Plasma cortisol-dehydroepiandrosterone (DHEA) ratios in schizophrenia and bipolar disorder

Peter Gallagher, B.Sc. (Hons), M.Phil.
Research Associate

Stuart Watson, M.B.B.S., M.D., M.R.C.Psych.
Consultant and Honorary Senior Lecturer in Psychiatry

Margaret S. Smith, R.G.N., S.R.C.N.
Research Nurse

Allan H. Young, M.B.,Ch.B., M.Phil., Ph.D., F.R.C.Psych.
Professor of Psychiatry

I. Nicol Ferrier, F.R.C.P., M.D. (Hons), F.R.C.Psych.
Professor of Psychiatry

From the School of Neurology, Neurobiology and Psychiatry; Newcastle University, UK

Address for correspondence:

Peter Gallagher,
School of Neurology, Neurobiology and Psychiatry,
Newcastle University,
Leazes Wing (Psychiatry),
Royal Victoria Infirmary,
Newcastle upon Tyne, NE1 4LP, UK.
Telephone: +44 (0)191 282 4065    Fax: +44 (0)191 222 6162
E-mail: peter.gallagher@newcastle.ac.uk
SUMMARY

Hypercortisolaemia is a feature of many severe psychiatric illnesses and has been suggested to be both a causal and exacerbating factor of clinical symptoms and neurocognitive impairment. The adrenal steroid dehydroepiandrosterone (DHEA) has antiglucocorticoid properties that may have regulatory effects on glucocorticoid action in the brain. However, there is a paucity of data on these steroids and their ratio in schizophrenia and bipolar disorder. We therefore sought to assess cortisol and DHEA levels and the cortisol-DHEA ratio in patients with schizophrenia (n=20) and bipolar disorder (n=20), on stable medication for a minimum of 6 weeks, and healthy age- and sex-matched control subjects (n=20). Steroid levels were measured from plasma samples collected at 30 minute intervals from 1:00 p.m. to 4:00 p.m. Cortisol levels were found to be significantly elevated in both patient groups compared with controls. DHEA levels were elevated in schizophrenic patients compared with bipolar patients and controls, but there was no evidence of a difference in the cortisol-DHEA ratio of the groups. These data suggest that afternoon hypercortisolaemia is evident in symptomatic bipolar and schizophrenic patients compared to controls. However, an elevation in DHEA levels may represent a specific endocrine marker in schizophrenia.
1. INTRODUCTION

Abnormalities of the hypothalamic-pituitary-adrenal (HPA) axis are hypothesised to play a central role in the pathogenesis and aetiology of severe psychiatric illnesses (Raison & Miller, 2003). These abnormalities are evident at several levels; including decreased glucocorticoid receptor (GR) mRNA post-mortem (Perlman et al., 2004; Webster et al., 2002); increased ACTH and cortisol responses to the dexamethasone/corticotropin-releasing hormone (dex/CRH) challenge test (Lammers et al., 1995; Watson et al., 2004) and cortisol non-suppression following dexamethasone challenge (DST; Dewan et al., 1982; Greden et al., 1982; Nelson & Davis, 1997). In many patients with schizophrenia and bipolar disorder these abnormalities lead to increased basal cortisol levels (Linkowski et al., 1985; Ryan et al., 2004).

Recently there has been increased interest in the role of other adrenal steroids such as dehydroepiandrosterone (DHEA) which, in its sulfated form (DHEA-S) is the most abundant in humans (Morfin, 2002). DHEA is a substrate for androstenedione and testosterone synthesis and may have a role as an adrenal androgen (Gurnell & Chatterjee, 2001). The precise mechanism of action of DHEA in the brain is less well known (for a comprehensive overview, see; Morfin, 2002) although it has been shown to have actions on membrane-bound receptors and is a gamma-aminobutyric acid type A (GABA_A) antagonist (Hansen et al., 1999) as well as a sigma-1 receptor agonist (Maurice et al., 2006).

