The assessment of serotonin function in major depression

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Abstract

Major depression is a common psychiatric disorder with considerable associated morbidity and mortality. Investigations to understand the causes of depression and how it can be more effectively treated are high priority. The serotonergic system has been implicated in the aetiology and treatment of depression by a wealth of preclinical and clinical evidence. This includes findings that drugs increasing serotonin neurotransmission have antidepressant action and inhibition of serotonin synthesis (via tryptophan depletion) can induce relapse of symptoms in depressed patients.

Neuroendocrine challenges are an established method of indirectly examining brain serotonergic function, utilising changes in the secretion of pituitary hormones influenced by tonic serotonergic activity at the level of the hypothalamus. This thesis describes the development of two such neuroendocrine challenge tests, using the selective serotonergic probes, zolmitriptan and citalopram.

Orally administered zolmitriptan, licensed for the treatment of migraine, clearly elevated plasma growth hormone in healthy subjects. This growth hormone response was antagonised by ketanserin suggesting mediation by postsynaptic 5-HT_{1D} receptors. The response to zolmitriptan was attenuated in melancholic depressed subjects, and further reduced following antidepressant treatment. This implies a dysfunction of postsynaptic 5-HT_{1D} receptor function in melancholia, and further 5-HT_{1D} functional downregulation following antidepressant treatment.

Citalopram, a selective serotonin reuptake inhibitor, administered at low dose intravenously was associated with an increase in plasma prolactin and cortisol in healthy subjects. The postsynaptic serotonin receptor subtype mediating these responses was not clearly elucidated from experiments using available 5-HT_2 and 5-HT_{1A} ligands. Depressed subjects demonstrated an attenuated prolactin response to citalopram suggesting impaired presynaptic 5-HT neuronal function.

These findings confirm impaired brain 5-HT function in depressed patients and assist in more clearly defining the nature of this impairment.

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Publications

The following publications have been based on the studies described in this thesis for which I was the primary investigator, and were conducted in collaboration with my co-authors below.

Bhagwager Z, Whale R, Cowen PJ. 2002. State and trait abnormalities in serotonin function in major depression. *British Journal of Psychiatry* 180: 24-28.

Attenburrow MJ, Mitter PR, Whale R, Terao T, Cowen PJ. 2001. Low-dose citalopram as a 5-HT neuroendocrine probe. *Psychopharmacology* 155: 323-326.

Whale R, Clifford EM, Bhagwager Z, Cowen PJ. 2001. Decreased sensitivity of 5-HT_{1D} receptors in melancholic depression. *British Journal of Psychiatry* 178: 454-457.

Whale R, Bhagwagar Z, Cowen PJ. 1999. Zolmitriptan induced growth hormone release in humans: mediation by 5-HT_{1D} receptors? *Psychopharmacology* 145: 223-226.

Whale R, Cowen PJ. 1998. Probing the function of 5-$HT_{1B/1D}$ receptors in psychiatric patients. *CNS Spectrums* 3: 40-45.

PART 1

INTRODUCTION

CHAPTER 1

5-HT AND DEPRESSION

INTRODUCTION

Depressive disorders are increasingly recognised as a major public health issue in current society (Scott and Dickey 2000) with around 16% of the population affected at some time in their lives (Kessler et al. 2003). The incidence of depressive disorders appears to be increasing, particularly in younger age groups (Klerman and Weissman, 1989). Up to 15% of more severely depressed subjects commit suicide (reviewed by Kreitman 1993). There is an obvious need to understand the aetiology of depressive disorders and develop effective identification and treatments.

The involvement of serotonin (5-hydroxytryptamine, 5-HT) in depressive disorders was first suggested in 1957 when the mood altering effects of imipramine and iproniazid, which modify serotonergic function, were reported (Ayuso-Gutierrez 2002). Following much investigation, 5-HT has been shown to have a critical role in the aetiology of depression and its treatment, which will be discussed in this thesis. The exact nature of this role of 5-HT, however, remains unclear.

This chapter will examine depressive disorders, known aetiological factors and treatments. The nature and function of 5-HT and the experiments that have been undertaken to elucidate its role in depressive disorders are then discussed.

DEPRESSIVE DISORDERS

CLINICAL FEATURES

Experiencing a range of emotions is part of normal human experience, and unhappiness is common. A more persistent or severe lowering of mood, however, may reach the clinical characteristics of a depressive disorder. Depression is known to occur secondarily to other disorders such as psychiatric (e.g. schizophrenia) (Foulds et al. 1975) or physical illnesses (e.g. substance misuse or hypothyroidism) (Lishman 1997). As depression in these situations appears to have a different aetiology and management to primary depression, the exclusion of such a cause is necessary.

Depressive disorder is a syndrome with cognitive and somatic features. Individuals may vary in which particular features are most manifest; classification of symptom groups using diagnostic guidelines such as the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV 1994) can be useful to predict the most beneficial treatments and predict prognosis. Validated scales, such as the Hamilton Rating Scale for Depression (HAM-D) are widely used to quantify depressive symptoms (Hamilton 1967). The following clinical description of depression is derived from Hamilton's account of this disorder (Hamilton 1980).

All depressed individuals experience **low mood**. This is often described subjectively as being depressed, sad, hopeless or helpless. Concurrent anxiety or irritability is common.

Complaints of physical aches and pains may often be the superficial presentation of such mood changes. It is however important to distinguish between hypochondriasis as a personality trait and as a symptom of depressive disorder. **Diurnal variation of mood** often occurs, classically with depressed mood being worse on wakening but improving during the day, although its significance has been debated (Haug and Wirz-Justice 1993). **Loss of interest or pleasure** (anhedonia) in activities they would usually enjoy and diminution of working capacity are pervasive features of depressive disorders. Patients reject 'hobbies' and avoid social encounters. They often describe reduced libido.

Appetite is often reduced but rarely in some 'atypical' depressive disorders this may be increased. Associated weight changes may be present. In severe disorders, life threatening starvation and weight loss may be present.

Sleep is commonly disturbed in depression, classically associated with early morning wakening, but also associated with difficulty getting off to sleep (particularly if anxiety features are present) and waking throughout the night. Excessive sleep (hypersomnolence) is again associated with 'atypical' depressive disorders.

Psychomotor changes associated with depression include agitation, with restlessness, or retardation, with slowed speech, thinking and movements. If severe, retardation can be manifest as speechlessness and catatonia with no observable movements by the patient.

Reduced **energy** and becoming easily fatigued are common. Self neglect may be associated with this.

Depressed patients describe an **inability to concentrate** on everyday tasks and may complain of **memory disturbances** (most likely associated with impairment of memory formation through concentration disturbance).

Cognitive biases of depressive disorder are always present to some degree, and may include hopelessness, helplessness, worthlessness, guilt, negative appraisal of oneself and others, and negative misinterpretation of events (Beck 1967). Other disturbances of thought such as suicidal ideas become more common with increasing severity of illness, and at an extreme, mood congruent delusions may arise. Similarly, insight may diminish with increasing severity of illness.

CLASSIFICATION AND HISTORICAL PERSPECTIVE

Current thinking about depression can be traced back to Hippocrates' (460-337BC) descriptions of melancholia, with features of 'aversion to food, sleeplessness, irritability and restlessness'. Hippocrates suggested that melancholia was caused by Saturn's influence on the spleen resulting in secretion of black bile, hence blackening the mood through effect on the brain (Hippokrates 1897). Later, the Romans (Aurelianus, translated in 1950) added further symptoms to this syndrome, such as aggression, suicide and delusions. In 1621 Robert Burton published his influential work, 'Anatomy of Melancholy' (reprinted 1850), and attempted to subgroup melancholic disorders and postulate a rational aetiology relating to environmental factors (diet, alcohol, biological rhythms, and intense emotions). Esquirol (1838) postulated that melancholia was due to a primary mood disturbance (not a form of insanity), influencing other European psychiatrists to propose the existence of milder states of melancholia without delusions.

Kraepelin's careful longitudinal observations gave rise to his concept of manic depressive

insanity, classifying all affective disorders within this heading whether having only depressive features or both manic and depressive features (Kraepelin 1913). This related to his identification of the cyclical nature of both manic and depressive states, and believing that they had common heredity. The flaws in this 'unitary affective disorder' hypothesis were primarily that depressive states, identical to the descriptions of Kraepelin, could arise in response to stress (and not be of hereditary or endogenous origin), and other depressive states not reaching the criteria of melancholia showed no clear boundary from melancholia, needing to be classified separately.

Kraepelin's concepts were successfully challenged by Angst (1966) and Perris (1966), demonstrating different features of recurrent manic disorders (e.g. age of onset, duration of episode, and incidence of illness in relatives) to recurrent depressive disorders through studies of patient groups. Leonhard's concept of unipolar and bipolar disorders subsequently developed (Leonhard 1957). Bipolar disorder described disorders with features of both mania and depression whilst unipolar described disorders of only recurrent mania or recurrent depression. This unipolar-bipolar distinction is further supported by a greater concordance of unipolar-unipolar and bipolar-bipolar features reported in a study of Kraepelinian manic depressive twins (Bertelsen et al. 1977).

In the 20th century, many dichotomous subcategories of unipolar depression were proposed including: endogenous-exogenous, neurotic-psychotic, autonomous-reactive and acute-chronic, however these have been largely unhelpful and have not been clarified as groups with different aetiological characteristics or been consistently useful predictors

of best treatment (Kendell 1993). Statistical methods such as multiple regression analysis, factor analysis and clustering have been adopted to attempt to subgroup clinical features but there have been few replicable outcomes. The strongest finding appears to be a cluster representing melancholic (endogenous) features (see appendix); however an opposing cluster (such as 'reactive') has not been shown (Kendell 1993). It should be noted that the term 'melancholic' refers to a particular symptom profile, with no assumption about causation, although there seem to be biological differences that distinguish this group which will be explored later in this thesis. Melancholic patients have been shown in some studies to have a greater response rate to antidepressant medication than non melancholic patients (eg. Heiligenstein et al. 1994), but more robustly, melancholic subjects have a lower response rate to placebo than patients without these features (Peselow et al. 1992). Atypical depression as described above, particularly with features of hypersomnia and hyperphagia, also appears to be a discrete subgroup with some differing demographics and clinical features from non atypical depression (Matza et al. 2003; Posternak and Zimmerman 2002), and is more likely to respond to monoamine oxidase inhibitors (MAOIs) than other antidepressant treatments (Posternak and Zimmerman 2002). Seasonal affective disorder (SAD), characterised by cyclical depression during winter months and subsequent remission, may represent a further subgroup (Magnusson and Boivin 2003). This disorder classically responds to bright light therapy but many patients also require antidepressant medication, which has established efficacy in SAD. Several studies have investigated the biological substrates of SAD, with particular focus on circadian rhythms, but findings have largely been inconsistent. Whether SAD is a valid depressive disorder subtype remains to be clarified.

The principal challenge that has arisen in later years is the categorical distinction of depressive disorders from other psychiatric disorders and the development of clinically useful diagnostic systems. DSM-IV retains the concept of unipolar and bipolar disorders, distinguishes between single episodes of illness, and terms recurrent illness episodes as recurrent depressive disorders. 'Major depressive disorder' appears with a clear categorical definition, and can occur with or without melancholic features (see appendix). Melancholic features are retained for reasons as described above. DSM-IV has been widely adopted amongst international researchers, standardising groups of patients examined, and will be used throughout this thesis.

EPIDEMIOLOGY OF DEPRESSIVE DISORDERS

The lifetime prevalence for DSM-III major depressive disorder from the first large community based epidemiological case finding study using fully structured interviews (NIMH Epidemiological Catchment Area studies for major depressive disorder, Robins and Reiger eds. 1991) was 3.0 to 5.9%. The latest such study identifying DSM-IV major depressive disorder found a lifetime prevalence of 16.2% (Kessler et al. 2003). From these findings and others (Klerman and Weissman, 1989), the incidence of depression appears to be increasing over time; however the influence of subjects being more willing to admit psychiatric disturbance and the effect of changing diagnostic systems cannot be excluded. A consistent gender difference is observed amongst such studies, with women having a greater prevalence. There is no clear biological or psychosocial cause for this

difference but it may be linked to a greater chronicity of illness in women (Bracke 1998). Depression commonly has an early age of onset and an increase in prevalence in younger groups has been observed (Kessler et al. 2003), possibly relating to psychosocial factors.

Urban communities and working class status have a greater association with depression than rural and middle class groups respectively (Brown and Harris 1978). There appears to be no racial variation in lifetime prevalence of depression (Jablensky et al. 1981) but the way in which depressive disorders present does vary. Chinese and Asian depressed subjects (for example) present with more somatic symptoms than subjective mood changes.

Depressive disorders have a high rate of comorbidity with other DSM-IV disorders, reported as 78.5% of cases by Kessler et al. (2003).

AETIOLOGY

The cause of depression remains obscure; however several factors have been shown to be important, from both biological and environmental viewpoints.

Biological aetiology

Genetic influence

It is clear that (Kraepelinian defined) affective disorders have a familial component. From

both Angst's and Perris' family studies in 1966, the distinction between unipolar and bipolar affective disorder was clarified. Both found a high risk of unipolar illness in first degree relatives of unipolar (depressed) patients and no increased risk (above population rates) of bipolar illness. Perris found that bipolar illness similarly 'bred true', however Angst found an increase of both bipolar and unipolar illness in relatives of bipolar patients.

Studies have reported a wide variation of rates of unipolar illness in first degree relatives of unipolar depressed subjects, probably due to varied diagnostic criteria, but the pooled risk from studies examined by McGuffin and Katz (1986) was 9.1% (range 6 to 40%), compared with general population rates of 3%.

Twin studies are similarly affected by diagnostic problems. A review of previous studies by Allen (1976) found a monozygotic concordance of unipolar depressive disorder of 40% and dizygotic of 11%. In comparison, for bipolar disorder the monozygotic to dizygotic ratio was 72% to 14%, demonstrating a relatively greater genetic influence in these disorders.

There are few adoption studies of unipolar depression. Cadoret and Gath (1978), however, found a higher rate of unipolar illness in adopted away children of biological parents with unipolar disorder than children of parents without such a disorder.

The association between genetic traits, such as human erythrocyte blood groups and

human leukocyte antigen, and depressive disorder has been investigated with no positive replicated findings (Propert et al 1981). A small association has been noted with depressive disorder and two different sites on the 5-HT transporter (5-HTT) gene (Furlong et al. 1998).

Particular interest has arisen with the identification of polymorphic areas within the genome that code for specific proteins or enzymes involved with the activity of amines implicated in the process of depressive illness. One of the most important findings has been a common 44 base pair insertion/deletion polymorphism in the promotor region of the 5-HTT gene, with long and short variants (Heils et al. 1995), which alter 5-HTT gene transcription. The short variant is associated with less cellular 5-HTT expression, more anxiety related traits (Lesch et al.1996) and a greater depressogenic effect to tryptophan depletion (Moreno et al. 2002; Neumeister et al. 2002). The long variant is associated with a greater plasma prolactin (PRL) response to acute clomipramine in healthy subjects (Whale et al. 2000) and a greater reduction in hippocampal volume in depressed subjects (Frodl et al. 2004). A recently reported large prospective cohort study indicated that subjects with the long variant were significantly less likely to report symptoms of depression or suicidality following life events, or be formally diagnosed with depression following such events (Caspi et al. 2003). Childhood maltreatment of subjects in this study also predicted adult depression only in those carrying the short allele. This study therefore displays a potential mediating effect of genes in the development of depressive disorders following environmental stressors. A 5-HTT knockout mouse has been developed which has overall reduced brain 5-HT and reduced 5-HT neurone firing, but

increased 5-HT synthesis and synaptic 5-HT (Gobbi et al. 2001; Murphy et al. 2004). Together, these findings indicate that the reduced expression (short allele) variant subjects have a greater vulnerability to depression, although the hippocampal findings described above (Frodl et al. 2004) do not appear consistent with this, and clearly neurotransmission is modulated by many other factors including autoreceptor function. These different concepts and experimental paradigms will be discussed later in this thesis.

Functional anatomy

Functional and structural imaging studies in recent years have attempted to localise brain structures and circuits involved with mood. Both patients and healthy subjects have been involved, with novel experimental designs including scanning during illness episodes and following treatment (Bench et al. 1995), and during psychological induction of depressive states (Schneider et al. 1997). Most studies have implicated several cortical and subcortical structures, such as orbitofrontal cortex, cingulate cortex and basal ganglia. A depressive state abnormality of over activity in amygdala, hippocampus and parts of the temporal lobes has also been found (Schneider et al. 1997). The findings from MRI volumetric studies have varied but reduction in hippocampal volume has been reported (Shah et al. 1998; Sheline et al. 1996).

Neuropathology findings

Findings from structural imaging studies have driven neuropathological investigations into mood disorders and there are now preliminary reports of cytoarchitectural alterations in anterior cingulate and prefrontal cortices. These alterations are characterised by a

decrease in the number or density of glia. Reductions in the size and density of neuronal populations have also been reported, which is postulated to be caused by abnormal neurodevelopment or impaired neuronal plasticity (reviewed by Harrison 2002). Drug treatment does not appear to be a causative factor.

The amine hypothesis

Since the 1960's, the 'amine hypothesis' of depressive disorders has been the primary proposed aetiology (Schildkraut 1965). It has been clear from this time that drugs enhancing amine systems can have antidepressant effects. Tricyclic antidepressants (TCAs) inhibit amine reuptake from the synaptic cleft into presynaptic terminals (increasing available monoamines for binding at postsynaptic receptor sites), and MAOIs block the breakdown of amines in presynaptic terminals (increasing the amount of amines available for release into the synapse). Both drug groups therefore enhance amine neurotransmission. Reserpine and tetrabenazine deplete presynaptic stores of monoamines and have been shown to be depressogenic (Muller et al. 1955). The three amines implicated in this theory are 5-HT, dopamine (DA) and noradrenaline (NA). The importance of 5-HT in depressive disorders will be dealt with in more detail in the dedicated section below.

DA has been implicated in depression for the following reasons. L-dopa, a precursor of DA, was shown to reverse the psychological effects induced by reserpine (Degkwitz et al. 1960). Bupropion, a drug reported to have dopaminergic properties, has antidepressant

effects and may be a useful addition to selective 5-HT reuptake inhibitor (SSRI) treatment in depressed partial responders (Bodkin et al. 1997). In Parkinson's disease (a disorder characterised by a primary dysfunction of dopaminergic function) there is a high rate of comorbid depression (of around 40%), and comparison studies have shown this rate in Parkinson's disease to be greater than in healthy controls, spouses and other physically disabled groups (as reviewed by Cummings 1992). Interestingly electroconvulsive therapy (ECT), which enhances DA transmission in rat striatum (McGarvey et al. 1993) and DA mediated neuroendocrine responses in humans (Costain et al. 1982), improves both depression and the motor symptoms of Parkinson's disease (Mann et al. 1995).

Cerebrospinal fluid (CSF) concentrations of DA's primary metabolite, homovanillic acid (HVA) are generally lower in depressed patients in comparison to healthy controls (eg. Kasa et al. 1982). A reduced CSF HVA has been associated specifically with psychomotor retardation (Wolfe et al. 1990) and suicidality (Engstrom et al. 1999). The rate at which CSF HVA accumulates after probenecid blockade (see later) is also reduced in depression, implying reduced DA turnover.

A limited number of imaging studies have examined DA function in depression. Agren and Reibring (1994) found a decreased uptake of [(11)C]L-DOPA over the blood brain barrier in depressed subjects, possibly implying reduced overall dopaminergic brain function. Two single photon emission computerised tomography (SPECT) studies of striatal D_2 receptor binding found no difference in mean ligand binding between unmedicated depressed and control subjects but reduced D_2 binding in treatment

responders in comparison to pretreatment and controls (Ebert et al. 1996 and Klimke et al. 1999). Striatal DA transporter binding affinity has also been shown to be increased in drug free depressed subjects in comparison to controls using SPECT (Brunswick et al. 2003). These findings go some way to support an abnormality of DA function in depressive disorders and a role of DA in treatment.

Clinically, the most significant evidence for specific involvement of NA in depression is the antidepressant efficacy of selective NA reuptake inhibitors, such as reboxetine with apparent equal efficacy to TCAs and SSRIs (Holm and Spencer 1999). The major metabolite of NA, 3-methoxy-4-hydroxyphenylglycol (MHPG), measured in urine, plasma and CSF has been shown to vary widely in depressed subjects, with studies reporting both increases and decreases (Delgado 2000). Patients with low urinary MHPG may be more responsive to TCAs than those with high MHPG levels (Maas et al. 1984). It is also claimed that the enzyme responsible for the breakdown of neuronally released NA, catechol-0-methyltransferase (COMT), has reduced activity in erythrocytes of depressed patients and their first degree relatives (Gershon and Jonas 1975); however, how far this correlates with central COMT activity is unknown.

Using a ligand that binds to the NA presynaptic transport mechanism, [^{3}H] nisoxetine, Klimek et al. (1997) found reduced transport sites in the midcaudal portion of the locus coeruleus in postmortem brains from subjects with major depression compared with healthy control subjects. Several groups have examined the numbers and functions of specific noradrenergic receptors in depression, particularly the presynaptic α_2

autoreceptor. The density and affinity of α_2 adrenoreceptors appear increased in the frontal cortex, hypothalamus, amygdala and hippocampus of depressed suicide victims (Meana et al. 1992), which theoretically could be linked with reduced NA release from presynaptic areas, and therefore a reduced overall noradrenergic function. An increase in density and affinity of platelet α_2 receptors in depressed subjects (Piletz 1990) and a decrease following antidepressant treatment have been reported (Garcia-Sevilla 1990) although not replicated. Studies of β-adrenoreceptor density and affinity in depressed subjects have varied (as reviewed by Leonard 1997).

Neuroendocrine studies (see Chapter 2) have examined the hypothalamic α_2 receptor mediated growth hormone (GH) response to clonidine, which is blunted in most studies of depressed patients compared with controls (eg. Checkley et al. 1981; Checkley et al. 1984). This blunted GH response persisted in one study of recovered depressed subjects, and may therefore represent a trait marker of depression and persistent α_2 receptor dysfunction (Mitchell et al. 1988). Miller et al. (1996) examined the effects of catecholamine depletion in groups of depressed patients in remission including depressed unmedicated patients, patients medicated with a noradrenergic antidepressant (desipramine) and patients medicated with a serotonergic antidepressant (fluoxetine). This method depletes central NA and DA by inhibiting the rate limiting enzyme in NA production using α-methyl-p-tyrosine (AMPT). They found no effect on mood in unmedicated patients or in patients on fluoxetine, but a significant relapse of mood symptoms in patients on desipramine. Understandably, this implies a need for central NA to maintain recovery in patients on a noradrenergic antidepressant. The fact that

unmedicated patients were not made worse by AMPT suggests that dysfunction of NA systems does not correlate with the severity of depression. However, AMPT did cause pronounced, temporary, depressive relapse in unmedicated patients who had recovered from depression and were clinically well. This suggests that in those vulnerable to depression, depletion of NA and /or DA can be sufficient to cause clinical symptomatology.

