Prednisolone suppression test in depression: prospective study of the role of HPA axis dysfunction in treatment resistance

Mario F. Juruena, Carmine M. Pariante, Andrew S. Papadopoulos, Lucia Poon, Stafford Lightman and Anthony J. Cleare

Background
People with severe depressive illness have raised levels of cortisol and reduced glucocorticoid receptor function.

Aims
To obtain a physiological assessment of hypothalamic–pituitary–adrenal (HPA) axis feedback status in an in-patient sample with depression and to relate this to prospectively determined severe treatment resistance.

Method
The prednisolone suppression test was administered to 45 in-patients with depression assessed as resistant to two or more antidepressants and to 46 controls, prior to intensive multimodal in-patient treatment.

Results
The patient group had higher cortisol levels than controls, although the percentage suppression of cortisol output after prednisolone in comparison with placebo did not differ. Non-response to in-patient treatment was predicted by a more dysfunctional HPA axis (higher cortisol levels post-prednisolone and lower percentage suppression).

Conclusions
In patients with severe depression, HPA axis activity is reset at a higher level, although feedback remains intact. However, prospectively determined severe treatment resistance is associated with an impaired feedback response to combined glucocorticoid and mineralocorticoid receptor activation by prednisolone.

Declaration of interest
None.

A substantial proportion of patients with depression respond poorly to treatment; this group accounts for about half the total treatment costs for this disorder.[1] Few data are available as to any specific biological substrate for this treatment resistance. One of the most consistent findings in depression is hypothalamic–pituitary–adrenal (HPA) axis dysfunction;[2] furthermore, glucocorticoid receptor resistance is particularly evident in patients with treatment-resistant depression.[3,4] It is not known whether HPA axis dysfunction contributes to treatment resistance, although persistent glucocorticoid receptor resistance in depression is associated with relapse.[5,6] A suppressive test of the HPA axis using prednisolone has now been developed;[7] this differs from the traditional dexamethasone suppression test in that whereas dexamethasone probes the function of glucocorticoid receptors only, the prednisolone suppression test (PST) probes both glucocorticoid and mineralocorticoid receptors. Since endogenous HPA axis feedback involves both glucocorticoid and mineralocorticoid receptors, and since there is some evidence that mineralocorticoid receptors can compensate for altered glucocorticoid receptor function,[8] prednisolone should provide a more valid test of the HPA axis in depression.[9] In a preliminary study of 18 participants with depression, we found a normal suppressive response to prednisolone (5 mg) even though the same individuals demonstrated non-suppression to dexamethasone.[9] In this paper we report the results of administering the PST to an extended cohort of 45 people receiving in-patient treatment for depression. Our aim was to understand more about the role of the HPA axis in severe depression, and specifically in the aetiology of treatment resistance, using the PST as a tool. Our hypothesis was that HPA axis impairment as elicited by the PST would be associated with more severe illness clinically and a higher level of treatment resistance. The finding of a link between the HPA axis and treatment resistance could also suggest new therapeutic targets in patients not responsive to current treatments.

Method

Study design

The study used a single-blind non-randomised placebo-controlled repeated-measures design, as previously used for the validation of the PST in healthy controls and patients with depression.[7,9] On day 1, participants received placebo capsules and on day 2, they received prednisolone capsules (5 mg), both at 22.00 h. No alcohol, coffee, tea or meals were allowed after each capsule. On the day following each capsule administration, saliva samples were collected at 09.00 h, 12.00 h and 17.00 h. Participants with depression underwent a full clinical assessment at baseline and after the completion of a period of intensive, multimodal in-patient treatment on the National Affective Disorders Unit as described below.

Participants

Two groups were recruited: a group of 45 individuals with major depression who were in-patients on the National Affective Disorders Unit of the Bethlem Royal Hospital, South London and Maudsley National Health Service (NHS) Trust, and a healthy control group ($n=46$) recruited from our database of controls from hospital staff, students and the local community. Patients and controls were matched according to age (to within a limit of 5 years), gender and body mass index (to within a range of 5 kg/m^2). Patients were included in this study if they were aged 18–75 years and diagnosed as having major depressive disorder according to DSM–IV criteria.[10] In addition, all patients had a

disorder that was moderately treatment-resistant on the basis of prior non-response to at least two different classes of anti-depressants. A thorough medical examination was performed to assess comorbidity, physical disorders, general medical conditions, lifestyle, psychosocial problems and stress. For practical reasons it was not possible to test most patients in a drug-free state; for those continuing to take medication, a switch in regimen was avoided for at least 7 days before the experimental procedures.

Exclusion criteria for the patient group were a history of hypersensitivity to corticosteroids or steroid use, heavy smoking (more than 25 cigarettes a day), a viral illness during the preceding 2 weeks, pregnancy or lactation, alcohol dependence and significant physical illness (severe allergy, autoimmune disease, hypertension, malignancy, or haematological, endocrine, pulmonary, renal, hepatic, gastrointestinal or neurological disease). Patients with bipolar affective disorder, psychotic symptoms unrelated to their depressive disorder or an organic cause for their depression were excluded.