Importantly, DHEA (or its active metabolites, see; Muller et al., 2006) may possess anti-glucocorticoid properties (Kalimi et al., 1994). For example, in animals it has been demonstrated that DHEA protects hippocampal neurons against neurotoxin-induced cell death, possibly by decreasing nuclear GR levels (Cardounel et al., 1999). DHEA(S) has also been shown to inhibit glucocorticoid-induced enzyme activity (Browne et al., 1992). In healthy humans, acute administration of DHEA has been shown to rapidly reduce circulating cortisol.
levels (Wolf et al., 1997). Consequently, since DHEA levels appear to have regulatory effects on glucocorticoid action in the brain, it has been argued that the ratio of cortisol to DHEA most accurately reflects the degree of ‘functional’ hypercortisolaemia (see; Wolkowitz et al., 2001).

While some studies have assessed cortisol or DHEA levels in patients with schizophrenia and severe mood disorders, relatively few have measured both these steroids in the same samples. There have been several reports of elevated molar cortisol-DHEA ratios in patients with major depressive disorder, in both medicated (Michael et al., 2000) and unmedicated (Young et al., 2002) patients. Recently it has been shown that cortisol-DHEA ratios are also elevated in schizophrenia (Ritsner et al., 2004). However, many of these studies have reported neuroendocrine measures from a single time point. The diurnal and ultradian variation in hormone levels creates difficulty in interpreting such data. To our knowledge no previous study has examined cortisol, DHEA and the cortisol-DHEA ratio over multiple time-points in symptomatic patients with schizophrenia and bipolar disorder.

In this study, we sought to compare plasma cortisol and DHEA levels and the cortisol-DHEA ratios of symptomatic patients with schizophrenia or bipolar disorder and matched controls. We hypothesised that the patient groups would exhibit hypercortisolaemia and a significantly elevated cortisol-DHEA ratio compared to healthy, age- and sex-matched control subjects.

2. METHODS

2.1 Subjects

Patients aged 18 to 65 years with a diagnosis of schizophrenia or bipolar disorder, confirmed using the Structured Clinical Interview for DSM-IV (SCID; First et al., 1995), were recruited from secondary and tertiary care services in North East of England. The study formed part of a research program examining the efficacy of
glucocorticoid antagonists in schizophrenia and bipolar disorder, the results of which are reported elsewhere (Gallagher et al., 2005; Young et al., 2004). A specific attempt was made to recruit those who were in a stable clinical condition, but who remained symptomatic. Illness characteristics, clinical ratings and medication history were determined by trained psychiatrists using full history, case-note and medication review and standardized rating scales.

All patients were medication at the time of testing which had remained stable for a minimum of 6 weeks. Of the schizophrenic patients; 2 were taking carbamazepine (as a mood stabilizer), 7 were taking an antidepressant, all 20 were taking at least one antipsychotic and 7 were taking anticholinergics. Of the bipolar patients; 17 were taking at least one mood stabilizer, with 13 taking at least one antidepressant and 11 taking an antipsychotic (see Appendix 1 for details).

Healthy control subjects were recruited by advertisement and from hospital/university staff. All were physically healthy and had no personal or family history of psychiatric illness.

After a complete description of the study, written informed consent was obtained from all participants; the study received full approval from the local ethics committee.

2.2 Procedure

In order to profile plasma cortisol and DHEA secretion, subjects were canulated in the antecubital fossa at 12:30 p.m. and blood samples (~5ml) collected at 30 minute intervals from 1:00 p.m. to 4:00 p.m. (Halbreich et al., 1982). Subjects fasted throughout this period, remained semi-supine and did not sleep. Following extraction of serum by centrifugation, samples were immediately frozen and stored at -20°C.
2.3 Biochemical assays

All assays were performed in Psychiatry Research Laboratories, Newcastle University, using validated protocols. Cortisol levels were determined using Corticotide radioimmunoassay kits (ICN Pharmaceuticals, Costa Mesa, California). DHEA was measured in extracted plasma using a modified DHEA tritium radioimmunoassay kit (ICN) (for detailed procedure see; Gallagher et al., 2006).