There are several findings that appear inconsistent with the amine hypothesis. Firstly, antidepressant efficacy in depressed patients is only apparent after several weeks of treatment. The expected immediate increase in monoaminergic function from blockade of amine reuptake, for example, should cause a rapid antidepressant effect under the amine hypothesis. The immediate increase in extracellular monoamines is actually likely to be diminished by the activity of presynaptic autoreceptors. Several studies have shown downregulation of postsynaptic (particularly 5-HT) receptors (see Chapter 2), which again would go against enhanced overall aminergic function. Tianeptine, a 5-HT uptake enhancer, reduces 5-HT induced behaviour in rats but has clear antidepressant effects in humans (reviewed by Wilde and Benfield 1995). Some drugs which alter monoamine levels, such as cocaine and amphetamine are not antidepressant. Similarly, some drugs that have minimal or no apparent effect on aminergic function have antidepressant properties, such as iprindole (Zis and Goodwin 1979) or MK 869 (Argyropoulos and Nutt 2000).

5-HT, DA and NA have been dealt with separately; however there is a well documented

interaction between these amines. For example: stimulation of α_1 receptors on serotonergic neurone cell bodies causes an increased firing of these neurones, stimulation of α_2 receptors on serotonergic terminal areas reduces 5-HT release, and in animal models, 5-HT_{2C} receptor stimulation causes a reduction in activity of mesocortical dopaminergic and adrenergic transmission (Gobert et al. 2000). Many other studies have detailed such interactions (see discussion of individual 5-HT receptor types in this Chapter). There is also evidence of interaction between neurotransmitter systems at an intracellular level, so that binding to one receptor influences activity at another (Manji 1992). These findings may explain why selectively blocking the reuptake of a single amine has the same antidepressant efficacy as using a non selective amine reuptake blocker.

In conclusion, monoamines clearly play a part in depressive disorders but the exact nature of disturbances in monoamine function, and how this can lead to such a clinical syndrome, remains unclear.

Endocrine abnormalities

Cortisol (CORT) output is increased in depressive disorders, but despite this being known since the 1960's, the functional significance remains unclear. Theories exist of CORT having both antidepressant qualities and being a causative factor in depression (Dinan 1994). The dexamethasone suppression test (DST) has been widely used as an index of this hypercortisolaemia. Dexamethasone potently inhibits the secretion of

adrenocorticotrophic hormone (ACTH), which in turn suppresses CORT secretion for 24 hours in normal subjects. Plasma CORT levels remain high after dexamethasone challenge (non suppression) in subjects with hypercortisolaemia. Carrol et al. (1981) claimed that DST non suppression was a specific test for melancholic depression. Other authors suggested this test predicts response to biological antidepressant treatments, such as TCAs or ECT. These findings have not been consistently replicated, but confounding factors for the DST, such as weight loss and insomnia, have been more clearly identified (Mullen et al. 1986). In association with raised CORT, adrenal gland hypertrophy, reversed on recovery from depression (Rubin et al. 1995), and decreased bone density in elderly depressed women have been observed (Michelson et al.1996). CORT has also been shown to be toxic to hippocampal neurones. The hippocampus has an inhibitory influence over the hypothalamic pituitary adrenal axis (HPA), so a link with depression and HPA overactivity may therefore by perpetuated by CORT induced hippocampal damage.

Thyroid dysfunction has been implicated as a potential cause of depressive disorder due to the common co-occurrence of depressive symptoms with hypothyroidism and the beneficial use of tri-iodothyronine as a useful adjunct to antidepressant treatment in euthyroid depressed patients (Aronson et al. 1996). The thyroid stimulating hormone (TSH) response to thyrotrophin releasing hormone (TRH) is abnormal in some depressed subjects (Duval et al. 1999), but the clinical significance of this is unclear. Other endocrine disorders, such as Cushing's syndrome, hyper and hypoparathyroidism, are associated with depression. Normalisation of the hormonal and metabolic abnormalities is

largely followed by resolution of the depressive state suggesting a cause and effect relationship (Kelly et al. 1983; Sonino et al. 1993).

Physical illness and change in physiological state

Many physical disorders and state changes are associated with depressive disorder, and some of these that have been specifically investigated are detailed below. The psychosocial stress of physical illness is an important aetiological factor in itself but the illness process may have a direct biological aetiological effect too.

Childbirth is significantly associated with depressive states. 'Postnatal blues' occurs in at least 50% of women within 10 days of childbirth, with features of crying, low mood and emotional lability. This state usually rapidly resolves but a higher rate of more severe depression is recorded in these subjects at a later postnatal stage. Some biochemical associations have been found with postnatal blues such as increased urinary cyclicAMP and reduced plasma concentrations of free tryptophan (TRP). Neurotic type personality features appear to be higher in this group. Approximately 13% of postnatal women experience a major depressive episode, with onset typically between 2 and 12 weeks postpartum. No consistent associations with depressive states and sex hormones have been observed (Harris et al. 1996).

An association between hysterectomy and depression has been reported, with 36% of postoperative women being treated for depression in one study, four times the rate of matched controls (Richards 1973). Gath et al. (1982), however, found that women who

undergo hysterectomy have higher rates of psychopathology pre and post operation than controls, suggesting that those who undergo surgery are more at risk of illness than the general population.

Many investigators have examined depression following stroke, demonstrating that it is a common consequence and is associated with excess disability, cognitive impairment and mortality (Whyte and Mulsant 2002). Studies have focussed on whether lesions in specific brain areas, particularly left anterior regions, are more associated with depression but this hypothesis has been discounted by a systematic review of 48 such reports (Carson et al. 2000).

Viral infections are often linked with depressive disorders, particularly during physical recovery. Few studies have systematically investigated this association. Depression is common in HIV positive subjects, with clear psychosocial reasons (Fenton 1987). Such groups respond as well to antidepressant medications as other physically healthy groups (Wagner et al. 1996). The organic changes associated with AIDS similarly can clearly be linked with psychiatric changes (Fenton 1987).

The mediator between physical pathology, stress and mood states may be cytokines (including the interleukins and interferons) which are a diverse group of proteins that regulate the immune response and also act as neuromodulators in the central nervous system (CNS). Cytokines are released following physical and psychological stress and have a stimulatory influence on the HPA, which could thereby alter mood via enhanced

CORT release as described above (reviewed by Kronfol and Remick 2000). Maes et al. (1993; 1995) have reported an increase in the plasma levels of acute phase proteins and the proinflammatoy cytokines interleukin-1, interleukin-6 and their respective receptors in patients with major depression, which correlated with severity of depression and measures of HPA hyperactivity. This has not been consistently replicated however (Weizman et al. 1994). Treatment of depressed subjects with clomipramine has been associated with an apparent paradoxical increase in the synthesis of interleukin-1 and interleukin-3-like activity in lymphocytes (Weizman et al. 1994). Treatment with interferon also has been reported to be associated with depression (Kronfol and Remick 2000).

Sleep

Disturbances of diurnal rhythms may be a causal factor for depressive disorders for several reasons. Disturbances of sleep, particularly early morning wakening, are a common feature of depressive disorders, particularly severe or melancholic types (DSM-IV 1994). A reasonably consistent finding on electroencephalogram (EEG) recording in this group is shortened rapid eye movement (REM) sleep latency and a reduction in total slow wave sleep (reviewed by Kendell 1993). Therapeutically, sleep deprivation has been shown to have antidepressant qualities in 50 to 70% of depressed subjects, and the brief duration of this effect may be extended by restricting sleep at critical times of the day (Riemann et al. 1996).

Environmental aetiology

Environmental factors are clearly involved in the aetiology of depression, as shown by the incomplete evidence of a genetic basis, as detailed above. Studies are beginning to show gene-environment interactions, such as a variant of a 5-HTT gene promotor region polymorphism being associated with adult depression following early life adversity (Caspi et al. 2003) (see above). Three focal areas of environmental stress have been particularly studied: early parental loss, life events and social support.

Parental loss

Parental loss has been investigated since the original proposals of Freud (1917) and Abraham (1924), that melancholia was a reaction to the loss of a 'love object' in childhood. Many studies have attempted to clarify this with inconsistent results. Methodological problems include matching control groups, and studies using known depressed subjects rather than all depressed subjects in a population (personality features being the confounding factor). In a large community study of women, Harris et al. (1986) found a relationship between loss of a mother (not father) before the age of 17 years and depression in the previous 12 months. 'Lack of care' appeared more important than the loss event itself in this group. The association was also not specific to depression hence early parental loss is likely to be commoner in all subjects with psychiatric illness.

Life events

Life events clearly have a role in the aetiology of depression; however investigations have been similarly marred by methodological problems. Studies before the mid 1980's had been retrospective, with the associated problem of depressed subjects attributing past events more negatively due to their current mood, and remembering more negative than positive events. Identifying events that occurred independently of a depressive illness (rather than as a result of it, for example losing a job due to depressive cognitive impairment) was also difficult. Paykel et al. (1969) found a three times greater number of life events (unpleasant, mainly social loss events) in referred depressed subjects than matched controls. Brown and Harris (1978) found an increased rate of events of all types (mostly threatening) in the 3 weeks preceding depressive illness. This group also identified vulnerability factors, which increase the likelihood of depressive illness only in combination with provoking (threatening) events. These vulnerability factors were: loss of mother before the age of 11 years, having three or more children at home below the age of 14 years, the lack of a confiding intimate relationship, and unemployment. They also point out a higher prevalence of depression in lower social classes (IV and V). Studies have attempted to replicate these findings, agreeing only with the social class and lack of confiding intimate relationship attributes (eg. Campbell et al. 1993). Two studies have prospectively examined the influence of vulnerability and life events, Brown et al. 1986, and Surtees et al. 1986. Brown found the most important vulnerability factor to be low self esteem, and highlighted the importance of a confiding relationship at the time of a stressful event. Surtees failed to replicate the importance of such vulnerability factors, and only found an increase of life events prior to illness episode that could be seen as

related to the illness. Surtees did however identify chronic stress (major difficulties) as an important aetiological factor. Several authors have more recently examined the influence of parental care on later depression and low maternal care (rated with the Parental Bonding Instrument) has been replicated as an important factor (Parker et al. 1995). Both childhood physical and sexual abuse are also associated with a greater risk of depression in later life (Bifulco et al. 1994; Vize and Cooper 1995).

Social support

As indicated above, Brown and Harris (1978) reported an importance of social support (presence of a confiding intimate relationship) as a vulnerability factor for depression. Methodological problems arise in investigating this area as depressive disorders, and possible associated personality attributes will influence social networks. Harris et al. (1999) investigated the effects of volunteers befriending patients with chronic depression (in a randomised manner). Although difficulties arose with a control group and 50% of potential subjects not being interested, a significant effect on remission rates was observed. Living with an over involved and critical family (measured as expressed emotion) has also been shown to increase illness relapse in depression (Vaughan and Leff 1976), as shown in schizophrenia.

Conclusion

A unitary theory of major depressive disorder aetiology needs to include the predisposing factors of genetic susceptibility and early life adversity, and the precipitating influence of acute and chronic stresses and some drugs. Psychosocial support appears to have a

protective influence. These combine to create a disorder with significant clinical heterogeneity, characterised by low mood and anhedonia, with biological features such as sleep and appetite disturbance, hypercortisolaemia, monoamine neurotransmitter dysfunction, and focal disturbance of brain function. This disorder responds in a significant proportion of people to manipulation of monoaminergic neurotransmission, and in milder disorders to psychological treatment alone.

Kendler et al. (1993) clarified the diverse importance of such factors, showing that 50% of the variance in liability to suffer major depression at follow up was accounted for by stressful life events, genetic factors, a previous depressive episode and neuroticism as a personality trait. The aetiology of depression is therefore complex with a variety of factors that interact with each other both directly and indirectly. In view of this complexity and probable biochemical heterogeneity it is perhaps rather remarkable that fairly simple neurotransmitter manipulations such as potentiation of 5-HT function can have antidepressant effects in a sizeable proportion of depressed subjects (see below).

TREATMENT OF DEPRESSIVE DISORDERS

The treatment of depressive disorders has both biological and psychosocial components, with efficacy studies showing significant benefits for specific treatments from both areas.

Biological Treatments

The first widely used specific antidepressant, imipramine, was introduced in 1957 by Roland Kuhn who published an open trial of its use in depressed subjects (reviewed by Ayuso-Gutierrez 2002). This tricyclic drug was discovered following the adaptation of the phenothiazine antipsychotic drug, promazine. Isoniazid, a treatment for tuberculosis, was also shown to have antidepressant properties by Nathan Kline in 1957, and was identified as an MAOI, but was not taken on for the treatment of depression due to liver toxic properties. Following this era, many variations of antidepressant have been discovered, and will be discussed by group.

TCAs, for example amitriptyline, imipramine and dothiepin, have been the most widely used antidepressants until recent years. They block the presynaptic reuptake of the monoamines, 5-HT and NA (Stahl 1998). This theoretically enhances the availability of monoamines to bind at postsynaptic receptor sites and thereby increases monoaminergic neurotransmission. The antidepressant effect, as with other antidepressant drugs, starts after about 2 to 3 weeks continuous administration (Blier et al. 1994). This time course

coincides with the onset of a variety of neuroadaptive effects, such as postsynaptic receptor downregulation (Stahl 1998). The side effects of TCAs are predicted by their effects at cholinergic, adrenergic and histaminergic receptors. The most dangerous side effect is their arrhythmogenic properties, leading to toxicity in overdose (Henry et al. 1995). This has been one of the driving forces to discover less toxic antidepressants.

SSRIs, such as fluoxetine, fluvoxamine, paroxetine, sertraline and citalopram, have been available for use as antidepressants from the late 1980s/early 1990s. Due to their greater tolerability than TCAs, and much lower toxicity in overdose (with the possible exception of citalopram), their popularity has risen dramatically. Their efficacy appears equal to TCAs (see below) but their cost initially limited their use. Their mechanism of action is the selective blockade of 5-HT reuptake into presynaptic terminals. More detailed receptor effects will be discussed below. Side effects are associated with increased serotonergic function, including nausea, decreased appetite, diarrhoea, anxiety, dizziness, headache and sexual dysfunction. The reported increased suicidality with SSRIs is discussed later in this chapter. There appear to be few differences between SSRIs in terms of efficacy, but tolerance may vary between subjects. Fluoxetine may be associated with greater agitation, a slightly longer time to onset of effect, but fewer withdrawal effects, citalopram has more associated deaths in overdose and paroxetine has the most troublesome withdrawal syndrome (Edwards and Anderson 1999). Escitalopram, the active S-isomer of citalopram, has been recently introduced (Owens et al. 2001) with claims of greater efficacy than citalopram (Gorman et al. 2002), but this will need to be examined in further studies.

The antidepressant efficacy of TCAs and SSRIs, in comparison to placebo, was examined in a meta analysis of 49 RCTs by Joffe et al. published in 1996. A clear benefit of drug treatment was observed, with 69% of patients taking placebo doing worse than the average person on drug treatment (effect size 0.50). Subsequently, several authors have questioned whether antidepressants have any benefit over placebo. Kirsch et al. (2002) undertook a systematic review of all trials submitted to the US Food and Drug Administration of antidepressants approved between 1987 and 1999 (fluoxetine, paroxetine, sertraline, venlafaxine, nefazodone and citalopram). This included many previously unpublished trials, so represents the most complete potential dataset. They found that approximately 80% of the response to medication was duplicated by placebo and the mean difference between drug and placebo was 2 points on the HAM-D scale, with no difference between high and low doses. They argued that this represents a negligible difference between these antidepressants and placebo in such trials and that different trial designs are necessary to clearly examine the placebo effect in depression. The 2 point HAM-D scale difference was a highly statistically significant benefit however and this may be an under estimation of the actual effect of these drugs in usual clinical practice for many reasons, including sample selection biases and the studies being set up to measure efficacy as opposed to effectiveness.

Several systematic reviews have examined the relative antidepressant efficacy of SSRIs as opposed to TCAs. The reviews by Geddes et al. (2000), Hotopf et al. (1997), and Song et al. (1993) revealed no significant difference between these drug groups. The

tolerability of SSRIs was significantly better than TCAs, demonstrated by a lower trial dropout rate of patients on SSRIs. Within the limits of these reviews, no difference was shown between the efficacy of SSRIs and TCAs in severe depressive disorders. Anderson (2000) also examined this comparison with a slightly larger number of pooled studies than Geddes et al. (2000) agreeing that there is no overall difference between efficacy of SSRIs and TCAs but found that TCAs had a greater effect in inpatients and that amitriptyline was more effective than SSRI comparators. Barbui and Hotopf (2001) also found that amitriptyline had a greater efficacy than SSRIs and reported that amitriptyline had a greater effect than other TCAs.

Other drugs which block the reuptake of monoamines more selectively than TCAs have been developed. Venlafaxine selectively blocks 5-HT and NA reuptake, and has been reported to have a greater efficacy than SSRIs (Smith et al. 2002), particularly in more severe depressive disorders (Hirschfeld 1999). Duloxetine has recently become available and is also a reuptake blocker of 5-HT and NA. It has an efficacy in depressive disorders greater than placebo and an apparent equivalence to paroxetine in acute and chronic treatment (Detke et al. 2004) but does not appear to have any greater efficacy in melancholic than non melancholic subjects (Mallinckrodt et al. 2005). Duloxetine has also has been shown to reduce reports of physical pain associated with depression (Brannan et al. 2005) but this could be a class property of all antidepressants. Reboxetine, which only blocks NA reuptake has been shown to have equal efficacy to SSRIs and may also improve social functioning rather more (Holm and Spencer 1999).

Monoamine oxidase inhibitors have been available since the 1950's, and those without hepatotoxic effects, such as phenelzine, isocarboxazid and tranylcypromine are still used clinically. Their mechanism of action is to block both forms (A and B) of the enzyme monoamine oxidase (MAO) which breaks down monoamines presynaptically. They thereby increase the availability of monoamines for release into the synapse. As this blockade is irreversible, dietary tyramine is not broken down hence a potentially dangerous hypertensive effect can be experienced if tyramine is ingested. Patients on irreversible, non selective, MAOIs therefore need to avoid tyramine containing foods such as: cheese, meat extracts, Chianti, pickles, game, offal and avocado. This side effect and perceived limited clinical efficacy have inhibited the widespread use of MAOIs. In the Clinical Trial of the Treatment of Depressive Illness (Medical Research Council Clinical Psychiatry Committee 1965), phenelzine was shown to offer no benefit over placebo, and indeed was worse than placebo in female subjects. A systematic review (Thase et al. 1995) compared MAOIs with TCAs, confirming clinical opinion that MAOIs are less effective in severe depressive disorders, but may be more effective in atypical depressive syndromes (with hypersomnia, increased appetite, mood reactivity and rejection sensitivity).

A reversible inhibitor of MAO A, moclobemide, was recently introduced, which at therapeutic doses does not have the tyramine pressor effect. The clinical efficacy data of moclobemide relative to other antidepressants is uncertain, perhaps explaining why it has had limited impact on clinical practice (Lotufo-Neto et al. 1999).

Several other drugs have been developed with more selective receptor action than TCAs, in an attempt to produce a better tolerated drug with equal efficacy to TCAs. Mirtazepine and nefazodone are two such drugs, with particular action on the 5-HT_2 receptor, a site implicated as important in antidepressant action (see below).

Other antidepressants worthy of mention include L-TRP. This is a precursor to 5-HT, which has some evidence supporting a benefit in the treatment of mild to moderate depression (Meyers 2000). Theoretically, loading the system with more precursor would boost the amount of presynaptically available 5-HT. L-TRP was withdrawn from regular clinical use due to the association with eosinophilia myalgia syndrome, but has returned on a named patient prescription basis. It is probably used most currently as an augmentor of antidepressant treatment in resistant disorders (Meyers 2000). The use of L-TRP in neuroendocrine challenges will be discussed in Chapter 2.

In the pharmacological management of depressive disorders, there has been much debate as to which drug group should be considered first line. TCAs have the disadvantage of toxicity in overdose and are less well tolerated than SSRIs. SSRIs have been felt to be less effective in severe disorders and have been expensive. SSRIs are now widely recommended as first line, particularly those off patent, and reasonably priced. In subjects not responding to initial drug treatment there is reasonable evidence to change to another drug. Patients are usually given 6 weeks on a medication without improvement before lack of response is accepted. Other monotherapy options at this stage may include venlafaxine, mirtazepine, or an MAOI. In depressive disorders not responding to

monotherapy, there is good evidence for the augmenting effect of lithium and some that tri-iodothyronine can augment TCAs (Joffe et al. 1993). Controlled evidence for the efficacy of other combinations of drugs (SSRI - TCA or MAOI - TCA) or augmentors (amphetamine, bupropion, pindolol, typical and atypical neuroleptic) are sparse. The National Institute for Clinical Excellence for England and Wales, has reviewed available data in depth and published guidelines for antidepressant use, available at www.nice.org.uk.

Case reports that pindolol augmentation of SSRIs reduced the time to onset and enhanced antidepressant effect, along with a convincing mechanism of action (Artigas 1994) has set off a flurry of research in recent years. Pindolol has been shown to have an affinity for 5-HT_{1A} receptors (alongside β-adrenergic receptor antagonism) and in theory might antagonise the presynaptic 5-HT_{1A} autoreceptor on the 5-HT cell body. 5-HT_{1A} autoreceptors would normally mediate the inhibition of 5-HT cell firing that follows the increase of extracellular 5-HT caused by an SSRI. Hence concomitant 5-HT_{1A} autoreceptor blockade might persistently enhance serotonergic neurotransmission, and may reduce the time to onset of clinical effect. Several randomised controlled trials (RCTs) have been undertaken to examine this effect of pindolol, and a great heterogeneity of response is observed. Taking these studies together, a positive overall antidepressant enhancing effect is observed up to 4 weeks of treatment (the number needed to treat statistic (NNT) at 2 weeks is 7), with a possible reduced time to onset of effect (Whale et al., The Cochrane Library, in preparation). This line of augmentation strategy, using presynaptic antagonists, appears hopeful in improving future antidepressant treatment.

