State and trait abnormalities in serotonin function in major depression

Z. BHAGWAGAR, R. WHALE and P. J. COWEN

Background  Neuroendocrine studies of brain serotonin (5-HT) function in depression generally show evidence of impaired 5-HT function but it is disputed whether or not this impairment resolves with clinical recovery.

Aims  To use the endocrine response to the selective 5-HT reuptake inhibitor, citalopram, to study brain 5-HT function in acute and recovered depressed subjects relative to healthy controls.

Method  We used a double-blind, placebo-controlled design to measure the prolactin and cortisol responses to citalopram (10 mg intravenously) in patients with major depression, in unmedicated subjects recovered from depression and in healthy controls.

Results  The prolactin responses to citalopram were blunted similarly in both acutely depressed and recovered subjects. The cortisol responses were blunted in the acutely depressed patients but not in the recovered subjects.

Conclusions  Our data support the proposal that some aspects of impaired 5-HT neurotransmission may be trait markers of vulnerability to depression. The recovery of the cortisol response to citalopram may indicate resolution of hypothalamic–pituitary–adrenal axis dysfunction.

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Serotonin (5-HT) neuroendocrine challenge tests have been used widely to assess brain 5-HT function in patients with acute major depression (Cowen, 1998). The consensus of these studies is that 5-HT neurotransmission is impaired in unmedicated depressed patients but it is unclear whether this abnormality persists following clinical recovery. The aim of the present study was to use a relatively new probe of 5-HT function, the selective 5-HT reuptake inhibitor citalopram (Joubert et al, 2000), to assess 5-HT neuroendocrine function in patients with acute depression and in those who had made a full clinical recovery from their illness.

METHOD

Subjects  A total of 46 subjects were recruited for the study over an 18-month period from the unit’s volunteer database, by advertisement or through out-patient clinics. All subjects gave written informed consent for the study, which was approved by the local ethics committee. The subjects included 16 controls (7 males and 9 females), 14 subjects who met the criteria for DSM-IV (American Psychiatric Association, 1994) major depression (7 males and 7 females) and 16 subjects recovered from at least two episodes of DSM-IV major depression (7 males and 9 females). All subjects were screened using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID; First et al, 1997). Subjects were excluded from the study if they had any history of an Axis I disorder (controls), any significant Axis I disorder apart from depression (acute and recovered groups), a history of a neurological disorder or a major physical illness.

The mean (s.e.) ages of the three groups were similar (controls, 38.6 (3.0) years; acute depression group, 44.5 (3.1) years; recovered depression group, 40.6 (3.1) years), as were their weights (controls, 70.1 (3.1) kg; acute group, 75.9 (3.6) kg; recovered group, 72.2 (3.8) kg). Patients with acute depression were drug-free for at least 3 months and had a mean (s.e.) score on the Hamilton Rating Scale for Depression (HRSD; Hamilton, 1960) of 20.9 (1.5) and a Beck Depression Inventory (BDI; Beck et al, 1961) score of 25.2 (3.3). Six subjects also met the criteria for melancholic depression. The subjects who were recovered from depression had been euthymic for at least 6 months and medication-free for at least 3 months. Their HRSD score had to be ≤7 (Frank et al, 1991). Their mean (s.e.) score on the HRSD was 1.5 (0.5) and on the BDI was 4.4 (1.3). Two in the recovered group and two in the acutely depressed group had a past history of suicide attempts.

Neuroendocrine testing  All subjects were tested on two separate days in a double-blind, balanced order, placebo-controlled design. The mean (s.e.) gap between the two tests was 10 (2) days, with no significant difference between the groups (data not shown). Subjects fasted after a light breakfast and came to the research unit at 12.00 h. This time was chosen to avoid the sharp morning decline in plasma prolactin and cortisol levels. An indwelling venous canula was inserted and maintained with heparinised saline. Subjects were tested reclining and were not allowed to sleep. After a 30-min rest period for removal of baseline venous samples for prolactin and cortisol estimation, 10 mg citalopram (diluted in 5 ml saline) or 5 ml saline was administered intravenously over 30 min. Blood sampling continued at 15-min intervals for a further 150 min. At 30-min intervals, subjects completed a 100-mm visual analogue rating scale (VAS) for ‘nausea’. All female subjects were tested in the first half of the menstrual cycle.

