Psychoneuroendocrinology (2005) **30**, 297–306

PNEC

www.elsevier.com/locate/psyneuen

Glucocorticoid receptor polymorphisms and post-traumatic stress disorder

Anthony W. Bachmann[a], Teresa L. Sedgley[a], Richard V. Jackson[c], John N. Gibson[b], Ross McD. Young[d], David J. Torpy[e,*]

[a]*Department of Medicine, University of Queensland, Greenslopes Private Hospital, Brisbane, Qld 4120, Australia*
[b]*Department of Psychiatry, Greenslopes Private Hospital, Greenslopes, Qld, Australia*
[c]*Department of Medicine, Logan Hospital, Meadowbrook, Qld 4131, Australia*
[d]*School of Psychology and Counselling, Faculty of Health, Queensland University of Technology, Carseldine, Qld 4034, Australia*
[e]*Endocrine and Metabolic Unit, Royal Adelaide Hospital, Adelaide, SA 5000, Australia*

Received 30 March 2004; received in revised form 22 July 2004; accepted 9 August 2004

KEYWORDS
Glucocorticoid receptor polymorphisms;
Glucocorticoid sensitivity;
Post-traumatic stress disorder;
Cortisol;
Low-dose dexamethasone suppression test;
Dermal vessel vasoconstrictor assay

Summary Post-traumatic stress disorder (PTSD) is reported in some studies to be associated with increased glucocorticoid (GC) sensitivity. Two common glucocorticoid receptor (GR) polymorphisms (N363S and *BclI*) appear to contribute to the population variance in GC sensitivity. There is some evidence that there may be a genetic predisposition to PTSD. Hence we studied 118 Vietnam war veterans with PTSD for (i) GR polymorphisms, particularly the N363S and the *BclI* polymorphisms which are thought to be GC sensitising, and (ii) two measures of GC sensitivity, the low-dose 0.25 mg dexamethasone suppression test (LD-DST) and the dermal vasoconstrictor assay (DVVA). The DST and GR polymorphisms were also performed in 42 combat exposed Vietnam war veterans without PTSD.

Basal plasma cortisol levels were not significantly different in PTSD (399.5 ± 19.2 nmol/L, $N=75$) and controls (348.6 ± 23.0 nmol/L, $N=33$) and the LD-DST resulted in similar cortisol suppression in both groups (45.6 ± 3.2 vs. $40.8 \pm 4.1\%$). The cortisol suppression in PTSD patients does not correlate with Clinician Administered PTSD Scores (CAPS), however there was a significant association between the *BclI* GG genotype and low basal cortisol levels in PTSD ($P=0.048$). The response to the DVVA was similar to controls (945 ± 122, $N=106$ vs. 730 ± 236, $N=28$, $P=0.42$). PTSD patients with the GG genotype, however, tended to be more responsive to DVVA and in this group the DVVA correlated with higher CAPS scores. The only exon 2 GR polymorphisms detected were the R23K and N363S. Heterozygosity for the N363S variant in PTSD, at 5.1% was not more prevalent than in other population studies of the N363S polymorphism in Caucasians (6.0–14.8%). The GG genotype of the *BclI* polymorphism found to be associated with increased GC sensitivity in many studies

* Corresponding author. Tel.: +61 8 8222 5183; fax: +61 8 8222 5908.
E-mail address: dtorpy@mail.rah.sa.gov.au (D.J. Torpy).

0306-4530/$ - see front matter
doi:10.1016/j.psyneuen.2004.08.006

showed a tendency towards increased response with DVVA and correlated with higher CAPS scores.

In conclusion, the N363S and *Bcl*I GR polymorphisms were not more frequent in PTSD patients than controls and reported population frequencies. Our PTSD group did not display GC hypersensitivity, as measured by the LD-DST and DVVA. In a subset of PTSD patients with the *Bcl*I GG genotype, CAPS scores and basal cortisol levels were negatively correlated.

1. Introduction

Post-traumatic stress disorder (PTSD) is a disabling syndrome that affects many combat exposed Vietnam war veterans. It appears to develop in biological predisposed individuals after exposure to life threatening stress. The stress system in PTSD patients is chronically dysregulated, characterized by a heightened reactivity of the sympathetic nervous system (Pitman et al., 1990) and alterations in the hypothalamic–pituitary–adrenal (HPA) axis resulting in relative hypocortisolism (Yehuda et al., 1990, 1995; Boscarino, 1996). Enhanced sensitivity to feedback inhibition by glucocorticoids (GC) could account for the low cortisol levels and several investigators have found that, in PTSD patients, cortisol levels fall excessively ('hypersuppressible') after low-dose dexamethasone (DEX) (Yehuda et al., 1995; Stein et al., 1997). Further evidence for increased GC sensitivity in PTSD comes from the findings of a blunted adrenocorticotropic hormone (ACTH) response to corticotrophin-releasing factor (CRF) in PTSD (Smith et al., 1989) and an exaggerated ACTH response to metyrapone in PTSD compared to normal subjects (Yehuda et al., 1996).

Evidence for a genetic predisposition to PTSD comes from results in twin studies of Vietnam war veterans where it was found that genetic factors accounted for up to 30% of variability in PTSD symptoms and monozygotic twins were more highly concordant for combat exposure than dizygotic twins (True et al., 1993). It has also been noted that there is a trend for increased GC sensitivity in unaffected first degree relatives of PTSD patients (Yehuda et al., 2002).

Recent studies have found a glucocorticoid receptor (GR) polymorphism in exon 2 (N363S) which alters the N-terminal transactivation domain to be associated with GC hypersensitivity, increased body mass index (BMI) and low bone mineral density (Huizenga et al., 1998; Lin et al., 1999). In addition, the *Bcl*I polymorphism located in intron 2, has been associated with high blood pressure, insulin sensitivity, body mass index and abdominal fat distribution (Weaver et al., 1992; Buemann et al., 1997) as well as increased GC sensitivity, determined by the skin blanching response to topical GCs and increased cortisol suppression after low-dose DEX (Panarelli et al., 1998; van Rossum et al., 2002).