Other apparently non aminergic antidepressant agents are being investigated, such as Substance P receptor antagonists. The substance P antagonist, MK-869, has been shown to have antidepressant properties in a placebo controlled trial in patients with moderate to severe major depression (Kramer et al. 1998) and to be of equivalent efficacy to paroxetine treatment (Nutt et al. 1998). Haddjeri and Blier (2000) recently demonstrated that such agents alter central NA and 5-HT activity in rats, possibly suggesting that while they are primarily non aminergic agents, Substance P antagonists may nevertheless act by modifying monoamine neurotransmission.

ECT has been the treatment of choice for severe, life threatening depressive illness for some time, with proven efficacy since the 1960's (The UK ECT Review Group 2003). The first recorded association between seizures (induced by oral camphor) and relief of melancholic symptoms was in 1785. Troublesome associated side effects, such as temporary memory impairment, and social stigma have limited its use. The efficacy of ECT as a rapid onset antidepressant treatment, and its benefit in resistant disorders, mean that it retains an important place in clinical management. The mode of action of ECT is unclear. Animal studies have shown that ECT down regulates β noradrenergic receptors (as with antidepressants) but up regulates 5-HT_2 receptors (opposite to antidepressants) (Leonard 1991). Further elucidation of the mechanism of action may lead to the discovery of more effective antidepressant treatments.

Repetitive transcranial magnetic stimulation (rTMS) is a technique in which small electrical currents are induced in brain tissue by placing a magnetic coil over the cortex.

This was originally used as a tool to map brain functions, but has recently been used experimentally as a treatment for psychiatric disorders. rTMS appears to improve the mood of depressed patients when applied over the dorsolateral prefrontal cortex. Controlled studies have demonstrated a clinically useful antidepressant effect (George et al. 1997; Pascual-Leone et al. 1996), although the optimal dosing schedule is unclear. As rTMS is well tolerated (in patients without a history of fits) and does not require concurrent anaesthesia, the potential for widespread use as an antidepressant and for insights into the aetiology of depression are evident.

Several authors have attempted to identify predictors of response to specific treatments. The clinical impression that response to physical treatments (drugs and ECT) is more likely in melancholic depressed subjects is not consistent in well controlled trials, but these subjects do appear less likely to respond to placebo than non melancholic subjects (Peselow et al. 1992). Patients with atypical depression have a greater response rate to MAOIs than non atypical depressed patients (Posternak and Zimmerman 2002). The presence of depressive delusions also appears to predict response to ECT (Johnstone et al. 1980). Genetic influences may affect response. Replicated studies of the insertion/deletion polymorphism in the promotor region of the 5-HTT gene, referred to above, have demonstrated poorer therapeutic response and greater side effects during SSRI treatment for the short variant homozygote (reviewed by Murphy et al. 2004), consistent with the neuroendocrine and tryptophan depletion findings described above.

Psychosocial Treatments

Many psychological treatments have been claimed to be effective in the treatment of depressive disorders, although few have undergone rigorous trials. Most psychological treatments compare favourably with placebo (or waiting list) but a limited number of trials have compared them directly with drug treatments. This will be briefly discussed for the most effective treatments. The effects of a supportive partner are discussed in the social support section above.

Psychoanalysis does not lend itself to studies of effectiveness of treatment of acute depression. Treatment goals are usually associated with change in underlying conflicts or deficits that may change the subject's vulnerability to depression. Therapeutic techniques used would be difficult to standardise for a meaningful trial. Although brief psychodynamic psychotherapy for major depressive disorder is more effective than waiting list control, it does not appear to be as effective as cognitive behavioural therapy (CBT) or interpersonal therapy (IPT) (meta-analysis finding, Depression guideline panel, 1993).

CBT is based on Beck's theory that depressive symptoms may arise from dysfunctional patterns of thinking and behaviour, which may be a result of early life experiences (Beck et al. 1979). The process of treatment involves identification of such abnormal patterns of thinking and behaviour, and underlying attitudes that may influence these. The therapist assists in challenging irrational thinking and dysfunctional assumptions, and behavioural

modification. Twenty RCTs were included in a systematic review comparing cognitive therapy with waiting list or placebo (Gloaguen et al. 1998). Significantly, 79% of patients in the placebo group were more symptomatic than the average patient treated with cognitive therapy. Seventeen studies were included in this review comparing drug treatment with cognitive therapy. 65% of patients receiving cognitive therapy were less symptomatic than the average patient receiving drug treatment. The subjects in these studies were from primary care settings, likely to have mild to moderate depression and drug dosages varied, possibly explaining the more favourable response to psychological than drug treatment. A recent 'mega analysis', including four selected RCTs of CBT vs antidepressant medication in severe depression, however, reported an equal efficacy of both treatments (DeRubeis et al. 1999).

The focus of IPT is current social context and relationships, and how interpersonal problems may be linked with depression. The areas of loss, role disputes, role transitions and interpersonal skill deficits are examined and tackled using practical problem solving techniques (Klerman et al. 1984). A large RCT of 16 weeks duration, including mild to moderate depressive disorders, compared IPT with placebo and imipramine treatment. The recovery rates were favourable for IPT: 21% recovery in the placebo group, 43% in the IPT group and 42% in the imipramine group.

Several small RCTs have examined the effect of problem solving therapy, in which the focus of therapy is the identification and practical management of specific life stresses (detailed in Hawton et al. 1989). Problem solving was shown to be as effective as drug

treatment in major depressive disorders (in a primary care setting), with no further benefit gained from adding drug to psychological treatment (Mynors-Wallis et al. 2000).

The addition of drug treatment to psychological treatments is more effective than psychological treatment alone in severe depressive disorders, but addition of drug treatment does not appear to add any significant benefit in milder depressive states (Thase et al. 1997).

PROGNOSIS

Less is known of the prognosis of depressive disorders than other severe psychiatric disorders as few long term follow up studies have been carried out, and many cases do not reach medical attention.

The short term prognosis is relatively good, but variable, with a mean duration of 16 weeks reported by Kessler et al. (2003). 20% of patients have symptoms which persist beyond 2 years (Keller et al. 1992). Depressive disorder is highly recurrent with 30% of patients experiencing a relapse within 3 months of recovery and 50% (in the absence of ongoing treatment) experience relapse within 2 years. In Lee and Murray's (1988) follow up of inpatients, 18 years after initial admission, only 11 of 89 patients had good outcome with no further depressive episodes, and those with severe (melancholic or psychotic) index episodes had by far the worst eventual outcome. Very low rates of adequate

community treatment of depressive disorder of 22% are observed (Kessler et al. 2003).

Suicide rates are relatively easy to measure and globally the annual incidence of suicide among depressed patients has been found to be 1%. About 15% of depressed patients treated as inpatients eventually die by suicide. History of depression is associated with a 30 fold increase in suicide risk (reviewed by Kreitman 1993). Elderly depressed patients (over 50 years old) and younger men appear to be at greater risk (Lejoyeux et al. 1994). Although there is ample evidence that available treatments significantly improve acute depressive disorders, and some reduce the likelihood of further episodes, there appears to be little impact on the general suicide rate (Kreitman 1993).

There have been many recent reports of SSRIs increasing suicidality in adults but more rigorous investigation has not confirmed this (eg. Khan et al. 2003). In 2003, the Committee on Safety of Medicines in the UK reviewed the safety and efficacy of SSRIs in the treatment of major depressive disorder in the under 18s, recommending that paroxetine, venlafaxine, sertraline, citalopram and escitalopram are contraindicated due to risks outweighing any benefits of treatment, particularly increased suicidality,. Fluoxetine was the only SSRI deemed to have a favourable balance of risks. In 2004 the Committee on Safety of Medicines concluded that there is no clear evidence of an increased risk of self harm and suicidal thoughts in young adults over 18 years, but as in all age groups, anyone treated with SSRIs should be closely monitored for the development of suicidal ideas, particularly at the time of initiation and dose changes. These reports are available at http://medicines.mhra.gov.uk.

If the above findings are valid, then a major public health problem will exist in the future. The World Health Organisation has predicted that by 2020, major unipolar depression will one of the leading causes of ill health related disability (Murray and Lopez 1997).

THE DISCOVERY AND BIOCHEMISTRY OF 5-HT

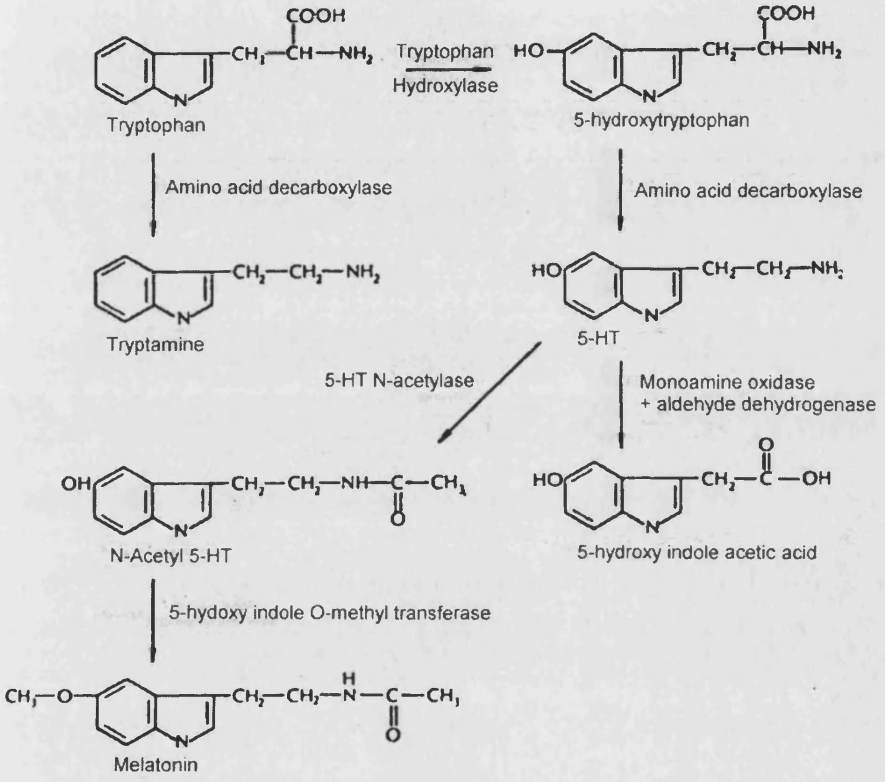
Scientists in the nineteenth century were aware of a component of human serum that caused contraction of smooth muscle. It was not until the years 1948 to 1953, however, that this substance was isolated, identified as 5-HT and synthesised. This work was carried out by Rapport, Green and Page. As 5-HT was a serum vasoconstrictor, released from platelets during blood clotting, they called it 'serotonin' (serum tonic factor) (Page 1976). Concurrently, Erspamer had been studying a smooth muscle contracting substance, that he called 'enteramine' (due to its extraction from gut enterochromaffin cells), which was later found to be identical to 5-HT (Erspamer 1963). In 1953 Twarong and Page detected 5-HT in extracts of brain (Twarong 1988), which has led to the investigation of its involvement in brain disorders.

5-HT is formed in the body by hydroxylation and decarboxylation of the essential amino acid TRP (figure 1.1). TRP is found in foods such as cheese, peanuts, chocolate and fruits such as banana and avocado. TRP is highly bound to plasma protein, with less than 20% being free in plasma. Normally TRP hydroxylase (the rate limiting step to 5-HT

formation) is not saturated, so an increase of available TRP in the brain will increase brain 5-HT concentration. The amount of available brain TRP is dependent on total serum TRP, unbound serum TRP and the concentration of other amino acids (tyrosine, valine, phenylalanine, isoleucine and leucine) competing for the same brain uptake mechanism. 5-HT synthesis occurs in nerve terminals. It is stored in vesicles in the presynaptic areas, and released into the synaptic cleft on nerve firing via a Ca^{2+} dependant mechanism (Stahl 1998).

Following release from serotonergic neurones, available 5-HT is taken back into the presynaptic neurone via an active transport mechanism, the 5-HTT. At this stage it is broken down by monoamine oxidase to the inactive, 5-hydoxyindoleacetic acid (5-HIAA). 5-HIAA can be measured in CSF (and urine), and may be helpful in estimating total serotonergic activity. In the pineal gland, 5-HT is converted to melatonin (N-acetyl-5-methoxytryptamine), which has diurnal control functions including sleep regulation (Forsling 2000).

Figure 1.1 5-HT synthesis and metabolism



BRAIN DISTRIBUTION OF 5-HT NEURONES

Serotonergic neurone cell bodies are located primarily in the midline raphe of the midbrain, pons and medulla oblongata. They have extensive projections throughout the brain and spinal cord.

Most ascending serotonergic pathways originate from raphe nuclei in the medulla, give branches to the substantia nigra and interpeduncular nucleus and supply the posterior and lateral hypothalamic nuclei and the neostriatum. The largest distribution of the ascending fibres is to the limbic system and neocortex.

Descending serotonergic pathways arise from the raphe nuclei of the pons and medulla and enter the dorsolateral spinal tract. These pathways synapse with other neurotransmitter associated neurones in the spinal tract, and may be involved with pain modulation. 5-HT neurones therefore have a very widespread distribution in the CNS.

5-HT RECEPTORS AND THEIR FUNCTIONAL SIGNIFICANCE

Over the last 20 years the identification of distinct 5-HT receptors has progressed dramatically. Initially, subtypes of 5-HT receptor were identified by pharmacological tools but the use of radioligand binding techniques, the development of more selective ligands and molecular biological techniques have assisted in subcharacterisation. Indeed functional correlates now lag behind molecular identification in that RNA has been identified for receptors that have no known functional significance (eg.5-ht_{1E}, 5-ht_{1F}, 5-ht_5).

An important early distinction between 5-HT receptors was made by Peroutka and Snyder who described 5-HT_1 and 5-HT_2 subtypes using the serotonergic ligands, [^{3}H] 5-HT, [^{3}H] LSD (lysergic acid diethylamide) and [^{3}H] spiperone. [^{3}H] 5-HT had high affinity for 5-HT_1 receptors and [^{3}H] spiperone had high affinity for 5-HT_2 receptors (Peroutka 1988). Many other radioligands have been introduced since, initially leading to subdivision of 5-HT_1 into 5-HT_{1A} to $_{1D}$ and the identification of 5-HT_3 receptors (Bradley et al. 1986). More recent molecular biological techniques have identified the protein structure of 5-HT receptors, enabled cloning and expression in cell lines, clarified known groups, expanded subtypes, identified further groups and allowed further pharmacological and functional classification. Identification of receptor DNA sequences has enabled antibody and antisense techniques to further clarify individual receptor function.

To date, seven families of 5-HT receptor (5-$HT_{1\ to\ 7}$) and 14 structurally and

pharmacologically distinct mammalian subtypes have been identified (TiPS Receptor and Ion Channel Nomenclature Supplement 2000). Some receptors still lack selective ligands. The families and subtypes of 5-HT receptors, their pharmacology, CNS distribution and function in human and animal models will be summarised. The physiological and behavioural function of 5-HT receptors has largely been localised to receptor groups so will be discussed under these headings. Receptors and their associated ligands are summarised in table 1.1.

5-HT_1 FAMILY

This family is characterised by high affinity binding of [^{3}H] 5-HT (Peroutka 1988). 5-HT_1 receptors are negatively coupled to adenylate cyclase via G proteins. Subdivisions are classified 5-HT_{1A} to $_{1F}$, excluding 5-HT_{1C} which was reclassified as 5-HT_{2C} (Pazos et al. 1984). 5-HT_1 pharmacological effects are mimicked by 5-CT, blocked by methiothepin and not blocked by selective 5-HT_2 and 5-HT_3 antagonists (Bradley et al. 1986).

5-$HT1_A$ receptor

The 5-HT_{1A} receptor is the most investigated of the 5-HT receptors to date. The identification of this receptor subtype (Pedigo et al. 1981) and discovery of a selective 5-HT_{1A} ligand, 8-OH-DPAT (Hjorth et al. 1982), enabled rapid progress in pharmacological characterisation.

As with other G protein linked receptors, this receptor has seven membrane spanning units. The 5-HT_{1A} receptor gene is located on chromosome 5 of the human genome and has a high sequence homology to the β_2 adrenoceptor gene. Several β receptor ligands, for example pindolol, also have activity at 5-HT_{1A} receptors (see below).

The brain distribution of this receptor has been investigated in humans using several radioligands in both postmortem specimens using autoradiography (Hall et al. 1997), and in vivo in humans using PET (Pike et al. 1996). The most popular 5-HT_{1A} ligand currently used for distribution studies is radiolabelled WAY-100635, a silent and selective antagonist of 5-HT_{1A} receptors, the binding of which is antagonised by the addition of other 5-HT_{1A} receptor ligands, 5-HT, buspirone, pindolol and 8-OH-DPAT (Hall et al. 1997). A high density of 5-HT_{1A} receptors is observed in the hippocampus, raphe nuclei and neocortex. Brain regions such as the amygdala, septum and claustrum have a low density of sites, with fewer 5-HT_{1A} receptor sites demonstrated in the basal ganglia, cerebellum and brain stem structures (except the raphe nuclei). Localisation of the 5-HT_{1A} receptors at the cellular level has been possible using light and electron microscopic immunocytochemistry with specific antibodies (Riad et al. 2000). 5-HT_{1A} receptors are found presynaptically in the raphe nuclei, on serotonergic neuronal cell bodies, dendrites, and along extrasynaptic portions of their plasma membrane. They are present postsynaptically to serotonergic neurones throughout other brain regions. 5-HT_{1A} heteroreceptors are probably located in cholinergic neurones of the septum and glutamatergic neurones in cortex and hippocampus.

5-HT_{1A} receptors are pharmacologically distinct from other 5-HT_1 receptors. Selective agonists include 8-OH-DPAT, dipropyl-5-CT, ipsapirone, gepirone and tandospirone. Buspirone is a weak, less selective, agonist but widely available and licensed as an anxiolytic. As above, the most useful experimental antagonist is WAY-100635, which is potent and has no intrinsic activity. β receptor antagonists, such as pindolol, penbutolol and tertatolol, are widely available for the treatment of hypertension, and may have 5-HT_{1A} antagonist properties. Pindolol is likely to be a partial agonist, however (Gartside et al. 1999).

The function of the 5-HT_{1A} receptor is diverse. As animal experiments have contributed significantly to the elucidation of receptor function, these studies will be referred to alongside human studies. Using transfected cell lines, 5-HT_{1A} receptors have been shown to couple negatively with adenylate cyclase via G proteins, alter intracellular calcium ions and activate phospholipase C (reviewed by Boess and Martin 1994). Electrophysiology studies have demonstrated a K^+ mediated neuronal hyperpolarisation resulting from 5-HT_{1A} receptor stimulation (Nicoll et al. 1990). These studies have also revealed differential effects of some weaker agonists at dorsal raphe and forebrain regions (pre and postsynaptic), possibly relating to drugs such as buspirone acting as a partial agonist at areas of low receptor density (forebrain) and a full agonist at areas of high receptor density (raphe). This may also indicate further subdivision of 5-HT_{1A} receptors (Barnes and Sharp 1999). Stimulation of 5-HT_{1A} receptors in the rat brain dorsal raphe by agonists such as 8-OH-DPAT and ipsapirone cause inhibition of serotonergic neurone cell firing (Sprouse and Aghajanian 1988). This effect is blocked by antagonists such as WAY-

100635. Microdialysis studies in rats are consistent with this finding, in that 5-HT_{1A} agonists reduce 5-HT release in forebrain areas (Sharp and Hjorth 1990). The dorsal raphe 5-HT_{1A} receptors are therefore acting as autoreceptors, modulating cell firing and subsequent 5-HT release. Studies of the effect of 5-HT_{1A} antagonists (e.g. WAY-100635) alone on cell firing rate and extracellular forebrain 5-HT are inconsistent (Sharp et al. 1996). This is probably due to an absence of basal tone at the 5-HT_{1A} receptor. In the presence of 5-HT reuptake inhibitors (e.g. paroxetine), cell firing is inhibited, but firing is restored when WAY-100635 is added (Gartside et al. 1997). The SSRI induced reduction in forebrain extracellular 5-HT in microdialysis studies is prevented by WAY-100635 (Gartside et al. 1995). This has been projected clinically to depressed patients with the use of pindolol augmentation of SSRIs, which in theory could induce a more rapid antidepressant response by diminishing the reduction of 5-HT release occurring when treatment with an SSRI alone is commenced (Artigas et al. 1994, as discussed above).

Cortical and hippocampal acetylcholine (ACh) and NA release is enhanced in the presence of 8-OH-DPAT in rats, and blocked by WAY-100635 (reviewed by Barnes and Sharp 1999). Both responses are likely to involve postsynaptic 5-HT_{1A} receptors. 5-HT may therefore play a part in modulation of responses known to involve both ACh and NA, such as attention, mood and cognition.

Many studies have examined behavioural and physiological responses in the rat using 5-HT_{1A} ligands (reviewed by Lucki 1992). 5-HT_{1A} agonists cause hyperphagia,

hypothermia, altered sexual behaviour, tail flick and the 5-HT behavioural syndrome (including hyperlocomotion, head weaving, flat body posture and forepaw treading). Only hyperphagia and hypothermia are likely to be mediated via presynaptic receptors. Human studies have linked 5-HT_{1A} receptors with temperature control, anxiety and depression. In healthy volunteers, the azapirones buspirone, ipsapirone, and gepirone decrease body temperature (Cowen et al. 1990). The hypothermic effect of buspirone is reduced by pindolol (Anderson and Cowen 1992), but pindolol alone also has a hypothermic effect (probably due to its partial agonist effects), (Meltzer and Maes 1996). Stahl (1998) proposed that presynaptic 5-HT_{1A} receptor hypofunction is linked with anxiety (downregulation of receptors due to an excess of 5-HT) and hyperfunction (upregulation of receptors due to a lack of 5-HT) with depression. Buspirone has proven benefit in the treatment of generalised anxiety disorder, and, similar to the time of onset of effect with antidepressant drugs, takes from two weeks to have therapeutic effect, suggesting a need for receptor adaptation for its anxiolytic effects (Ninan 1999). Buspirone also possesses antidepressant effects in patients with major depression. In addition it is used to augment antidepressant medication in resistant depression, but the only randomised trial found no significant benefit (Landen et al. 1998). It has been speculated that buspirone may be effective in both anxiety and depression because of its partial agonist properties, enabling it to act as an antagonist (upregulating receptors) in anxiety, and an agonist in depression (desensitising receptors). This results in a net normalisation of 5-HT function in both conditions. This hypothesis seems rather implausible and in any case it is now increasingly recognised that antidepressant drugs such as SSRIs are also effective in a range of anxiety disorders. The neuroendocrine

studies of human 5-HT_{1A} receptor function will be discussed in Chapter 2, particularly with reference to depressive disorders.