Biochemical measurements  Following blood collection, plasma was separated by centrifugation and stored at −20°C. Plasma prolactin was measured using a standard immunoradiometric assay (reagents provided by Netria, London, UK). The inter- and intra-assay coefficients of variation of the prolactin assays over the range encompassed by the standard curve were 5% and 1%, respectively. Cortisol was analysed using radioimmunoassay
(RIA) (reagents provided by Amersham International, Amersham, and Biosil, Cardiff, UK) with inter- and intra-assay coefficients of variation over the range encompassed by the standard curve of 10% and 1%, respectively.

**Statistical analysis**

All data were analysed using SPSS for Windows (version 9.0). Prolactin and cortisol responses to citalopram were analysed as area under the curve (AUC) using the trapezoid method, with subtraction of baseline secretion extrapolated from time zero. Change in AUC (ΔAUC) was measured by subtracting the AUC of prolactin and cortisol following placebo challenge from the respective AUC following citalopram challenge. The ΔAUC data were rank transformed in order to approximate satisfactorily to a normal distribution and then subjected to standard parametric analyses (Conover & Iman, 1981) with univariate analysis of variance (ANOVA), where ‘group’ (control, acute depression and recovered depression) was the between-subject factor. Gender, weight and age were added to the ANOVA as covariates. Significant differences on the ANOVA were followed by post hoc unpaired t-tests. In the text, for comprehensibility, the hormonal responses are illustrated by the raw data rather than the rank-transformed results. Correlations also were carried out on rank-transformed ΔAUC data using Pearson’s product moment. Baseline levels of prolactin and cortisol were calculated for each group using the average of the baseline values (at time zero) on the placebo and citalopram days and analysed by univariate ANOVA. The VAS scores of nausea were measured as peak change from baseline and also were analysed with an ANOVA.

**RESULTS**

Citalopram generally was well tolerated except for one female subject with acute depression who vomited after the infusion. This subject was excluded from further analysis. There was no change in VAS nausea ratings after placebo infusion (data not shown). Following citalopram, nausea ratings increased but there was no significant difference in mean (s.e.) peak nausea scores between the groups (F=0.29; d.f.=2.42; P=0.75); controls, 9.7 (5.7) mm; acute group, 9.2 (3.8) mm; recovered group, 14.4 (5.8) mm.

There was no significant difference between mean (s.e.) baseline prolactin levels across the three groups (F=0.60; d.f.=2.42; P=0.55); controls, 174.1 (14.0) mU/l; acute group, 162.0 (16.9) mU/l; recovered group, 151.0 (15.3) mU/l. The ANOVA on the ΔAUC prolactin data showed significant effect of group (F=3.53; d.f.=2.39; P=0.039). Post hoc analysis showed that, relative to controls, prolactin responses were decreased significantly in both the acute and recovered depression groups, with no significant difference between these two groups (Fig. 1). The covariates of gender (F=0.003; d.f.=1.39; P=0.96), weight (F=1.1; d.f.=1.39; P=0.31) and age (F=0.51; d.f.=1.39; P=0.48) were not significant in the ΔAUC prolactin response.

There was no difference between baseline cortisol levels across the three groups (F=1.618; d.f.=2.42; P=0.210): controls, 16.2 (1.9) µg/100 ml; acute group, 17.2 (2.03) µg/100 ml; recovered group, 13.2 (0.73) µg/100 ml. The ANOVA of the ΔAUC cortisol data showed a significant effect of group (F=6.03; d.f.=2.39; P=0.005). The post hoc analysis showed that, relative to controls, subjects with acute depression had significantly blunted cortisol responses whereas the recovered subjects did not. In addition, the responses of the recovered subjects were significantly greater than those in the acute group (Fig. 2). The covariates of gender (F=0.47; d.f.=1.39; P=0.50), weight (F=0.01; d.f.=1.35; P=0.95) and age (F=0.29; d.f.=1.39; P=0.60) were not significant in the ΔAUC cortisol response.