The acute cortisol response to a standardized psychosocial stressor was enhanced in 10 N363S carriers and diminished in 18 *Bcl*I GG carriers compared to controls, suggesting that these common GR polymorphisms may alter real-life stress responses (Wust et al., 2004).

We therefore hypothesised that the mechanism of central GC hypersensitivity observed in PTSD might involve altered GR function, due to inherited polymorphisms, leading to increased receptor sensitivity. We determined the genotypes for the GR polymorphisms N363S and *Bcl*I in Vietnam veterans with PTSD and combat exposed controls and then examined the relationship between the genotypes and measures of central (hypothalamic-pituitary unit) and peripheral (microvasculature) GC sensitivity as assessed by low-dose DEX suppression test (DST) and the dermal vessel vasoconstrictor assay (DVVA).

2. Materials and methods

2.1. Subjects

One hundred and eighteen combat exposed Vietnam veterans (mean age $\pm$ SD; 55.7 $\pm$ 4.2; 42–68 yr) were recruited from a nationally accredited inpatient and outpatient PTSD treatment program conducted at the Keith Payne Unit, Greenslopes Private Hospital, Brisbane, Australia. Diagnosis of PTSD was made using the Clinician Administered PTSD Scale (CAPS) for DSM-IV (current diagnostic version) and psychiatric interview. The mean CAPS score obtained for this group was 79.4 $\pm$ 14.4 (SD) with a range of 36–109 (maximum possible score = 136). Patients diagnosed with other active psychiatric disorders were excluded from the program. Patients abstained from substance abuse during

the program, however, medication was not ceased. Psychotropic medications do not influence the molecular studies but might affect neuroendocrine tests; however, previous studies have indicated little effect from the commonly employed selective serotonin re-uptake inhibitors (SSRIs) (Torpy et al., 1997).

Combat exposed non-PTSD volunteers were recruited by newspaper advertisement and through the co-operation of Vietnam veterans' regiment associations. Forty-two subjects (mean age $\pm$SD; 61.2 $\pm$ 7.1; 53–76 yr) were recruited. They were interviewed for general medical history and health status was assessed with routine blood tests. Features of metabolic syndrome (diabetes, hypertension, central adiposity and hyperlipidaemia) were noted. Exclusion of PTSD and other psychiatric disorders was determined by CAPS and clinical interview. A CAPS score range of 0–28 (mean $\pm$SD; 8.5 $\pm$ 7.9) was obtained for this group. The study was approved by the Greenslopes Private Hospital and University of Queensland Human Ethics Committees and written informed consent was obtained from all subjects.

2.2. Study protocol

On day 1 between 1500 and 1700 h a skin patch for the DVVA was applied and removed the following morning at 0800 h for reading. After an overnight fast blood was collected on day 3 from an antecubital vein for DNA and basal cortisol levels. After taking DEX at 2300 h on day 3, and a post-DEX blood sample was collected at 0800 h on day 4.

2.3. Dermal vessel vasoconstrictor assay (DVVA)

The dermal vessel vasoconstrictor assay (DVVA) was used as it is one of the few reported in vivo tests of peripheral GC sensitivity. DVVA responses have also been shown to vary in relation with the *Bcl*I GR polymorphism (Panarelli et al., 1998). Solutions of beclomethasone dipropionate (Sigma-Aldrich Pty Ltd, Castle Hill, NSW, Australia) were prepared in propylene glycol/water/ethanol (49:49:2, vol/vol) at concentrations of 0, 5, 10, 50 and 100 ug/mL. A template containing five circles to accommodate 18 mm diameter Whatman^{R} filter paper (Whatman International Ltd, Maidstone, England) was fixed on the volar aspect of the non-dominant forearm. Between 1500 and 1700 h a pre-test erythema index was measured at each site by reflectance spectrophotometry (Erythemameter, Diastron, UK). The beclomethasone solutions (50 μL) were then applied to five separate filter papers in random order and covered with a waterproof transparent Tegaderm™ dressing (3M Health Care, St Paul, MN). The dressing was removed the following morning at 08:00 h and the blanching response measured as a decrease in erythema index. A corrected erythema index response was calculated by subtracting the pre-test difference between each concentration site and the zero beclomethasone site from the comparable difference measured on the day following application of the steroid. This corrects for forearm site differences due to variation in blood flow and variations with time. From these delta values area under the curve (AUC) estimations were calculated for each subject over the full range of steroid concentrations using the trapezoidal rule. A small number of tests were excluded from analysis due to a complete lack of blanching response or an extreme erythematous reaction on Tegaderm removal. Subjects using systemic or topical GCs were also excluded from test.

2.4. Low-dose DST

Initial studies with 0.5 mg DEX produced complete cortisol suppression ($<$25 mmol/L) in controls so the 0.25 mg dose was used as described in a previous report of PTSD patients (Yehuda et al., 1995). After an overnight fast subjects had venous blood collected into EDTA-containing tubes at 08:00 h for plasma cortisol measurement. Subjects were given 0.25 mg DEX (half a 0.5 mg tablet of DEX) and instructed to ingest it at 2300 h that evening. The following morning a post-DEX blood sample was collected at 0800 h. Pre- and post-DEX plasma cortisol concentrations were determined by RIA (Diagnostic Systems Laboratories Inc., Webster, TX). Intra- and inter-assay variations of the assay were 6.1 and 8.0%, respectively, and the cortisol sensitivity was 3 nmol/L. Subjects diagnosed with diabetes were excluded from this test.