5-HT_{1B} receptor

Due to significant structural similarity between 5-HT_{1B} and 5-HT_{1D} receptors, and differing pharmacological properties between humans and animal models, much confusion has arisen in the classification and investigation of these receptors.
The 5-HT_{1B} receptor was first characterised pharmacologically in the rat (Pedigo et al. 1981). This receptor was subsequently identified in mouse and hamster. A further site, binding [^{3}H] 5-HT was identified in bovine brain and subsequently in guinea pig, dog and human. This latter receptor was named 5-HT_{1D}. Distribution of these 5-HT_{1B} and 5-HT_{1D} receptors (in neuronal terminal regions) between species was identical, however, which brought about the suggestion that these were species equivalents of the same receptor (Hoyer and Middlemiss 1989). Human genomic investigation identified two related receptor genes, with high sequence homology, which when expressed had the pharmacological characteristics of the 5-HT_{1D} receptor (Hartig et al. 1992). These were labelled 5-$HT_{1D\alpha}$ and 5-$HT_{1D\beta}$ receptors. Rat 5-HT_{1B} receptors were later cloned and found to have high sequence homology (96%) with the human 5-$HT_{1D\beta}$ gene (Jin et al. 1992). The receptor nomenclature was subsequently reassessed, taking into account that, despite pharmacological differences, human 5-$HT_{1D\beta}$ receptors have the same function as rat 5-HT_{1B} receptors. Human 5-$HT_{1D\beta}$ receptors were therefore relabelled as 5-HT_{1B}

receptors and 5-$HT_{1D\alpha}$ as 5-HT_{1D} receptors (Hartig 1996). The gene encoding human 5-HT_{1B} receptors is located on chromosome 9 (Saudou and Hen 1994).

In human studies, 5-HT_{1B} receptors have been identified at highest densities in the substantia nigra and globus pallidus and moderate densities in the caudate nucleus, putamen, nucleus accumbens, central grey and hippocampal formation (Bonaventure et al. 1997). 5-HT_{1B} receptors are located both presynaptically and postsynaptically on serotonergic neurones. Presynaptically they are found in the terminal regions, acting as autoreceptors. They are also found on non serotonergic neurones, suggesting action as heteroreceptors controlling neurotransmitter release (for review, Barnes and Sharp 1999). An association with GABAergic neurones in caudate and hippocampus has been identified (Boschert et al. 1994).

The 5-HT_{1B} receptor is pharmacologically characterised by affinity for [^{3}H] 5-HT and low affinity for spiperone and 8-OH-DPAT (in rats). Few selective ligands are available. Agonists include L-694247, RU 24969, 5-CT and CP 93129 and methiothepin is an antagonist. GR 127935 is a useful antagonist at 5-$HT_{1B/D}$ receptors, and more recently developed drugs, more potent at 5-HT_{1B} than 5-HT_{1D} receptors include SB-224289 and SB-216641. Problems have arisen in the past in differentiating the function of these receptors, but such specific antagonists will help clarify the picture. The 5-HT_2 receptor antagonists ketanserin and ritanserin also have a much greater affinity for 5-HT_{1D} than 5-HT_{1B} receptors. Several 'triptans' such as sumatriptan, zolmitriptan, naratriptan and rizatriptan are agonists at 5-$HT_{1B/D}$ receptors, and are available for the treatment of

migraine. A significant difference between human and rat 5-HT_{1B} receptors is their affinity for beta blockers. Pindolol has significant affinity for both 5-HT_{1A} and 5-HT_{1B} receptors in rats but just 5-HT_{1A} receptors in humans (Parker et al. 1993), which has important implications in using animal data to help interpret findings with pindolol in humans.

Like other 5-HT_1 receptors, 5-HT_{1B} receptors couple negatively with adenylate cyclase. Some 5-HT_{1B} receptors are located presynaptically in nerve terminal regions where they act as autoreceptors, reducing the release of 5-HT. In vitro, 5-HT agonist induced reduction of 5-HT release is inhibited by the 5-HT_{1B} selective antagonist SB-216641 (Schlicker et al. 1997). 5-HT_{1B} receptor antagonists alone may enhance 5-HT release, although this appears to vary between different brain regions, possibly depending on variations in neuronal tone (Barnes and Sharp 1999). Guinea pig 5-HT_{1B} receptors appear to be a good pharmacological model of human 5-HT_{1B} receptors (Pauwels et al. 1998). 5-HT_{1B} receptors also appear to act as heteroceptors, being present in terminal regions of non serotonergic neurones, and influencing neurotransmitter release. 5-HT_{1B} ligands have been shown to affect cholinergic (Cassel et al. 1995), dopaminergic (Iyer and Bradberry 1996), glutamatergic (Boeijinga and Boddeka 1996) and GABAergic function (Johnson et al. 1992).

Behavioural and physiological effects of 5-HT_{1B} ligands in animal models include stimulation of locomotion, hypophagia, hypothermia, penile erection, and corticosterone and PRL secretion (Middlemiss and Hutson 1990). A 5-HT_{1B} knockout mouse has been

developed (Saudou et al. 1994) which has increased aggression and an absent locomotor response to 5-HT_{1B} agonists. The greatest drive in the development of 5-$HT_{1B/D}$ ligands is currently for the treatment of migraine. The mechanism of action against migraine relates to controlling blood vessel dilation and protein extravasation. (Deleu and Hanssens 2000).

5-HT_{1D} receptor

The discovery and nomenclature problems of 5-HT_{1D} receptors are described above. The 5-HT_{1D} gene is located on chromosome 1.

The brain location of 5-HT_{1D} receptors has been investigated by several groups. Castro et al. (1997) examined radiolabelled sumatriptan binding that is inhibited by ketanserin in human brains, indicating the presence of 5-HT_{1D} receptors in globus pallidus, substantia nigra, periaqeductal grey and spinal cord. Bonaventure et al. 1997 and Varnas et al. 2001 also examined 5-HT_{1D} receptor distribution in human postmortem brain using radiolabelled 5-$HT_{1B/1D}$ receptor antagonists (alniditan and GR 125743 respectively). They compared this 5-$HT_{1B/1D}$ binding in the presence and absence of ketanserin to more selectively inhibit 5-HT_{1D} binding and SB 224289 to inhibit 5-HT_{1B} binding (although these are not highly selective ligands). They concluded that the numbers of 5-HT_{1D} receptors in human brain were low (in comparison to 5-HT_{1B} receptors) and were localised primarily in the basal ganglia (more specifically in the ventral pallidum). 5-HT_{1D} mRNA has been identified in rat brain at low levels in areas such as caudate,

putamen, accumbens, dorsal raphe and locus coeruleus, but not in globus pallidus, or substantia nigra (Brunivels et al. 1994). This may indicate the presence of 5-HT_{1D} receptors in non serotonergic neurones.

The pharmacology of 5-HT_{1D} receptors is very similar to that of 5-HT_{1B} receptors due to the high receptor structure homology. Several drugs have been developed with high specificity for 5-$HT_{1B/D}$ receptors, such as GR 127935. The most useful distinguishing tools are ketanserin and ritanserin which both have a greater affinity for 5-HT_{1D} receptors (Pauwels et al. 1996).

Concerning function, it is possible that 5-HT_{1D} receptors have a role as autoreceptors on serotonergic and non serotonergic neurones, as implicated by their location (see above). Pineyro et al. (1995) demonstrated that 5-HT_{1D} receptor stimulation in rat dorsal raphe, in vitro and in vivo, negatively modulated the somatodendritic release of 5-HT.
Other 5-HT_{1D} receptors are located postsynaptically and may act as heteroreceptors, controlling, for example, the release of glutamate. This thesis will comment further on 5-HT_{1D} function in Part 2.

5-ht_{1E} receptor

This receptor was originally defined pharmacologically, with significant affinity for [^{3}H] 5-HT, no affinity for other known 5-HT_1 subtype antagonists and low affinity for 5-CT (Leonhardt et al. 1989), although we now know that 5-ht_{1F} and 5-ht_6 receptor

subtypes share these properties. The human gene encoding the 5-ht_{1E} receptor was subsequently identified (Zgombick et al. 1992) and is located on chromosome 6 (Levy et al. 1992).

No selective 5-ht_{1E} receptor ligands are available, so location of these receptors is unclear. 5-ht_{1E} mRNA is present predominately in cortical areas, caudate and putamen and detectable in amygdala and hypothalamus (Bruinvels et al. 1994).

Pharmacologically, 5-ht_{1E} receptors have most in common with 5-$HT_{1B,}$ 5-HT_{1D} and 5-ht_{1F} receptors. 5-ht_{1E} are distinguished from 5-ht_{1F} by the latter having a greater affinity for sumatriptan. The function of this receptor is not yet known.

5-ht_{1F} receptor

The 5-ht_{1F} receptor was discovered following identification of a mouse gene sequence with homology to the 5-$HT_{1B/D}$ receptor genes. Identification of the human gene followed soon after (Adham et al. 1993). This gene is located on chromosome 3 (Saudou and Hen 1994).

The location of 5-ht_{1F} mRNA is clearly different to 5-ht_{1E} mRNA. Using in situ hybridisation, 5-ht_{1F} mRNA is found in guinea pig cortex, hippocampus and to a lesser extent in the dorsal raphe nucleus (Brunivels et al. 1994). Autoradiography using radio labelled sumatriptan and 5-CT in the human brain has shown 5-ht_{1F} receptor sites (with

no displaced sumatriptan binding) that correlate highly with guinea pig 5-ht_{1F} mRNA location (Pascual et al. 1996).

Pharmacologically, 5-ht_{1F} receptors have high affinity for 5-HT and sumatriptan, but low affinity for 5-CT. Selective ligands are being developed, including LY344864 (Phebus et al. 1997) and LY334370 (Johnson et al. 1997).

The function of 5-ht_{1F} receptors is unclear. From their location, it could be suggested that they may be involved as 5-HT autoreceptors and in visual and cognitive function. The selective agonist LY334370 does not evoke behavioural changes but does influence dural protein extravasation, possibly having a role in migraine treatment (Johnson et al. 1997). Several of the triptans used as acute antimigraine treatments have a high affinity for both the 5-HT_{1D} and the 5-ht_{1F} receptor

5-HT_2 FAMILY

The 5-HT_2 receptor family now has 3 members, 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C}, which have a high degree of structural homology, are coupled positively to inositol phosphate and mobilise intracellular calcium. The 5-HT_2 receptor described by Peroutka and Snyder with a high affinity for spiperone is now known as the 5-HT_{2A} receptor. The 5-HT_{1C} receptor was renamed the 5-HT_{2C} receptor, and the 5-$HT_{2\text{-like}}$ receptor (located in the stomach) as 5-HT_{2B}. Pharmacologically, this family have a relatively low affinity for

5-HT, and a high affinity for the 5-HT_2 agonists DOI, DOB and DOM and antagonists such as ritanserin. mCPP and TFMPP are non selective, less potent 5-HT_2 agonists. 5-HT_2 receptors mediate several behavioural and physiological responses, such as increased motor activity, hyperthermia, head twitches and 'wet dog shakes' (for review see Koek et al. 1992). The mediation of certain neuropsychological processes is also likely and is discussed.

5-HT_{2A} receptor

The human 5-HT_{2A} receptor gene is located on chromosome 13. The CNS distribution has been identified as predominately cortical areas, caudate nucleus, nucleus accumbens, olfactory tubercle, hippocampus and forebrain (Pazos et al. 1985). 5-HT_{2A} receptors are located postsynaptically to serotonergic neurones. The distribution of these postsynaptic sites is identical to the brain innervations of neurones from the dorsal raphe nucleus (Blue et al. 1988), although electrophysiological stimulation of the median raphe nucleus also induces 5-HT_{2A} receptor mediated responses (Godbout et al. 1991). 5-HT_{2A} receptors are also located extraneuronally in glial cells. Other neurotransmitter systems, such as GABAergic, glutamatergic and cholinergic neurones have been confirmed to contain 5-HT_{2A} heteroreceptors (reviewed by Barnes and Sharp 1999).

Pharmacologically, there have been problems distinguishing 5-HT_2 receptor subtypes, until the recent introduction of more selective antagonists. Ketanserin and spiperone have a greater affinity for 5-HT_{2A} over 5-HT_{2B} and 5-HT_{2C}. Antagonists such as MDL100907

distinguish 5-HT_{2A}, but no selective agonists are available.

Functionally, 5-HT_{2A} stimulation has been shown to activate specific gene expression including brain derived neurotrophic factor (BDNF) (Vaidya et al. 1997). There may be an association between the increased expression of BDNF seen during antidepressant treatment, 5-HT_{2A} activity, and antidepressant efficacy (further discussed in chapter 8). Using electrophysiology techniques in rats, 5-HT_{2A} receptors have been shown to mediate cortical excitation (Marek and Aghajanian 1994). Electrophysiology has also demonstrated that 5-HT_{2A} stimulation inhibits noradrenergic neurotransmission at the level of the locus coeruleus; this has been confirmed by microdialysis (Done and Sharp 1992). Behaviourally, 'head twitches' and 'wet dog shakes' in rats induced by non specific 5-HT_2 ligands, 5-HT releasing drugs and 5-HT precursors, are mediated by 5-HT_{2A} receptors (blocked by selective antagonists, such as MDL 100907, Schreiber et al. 1995). Hyperthermia induced by 5-HT_2 ligands is probably mediated by 5-HT_{2A} receptors (Gudelsky et al. 1986).

Drugs acting via serotonergic systems to induce hallucinations have a mechanism of action probably involving activation of 5-HT_2 receptors (reviewed by Glennon 1990). This is likely to be mediated predominantly by 5-HT_{2A} receptors, as mCPP (acting predominately at 5-HT_{2C} receptors) is not hallucinogenic in humans. Several antipsychotic medications (such as clozapine and risperidone) have a high affinity for 5-HT_{2A} receptors (reviewed by Leysen et al. 1993). There has obviously been much interest in developing more selective 5-HT_{2A} receptor ligands for the treatment of

schizophrenia.

5-HT_{2B} receptor

The 5-HT_{2B} receptor (as it is now known) was originally identified in the rat stomach fundus. The human 5-HT_{2B} receptor gene is located on chromosome 2, with DNA and receptor structure very similar to the other 5-HT_2 receptors. Low levels of 5-HT_{2B} mRNA have been found in human brain (Bonhaus et al. 1995), with no clear localisation. Drugs such as spiperone and ketanserin have a lower affinity and yohimbine a higher affinity for 5-HT_{2B} receptors in comparison to other 5-HT_2 receptors. SB204 741 has been described as a selective 5-HT_{2B} receptor antagonist.

Functionally, 5-HT_{2B} receptors have been shown to mediate the action of 5-HT on embryonic neuronal morphogenesis in mice, by using 5-HT_{2B} antagonists in early stages of cell division (Choi et al. 1997). 5-HT_{2B} receptors may also mediate anxiety, as shown by anxiogenesis in response to the 5-HT_{2B} agonist BW 723C86 (Kennet et al. 1996) and anxiolysis in response to the 5-$HT_{2B/2C}$ antagonist SB200646A (Kennet et al. 1995), in rats.

5-HT_{2C} receptor

This receptor was initially grouped with the 5-HT_1 receptors due to its high affinity for [^{3}H] 5-HT but following cloning, and further investigation, a reclassification as 5-HT_{2C}

was necessary (Humphrey et al. 1993). The 5-HT_{2C} receptor is X linked and is a G protein coupled receptor. A splice variant of this receptor has been identified but the resulting protein does not possess a 5-HT binding site. Several receptor isoforms have been identified, created by post transcriptional editing of receptor mRNA, with different brain distributions (Burns et al. 1997). This may indicate further functional subdivision of the 5-HT_{2C} receptor.

5-HT_{2C} receptors have high density in the choroid plexus and are found widely in the cortex, limbic system and basal ganglia (reviewed by Barnes and Sharp 1999). 5-HT_{2C} receptors do not appear to exist outside the CNS. There is good concordance between 5-HT_{2C} receptor mRNA distribution and 5-HT_{2C} binding sites (Mengod et al. 1990).

The 5-HT_{2C} receptor can be labelled with [^{3}H] 5-HT, [^{3}H] mesulergine, [^{3}H] LSD but not [^{3}H] ketanserin. Other 5-HT_2 agonists such as DOI and mCPP and antagonists such as ritanserin, LY 53857 and mianserin, non specifically bind to 5-HT_{2C} receptors. Two ligands described by Martin et al. (1998), including (S)-2-(chloro-5-fluoro-indol-1-yl)-1-methylethylamine 1:1 $C_4H_4O_4$, exhibit high affinity for the 5-HT_{2C} receptor, with much lower affinity for other 5-HT receptors. Antagonists such as SB242 084 also specifically distinguish 5-HT_{2C} (reviewed by Barnes and Sharp 1999).

Functionally, 5-HT_{2C} receptors in the choroid plexus may regulate CSF production (Kaufman et al. 1995). In rats, 5-HT_{2C} receptors appear to mediate hypolocomotion,

hypophagia, anxiety, penile erection, and hyperthermia (Koek et al. 1992). In humans, regular administration of mCPP has been shown to reduce body weight (Sargent et al. 1997a). Blockade of 5-HT_{2C} receptors appears to increase slow wave sleep (Sharpley et al. 1994). The PRL response to mCPP administration appears to be mediated by 5-HT_{2C} receptors (see below, Cowen et al. 1996). In several animal models, 5-HT_{2C} receptor agonists have activity in models of compulsive behaviour (Martin et al. 1998), and antagonists have anxiolytic properties (Kennet et al. 1997). 5-HT_{2C} antagonists have been shown to increase cortical NA and DA in animal models, suggesting 5-HT_{2C} receptors tonically inhibit neuronal firing in these systems (Di Mateo et al. 1998; Millan et al. 1998).

Several polymorphisms of the human 5-HT_{2C} receptor have been identified. Reynolds et al. (2002) identified an association between a polymorphism in the promotor region of the 5-HT_{2C} receptor gene and atypical antipsychotic induced weight gain. An association between 5-HT_{2C} polymorphic variation and response to clozapine in schizophrenic subjects has also been described (Sodhi et al. 1995).

5-HT_3 FAMILY

5-HT_3 receptors are the only serotonergic receptors that are not G protein linked. Instead, this receptor is a ligand gated ion channel. The 5-HT_3 receptor gene has been identified on chromosome 11. Two 5-HT_3 receptor subunits have been identified (nominally 5-HT_{3A} and 5-HT_{3B}), with receptors having the structure 5-HT_{3A} alone or 5-HT_{3A}/ 5-HT_{3B}

combined. The 5-HT_{3B} subunit expressed alone does not form a functional receptor (Davies et al. 1999).

5-HT_3 receptors have their greatest density in the dorsal vagal complex of the brainstem, but have been located in human forebrain areas such as the hippocampus, amygdala, caudate, putamen and superficial cortex. In the rat cortex and hippocampus, 5-HT_3 receptors are associated with GABAergic neurones. In humans, 5-HT_3 receptors in the basal ganglia are also probably located on GABAergic neurones as opposed to dopaminergic neurones (reviewed by Barnes and Sharp 1999).

Several 5-HT_3 selective ligands are now available enabling the pharmacology of 5-HT_3 receptors to be more clearly defined. Selective antagonists include ondansetron, granisetron and topisetron. Substances that act at other ligand gated ion channel receptors, such as alcohol, barbiturates and steroids, also act at the 5-HT_3 receptor.

The most important psychiatric functional attributions of the 5-HT_3 receptor are mediation of anxiety, cognition, and possibly psychotic symptoms. Ondansetron is licensed for use as an antiemetic following chemotherapy, radiotherapy or post operatively, an effect probably mediated via the dorsal vagal complex. In animal models, 5-HT_3 antagonists (such as ondansetron) have been shown to reduce and 5-HT_3 agonists to enhance anxiety associated behaviours. The amygdala is thought to mediate this response (Higgins et al. 1991). 5-HT_3 receptor stimulation also enhances release of 5-HT (Martin et al. 1992), which may explain the association with anxiety. Cholecystokinin

(CCK) release is also modulated by 5-HT_3 receptors (Raiteri et al. 1993), which may be a further association of 5-HT_3 with anxiety, as raised CCK induces anxiety in humans. No clinical studies have shown anxiolytic effects of ondansetron to date. 5-HT_3 antagonists have been shown to enhance cognitive function in animal models (for review, Bentley and Barnes 1995). This effect could be explained by enhanced cortical ACh function, an effect indirectly mediated by 5-HT_3 receptors expressed on GABAergic neurones (reviewed by Barnes and Sharp 1999). In animal models using behavioural, neurochemical and electrophysiological paradigms, the 5-HT_3 receptor modulates dopaminergic activity. For example, 5-HT_3 agonists induce release of DA in rat accumbens and striatum in vitro (Blandina et al. 1989). This underpins the excitement about 5-HT_3 antagonists having antipsychotic properties. However, initial randomised trials with ondansetron in schizophrenia were inconclusive. More recent randomised studies have indicated a possible role for ondansetron in decreasing alcohol use in alcohol dependent humans (Sellers et al. 1994); this could be attributable to a link between 5-HT_3 receptors and DA mediated reinforcement processes.

5-HT_4 FAMILY

The 5-HT_4 receptor was identified initially using a functional assay of adenylate cyclase activity in animal brain tissue (Dumuis et al. 1988). 5-HT_4 receptor cDNA was discovered by scanning sequences using polymerase chain reaction (PCR) primers based on the structure of other G protein coupled 5-HT receptors with the characteristic 7 putative transmembrane domain. Four isoforms of the 5-HT_4 receptor have now been identified,

nominated the 5-$HT_{4(a)}$, 5-$HT_{4(b)}$, 5-$HT_{4(c)}$ and 5-$HT_{4(d)}$ receptors, resulting from variations in splicing of 5-HT_4 mRNA (with 387, 406, 380 and 360 aminoacids per isoform respectively), (reviewed by Barnes and Sharp 1999). The 5-HT_4 gene is located on chromosome 5.

Brain distribution of 5-HT_4 receptors has been investigated using available selective 5-HT_4 radioligands, indicating high densities in human nigrostriatal and mesolimbic areas. Information concerning distribution of specific isoforms is not available.