The control group participants were physically healthy on the basis of a complete medical history and examination, were not taking any psychotropic medication, were not taking any hormonal medication (including oral contraceptives) and had no history of hypersensitivity to corticosteroids. Urine tests for illicit drug use and pregnancy were conducted before the start of the study. Healthy individuals were excluded if they had a personal history or first-degree relative history of a DSM–IV Axis I disorder. The Beck Depression Inventory II (BDI–II) and the 21-item Hamilton Rating Scale for Depression (HRSD) provided information on the severity of control participants' depressive symptoms.[11,12] Inclusion in the control group required a BDI–II score of less than 9 (in fact, none of the group scored above 6).

The study protocols were all approved by the research ethical committee of the Institute of Psychiatry and South London and Maudsley NHS Trust. Written informed consent was obtained from all participants.

Clinical assessment of the patient sample

The Affective Disorders Unit receives referrals of many patients with long-standing or difficult to treat depressive illness, usually with a history of not responding to pharmacotherapy or psychotherapy. Patients underwent detailed assessment using the tools described below in order to clarify the main features of their illness. Many of these measures are already part of the unit's normal assessment process, but some were added for this research protocol. For diagnostic assessment we used the Structured Clinical Interview for DSM–IV Axis I disorders (SCID–I) and the Structured Clinical Interview for DSM–III–R Personality Disorders (SCID–II).[13,14]

Treatment resistance

For assessment of treatment resistance we used the Antidepressant Treatment History Form to assess the number of prior treatments of adequate dosage and duration to which the patient had adhered.[15] According to Sackeim, resistance to a given treatment could also be concluded if, despite continued adherence to the same medication and dosage that produced an initial response, a patient experienced relapse or recurrence of a depressive episode.[15] We also used Thase & Rush's staging criteria, which recognise five stages of treatment resistance according to the number of treatment trials adequately delivered.[16]

Clinical severity

For clinical severity of depression we used the 21-item HRSD, the Montgomery–Åsberg Depression Rating Scale (MADRS), the Inventory of Depressive Symptomatology (IDS) self-report version, the BDI–II and the Beck Anxiety Inventory (BAI).[17–19]

Other measures

The following measures were also used:

(a) for suicide assessment we used the Beck Scale for Suicide Ideation (BSI) and the Beck Hopelessness Scale;[20,21]

(b) for cognitive function we used the Mini-Mental State Examination (MMSE);[22]

(c) for functional capacity and disability we used the Social Adaptation Self-evaluation Scale (SASS), the Golombok–Rust Inventory of Marital State (GRIMS) and the Dysfunctional Attitudes Scale (DAS);[23–25]

(d) for sleep disturbance we used the Pittsburgh Sleep Quality Index (PSQI);[26]

(e) for environmental stress we used the Recent Life Changes Questionnaire (RLCQ), the Childhood Experience of Care and Abuse (CECA) interview and the Childhood Experience of Care and Abuse Questionnaire (CECA–Q).[27–29]

In-patient treatment protocol

All patients underwent intensive in-patient treatment for their depression for a mean of 20 weeks. Treatment consisted of an individualised combination of the following therapies as clinically indicated for each patient: intensive psychopharmacology, using combinations of medications as indicated by the Maudsley prescribing guidelines;[30] weekly cognitive–behavioural therapy; daily occupational therapy; fortnightly couple and sexual therapy; alleviation of any physical health consequences or corollaries of depression (such as hypercholesterolaemia, hypertension, obesity, malnutrition and dental problems); and supportive and enabling nursing care including group sessions for anxiety management and behavioural activation. The patients' response to treatment was carefully assessed by repeating shortly before discharge the same psychometric measures that were administered at baseline. Responders and non-responders to treatment were defined using the *a priori* definition of a reduction in HRSD score of 50% or greater.

Endocrine protocol

The prednisolone suppression test was administered shortly after admission for patients (range 5–21 days). Both patients and controls were admitted to the research rooms of the Affective Disorders Unit, where they spent the period from 08.45 h to 17.15 h engaged in sedentary activities. Snacks, meals and drinks were standardised throughout the day. Saliva samples were collected using untreated cotton swabs (Salivettes, Sarstedt, Leicester, UK). Participants were asked to place the swab in their mouth and move it around with their tongue until it was saturated with saliva; the swab was then replaced in the vial without being touched. Saliva was separated from the cotton roll by quick centrifugation (3500 rev/min for 10 min) and samples were stored in a freezer at −40 °C until assayed. Samples were always collected at the same time of day to control for circadian variations. In addition to saliva samples, blood samples were taken by venepuncture at 09.00 h on the day after administration of prednisolone and placebo in order to measure plasma prednisolone levels. The saliva samples were always collected before blood sampling or meals to avoid confounding effects of blood collection or eating.

Saliva cortisol assay

Salivary cortisol level was measured using a time-resolved immunofluorescent assay as previously described.[7,9] The intra-assay precision was 8.8% at 0.3 nmol/l, 8.9% at 1.0 nmol/l and 6.6% at 4.6 nmol/l. The inter-assay precision was 7.7% at 2.1 nmol/l and 5.9% at 9.2 nmol/l. The minimal detection concentration was 0.1 nmol/l and there was no 'drifting' evident in assays up to 200 wells. The cross-reactivity of the antiserum was prednisolone 28%, 11-deoxycortisol 10%, cortisone 1% and corticosterone 1%.