2.5. Genetic studies

Amplification of the GR exon 2. Genomic DNA was extracted from peripheral blood leukocytes using a commercial method (Nucleon BACC2, Amersham Biosciences Pty Ltd, Baulkham Hills, NSW, Australia). Exon 2 of the GR was amplified by PCR using 1 μg of genomic DNA in a reaction volume of 100 μL containing sterile ultra-pure water, 10$\times$ buffer, 3 mM $MgCl_2$, 2 U *Taq* DNA polymerase (Qiagen Pty Ltd, Clifton, Vic., Australia), 200 μM each of dNTPs and 300 nM each of specific exon 2 GR primers 5′-TCG GAT CAG GAA GAT AAT GTG A-3′ (forward)

and 5′-CAA AAG GCC ACT TAA ACT TAT TCA-3′ (reverse). The PCR conditions used were one cycle of denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1.5 min, extension at 72 °C for 1.5 min and a final cycle at 72 °C for 5 min. The PCR product (1404 bp) was purified for sequencing using the NucleoSpin^{R} Extract kit (Macherey-Nagel, Duren, Germany).

Exon 2 GR polymorphisms. GR polymorphisms were detected by automated direct sequencing using an ABI 377 DNA sequencer (PE Applied Biosystems, Foster, CA) and a dideoxy cycle sequencing protocol in the presence of specific primers and fluorescent labelled dideoxy terminators (Sanger et al., 1977). To ensure accuracy of mutation detection in exon 2 of the GR four overlapping sequence reactions were employed. In addition to the GR PCR primers the following sequencing primers were used: 5′-CGG TAA AAT GAG AGG CTT GC-3′ and 5′-GGC GGG AGA AGA CGA TTC-3′.

Genotyping of GR BclI RFLP. Intron 2 primers were designed for a PCR product (335 bp) to include the *Bcl*I RFLP C to G mutation 646 bp downstream of exon 2. PCR was performed using 0.5 μg genomic DNA in a 50 μL reaction containing sterile water, PCR buffer (10×), Q-solution (5×), 1 U *Taq* DNA polymerase (Qiagen), 200 uM each of dNTPs and 300 nM each of the following primers 5′-TGC TGC CTT ATT TGT AAA TTC GT-3′ (forward) and 5′-AAG CTT AAC AAT TTT GGC CAT C-3′. The PCR conditions used were the same as those employed for the GR PCR. The genotypes were determined by *Bcl*I digestion (15 U) for 1.5 h at 50 °C and subsequent separation on 2% agarose gels. The CC genotype produced two bands (221 and 117 bp), the CG genotype three bands and the GG genotype remained undigested (Fig. 1).

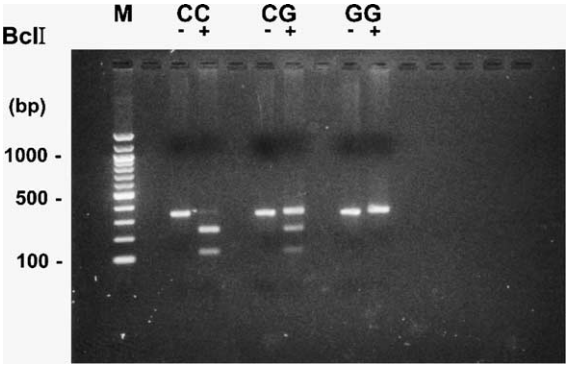

Figure 1 Results of a *Bcl*I restriction enzyme digest of a 335 bp PCR product from intron 2 of the GR. This DNA product contains a C to G mutation 646 nucleotides downstream from exon 2. Lane 1 shows a DNA ladder with as 100 bp interval. Lanes 2, 4 and 6 show undigested product, lane 3 the two bands (117 and 222 bp) of the CC homozygote, lane 5 the three bands (117, 222 and 335 bp) of the CG heterozygote and lane 7 the undigested GG homozygote.

2.6. Statistical analyses

All descriptive statistics and analyses were performed using the Statistica computer application (Statsoft, Tulsa, OK). Differences in measures of baseline cortisol and responses to low-dose DST between PTSD and controls were compared with two-tailed, unpaired *t*-tests. Correlations between CAPS and various parameters were measured using the Pearson product-moment correlation coefficient. The AUC for erythema index from the DVVA was calculated by the trapezoidal method and differences in genotypes between the study groups were analysed with two-tailed, unpaired *t*-tests. Allelic frequencies for GR polymorphisms in PTSD and controls were compared by computing a R by C table chi-square statistic. All results are expressed as mean ± SE, unless otherwise indicated as standard deviation (SD). *P* values ≤ 0.05 were considered to be significant.

3. Results

3.1. GR polymorphisms

Sequence analyses of the GR exon 2 were performed in 118 PTSD and 42 control subjects. Allelic frequencies for PTSD and controls for previously reported mutations (5 missense and 2 silent polymorphisms) are shown in Table 1. N363S polymorphism carriers were detected at a rate of 5.1% in PTSD compared to 2.4% in controls ($\chi^2 = 0.54$, $P = 0.46$). Similarly the R23K polymorphism was found only in heterozygous form and occurred in linkage disequilibrium with a silent nucleotide substitution in codon 22 (Koper et al., 1997). The R23K carrier frequency in PTSD was not significantly different to that in controls (5.1 vs 9.5%, $\chi^2 = 1.04$, $P = 0.31$). No additional mutations in exon 2 were detected and the variants F29L, L112F and D233N first reported in puerperal psychosis and schizophrenia (Feng et al., 2000) were not identified in our study populations. The GR genotypes (CC, CG and GG) resulting from the *Bcl*I polymorphism were determined in 118 PTSD and 42 control subjects. The genotype frequencies for both PTSD (CC, 0.47; CG, 0.42; GG, 0.11) and controls (CC, 0.36; CG, 0.48; GG, 0.16) were consistent with the Hardy-Weinberg equilibrium

Table 1 Glucocorticoid receptor polymorphisms in exon 2 and allelic frequency in PTSD and controls.