2.4 Statistical analysis

Data were not normally distributed and were therefore log (base 10) transformed to meet the statistical assumptions permitting parametric analysis. Untransformed data summaries are reported for clarity. Cortisol levels, DHEA levels and molar cortisol-DHEA ratios were examined in separate repeated measures ANOVAs with log10 transformed sample (the 7 time points from 1:00 p.m. to 4:00 p.m.) as a within subjects factor and diagnosis (bipolar, schizophrenic or control) as a between subjects factor. Where sphericity was violated, within subject degrees of freedom were adjusted using the Huynh-Feldt correction. The adjusted significance values are reported, though the original degrees of freedom are reported for clarity. Post hoc analyses were performed using Fisher's LSD (protected t-test).

3. RESULTS

3.1 Demographics

Twenty schizophrenic patients, 20 bipolar patients and 20 healthy controls were recruited (18 males, 2 females in all groups). There was no significant difference in the mean age in the groups (schizophrenic patients: 42.1 years, SD=10.3; bipolar patients: 48.6 years, SD=10.8; controls: 45.3 years, SD=12.4; F=1.705, df=2,57, p=0.191). Schizophrenic and bipolar patients did not differ in severity of
symptoms on the Brief Psychiatric Rating Scale (BPRS; schizophrenic patients: 29.7, SD=9.8; bipolar patients: 30.1, SD=7.1; t=0.666, df=38, p=0.869).

At the time of testing, all schizophrenic patients were symptomatic at the time of testing (see BPRS above). In addition, the mean Montgomery-Åsberg Depression Rating Scale (MADRS) score of the group was 12 (SD=9) and the mean 17-item Hamilton Depression Rating Scale (HDRS17) score was 11 (SD=8). The Calgary Depression Scale (CDS; Collins et al 1996) was also administered. Its use is preferential in schizophrenia because of its independence from negative symptoms and extra pyramidal side effects. The mean CDS score in the group was 3 (SD=4).

All bipolar patients had persistent depressive symptoms, with 17 fulfilling full DSM-IV (SCID) criteria for a depressive episode. The mean Montgomery-Åsberg Depression Rating Scale (MADRS) score of the group was 23 (SD=10) and the mean 17-item Hamilton Depression Rating Scale (HDRS17) score was 18 (SD=10). The mean MADRS and HDRS17 scores of the three patients who did not meet SCID criteria for a current depressive episode were 8 (SD=5) and 4 (SD=1), respectively.

3.2 Neuroendocrine data

The DHEA level at a single time point (3:30 p.m.) was missing for one bipolar patient. This was replaced using the calculated midpoint between the 3:00 p.m. and 4:00 p.m. samples for this subject. The initial sample (both cortisol and DHEA) was missing for one schizophrenic patient; values were replaced using the mean levels of the group.

3.2.1 Cortisol

See figure 1.

A main effect of diagnosis was observed for cortisol (F=4.975, df=2,57,
p = 0.010). Post hoc (LSD) analysis revealed that schizophrenic patients (mean log\(_{10}\) difference = 0.126, 95%CI = 0.044 to 0.207; p = 0.003) and bipolar patients (mean log\(_{10}\) difference = 0.087, 95%CI = 0.005 to 0.169; p = 0.037) exhibited significantly higher afternoon cortisol levels compared to controls. There was no difference between the two patient groups (mean log\(_{10}\) difference = 0.039, 95%CI = -0.043 to 0.120; p = 0.350).

A significant main effect of sample was also observed (F = 17.878, df = 6,342, p < 0.001), indicating the diurnal change in cortisol levels although there was no diagnosis by sample interaction (F = 0.537, df = 12,342, p = 0.797).

3.2.2 DHEA

See figure 1.

A main effect of diagnosis was observed for DHEA (F = 6.783, df = 2,57, p = 0.002). Post hoc (LSD) analysis revealed that schizophrenic patients had significantly higher afternoon DHEA levels compared to both controls (mean log\(_{10}\) difference = 0.187, 95%CI = 0.080 to 0.294; p = 0.001) and bipolar patients (mean log\(_{10}\) difference = 0.146, 95%CI = 0.040 to 0.253; p = 0.008). There was no significant difference in DHEA levels between bipolar patients and controls (mean log\(_{10}\) difference = 0.041, 95%CI = -0.066 to 0.147; p = 0.451).