Plasma prednisolone assays

Plasma levels of prednisolone were measured by high-performance liquid chromatography (Hewlett–Packard UV Detector linked to a ChemStation collection system; Agilent Technologies, www.chem.agilent.com). The calibration graph of the method was in the range 5–500 ng/ml. The intra-assay precision for prednisolone was 11.2% at 5 ng/ml, 5.2% at 18 ng/ml and 2.0% at 225 ng/ml. The inter-assay precision was 10.7% at 5 ng/ml, 9.6% at 18 ng/ml and 3.1% at 225 ng/ml.

Statistical analysis

The general linear model analysis for repeated measures was used to examine both between-group differences (patients *v.* controls) and within-group differences (placebo *v.* prednisolone) in salivary cortisol levels for all time points. We also used as summary measures the total salivary cortisol output, calculated as the area under the curve (AUC) using the trapezoidal method, after placebo ($AUC_{PLACEBO}$) and prednisolone (AUC_{PRED}), and further calculated the percentage suppression of salivary cortisol for each individual. The percentage suppression represented the AUC_{PRED} as a percentage of the $AUC_{PLACEBO}$ based on the formula –

$$\text{Percentage suppression} = \left(\frac{AUC_{PLACEBO} - AUC_{PRED}}{AUC_{PLACEBO}} \right) \times 100$$

We used *t*-tests to compare the AUC values, percentage suppression, clinical data and prednisolone plasma levels. Correlations between the AUC values and psychometric measures were examined using Pearson's product-moment correlation coefficients. Chi-squared tests were used to analyse categorical variables. The relationship between endocrine status and subsequent treatment response was tested by comparing the AUC values between treatment responders and non-responders using an independent *t*-test. All analyses were conducted using the Statistical Package for the Social Sciences, SPSS for Windows, release 13.0. All values are presented as means and standard error of the mean. All probability values reported are two-tailed. A value of *P*<0.05 was considered statistically significant.

Results

Clinical assessment

At baseline, patients had a mean BDI–II score of 38 (s.e.m.=1.3) and a mean HRSD score of 23.4 (s.e.m.=0.9). As expected, the mean scores on these scales were lower in the control group (BDI–II mean score 1.9, s.e.m.=0.2, $t=-26.13$, d.f.=1,89, *P*<0.001; HRSD mean score 4.1, s.e.m.=0.3, $t=-19.31$, d.f.=1,89, *P*<0.001). There was a wide range of Axis I comorbidity (Table 1). According to the SCID–II, almost half (22/45; 49%) of the patient group had some degree of comorbidity in Axis II. It is also noteworthy that more than two-thirds of the patient group (31/45; 69%) had experienced some form of early life stress according to

the CECA–Q: specifically, 25 (55%) had experienced parental neglect or emotional abuse, 10 (22%) had experienced physical abuse and 9 (20%) had experienced sexual abuse. Among the 45 patients, 38 were taking medication at the time of testing (Table 1). Seven (16%) were drug-free for at least 14 days before testing.

Using the *a priori* definition of treatment response, 24 of 45 patients showed a response to treatment (and were designated 'responders') and 21 did not (designated 'non-responders'). Among the responders, all the scales measuring severity of depression or related symptoms showed significant improvement between admission and discharge (Table 2). The non-responders group showed no significant improvement on any of the scales, although there was a trend towards improvement in HRSD,

Table 1 Demographic and clinical features of the study sample

	Depression group (*n*=45)	Control group (*n*=46)
Female gender, *n* (%)	37 (82)	29 (63)
Age, years: mean (s.e.m.)	50.9 (1.5)	46.5 (2.1)
BMI, kg/m^2: mean (s.e.m.)	29.7 (0.9)	26.9 (0.7)
Years of education: mean (s.e.m.)	12.4 (0.4)	12.8 (0.6)
SCID–I primary diagnosis of major depressive disorder, *n* (%)	45 (100)	0 (0)
Comorbidity, *n* (%)		
Generalised anxiety disorder	15 (33)	
Atypical depression	5 (11)	
Social phobia	5 (11)	
PTSD	5 (11)	
Eating disorder	4 (8)	
Sleep disorder	4 (8)	
Dysthymia	4 (8)	
Panic disorder and agoraphobia	2 (4)	
Somatisation disorder	2 (4)	
Age at onset, years: mean (s.e.m.)		
First MDE	29.3 (1.6)	
Current episode	50.9 (1.5)	
No. of prior MDE: mean (s.e.m.)	7.9 (0.7)	
Duration of current MDE, months: mean (s.e.m.)	38.4 (4.5)	
Duration of illness, years: mean (s.e.m.)	20.2 (2.0)	
No. of previous hospital admissions: mean (s.e.m.)	3.9 (0.6)	
No. of prior adequate treatment trials: mean (s.e.m.)	12.8 (1.2)	
Duration of current admission, weeks: mean (s.e.m.)	20.3 (2.2)	
Current medication, *n* (%)		
Mood stabiliser	35 (78)	0 (0)
SSRI/SNRI	35 (78)	0 (0)
Benzodiazepine	19 (42)	0 (0)
Atypical antipsychotic	16 (36)	0 (0)
Tricyclic antidepressant	11 (24)	0 (0)
MAOI	11 (24)	0 (0)
Drug free	7 (16)	46 (100)
Other antipsychotics	3 (7)	0 (0)
ECT in the past, *n* (%)	23 (51)	0 (0)
Treatment resistance stage[a] *n* (%)		
Stage 5	34 (76)	
Stage 4	9 (20)	
Stage 3	2 (4)	

