Evaluation of central serotonergic function in affective and related disorders by the fenfluramine challenge test: a critical review

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Abstract

Plasma prolactin levels following oral administration of the serotonin (5-HT) releasing agent, fenfluramine hydrochloride, have been extensively used to evaluate central serotonergic function in affective and related disorders. Cortisol responses to fenfluramine have generally been a less informative measure. In healthy subjects, prolactin release by fenfluramine is dose-dependent, blocked by antagonists of serotonin receptors of the 5-HT-2α/2c type, negatively correlated with age and increased in young females. In major depression, a preponderance of studies have found blunted prolactin responses compared to matched normal controls. Although a significant minority of studies have not found blunting, increased prolactin release has not been observed. The blunted prolactin release is not due to a deficient secretory capacity of pituitary lactotrophs and is congruent with other evidence for reduced central serotonergic function in major depression. Blunting of the prolactin response may be associated with severity of depression and with elevated baseline cortisol levels. Treatment with antidepressant drugs and electroconvulsive therapy has been reported to increase the prolactin response but this has not been replicated in all studies. Blunted prolactin responses to fenfluramine have been fairly consistently associated with impulsive aggression in different personality disorders and with severity of suicide attempts in depressed patients. A number of studies employing the fenfluramine challenge test (FCT) have been conducted in obsessive compulsive disorder but their results have been variable. Prolactin responses to fenfluramine may be enhanced in panic disorder and chronic fatigue syndrome but the number of studies in these conditions is small as is the case for seasonal affective disorder. Since the therapeutic administration of fenfluramine as an appetite suppressant has been suspended because of reports of cardiac complications, further use of this compound as a challenge agent is not anticipated. Future studies are likely to employ agents acting on specific serotonin receptors and should apply methodological insights from the use of the FCT, which are considered in this review. Use of concomitant brain imaging to evaluate the central effects of challenge agents directly is likely to become more prevalent and may supplant neuroendocrine challenge paradigms such as the FCT which have been remarkably heuristic but are limited in scope and methodologically complex.

Received 5 February 1998; Reviewed 24 February; Revised 9 March 1998; Accepted 22 March 1998
Key words: Fenfluramine, prolactin, cortisol, depression, serotonin.

Introduction

Neuroendocrine challenge tests are a widely used research strategy in biological psychiatry and neuropsychopharmacology. Their basic premise is that neurotransmitters and receptors which control hormone release may also be implicated in the pathogenesis and treatment of neuropsychiatric disorders. Putatively, perturbations in the neurotransmitters and receptors which underlie neuro-psychiatric disorders and the changes effected by treatment will be reflected in the levels of hormones released by a challenge agent.

The widespread use of neuroendocrine challenges is primarily due to the fact that they provide an index of brain function, albeit in a specific brain area with unique characteristics. In this regard, neuroendocrine challenges have a strong advantage over strategies employing measurements of plasma levels of neurotransmitters and their metabolites, receptors on peripheral blood cells and urinary metabolite levels. Most challenges are easy to administer, are not associated with significant adverse effects, and provide the opportunity for observation of behavioural changes, subjective experiences and effects on physiological measures such as blood pressure, pulse...
and temperature. Many of the drugs used in neuroendocrine challenges are approved for regular clinical use and are thus easily accessible.

These advantages are offset by significant limitations. One which is often ignored by investigators in interpreting their data is that findings referring to the hypothalamus and/or pituitary cannot necessarily be generalised to areas of greater relevance to the etiology and treatment of the disorder under scrutiny. Interpretation of neuroendocrine challenge data is fraught with complexity for other reasons as well. It must be established that an abnormality in hormone release by a challenge agent acting via a particular neurotransmitter is not due to dysfunction of the gland secreting the hormone. It must also be considered that few pharmacological agents are completely specific for a particular neurotransmitter or receptor, and that the release of most hormones is controlled by multiple inputs. A number of crucial methodological issues must also be addressed. Plasma levels of the challenge agent should be measured and covaried with hormone release data so as to differentiate pharmacokinetic and pharmacodynamic effects. A placebo challenge is important to separate effects of the challenge agent from those due to the stress of the procedure. Finally, individual factors which influence hormone response to a particular challenge, such as age, gender and body weight must be taken into account.

In spite of these complexities, neuroendocrine challenge tests have had an important impact in biological psychiatry and neuropsychopharmacology and have yielded fairly consistent results in some cases. The fenfluramine challenge test (FCT) has been particularly widely used. Since the recent withdrawal of this agent from clinical use, an intermission in the use of the procedure has been forced and will probably become permanent. This is an opportune time to review critically the cumulated body of research employing the FCT, to evaluate whether there are substantive conclusions to be drawn, and to consider their implications for future research.

This review covers the period 1966–1997 and includes papers yielded by a MEDLINE search employing the terms ‘fenfluramine’, ‘prolactin’ and ‘cortisol’. The emphasis of the review is on affective disorders, particularly depression, in which the FCT has been primarily applied. Conditions such as obsessive-compulsive disorder (OCD), panic disorder (PD), and chronic fatigue syndrome (CFS) are also considered, as is application of the FCT to impulsive aggression. All of the above may share pathophysiological underpinnings with affective disorder and are variably responsive to antidepressant (AD) treatment.

Previous reviews in which the FCT is considered in the context of other serotonergic challenges (Power and Cowen, 1992; Yatham and Steiner, 1993) should be noted. Serotonergic challenges have been performed with the 5-HT precursor L-tryptophan, which increases secretion of prolactin and growth hormone (GH); with 5-hydroxytryptophan, which increases cortisol; with clomipramine, which increases synaptic 5-HT by inhibiting its uptake and results in increased prolactin; with the non-selective 5-HT receptor agonist m-chlorophenylpiperazine (mCPP), which increases secretion of prolactin, ACTH and cortisol; and with the postsynaptic 5-HT-1A receptor agonists ipsapirone, buspirone and gepirone, which increase ACTH, cortisol and growth hormone (for reviews see van Praag et al., 1986; Coccaro and Kavoussi, 1994). Fenfluramine acts both presynaptically to increase 5-HT release and postsynaptically as a receptor agonist (see below). Because of these dual properties, challenge tests using fenfluramine provide an overall indication of the ‘net’ activity of the serotonergic system, in contrast to other challenge agents where the information which can be derived is limited to an assessment either of 5-HT release or of activity at one of the many types of postsynaptic receptors for 5-HT. The relationship of serotonergic function to the hypothalamic-pituitary-adrenal axis has recently been reviewed (Levy and van de Kar, 1992; Dinan, 1996), as have the factors affecting interpretation of data in neuroendocrine challenge tests (Coccaro and Kavoussi, 1994).

Clinical background

Fenfluramine (N-ethyl-α-methyl-m-(trifluoromethyl)phenylethylamine) was approved as an anorectic drug for the treatment of obesity by the U.S. Food and Drug Administration (FDA) in 1973. In 1992 it was shown that a combination of fenfluramine with phentermine enabled loss of weight to occur more rapidly with fewer side effects. The d-isomer of fenfluramine (Redux) was approved by the FDA in April 1996, and by June of that year U.S. doctors had written almost 2.5 million prescriptions, with an estimated number of 60 million prescriptions worldwide. In August 1997, Connolly et al. reported heart valve pathology in 24 patients who had been taking the fenfluramine-phentermine combination. In the same issue of the New England Journal of Medicine, Mark et al. (1997) reported fatal pulmonary hypertension in a 29-year-old woman who had been taking the fenfluramine-phentermine combination for only 23 days. The release of these findings before publication led to the reporting of many more cases to the FDA. An earlier report linking fenfluramine to primary pulmonary hypertension had
Pharmacology

Effects of fenfluramine on 5-HT release

The action of fenfluramine was suggested by Garattini et al. (1979) to involve stimulation of 5-HT release from nerve terminals and blockade of its reuptake. This results in a decrease in brain tissue levels of 5-HT. A direct effect of d-fenfluramine to increase 5-HT release was first shown in vitro in rat brain slices by Mennini et al. (1985), and subsequently using in vivo microdialysis in rat frontal cortex by LaFerrere and Wurtman (1989) and in lateral hypothalamus by Schwartz et al. (1989). Microdialysis studies showing the stimulating effect of fenfluramine on 5-HT release have now been performed in several brain areas, including frontal cortex (Carboni and di Chiara, 1989), hippocampus (Sabol et al., 1992), and striatum (Kreiss et al., 1993). Carboni and di Chiara (1989) showed that the 5-HT releasing action of fenfluramine was independent of nerve terminal depolarization, since tetrodotoxin increased rather than decreased the amount of 5-HT released. The increase was explained by the blockade of physiological release by tetrodotoxin resulting in accumulation of 5-HT in the nerve terminals, thus increasing the amount of 5-HT available for release by fenfluramine.