There were no significant correlations between HRSD and BDI scores and ΔAUC cortisol or ΔAUC prolactin responses in either acute or recovered subjects (all P values >0.05). Endocrine responses did not differ in the small number of acutely depressed melancholic subjects (n=6) compared with non-melancholic subjects. In all subjects considered together there were no significant correlations between baseline prolactin and ΔAUC prolactin responses or between baseline cortisol and ΔAUC cortisol responses. In addition, baseline cortisol did not correlate with ΔAUC prolactin responses (all P values >0.5). However, baseline cortisol and prolactin responses correlated with each other (r=0.30, P=0.047) and the ΔAUC cortisol response correlated with the ΔAUC prolactin response (r=0.46; P=0.002). Finally, there was no correlation between peak nausea score after citalopram and ΔAUC cortisol or ΔAUC prolactin response (all P values >0.05).

**Fig. 1** The ΔAUC prolactin responses of healthy controls (n=16), acutely depressed subjects (n=13) and recovered subjects (n=16) to citalopram (10 mg intravenously). Bars show the median values. The responses of the controls are significantly greater than the responses of those in the acute (P=0.014) and recovered groups (P=0.031) (unpaired test on rank-transformed data).

25
DISCUSSION

Our findings indicate that the prolactin and cortisol responses to citalopram are blunted in patients with acute major depression. Following clinical recovery, citalopram-induced prolactin release remains attenuated whereas the cortisol responses return towards normal values.

Citalopram as 5-HT neuroendocrine probe

Citalopram is a highly selective 5-HT reuptake inhibitor that, when administered intravenously, produces dose-related increases in prolactin and cortisol (Seifritz et al., 1996; Attenburrow et al., 2001). Its neuroendocrine profile in this respect is the same as that of other 5-HT reuptake inhibitors such as clomipramine and indalpine (Laakmann, 1990). The 5-HT releasing agent d-fenfluramine produces very similar changes (Newman et al., 1998). Therefore, the ability of citalopram to increase plasma prolactin and cortisol is likely to be mediated via acute increases in 5-HT neurotransmission in the hypothalamus. The fact that prolactin and cortisol responses to citalopram correlated significantly with each other supports the proposal that they are mediated by a common (probably serotonergic) mechanism.

The most appropriate intravenous dose of citalopram for neuroendocrine challenge has not yet been established clearly. Other studies of citalopram in depressed and healthy subjects have used doses of 20 mg (Seifritz et al., 1996; Kapitany et al., 1999) but in healthy volunteers we found that doses of 5 mg and above produce significant increases in prolactin and cortisol relative to placebo (Attenburrow et al., 2001). We therefore chose a dose of 10 mg as likely to represent a reasonable balance between tolerability and the production of reliable endocrine responses. The identity of the post-synaptic 5-HT receptors that mediate citalopram-induced prolactin and cortisol release has not yet been determined. Our preliminary work suggests that they are unlikely to be of the 5-HT₂A receptor subtype (Attenburrow et al., 2001).

Serotonin neuroendocrine responses in acute depression

Our findings with citalopram are consistent with a large body of data indicating that the endocrine responses to 5-HT challenges, particularly those that act via facilitation of presynaptic 5-HT function, are reliably blunted in patients with acute major depression (Cowen, 1998). In particular, our data are consistent with studies that have found blunted prolactin responses to the 5-HT reuptake inhibitor clomipramine (Anderson et al., 1992; Golden et al., 1992), as well as one previous investigation using 20 mg of intravenous citalopram (Kapitany et al., 1999). In the latter investigation, citalopram-induced cortisol release was not decreased significantly in patients with depression relative to healthy controls, although a trend in this direction was apparent.

Less-consistent effects in depression have been noted with the prolactin response to the 5-HT releasing agent fenfluramine, where blunted responses may be more apparent in subjects with melancholic depression and in those with impulsive personality traits (Cowen, 1998). This latter observation suggests that features other than current major depression may contribute to blunted endocrine responses to 5-HT challenge, a notion supported by our findings in subjects recovered from depression (see below).