BMI, body mass index; ECT, electroconvulsive therapy; MAOI, monamine oxidase inhibitor; MDE, major depressive episode; PTSD, post-traumatic stress disorder; SCID–I, Structured Clinical Interview for DSM–IV Axis I disorders; SSRI, selective serotonin reuptake inhibitor; SNRI, serotonin and noradrenaline reuptake inhibitor.
a. Categorised according to Thase & Rush treatment resistance criteria.[16]

Table 2 Psychometric scores on admission and discharge in the patient sample

	All patients (n=45) Mean (s.e.m.)	Non-responders (n=21) Mean (s.e.m.)	Responders[a] (n=24) Mean (s.e.m.)
HRSD admission	23.4 (0.9)	21.5 (1.3)	25.0 (1.2)
HRSD discharge	14.5 (1.1)	19.8 (1.3)	9.8 (0.9)
P	<0.001	0.074	<0.001
BDI–II admission	38.0 (1.4)	39.3 (1.9)	36.8 (1.9)
BDI–II discharge	30.9 (1.9)	33.1 (2.5)	28.8 (3.1)
P	0.004	0.064	0.028
MADRS admission	35.2 (1.8)	34.8 (2.3)	35.8 (3.2)
MADRS discharge	27.9 (3.6)	33.0 (4.2)	21.8 (5.3)
P	0.05	0.682	0.033
IDS–SR admission	49.8 (2.5)	50.8 (3.4)	48.9 (3.6)
IDS–SR discharge	36.2 (5.8)	40.7 (8.7)	31.0 (7.8)
P	0.017	0.315	0.033
BHS admission	16.7 (0.7)	15.9 (1.2)	17.4 (0.7)
BHS discharge	13.7 (1.4)	13.7 (1.5)	13.7 (3.5)
P	0.044	0.278	0.105
BSI admission	22.7 (1.4)	24.0 (2.0)	21.5 (1.9)
BSI discharge	13.6 (1.7)	16.0 (2.2)	11.1 (2.3)
P	<0.001	0.063	0.003
BAI admission	25.1 (2.1)	23.7 (2.8)	26.4 (3.0)
BAI discharge	23.6 (3.6)	27.3 (2.3)	18.4 (7.9)
P	0.718	0.339	0.267
DAS admission	104.6 (5.6)	104.4 (8.7)	104.7 (7.5)
DAS discharge	109.4 (14.8)	112.0 (21.3)	105.0 (22.7)
P	0.709	0.694	0.989
MMSE admission	28.1 (0.4)	28.5 (0.6)	27.6 (0.7)
MMSE discharge	27.8 (0.5)	28.3 (0.7)	27.3 (0.8)
P	0.744	0.837	0.777
PSQI admission	12.1 (0.7)	12.0 (1.1)	12.1 (0.8)
PSQI discharge	10.9 (1.1)	11.1 (1.2)	10.5 (2.3)
P	0.338	0.603	0.418
SASS admission	31.4 (4.0)	37.2 (7.1)	29.0 (4.8)
SASS discharge	33.5 (1.9)	35.0 (2.3)	31.7 (3.1)
P	0.638	0.779	0.698
RLCQ admission	321.5 (29.4)	354.4 (42.8)	279.5 (37.1)
GRIMS admission	31.5 (4.0)	25.6 (4.9)	36.6 (5.7)

BAI, Beck Anxiety Inventory; BDI–II, Beck Depression Inventory–II; BHS, Beck Hopelessness Scale; BSI, Beck Scale for Suicidal Ideation; DAS, Dysfunctional Attitudes Scale; GRIMS, Golombok–Rust Inventory of Marital State; HRSD, Hamilton Rating Scale for Depression; IDS–SR, Inventory of Depressive Symptomatology – Self Rated; MADRS, Montgomery–Åsberg Depression Rating Scale; MMSE, Mini-Mental State Examination; PSQI, Pittsburgh Sleep Quality Index; RLCQ, Recent Life Change Questionnaire; SASS, Social Adaptation Self-evaluation Scale.
a. Response defined as a reduction in HRSD score of 50% or more.

Fig. 1 Salivary cortisol levels (nmol/l) in healthy controls (n=46) and in-patients with depression (n=45) at 09.00 h, 12.00 h and 17.00 h, after the administration at 22.00 h the previous night of placebo or 5 mg prednisolone.

BDI–II and BSI scores (Table 2). There was no significant difference in clinical features between the responder and non-responder groups. Both groups of patients followed the same treatment guidelines and there was no significant difference between them in the composition of treatments administered during the admission.