The pharmacology of the 5-HT_4 receptor isoforms is very similar. The only known difference is that renazapride acts as a partial agonist at the 5-$HT_{4(c)}$ receptor and a full agonist at the other receptors. Highly selective antagonists, such as GR113808 and SB204070 are available.

Functionally, 5-HT_4 receptors are coupled positively with adenylate cyclase. 5-HT_4 receptors have been shown to modulate the activity of cholinergic, dopaminergic and serotonergic systems. Using microdialysis in rats, frontal cortex acetylcholine release has been shown to increase following administration of selective 5-HT_4 agonists (BIMU1 and BIMU8), and the response is blocked by selective antagonists such as GR113808 (Consolo et al. 1994). DA release in the striatum and substantia nigra is similarly increased by 5-HT_4 agonists and the response is blocked by selective antagonists (Steward et al. 1996; Thorre et al. 1998). 5-HT_4 receptors do not; however, appear to be located on dopaminergic neurones in humans and the changes in DA release are likely to

be mediated by 5-HT_4 receptors located on the terminals of GABAergic neurones. 5-HT release is also enhanced by selective 5-HT_4 receptor agonists (and blocked by antagonists) in hippocampus and substantia nigra (Ge and Barnes 1996; Thorre et al. 1998).

5-HT_4 ligands do not affect overt behaviour in rats, which is surprising in view of their DA releasing properties. Cognition enhancing properties of 5-HT_4 agonists have been demonstrated in rat and primate models. A notable performance improvement in the delayed matching task was demonstrated in older macaque monkeys on RS17017 (Terry et al. 1998). This effect may be attributable to enhanced cortical ACh release. 5-HT_4 receptor agonists may therefore offer a novel means of boosting central cholinergic function and could thereby ameliorate the cholinergic deficit seen in some memory disorders. There are no reports to date on the central effects of cisapride (a brain penetrating 5-HT_4 receptor agonist licensed for use in gastric motility disorders) (Barnes and Sharp 1999). The use of these ligands in humans may be restricted, however, due to peripheral side effects such as abdominal cramps, diarrhoea and ventricular arrhythmias. Several groups have suggested a role of 5-HT_4 receptors in anxiety having found in animal models that selective antagonists (eg. GR113808) block the anxiolytic effects of diazepam (Costall and Naylor 1997) and paradoxically that selective antagonists (eg. GR113808 at higher doses) alone have anxiolytic effects in certain experimental paradigms (Silvestre at al. 1996). This effect may be mediated via 5-HT modulation in the dorsal hippocampus, an area suggested being involved with rat social anxiety (Andrews et al. 1994).

5-ht_5 FAMILY

The 5-ht_5 receptor was identified from DNA sequence screening, with two subtypes identified in rat brain (5-ht_{5A} and 5-ht_{5B}) (reviewed by Barnes and Sharp 1999). To date, only 5-ht_{5A} expression has been identified in humans, and there is no evidence of functional 5-ht_5 receptors in vivo. The structure of 5-ht_5 receptors is unclear. The 5-ht_{5A} gene is on human chromosome 7 and 5-ht_{5B} on chromosome 2. The receptors are probably G protein linked.

The brain distribution of 5-ht_{5A} receptor mRNA is widespread. Focal areas of 5-ht_{5A} mRNA in human forebrain and cerebellum have been described. No 5-ht_{5B} mRNA has been found in human brains.

Pharmacologically, 5-ht_5 receptors behave similarly to 5-HT_{1D} receptors, with relatively high affinity for 5-CT, LSD, ergotamine, methiothepin and sumatriptan. The transduction system associated with 5-ht_5 receptors is unclear and their functional significance unknown.

5-HT_6 FAMILY

The 5-HT_6 receptor was identified from DNA sequence screening (reviewed by Barnes and Sharp 1999). No functional gene variants (receptor subtypes) have been identified.

This is similarly a G protein coupled receptor with 440 amino acids and 7 membrane spanning regions. The 5-HT_6 receptor gene is located on chromosome 1.

5-HT_6 receptor mRNA is found in the human striatum, olfactory tubercles, nucleus accumbens and hippocampus. Studies with radioligands for the 5-HT_6 receptor have not been fruitful due to diffuse, non specific, low levels of binding. Using radiolabelled receptor antibodies, receptors have been found in the same distribution as 5-HT_6 mRNA. Electron microscopy studies indicate that hippocampal and striatal 5-HT_6 receptors are postsynaptic to 5-HT neurones. 5-HT_6 receptors have also been identified on dendritic processes of GABAergic neurones terminating in the basal ganglia.

The pharmacology of 5-HT_6 receptors is in its infancy, with only recently the identification of two selective antagonists (Sleight et al. 1998). Interestingly, several antidepressant and antipsychotic drugs (including clozapine) interact with this receptor, and may have therapeutic actions or adverse effects mediated by 5-HT_6 (Roth et al. 1994). Functionally, 5-HT_6 receptors are positively coupled to adenylate cyclase. Due to a lack of selective ligands, 5-HT_6 mediated responses have not been clearly characterised. An interaction between 5-HT_6 receptors and ACh systems has been demonstrated in rats (Bourson et al. 1998). Yau et al. (1997) demonstrated that 5-HT_6 mRNA expression (along with 5-HT_1 mRNA and 5-HT_7 mRNA) is increased in specific hippocampal areas (CA1) following pharmacological adrenalectomy. This may imply an inhibitory influence of corticosteroids on 5-HT_6, 5-HT_1 and 5-HT_7 expression, which may be enhanced in depressive disorder with associated hypercortisolaemia (as described above). This is a

further interesting insight into the associations between CORT and monoamine changes observed in depressive disorders.

5-HT_7 FAMILY

The 5-HT_7 receptor is the most recent 5-HT receptor to be identified (reviewed by Barnes and Sharp 1999). The 5-HT_7 gene resides on chromosome 10 and contains two introns. Although 4 gene variants have been identified in total, only three appear to be expressed in human tissue: 5-HT_{7a}, 5-HT_{7b} and 5-HT_{7d}. This G protein coupled receptor group predictably has amino acid sequences with seven membrane spanning regions. In the rat and guinea pig, 5-HT_7 receptors are located predominately in the thalamus, hypothalamus and hippocampus. Lower densities are found in cortex and amygdala. Receptor subgroups do not appear to have different distributions.

The pharmacology of this receptor is still to be explicitly defined but one selective antagonist has recently been reported. 8-OH-DPAT at high concentrations is a 5-HT_7 agonist, which has caused some confusion about whether some of the effects previously attributed to 5-HT_{1A} receptor activation are in fact due to stimulation of 5-HT_7 receptors. Several psychotropic drugs, such as antidepressants (fluoxetine) and antipsychotics (including clozapine) appear to bind to 5-HT_7 receptors (Roth et al. 1994), highlighting the need for further investigation into this receptor group. No pharmacological differences have been found between subtypes.

Functionally, 5-HT_7 receptors are positively linked with adenyl cyclase, and appear to induce an increase in intracellular Ca^{2+}. Several authors have described a role of 5-HT_7 receptors in the regulation of circadian rhythms (agonists inducing phase shifts), and suprachiasmatic nucleus activity (Lovenberg et al. 1993). Indeed 5-HT_7 mRNA is found in the suprachiasmatic nucleus. Bourson et al. (1997) have suggested a role of 5-HT_7 ligands in seizure control.

Table 1.1 5-HT receptors and selective agonists / antagonists

RECEPTOR	SELECTIVE AGONIST	SELECTIVE ANTAGONIST
$5\text{-}HT_{1A}$	Ipsapirone Gepirone Tandospirone	WAY-100635 Pindolol Penbutolol Tertatolol
$5\text{-}HT_{1B/1D}$	Methiothepin Sumatriptan Naratriptan Rizatriptan Zolmitriptan	GR127935 SB224289
$5\text{-}ht_{1E}$	-	-
$5\text{-}ht_{1F}$	LY344864 LY344370	-
$5\text{-}HT_{2A}$	-	SB200646A
$5\text{-}HT_{2B}$	-	SB204741
$5\text{-}HT_{2C}$	(S)-2-(chloro-5-fluoro-indol-1-yl)-1-methylethylamine 1:1 - $C_4H_4O_4$	SB242084
$5\text{-}HT_3$	-	Ondansetron Granisetron Topisetron
$5\text{-}HT_4$	Renazepride BIMU1 BIMU8	GR113808 SB204070
$5\text{-}ht_5$	-	-
$5\text{-}HT_6$	-	-
$5\text{-}HT_7$	-	-

5-HT FUNCTION AND DEPRESSION

Following the detection of 5-HT in brain extracts in 1953 (Twarong 1988), the interest in the involvement of 5-HT in brain disorders has flourished. Several drugs with established effects on mood and behaviour were later found to influence serotonergic neurotransmission, such as LSD, psilocybin, and bufotenine (McBride 2000).

At an early stage of investigation, the main breakdown product of 5-HT, 5-HIAA was found to be reduced in the CSF of depressed subjects (Ashcroft 1966). In the presence of probenecid, which blocks transport from the CSF, the rate of CSF 5-HIAA accumulation was also reduced in this group (van Praag 1977). Overall, more recent studies have not supported a difference in CSF 5-HIAA between depressed subjects and controls (Asberg 1984, Gjerris et al. 1987). Potential biases in the early studies included concurrent antidepressant use, amount of CSF drawn and the control group (Asberg 1984). 5-HIAA produced from spinal cord as opposed to brain tissue may also bias these findings. A consistent finding however has been that of reduced CSF 5-HIAA in suicide attempters. Asberg et al. (1976) studied CSF 5-HIAA in a group of depressed patients, finding a bimodal distribution of 5-HIAA levels, with subjects in the lower mode attempting suicide significantly more often than those in the high mode, and using more violent means. A further study of suicide attempters by violent means suggested that the lower CSF 5-HIAA levels in this group were associated entirely with high impulsivity scores (Cremniter et al. 1999).

Several early studies reported reduced 5-HT uptake sites, labelled with [^{3}H] imipramine, on blood platelets in untreated depressed patients (as reviewed by Elliott 1991). More recent, larger studies however have not shown such differences between depressed patients and controls (eg Healy et al. 1990; Mellerup and Langer 1991). This between study variation may be due to patient selection and biochemical methods and is further complicated by [^{3}H] imipramine not solely binding to 5-HT uptake sites (Hrdina 1984). Lawrence et al. (1994) examined platelet binding of [^{3}H] paroxetine, which appears to only bind to 5-HT uptake sites, also finding no difference between depressed subjects and controls. Many studies have examined the platelet 5-HT_{2A} receptor which mediates 5-HT activation of the platelet (reviewed by Mendelson 2000). Although study findings are inconsistent, 5-HT_{2A} receptor density appears to increase in depressive disorders with the clearest increase in those attempting suicide. Studies have examined platelet 5-HT_{2A} receptor density pre and post SSRI treatment, overall with no difference observed (eg. Hrdina et al. 1997). It remains unclear whether these platelet sites have functional similarities to brain uptake sites.

Postmortem studies initially demonstrated low brainstem 5-HT and diminished [^{3}H] imipramine binding in the brains of non psychiatrically defined suicide victims (Stanley 1982). Subsequent studies in postmortem brains of previously depressed suicides have shown a wide variation of results, with decreases, increases and no difference in [^{3}H] imipramine binding reported. Lawrence et al. (1998) demonstrated no overall difference in [^{3}H] imipramine binding between retrospectively diagnosed depressed brains and controls, but the group of subjects who undertook non violent suicide had

significantly less binding in the putamen than matched controls. This finding remained consistent when specific 5-HT uptake was examined (ie. Na^+ dependent [^{3}H] imipramine binding). Most studies examining [^{3}H] paroxetine binding in this previously depressed group have also shown no difference from controls. One group, primarily investigating schizophrenia, found significantly less [^{3}H] paroxetine binding in the prefrontal cortex of non psychotic suicides (Laurelle et al. 1993).

Investigation of 5-HT receptor subtype binding in previously depressed postmortem samples has also been undertaken. Lowther et al. (1997a) examined 5-HT_{1A} receptor binding with [^{3}H] 8-OH-DPAT, finding no difference between depressed suicides and controls (and no significant findings in sub analysis of method of suicide or treatment). This group also examined 5-HT_{2A} binding using [^{3}H] ketanserin, similarly with no difference between diagnosed groups (Lowther et al. 1994), a finding replicated by Stockmeier et al. (1997). Some previous studies of 5-HT_2 binding suggested higher binding in frontal and prefrontal cortex of depressed suicides, although subject numbers were smaller (eg. Arora and Meltzer 1989). Studies comparing treated with untreated depressed subjects showed a reduced 5-HT_2 binding in the treated group (equal to control levels); suggesting treatment may decrease 5-HT_2 receptor density in depressed subjects (eg. Yates et al. 1990). Prefrontal 5-HT_{2A} binding does appear higher in suicide victims irrespective of diagnosis, consistent with the platelet findings (eg Turecki et al. 1999). Lowther et al. (1997b) used [^{3}H] 5-HT with specific antagonists to examine 5-$HT_{1B/D}$ and 5-$HT_{1E/F}$ binding in suicides with retrospective depression diagnoses. The significant finding was of greater 5-$HT_{1B/D}$ binding sites in the globus pallidus of antidepressant free

depressed suicides in comparison to controls. This gives further potential evidence to the importance of 5-$HT_{1B/D}$ receptors in the aetiology of depression, which will be explored, further in chapters 4 and 5.

More recently, techniques to investigate the influence of 5-HT in depression using imaging technology have been developed. Progress has been restricted by the availability of appropriate radiotracers for use in vivo, but reports using ligands that bind to 5-HT_{1A} receptors, 5-HT_2 receptors and the 5-HTT are growing.

5-HT_{1A} binding using PET has largely been examined with the ligand [^{11}C] WAY 100635. Sargent et al. (2000) measured [^{11}C] WAY 100635 binding in treated and untreated depressed subjects and healthy controls. Binding was reduced across many brain regions, including frontal, temporal, and limbic cortex in all depressed patients compared with healthy volunteers. This finding replicated that of Drevets et al. 1999, which is significant in such a field of conflicting findings. Binding potential values in the Sargent study were similar in treated and untreated subjects suggesting no change in 5-HT_{1A} binding with SSRI treatment. This group further examined the ability of pindolol to bind to 5-HT_{1A} receptors in depressed subjects (used therapeutically as described above), finding that the commonly used dose of pindolol 2.5mg three times daily did not achieve significant 5-HT_{1A} binding but the higher dose of 5mg three times daily did (Rabiner et al. 2001). Pindolol appeared to bind preferentially to the presynaptic 5-HT_{1A} receptor. This is a good example of how PET can inform clinical practice.

The first studies looking at 5-HT_2 binding in depressed subjects used unspecific ligands and SPECT so findings such as higher parietal binding (D'Haenen et al. 1992) must be treated with caution. [^{18}F] altanserin, a more specific (but rapidly metabolised) 5-HT_2 ligand and PET was used by Biver et al. (1997) in unipolar drug free depressed subjects showing decreased binding in right posterolateral, orbitofrontal and anterior insular cortex in comparison to controls. Setoperone has been the most widely used 5-HT_2 ligand as it has high affinity and specificity for cortical 5-HT_{2A} receptors, with 100 fold preference over 5-HT_{2C} receptors and 10 to 50 times preference over D_2 receptors (which have minimal presence in the cortex) (Meyer et al. 2001a). Massou et al. (1997) using PET demonstrated an increase in [^{18}F] setoperone binding in the frontal cortex when comparing chronic SSRI treated depressed patients to untreated patients (not a within subject design), suggesting chronic treatment causes 5-HT_2 receptor up regulation, which may contribute to the therapeutic effect. Yatham et al. (1999) examined the effects of desipramine on 5-HT_2 receptors in depressed subjects using PET and [^{18}F] setoperone, finding a significant decrease in 5-HT_2 receptor binding following treatment in several brain areas, particularly frontal cortex. This is consistent with other studies indicating postsynaptic 5-HT receptor downregulation following treatment as elaborated later in this thesis. Meyer et al. (2001a) examined [^{18}F] setoperone binding in depressed subjects pre and post SSRI treatment, a within subjects design, in comparison to matched controls. No difference in binding was observed between depressed subjects and controls but they found decreased binding in cortical regions of depressed subjects post treatment only in younger subjects, with no association with treatment response. The results of this study may be more valid than the Massou et al. study due to more rigid design. Messa et al.

(2003) examined 5-HT_{2A} receptor binding in depressed subjects and controls using [^{18}F] fluoroethylspiperone, also a D_2 antagonist, by examining the ratio of binding in cortex and basal ganglia. They found a significant reduction in binding ratio in pretreated patients in comparison to controls in several areas including frontal, temporal and cingulate cortices but not the striatum. No difference in binding ratio was observed in another group of successfully treated euthymic patients from controls, suggesting reduced 5-HT_{2A} receptor binding may be a state dependant marker of depression that resolves with treatment. This would need to be clarified in a within subjects design. Yatham et al. (2001) also examined the effects of TRP depletion (as described below) on [^{18}F] setoperone binding in healthy subjects. TRP depletion induced a significant reduction in 5-HT_2 binding in several cortical regions, independent of mood effects in this group, which the authors argue is akin to the adaptive downregulation seen following antidepressant treatment.

Malison et al. (1998) first studied the 5-HTT in vivo in drug free depressed subjects using SPECT imaging of [^{123}I] β-CIT binding, a cocaine derivative which binds to the 5-HTT molecule exclusively in the brainstem (but the DA transporter in other brain areas). They showed reduced brainstem binding in depressed subjects in comparison to controls, suggesting that depressed subjects have a reduced density of the 5-HTT at least in the brainstem. It remains unclear whether this is a state or trait phenomenon. Meyer et al. (2001b) examined 5-HTT binding potential in depressed subjects pre and post SSRI treatment using [^{11}C] DASB binding measured with PET. They found a significant decrease in striatal 5-HTT binding following treatment, indicating approximately 80% of

these receptors are occupied by the SSRI.

The methodologies and specificity of ligands involved in these scanning experiments are still being developed, which may explain the conflicting results found so far. The variation of findings between postmortem and in vivo scanning studies could be explained by many factors including problems with retrospective diagnosis of depression in postmortem samples and that the neurochemical aetiology of suicide may be different from depression. Further clarification of the findings discussed is required and may only happen with improving technology.

The use of serotonergic drugs as effective antidepressants is discussed above. L-TRP and 5-hydroxytryptophan (5-HTP) are both precursors to 5-HT. As the brain capacity to produce 5-HT is limited by precursor availability (see above), the addition of these precursors induces a greater synthesis of 5-HT, with associated antidepressant effect in milder depressive states (Thompson et al. 1982; Meyers 2000). As brain 5-HT concentration is dependent on plasma levels of TRP, experimentally reducing TRP has been used to examine the effects of reduced serotonergic function. Rapid depletion of plasma TRP is achieved by consuming a protein drink lacking in TRP. This intervention briefly reverses antidepressant response in 67% of recently remitted depressed patients on medication (Delgado et al. 1990). This is only observed in subjects on serotonergic antidepressants, however. Those subjects treated with predominately noradrenergic drugs such as desipramine are largely not affected by TRP depletion (Delgado 1999). This demonstrates the importance of the serotonergic system in maintaining a euthymic mood

in recovered subjects treated with serotonergic drugs. TRP depletion also precipitated clinical depressive symptoms in well, untreated women, vulnerable to major depressive disorder (Smith et al. 1997). The findings support a key role for deficient 5-HT function in the aetiology of depression in this group. Mood response induced by TRP depletion reliably predicted a further major depressive episode in drug free remitted patients during the subsequent year, in a small randomised controlled trial (Moreno et al. 2000). Other factors predisposing to depressive relapse after TRP depletion were more than one previous episode, female gender, and history of suicidal behaviour.

In summary, the best evidence that 5-HT is involved in depressive disorder comes from evidence that drugs that selectively increase 5-HT are antidepressant. However, this is not direct evidence for a role of 5-HT in the pathophysiology of depression. Evidence that 5-HT function is abnormal in untreated patients in the studies so far described is inconsistent, probably because most study paradigms are indirect and models such as postmortem are beset with methodological difficulties. Developments in brain imaging offer promise and the consistent reduction in 5-HT_{1A} binding in depression appears to be an important finding. The extent to which this contributes to the psychopathology of depressive syndrome however is uncertain. This thesis will go on to describe more dynamic tests of 5-HT function which in general yield more consistent changes in depressed patients.

CHAPTER 2

NEUROENDOCRINE STUDIES AND FINDINGS IN DEPRESSED SUBJECTS

INTRODUCTION

Neuroendocrine techniques are a useful method to examine central amine neurotransmission. This experimental paradigm can be used to study differences in neurotransmission in disorders of brain function and the mechanisms of action of centrally active drugs.

The investigation of neuroendocrine abnormalities in depressive disorders followed from the identification of higher rates of depression in endocrine disorders, such as Cushing's disease and thyroid disorders (Sachar 1973). Pituitary hormones were subsequently investigated in basal and stimulated states in depressive disorders and the regulation of pituitary hormones by central amines was more explicitly defined (Wurtman 1970). Neuroendocrine challenge tests rely on this influence of monoamines on the release of anterior pituitary hormones. Serotonergic neurones from the raphe nucleus have direct synaptic contact with cell bodies of secretory neurones in the hypothalamus (Tork 1990). These include cell bodies in the arcuate, infundibular, paraventricular, suprachiasmatic and ventromedial nuclei. Stimulation of these neurones leads to secretion of anterior pituitary releasing hormones, and other substances, from these hypothalamic secretory neurones into the capillaries of the hypothalamic-pituitary portal system to which they project. These releasing hormones and substances pass to the pituitary through the portal vessels and act on secretory cells in the pituitary to influence the release of PRL, GH and ACTH into the blood stream. Drugs which activate the serotonergic system can be used to measure the function of this hypothalamic mediated system by measuring the specific

pattern of hormone release following their administration (Sachar 1973; Wurtman 1970). There are many factors that influence the secretion of these hormones however, that must be taken into account before a direct link can be made between the action of the drug and the subsequent hormone release. The specific mediators of and influences on the release of these hormones will be discussed individually.

