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Title	Steroid Regulation of T Cell Function Appears Unaltered in Borderline Personality Disorder
Type	Article
URL	https://clock.uclan.ac.uk/17033/
DOI	##doi##
Date	2015
Citation	Fischer, Anja  ORCID: 0000-0002-7872-1292, Grundmann, Johanna, Gold, Stefan M, Spitzer, Carsten and WIngenfeld, Katja (2015) Steroid Regulation of T Cell Function Appears Unaltered in Borderline Personality Disorder. <i>Journal of Personality Disorders</i> , 29 (2). ISSN 0885-579X
Creators	Fischer, Anja, Grundmann, Johanna, Gold, Stefan M, Spitzer, Carsten and WIngenfeld, Katja

It is advisable to refer to the publisher's version if you intend to cite from the work. ##doi##

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STEROID REGULATION OF T CELL FUNCTION APPEARS UNALTERED IN BORDERLINE PERSONALITY DISORDER

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Borderline personality disorder (BPD) is characterized by instability of interpersonal relationships and affection, impulsivity, and cognitive disruptions. Increasing evidence suggests hypothalamic-pituitary-adrenal (HPA) axis alterations in BPD. Changed glucocorticoid sensitivity of peripheral blood mononuclear cells is known in mood and posttraumatic stress disorders, representing frequent comorbidities in BPD. However, to the authors' knowledge, in BPD glucocorticoid sensitivity at the receptor level remains unexplored. Sixteen age-matched female BPD patients were compared to sixteen female healthy controls. In vitro steroid sensitivity of T cell proliferation was tested using aldosterone, dexamethasone, and hydrocortisone. Steroid sensitivity of BPD patients and healthy controls appeared comparable. Psychiatric comorbidities such as major depressive disorder or posttraumatic stress disorder and early life stress seemed to have had no influence on steroid sensitivity parameters. The data suggest unaltered GC sensitivity of T cell function in BPD.

Borderline personality disorder (BPD) is characterized by instability of interpersonal relationships and affection as well as cognitive disruptions and impulsivity beginning in early adulthood. It frequently co-occurs with posttraumatic stress disorder (PTSD) and mood disorders, which are characterized by altered stress-response systems such as the hypothalamic-pituitary-adrenal

This article was accepted under the editorship of Robert F. Krueger and John Livesley.

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Stefan M. Gold is supported by a Heisenberg-Fellowship from the German Research Society (DFG, grant GO 1357/4-1). The research leading to these results has received funding from the European Union Seventh Framework Programme (FP7/2007–2013) [grant number 268381 to Stefan M. Gold]. Katja Wingefeld receives funding from the German Research Society (DFG). This study was supported by grant WI 3396/2-1.

The Inims is supported by the Gemeinnützige Hertie-Stiftung.

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(HPA) axis (Wingenfeld, Spitzer, Rullkotter, & Löwe, 2010). Enhanced basal cortisol concentrations and reduced feedback sensitivity have been frequently but inconsistently reported in BPD. Carvalho Fernando and colleagues (2012) have found evidence for HPA axis involvement in the pathogenesis of BPD, suggesting neuroendocrine similarities to major depressive disorder (MDD) by means of increased basal cortisol concentrations. But importantly, despite elevated cortisol levels in the dexamethasone suppression test (DST), the hypothesized decrease in glucocorticoid (GC) feedback inhibition was not confirmed. Abnormalities in HPA axis challenge tests (such as the Dex-CRH suppression test) or altered circadian cortisol levels are frequently interpreted as indicators of dysfunction at the receptor level (i.e., glucocorticoid receptor [GR] or mineralocorticoid receptor [MR]). However, the multiple systems involved in the complex regulatory feedback loop of the HPA axis complicate the interpretation in terms of receptor function. Intriguingly, human lymphocytes express mineralocorticoid and glucocorticoid receptors (Armanini, Endres, Kuhnle, & Weber, 1988) while the immune system is a major target of endogenous glucocorticoids (Baschant & Tuckermann, 2010). Thus, immune cells can be used to directly examine steroid receptor function *ex vivo*. This approach has been used to study glucocorticoid sensitivity in psychiatric disorders and thus far increased as well as decreased sensitivity has been observed (Pariante, 2004; Rohleder, Wolf, & Wolf, 2010).

To our knowledge, studies of GC sensitivity in the immune system in the context of BPD have not yet been conducted. Therefore, we investigated steroid sensitivity of T cell responses as a potential biological substrate of BPD.