Endocrine assessment

In the between-participants analyses, the patient group had higher salivary cortisol levels compared with controls, both after placebo and after prednisolone (Fig. 1). The following main factors were entered into a general linear model: challenge (placebo v. prednisolone), group (patients v. controls) and time (09.00 h, 12.00 h and 17.00 h). According to the general linear modeal analysis there was a significant difference between groups ($F=26.19$, d.f.=1,267, $P<0.001$; i.e. overall higher cortisol levels in patients), a between-challenge effect ($F=335.19$, d.f.=1,267, $P<0.001$; i.e. overall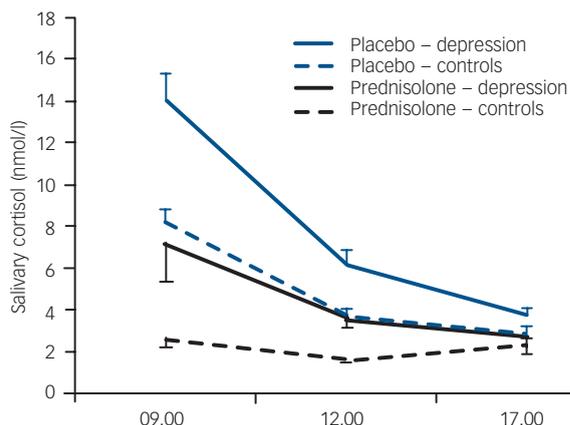 higher cortisol levels after placebo than prednisolone), an effect of time ($F=34.63$, d.f.=2,267, $P<0.001$; i.e. overall higher cortisol concentration in the morning than in the afternoon) and a group × time interaction ($F=6.00$, d.f.=2,267, $P=0.003$; i.e. the fall in cortisol levels over time was larger in the patient group owing to the higher 09.00 h values). There was also a challenge × time interaction ($F=17.6$, d.f.=2,267, $P<0.001$; i.e. greater suppression by prednisolone in the morning than the afternoon, due to the higher absolute values in the morning).

Subsequent pairwise analyses within groups were conducted separately in patients and controls. In controls there was a main effect of challenge (placebo v. prednisolone, $F=76.8$, d.f.=1,135, $P<0.001$) and a challenge × time interaction ($F=24.3$, d.f.=1,135, $P<0.001$). In patients there were also a main effect of challenge (placebo v. prednisolone, $F=23.06$, d.f.=1,132, $P<0.001$) and a challenge × time interaction ($F=5.96$; d.f.=2,132, $P=0.003$).

The results of the general linear model analysis were confirmed by the analysis of the total cortisol output, measured using the AUC. Patients had larger mean AUC cortisol compared with controls both after placebo ($AUC_{PLACEBO}$ was approximately 1.6 times higher) and after prednisolone (AUC_{PRED} was approximately twice as high) (Table 3). Patients and controls showed similar percentage suppression by prednisolone (Table 3).

In summary, these results showed that in-patients with depression and a history of moderate prior treatment resistance have marked hypercortisolism both before and after administration of prednisolone, but a similar percentage suppression of salivary cortisol to healthy controls.

Prediction of treatment response using the PST

The cortisol profiles after placebo and the prednisolone suppression test are shown in Fig. 2, divided into those who went on to respond to treatment and those who did not. There was a significant difference in the AUC_{PRED} between those who subsequently responded to treatment and those who did not: responders 23.5 nmol/l per hour (s.e.m.=4.2) v. non-responders 41.9 nmol/l per hour (s.e.m.=7.7); $t=2.1$, d.f.=43, $P=0.046$. On the other hand, the comparison of $AUC_{PLACEBO}$ did not show a significant difference between these patient subgroups: responders 53.1 nmol/l per hour (s.e.m.=8.2) v. non-responders 57.2 nmol/l per hour (s.e.m.=5.7); $t=0.4$, d.f.=43, $P=0.694$ (Table 3, Fig. 3).

Table 3 Prednisolone suppression test summary values, calculated as total salivary cortisol output (area under the curve) after placebo (AUC$_{PLACEBO}$) and prednisolone 5 mg (AUC$_{PRED}$)

	AUC$_{PLACEBO}$ Mean (s.e.m.)	AUC$_{PRED}$ Mean (s.e.m.)	Suppression,[a] % Mean (s.e.m.)	Plasma prednisolone levels, ng/ml Mean (s.e.m.)
Group				
Controls (n=46)	33.8 (2.5)	16.1 (1.6)	−49.6 (4.0)	66.5 (10.9)
Depression (n=45)	55.1 (5.1)	32.1 (4.4)	−42.2 (4.8)	56.1 (5.1)
P	<0.001	<0.001	0.24	0.40
Patients with depression				
Responding to subsequent treatment (n=24)	53.1 (8.2)	23.5 (4.2)	−52.5 (4.7)	74.9 (17.3)
Not responding to subsequent treatment (n=21)	57.2 (5.7)	41.9 (7.7)	−30.6 (8.2)	54.8 (10.1)
P	0.69	0.046	0.022	0.34
a. Percentage suppression by prednisolone in relation to placebo.				

Furthermore, comparing the percentage suppression of cortisol output after prednisolone, there was a significant difference between subsequent treatment responders and non-responders: responders –52.5% (s.e.m.=4.7) *v.* non-responders –30.6% (s.e.m.=8.2); t=2.4, d.f.=43, P=0.022 (Table 3). Indeed, as can be
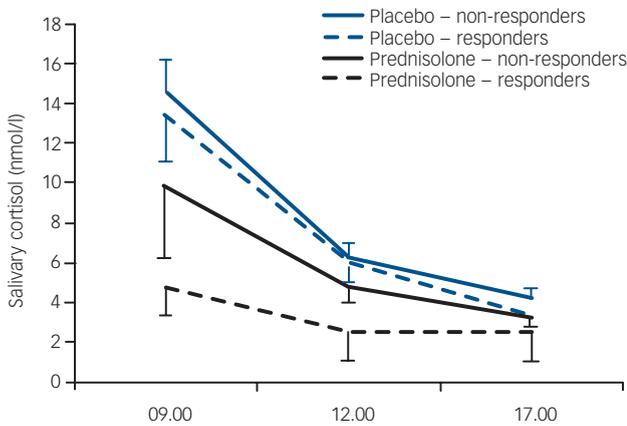