Sarkissian et al. (1990), Sabol et al. (1992) and Kreiss et al. (1992) showed that in the presence of the 5-HT uptake blockers fluoxetine or citalopram, the effect of fenfluramine to stimulate 5-HT release was blocked. These results indicated that transport of fenfluramine into serotonergic neurons is necessary to increase 5-HT release. Depletion of neuronal stores of 5-HT with the neurotoxin, p-chloroamphetamine (PCA), reduced d-fenfluramine-induced 5-HT release in frontal cortex by more than 90% (Series et al., 1994), while KCl-induced release was only reduced by about 60%. These results were interpreted as showing that fenfluramine induced 5-HT release preferentially from ‘fine’ axon terminals which are completely destroyed by PCA, while the depolarization-mediated 5-HT release induced by KCl involves both ‘fine’ and ‘beaded’ axon terminals, the latter being resistant to PCA-induced degeneration. Studies performed both on fresh human neocortex (Bonnano et al., 1984) and on rat hippocampus (Gobbi et al., 1993) or hypothalamus (Raiteri et al., 1995) have indicated that fenfluramine may induce 5-HT release by two different mechanisms. At a fenfluramine concentration of 5 μM, 5-HT release was blocked by fluoxetine and was Ca²⁺-independent and insensitive to inhibition by the 5-HT-1d agonist sumatriptan or the 5-HT-1b agonist RU 24969 (in human and rat tissue respectively), while at a fenfluramine concentration of 0.5 μM, release was still blocked by fluoxetine but was Ca²⁺-dependent and sensitive to inhibition by sumatriptan or RU 24969. It was suggested that at high concentrations, fenfluramine diffuses into serotonergic terminals and causes release of 5-HT via the 5-HT uptake or transporter site working in the inside-to-outside direction, while at lower concentrations fenfluramine enters the terminal via the carrier and elicits 5-HT release by an exocytosis-like mechanism.

The ability of the various isomers of fenfluramine to interact with different monoamines in the brain of the rat was investigated by Invernizzi et al. (1986). d-Fenfluramine was more potent than the l-isomer in reducing tissue levels of 5-HT and its metabolite 5-HIAA 4 h after administration, while l-fenfluramine was more potent in decreasing noradrenaline and dopamine levels. The effects of repeated injections of the various isomers on 5-HT levels in rat brain were studied by Kleven and Seiden (1989). Dose-related depletions of 5-HT were produced in somatosensory cortex, striatum, hypothalamus and hippocampus, with the d-isomer being more potent at low doses. At a dose of 12.5 mg/kg s.c., d-fenfluramine given twice daily for 4 days produced a greater than 80% reduction in cortical tissue 5-HT levels.

Fenfluramine and anorexia

Since both 5-HT and the fenfluramine metabolite d-norfenfluramine decrease feeding when injected into the paraventricular nucleus of the hypothalamus (Shor-Posner et al., 1986), it was assumed for many years that fenfluramine induces hypophagia by releasing 5-HT from hypothalamic stores. However, 5-HT uptake blockers were shown in two studies (Raiteri et al., 1995; McCann et al., 1995), to have no effect on the hypophagic or anorectic action of fenfluramine, suggesting that this effect was independent of 5-HT release. Further evidence for this was provided by the observations that different doses of fenfluramine were required to induce 5-HT release and to induce hypophagia (Raiteri et al., 1995), while the 5-HT depleting agent p-chlorophenylalanine (PCPA) had no effect on hypophagia but completely
prevented fenfluramine-induced 5-HT release (Gibson et al., 1993; Oluyomi et al., 1994). The anorectic effect in animals could be inhibited by several 5-HT receptor antagonists, including the non-specific antagonist metergoline, the 5-HT-1a/1b antagonist cyanopindolol, and the 5-HT-2 antagonist ritanserin (Neill and Cooper, 1989; Raiteri et al., 1995), and a similar antagonism by ritanserin was also shown in human volunteers (Goodall et al., 1993). The anorectic effect therefore appears to be independent of the effect of fenfluramine on 5-HT release, and to proceed via direct activation of postsynaptic receptors, although exactly which receptors are involved is still under debate (Curzon et al., 1997).

Neuroendocrinology

Effects of fenfluramine on hormone release

Fenfluramine was first shown to increase prolactin secretion in animals by Quattrone et al. (1978). The effect of low doses of d-fenfluramine on prolactin release was blocked by prior administration of either the non-specific 5-HT receptor antagonist, metergoline, or PCPA, indicating that the effect was dependent both on release of endogenous 5-HT and on stimulation of postsynaptic 5-HT receptors. Acute lesioning of 5-HT neurones with the neurotoxin 5,7-dihydroxytryptamine also abolished the hormone effect (Quattrone et al., 1979). Further evidence for involvement of postsynaptic receptors was provided by the finding of Kuhn et al. (1981) that chronic 5-HT depletion produced an extremely robust response, presumably due to receptor up-regulation.

Fenfluramine (the racemic or L-form) was first shown to increase prolactin secretion in normal human volunteers by Quattrone et al. (1983). The effect was dose-dependent, reached a peak 4 h after administration of the drug, and was blocked by prior administration of metergoline. Lewis and Sherman (1984) showed a similar dose-dependent, stimulating effect of d-fenfluramine on secretion of ACTH and cortisol in normal men. These effects were blocked by the 5-HT antagonist cyproheptadine.

It has been suggested that different mechanisms are involved in the effects of fenfluramine to release prolactin and cortisol. In animal studies, Van de Kar et al. (1985) found that the prolactin effect, which peaked 30 min after injection of fenfluramine, was inhibited by either of the 5-HT uptake blocking drugs, fluoxetine and indalpine, while the effect on corticosterone levels, which peaked later at 2 h, was unchanged. Similarly, the 5-HT precursor, l-tryptophan, potentiated the effect of fenfluramine on prolactin but not on corticosterone. McCann et al. (1996) found that fluoxetine pretreatment of rats did not prevent the fenfluramine-induced increase in prolactin, and concluded from this that the effect did not involve 5-HT release. The dose of fluoxetine used by these authors (5 mg/kg) was lower than that used by Van de Kar et al. (1985; 10 mg/kg). It should be pointed out, however, that inhibition of 5-HT release from the hypothalamus in the in vivo microdialysis experiments of Raiteri et al. (1995) was already achieved at a fluoxetine dose of 1 mg/kg.

While the effect of d-fenfluramine to increase prolactin has been shown by several investigators, there is less consistent data on the effects of the drug on cortisol. In two studies where the effect of d-fenfluramine was investigated in normal volunteers acting as a control group for patients with psychiatric illness, significant increases in cortisol were observed (Lucey et al., 1992; O’Keane et al., 1992). These studies were not placebo-controlled. Placebo-controlled studies have reported no significant increase in cortisol (Oliver et al., 1990), a clear increase (Bond et al., 1995; Palazidou et al., 1995; Park et al., 1996) or a slight increase consisting only of an attenuation of the circadian fall in cortisol seen with placebo (Lerer et al., 1988; Gorard et al., 1993). Goodwin et al. (1994) found no effect of 30 mg d-fenfluramine on levels of either prolactin or cortisol in 10 healthy male volunteers, when compared to the effect of placebo. These findings cast doubt on the validity of the increases reported in studies in which no placebo control was present.

Receptor mediation of fenfluramine-induced hormone release

Many studies in animals and human subjects have been devoted to elucidating which of the 5-HT receptor subtypes mediate the hormone responses to fenfluramine. Di Renzo et al. (1989) showed that the prolactin response to fenfluramine in rats was completely blocked by the specific 5-HT-2a/2c receptor antagonist ritanserin. Van de Kar et al. (1989) concluded, however, on the basis of the lack of inhibition of fenfluramine-stimulated prolactin secretion by the 5-HT-2a antagonist LY 53857, that the response was mediated by 5-HT-1b receptors. A possible explanation for this discrepancy is that the receptor involved in mediating the effect of fenfluramine is the 5-HT-2c receptor. This was the conclusion reached by Albinsson et al. (1994), who found no effect of the drug, amperozide, which has a high affinity for the 5-HT-2a receptor but a very low affinity for the 5-HT-2c receptor, on fenfluramine-induced prolactin secretion in rats.