Prolactin

The nature of PRL and its regulation is reviewed by Freeman et al. (2000). PRL is released from the pituitary lactotrophs which are widely distributed throughout the pars distalis of the pituitary gland. The normal pituitary holds 100 micrograms of PRL, 50 times less content than GH. Lactotrophs have spontaneously high secretory activity which is regulated by hypothalamic DA and other factors. PRL is the only pituitary hormone to have release inhibited by the hypothalamus, which is mediated by DA produced from tuberoinfundibular DA neurones. These neurones have cell bodies in the arcuate nucleus of the hypothalamus and their short axons end in the median eminence where DA is secreted into the portal capillaries. The subsequent action of DA on the lactotroph is a tonic inhibitory influence, via D_2 receptors. Other inhibitory factors of PRL release include somatostatin and GABA, and stimulatory factors include thyrotrophin releasing hormone (TRH), vasoactive intestinal peptide, oxytocin and neurotensin, all released from the hypothalamus into the portal system. Lactotrophs are also influenced by stimulatory and inhibitory factors from neighbouring cells (paracrine regulation) or from the lactotrophs themselves (autocrine regulation). Normal PRL plasma levels are less than 20 micrograms per litre. PRL is released in pulses, with lowest circulating levels at mid

day, a rapid increase at the onset of sleep and peak levels from the middle to the end of the night. PRL has an inhibiting action on its own secretion, via increasing hypothalamic secretion of DA, so subjects with high baseline PRL should be excluded from neuroendocrine tests as their subsequent PRL release will be inhibited and not reflect a true influence of the drug probe. Release is enhanced by stress, suckling, other nipple stimulation and orgasm.

DA antagonists increase the release of PRL by reducing the tonic inhibitory control at the level of the pituitary. Adrenergic α_2 agonists may inhibit PRL release. In vitro, 5-HT does not cause PRL release from the lactotrophs, suggesting 5-HT purely mediates PRL release via the raphe-hypothalamic neurones. Histaminergic drugs enhance PRL secretion at the level of the hypothalamus, likely via H_2 receptors and subsequent influence on portal DA levels. Opioids, both endogenous and administered, also enhance PRL release. Hyperprolactinaemia is seen in primary hypothyroidism due to raised TRH. Oestrogen enhances PRL release at the levels of both the hypothalamus and pituitary. The least time of oestrogen influence on PRL release is in the early stages of the menstrual cycle (O'Keane et al. 1991) hence neuroendocrine tests should ideally be carried out during this period of the cycle. For within subject comparisons, test days need to be at the same stage of the menstrual cycle. PRL acts on many organs including the liver, ovary, testis and prostate, but has its main role in initiating and maintaining lactation.

Growth hormone

The nature of GH and its regulation is reviewed by Muller et al. (1999). GH is secreted in

a pulsatile manner from the somatotrophs, which occupy the lateral wings of the pituitary gland. GH synthesis and release is stimulated by GH releasing hormone (GHRH), released into the portal circulation. GHRH secreting neurones have cell bodies in the infundibular nucleus which project to the median eminence, in close contact with portal vessels. GHRH neurone cell bodies are also present in other medial nuclei and project to other parts of the hypothalamus and sparsely to other brain regions. Other endogenous GH releasing peptides include TRH and gonadotrophin releasing hormone (GnRH). Somatostatin inhibits GH release, and is particularly important in the timing and magnitude of GH pulses, but has no effect on synthesis. Cell bodies of somatostatin secreting neurones are present in the ventromedial nucleus of the hypothalamus. GH inhibits its own release by action at the level of the hypothalamus, so as for PRL, subjects with high baseline GH should be excluded from neuroendocrine tests. GH release is also inhibited at the level of the pituitary by insulin like growth factor. The amount of GH secreted is greatest during adolescence and decreases with age. Females on the whole have greater GH secretion than males.

GH secretion is enhanced by slow wave sleep, chronic hyperglycaemia (diabetes), hypoglycaemia (starvation or anorexia), hepatic cirrhosis, exercise and stress. Certain amino acids, particularly arginine and leucine enhance release. Clonidine (and other α adrenergic agonists), DA agonists, ACh agonists and glucagon also stimulate release. Adrenergic β receptor antagonists augment GH stimulation, but have no effect alone. Many of these GH responses are blocked by muscarinic antagonists. GH release is

reduced by acute hyperglycaemia, acute increase in plasma free fatty acids, obesity and emotional deprivation in children. All of these influencing factors appear to act at the level of the hypothalamus. Opioids, both endogenous and administered, do not appear to influence GH release. Oestrogen augments GH responses, and as for PRL, neuroendocrine tests in females should be undertaken in the early stages of the menstrual cycle (O'Keane et al. 1992). CORT inhibits the release of GH, so particularly when measuring GH release in depressed subjects it is important to concurrently measure baseline CORT levels, because depressed patients may have hypercortisolaemia. In rats 5-HT has a stimulatory effect on GH release but in humans, 5-HT altering drug probes have a more varied influence, as covered later in this chapter. Histamine antagonists overall have an inhibitory influence over stimulated GH secretion. Depressive disorder itself does not appear to influence GH secretion as the GH response to GnRH (Amsterdam et al. 1982) and buspirone in melancholic subjects (Cowen et al. 1994) is not different from healthy controls. Physiologically GH enhances cell growth and influences carbohydrate and lipid utilisation in the body.

Adrenocorticotrophic hormone and cortisol

The nature of ACTH and CORT and their release is reviewed by Holsboer and Barden (1996). ACTH is released in a pulsatile manner from the pituitary coticotrophs into the blood stream. The synthesis and release of ACTH is controlled by corticotrophin releasing hormone (CRH), released into the portal system by hypothalamic neurones with cell bodies in the paraventricular nucleus. 5-HT neurones from the raphe have been shown to synapse directly onto these CRH containing cells. At least 2 rhythms of ACTH

release operate with pulses of secretion every 1-3 hours and at meal times, superimposed on a 24 hour pattern with lowest levels following sleep onset and highest levels just before waking. Physical or mental stress will also initiate ACTH secretion. ACTH released into the blood stream subsequently acts on the adrenal gland to release CORT (particularly from the adrenal zona fasciculata) into the blood stream. Plasma CORT levels therefore follow the pattern of ACTH secretion. CORT is partially bound in plasma to a specific binding protein and only the unbound fraction is active. CORT inhibits release of ACTH at the levels of the hypothalamus and pituitary and also via the hippocampus.

Release of ACTH is stimulated by hypoglycaemia. Histamine, NA and ACh enhance and GABA inhibits ACTH release. Serotonergic drugs enhance ACTH release which is reviewed below. ACTH levels vary throughout the menstrual cycle (Genazzani et al. 1975). As discussed above, in some depressed subjects the HPA appears hypersensitised and ACTH and CORT levels are increased. Replicated studies have shown an enlargement of adrenal glands in depressive disorders which resolves on treatment (Rubin et al. 1995). Plasma CORT is easier to measure than ACTH, so many studies use CORT as an endocrine measure of central 5-HT activity although this may not be as accurate, as peripheral 5-HT may directly influence the adrenal gland to release CORT. Physiologically ACTH acutely enhances the conversion of cholesterol to pregnenolone, it enhances synthesis and release of CORT, and maintains adrenal growth. ACTH also stimulates skin melanocytes. CORT has a wide ranging influence, including a catabolic effect, promotion of gluconeogenesis, control of water metabolism and modulation of

responses to injury and stress.

The posterior pituitary hormones, oxytocin and vasopressin are synthesised in the cell bodies of neurones in the supraoptic and paraventricular nuclei of the hypothalamus. They are directly transported along axons for release from the pituitary. Investigators have examined oxytocin in neuroendocrine paradigms but on the whole the response is less reliable than those of the anterior pituitary hormones. For example Cleare et al. (1998a) found the oxytocin response to ipsapirone was not robust and had significant baseline variation.

Weight loss has a significant influence on neuroendocrine responses, and is obviously a common feature of depressive disorders, particularly with melancholia (see appendix). Goodwin et al. (1987) demonstrated that recent weight loss enhanced the PRL response to L-TRP. In a group of low calorie dieters, Anderson et al. (1990b) also demonstrated an enhanced PRL response to L-TRP but only in female subjects, despite a similar percentage weight loss in both sexes. Female subjects showed a greater reduction in plasma TRP in this study.

Physical illness and pregnancy also influence hormone secretion. Aside from the stressful influence of physical disorders, other direct action on neuroendocrine systems may occur. Recently Muldoon et al. (2004) found a reduced PRL response to fenfluramine in a

sample of patients with metabolic syndrome. A low PRL response was associated with greater body mass index, higher concentrations of triglycerides, glucose, and insulin, higher systolic and diastolic blood pressure, greater insulin resistance, and less physical activity.

Recent psychoactive drug exposure also has an important influence on neuroendocrine challenge outcome. Ensuring a long enough time period for any drug to be totally cleared from the subject's system is essential to exclude any effect. The influence of drugs on system reactivity may last for longer than the drug is present in the plasma however. Gilmore et al. (1993) demonstrated that if healthy subjects are rechallenged with clomipramine following 2 weeks, a blunting of the PRL response is observed, which does not occur if the rechallenge is at 4 weeks, indicating that rechallenges should be at least 4 weeks apart.

Season of the year may affect responses. Cappiello et al. (1996) demonstrated a seasonal variation in the PRL response to L-TRP in depressed subjects, particularly females. Rechallenging subjects several months after the first test may therefore give skewed findings.

To validly interpret the hormonal response to a neuroendocrine probe as due to its activity on that system, it is therefore important to minimise any other influence on hormone release. Hence combining the factors discussed above, subjects should be rested, not stressed, not sleeping or eating, not taking any medications or smoking during the trials,

be physically well and not had recent weight loss. Females should be tested in the early phase of their menstrual cycle. These findings also highlight the importance of matching control subjects for age, sex and weight.

The other problematic issue is the selectivity of the neuroendocrine probe for the 5-HT system, to exclude influence of activity at other receptors. Many available drugs do not have such selectivity. As new, more specifically acting drugs are developed, more effective drug probes will become available. At present, it is often unclear exactly how, and at what anatomical level, drugs influence the release of hormones. When comparing neuroendocrine responses between different groups (as opposed to within subject comparisons), pharmacokinetic variables become important, again highlighting the need for accurate control matching for age, weight and sex at the very least. Some patient groups could also have reduced absorption rates of the drug used following oral administration (overcome by using an IV preparation), and could also theoretically have reduced brain uptake. Whilst stress can be minimised through environmental manipulations, the influence of the drug itself may be stress inducing for the subject, for example by causing nausea and dizziness, further biasing hormonal responses. Probes should therefore be well tolerated (Cowen 1998).

An ideal neuroendocrine probe of 5-HT function therefore has the following characteristics (Yatham and Steiner 1993):

1. A large, robust and consistent hormone response.
2. A dose dependent relationship between the drug and the hormone response

3. Hormone responses are due to a direct effect of the drug on the 5-HT system
4. Hormone responses due to other influences are abolished
5. The probe is well tolerated and does not lead to stressful side effects

The striking benefits of this method of investigation are that it is a functional, dynamic analysis of aminergic activity, minimally invasive, relatively easy to carry out and as well tolerated as the probe used (Delgado and Charney 1991). The most important limitation in human studies is extrapolating changes in neuroendocrine function to changes in aminergic function at other parts of the brain outside the hypothalamus. It must also be acknowledged that the system being tested has many levels and interpretation must take into account possible abnormalities at any one of these levels. There is also a large variability of hormone responses between subjects and small changes in responses could be overlooked. This highlights the importance of ensuring adequate power and statistical interpretation of these studies and replication of results between studies.

Several pharmacological probes have been used to date to examine 5-HT activity in depression, including precursors of 5-HT (L-TRP, 5-HTP), 5-HT releasers (fenfluramine), 5-HT reuptake inhibitors (clomipramine) and direct acting 5-HT receptor agonists (mCPP, gepirone, ipsapirone) and antagonists (ritanserin, ketanserin, metergoline). Not all fulfil the criteria above. These groups of neuroendocrine challenge tests are dealt with specifically in this thesis and are summarised in table 2.1 (on page 115).

Probes of noradrenergic function (clonidine and desipramine) and dopaminergic function (apomorphine and haloperidol) are available, but are not covered in this thesis (see Cowen 1998 for review).

PRECURSORS OF 5-HT

L-Tryptophan

Administration of L-TRP intravenously, in doses above 5g, produces dose related increases in plasma PRL and GH (Cowen et al. 1985). These responses are blocked by pindolol (Smith et al. 1991), which has 5-HT_{1A} antagonist properties (as well as being a β-adrenoreceptor antagonist) (Guan et al. 1992). Selective 5-HT_2 and 5-HT_3 antagonists do not influence these hormonal changes (Cowen et al. 1985). Pretreatment with clomipramine (a 5-HT reuptake inhibitor) further enhances the PRL and GH response to LTP (Anderson and Cowen 1986). These findings imply that although L-TRP would theoretically enhance overall serotonergic function, the endocrine responses are mediated by indirect activation of postsynaptic 5-HT_{1A} receptors.

Dieting has been shown to reduce plasma TRP in women and enhances the plasma PRL response following intravenous L-TRP (Anderson et al. 1990b). It is therefore important to exclude such subjects with recent weight loss when examining neuroendocrine responses to L-TRP.

Several studies have shown a blunting of PRL and GH following TRP infusion in drug free depressed subjects in comparison to normal controls (Cappiello et al. 1996; Cowen and Charig 1987; Deakin et al. 1990; Heninger 1984; Price et al.1985; Price et al. 1991). PRL secretion does not appear to be otherwise impaired in depression as the PRL response to thyrotropin releasing hormone (a direct lactotroph stimulant) (Rubin et al. 1989) and metoclopramide (a DA antagonist) (Anderson and Cowen 1991), are unimpaired. This blunted response to L-TRP in depression appears to be a state dependant abnormality, improving alongside the recovery of depressive symptoms (Upadhyaya et al. 1991). These findings imply a dysfunction of postsynaptic 5-HT_{1A} receptors during the process of a major depressive episode. Whether melancholic subjects have a greater blunting of hormonal response in comparison to non melancholic is unclear (Price et al. 1991).

Such blunting of responses to L-TRP have not been demonstrated in other psychiatric disorders (panic disorder (Charney and Heninger 1986), obsessive compulsive disorder (Fineberg et al. 1994) and bulimia nervosa without depressive symptoms (Brewerton et al. 1992)), so reduced postsynaptic 5-HT_{1A} receptor function may be solely associated with depressive disorders.

Studies to examine the influence of antidepressant medication on the PRL and GH response to L-TRP have also been undertaken. Chronic treatment with desipramine, amitriptyline, clomipramine, fluvoxamine, tranylcypromine, carbamazepine or lithium (reviewed by Price et al 1990b) all enhance the PRL response to L-TRP. This supports the

hypothesis that 5-HT_{1A} receptor function mediates depressive disorders and their resolution. Bupropion, mianserin, trazodone (reviewed by Delgado and Charney 1991), and mirtazepine (Whale et al. 2000) do not appear to alter these hormonal responses, suggesting that these drugs do not enhance serotonergic function (or at least 5-HT_{1A} neurotransmission). These drugs do however have antidepressant properties, suggesting mechanisms involving other 5-HT receptor subtypes or other aminergic mechanisms. Studies with TRP depletion and AMPT would help clarify the influence of these drugs on 5-HT and NA function. Indeed, Delgado et al. (2002) demonstrated that in a group of depressed patients receiving mirtazapine, a partial return of depression in most was seen following tryptophan depletion and AMPT administration (undertaken 1 week apart), implying that both systems are involved in mirtazapine's action .

5-Hydroxytryptophan

5-HTP is the immediate metabolic precursor of 5-HT. The neuroendocrine response to oral 5-HTP has been seen as unreliable and independent of dose (Westenberg et al. 1982). This may be explained by metabolism of 5-HTP into 5-HT both in and outside the CNS and a possible direct adrenal influence of 5-HTP to release CORT (reviewed by Delgado and Charney 1991). Oral 5-HTP has been observed to increase CORT, which is attenuated by the 5-HT_2 antagonist ritanserin (Lee et al. 1991). The unreliable increase of PRL following 5-HTP has been shown to be attenuated by both ritanserin and pindolol (Meltzer and Maes 1994), but pindolol has no effect on CORT. The CORT response therefore appears to be mediated by 5-HT_2 receptors and the PRL response by 5-HT_{1A} receptors and 5-HT_2 receptors.

In untreated depressed subjects, the CORT response to oral 5-HTP is enhanced in comparison to controls (Meltzer et al.1984), which appears to correlate positively with the severity of depression (Meltzer et al.1984b, Maes et al. 1987a). The enhanced CORT response correlated negatively with CSF 5-HIAA levels, and not with other monoamine metabolites, suggesting that this CORT response is CNS mediated (Koyama et al. 1987).

The CORT response to 5-HTP has been shown to be elevated following treatment with the serotonergic antidepressants fluoxetine, clomipramine and paroxetine in depressed patients (Meltzer et al. 1997, Sargent et al. 1998 a and b). This suggests enhanced 5-HT_2 receptor function following these antidepressant treatments. This is unlikely to explain the therapeutic effect of all antidepressants, as TCAs do not demonstrate such effects

(Meltzer et al. 1984c), but may relate to benefits seen in the treatment of other conditions (such as obsessive compulsive disorder) and observed side effects (such as sexual dysfunction).

5-HT RELEASERS

Fenfluramine

Fenfluramine is an amphetamine derivative that reliably induces an increase in PRL, ACTH, and CORT but not GH (Lewis and Sherman 1984; Quattrone et al. 1983). The isomer, d-fenfluramine has specific action as a 5-HT releaser and a 5-HT uptake blocker (unlike the racemic mixture, which also has dopaminergic activity). The PRL response to fenfluramine is dose dependent and blocked by ritanserin and amysergide, but not by pindolol (reviewed by Newman et al. 1998), suggesting this response is mediated by postsynaptic 5-HT_{2A} or 5-HT_{2C} receptors. The active metabolite of fenfluramine, norfenfluramine, may have direct activity at 5-HT_2 receptors in rats and guinea pigs (Mennini et al. 1991), possibly explaining the influence of ritanserin.

Several studies have found a blunted PRL and GH response to fenfluramine in medication free depressed subjects in comparison to normal controls, suggesting an impairment of 5-HT_2 receptor function (eg. Stahl et al. 1993; for review see Power and Cowen 1992). A minority of studies however have not replicated these findings. The CORT response to fenfluramine appears unaltered in most studies (Newman et al. 1998).

The PRL response to fenfluramine is enhanced after TRP infusion (Price et al. 1990b), chronic treatment with lithium (Muhlbauer 1984) and imipramine (Shapira et al. 1989) and possibly after ECT treatment (Shapira et al. 1992). This suggests increased postsynaptic 5-HT_2 receptor function following these antidepressant interventions, and the possibility that specific 5-HT_2 receptor ligands may have antidepressant activity. The PRL response following SSRI treatment in depressed subjects does not appear enhanced however (Kavoussi et al 1999). Fenfluramine has been a very useful neuroendocrine probe, but was withdrawn from use in 1997 due to cardiotoxicity (Newman et al. 1998). This has left a poverty of reliable, available probes.

5-HT REUPTAKE INHIBITORS

Clomipramine

Clomipramine administered intravenously has been widely used as a neuroendocrine challenge. It acts as a potent and selective inhibitor of 5-HT reuptake, but is metabolised to desmethylclomipramine, which significantly blocks NA reuptake. During the period of an intravenous neuroendocrine challenge, however, levels of this metabolite are negligible, giving apparent selectivity to testing the serotonergic system (Golden et al. 1989). Clomipramine increases PRL, ACTH and CORT in healthy subjects and there is no consistent GH response (Golden et al. 1989, 1990). The PRL response is blocked by the non selective 5-HT antagonist methysergide (Laakmann et al. 1983), but further receptor specific mediation of this response has not been established. Clomipramine can

induce nausea and vomiting at similar doses to those required to induce a PRL response (Anderson et al. 1992b), which may cause problems in interpretation of response as nausea can also increase PRL. Several studies have reported a blunted PRL response to clomipramine in depressed subjects in comparison with healthy subjects (Anderson et al. 1992b; Golden et al. 1990; Lopez-Ibor et al. 1989). In healthy subjects, the PRL response to clomipramine is enhanced following 4 days of lithium treatment, with no change in CORT response (McCance et al. 1989), but no significant hormone response to clomipramine is seen after 2 weeks of lithium treatment (Manji et al. 1991). Clear interpretation of these findings has not been possible.

5-HT RECEPTOR AGONISTS

5-HT_{1A} Agonists

The neuroendocrine profile of several 5-HT_{1A} agonists has been examined, including buspirone, ipsapirone, gepirone and tandospirone.

Buspirone

Buspirone is the most widely available 5-HT_{1A} agonist, marketed for the treatment of anxiety disorders. Oral buspirone (up to 30mg) induces an acute increase in GH, PRL, ACTH and CORT, and a reduction in body temperature in healthy subjects (Cowen et al. 1990; Meltzer et al. 1983). Buspirone however has some DA antagonist activity that confounds the interpretation of these responses, and is likely to explain the PRL response

(Meltzer et al. 1982).

Hypothermic responses to buspirone have been found to be blunted in drug free depressed subjects, particularly in subjects with melancholia, however the GH response was unchanged (Cowen et al. 1994). This implies an abnormality in presynaptic but not postsynaptic 5-HT_{1A} receptor activity (see below), and suggests that the blunted L-TRP induced GH release in depressed subjects is likely to be related to a presynaptic abnormality.

Ipsapirone

Ipsapirone has less intrinsic activity for DA receptors than buspirone (Peroutka 1988) and therefore is likely to be a more reliable probe of 5-HT_{1A} receptors. Ipsapirone (up to 3mg/kg) induces an increase in CORT in healthy subjects, but has no effect on PRL or GH (Lesch et al. 1989). Ipsapirone also causes a reduction in body temperature in healthy subjects.

The hypothermic response to ipsapirone is blunted in depressed subjects (Lesch et al. 1990). Chronic antidepressant treatment of depressed subjects (with fluoxetine) further blunted this hypothermic effect (Lerer et al. 1999). These findings suggest a functional reduction in presynaptic 5-HT_{1A} activity in depressed subjects (see below), which is further reduced in the presence of SSRIs, consistent with preclinical findings (Blier and de Montigny 1990). Blunted CORT responses following ipsapirone have also been found in depressed subjects (Lesch et al. 1990), conflicting with the buspirone findings,

however this may reflect dysregulation of the HPA axis in depression rather than 5-HT_{1A} function. The study by Lerer et al. (1999) found ACTH, CORT and GH responses were significantly blunted by fluoxetine treatment in depressed subjects, suggesting a functional down regulation of postsynaptic 5-HT_{1A} receptors following this intervention.