Fig. 2 Salivary cortisol levels (nmol/l) in 45 in-patients with depression at 09.00 h, 12.00 h and 17.00 h, after the administration at 22.00 h the previous night of placebo or 5 mg prednisolone. Patients are divided into those who subsequently responded to treatment and those who did not.
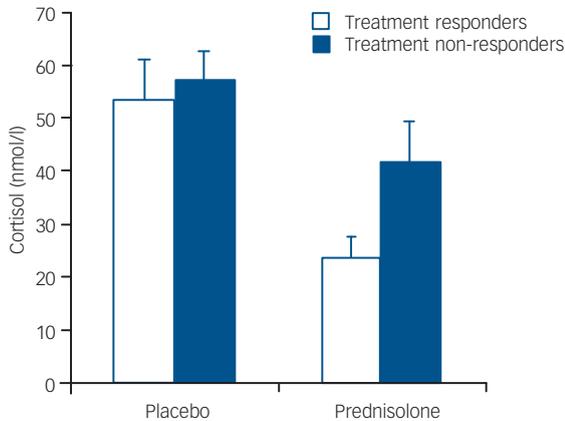
seen in Table 3 and Fig. 4, responders had a percentage suppression (−52.5%) virtually identical to that of healthy controls (−49.6%; t=−0.44, d.f.=68, P=0.66), whereas that of non-responders was lower than that of healthy controls (−30.6%; t=2.3, d.f.=65, P=0.02).

These findings indicate that the results of the PST – both absolute salivary cortisol values after prednisolone and the percentage suppression after prednisolone compared with placebo – on admission to the in-patient unit differed between those who went on to respond to treatment and those who did not (Fig. 3).

Relationship between PST and psychometric measures

We correlated the AUC values and psychometric measures in the patient group. Given the number of psychometric measures taken, we corrected for multiple comparisons using the rough false discovery rate – i.e. the α-value was adjusted by $(n+1)/2n$, which for 13 tests gives an adjusted significance level of $P < 0.027$. There was a significant negative correlation between the AUC$_{PRED}$ and the Beck Hopelessness Scale (r=−0.50, P=0.003); thus, higher post-prednisolone cortisol was associated with lower level of hopelessness. The correlations between AUC$_{PRED}$ and the other psychometric measures were not significant in patients. Mirroring the post-prednisolone data, there was a significant negative
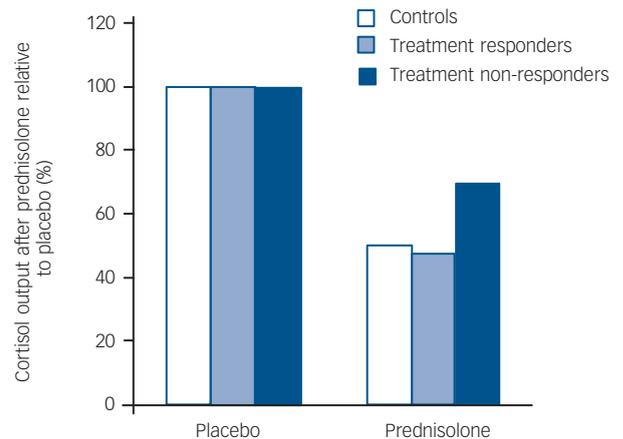

Fig. 3 Cortisol output (measured as area under the curve) after placebo and 5 mg prednisolone in in-patients with depression divided into those who subsequently responded to treatment (n=24) and those who did not (n=21).

Fig. 4 Cortisol output (measured as area under the curve) after 5 mg prednisolone relative to placebo (rebased to 100%) in 46 healthy controls and 45 patients. Patients who subsequently responded to treatment (n=24) showed the same sensitivity to the suppressive effects of prednisolone as controls (P=0.66), whereas treatment non-responders (n=21) showed lesser sensitivity (P=0.02).

correlation between the $AUC_{PLACEBO}$ and the Beck Hopelessness Scale ($r=-0.46$, $P=0.006$). There was no significant correlation between the percentage suppression of AUC_{PRED} and any of the psychometric measures using the adjusted significance threshold of $P<0.027$. There was no difference in $AUC_{PLACEBO}$ and AUC_{PRED} between patients with and without any comorbid personality disorder, or with and without individual personality disorder diagnoses. Similarly, there was no difference in $AUC_{PLACEBO}$ and AUC_{PRED} between patients with and without early life stress, either taken as a whole or separated into emotional, physical or sexual abuse. Finally, the presence of a comorbid Axis I anxiety disorder did not affect significantly the $AUC_{PLACEBO}$ or the AUC_{PRED}.

Plasma prednisolone levels

Plasma prednisolone levels during the PST did not differ between patients and controls ($t=-0.86$, $P=0.40$) or between treatment responders and non-responders ($t=-1.00$, $P=0.34$; Table 3).