In human volunteers, the 5-HT-1a/1b receptor antagonist, pindolol, when given in a single dose of 30 mg,
lowered baseline prolactin levels but did not significantly attenuate the response to d-fenfluramine (Park and Cowen, 1995). A different result was obtained by Palazidou et al. (1995) who gave 5 mg pindolol three times a day for 2 days to human volunteers and found attenuation of the prolactin but not of the cortisol response to a subsequently administered fenfluramine challenge. More consistent results have been obtained with 5-HT-2a/2c antagonists, since both ritanserin (Goodall et al., 1993) and amysgeride (Coccaro et al., 1996a) completely blocked the prolactin response. The 5-HT-3 antagonist, ondansetron, had no effect on d-fenfluramine-induced prolactin release (Coccaro et al., 1996b).

The nature of the receptors controlling corticosteroid secretion is less clear. Work in dogs (Barbieri et al., 1984) suggested the involvement of 5-HT-2 receptors since ketanserin blocked the action of a high dose of fenfluramine. Both human and animal work have also suggested that peripheral mechanisms may be important in controlling corticosteroid release. This was based on the finding of McElroy et al. (1984) that i.p. but not i.c.v. fenfluramine increases plasma corticosterone in rats, while in OCD patients, Hollander et al. (1993) found that cortisol levels peaked significantly earlier after fenfluramine than did prolactin levels. However, Schurmeyer et al. (1996), who administered fenfluramine repeatedly to healthy volunteers and measured responses over a 24-h period, concluded that fenfluramine stimulated the activity of the hypothalamic-pituitary-adrenal axis at a suprapituitary level by increasing the amplitude of the secretory bursts of ACTH and cortisol.

Park and Cowen (1995) consider the question of why, if the fenfluramine-induced increase in hormone levels is mediated at least in part by release of 5-HT, all postsynaptic receptors controlling hormone secretion should not be stimulated by fenfluramine to an equal extent. The explanation may be related to increased incidence of 5-HT-2a or 5-HT-2c receptors found postsynaptically to the ‘fine’ serotonergic terminals which are selectively destroyed by PCA and are associated with fenfluramine-induced 5-HT release (Series et al., 1994). A greater proportion of the 5-HT released from nerve terminals by fenfluramine is thus available for activation of these 5-HT-2 receptors than is the case for other 5-HT receptor subtypes. However, an alternative possibility is that the action of fenfluramine is predominantly postsynaptic, as suggested by McCann et al. (1996). This possibility is supported by the high affinity of the fenfluramine metabolite norfenfluramine for 5-HT-2c receptors (Gibson et al., 1993), and the fact that treatment of healthy subjects or depressed patients with 5-HT uptake blocking drugs did not reduce fenfluramine-stimulated hormone secretion but in some cases even increased it (see below).

**FCT in normal human volunteers**

The standard dose of the racemic mixture, dl-fenfluramine, has been 60 mg, administered orally. More recent neuroendocrine challenges have been performed with the d-isomer because of its greater specificity for the serotonergic system, with a routine dose of 30 mg. Several variables other than disease state must be taken into account if data obtained from neuroendocrine challenge tests are to be correctly interpreted (Coccaro and Kavoussi, 1994). These include age, gender, body weight, circadian rhythms and menstrual status for women. McBride et al. (1990) found greater prolactin responses to dl-fenfluramine in persons under 30 years of age compared to older subjects. Within the 30 years and younger age group, the responses were greater in women than in men, possibly due to weight differences. Broadly similar conclusions regarding the effects of age and gender were reached by Lerer et al. (1996), except that in this study there was an inverse relationship between the prolactin response and age over the entire age range studied.

Muldoon et al. (1996) found that plasma fenfluramine concentrations and prolactin responses were inversely proportional to body weight. When fenfluramine doses were calculated as functions of body weight (weight relative dose), the drug levels and the prolactin responses were directly proportional to this parameter.

O’Keane et al. (1991) measured prolactin responses to fenfluramine at different times throughout the menstrual cycle in 9 healthy women. Responses were maximal at mid-cycle and lowest during follicular phase, with responses premenstrually being midway between the two. Plasma estradiol levels varied in parallel with the neuroendocrine responses. In related work, two groups have studied women with menstrual cycle-related disorders. FitzGerald et al. (1997a) found a blunted prolactin response in 9 women with premenstrual dysphoric disorder compared to 11 healthy women in the luteal phase, while Bancroft and Cook (1995) found no change in the effect of d-fenfluramine on either prolactin or cortisol in women with premenstrual depression. A gender difference with respect to the influence of plasma tryptophan levels on the prolactin response to dl-fenfluramine was shown by FitzGerald et al. (1997b). In healthy female subjects, a lower total plasma tryptophan was associated with an increased response, while in male subjects tryptophan levels had no effect.

A direct correlation between prolactin and cortisol responses to dl-fenfluramine was shown in normal
responses to report found a high correlation between the prolactin fenfluramine, in keeping with the animal data presented above, is not specific for the serotonergic system, a recent report found a high correlation between the prolactin responses to DL-fenfluramine and to D-fenfluramine (Coccaro et al., 1996c).

Monteleone et al. (1997a) measured prolactin and cortisol after administration of 30 mg D-fenfluramine or placebo both in the morning and the afternoon. Prolactin levels after fenfluramine were increased on both occasions compared to placebo, with the effect in the afternoon being slightly but not significantly greater. Cortisol was increased relative to placebo only in the afternoon. These findings are most easily explained by stimulation of the HPA axis being more easily observed at the trough of cortisol levels in the afternoon. However, a circadian rhythm in plasma tryptophan levels with a peak in the afternoon might also explain this observation.

FCT in affective disorders

Depression

Table 1 lists 16 studies, 9 with DL-fenfluramine and 7 with the D-isomer, in which central serotonergic function in depressed patients was assessed by means of the FCT. Only 5 of the studies (Siever et al., 1989; Lichtenberg et al., 1992; Mann et al., 1995; Park et al., 1996) were placebo-controlled, and only four (Coccaro et al., 1989; Lichtenberg et al., 1992; Mann et al., 1995; Park et al., 1996) measured plasma fenfluramine levels. All the DL-fenfluramine studies employed a 60 mg oral dose irrespective of body weight, and all the D-fenfluramine studies administered 30 mg p.o. except for one which used 45 mg (Maes et al., 1991).

The studies listed in Table 1 show an overall trend towards reduced prolactin responses to fenfluramine in depressed patients. Eleven out of 16 studies which compared depressed patients to normal controls found lower prolactin responses in the depressed patients, the rest finding no significant difference. Four out of the 5 placebo-controlled studies (Siever et al., 1984; Coccaro et al., 1989; Lichtenberg et al., 1992; Mann et al., 1995) found blunting. This group includes three of the studies which took plasma fenfluramine levels into account.

In addition to the studies listed in Table 1, four studies (Lopez-Ibor et al., 1989; Maes et al., 1989; Mitchell et al., 1990; Malone et al., 1996) have compared prolactin responses in various groups of depressed patients, in some cases using dysthymic patients as a control group. Of these, the study by Maes et al. (1989) reported an increased prolactin response to DL-fenfluramine in major as opposed to minor depression, while all the other studies reported decreased responses in the more severely depressed groups.

Results for the cortisol response are more variable. Five studies (versus normal controls) show a reduced response, and 5 no difference. Only 1 study employed a placebo control group (Park et al., 1996). An increased cortisol response in depression was found in one study where 14 patients with major depressive disorder and melancholia, and 12 patients with major depressive disorder without melancholia were compared with 8 patients with dysthymic disorder (Lopez-Ibor et al., 1989). As discussed above, results from those studies in which the cortisol response to fenfluramine was measured in the absence of a challenge with placebo should be interpreted with caution. At the dose normally used, fenfluramine serves only to attenuate the circadian fall in cortisol levels, and no real increase occurs.