A small but significant main effect of sample was also observed (F = 2.452, df = 6,342, p = 0.046), indicating a more subtle diurnal change in DHEA levels than that seen in cortisol levels, although again there was no diagnosis by sample interaction (F = 0.266, df = 12,342, p = 0.978).

3.2.3 Cortisol-DHEA ratios

See figure 2.

There was no significant main effect of diagnosis in the molar cortisol-DHEA ratio (F = 1.729, df = 2,57, p = 0.187). A significant main effect of sample was observed
although there was no diagnosis by sample interaction (F=0.828, df=12,342, p=0.563).

3.2.4 Exploratory analyses: effects of age

The primary analyses were repeated with the inclusion of age as a covariate. In the analysis of cortisol levels, age was not a significant covariate (F=0.216, df=1,56, p=0.644). In the analysis of DHEA, age was found to be a significant covariate (F=19.890, df=1,56, p<0.001) although the effect of diagnostic group remained highly significant (F=6.068, df=2,56, p=0.004). Similarly, age was a significant covariate in the analysis of cortisol-DHEA ratios (F=12.421, df=1,56, p=0.001), although the main effect of group remained non-significant (F=0.658, df=2,56, p=0.522).

3.2.5 Exploratory analyses: effects of medication

The primary analyses were repeated to examine the effects of lithium. As no patients with schizophrenia were taking lithium, the comparison was made between bipolar patients on lithium (n=11), bipolar patients not on lithium (n=9), schizophrenic patients (n=20) and controls (n=20).

For cortisol levels, the between group comparison revealed a significant main effect (F=3.920, df=3,56, p=0.013) with the bipolar patients not on lithium (mean log\textsubscript{10} difference=0.128, 95%CI=0.025 to 0.232; p=0.016) and schizophrenic patients (mean log\textsubscript{10} difference=0.126, 95%CI=0.044 to 0.207; p=0.003) having higher cortisol levels than control. No other significant differences were found.

For DHEA levels, there was a significant between group main effect (F=4.567, df=3,56, p=0.006). Schizophrenic patients had higher DHEA levels than all other groups (p<0.05) although there were no other differences.

Finally, for cortisol-DHEA ratios, there was no overall ANOVA main effect
observed (F=1.851, df=3,56, p=0.148). However, it is of note that the bipolar patients not on lithium had the highest cortisol-DHEA ratios (untransformed marginal mean collapsed across time: bipolar patients not on lithium, mean=11.01, SEM=1.27; controls, mean=9.05, SEM=0.85; bipolar patients on lithium, mean=8.16, SEM=1.15; schizophrenic patients, mean=7.40, SEM=0.85). In an exploratory pairwise comparison, the cortisol-DHEA ratios of bipolar patients not on lithium were significantly higher than the schizophrenic patients (mean log_{10} difference=0.172, 95%CI=0.026 to 0.319; p=0.022).

4. DISCUSSION

The present study sought to examine afternoon plasma cortisol and DHEA levels and the cortisol-DHEA ratio in matched groups of schizophrenic and bipolar patients and healthy controls. In line with our original hypothesis, cortisol levels were significantly elevated in both patient groups compared with controls. However there was no evidence of a difference in the cortisol-DHEA ratio of the groups, despite an elevation in DHEA levels in schizophrenic patients compared with bipolar patients and controls.

To our knowledge there are no previous reports of cortisol-DHEA ratios in bipolar disorder. One post-mortem study that assessed neurosteroid levels in posterior cingulate and parietal cortex tissue from the Stanley Foundation Neuropathology Consortium collection found that pregnenolone and DHEA levels were higher in subjects with schizophrenia and bipolar disorder compared to control subjects (Marx et al., 2006).