Nucleotide change	Codon change	Amino acid change	Allele frequency[a]	
			PTSD ($N=118$)	Controls ($N=42$)
G198A	GAG > GAA	E22E	6/236 (2.5)	4/84 (4.8)
G200A	AGG > AAG	R23K	6/236 (2.5)	4/84 (4.8)
C219G	TTC > TTG	F29L	0/236 (0)	0/72 (0)
C446T	CTT > TTT	L112F	0/236 (0)	0/72 (0)
G829A	GAT > AAT	D233N	0/236 (0)	0/72 (0)
G1011A	AAG > AAA	K293 K	0/236 (0)	0/72 (0)
A1220G	AAT > AGT	N363S	6/236 (2.5)	1/84 (1.2)

[a] Shown as the number of affected alleles compared to total alleles with percentage in parentheses.

and the genotype distributions were not significantly different between the two groups ($\chi^2=1.83$, $P=0.40$).

3.2. Low-dose DST and GR polymorphisms

The morning fasting baseline cortisol concentrations in PTSD and controls were not significantly different (Table 2, 399.5±19.2 nmol/L, $N=75$ vs. 348.6±23.0 nmol/L, $N=33$) and over 50% of measurements were below 400 nmol/L in both groups. The Δ cortisol response to low dose DEX (0.25 mg) was not significantly different to controls (Table 2, 192.8±16.9 vs. 146.0±17.8 nmol/L). A comparable range of % cortisol suppression was observed in PTSD (−26.0 to 92.3%) compared to controls (−33.6 to 83.1%). The difference between the number of hyper-suppressors, that is, subjects suppressing cortisol below 140 nmol/L observed in PTSD (37.3%) and controls (27.3%) was not significant ($\chi^2=1.03$, $P=0.31$). For the PTSD group no significant correlations were found between CAPS scores, basal and post-DEX plasma cortisol levels or % cortisol suppression.

Table 3 shows cortisol levels in PTSD before and after administration of 0.25 mg DEX for the N363S alleles and the *Bcl*I genotypes, respectively. For both polymorphisms there was no difference in baseline cortisol levels. However, CAPS scores tended to be greater for the GG genotype compared to CC and CG (84.3 vs. 79.6 and 78.7) and a significant negative correlation was found between CAPS and basal cortisol levels for the GG genotype ($r=-0.71$, $P=0.048$). The mean decrease in cortisol for the N363S carrier was not significantly different from that observed in the non-N363 carrier (68.6±86.2 vs. 199.8±17.0 nmol/L). Similarly no significant difference in Δ cortisol levels between the CC (196.9±30.2 nmol/L), CG (188.7±23.6 nmol/L) and GG (195.0±31.0 nmol/L) *Bcl*I genotypes was found in PTSD.

3.3. DVVA and GR polymorphisms

The blanching response of the forearm vasculature to topical beclomethasone as measured by a decrease in erythema index (an increased AUC) was not significantly different in PTSD than in controls (945±122 vs 730±236, $P=0.42$). N363 allelic status did not alter the response to DVVA in the PTSD group (Table 3). In PTSD the *Bcl*I GG genotype (1400±300) was not significantly different to beclomethasone than the CC (873±169) and CG (901±206) genotypes (Table 3). The GG genotype did not show a positive correlation between the CAPS score and the DVVA response ($r=0.55$, $P=0.12$).

4. Discussion

This is the first study examining the possibility that PTSD may be associated with altered frequencies of GR polymorphisms. We did not find altered

Table 2 Cortisol responses to low-dose DEX and changes in erythema index after DVVA as measures of glucocorticoid sensitivity in PTSD and controls.

	PTSD ($N=75$)	Controls ($N=33$)
Fasting cortisol (nmol/L)	399.5±19.2	348.6±23.0
Post DEX cortisol (nmol/L)	206.7±14.0	202.6±16.4
Δ Cortisol (nmol/L)	192.8±16.9	146.0±17.8
% Cortisol suppression	45.6±3.2	40.8±4.1
Δ Erythema index (AUC)[a]	945±122 ($N=106$)	730±236 ($N=28$)

Values are mean±SE

[a] Area under the curve (AUC) calculated using the trapezoidal method and represents the decrease in erythema index of test beclomethasone concentrations from zero corrected for differences occurring with both time and test sites. These were not different between PTSD patients and controls.

Table 3 Measures of glucocorticoid sensitivity for GR polymorphisms N363S and *Bcl*I in PTSD.

	N363N ($N=71$)		N363S ($N=4$)
Fasting cortisol (nmol/L)	403.3±20.2		332.9±18.5
Post DEX cortisol (nmol/L)	203.4±14.2		264.2±77.4
Δ Cortisol (nmol/L)	199.8±17.0		68.6±86.2
% Cortisol suppression	47.1±3.0		18.1±25.0
CAPS scores[a]	79.2±15.0		85.5±16.9
Δ Erythema index (AUC)[b]	966±127 ($N=100$)		596±461 ($N=6$)
	*Bcl*I genotypes		
	CC ($N=31$)	CG ($N=35$)	GG ($N=9$)
Fasting cortisol (nmol/L)	402.3±28.3	401.1±32.3	383.9±28.9
Post DEX cortisol (nmol/L)	205.4±18.5	212.4±23.6	188.9±38.9
Δ Cortisol (nmol/L)	196.9±30.2	188.7±23.6	195.0±31.0
% Cortisol suppression	42.8±5.8	46.3±4.0	52.6±7.7
CAPS score[a]	79.6±16.2	78.7±13.7	83.4±17.2
Δ Erythema index (AUC)[b]	873±169 ($N=49$)	901±206 ($N=45$)	1400±300 ($N=12$)

A significant negative correlation was found between CAPS and basal cortisol levels for the GG genotype ($r=-0.71$, $P=0.048$). All values except CAPS scores (mean±SD) are mean±SE.