Several factors have been reported to affect the outcome of fenfluramine challenge tests and must be taken into account in the interpretation of the above results:

(a) Age, gender and body weight. A negative correlation between age and prolactin response to fenfluramine in normal volunteers has been discussed above. Asnis et al. (1988) also found that the prolactin response to fenfluramine in normal controls was negatively correlated with age, while in the depressed patients the cortisol response was positively correlated with age. Mann et al. (1995) divided their sample into two groups according to age, with a cut-off of 30 years. The younger depressed patients showed a significant 38% reduction in the prolactin response compared to controls, while there was no difference between patients and controls in the older age group. Broadly similar results were obtained by Lerer et al. (1996). There was no relationship between age and the prolactin response in depressed patients, in contrast to the inverse relationship seen in controls, and the degree of blunting of the prolactin response in the older depressed patients was less marked than in the younger ones. Since the age-dependent decline in serotonergic functioning resembles the decline seen in depressed patients at a younger age, Lerer et al. (1996) postulated that the susceptibility of older people to depression and the more severe clinical picture they often present may be due to an additive effect of age and depression on serotonergic transmission.

In keeping with the increased prolactin responses to DL-fenfluramine found in young normal women compared to men (McBride et al., 1990), three studies found increased
### Table 1. Fenfluramine challenge tests in depressed patients vs. normal controls

<table>
<thead>
<tr>
<th>Reference</th>
<th>Controls</th>
<th>Patients</th>
<th>Dose</th>
<th>Prolactin</th>
<th>Cortisol</th>
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<tr>
<td></td>
<td>(n)</td>
<td>(n) (diagnosis)</td>
<td>(mg)</td>
<td>response</td>
<td>response</td>
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<tr>
<td><strong>(a) d-fenfluramine</strong></td>
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<tr>
<td>Siever et al., 1984*</td>
<td>10</td>
<td>9/18 (RDC-MDD)</td>
<td>60</td>
<td>Reduced</td>
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<td></td>
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<td>(12 BP)</td>
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<tr>
<td>Asnis et al., 1988</td>
<td>10</td>
<td>15 (RDC-MDD)</td>
<td>60</td>
<td>No difference</td>
<td>No difference</td>
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<td></td>
<td></td>
<td>(12 BP)</td>
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<td></td>
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<tr>
<td>Weizman et al., 1988</td>
<td>8</td>
<td>8 (RDC-MDD)</td>
<td>60</td>
<td>No difference</td>
<td>Reduced</td>
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<td></td>
<td></td>
<td>(All UP)</td>
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<tr>
<td>Coccaro et al., 1989†</td>
<td>18</td>
<td>15 (RDC-MDD)</td>
<td>60</td>
<td>Reduced</td>
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<td>(7 BP)</td>
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<tr>
<td>Mitchell and Smythe,</td>
<td>14</td>
<td>14/27 (RDC-MDD)</td>
<td>60</td>
<td>Reduced</td>
<td>No difference</td>
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<tr>
<td>1990</td>
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<td>(3/4 BP)</td>
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<tr>
<td>Lichtenberg et al.,</td>
<td>12</td>
<td>27 (DSM III-R-MD)</td>
<td>60</td>
<td>No difference</td>
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<tr>
<td>1992*†</td>
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<td>24 (RDC MDD)</td>
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<td>(6 BP)</td>
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<td>Gerra et al., 1995</td>
<td>9</td>
<td>9‡</td>
<td>60</td>
<td>Reduced</td>
<td>Reduced</td>
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<tr>
<td>Mann et al., 1995*‡</td>
<td>26</td>
<td>26 (RDC MDD)</td>
<td>60</td>
<td>Reduced (age &lt; 30 only)</td>
<td>Reduced</td>
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<td></td>
<td></td>
<td>(All UP)</td>
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<tr>
<td><strong>(b) d-fenfluramine</strong></td>
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<tr>
<td>Maes et al., 1991</td>
<td>10</td>
<td>(DSM III-R-17 Minor D. 21 MD, 20 Melancholic)</td>
<td>45</td>
<td>No difference</td>
<td>No difference</td>
</tr>
<tr>
<td>O’Keane and Dinan, 1991</td>
<td>16</td>
<td>23 (DSM III-R-MD)</td>
<td>30</td>
<td>Reduced</td>
<td>Reduced</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lucey et al., 1992</td>
<td>10</td>
<td>10 (DSM III-R-MD)</td>
<td>30</td>
<td>Reduced</td>
<td>Reduced</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cleare et al., 1995</td>
<td>25</td>
<td>15 (DSM III-R-MD)</td>
<td>30</td>
<td>Reduced</td>
<td>Reduced</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cleare et al., 1996</td>
<td>19</td>
<td>19 (DSM III-R-MD)</td>
<td>30</td>
<td>Reduced</td>
<td>Reduced</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(All UP)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Park et al, 1996*†</td>
<td>29</td>
<td>31 (DSM III-R-MD)</td>
<td>30</td>
<td>No difference</td>
<td>No difference</td>
</tr>
<tr>
<td>Abel et al., 1997</td>
<td>15</td>
<td>15 (DSM III-R-MD)</td>
<td>30</td>
<td>Reduced</td>
<td>No difference</td>
</tr>
</tbody>
</table>

* Placebo controlled studies; †fenfluramine levels measured; ‡Heroin addicts with comorbid DSM III-R-MD.

**RDC MDD** = Research Diagnostic Criteria – Major Depressive Disorder.

**Notes**

1. When the breakdown of the groups into UP and BP patients is explicitly stated in the paper, this is noted. In most studies, diagnoses are reported as DSM III-R Major Depression (DSM III-R-MD), which implies that BP patients were not included.

2. In two studies (Siever et al., 1984; Mitchell and Smythe, 1990) a group of depressed patients matched to controls from within a larger group of depressed patients, is reported. The total number of depressed patients is given after the number matched (i.e. n matched/n total).

Proline responses in depressed female patients compared to depressed males (O’Keane and Dinan, 1991; O’Keane et al., 1992a; Mann et al., 1995), although no gender difference was reported in the study of Lichtenberg et al. (1992).

Mitchell and Smythe (1990) found that blunting of the prolactin response to fenfluramine occurred to a greater extent in patients who showed a greater degree of weight loss. Lichtenberg et al. (1992) showed the opposite i.e. a greater degree of blunting in patients who did not show weight loss.

(b) **Severity of depression and suicidality.** Severity of depression may explain some of the variation in the results observed. Both Lopez-Ibor et al. (1989) and Mitchell and Smythe (1990) found greater blunting of the prolactin response to fenfluramine in endogenous depressives, and concluded that the degree of blunting was directly related to the severity of depression. A similar result was obtained by Mitchell et al. (1990) in a study in which different groups of depressed patients were assessed in the absence of controls. O’Keane and Dinan (1991), however, found that patients with en-
dogenous depression had a lesser degree of blunting than non-endogenously depressed patients. Maes et al. (1991) examined patients with minor (including dysthymia), major and melancholic depression separately and found no difference from controls in any of the groups.

Other possible indices of severity are difficult to discern from the papers reviewed. There appears to be a trend for patients showing blunted prolactin responses to have higher Hamilton Depression Scale scores, although this cannot be said definitively since the 17 and 21 Hamilton scales were both used and some studies do not state which was administered. A relationship between Hamilton scores and prolactin response to fenfluramine was not observed in those studies which examined this correlation. Hospitalisation status may also give an indication of severity. In many of the papers it is not clear whether inpatients or outpatients were studied. Nevertheless, it may be noted that those studies which definitively stated that all or most of their depressed patients were hospitalised, found blunted prolactin responses (Coccaro et al., 1989; Mitchell and Smythe, 1990; O'Keane and Dinan, 1991; Lichtenberg et al., 1992; Mann et al., 1995).

Coccaro et al. (1989) found that blunted prolactin responses correlated with a history of suicidal behaviour in patients with affective disorders. Lopez-Ibor et al. (1990) examined a group of 17 suicidal patients from which those suffering from depression or psychotic disorders had been excluded. In comparison with an age-and sex-matched control group, the suicide attempters had significantly lower prolactin and cortisol responses to d-fenfluramine, and also showed significantly higher basal cortisol concentrations. In the study by Mann et al. (1995), there was no correlation between reduced prolactin response and severity of depression or previous suicide attempts. However, the younger depressed patients, amongst whom a decreased response was found, had earlier onset of the first lifetime episode of major depression, greater degree of suicidal intent during a recent suicide attempt, and a higher rate of comorbid borderline personality disorder. In another recent study by the same group (Malone et al., 1996), prolactin responses were compared in two groups of suicidal patients with major depression. Those who had attempted more potentially lethal and highly planned suicide attempts had a significantly lower response than those whose attempts at suicide had been less potentially lethal. Attenuation of the cortisol response to d-fenfluramine in patients with a history of suicide attempts was reported by Cleare et al. (1996). These authors matched their depressed patients with control subjects for age, sex, weight and menstrual status, and concluded on the basis of the reductions seen in the patients both in prolactin and cortisol, that decreased serotonergic transmission occurs in depression and is further reduced in suicide.