A potential confounding factor in this study could be the differing medication regimes of the two patient groups. The majority of bipolar patients were taking a mood stabilizer at the time of testing while all of the schizophrenic patients were taking antipsychotics (see Appendix 1). Some differences were observed in relation to the effects lithium treatment on cortisol levels and the
cortisol-DHEA ratio. However, it should be noted that this was part of an exploratory analysis only (see section 3.2.5) and the implication of this finding is unclear as previous animal work has shown that chronic lithium administration can lower frontal cortex and hippocampus DHEA(S) levels, and increase serum DHEA peripherally (Maayan et al., 2004). It should also be noted that the atypical antipsychotic clozapine has been found to decreased rat brain cortical DHEA(S) levels (Nechmad et al., 2003). Our results require replication in a drug-free sample.

A further potential weakness is the sampling timeframe utilised in the present study. We carried out sampling at 30 minute intervals to account for the pulsatile nature of the HPA axis. However, the afternoon period (1:00 p.m. to 4:00 p.m.) may be a relatively insensitive time to detect abnormalities between patients and controls and future studies should consider sampling around the morning peak or evening trough.

Several recent studies have examined DHEA(S) levels in schizophrenia. Findings are consistent with those of the present study. Di Michele and colleagues found that plasma levels of DHEA were significantly elevated in 23 medicated patients with schizophrenia compared to matched controls (di Michele et al., 2005). Importantly there was little evidence of an effect of medication as there was no significant relationship between DHEA levels and olanzapine equivalents, although there was a strong negative relationship between clozapine dosages and DHEA plasma levels in the group of patients treated with clozapine only (however there were only 3 patients in this comparison). In a series of studies, Ritsner and colleagues have also shown that plasma cortisol-DHEA and cortisol-DHEAS ratios were significantly higher in schizophrenia patients than in healthy comparison subjects. Again, no significant association was found between these ratios and severity of psychopathology and type or dose of antipsychotic agent (Ritsner et al., 2004). Interestingly, elevated cortisol levels and an elevated cortisol-DHEA(S) ratio have been shown to be predictive of a positive short-term response to
antipsychotic treatment (Ritsner et al., 2005). Similarly, elevated DHEA(S) levels have been found in first-episode patients, along with a trend for higher DHEAS levels to be associated with shorter hospitalizations (Strous et al., 2004).

It is important to note the temporal evolution of structural changes within the HPA axis in psychotic illness. In a series of studies, Pariante and colleagues demonstrated that pituitary volumes are enlarged (by 10% compared to controls) in patients with first-episode psychosis while volumes are decreased (by 17% compared to controls) in those with ‘established’ schizophrenia (Pariante et al., 2004). Separated by diagnosis, the numerically largest increases have been found in schizophrenia/schizophreniform disorder (24%), compared to 19% larger in depressed patients, 16% larger in bipolar patients and 12% larger in those with other psychoses (Pariante et al., 2005). The patients in our study were recruited from secondary and tertiary care services and therefore had longer-term, chronic illnesses. An elevation of both cortisol (Ryan et al., 2003; Ryan et al., 2004) and DHEA (Strous et al., 2004) levels has been found in first-episode schizophrenia therefore, as we observed a similar neuroendocrine profile in our study, this may suggest these abnormalities are stable or even represent trait markers of illness. However, this – and the relationship between the structural changes and neuroendocrine profile – remains to be confirmed in longitudinal studies.

The mechanism of action for dissociable changes in cortisol and DHEA levels is unclear. Both steroids are mainly synthesized and secreted by the adrenal cortex. However, within the adult adrenal the zona fasciculata produces cortisol and the zona reticularis both DHEA and DHEAS (Rainey et al., 2002). It is therefore possible that there are differential alterations in the function or structure of these distinct morphological zones.