Gepirone and tandospirone

Gepirone and tandospirone are also relatively selective 5-HT_{1A} agonists without significant intrinsic dopaminergic activity. Their neuroendocrine profiles differ, however, as gepirone (up to 20mg) causes robust increases in ACTH, CORT, PRL, GH and reduced temperature (Anderson et al. 1990a) and tandospirone (60mg) only appears to increase GH and decrease temperature (Nakayama et al. 2002).

SSRI treatment has been shown to blunt the hypothermic, CORT and GH responses to gepirone (Sargent et al. 1997b), consistent with the effect of fluoxetine on ipsapirone response.

These differences in the responses to 5-HT_{1A} ligands many relate to differing intrinsic activity, receptor selectivity, and activity of metabolites. Pindolol (which exhibits 5-HT_{1A} antagonist activity) has been demonstrated to block the ACTH, GH and hypothermic responses to 5-HT_{1A} ligands (Cowen 1993), suggesting it is these neuroendocrine responses that are most reliable indicators of 5-HT_{1A} function. The hormonal responses to 5-HT_{1A} agonists are hypothesised to be mediated by activity at postsynaptic 5-HT_{1A} receptors, as these findings are replicated in animal models by directly injecting the

5-HT_{1A} agonist 8-OH-DPAT into the hypothalamus. The hypothermic response appears to be mediated through presynaptic 5-HT_{1A} receptors as this is replicated by injecting 8-OH-DPAT into the dorsal raphe (Cowen 1993). However, in the rat (but not the mouse) a component of the hypothermic response may be mediated via activation of postsynaptic 5-HT_{1A} receptors.

5-HT_{1B} and 5-HT_{1D} Agonists

A number of drugs (collectively known as 'triptans') recently introduced for the acute treatment of migraine have a high affinity for human 5-HT_{1B} and 5-HT_{1D} receptors (Johnson et al. 1997), and have been used as neuroendocrine probes. The triptans include sumatriptan, naratriptan, rizatriptan and, more recently, zolmitriptan. The development of zolmitriptan as a neuroendocrine probe will be detailed in Chapters 5 and 6.

The triptans

Several investigators have found that acute administration of sumatriptan increases plasma GH in healthy men and women (Herdman et al. 1994; Boeles et al. 1997; Facchinetti et al. 1994; Franceschini et al. 1994). However the magnitude of this response is small and inconsistent (only 25 of 49 subjects pooled from different studies showed a GH response to sumatriptan greater than 5 mIU/l, Whale and Cowen 1998b).

Sumatriptan can also lower plasma PRL, although this effect is even less consistent (Herdman et al. 1994; Whale and Cowen 1998a). Recently it has been noted that rizatriptan can increase plasma GH (Sciberras et al. 1997). Animal studies suggest that

5-HT_{1B} receptor agonists can cause hypothermia (Hagan et al. 1997) although such an effect has been documented only rarely in humans (Wing et al. 1996).

Two studies have reported blunted GH responses to sumatriptan in patients with major depression (Cleare et al. 1998b; Yatham et al. 1997a), although these results are difficult to interpret because of the number of healthy subjects who fail to produce useful GH responses. Wing et al. (1996) investigated the effect of repeated SSRI administration in healthy subjects, finding no change in plasma PRL responses post treatment. These findings will be explored further in Chapter 5.

5-HT_2 Agonists

5-HT_2 receptor ligands, such as mCPP have also been used in neuroendocrine studies.

mCPP

mCPP is a metabolite of the antidepressant drug trazodone, and acts as a rather non selective 5-HT agonist, with greatest affinity for 5-HT_{2C} receptors. It also binds to the 5-HT_{2A} receptor but acts there as an antagonist. It has been shown to elevate plasma PRL, ACTH and CORT and have a hyperthermic effect (Kahn and Wetzler 1991). This response is more reliable if mCPP is administered intravenously. IV mCPP (but not oral) inconsistently elevates plasma GH levels (Kahn and Wetzler 1991). The PRL and CORT responses (but not GH) are blocked by metergoline and ritanserin, implying these responses are mediated by postsynaptic 5-HT_{2C} receptors (Seibyl et al. 1991).

The CORT and PRL responses to mCPP in unmedicated depressed subjects did not differ from those of controls (Anand et al. 1994; Kahn et al. 1990). This conflicts with findings with the fenfluramine challenge (see above), and suggests that any abnormality in 5-$HT_{2A/2C}$ mediated PRL release may be due to a defect in presynaptic 5-HT neurones rather than at postsynaptic 5-HT_{2C} receptors. mCPP has been also used to examine the effect of SSRI treatment on 5-HT_{2C} receptor function in healthy subjects. Quested et al. (1997) found blunted PRL and hyperthermic responses to mCPP following SSRI administration, suggesting that SSRIs lower the sensitivity of post synaptic 5-HT_2 receptors in healthy subjects.

Table 2.1 Neuroendocrine challenges of serotonergic neurotransmission

DRUG	HORMONE RESPONSE	RESPONSE MEDIATED BY	EFFECT IN DEPRESSION*	EFFECT OF SSRI **
L-TRP	↑ PRL GH	post synaptic 5-HT_{1A}	blunted PRL GH	enhanced PRL
5-HTP	↑ CORT (↑PRL)	post synaptic 5-HT_2	enhanced CORT	enhanced CORT
Fenfluramine	↑ PRL ACTH CORT	post synaptic 5-HT_2	blunted PRL	PRL→
Clomipramine	↑ PRL ACTH CORT	?	blunted PRL	
Buspirone	↑ PRL GH ACTH CORT	post synaptic 5-HT_{1A}	GH→	
	↓ Temp	pre synaptic 5-HT_{1A}	blunted Temp	
Ipsapirone	↑ CORT	post synaptic 5-HT_{1A}	blunted CORT	blunted CORT
	↓ Temp	pre synaptic 5-HT_{1A}	blunted Temp	blunted Temp
Gepirone	↑ PRL GH ACTH CORT	post synaptic 5-HT_{1A}		blunted CORT GH
	↓ Temp	pre synaptic 5-HT_{1A}		blunted Temp
Tandospirone	↑ GH	post synaptic 5-HT_{1A}		
	↓ Temp	pre synaptic 5-HT_{1A}		
Sumatriptan	↓ PRL	pre synaptic 5-HT_{1B}		PRL→
	↑ GH	post synaptic 5- $HT_{1B/1D}$	blunted GH	
m-CPP	↑ PRL ACTH CORT	post synaptic 5- HT_{2C}	CORT→	blunted PRL
	(↑GH) ↑ Temp		PRL→	blunted Temp

Inconsistent hormone responses are in brackets

* Effect on hormone response in a depressed group in comparison to healthy controls

** Further effect on the hormone response following SSRI treatment

AIMS OF THE THESIS

Use of 5-HT neuroendocrine challenge tests has begun to give investigators insight into the function of 5-HT systems, the impact of illness states on these systems and the effect of drug treatments. The tests described are summarised in table 2.1 above. There are a number of areas where improvements would help develop neuroendocrine research.

This thesis describes the development of two new neuroendocrine challenges and their use in investigating depressive disorder:

I. Zolmitriptan as a probe of 5-$HT_{1B/1D}$ receptors.

II. Low dose citalopram as a probe of presynaptic 5-HT function

Aims of the zolmitriptan studies

Previous studies, as indicated above, have used sumatriptan to investigate 5-$HT_{1B/1D}$ receptor function. This receptor group is of particular interest in depressive disorders due to acting both as presynaptic autoreceptors and post synaptic receptors on 5-HT neurones (Barnes and Sharp 1999). The 5-HT_{1A} receptor has been investigated extensively due to its autoreceptor function and ligands acting at this receptor have potential benefits in antidepressant treatment. It has been suggested that the 5-HT_{1A} autoreceptor is hypersensitised in depressive disorders, leading to a reduction in synaptic 5-HT, reduced 5-HT neurotransmission and changes in mood (Blier et al. 1990). If the temperature

reduction to 5-HT_{1A} neuroendocrine probes is a measure of presynaptic receptor function as proposed, then the findings in depressed subjects of a blunted temperature response is not consistent with this hypothesis. SSRIs have been shown to desensitise the 5-HT_{1A} autoreceptor in rats (Blier et al.1990) and enhance synaptic 5-HT levels and neurotransmission, with likely associated antidepressant effects. The addition of 5-HT_{1A} antagonists to SSRI treatment in humans does not appear to have as dramatic an effect as expected, however (Whale et al. in preparation). This may be because 5-$HT_{1B/1D}$ autoreceptors further modulate the synaptic availability of 5-HT counteracting any benefits of 5-HT_{1A} autoreceptor antagonism. Indeed, Roberts et al. (1999) demonstrated that to increase extracellular 5-HT in guinea pig frontal cortex, antagonism at both 5-HT_{1A} and 5-$HT_{1B/1D}$ receptors is required, but in the dorsal hippocampus a 5-HT_{1B} antagonist alone is enough to increase 5-HT. Sharpe et al. (1997) also demonstrated in rats that enhanced frontal cortex 5-HT was seen with an SSRI plus a 5-HT_{1A} and a 5-HT_{1B} antagonist, an SSRI plus a 5-HT_{1B} antagonist, but not with a 5-HT_{1A} or 5-HT_{1B} antagonist alone. 5-$HT_{1B/1D}$ autoreceptors could also be hypersensitised in depressive disorders, contributing to the pathology. If neuroendocrine findings have not shown 5-HT_{1A} hypersensitivity, it could be that 5-$HT_{1B/1D}$ autoreceptors alone have this enhanced activity in depression. It is therefore of significant interest to examine this 5-$HT_{1B/1D}$ autoreceptor function in depression, and examine the influence of antidepressant treatment on this function.

The postsynaptic function of 5-$HT_{1B/1D}$ receptors could also play a role in the development of depression and its treatment and similarly would be useful to examine.

TCAs and ECT have been shown to hypersensitise postsynaptic 5-HT_{1A} receptors in rats (Blier et al. 1990), theoretically enhancing neurotransmission and giving a potential mechanism for their action. As described above, studies with sumatriptan in healthy human subjects have shown a reduction in PRL and temperature (suggested to be presynaptic mediated responses) and increased GH (suggested to be a postsynaptic mediated response) following its administration (Whale and Cowen 1998a). These responses are inconsistent in subjects however, likely due to the pharmacokinetics of sumatriptan. In depressed subjects the proposed presynaptic responses (PRL and temperature) have not been described so no conclusions have been drawn. The GH response has been shown to be blunted in depression however (Cleare et al. 1998b, Yatham et al. 1997a), suggesting reduced postsynaptic 5-$HT_{1B/1D}$ receptor function, and consistent with the hypothesis of reduced 5-HT neurotransmission in depression. SSRI treatment for 16 days in healthy subjects did not change the PRL and temperature response to sumatriptan (Wing et al. 1996), suggesting no change in presynaptic 5-$HT_{1B/1D}$ receptor function with treatment. It is therefore of significant interest to use a 5-$HT_{1B/1D}$ receptor probe with better brain penetration than sumatriptan, to examine whether the findings with sumatriptan can be more reliably replicated and examine the function of this receptor group at both pre and postsynaptic levels in depressed subjects and following antidepressant treatment. The introduction of zolmitriptan following sumatriptan offered an ideal opportunity to explore this area.

I also proposed to examine if the neuroendocrine responses to zolmitriptan could be isolated to either 5-HT_{1B} or 5-HT_{1D} receptors. This is of potential importance if just one

of these receptor groups is dysfunctional in depression, and could influence selective ligand testing for therapeutic benefits with potentially less side effects of dually acting ligands.

Aims of the citalopram studies

The withdrawal of fenfluramine for use in neuroendocrine challenges was a great loss, particularly since its neuroendocrine responses were reasonably reliable and it had been worked up to a high level, with good information on the mechanism of the endocrine responses (Newman et al. 1998). The other hypothesised tests of presynaptic function, or possible net 5-HT activity, are L-TRP, 5-HTP and clomipramine, as discussed above. L-TRP has some differing neuroendocrine responses to fenfluramine which may represent the difference between their receptor mediated effects (5-HT_{1A} and 5-HT_2 respectively), the responses to 5-HTP are inconsistent and clomipramine is not always well tolerated. There was therefore much interest in using other drugs as potential presynaptic probes. Citalopram would appear to have such potential as it acts presynaptically, is available for IV administration, and SSRIs are in general better tolerated than TCAs. Preliminary studies with citalopram 20mg IV did not indicate that it was well tolerated however (Seifritz et al. 1996). I therefore set out to examine lower doses of citalopram to see if these had utility as a neuroendocrine probe with good tolerability and reliable hormonal responses. I then proposed to examine which receptors had influence in mediating the hormonal responses to citalopram and examine these responses in depressive disorder. As table 2.1 demonstrates, the responses to probes with similar actions on the 5-HT system or similar receptor mediated responses (direct or indirect), are not fully consistent. There

is therefore much benefit to be gained by the introduction of further neuroendocrine probes to clarify which are common responses to these probes and further validate their interpretation.

PART 2

EXPERIMENTAL STUDIES

CHAPTER 3

GENERAL METHODS

SELECTION AND RECRUITMENT OF SUBJECTS

Normal volunteers

Normal volunteers were selected from a volunteer register that had been compiled through newspaper advertisement and recruitment from blood transfusion donor sessions. They underwent a brief telephone screening to exclude those with any history of psychiatric disorder, or significant medical disorder. They were invited to the laboratory and a more detailed examination was undertaken. They received a Structured Clinical Interview for DSM-IV (SCID) to ensure that none met criteria for any Axis I psychiatric disorder on DSM-IV. A physical examination, routine blood tests (full blood count, urea and electrolytes, liver and thyroid function tests) and electrocardiogram were undertaken. Subjects were excluded if they had any abnormalities on these investigations, substance misuse, or used psychotropic medication within the last 3 months. All subjects were in the age range 18 to 65 years.

Depressed subjects

Patients were recruited from clinics set up in the primary care setting and secondary care outpatient clinics at the Warneford Hospital, Oxford. Patients were incorporated into studies if they met criteria for Major Depression on the basis of the SCID. It was noted whether they also fulfilled criteria for Major Depression with Melancholia (DSM-IV) (see

appendix). Severity of depression was assessed using the HAMD (Hamilton, 1967) (rated by the investigator or a research nurse) and the self rated Beck Depression Inventory (BDI) (Beck et al. 1961). Patients were accepted whether they had recurrent depressive episodes or not. A period of being free from psychotropic medication for at least 2 weeks (6 weeks if they had been on fluoxetine) was ensured prior to starting the studies. They underwent physical examination and exclusion as appropriate, as described for normal volunteers.

Patients who received treatment as part of the studies were prescribed an SSRI (including venlafaxine at lower dose) for at least four weeks before rechallenging with the neuroendocrine probe. During the treatment, patients were monitored as in usual clinical care, at most on a weekly basis, with sessions lasting up to 30 minutes. If psychological treatment was necessary then this was restricted to supportive psychotherapy incorporating problem solving (Hawton 1989).

Controls for depressed subjects

Controls were taken from the group of normal volunteers and matched individually to depressed subjects for gender, menstrual status (stage of the menstrual cycle or postmenopausal), age (+/- 5 years), weight (+/- 5kg) and hormonal medication (eg. oral contraceptives, hormone replacement therapy).

Consent

All subjects gave written informed consent to the studies that could be withdrawn at any stage throughout the investigation. These studies were approved by the local ethics committee.

GENERAL PROCEDURE FOR NEUROENDOCRINE STUDIES

Subjects were brought to the laboratory having fasted for at least four hours. An indwelling venous cannula was inserted and maintained with heparinised saline. Subjects rested for a period of 30 minutes before drug administration to allow any stress effects of getting to the laboratory and cannulation on the baseline hormone levels to be minimised. Blood samples were removed before and at regular intervals following drug administration at time '0'. Subjects remained at rest throughout the test procedure and were not allowed to eat or smoke. If thirsty they were given sips of water. It was ensured that subjects remained awake throughout the procedure as sleep influences hormonal secretion. Female subjects underwent neuroendocrine challenge only in the early follicular phase of their menstrual cycle (if they were menstruating). The follicular stage starts on the first day of menstrual bleeding and continues for 15 days. Oestrogen levels remain low up to around day 10. The results of a rechallenge with drug at a later occasion were therefore comparable to the first, with minimised influence of oestrogen on hormone levels (O'Keane et al. 1991).

Study design

Subjects were recruited to double blind, randomised, balanced order, placebo controlled, cross over designed trials. Randomisation was carried out by an independent researcher by coin toss. This researcher dispensed drug or placebo into sealed envelopes, labelled with the trial day and subject code. This method of randomisation is criticised firstly due to problems with full concealment from the investigators. This was attained in these studies in terms of the randomisation code but investigators could obviously try to predict which arm the subjects were in from side effects experienced with the probes. Proving the validity of the coin toss as purely random, completely excluding human influence, is more difficult and ideally random number generators should have been used. To ensure reproducibility at the end of the trial and following publication, the randomisation code was recorded in a permanent document and stored in a sealed envelope in a filing cabinet.

Zolmitriptan test

The nature and use of zolmitriptan and reasons for the dose chosen in these experiments is described in detail in Chapter 4. Zolmitriptan tablets (Astra Zeneca) 5 mg or placebo were encapsulated and administered at time 0. Blood samples (10ml up to 20ml (if a plasma drug analysis was taken at that time point)) were removed from the cannula at 15 minute intervals from 30 minutes before, up to 180 minutes after drug administration.

Citalopram test

The nature and use of citalopram is described in detail in Chapter 6. Intravenous citalopram (Lundbeck), 5mg or 10mg, was made up to 10ml with saline. Citalopram or placebo (10ml saline) were infused over a 30 minute period using a syringe driver. Blood samples (10ml to 20ml) were taken at 15 minute intervals from 30 minutes before, up to 180 minutes after drug administration.

Monitoring of vital signs and side effects

The subjects' well being was monitored closely throughout the tests. The intention at the start of the studies was to give subjects 100mm visual analogue scales (VAS) to rate the subjective experiences of dizziness, drowsiness and nausea at 30 minute intervals throughout the testing, anchoring 0 as the least and 100 as the most they have ever experienced these effects. Oral temperature was measured in some studies, at 30 minute intervals, with a glass mercury clinical thermometer that remained in situ for ten minutes.

If subjects reacted to drug administration in a manner that would skew hormonal results (such as extreme nausea or vomiting) they were excluded from further analysis.

Plasma samples

Following removal from the cannula, blood samples were labelled and stored in lithium heparin bottles on ice until the end of the individual test period. Plasma was separated from the blood samples by centrifugation at 200g for 10 minutes at 4°C. Plasma samples were then frozen at -20 °C and stored until assay.

Biochemical assays

All assays were carried out by the laboratory staff of the Neuroscience Department, Warneford Hospital. Plasma PRL and GH were measured using standard immunoradiometric assays (reagents provided by Netria, London). The inter and intra assay coefficients of variation of the PRL assays over the range encompassed by the standard curve were 4.8% and 2.4%. The corresponding values for GH were 4.1% and 2.6%. Because GH can inhibit its own secretion, we excluded subjects whose tests demonstrated elevated baseline GH secretion (>10 mIU/l) at time '0'.

Plasma CORT was determined by direct specific radioimmunoassay (RIA). The intra and inter assay coefficients of variation over the range encompassed by the standard curve were 4.3% and 5.8%.

ACTH was not measured during these studies as the laboratory did not have the necessary reagents available, although this would ideally have been undertaken as a more direct

measure of hypothalamic / pituitary function than CORT.

Plasma drug levels were measured by a high performance liquid chromatography (HPLC) procedure that utilised coulometric end point detection, and solid phase extraction (Clement and Franklin 2002).

Analysis of samples from each individual at each time point was always carried out in the same assay to remove intra assay variation as a confounding variable from within subject analyses. Assays were carried out blind to subject diagnosis and treatment.

Analysis of results

All statistical analyses were carried out using SPSS for Windows (version 11). Hormone and temperature responses are shown graphically, plotted against time. Hormone change data following neuroendocrine challenge are displayed as area under the plasma concentration versus time curve (AUC), calculated by the trapezoid method using plasma hormone values with baseline scores subtracted. Hormonal responses and drug levels were also measured as peak change from baseline where appropriate and correlations between peak change and AUC examined. AUC is a useful, sensitive measure of hormonal response to a challenge which takes into account the magnitude and duration of the response, and reflects individual pharmacokinetic variation. Peak hormone response is less useful as it just measures magnitude. The AUC is less worthwhile using if responses are absent (as can occur with GH) and gives erroneous values for responses that vary

above and below the baseline. Change in hormone response AUC (ΔAUC) between active drug and placebo challenges was calculated to give simpler statistics in studies with placebo arms comparing patients and controls.

Data was examined for normal distribution using the one sample Kolmogorov Smirnov test, before using parametric analyses. The results will only be shown for this test if data do not conform to normal distribution.

Comparison of data using raw, AUC and ΔAUC values was undertaken as appropriate using analysis of variance (ANOVA) both within subjects for repeated challenges (active drug vs placebo or active drug vs active drug if rechallenged after a period of time) and between subjects for comparison between different subject groups (depressed subjects vs controls). ANOVA assesses whether the means of results on different test days or between subjects are from the same normal distribution and have equal variances. ANOVA is a powerful statistical tool to test this but if data are correlated (termed 'sphericity is violated') then the critical value of F is too low and therefore biases the result. This can occur in ANOVAs with 3 or more levels. Thus the Huyhn-Feldt correction was used in all calculations to remove this potential bias. ANOVAs on raw values were omitted if there were too many levels within the calculation that would bias the result. Post hoc testing following significant ANOVA results used Student's paired and unpaired t tests (2 tailed). Graphically, Fisher's test of least significant difference was used to show significant differences in data at different time points.

Exploratory ANOVAs were used to examine main or interactive effects of order of administration of drug or placebo, or gender on the hormone responses.

Correlations between variables were assessed by Pearson's product moment correlation (r). Post hoc power analyses were conducted as necessary using the statistical program NCSS (Hintze 2004).