(c) Baseline cortisol levels. An indication of the degree of depression may be provided by basal cortisol levels. Mitchell and Smythe (1990) found both elevated cortisol and reduced prolactin in their group of depressed patients. Covarying for these baselines resulted in a loss of significance of the difference in prolactin response to fenfluramine between controls and the depressed patients. However, Lichtenberg et al. (1992) found no differences in basal levels of either hormone and significant blunting of the prolactin response to fenfluramine was still present after covarying for baseline cortisol. An inverse correlation between baseline cortisol and the prolactin response to d-fenfluramine was found by Cleare et al. (1995) and the same authors also reported an inverse correlation between baseline cortisol and the cortisol response to d-fenfluramine in patients who had received antidepressant (AD) treatment (Cleare et al., 1997). O’Keane and Dinan (1991) found a highly significant correlation between high baseline cortisol levels, presence of weight loss, and severity of depression according to the Hamilton scale. Although not stated in the original paper, these authors in subsequent correspondence (Dinan and O’Keane, 1992) confirmed an inverse correlation between basal cortisol levels and the prolactin response to fenfluramine. However, there was no direct relationship between severity of depression and blunting of the prolactin response. Indeed, patients with weight loss tended to have non-blunted responses. The patients in the study of Park et al. (1996) did not show increased basal cortisol levels, possibly suggesting that the absence of blunting of the response was because these patients were less depressed. However, other studies, e.g. Weizmann et al. (1988) found little evidence of blunting of the prolactin response in the presence of clearly elevated basal cortisol levels in the depressed patients.

(d) Unipolar vs. bipolar depression. A shortcoming of some of the studies listed in Table 1 is that their depressed samples included bipolar as well as unipolar patients, and did not differentiate between them. In others, the diagnosis is given as major depression according to DSM-III-R (American Psychiatric Association), which implies that the patients were not bipolar (although they could have been in their first episode of affective illness). The only study that directly compared unipolar and bipolar depressives (Mitchell et al., 1990) found reduced basal prolactin levels in the bipolars, although the response to fenfluramine was similar in both groups. Overall, blunted prolactin responses to fenfluramine do not appear to be specific to bipolar depression, as suggested by Asnis et al. (1991). There have been two studies employing the FCT in the
Table 2. Effects of antidepressant and mood stabilising treatments on responses to fenfluramine challenge in depression

<table>
<thead>
<tr>
<th>Reference</th>
<th>n</th>
<th>Diagnosis</th>
<th>Treatment</th>
<th>Fenfluramine</th>
<th>Prolactin response</th>
<th>Cortisol response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muhlbauer, 1984</td>
<td>8</td>
<td>Bipolar†</td>
<td>Lithium</td>
<td>αt, 60 mg</td>
<td>No difference</td>
<td></td>
</tr>
<tr>
<td>Muhlbauer et al., 1985</td>
<td>11</td>
<td>Bipolar†</td>
<td>Lithium</td>
<td>αt, 60 mg</td>
<td>Increased</td>
<td></td>
</tr>
<tr>
<td>Asnis et al., 1988</td>
<td>5</td>
<td>Depression</td>
<td>Desipramine</td>
<td>αt, 60 mg</td>
<td>No difference</td>
<td>No difference</td>
</tr>
<tr>
<td>Shapira et al., 1989*</td>
<td>10</td>
<td>Depression</td>
<td>Imipramine</td>
<td>αt, 60 mg</td>
<td>Increased</td>
<td>No difference</td>
</tr>
<tr>
<td>Kasper et al., 1990†</td>
<td>31</td>
<td>Depression</td>
<td>Mixed</td>
<td>αt, 60 mg</td>
<td>Reduced</td>
<td></td>
</tr>
<tr>
<td>Shapira et al., 1992a‡</td>
<td>18</td>
<td>Depression</td>
<td>ECT</td>
<td>αt, 60 mg</td>
<td>Increased</td>
<td></td>
</tr>
<tr>
<td>O’Keane et al., 1992a</td>
<td>21</td>
<td>Depression</td>
<td>ECT (5)</td>
<td>αt, 30 mg</td>
<td>No difference</td>
<td>No difference</td>
</tr>
<tr>
<td>Shapira et al., 1992b*</td>
<td>9</td>
<td>Depression</td>
<td>Clomipramine</td>
<td>αt, 60 mg</td>
<td>Increased</td>
<td>No difference</td>
</tr>
<tr>
<td>Stahl et al., 1993*</td>
<td>6</td>
<td>Depression</td>
<td>Nortriptyline</td>
<td>αt, 100 mg</td>
<td>Reduced</td>
<td>Reduced (trend)</td>
</tr>
<tr>
<td>Cleare et al., 1997</td>
<td>10</td>
<td>Depression</td>
<td>ORG-4428 (5)</td>
<td>αt, 30 mg</td>
<td>Increased</td>
<td></td>
</tr>
<tr>
<td>Mannel et al., 1997†</td>
<td>17</td>
<td>Bipolar</td>
<td>Lithium</td>
<td>αt, 60 mg</td>
<td>No difference</td>
<td>No difference</td>
</tr>
<tr>
<td>Monteleone et al., 1997b*</td>
<td>10</td>
<td>OCD</td>
<td>Carbamazepine</td>
<td>αt, 30 mg</td>
<td>Increased</td>
<td></td>
</tr>
</tbody>
</table>

* Placebo controlled studies. † Compared with normal control group. ‡ Fenfluramine levels measured.

Manic phase of bipolar disorder. Yatham (1996) found that prolactin or cortisol responses to α-fenfluramine in manic patients were not different from those in controls. Thakore et al. (1996) found increased basal cortisol levels and reduced prolactin responses to α-fenfluramine in manic patients, suggesting mania to be associated with a state of decreased 5-HT responsivity similar to that found in the depressed state.

**Effects of antidepressant drugs, mood stabilising drugs and ECT**

Table 2 lists 8 studies which examined the effect of sub-chronic administration of ADs on the prolactin and/or cortisol responses to fenfluramine challenge. One of these studies (Monteleone et al., 1997b) examined patients with OCD but is included here for completeness. Various ADs were used, as indicated in Table 2. Two studies examined patients before and after treatment with a course of electroconvulsive therapy (ECT) (Shapira et al., 1992a; O’Keane et al., 1992a). Six studies incorporated a placebo challenge in the research design but only two measured fenfluramine levels. αt-fenfluramine was administered in all but three studies (O’Keane et al., 1992a; Cleare et al., 1997; Monteleone et al., 1997b).

Five of the 8 studies which examined the effect of ADs found an increased prolactin response to fenfluramine when comparing responses under treatment to those at baseline. One (Asnis et al., 1988) found no change, and two (Kasper et al., 1990; Stahl et al., 1993) found a decrease. In the study of Kasper et al. (1990), plasma levels of fenfluramine and norfenfluramine were lower when patients were tested after receiving ADs, suggesting a pharmacokinetic explanation for the reduced prolactin response. The study by Cleare et al. (1997) showed a distinct increase in the prolactin response to α-fenfluramine after treatment with noradrenergic as opposed to serotonergic drugs. The overall results quoted in this paper, however, are marred by the inclusion of data from 3 patients treated with placebo instead of an AD drug. It should be noted that the number of patients receiving a particular AD in this as well as many of the other studies was small.

The two studies which examined the effect of ECT found different results. That by Shapira et al. (1992a) was substantially larger, included a placebo challenge and measured fenfluramine levels. An enhanced prolactin response to fenfluramine was observed after ECT. O’Keane et al. (1992a) examined only 5 patients before and after ECT and found no change. A common feature of
all the studies in which clinical ratings were performed (Kasper et al., 1990; Shapira et al., 1992a; O’Keane et al., 1992; Monteleone et al., 1997b; Cleare et al., 1997) was that the changes in hormone secretion did not correlate with drug response criteria and were thus independent of clinical improvement in the patients.