[a] Missing CAPS data; N363N ($N=64$), CC ($N=29$), CG ($N=31$), GG ($N=8$).

[b] Area under the curve (AUC) calculated using the trapezoidal method and represents the decrease in erythema index of test beclomethasone concentrations from zero corrected for differences occurring with both time and test sites.

frequencies of common GR polymorphisms in PTSD. In addition, the measures of GC sensitivity in our PTSD patients, the LD-DST and the DVVA test, were normal, in contrast to expectation based on other reports (Yehuda, 1997). A subgroup of PTSD patients with the *Bcl*I GG genotype tended to be more responsive to DVVA and have higher Clinician Administered PTSD Scale (CAPS) scores that were significantly negatively correlated with basal plasma cortisol levels. However, the *Bcl*I GG genotype group was small and this finding needs verification in a different patient group.

Lower urinary and plasma cortisol levels in PTSD compared to controls have been documented but a number of studies have not shown this difference (Yehuda, 2002). In this study baseline plasma cortisol levels in 75 subjects with PTSD and 33 controls were similar (399.5±19.2 vs 348.6± 23.0 nmol/L) and comparable with mean values (range 127–596 nmol/L) obtained from other PTSD studies (Yehuda, 2002). Single plasma measurements are not regarded as the most reliable estimates of cortisol because of some variability caused by transient stressors, however this is unlikely to be responsible for the above finding as all samples were collected under carefully controlled fasting conditions.

In this study an increased sensitivity to DEX in PTSD was not found. We observed similar levels of cortisol suppression to 0.25 mg DEX in PTSD (45.6± 3.2%) and to controls (40.8±4.5%); the percentage of hyper-responders (suppression <140 nmol/L) also did not differ significantly. We found that the 0.25 mg DEX dose was more sensitive in distinguishing hyper-responders compared to 0.5 mg DEX used in our initial studies where most subjects suppressed by greater than 80% (results not shown). Enhanced suppression of cortisol in trauma survivors with PTSD compared to non-exposed subjects has been documented in a number of studies after administration of both 0.25 and 0.5 mg DEX (Yehuda et al., 1995, 2002; Stein et al., 1997). However, in another study on adolescent earthquake victims enhanced suppression to DEX was observed at 4 pm in the group with more severe PTSD symptoms who had been closer to the epicentre compared to those further from the epicentre (Goenjian et al., 1996). The degree of suppression for these two groups was similar at 8 am. This finding suggests that the DEX suppression may be prolonged in PTSD. In a comparable study to ours using 0.25 mg DEX (Yehuda et al., 1995) the level of cortisol suppression in combat veterans with PTSD was higher than that observed in our combat veterans (54.4 vs 45.6%) and lower (36.7 vs 40.8%) for combat veterans without PTSD. The results observed in our study may be a reflection on the different modes of recruitment of the two groups. The controls were generally an older group who might have had a reduced metabolic clearance of DEX compared to PTSD veterans resulting in relatively greater suppression. This was not confirmed in this

study as circulating DEX levels were not measured. However in a study on the variability and sensitivity of a low-dose DST in an elderly population the relationship between pre- and post-DEX cortisol concentrations was found to be strongly associated with the actual DEX levels (Huizenga et al., 1998b). Even though the DST has been fairly ubiquitously applied to evaluate GC feedback sensitivity some reservations about its validity have been expressed. These concerns relate to the different affinities of DEX and cortisol for GR subtypes (de Kloet, 1991) and the level of the HPA axis at which inhibition is recognised being primarily at the pituitary for DEX as it does penetrate well into the brain (Miller et al., 1992).

There is some evidence that variability in GC sensitivity is tissue-specific and not a trait affecting all tissues equally (Ebrecht et al., 2000). Hence, we performed a central measure of GC sensitivity (DST), and a peripheral assessment of GC sensitivity (DVVA). As a measure of GC-induced dermal vasoconstriction the resultant skin blanching has proved useful in assessing GC potency and sensitivity. Using this methodology patients with essential hypertension have been shown to exhibit increased skin blanching compared to normotensive subjects (Walker et al., 1996) and in another study it was found that the intensity of blanching correlated with the therapeutic sensitivity to GCs in asthma (Brown et al., 1991). We did not find a significant difference in the DVVA response between veterans with PTSD and the control group even though the overall response to topical beclomethasone was greater in PTSD. Consistent with the notion that considerable variation in GC sensitivity exists across individuals we observed a wide range of inter-individual responses to DVVA in both groups.