METHODS

SUBJECTS

Age- and education-matched female patients with BPD ($n = 16$) and healthy female control subjects (HC; $n = 16$) were recruited from the Department of Psychosomatic Medicine and Psychotherapy, Schön Klinik Hamburg Eilbek, or via local advertisement (posters at the University Hospital and via the Department home page) and received financial remuneration. All subjects

provided written informed consent. Physical health status was assessed by medical history and careful clinical examination by a staff physician.

Exclusion criteria were a history of severe somatic diseases (e.g., neurological diseases), metabolic diseases (e.g., diabetes), endocrine disorders (e.g., Cushing's syndrome), immune-mediated diseases, hypertension, or current infections. Furthermore, pregnancy, current anorexia, current or lifetime schizophrenia, alcohol or drug dependence, bipolar disorder, schizoaffective disorder, major depression with psychotic symptoms, attention-deficit/hyperactivity disorder, and cognitive impairment resulted in exclusion. The study was approved by the Ethics Committee of the Chamber of Physicians, Hamburg, Germany.

CLINICAL ASSESSMENTS

Patients underwent structured clinical interviews administered by trained psychologists (J.G., K.W.) (Structured Clinical Interview for *DSM* Disorders; SCID; First, Spitzer, Gibbon, & Williams, 1996). Furthermore, patients completed German versions of the Beck Depression Inventory (BDI; Beck, Ward, Mendelson, Mock, & Erbaugh, 1961), the Posttraumatic Diagnostic Scale (PDS; Foa, 1995), and the Borderline Symptom List (BSL; Bohus et al., 2007). To screen for childhood maltreatment, the German Childhood Trauma Questionnaire (CTQ; Klinitzke, Rompell, Häuser, Brähler, & Glaesmer, 2012) was administered.

STEROID SENSITIVITY ASSAY

All blood samples were taken between 8:30 and 9:30 a.m. and processed immediately for cryopreservation. Samples were processed according to standard operating procedures of the biobank of the Institute for Neuroimmunology and Clinical Multiple Sclerosis Research (Inims). Peripheral blood mononuclear cells (PBMCs) were isolated using the Ficoll-Hypaque method and cryopreserved until assayed. After density centrifugation, PBMCs were resuspended in a stepwise procedure in medium 1 (RPMI-1640 + 10% FCS) and medium 2 (40% RPMI-1640, 40% FCS, 20% DMSO) at a concentration of 10^7 viable PBMCs per aliquot (1 mL) and then slowly frozen at an optimal cooling rate of $-1^{\circ}\text{C}/\text{minute}$ using Mr. Frosty® freezing containers (ThermoFisher Scientific, Waltham, MA). After overnight cooling, aliquots were moved to an N₂ storage facility (-180°C). Using this procedure, viability of cells as

determined by trypan blue staining is > 95% for storage times of up to 5 years. For the current study, maximum storage time was 7 months. Samples from BPD patients and HC were run in parallel using the same batch of reagents. Functional steroid sensitivity of T cell function was assessed as previously described (Fischer et al., 2012). Cells were seeded at 2×10^6 viable cells/mL in RPMI-1640 (PAA Laboratories, Pasching, Austria) supplemented with 2 mM L-glutamine and penicillin/streptomycin and stimulated with phytohemagglutinin (PHA) at a concentration of 1 μ g/mL alone or in combination with one of seven concentrations of the agonists dexamethasone (10^{-12} M to 10^{-6} M), aldosterone, or hydrocortisone (each 10^{-11} M to 10^{-5} M). PBMCs were incubated (37°C, 5% CO₂) for 48 hours and then pulsed with 1 μ Ci/well [methyl-³H]thymidine. After another 24 hours, cells were harvested with a Tomtec Harvester 96® Mach III M (Tomtec, Inc., Hamden, CT), and thymidine incorporation was determined by liquid scintillation (Wallac 1450 microbeta, Trilux, PerkinElmer, Rodgau, Germany). Results were averaged from triplicate wells for each condition expressed as counts per minute (cpm).

STATISTICAL ANALYSIS

The inhibitory concentration 50 (IC-50) values representing concentrations inhibiting 50% of the PHA-induced proliferation for each subject and ago-

TABLE 1. Clinical Characteristics as well as Inhibitory Concentration (IC-) 50 for Dexamethasone (DEX), Aldosterone (Aldo), and Hydrocortisone (Cort) of Borderline Personality Disorder (BPD) Patients and Matched Healthy Controls (HC)

