthy subjects (to 45.8±8.2%). The stronger decrease of glucocorticoid receptors in the group of patients ($p=0.0001$; t-test) was independent of the duration of illness and medication, suggesting a trait-like characteristic of the response.

1. Introduction

The hypothalamic–pituitary–adrenal (HPA) axis is modulated by neuronal and endocrine interactions. A dysfunction of the HPA axis in terms of hypercortisolism has been described for major depressive (Holsboer, 2001) and bipolar disorders (Rush et al., 1996; Rybakowski and Twardowska, 1999) and seems to be accompanied by the dysregulation of glucocorticoid receptors (GCRs) (McQuade and Young, 2000). As an aid to the diagnosis of abnormal neuroendocrine function, especially with respect to GCRs in vivo, the combined dexamethasone suppression/corticotropin-releasing hormone challenge test was introduced (e.g. Heuser, 1998).

Under continuous stress accompanied by high glucocorticoid levels in the blood, feedback regulation of HPA axis activity is mediated by GCRs (De Kloet et al., 1998; Spencer et al., 1998). A decreased GCR transcription activity has been reported from post-mortem studies of patients suffering from affective disorders (Webster et al., 2002). Armanini (1994) demonstrated a corresponding regulation of GCRs in brain and blood cells. Studies of GCR binding in blood cells of patients with major depression showed altered GCR levels, which might be attributed to the technique used for GCR measurement (whole-cell binding versus cytosolic binding). The majority of investigations have revealed no differences between healthy subjects and patients (for details, see Pariante and Miller, 2001).

The immortalization of native human B-lymphocytes by Epstein–Barr virus (EBV) infection is commonly used to establish B-lymphoblastoid cell lines from human individuals (Taylor et al., 1983). In a pilot-study, we used this technique for the quantification of GCRs of B-lymphoblastoids derived from 13 healthy subjects and 14 persons from families with multiple members affected with affective disorders in a steady state condition after cell culture for at least 12 weeks and after short-term incubation with 0.1 μM hydrocortisone for 48 h.

2. Methods

2.1. Subjects

Fourteen patients (11 female, three male; mean age 57.21±16.65 years) with diagnoses of major depressive disorder, recurrent (MDD rec; $n=8$), bipolar disorder I (BPI; $n=4$) and BPII ($n=2$) were included in the study. All patients were interviewed with the Schedule for Affective Disorders and Schizophrenia Lifetime Version (Endicott and Spitzer, 1978). Lifetime 'best estimate' diagnoses according to DSM-IV criteria (APA, 1994) were based on multiple sources of information including the structured interviews, medical records, and family history method. Consensus diagnoses were performed by two psychiatrists/psychologists, and whenever necessary, a further psychiatrist was involved in the decision process. All patients were of Spanish descent and recruited from six large families which lived in a mountainous region of less than 50 km^2 in the southern area of Spain. Diagnosis of BPI requires the occurrence of at least one episode of severe mania during the life time. In BPII a milder form of mania (hypomania) occurs. Patients with MDD rec suffer from depression without ever experiencing episodes of pathologically raised mood. Patients were treated with paroxetine (40 mg/day) and mianserin (10 mg/day), amitriptyline (25–50 mg/day) with or without flupenthixol (1 mg/day) and melitracen (20 mg/day) or with alprazolam (3 mg/day) and lormetazepam (1 mg/day). One patient was under unknown medical treatment and four patients (three MDD, one BPII) were not receiving medication at the time of investigation.

Thirteen non-Spanish healthy controls (six females, seven males; 45.83±9.65 years) were included. Control subjects were healthy according to medical and family history. The ethical committee of the Medical Faculty of the University of Bonn approved the protocol for the genetic study, and the ethical committee of the Medical Faculty of the University of Duesseldorf approved the protocol for the biochemical study.