A series of studies have evaluated the effect of lithium in a parallel design comparing bipolar patients in remission with normal controls (Mühlbauer, 1984; Mühlbauer and Muller-Oerlinghausen, 1985; Mannel et al., 1997). The first study measured the effect of Li on the prolactin response to fenfluramine and found no difference between euthymic bipolar patients on the drug and normal controls (Mühlbauer, 1984). A greater cortisol response in the patients on long-term lithium was found in a subsequent report (Mühlbauer and Muller-Oerlinghausen, 1985) but when examined after 9 months of prophylaxis with lithium or carbamazepine, the prolactin and cortisol responses of the patients were not different from those measured under drug-free conditions prior to prophylactic treatment (Mannel et al., 1997). Shapira et al. (1992b) examined patients treated with clomipramine for 4 wk who were re-challenged following additional lithium treatment for 3 wk. No further increase in the prolactin response to fenfluramine compared to the pre-treatment baseline was observed beyond that induced by clomipramine. In the only comparable study performed in animals, Aulakh et al. (1991) showed that short-term treatment with lithium but not clorgyline or imipramine, potentiated fenfluramine-induced increases in prolactin but not corticosterone, while long-term treatment with clorgyline but not imipramine or lithium attenuated the effect on prolactin secretion. A lack of effect of lithium on the prolactin response to d-fenfluramine was shown in healthy controls (Power et al., 1993). In the animal study (Aulakh et al., 1991), long-term lithium potentiated the effect of fenfluramine on corticosterone secretion.

One study has examined the effect of administration of an AD to normal healthy individuals on the FCT. Sommers et al. (1994) administered either a single 40 mg dose of fluoxetine 8 h before the FCT or 20 mg fluoxetine daily for 14 days prior to the FCT. There were no differences in prolactin responses to either acute or sub-chronic fluoxetine, although basal prolactin levels were reduced after 14 days of fluoxetine, suggesting a decrease in 5-HT turnover.

If it is assumed that the effect of fenfluramine is mediated by receptors of the 5-HT-2 family, the studies noted above, which found increased prolactin responses to fenfluramine after AD treatment, are consistent with recent findings of Li et al. (1993) in rats. These authors found that the ADs, fluoxetine and desipramine, both potentiated hormone responses to the 5-HT-2a/2c agonist DOI. The potentiation was accompanied by an increase in the number of 5-HT-2a binding sites in the hypothalamus, while the number of sites in cortex was unchanged.

**FCT in remitted depressed patients**

In a follow-up study of patients who had been challenged before and after treatment with ECT or with clomipramine supplemented with lithium (Shapira et al., 1992a,b), prolactin responses to d-fenfluramine were examined during remission, 6 months after recovery from the major depressive episode and 2 wk following discontinuation of maintenance pharmacotherapy (Shapira et al., 1993). The responses in these remitted patients were elevated compared to those seen prior to treatment, and identical to those seen immediately following the ECT series or after treatment with clomipramine and lithium. These results imply that the increased prolactin response is a state-dependent phenomenon. Coccaro et al. (1989) measured prolactin responses in 10 remitted depressed patients and found them to be not significantly different from the responses of acutely depressed patients. However, they were not blunted compared to controls, while the responses of acutely depressed patients were.

**Seasonal affective disorder**

Of the two studies in which hormone responses in seasonal affective disorder (SAD) were examined, one (Yatham and Michalson, 1995) showed no difference from controls, while in the other (Coiro et al., 1993), the responses of the SAD patients were reduced both in summer and winter, suggestive of a trait phenomenon. No firm conclusion as to serotonergic function in SAD can be drawn from this data.

**FCT in related disorders**

**Obsessive-compulsive disorder**

Table 3 shows the results of the seven studies which have been performed on patients with OCD. Four of these incorporated a placebo challenge, and one reported plasma fenfluramine levels. No firm conclusion as to central serotonergic function in OCD can be drawn from the FCT. Concurrent depression occurs frequently in OCD patients and may be thought to have influenced the results, particularly those in which a reduced response compared to controls was observed. However, Hewlett et al. (1992) found no relationship between the prolactin response and degree of depression as determined by the Hamilton scale in their OCD patients, while Lucey et al.
Table 3. Fenfluramine challenge tests in obsessive-compulsive disorder

<table>
<thead>
<tr>
<th>Reference</th>
<th>Controls</th>
<th>Patients</th>
<th>Fenfluramine</th>
<th>Prolactin response</th>
<th>Cortisol response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hewlett et al., 1992</td>
<td>20</td>
<td>26</td>
<td>dl-., 60 mg</td>
<td>Reduced</td>
<td></td>
</tr>
<tr>
<td>McBride et al., 1992*</td>
<td>27</td>
<td>21</td>
<td>dl-., 60 mg</td>
<td>No difference</td>
<td></td>
</tr>
<tr>
<td>Hollander et al., 1992†</td>
<td>10</td>
<td>20</td>
<td>dl-., 60 mg</td>
<td>No difference</td>
<td>No difference</td>
</tr>
<tr>
<td>Lucey et al., 1992</td>
<td>10</td>
<td>10</td>
<td>d-, 30 mg</td>
<td>Reduced</td>
<td>Reduced</td>
</tr>
<tr>
<td>Monteleone et al., 1997*</td>
<td>13</td>
<td>13</td>
<td>d-, 30 mg</td>
<td>Reduced</td>
<td>Reduced</td>
</tr>
<tr>
<td>Monteleone et al., 1997†</td>
<td>20</td>
<td>20</td>
<td>d-, 30 mg</td>
<td>Reduced</td>
<td>Reduced (female only)</td>
</tr>
<tr>
<td>Fineberg et al., 1997</td>
<td>14</td>
<td>14</td>
<td>d-, 30 mg</td>
<td>Increased</td>
<td></td>
</tr>
</tbody>
</table>

* Placebo controlled study.
† Fenfluramine levels measured.

(1992) state that their OCD patients were not depressed, although their baseline cortisol levels were elevated. In the study by Monteleone (1997c), baseline plasma cortisol was significantly increased in patients with OCD, but, in agreement with the study of Goodwin et al. (1994) quoted above, a cortisol response to fenfluramine was observed in women but not in men. This response was significantly reduced in the female patients compared to normal women.

**Panic disorder**

Two groups have performed FCTs in patients with panic disorder. Apostolopoulos et al. (1993) found increased prolactin responses to dl-fenfluramine in 11 patients compared to 12 controls, but could not repeat this finding using d-fenfluramine in a larger sample of 16 patients and 14 controls (Judd et al., 1994). Targum, however, in two studies (Targum and Marshall, 1989; Targum, 1990), observed increased responses in panic disorder patients regardless of the incidence of concurrent depression.

**Chronic fatigue syndrome**

Bearn et al. (1995) performed challenge tests with 30 mg dl-fenfluramine on 9 patients with chronic fatigue syndrome (CFS). The prolactin and cortisol responses did not differ but the ACTH response was increased relative to that in 10 controls. Sharpe et al. (1997) found an increased prolactin response in 10 men with CFS. This together with reports of increased prolactin responses to the directly-acting 5-HT-1a receptor agonist buspirone in this condition (Sharpe et al., 1996) suggest that it is characterised by serotonergic hyperactivity. However, in another study (Yatham et al., 1995), no differences in either basal levels or prolactin or cortisol responses to dl-fenfluramine were found.

**Impulsive aggression**

Several studies have used the FCT to evaluate serotonergic function in impulsive aggression in the context of various diagnostic categories (Table 4). Coccaro et al. (1989) evaluated patients with borderline personality disorder compared to controls. Prolactin responses were reduced in the personality disorder patients and correlated inversely with scores on the Buss–Durkee hostility inventory, the Brown–Goodwin aggression scale, and the Barratt total impulsiveness scale. O'Keane et al. (1992b) studied 9 drug-free male offenders in a forensic hospital who had been convicted of murder, and were diagnosed as antisocial personality disorder. These subjects showed a reduced prolactin response to d-fenfluramine when compared to controls. Coccaro et al. (1994a) examined first-degree relatives of patients with a primary DSM-III-R diagnosis of personality disorder. Reduced prolactin responses to fenfluramine in the probands were associated with an increased morbid risk of impulsive personality disorder traits in the relatives, although there was no correlation between these measures in the relatives and the impulsive aggression scores of the probands. Recently, Coccaro et al. (1997a) determined the relationship between aggression and the prolactin response to d-fenfluramine in 24 subjects with personality disorders. Aggression was again found to be significantly and inversely correlated with the prolactin response. That impulsivity and aggression correlate inversely with prolactin responses was also suggested by another series
of experiments of Coccaro et al. (1996d), in which they used a direct laboratory measure of aggressive behaviour as well as scores on two different inventories to measure aggression in subjects with personality disorder. Similar results were obtained in healthy subjects by Cleare and Bond (1997). In male subjects there was an inverse correlation between the α-fenfluramine-mediated cortisol response and both the Buss–Durkee hostility inventory total score and the aggression factor, while no such correlation was found either in females or with the prolactin responses. An inverse relationship therefore appears to exist between central serotonin function and aggression/hostility in healthy males, similar to that seen in violent or highly aggressive populations. In a study of 97 patients with DSM-III-R personality disorder, those who had shown self-injurious behaviour, sometimes in the course of suicide attempts, showed blunted prolactin and cortisol responses to α-fenfluramine compared to the responses in patients who had not inflicted self-injury or attempted suicide (New et al., 1997). These results suggest that self-directed violence, like aggressiveness and violent behaviour directed towards others, may be associated with reduced responsivity of the serotonergic system.