Discussion

We have, for the first time, assessed the relationship of endogenous HPA activity to prospectively defined severe treatment resistance in a cohort of in-patients with depression. We also used a novel test of HPA activity – the prednisolone suppression test – which allowed us to test the feedback function of both glucocorticoid and mineralocorticoid receptors on the HPA axis, and related the response to the clinical status of our patients.

The HPA axis in in-patients with depression

In this study, in-patients with severe depression and a moderate degree of retrospectively defined treatment resistance had higher salivary cortisol levels compared with controls, both after placebo and after prednisolone administration. This confirms previous findings reporting that people with severe depression have a hyperactive HPA, leading to high cortisol levels.[8,31] Despite the marked basal hypercortisolism in these patients, the mean suppressive effect of prednisolone was similar to that seen in the healthy controls. This confirms our earlier data[9] and focuses attention on why these patients show reduced suppression to the pure glucocorticoid receptor agonist dexamethasone but not to the mixed glucocorticoid receptor and mineralocorticoid receptor agonist prednisolone.[9]

Previous studies in depression with the dexamethasone suppression test and the dexamethasone-suppressed corticotrophin releasing hormone (Dex–CRH) test suggest impaired glucocorticoid receptor function,[5,32] whereas other studies suggest that mineralocorticoid receptor function is upregulated.[33] Both mineralocorticoid and glucocorticoid receptors are active in the negative feedback of the HPA axis. Since prednisolone is active at both receptor sites, our results taken together with these previous studies are compatible with the notion that in severe, treatment-resistant depression there is a change in differential responsiveness of the HPA axis to glucocorticoid and mineralocorticoid receptors, with increased mineralocorticoid receptor signalling compensating for impaired glucocorticoid receptor function. We conclude that in severe depression, rather than generalised glucocorticoid resistance there is an imbalance in the normal physiology of the regulation of the HPA axis characterised by glucocorticoid receptor resistance and increased mineralocorticoid receptor sensitivity. This is seen within a general resetting of HPA activity with markedly raised basal cortisol levels, suggesting a new set-point for HPA function but with intact negative feedback when this is measured using a more 'physiological' challenge able to activate both glucocorticoid and mineralocorticoid receptors.[34]

As expected for a group of patients with severe depression, there was a wide range of Axis I comorbidity, mainly anxiety disorders. Substantial data suggest that patients with depression comorbid with anxiety diagnoses have more severe depressive symptoms, a worse clinical course, a higher risk of suicide and possibly a different family history.[35] However, the influence of comorbid anxiety disorders on the neuroendocrine picture of major depression has not been well studied. Although Young *et al* noted that patients with depression and comorbid anxiety disorders show even greater impairment to the negative feedback on the HPA axis than those without such comorbidity,[36] this was not observable in our study.

Relation to treatment response

A particularly interesting aspect of our findings was that although as a whole this group of patients with depression showed preserved negative feedback, this did not apply to all patients. After completing the PST, patients underwent a period of intensive in-patient therapy. After this treatment, just over half the participants (53%) were classified as treatment responders, with a concomitant improvement in several clinical measures. Those classed as non-responders had been prospectively treated with an intensive, evidence-based treatment package and thus represent a well-defined and truly treatment-resistant population (rather than an insufficiently treated population). We found that there was a significant difference in the AUC_{PRED} between these severely treatment-resistant patients and those who did eventually respond to treatment, in that a higher AUC_{PRED} was associated with absence of clinical response to subsequent treatment. In other words, there was a higher post-prednisolone cortisol release (representing impaired suppression) in the severely treatment-resistant group compared with the treatment-responsive group. In contrast, no such relationship with clinical response was found for $AUC_{PLACEBO}$. Using the measure of percentage suppression, again there was significantly impaired suppression in the severely treatment-resistant group compared with the treatment responder group. The implication of this is that there may be a subgroup of patients within those who are severely depressed who have significant neuroendocrine dysfunction, represented by a disturbed HPA axis feedback and an imbalance in the ratio of mineralocorticoid/glucocorticoid receptor signalling, who are less responsive to the treatments currently available for depression and offered in an in-patient affective disorders unit. It may be that the underlying difference in these patients is an inability to compensate for glucocorticoid receptor resistance by increased mineralocorticoid receptor function. This would suggest that other treatment options need to be sought for such patients, and it could be that targeting the HPA axis is a fruitful area for future study in these patients.

Although this is the first study to use the PST to predict treatment response or resistance in depression, other HPA axis tests have been studied as predictors of treatment response. Baseline dexamethasone suppression test status did not predict response to antidepressant treatment or outcome after hospital discharge.[5] Zobel *et al* found that patients who showed an increase in cortisol levels after the Dex–CRH test between admission and discharge tended to relapse during the follow-up period.[6] Similarly, attenuation of the adrenocorticotrophic hormone response to the Dex–CRH test early during in-patient admission

was linked with a positive treatment response after 5 weeks and a higher remission rate at the end of hospitalisation.[37]

The potential advantages of the PST are that it is simple to administer and tests both glucocorticoid and mineralocorticoid receptors rather than just glucocorticoid receptor alone, an important factor given our improved understanding of the HPA axis in recent years. Furthermore, we are not aware of data from other tests of the HPA axis that have been applied specifically to patients with severe depression with retrospectively defined treatment resistance. Given the expense of in-patient treatment programmes and the scarcity of available expertise, any advance in predicting which patients are most likely to benefit from these programmes could be important clinically.