A number of mutations in the GR have been identified that show associations with GC sensitivity (Huizenga et al., 1998a; Panarelli et al., 1998; van Rossum et al., 2002). In particular the N363S and the *Bcl*I polymorphisms were investigated to see whether these mutations might predispose to an increased GC sensitivity observed in some PTSD subjects. To determine the frequency of these GR mutations, exon 2 containing the N-terminal domain involved in modulating transcriptional activation was sequenced. The only polymorphisms detected were the R23K linked to phenotypes ranging from asymptomatic to GC resistant (Koper et al., 1997) and the N363S carrier associated with GC hypersensitivity (Huizenga et al., 1998a). In our groups we did not detect the F29L, L112F and D233N variants first described in study on patients diagnosed with puerperal psychosis and schizophrenia (Feng et al., 2000) or any other new mutations. The allelic frequencies of these polymorphisms were not significantly different between the PTSD and control groups and we did not find an enhanced presence of the N363S carrier genotype in PTSD (5.1%) compared to those observed (6–9.6%) in other studies (Huizenga et al., 1998a; Lin et al., 1999; Dobson et al., 2001; Rosmond et al., 2001). Using two measures of GC sensitivity (low-dose DST and DVVA) no significant associations between N363S carrier status and increased sensitivity to GCs were found in the PTSD group. It is difficult to interpret the results of the control group as only one N363S carrier was detected. In the literature there are mixed findings linking this asparagine to serine point mutation to increased GC sensitivity. Studies in an elderly Dutch population found the N363S was associated with enhanced suppression of cortisol to DEX, increased BMI and a lowered bone mineral density in the lumbar spine (Huizenga et al 1998a). Similarly in British (Dobson et al., 2001) and Australian studies (Lin et al., 1999) significant associations between the N363S polymorphism and greater waist–hip ratio in males and obesity (BMI $>$ 25), respectively. In contrast other studies in Danish (Echwald et al., 2001) and Swedish men (Rosmond et al., 2001) did not find any association between the N363S mutation, obesity, and the degree of cortisol suppression with DEX. The different genetic backgrounds and/or the gender composition of the populations studied could have contributed to these disparate findings. These variable results therefore make it difficult to conclude whether the N363S GR polymorphism is a reliable genetic marker of GC sensitivity. The exact molecular mechanism of how the N363S variant might cause increased GC sensitivity is not known; however, it is proposed that new serine residue allows for modulation of the phosphorylation state of the receptor and thereby altering protein interactions with transcription factors. Even though there is good evidence of increased GC sensitivity in the N363S from a number of in vivo studies, in vitro studies on the N363S polymorphism found no effects on its ability to bind hormone or activate transcription (Gaitan et al., 1995; de Lange et al., 1997).

The *Bcl*I polymorphism in the GR previously characterised as the large (4.5 kb) and small (2.3 kb) restriction fragments has recently been identified as a C to G mutation in intron 2, 646 nucleotides downstream from exon 2 (van Rossum et al., 2002). A number of investigations have found associations between the large allele (4.5 kb) or the GG genotype and parameters indicative of increased GC sensitivity such as increased abdominal visceral fat (Buemann et al., 1997), hyperinsulinaemia

(Weaver et al., 1992), body mass index (van Rossum et al., 2002) and evidence for increased sensitivity to topical budesonide (Panarelli et al., 1998) and increased sensitivity of the HPA negative feedback axis (van Rossum et al., 2002). In this study the *Bcl*I allelic frequencies for PTSD and controls (C = 0.68 and 0.60, G = 0.32 and 0.40) were not significantly different and were comparable to other studies (Weaver et al., 1992; Buemann et al., 1997; Panarelli et al., 1998; van Rossum et al., 2002). Even though no significant associations between the *Bcl*I genotypes and the measures of GC sensitivity were found there was a trend for the GG genotype in PTSD to show increased responsiveness to DVVA and correlate with the severity of PTSD symptoms. In addition, the CAPS score in the GG genotype showed a significant negative correlation with basal plasma cortisol levels. Taken together these findings are suggestive of increased GC sensitivity in the subset of PTSD possessing the GG *Bcl*I genotype. As this *Bcl*I polymorphism is intronic, its effect on GR gene activity may be indirect, such as acting as a marker in a GR function altering mutation. Alternatively, it has been suggested that the *Bcl*I polymorphism might affect the GR gene promoter by selectively acting either on repressor or enhancer sites within the promoter thereby altering GC sensitivity (Panarelli et al., 1998).

Recent studies into PTSD have uncovered a number of biological abnormalities including a possible dysfunctional HPA axis. A substantial body of studies have documented low circulating cortisol levels coupled with evidence of increased negative feedback inhibition (Yehuda et al., 1995, 2002; Stein et al., 1997; Heim et al., 1998) or decreased responsiveness of the adrenal cortex (Kanter et al., 2001). However not all studies have confirmed lower cortisol levels in PTSD compared to a control group (Halbreich et al., 1989; Liberzon et al., 1999). At the moment it is not clear whether hypocortisolism is a consistent finding in PTSD and if it is what role it plays in the development of PTSD. Certainly low cortisols could be a general marker of stress system dysregulation and support for this comes from reports of hypocortisolism in other stress related disorders such as chronic fatigue syndrome and fibromyalgia (Heim et al., 2000).

In this study, patients volunteered from a representative group of well-studied PTSD sufferers (Vietnam war veterans), and age matched controls were selected from non-PTSD affected combat-exposed Vietnam veterans. The PTSD group had been diagnosed prior to the study and were participating in therapy/support groups at a dedicated psychiatric facility. The severity of the participants' PTSD was assessed using CAPS. Other psychiatric diagnoses including active major depression, psychosis organic brain syndromes were excluded by a psychiatrist experienced in PTSD. Controls were recruited by advertisement and had PTSD excluded by interview with a psychologist (TS). The control group were smaller than the patient group and neither group was very large, although the study groups were comparable to other recent studies of the effect of genetic polymorphisms on psychiatric-neuroendocrine phenomena (Feng et al., 2000; Wust et al., 2004). Nevertheless, small genotypic effects on the risk of PTSD may have been undetected.

In conclusion, the N363S and *Bcl*I GR polymorphisms were not more frequent in PTSD patients than controls and reported population frequencies. Our PTSD patient group did not display GC hypersensitivity, as measured by the LD-DST and DVVA. In a subset of PTSD patients with the *Bcl*I GG genotype, CAPS scores and basal cortisol levels were negatively correlated. Overall, the results do not support the hypothesis that common GR polymorphisms act as risk factors for the development of PTSD.