Fishbein et al. (1989) studied the effect of α-fenfluramine in 24 substance abusers with differing levels of aggressiveness and impulsivity. The subjects had been drug-free for 5 days. Baseline prolactin and both the prolactin and cortisol responses were higher in subjects who self-reported higher levels of impulsivity and aggressiveness, with a significant correlation between impulsivity and the peak prolactin and cortisol levels. Coccaro et al. (1997a) suggested that the increased responses in the drug abusers reported by Fishbein et al. (1989) may have been due to the presence of cocaine, since although the subjects reported no cocaine use during 2 wk prior to admission, 2 of them who had used over 1g cocaine daily had elevated basal prolactin levels. However, in an animal study by Levy et al. (1994), reduced ACTH responses to α-fenfluramine were observed in rats which had received 15 mg/kg cocaine twice daily for 7 days.

Stoff et al. (1992) reported no relationship between aggression and prolactin responses to α-fenfluramine in a group of children with disruptive behavioural disorders. Halperin et al. (1994) reported elevated prolactin responses to α-fenfluramine in aggressive compared with non-aggressive children with attention deficit hyperactivity disorder. However, in a follow-up study, Halperin et al. (1997) failed to replicate this finding. The discrepancy between the two findings was due to a significant

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**Table 4. Fenfluramine challenge tests in impulsive aggression**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Controls (n)</th>
<th>Diagnosis</th>
<th>Patients (n)</th>
<th>Fenfluramine</th>
<th>Prolactin response</th>
<th>Cortisol response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coccaro et al., 1989§</td>
<td>18</td>
<td>Personality disorder</td>
<td>20</td>
<td>α-, 60 mg</td>
<td>Reduced</td>
<td></td>
</tr>
<tr>
<td>Fishbein et al., 1989*</td>
<td>24</td>
<td>Substance abuse†</td>
<td>24</td>
<td>α-, 60 mg</td>
<td>Increased</td>
<td>Increased</td>
</tr>
<tr>
<td>O'Keane et al., 1992b</td>
<td>9</td>
<td>Antisocial personality disorder</td>
<td>9</td>
<td>α-, 30 mg</td>
<td>Reduced</td>
<td></td>
</tr>
<tr>
<td>Stoff et al., 1992§</td>
<td>8</td>
<td>Disruptive behaviour disorder</td>
<td>8</td>
<td>α-, 1 mg/kg</td>
<td>No difference</td>
<td>No difference</td>
</tr>
<tr>
<td>Halperin et al., 1994</td>
<td>14</td>
<td>Aggression (boys 7–11)</td>
<td>10</td>
<td>α-, 1 mg/kg</td>
<td>Increased</td>
<td></td>
</tr>
<tr>
<td>New et al., 1997</td>
<td>72</td>
<td>Personality disorder#</td>
<td>24</td>
<td>α-, 60 mg</td>
<td>Reduced</td>
<td>Reduced</td>
</tr>
<tr>
<td>Halperin et al., 1997</td>
<td>12</td>
<td>Aggression (boys 7–11)</td>
<td>13</td>
<td>α-, 1 mg/kg</td>
<td>No difference</td>
<td></td>
</tr>
</tbody>
</table>

* Placebo controlled studies.
† Comparison between two groups of subjects divided according to impulsivity and aggression.
‡ Comparison between two groups of subjects divided according to history of incidence of attempted suicide or self-mutilation.
§ Fenfluramine levels measured.
interaction between the age of the boys and degree of aggression, with young aggressive boys showing a significantly greater prolactin response than young non-aggressive boys, while there was no difference in the older age group. Similar findings were obtained by Pine et al. (1997), who examined younger brothers of convicted delinquents in relation to socially adverse rearing conditions. Increasing degrees of aggressive behaviour and the incidence of adverse rearing conditions were each positively correlated with the prolactin response.

It is clear that the majority of studies support the concept of an association between reduced serotonergic activity and increased impulsiveness or aggression in adults, although the reverse relationship, namely a direct positive correlation between 5-HT function and violence, may exist in young boys.

Simultaneous FCTs in different disorders

Several studies have simultaneously compared more than one group of patients with affective and other disorders with a single control group. Comparisons of the individual groups with controls in such studies have been considered in the previous sections and are listed in the appropriate Tables. Because additional insights can be obtained from the cross-diagnostic comparisons, some of these studies are briefly reconsidered in this section. Coccaro et al. (1989) studied 20 patients with personality disorder in addition to 25 with major affective disorder. Although the prolactin response was reduced in both groups compared to normal controls, a decreased response was correlated with irritable impulsive aggression in the personality disorder patients only, while there was no correlation with degree of depression in either group. Targum and Marshall (1989), and Targum (1990) studied patients with panic disorder, in some cases co-existing with major depressive disorder, and patients with major depressive disorder alone, and found increased prolactin and cortisol responses in patients with panic disorder, regardless of co-existing depression. The depressed patients without panic disorder did not differ from controls. Lucey et al. (1992) studied 10 depressives as well as 10 OCD patients and 10 controls. They found reduced prolactin and cortisol responses in both groups of patients, and suggested that blunting of hormone responses may be a trait-dependent marker for the whole spectrum of affective disorders. However, no direct correlation between the degree of blunting and severity of the symptoms for either condition could be shown.

In the study by Cleare et al. (1995), prolactin responses to D-fenfluramine were compared in 25 healthy controls, 15 patients with major depression and 10 patients with CFS. Baseline cortisol levels were highest in the depressed, lowest in the CFS and intermediate in the control group, while the prolactin response showed an inverse pattern; highest in CFS, lowest in depression and intermediate in controls. Abel et al. (1997) compared responses in 15 patients with major depressive disorder and in 13 patients with schizophrenia with controls individually matched for gender, age, weight and phase of the menstrual cycle. The prolactin responses of the depressed group were significantly lower than those of the control group, while those in the schizophrenic group were significantly higher. Cortisol responses did not differ.

Two studies have examined responses in drug addicts with or without comorbid depression. Gerra et al. (1995, 1997) found reduced prolactin and cortisol responses to fenfluramine only in heroin abusers who had comorbid depression, and a similar effect was found when the mothers of the addicts were tested.

FCT and other serotonergic markers

The relative value of the prolactin response to fenfluramine as a marker of central serotonergic activity, in comparison with other markers, has recently been evaluated in several studies. Mann et al. (1992) found a positive correlation in depressed patients between the maximum prolactin response to fenfluramine and CSF levels of 5-HIAA and also platelet 5-HT-2 receptor number, although the correlation with the latter was only valid in patients aged 30 or more. The relationship with CSF 5-HIAA levels suggests that fenfluramine-induced prolactin release may reflect serotonergic activity in brain areas other than the hypothalamus, since the metabolite in CSF is derived from multiple regions including prefrontal cortex. The same group (Mann et al., 1996) has used fenfluramine as a probe for visualising serotonergic effects on glucose metabolism in different brain areas of living patients and controls, by means of positron emission tomography (PET) scans after administration of 18F-fluorodeoxyglucose. In normal controls, regional cerebral glucose metabolic rate was increased by D/L-fenfluramine in left prefrontal and temporoparietal cortex, while in right prefrontal cortex, metabolic rate was decreased by fenfluramine. Depressed patients showed no changes in metabolic rate in response to fenfluramine in any brain area. These results provide a direct indication of reduced responses to 5-HT release in depression. Since prolactin responses to fenfluramine were not significantly reduced in the same small sample of depressed patients compared to controls, the results suggest that direct measures of brain activation by serotonergic stimulation such as the deoxyglucose-PET method could be superior to a neuro-
endocrine challenge test in terms of ability to reflect brain serotonergic activity.