The nature of the impairment in brain 5-HT pathways that is responsible for the blunted endocrine responses to presynaptic 5-HT challenge in acute depression has not been established clearly. However, brain imaging studies have identified decreased numbers of post-synaptic 5-HT₁A (Sargent et al., 2000) and 5-HT₁A receptors (Yatham et al., 2000) in patients with major depression. Perhaps most pertinent to challenge studies with 5-HT reuptake inhibitors are reports of decreased availability of 5-HT reuptake sites in mid-brain and brain-stem regions of patients with depression (Malison et al., 1998; Willeit et al., 2000). These abnormalities could contribute to the decreased functional responses to 5-HT challenge identified by neuroendocrine tests.

Serotonin neuroendocrine function in subjects recovered from depression

We found that the prolactin response to citalopram remained blunted in subjects who had recovered from depression and were off medication. Because people who have previous episodes of depression are at high risk of future episodes (Kendler et al., 1993), it is possible that blunted 5-HT-mediated prolactin release may be a trait marker indicating vulnerability to major depression. It is possible also that blunted 5-HT-mediated prolactin release could be a consequence of having experienced an episode of major depression.

To resolve this issue, future investigations will need to study larger numbers of subjects and identify as accurately as possible in each individual the number of previous depressive episodes as well as the length of symptomatic remission. Other studies have suggested that altered...
5-HT function may be linked to particular clinical correlates, such as liability to suicidal and aggressive behaviour (Kavoussi et al., 1997). Again, larger studies will be needed to explore this kind of possibility. However, in our study the small number of subjects who had made suicide attempts suggest that this may not be a prominent factor underlying blunted prolactin responses to citalopram. Recent weight loss also may complicate interpretation of endocrine responses to 5-HT challenge (Cowen, 1998). However, again this is unlikely to be a factor in the blunted prolactin responses in subjects recovered from depression, who did not describe any recent appetite change or weight loss on the clinical rating scales.

Our data are consistent with two of the three studies that have examined prolactin responses to fenfluramine in unmedicated subjects who had recovered from depression (Coccaro et al., 1987; Shapira et al., 1993; Flory et al., 1998). However, in two previous studies we have found that recovered patients have normal prolactin responses to the 5-HT precursor l-tryptophan (Upadhyaya et al., 1991; Smith et al., 2000). This suggests that some 5-HT abnormalities in depression are reversible with recovery, whereas others are not. Specifically, the prolactin response to l-tryptophan appears to be mediated via post-synaptic 5-HT1A receptors (Cowen, 1998), whereas the prolactin responses to citalopram are not attenuated by penbutolol, a β-adrenoceptor antagonist with 5-HT1A receptor antagonist properties (further details available from the author upon request).

Unlike citalopram-induced prolactin release, the cortisol responses in euthymic patients did show significant recovery compared with the acute depressed state. This again may reflect differential recovery between distinct 5-HT sub-systems. Another possibility is that blunted cortisol responses to 5-HT challenge are, in fact, partly dependent on the abnormal cortisol regulation often associated with the depressed state. The function of the hypothalamic–pituitary–adrenal axis shows extensive recovery with clinical remission (Zobel et al., 1999) and this could account for the relative normalisation of citalopram-induced cortisol release in recovered patients.

In conclusion, our findings confirm that acute major depression is associated with blunted 5-HT-mediated prolactin release and that this abnormality persists into clinical remission. Further studies will be needed to establish whether this represents a trait marker of vulnerability to depression and, if so, whether its origin is predominantly genetic or environmental.

CRITICAL IMPLICATIONS

- People with a history of recurrent depression have enduring abnormalities in some aspects of brain 5-HT function.
- Impaired 5-HT function could be a marker of vulnerability to depressive relapse.
- Measures designed to increase brain 5-HT function could decrease the risk of relapse in vulnerable individuals.

LIMITATIONS

- Abnormalities in 5-HT neuroendocrine mechanisms do not necessarily reflect changes in 5-HT function in brain regions implicated in the depressive syndrome.
- The impaired prolactin response to citalopram in recovered subjects could be a consequence of having been depressed, rather than a marker of vulnerability.
- The sample size was too small for men and women to be studied independently.

ZUBIN BHAGWAGAR, MRCPsych, RICHARD WHALE, MRCPsych, PHILIP J. COWEN, FRCPsych, University Department of Psychiatry, Warneford Hospital, Oxford, UK

Correspondence: Professor P.J. Cowen, University Department of Psychiatry, Warneford Hospital, Oxford OX3 7JX, UK

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