Acknowledgements

The authors would like to thank the staff of the Keith Payne Unit, Department of Psychiatry, Greenslopes Private Hospital for their assistance in coordinating the studies on the PTSD patients admitted for the treatment program. This research was supported by grants from the Greenslopes Private Hospital Veterans Research Foundation and a University of Queensland Research and Development Scheme.

References

Boscarino, J.A., 1996. Posttraumatic stress disorder, exposure to combat, and lower plasma cortisol among Vietnam veterans: findings and clinical implications. J. Consult. Clin. Psychol. 64, 191–201.

Brown, P.H., Teelucksingh, S., Matusiewicz, S.P., Greening, A.P., Crompton, G.K., Edwards, C.R.W., 1991. Cutaneous vasoconstrictor response to glucocorticoids in asthma. Lancet 337, 576–580.

Buemann, B., Vohl, M.C., Chagnon, M., Chagnon, Y.C., Gagnon, J., Perusse, L., Dionne, F., Depres, J.P., Tremblay, A., Nadeau, A., Bouchard, C., 1997. Abdominal visceral fat is associated with a *Bcl*I restriction fragment length polymorphism at the glucocorticoid receptor gene locus. Obes. Res. 5, 186–192.

de Kloet, E.R., 1991. Brain corticosteroid receptor balance and homeostatic control. Front. Neuroendocrinol. 12, 95–164.

de Lange, P., Koper, J.W., Huizenga, N.A.T.M., Brinkmann, A.O., de Jong, F.H., Karl, M., Chrousos, G.P., Lamberts, S.W.J.,

1997. Differential hormone-dependent transcriptional activation and -repression by naturally occurring human glucocorticoid receptor variants. Mol. Endocrinol. 11, 1156–1164.

Dobson, M.G., Redfern, C.P.F., Unwin, N., Weaver, J.U., 2001. The N363S polymorphism of the glucocorticoid receptor: potential contribution to central obesity in men and lack of association with other risk factors for coronary heart disease and diabetes mellitus. J. Clin. Endocrinol. Metab. 86, 2270–2274.

Ebrecht, M., Buske-Kirschbaum, A., Hellhammer, D., Kern, S., Rohleder, N., Walker, B., Kirschbaum, C., 2000. Tissue specificity of glucocorticoid sensitivity in healthy adults. J. Clin. Endocrinol. Metab. 85, 3733–3739.

Echwald, S.M., Sorensen, T.I.A., Andersen, T., Pedersen, O., 2001. The Asn363Ser variant of the glucocorticoid receptor gene is not associated with obesity or weight gain in Danish men. Int. J. Obes. 25, 1563–1565.

Feng, J., Zheng, J., Bennett, W.P., Heston, L.L., Jones, I.R., Craddock, N., Sommer, S.S., 2000. Five missense variants in the amino-terminal domain of the glucocorticoid receptor: no association with puerperal psychosis or schizophrenia. Am. J. Med. Genet. 96, 412–417.

Gaitan, D., DeBold, C.R., Turney, M.K., Zhou, P., Orth, D.N., Kovacs, W.J., 1995. Glucocorticoid receptor structure and function in an adrenocorticotropin-secreting small cell lung cancer. Mol. Endocrinol. 9, 1193–1201.

Goenjian, A.K., Yehuda, R., Pynoos, R.S., Steinberg, A.M., Tashjian, M., Yang, R.K., Najarian, L.M., Fairbanks, L.A., 1996. Basal cortisol, dexamethasone suppression of cortisol and MHPG in adolescents after the 1988 earthquake in Armenia. Am. J. Psychiatry 153, 929–934.

Halbreich, U., Olympia, J., Carson, S., Glogowski, J., Yeh, C.M., Axelrod, S., Desu, M.M., 1989. Hypothalamo-pituitary-adrenal activity in endogenously depressed post-traumatic stress disorder patients. Psychoneuroendocrinology 14, 365–370.

Heim, C., Ehlert, U., Hanker, J.P., Hellhammer, D.H., 1998. Abuse-related posttraumatic stress disorder and alterations of the hypothalamic–pituitary–adrenal axis in women with chronic pelvic pain. Psychosom. Med. 60, 309–318.

Heim, C., Ehlert, U., Hellhammer, D.H., 2000. The potential role of hypocortisolism in the pathophysiology of stress-related bodily disorders. Psychoneuroendocrinology 25, 1–35.

Huizenga, N.A.T.M., Koper, J.W., de Lange, P., Pols, H.A.P., Stolk, R.P., Burger, H., Grobbee, D.E., Brinkmann, A.O., de Jong, F.H., Lamberts, S.W.J., 1998a. A polymorphism in the glucocorticoid receptor gene may be associated with an increased sensitivity to glucocorticoids in vivo. J. Clin. Endocrinol. Metab. 83, 144–151.

Huizenga, N.A.T.M., Koper, J.W., de Lange, P., Pols, H.A.P., Stolk, R.P., Grobbee, D.E., de Jong, F.H., Lamberts, S.W.J., 1998b. Interperson variability but intraperson stability of baseline plasma cortisol concentrations, and its relation to feedback sensitivity of the hypothalamo–pituitary–adrenal axis to a low dose of dexamethasone in elderly individuals. J. Clin. Endocrinol. Metab. 83, 47–54.

Kanter, E.D., Wilkinson, C.W., Radant, A.D., Petrie, E.C., Dobie, D.J., McFall, M.E., Peskind, E.R., Raskind, M.A., 2001. Glucocorticoid feedback sensitivity and adrenocortical responsiveness in posttraumatic stress disorder. Biol. Psychiatry 50, 238–245.

Koper, J.W., Stolk, R.P., de Lange, P., Huizenga, N.A.T.M., Molijn, G.J., Pols, H.A.P., Grobbee, D.E., Karl, M., de Jong, F.H., Brinkmann, A.O., Lamberts, S.W.J., 1997. Lack of association between five polymorphisms in the human glucocorticoid receptor gene and glucocorticoid resistance. Hum. Genet. 99, 663–668.