Relation to psychometric measures

We found a higher level of hopelessness to be associated with both a lower AUC_{PRED} and a lower $AUC_{PLACEBO}$. The hopelessness theory of depression is a cognitive vulnerability–stress model that attempts to understand risk factors for suicide behaviour.[38] In this model certain vulnerable patients experience increased symptoms of hopelessness and depression when they experience negative life events.[39] Two studies have used this model to investigate the link between the HPA axis and hopelessness; both showed that lower HPA axis activation – assessed either by free cortisol levels or with dexamethasone suppression – is associated with greater hopelessness, consistent with our finding.[40,41] The interpretation of this finding might be that there is maladaptive, enhanced negative feedback regulation of cortisol in patients at risk of suicide. If overactive negative feedback were a risk factor for becoming hopeless in the face of life events, it would be important to investigate whether this is a trait variable that persists in patients, even when recovered.

Early life stress

Around 70% of our sample of patients had early life stress according to the items of the CECA–Q. However, there was no significant difference between AUC_{PRED} and $AUC_{PLACEBO}$ in patients with or without early life stress, perhaps due to the high rate in this sample. Others have reviewed the literature in this area and concluded that early life stress may lead to disruptions in HPA axis functioning, and that factors such as the age when maltreatment occurred, parental responsiveness, subsequent exposure to stressors, type of maltreatment and type of psychopathology or behavioural disturbance displayed may influence the degree and pattern of HPA disturbance.[42,43]

Limitations

This study has some limitations. First, our sample size was modest, although this is the largest study of the PST to date and is comparable in size to previous studies using other HPA axis tests such as the Dex–CRH test to predict outcome.[6,37] Second, all participants in the depression group were in-patients who were chronically ill with moderate prior treatment resistance. The sensitivity of the prednisolone test might be different in an out-patient group, as reported for other tests of HPA axis function.[44,45] Third, the use of medication might have affected results. One mechanism for this might be through pharmacokinetic interactions altering the metabolism of prednisolone, as has been demonstrated for dexamethasone in some studies. However, we demonstrated that the prednisolone plasma levels were similar not only between patients and controls but also, importantly, between responders and non-responders, excluding such an effect. Another mechanism could be the direct effect of medication on

the HPA axis. Although this is possible, Kunugi *et al* demonstrated that hormonal measures did not differ between patients receiving medication and patients without medication on admission, indicating that medication status did not affect Dex–CRH test results.[46] This observation is in line with the finding that the presence or absence of antidepressant treatment and the type and number of antidepressant treatments during the index episode had no effect on hormonal responses to the Dex–CRH test.[47]

Clinical implications

This study confirms that there is HPA axis overactivity in in-patients with severe depression, characterised by raised basal cortisol levels. Although we find an intact negative feedback system reset to this higher level, our results taken together with prior studies suggest that this intact feedback depends on enhanced mineralocorticoid receptor sensitivity compensating for glucocorticoid receptor resistance. However, in prospectively defined severely treatment-resistant patients who do not respond to an intensive evidence-based treatment package, this compensatory mechanism is not functional and these patients demonstrate a combination of high cortisol levels and impaired negative feedback. It is, therefore, the patients who show the greatest neuroendocrine dysfunction on admission (i.e. non-suppression to prednisolone) who prove to be the least responsive to treatment.

The categorisation of depressive illnesses continues to develop and many have suggested that at some stage the addition of reliable biomarkers would advance this process. This study adds to evidence that HPA axis changes have an important role in depression and, we suggest, in the aetiology of treatment resistance in depression. However, we should learn from the mistakes of the past, when the dexamethasone suppression test was pursued as a 'diagnostic test' for depression or used as a proxy for an 'endogenous' subtype of depressive illness; any model would best incorporate markers of neuroendocrine dysfunction such as the PST alongside psychopathological and other indicators of treatment response and prognosis. Such improvements in the categorisation of depression to incorporate biomarkers should eventually open new therapeutic avenues and ultimately improve the outcome for patients with this often incapacitating and persistent illness.

Mario F. Juruena, MD, MPhil, MSc, PhD, Section of Neurobiology of Mood Disorders, and Stress, Psychiatry and Immunology Laboratory (SPI-Lab), Institute of Psychiatry, London, and National Affective Disorders Unit, Bethlem Royal Hospital, Beckenham; **Carmine M. Pariante**, MD, MRCPsych, PhD, SPI-Lab, Institute of Psychiatry, London; **Andrew S. Papadopoulos**, PhD, MSc, CChem, MRGC, Csci, **Lucia Poon**, RGN, SCM, RMN, National Affective Disorders Unit, Bethlem Royal Hospital, Beckenham; **Stafford Lightman**, PhD, FRCP, FMedSci, Henry Wellcome Laboratories for Integrative Neuroscience and Endocrinology University of Bristol; **Anthony J. Cleare**, BSc, MBBS, MRCPsych, PhD, Section of Neurobiology of Mood Disorders, Institute of Psychiatry, London, and National Affective Disorders Unit, Bethlem Royal Hospital, Beckenham, UK

Correspondence: Dr Anthony J. Cleare, Section of Neurobiology of Mood Disorders, PO 74, Institute of Psychiatry, 103 Denmark Hill, London SE5 8AF, UK. Email: a.cleare@iop.kcl.ac.uk

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