	Age	Body height (cm)	Weight (kg)	Smoking (daily consumption)	MDD (y/n)	BDI score	PTSD (y/n)	PDS score	Childhood trauma score
IC-50 Dex [log M]									
IC-50 Aldo [log M]									
IC-50 Cort [log M]									
BPD (n = 16)									
	26.13 + 1.12	170.31 + 1.60	62.84 + 3.16	10.97 + 1.84	08/16	21.85 + 2.84	06/16	21.8 + 3.99	59.08 + 5.18 (n = 16) -8.74 + 0.12 -6.30 + 0.1 -7.24 + 0.12
HC (n = 16)									
	25.81 + 1.23	169.31 + 1.81	69.25 + 3.47	6.19 + 1.62	0/16	6.03 + 1.62	0/16	7.27 + 2.92	38.00 + 2.97 (n = 16) -8.71 + 0.12 -6.2 +

0.08 7.34 + 0.14

***p* value**

.72 .52 .14 .06

.00

.02 < .01

p* value *d .89 0.06 .42 0.03 .59 0.02

Note. Data are presented as mean + standard error of mean (SEM); BDI = Beck Depression Inventory, MDD = major depressive disorder, PTSD = posttraumatic stress disorder score, yes/no, PDS = Posttraumatic Stress Diagnostic Scale.

nist were determined by nonlinear sigmoidal curve fitting utilizing Prism® Software as described by Fischer et al. (2012). All variables were tested for normality and equality of variance. Student *t* tests were conducted in order to detect differences between the BPD and healthy control (HC) groups using PASW Statistics 18 software, with *p* values < 0.05 considered significant.

RESULTS

Patients with BDP and HC were well matched for age, body height, and weight, while there was a marginally significant difference for smoking (Table 1). In the patient group, patients met, on average, six of the *DSM-IV* criteria for BPD. In addition, eight BPD patients met *DSM-IV* criteria for MDD and six were diagnosed with PTSD (Table 1). Four of these BPD patients had both comorbidities. While seven of the BDP patients were untreated, several participants in the patient group were taking psychotropic medication. These included atypical antipsychotics (Quetiapine, $n = 4$; Olanzapine, $n = 1$) as well as antidepressive treatment (noradrenergic and specific serotonergic antidepressants, $n_{\text{Mirtazapine}} = 1$; Selective serotonin reuptake inhibitors, $n_{\text{Citalopram}} = 2$, $n_{\text{Fluoxetine}} = 1$; tricyclic antidepressants, $n_{\text{Trimipramine}} = 1$; serotonin and norepinephrine reuptake inhibitors, $n_{\text{Venlafaxine}} = 1$). HC were free of psychiatric disorders according to SCID.

As expected, BPD patients had significantly higher scores on self-report measures of depression (BDI), childhood trauma (CTQ), and PTSD (PDS) compared to HC. There were no significant differences between BPD and HC in T cell steroid sensitivity as measured by IC-50 values with any of the three agonists (see Table 1 and Figure 1). Exploratory analyses revealed

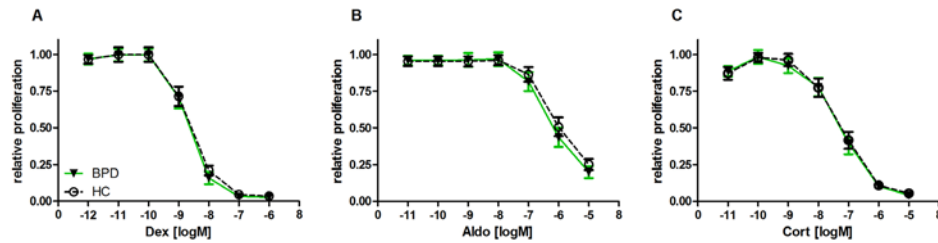


FIGURE 1. Steroid sensitivity in borderline personality disorder and healthy controls. Relative proliferation (counts per minute [cpm]/ PHA control) in the presence of PHA (present in all conditions) and different concentrations of (a) dexamethasone (Dex) (b) aldosterone (Aldo), and (c) hydrocortisone (Cort) both in individuals with Borderline personality disorder (BPD) and in healthy controls (HC).

no correlations between IC-50 values and measures of BPD symptoms as measured by the BSL ($p_{\text{dex}} = .68$, $p_{\text{aldo}} = .32$, $p_{\text{Cort}} = .99$), depression severity as indicated by BDI scores ($p_{\text{dex}} = .79$, $p_{\text{aldo}} = .38$, $p_{\text{Cort}} = .94$), the occurrence of childhood trauma according to the CTQ score ($p_{\text{dex}} = .32$, $p_{\text{aldo}} = .77$, $p_{\text{Cort}} = .93$), or PTSD symptoms (PDS score, $p_{\text{dex}} = .48$, $p_{\text{aldo}} = .68$, $p_{\text{Cort}} = .85$).