Liberzon, I., Abelson, J.L., Flagel, S.B., Raz, J., Young, E.A., 1999. Neuroendocrine and psychophysiologic responses in PTSD: a symptom provocation study. Neuropsychopharmacology 21, 40–50.

Lin, R.C.Y., Wang, W.Y.S., Morris, B.J., 1999. High penetrance, overweight, and glucocorticoid receptor variant: case-control study. Br. Med. J. 319, 1337–1338.

Miller, A.H., Spencer, R.L., Pulera, M., Kang, S., McEwen, B.S., Stein, M., 1992. Adrenal steroid receptor activation in rat brain and pituitary following dexamethasone: implications for the dexamethasone suppression test. Biol. Psychiatry 32, 850–869.

Panarelli, M., Holloway, C.D., Fraser, R., Connell, J.M.C., Ingram, M.C., Anderson, N.H., Kenyon, C.J., 1998. Glucocorticoid receptor polymorphism, skin vasoconstriction, and other metabolic intermediate phenotypes in normal human subjects. J. Clin. Endocrinol. Metab. 83, 1846–1852.

Pitman, R.K., Orr, S.P., Forgue, D.F., Altman, B., de Jong, J.B., Herz, L.R., 1990. Psychophysiologic responses to combat imagery of Vietnam veterans with posttraumatic stress disorder versus other anxiety disorders. J. Abnorm. Psychol. 99, 49–54.

Rosmond, R., Bouchard, C., Bjorntorp, P., 2001. Tsp509I polymorphism in exon 2 of the glucocorticoid receptor gene in relation to obesity and cortisol secretion: cohort study. Br. Med. J. 322, 652–653.

Sanger, F., Nicklen, S., Coulson, A.R., 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. 74, 5463–5467.

Smith, M.A., Davidson, J., Ritchie, J.C., Kudler, H., Lipper, S., Chappell, P., Nemeroff, C.B., 1989. The corticotropin-releasing hormone test in patients with PTSD. Biol. Psychiatry 26, 349–355.

Stein, M.B., Yehuda, R., Koverola, C., Hanna, C., 1997. Enhanced dexamethasone suppression of plasma cortisol in adult women traumatized by childhood sexual abuse. Biol. Psychiatry 42, 680–686.

Torpy, D.J., Grice, J.E., Hockings, G.I., Walters, M.M., Crosbie, G.V., Jackson, R.V., 1997. Diurnal effects of fluoxetine and naloxone on the human hypothalamic–pituitary–adrenal (HPA) axis. Clin. Exp. Pharmacol. Physiol. 24, 421–423.

True, W.R., Rice, J., Eisen, S.A., Heath, A.C., Goldberg, J., Lyons, M.J., Nowak, J., 1993. A twin study of genetic and environmental contributions to liability for posttraumatic stress symptoms. Arch. Gen. Psychiatry 50, 257–264.

van Rossum, E.F.C., Koper, J.W., Huizenga, N.A.T.M., Uitterlinden, A.G., Janssen, J.A.M.J.L., Brinkmann, A.O., de Jong, F.H., van Duyn, C.M., Pols, H.A.P., Lamberts, S.W.J., 2002. Identification of a restriction site polymorphism in the glucocorticoid receptor gene: association with increased sensitivity to glucocorticoids in vivo, corticosteroid-binding globulin and systolic blood pressure. Proc. 84th Annu. Sci. Meeting Endocr. Soc., San Francisco, CA 2002; Oral 49–1.

Walker, B.R., Best, R., Shackleton, C.H.L., Padfield, P.L., Edwards, C.R.W., 1996. Increased vasoconstrictor sensitivity to glucocorticoids in essential hypertension. Hypertension 27, 190–196.

Weaver, J.U., Hitman, G.A., Kopelman, P.G., 1992. An association between a BclI restriction fragment length

polymorphism of the glucocorticoid receptor locus and hyperinsulinaemia in obese women. J. Mol. Endocrinol. 9, 295–300.

Wust, S., van Rossum, E.F.C., Federenko, I.S., Koper, J.W., Kumsta, R., Helllhammer, D.H., 2004. Common polymorphisms in the glucocorticoid receptor gene are associated with adrenocortical responses to psychosocial stress. J. Clin. Endocrinol. Metab. 89, 565–573.

Yehuda, R., 1997. Sensitization of the hypothalamic–pituitary–adrenal axis in posttraumatic stress disorder. Ann. N. Y. Acad. Sci. 821, 57–75.

Yehuda, R., 2002. Current status of cortisol findings in post-traumatic stress disorder. Psychiatr. Clin. N. Am. 25, 341–368.

Yehuda, R., Southwick, S.M., Nussbaum, G., Wahby, V., Giller, E.L., Mason, J.W., 1990. Low urinary cortisol excretion in combat-related posttraumatic stress disorder. J. Nerv. Ment. Dis. 178, 366–369.

Yehuda, R., Boisoneau, D., Lowry, M.T., Giller, E.L., 1995. Dose–response changes in plasma cortisol and lymphocyte glucocorticoid receptors following dexamethasone administration in combat veterans with and without posttraumatic stress disorder. Arch. Gen. Psychiatry 52, 583–593.

Yehuda, R., Levengood, R., Schmeidler, J., Wilso, S., Guo, L.S., Gerber, G., 1996. Increased pituitary activation following metyrapone administration in PTSD. Psychoneuroendocrinology 21, 1–16.

Yehuda, R., Halligan, S.L., Bierer, L.M., 2002. Cortisol levels in adult offspring of Holocaust survivors: relation to PTSD symptom severity in the parent and child. Psychoneuroendocrinology 27, 171–180.