Recent studies have explored the use of antiglucocorticoid agents (Gallagher & Young, 2006), including DHEA, in the treatment of severe psychiatric illness. Strous and colleagues found that DHEA augmentation in
medicated schizophrenic patients resulted in a significant improvement in negative-, depressive- and anxiety symptoms which, in the case of the negative symptoms, correlated with the increase in plasma DHEA(S) levels (Strous et al., 2003). Improvements in extrapyramidal symptomatology, specifically parkinsonism, have also been demonstrated following adjunctive DHEA treatment (Nachshoni et al., 2005), although it should be noted that recently Ritsner and colleagues found that augmenting standard treatment with DHEA (200 mg/day for 6 weeks) did not improve symptoms, side effects, or quality-of-life impairment in schizophrenia, but did improve neurocognitive functioning and PANSS scores (from baseline) (Ritsner et al., 2006). Interestingly, the patients examined in the present study went on to take part in a trial of the GR-antagonist mifepristone (RU-486) where positive effects were observed in neurocognitive function and mood in the patients with bipolar disorder (Young et al., 2004) but not in the patients with schizophrenia (Gallagher et al., 2005) – i.e. the group with elevated levels of the ‘endogenous antiglucocorticoid’ DHEA. To date there have been no trials of DHEA in bipolar disorder although there are case reports that have suggested that DHEA may induce mania in some individuals with (Dean, 2000; Vacheron-Trystram et al., 2002) or without (Markowitz et al., 1999) previous history of bipolar disorder. It should also be noted that aside from the putative anti-glucocorticoid actions of DHEA(S), other properties of this steroid have potentially important therapeutic implications. DHEA replacement in patients with Addison’s disease (primary adrenal insufficiency) who were on standard glucocorticoid and mineralocorticoid replacement has been shown to improve self-esteem and mood, and a decrease in fatigue (Hunt et al., 2000). Improvements in insulin sensitivity in hypoadrenal women have also been described (Dhatariya et al., 2005). While the efficacy of DHEA has been examined in other diverse clinical conditions (Cameron & Braunstein, 2005), these effects have not been demonstrated consistently and may warrant further examination in larger randomised clinical trials.
In summary, our data demonstrates that afternoon hypercortisolaemia is evident in symptomatic bipolar and schizophrenic patients compared to controls. However, the elevation in DHEA levels may represent a dissociable endocrine marker in schizophrenia, although there was no overall change in cortisol-DHEA ratio in any group. Assessing the influence of individual drug treatments and profiling in medication-free groups should be a priority for future studies to increase our understanding of the pathophysiology of these illnesses and elucidate new avenues for treatment.
5. REFERENCES


schizophrenia. Archives of General Psychiatry 60, 133-141.


Figure 1: Afternoon cortisol and DHEA levels in bipolar patients (n=20), schizophrenic patients (n=20) and controls (n=20).

*Untransformed values are presented*
**Figure 2:** Molar cortisol-DHEA ratios in bipolar patients (n=20), schizophrenic patients (n=20) and controls (n=20).
**Appendix 1.** Medication details of bipolar and schizophrenic patients

<table>
<thead>
<tr>
<th></th>
<th>Bipolar patients (n)</th>
<th>Schizophrenic patients (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mood stabilizers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>carbamazepine</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>gabapentin</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>lamotrigine</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>lithium</td>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td>valproate</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td><strong>Antipsychotics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>amisulpride</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>clozapine</td>
<td>-</td>
<td>3</td>
</tr>
<tr>
<td>fluphenazine</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>haloperidol</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>olanzapine</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>quetiapine</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>risperidone</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>sulpiride</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>zuclopenthixol</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td><strong>Antidepressants</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>amitriptyline</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>citalopram</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>fluoxetine</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>lofepramine</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>mirtazapine</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td>paroxetine</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>reboxetine</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>sertraline</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>trazodone</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>venlafaxine</td>
<td>6</td>
<td>1</td>
</tr>
</tbody>
</table>

*a included are 9 bipolar patients on combination therapies:*
- lithium + lamotrigine: n=5
- valproate + lamotrigine: n=1
- lithium + carbamazepine + gabapentin: n=1
- carbamazepine + gabapentin: n=1
- lithium + valproate + carbamazepine: n=1

*b included are 2 schizophrenic patients on combination therapies:*
- olanzapine + haloperidol: n=1
- risperidone + fluphenazine: n=1

*c included are 4 bipolar patients on combination therapies:*
- venlafaxine + mirtazapine: n=3
- citalopram + mirtazapine: n=1
Acknowledgements

We thank the Mental Health Foundation (North-East of England branch) and the Peel Medical Research Trust for their generous financial support of this study. We thank Shirley Dodds and Mel Leitch for their technical support and for performing the biochemical analysis.