DISCUSSION

To our knowledge, this is the first study assessing steroid sensitivity in the immune system in the context of BPD. Our results indicate no apparent alterations in T cell steroid sensitivity in BPD. As expected, MDD and PTSD comorbidity was frequent in the patient group. However, GC sensitivity was not associated with depressive symptoms, PTSD severity, or early life stress (ELS). The cortisol suppression capacity in the DST is considered a measure of the HPA axis GR-mediated negative feedback function. HPA axis hyporesponsiveness with lower basal cortisol levels and pronounced cortisol suppression in the DST have also been reported in BPD, but the majority of studies support the notion of a hyperreactive HPA system (Carvalho Fernando et al., 2012). Furthermore, HPA hyperresponsiveness as measured by the Dex-CRH test was found in BPD patients with a history of chronic abuse in early childhood (Rinne et al., 2002). The authors hypothesized that ELS accounts for this altered HPA state in BPD. Importantly, Carvalho Fernando et al. (2012) have found increased cortisol levels in the DST in BPD, as well as an association between cortisol release and childhood trauma. In contrast to basal and stimulated cortisol (i.e., regulation of the HPA axis itself), we did not find evidence for an association between childhood trauma and GC sensitivity in

peripheral immune cells (i.e., a major target tissue of endogenous glucocorticoids). Our data suggest that GC sensitivity of T cell function is unaltered in BPD, which may indicate a tissue-specific dysregulation of steroid signaling. In vitro inhibition of cell proliferation has previously been used to reveal differences in GC sensitivity of T cells as a biological substrate of depressive symptoms in patients with multiple sclerosis, a neuro-inflammatory disease of presumably T cell-driven origin (Fischer et al., 2012). Similarly, studies have shown altered GC sensitivity of T cell proliferation in patients with PTSD (de Kloet et al., 2007) and major depression (Lowy, Reder, Gormley, & Meltzer, 1988; Wodarz et al., 1991). This would suggest that the assay system is specific and sensitive to pick up GC sensitivity differences in T cell function in autoimmune and psychiatric disorders, but that T cell regulation by GCs is unaltered in BPD. However, our results do not rule out the possibility that other immune cell subpopulations of the innate (e.g., monocytes, NK cells) or adaptive immune system (e.g., B cells) may show altered GC regulation in BPD. This remains to be elucidated with appropriate functional assay systems.

BPD is a very heterogeneous disorder, and it becomes increasingly clear that comorbidities and clinical features such as trauma history may have variable, interacting influences on the neuroendocrine profile in BPD (Zimmerman & Choi-Kain, 2009). However, GC sensitivity measures were not correlated to severity of depression, severity of PTSD symptoms, or CTQ scores in our study. It has been previously reported that elevated free cortisol levels in BPD patients are positively correlated to MDD severity and negatively associated with PTSD symptomatology (Wingenfeld, Driessen, Adam, & Hill, 2007). In a longitudinal study, DST results were identified as a stable marker of abnormal HPA axis function in BPD with comorbid PTSD, resulting in more pronounced suppression (Wingenfeld, Lange, et al., 2007). In another study, it was shown that acute hydrocortisone administration had beneficial effects on hippocampus-relevant memory in BPD, while in healthy controls hydrocortisone impaired memory retrieval (Wingenfeld et al., 2013). These results were interpreted in terms of a cortisol-induced enhancement of hippocampal functioning, which might be related to a sensitization of GR function. Possibly, in BPD, altered GR function might be more tissue specific and restricted to CNS GR (e.g., in the hippocampus and hypothalamus) as well as the pituitary.

The present study has several limitations. Due to the small sample size, lack of

power is obviously an issue, and the absence of statistically significant differences could reflect this fact rather than absence of a biological effect. Therefore, we have also provided effect sizes, which were all well below the accepted thresholds for small effects (Table 1). In addition, the overlapping steroid sensitivity curves and the small error bars observed (Figure 1) argue against a biologically significant effect. Given these considerations and the very low effect sizes obtained in this pilot study, we believe that BPD is unlikely to be linked to altered steroid sensitivity of T cell responses. In addition, in this small pilot study, we did not obtain measures of GC regulation in other systems, most notably in the HPA axis itself. Therefore, future studies should assess HPA axis regulation (such as the Dex-CRH test) in parallel with functional assays of GC sensitivity in important target tissues of endogenous cortisol, such as cells of the innate and adaptive immune system.

In conclusion, our study suggests unaltered GC sensitivity in T cells in BPD, a major compartment of the adaptive immune system.

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