Coccaro et al. (1997b) studied the relationships between hormonal responses to serotonergic challenge agents, levels of aggression, and CSF 5-HIAA levels in 47 patients with personality disorder and 12 with major affective disorder. While there was a positive correlation between fenfluramine and mCPP-induced prolactin responses, both these responses correlated inversely with CSF 5-HIAA levels in the personality disorder patients. This correlation was in the opposite direction to that reported by Mann et al. (1992). The difference would seem to be primarily due to differences in the populations studied. Mann et al. (1992) examined both male and female subjects who were principally suicide attempters, characterized by low CSF 5-HIAA levels, while in the study of Coccaro et al. (1997b) only males with a diagnosis of personality disorder were examined. This differentiation is strengthened by the fact that Coccaro et al. (1997b) observed a positive, although not significant, correlation between degree of aggression as measured by the Buss–Durkee Assault scale and CSF 5-HIAA levels.

**Discussion**

At what appears to be the end of the fenfluramine challenge test ‘era’, it is interesting to note how extensively this procedure has been applied in neuro-psychiatry. The two primary issues addressed in this review are the mechanism of action of fenfluramine and what changes if any occur in the hormone-releasing actions of fenfluramine in affective disorders and after drug treatment. Regarding the first issue, recent data are consistent with mediation of the hormone-releasing action of fenfluramine by 5-HT-2c receptors. This can occur by one of two mechanisms; release of endogenous 5-HT which then selectively activates 5-HT-2c receptors, or a direct post-synaptic action of fenfluramine. It is not clear why 5-HT re-uptake blockers were found to inhibit the action of fenfluramine to induce hormone release in some animal studies, while such an effect has not been reported in humans.

Regarding the effects of fenfluramine in affective disorders, it is worthwhile to reiterate certain methodological caveats before going on to draw general conclusions from the substantial, if somewhat confusing body of data, available.

Firstly, in order to show that a change in the amount of hormone released as a result of fenfluramine stimulation is due to a change in central serotonergic activity, it is necessary to show that no changes in hormone secretory capacity are responsible for observed differences in hormone release induced by fenfluramine. This criterion has been met for many of the conditions reviewed here by performing challenge tests with substances such as thyrotropin releasing hormone (TRH), which acts directly on pituitary lactotrophs to release prolactin and thus bypasses serotonergic neurones. Prolactin responses to TRH were unaltered in patients with depression (Baumgartner et al., 1988). OCD (Lucey et al., 1993), or personality disorder (Coccaro et al., 1994b). Similarly, in a recent study by Volpi et al. (1997) in Parkinsonian patients, ACTH and cortisol responses to DL-fenfluramine were completely abolished, while the responses of the same hormones to exogenously administered corticotrophin releasing hormone (CRH) were intact, indicating a selective disturbance of serotonergic neurones in the hypothalamus. A further point concerns the effect of repeated fenfluramine challenges in the same individual. This is particularly relevant to studies in which patients are examined before and after treatment with drugs (Table 2). Coccaro et al. (1987) indeed showed a reduced prolactin response to DL-fenfluramine in psychiatric patients when the challenge was repeated within 2–12 days of a previous challenge, but not after a longer interval. In a similar study involving boys with disruptive behaviour disorders, Stoff et al. (1992) found that 2 acute administrations of DL-fenfluramine (1 mg/kg p.o.) at a 1-wk interval produced consistent effects on prolactin but not on cortisol responses. The period of drug administration in all the studies in Table 2 was longer than 12 days.

Other methodological concerns have been noted in the body of the review. Prominent among these is the use of placebo challenge in only a minority of studies and measurement of plasma levels of fenfluramine and its metabolite norfenfluramine in even fewer. Diagnostic boundaries have been a problem in studies of depression, with a clear differentiation between unipolar and bipolar depressed patients absent from some key studies. Similarly, indices of the severity of depression have not been consistently examined or reported. Control for variables known to influence hormone responses to fenfluramine has been sporadic. While index and control groups were generally matched for age, hormone responses to fenfluramine were differentially examined in older and younger patients in only 2 studies. Similarly, the influence of weight and recent weight loss has been only sporadically considered. The influence of baseline hormone levels, particularly cortisol, on responses has been addressed slightly more frequently and has yielded intriguing results.

The most likely consequence of sporadic attention to potential confounding variables is to decrease the signal to noise ratio in the data, an effect which is amplified in the small samples used in most of the studies reviewed here. In such a situation, the emergence of consistent trends in
a minority of the disorders studied by the FCT is all the more impressive. Depression has been the condition most extensively studied with the FCT. As summarised in Table 1, and as reviewed in the appropriate section, 11 out of 16 studies found blunted prolactin responses to fenfluramine in depressed patients compared to controls, and none found increased responses. This accumulation of data is indeed supportive of the view that net serotonergic function is reduced at the synapses which control prolactin release by 5-HT in depressed patients, in keeping with the classical indoleamine hypothesis of depression (see Mann et al., 1995). However, a reverse interpretation is also possible. Fishein et al. (1989) point out that decreased presynaptic activity, i.e. lower neuronal firing and release would lead to an increase in the amount of intracellular presynaptic 5-HT available for release as a result of fenfluramine stimulation. This was held to account for the increased prolactin and cortisol responses seen in impulsive and aggressive substance abusers by these authors, despite the reduction in CSF levels of 5-HIAA in this condition, which is generally taken as evidence of decreased serotonergic transmission. A similar rationale was used above to explain the increase in fenfluramine-stimulated 5-HT release induced by tetrodotoxin in animal experiments (Carboni and di Chiara, 1989). According to this interpretation, blunted prolactin responses to fenfluramine could be indicative of reduced availability of 5-HT for release from presynaptic stores, and might be a consequence of increased neuronal firing in depression, with resultant depletion of the neurotransmitter.

An area where the FCT has yielded reasonably consistent results has been that of impulsive aggression, at least in adults. With one exception, studies have shown an association between such behaviours and reduced prolactin responses to fenfluramine across diagnostic categories. An inverse association has also been demonstrated in normal males between hostility and aggression scores and cortisol responses to fenfluramine.

An inverse relationship between basal cortisol levels and the prolactin response to fenfluramine has been reported, and may be valid across different diagnostic categories. It has been proposed (Dinan, 1994) that impairment of brain 5-HT function in depression is secondary to elevated cortisol levels. Evidence for this was provided by administering the cortisol synthesis inhibitor ketoconazole to 8 depressed patients for 4 wk (Thakore and Dinan, 1995). This treatment resulted in normalization of the previously blunted prolactin responses to d-fenfluramine in all the patients, and recovery from depression in 5 of them. In another study (Dinan and Scott, 1996), 5 healthy male subjects, administered the cortisol synthesis inhibitor metyrapone, experienced an enhanced spontaneous nocturnal prolactin surge and also an increased prolactin response to d-fenfluramine. Another 6 subjects treated for 24 h with hydrocortisone showed no nocturnal increase in prolactin and a reduced response to d-fenfluramine. Six other subjects were treated with TRH, which acts directly on the pituitary to induce prolactin release, and either metyrapone or placebo. The TRH-induced rise in prolactin following metyrapone was significantly greater than that following placebo, suggesting that cortisol influences both spontaneous and 5-HT-stimulated prolactin release by acting at the pituitary level. However, in another study, administration of cortisol for 10 days to normal subjects did not produce any change in their prolactin responses to fenfluramine (Young et al., 1998). Whether or not cortisol hypersecretion accounts for the reduced prolactin responses to fenfluramine seen in depression is thus still an open question.

In terms of future research directions, closure of the FCT ‘era’, while ostensibly premature, may well turn out to be timely. In conditions such as depression, where results with the challenge procedure have been reasonably uniform, additional studies would add little unless they encompass samples large enough to control for potential confounding variables and address numerous methodological concerns. The same applies to studies of impulsive aggression, where findings with the FCT have been even more consistent. In these conditions and others, use of agents with an action at specific receptors would appear to be more highly indicated at this time. Methodological insights gained from the experience with fenfluramine should certainly be applied to studies of this type.

Concurrent evaluation of the effect of challenge agents on cerebral metabolism is clearly an important direction to follow, since this approach provides an insight into serotonergic function in brain areas other than those directly tested by the neuroendocrine paradigm. An even more direct approach would be to directly assess biochemistry of 5-HT in the brain by the use of imaging techniques such as PET and SPECT in combination with radioligands which already exist or are under development. Ultimately, these approaches can be expected to supplant neuroendocrine challenge techniques by providing a panoramic view of brain function, as compared to the intriguing and highly heuristic, but nevertheless limited, window opened by procedures such as the FCT.

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