CHAPTER 4

THE ZOLMITRIPTAN CHALLENGE IN NORMAL SUBJECTS

THE NEUROENDOCRINE EFFECTS OF ZOLMITRIPTAN IN NORMAL SUBJECTS

INTRODUCTION

The release of various anterior pituitary hormones is under the control of 5-HT neurones and hormonal responses to selective 5-HT ligands provide a useful means of determining the sensitivity of distinct 5-HT receptor subtypes in humans (Cowen 1993), as discussed in Chapter 2. The triptan group of drugs (including sumatriptan, rizatriptan, naratriptan and zolmitriptan) have a high affinity for human 5-HT_{1B} and 5-HT_{1D} receptors (Johnson et al. 1997) and have potential use as probes of the function of these receptors (as discussed in Chapter 2). Measurement of the sensitivity of 5-HT_{1B} and 5-HT_{1D} receptors is of interest because they may be involved in the pathophysiology of major depression as well as mediation of the effects of antidepressant drug treatment (Briley and Moret 1998) (see aims of the thesis, chapter 2). For example, desensitisation of presynaptic 5-HT_{1B} receptors on 5-HT nerve terminals may be involved in the ability of SSRIs to increase 5-HT neurotransmission (Moret and Briley 1990). In addition, administration of the selective 5-HT_{1B} receptor antagonist SB-224289 increased 5-HT release in the guinea pig dentate gyrus, which would be explained by antagonism of the presynaptic 5-HT_{1B} autoreceptor, suggesting possible antidepressant activity as a sole agent (Roberts et al. 1998). Administration of a 5-$HT_{1B/1D}$ receptor agonist such as sumatriptan or zolmitriptan could either reduce 5-HT secretion from the presynaptic area by agonism of the

presynaptic autoreceptor or stimulate 5-HT neurotransmission by agonism of the postsynaptic membrane receptors. This would be reflected in a neuroendocrine paradigm by a reduction or increase in resultant hormonal secretion respectively.

The use of sumatriptan as a neuroendocrine probe is reviewed in Chapter 2. In summary, sumatriptan increases plasma GH in healthy men and women and can also lower plasma PRL, although this effect is less consistent (Herdman et al. 1994; Whale and Cowen 1998a). A hypothermic effect of sumatriptan can occasionally be seen in humans (Wing et al. 1996). This suggests a potential ability to measure both pre and postsynaptic $5\text{-}HT_{1B/1D}$ mediated responses.

The purpose of the present study was to assess the effects of the more recently developed $5\text{-}HT_{1B/1D}$ receptor agonist, zolmitriptan (Martin 1997), on plasma GH and PRL and on oral temperature in healthy volunteers. Zolmitriptan is of particular interest to study because it has greater brain penetration than sumatriptan (Martin 1997; Sleight et al. 1990) and its neuroendocrine effects might therefore be expected to be elicited more reliably.

Zolmitriptan's chemical structure is shown in figure 4.1. Zolmitriptan is indicated for the acute treatment of migraine. The recommended dose to treat such an event is 2.5mg, which can be increased to 5mg with beneficial effect if a subsequent migraine occurs within 24 hours. It is well tolerated, with typically mild to moderate adverse reactions occurring within 4 hours of administration, which resolve spontaneously. The most

common adverse reactions are nausea, dizziness, somnolence, warm sensation, asthenia and dry mouth. Associated cardiovascular changes are minimal and not clinically significant (Seaber et al 1996).

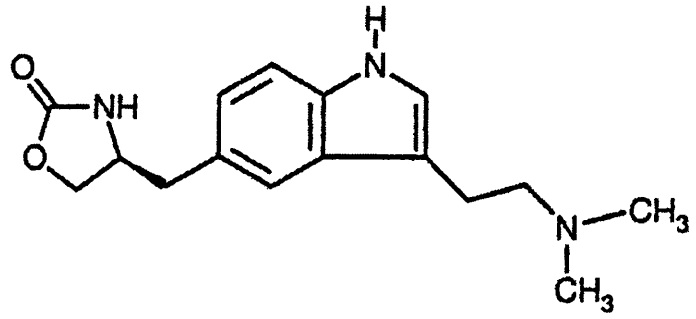


Fig 4.1 The chemical structure of zolmitriptan.

Zolmitriptan has high affinity and full agonist activity at transfected human 5-HT_{1B} and 5-HT_{1D} receptors, with some preference for 5-HT_{1D}. Other than moderate affinity for 5-HT_{1A} and 5-$ht_{1E/F}$ receptors, the drug has no or negligible affinity at other receptor types (including other 5-HT receptors) (Martin 1997). The 5-HT_1 binding profile of zolmitriptan in comparison to sumatriptan is shown in table 4.1 (on page 154).

The antimigraine effects of zolmitriptan appear to be mediated by constriction of specific blood vessels (including cranial arteries), and inhibition of excitability of cells in the trigeminal nucleus caudalis via a reduction of the neurogenic inflammatory response causing protein extravasation. This reduction of trigeminal excitability reduces sensitivity to mechanical and chemical stimuli that would normally be processed as pain by higher

centres (as reviewed by Martin 1997).

Zolmitriptan has a higher oral bioavailability than sumatriptan (40% and 14% respectively) and studies with triptans have shown that this correlates with increased brain penetration (Martin 1997). Zolmitriptan is rapidly absorbed, with peak plasma levels occurring at 2 to 4 hours, and has high plasma clearance, with a half life of 2.5 to 3 hours (Seaber et al. 1996). It is largely eliminated by hepatic biotransformation followed by urinary excretion. It has three main metabolites, the indole acetic acid, the N-oxide and the N-desmethyl analogues. The latter metabolite has 5-$HT_{1B/1D}$ receptor activity and is likely to contribute to the clinical effect. This metabolite has peak levels up to 45 minutes following the parent drug, with similar half life (Seaber et al 1996).

This profile of good oral bioavailability, well tolerated, reasonably selective receptor activity, peak levels within 4 hours and fast elimination render zolmitriptan an attractive drug for potential use as a neuroendocrine probe.

METHODS

Subjects

Twelve healthy subjects were studied (6 men and 6 women, mean age 34, range 18 to 46), recruited as described in Chapter 3. On the basis of a semi structured clinical interview they were determined to have no personal history of psychiatric disorder. They had taken

no psychotropic medication for at least 3 months. Two women were taking the contraceptive pill, and one was taking hormone replacement therapy.

Neuroendocrine tests

Procedures are described in chapter 3. Subjects were tested on two occasions receiving either zolmitriptan (5mg orally) or identical placebo in a double blind, placebo controlled, cross over, balanced order design. This dose of zolmitriptan was chosen as it is a well tolerated, clinically effective dose for the treatment of migraine and corresponded with the clinically effective dose (for migraine) of subcutaneous sumatriptan (6mg) which also demonstrated a reasonable neuroendocrine response (Whale and Cowen 1998a). Test days were separated by a mean of 11.6 days (range 2 to 29). Female subjects were tested within the follicular phase of their menstrual cycle. Subjects were studied at midday after a four hour fast when an indwelling venous cannula was inserted. After a 30 minute rest period, zolmitriptan or placebo were administered and blood samples removed at 15 minute intervals for the following 180 minutes. Oral temperature was measured at 30 minute intervals with a glass mercury clinical thermometer that remained in situ for ten minutes.

Biochemical measurements

Assay methods are described in Chapter 3. The plasma zolmitriptan level assay was unfortunately not available at the time of this study but would have been ideally undertaken to control for pharmacokinetic variation between subjects.

Analysis of results

Hormone and temperature responses were analysed using a two way repeated measures ANOVA with 'occasion' (zolmitriptan vs placebo) and 'time' as the main within subject factors. Plasma GH responses were also measured as AUC using the trapezoid method and analysed using a paired samples t test.

RESULTS

Zolmitriptan was well tolerated. The mean peak VAS scores (total possible score 100) for nausea, dizziness and drowsiness were 2, 5 and 9 on zolmitriptan test days and 0, 0 and 7 on placebo test days respectively (ANOVAs of all measures between tests days were non significant with p values >0.2) . The number of subjects reporting no change in nausea, dizziness and drowsiness on the zolmitriptan challenge day was 10, 9 and 6 respectively. Three subjects were excluded from the GH analysis because at least one of their baseline GH levels at time '0' was high. Following zolmitriptan, but not placebo, there was a marked increase in plasma GH (Fig 4.2). The two way ANOVA of individual GH data points showed a significant main effect of occasion ($F= 14.27$; df 1, 8; $p= 0.005$) and time ($F= 5.49$; df 4, 29; $p= 0.003$) with a significant occasion by time interaction ($F= 5.62$; df 3, 25; $p= 0.004$). The peak GH response occurred between 60 and 120 minutes (Fig 4.2). Eight subjects had a clear GH response to zolmitriptan above 5mIU/l and the other subject had no response at all.

Mean ± SEM GH AUC values on zolmitriptan and placebo test days were 24.8 ± 6.9 and -1.1 ± 1.5 mIUxHr/l respectively (p=0.009). GH AUC values were highly correlated with peak GH change from baseline (r= 0.95; p= 0.001). Corresponding mean ± SEM peak GH positive change from baseline was 16.1 ± 6.0 mIU/l and 0.4 ± 1.0 mIU/l respectively.

In contrast, zolmitriptan did not alter plasma PRL concentration relative to placebo (Fig 4.3). The ANOVA showed no main effect of occasion (F= 0.14; df 1, 11; p=0.72) but a significant effect of time (F= 9.19; df 3, 30; p< 0.001). However, there was no occasion by time interaction (F= 0.84; df 3, 38; p=0.49). Similarly there was no significant change in oral temperature following zolmitriptan, although from Fig 4.4 it is observed that mean temperature appeared lower on the zolmitriptan test days. This ANOVA showed no main effect of occasion (F= 2.71; df 1, 11; p= 0.13), or of time (F= 1.47; df 4, 41; p= 0.23) and no occasion by time interaction (F= 1.39; df 5, 51; p= 0.25).

Sex of subject had no significant effect as a covariate in the two way ANOVA of hormone responses (for GH response, p=0.85). Mean peak ± SEM GH response on zolmitriptan test days for men and women was 22.4 ± 5.9 mIU/l and 24.5 ± 11.7 mIU/l respectively. Order of administration of zolmitriptan or placebo similarly had no significant effect as a covariate in the two way ANOVA of hormone responses (for GH response, p=0.50).

These results are discussed following the next section on defining the mode of action of the GH response.

Fig. 4.2 Mean ± SEM plasma GH concentrations in 9 healthy volunteers following administration of zolmitriptan (5mg orally) (open squares) and placebo (closed circles). Subjects were tested on two occasions in a double blind, cross over design. *p<0.05, **p<0.01 (Fisher's test of least significant difference).

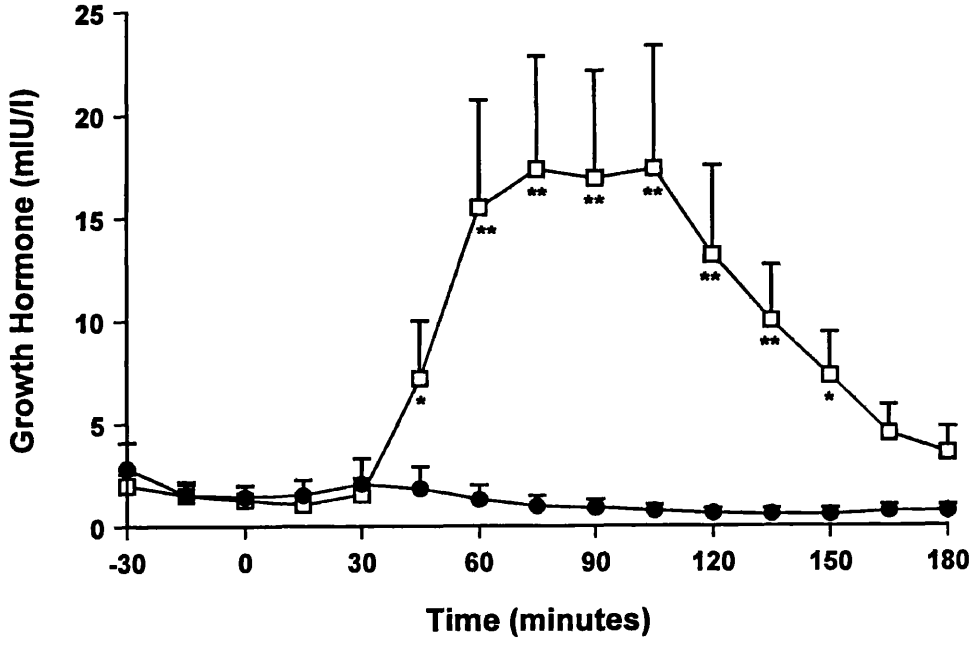


Fig. 4.3 Mean ± SEM plasma PRL concentrations in 12 healthy volunteers following administration of zolmitriptan (5mg orally) (open squares) and placebo (closed circles). Subjects were tested on two occasions in a double blind, cross over design. No statistical difference between the two tests is observed (ANOVA).

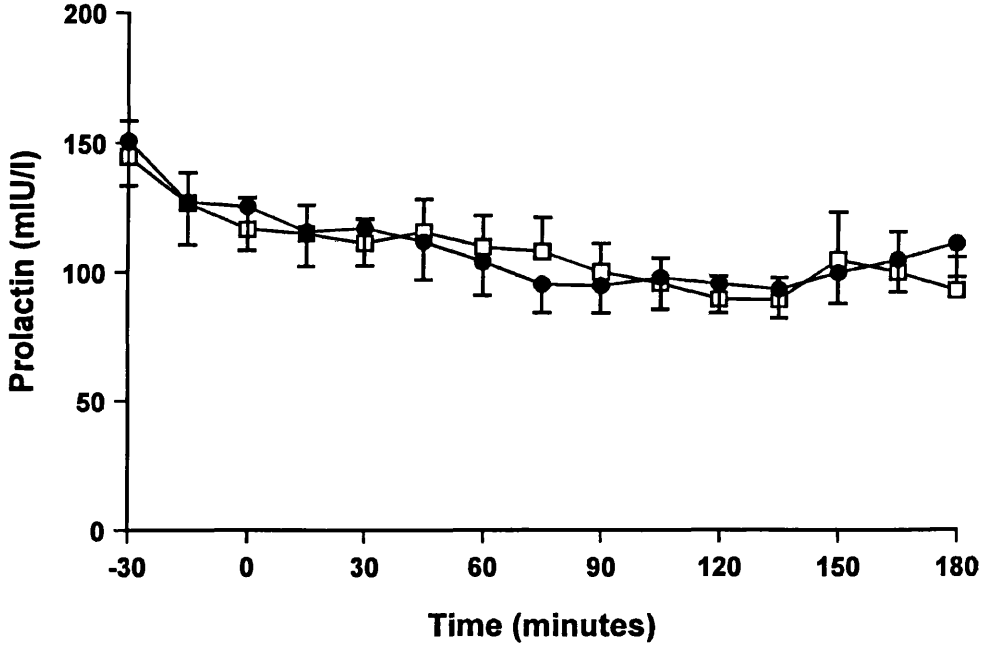
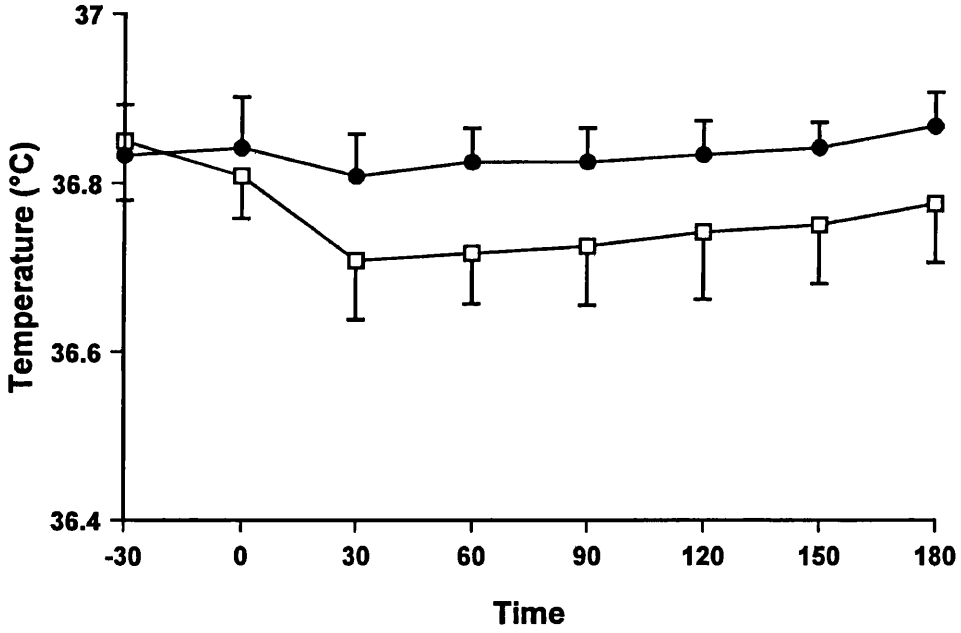


Fig 4.4 Mean ± SEM oral temperature in 12 healthy volunteers following administration of zolmitriptan (5mg orally) (open squares) and placebo (closed circles). Subjects were tested on two occasions in a double blind, cross over design. No statistical difference between the two tests is observed (ANOVA).



DEFINING THE MODE OF ACTION OF THE NEUROENDOCRINE RESPONSE TO ZOLMITRIPTAN

INTRODUCTION

Having established the neuroendocrine properties of zolmitriptan, elucidating which receptor/s are involved in the mediation of the GH response is the next priority in developing a useful neuroendocrine probe. Zolmitriptan has highest affinity for 5-HT_{1B} and 5-HT_{1D} receptors, with moderate preference for 5-HT_{1D} (Martin 1997, Table 4.1). These receptors have likely differing brain location and function (as described in Chapter 1). Several antagonist drugs have activity which can be used to discriminate between 5-HT_{1B} and 5-HT_{1D} receptors, including the classical 5-HT_{2A} receptor antagonist and α_1 adrenoceptor antagonist ketanserin, and the 5-$HT_{2A/2C}$ receptor antagonist ritanserin. Ritanserin was not available for use at the time of this experiment. In vitro and in vivo studies in animals show that ketanserin blocks the human 5-HT_{1D} receptor with some preference over the 5-HT_{1B} receptor (Pauwels et al. 1996). With recombinant human 5-HT_{1D} and 5-HT_{1B} receptors, ketanserin has moderate affinity for 5-HT_{1D} (pKi = 7.17) and 71 fold selectivity for recombinant human 5-HT_{1D} over 5-HT_{1B} receptors (Zgombick et al. 1995). The affinity of ketanserin for 5-HT_2 receptors is 5 times greater than for NA and histamine receptors and 100 times greater than for DA receptors (Leysen et al. 1981). Sharpley et al. (1994) used ketanserin 40mg orally in a study of the effect of blocking

5-HT_2 receptors on slow wave sleep in humans, demonstrating that it does indeed enhance slow wave sleep (although not as effectively as ritanserin) and was well tolerated.

Ketanserin has been used for the treatment of arterial hypertension although is not currently licensed for such treatment in the UK (Electronic medicines compendium). The commonly used dose is 40mg twice daily. Following oral administration more than 98% is rapidly absorbed and peak plasma levels are reached within 0.5 to 2 hours. Bioavailability is around 50%. Due to a high level of plasma protein binding, the free fraction is 5%. Ketanserin is primarily metabolized by ketone reduction, and subsequently excreted in the urine. This metabolite does not add to ketanserin's pharmacological effect. The terminal half life of oral ketanserin is 14.3 hours. Ketanserin's pharmacokinetics are not influenced by age or sex. Plasma levels are higher in severe liver disease. There have been no clear reports of influence on the metabolism of coadministered drugs. The only common side effects at usual therapeutic doses are sedation (mediated via histamine receptor antagonism) and reduced saliva production. Higher doses have been associated with cardiac QT interval prolongation (reviewed by Persson et al. 1991).

METHODS

Subjects

Six healthy male subjects we recruited from the volunteer register (mean age 36, range 26 to 44) as described in Chapter 3. One subject took part in both this study and the above zolmitriptan / placebo study. It was acknowledged that this was a small sample but such numbers were adequate in other studies which examined block of neuroendocrine responses (eg. Lewis and Sherman 1985) and it was predicted that if the large, robust GH response was purely mediated by 5-HT_{1D} receptors then even a small amount of receptor blockade would reflect a GH change that could be picked up with this sample.

Neuroendocrine tests

Subjects were brought to the laboratory at midday after a 4 hour fast. Each subject was studied on two occasions, receiving either zolmitriptan 5mg plus ketanserin 40mg, or zolmitriptan 5mg plus placebo, in a double blind, cross over, balanced order design. This dose of ketanserin was chosen due to the tolerability demonstrated by Sharpley et al. (1994), which produced near maximal blockade of brain 5-HT_{2A} receptors. An indwelling venous cannula was inserted and ketanserin or placebo was given at -60 minutes to ensure optimum plasma ketanserin levels at the time of peak GH response to zolmitriptan. Zolmitriptan was administered at time '0'. Blood samples were removed at 15 minute intervals from -60 minutes to 180 minutes. Plasma samples for zolmitriptan levels were taken at 30 minute intervals, to examine any effect of ketanserin on zolmitriptan levels.

Subjects remained at rest throughout the test procedure. Test days were separated by a mean of 28.5 days (range 14 to 61).

Biochemical Measurements and Analysis of Results

Plasma zolmitriptan levels were measured by HPLC (Clement et al. 2002).

Plasma GH and zolmitriptan levels were analysed using a two way repeated measures ANOVA with 'occasion' (ketanserin vs placebo) and 'time' as the main within subject factors. AUC values for GH and zolmitriptan levels were also calculated and analysed with paired t tests.

RESULTS

All subjects tolerated the drug challenges well with no associated significant adverse events. VAS scales were unfortunately not consistently used. Zolmitriptan induced GH responses were lower following ketanserin pretreatment (Fig 4.5). The two way ANOVA of GH responses showed a main effect of occasion ($F= 11.07$; df 1, 5; $p= 0.021$) and time ($F= 5.89$; df 3, 16; $p= 0.007$) but only a trend to a significant occasion by time interaction ($F= 2.61$; df 4, 18; $p= 0.074$).

The mean ± SEM AUC GH response was 32.4 ± 9.9 mIUxHr/l to zolmitriptan alone and 13.3 ± 6.4 to zolmitriptan with ketanserin, for paired t test $p= 0.013$, (corresponding

mean ± SEM peak GH response was 27.8 ± 8.1 mIU/l and 16.1 ± 7.3, p=0.015). The ANOVA of AUC GH response was significant between test days (F= 14.51; df 1, 5; p= 0.013), with a power of 0.86 (α being set at 0.05). Hence despite a small sample size, this comparison had sufficient statistical power to demonstrate a difference between test days.

Plasma zolmitriptan levels tended to be lower after ketanserin treatment although not significantly so (Fig 4.6). The two way ANOVA of zolmitriptan levels showed no main effect of occasion (F= 2.86; df 1, 5; p= 0.15) but a significant effect of time (F= 8.74; df 3, 15; p= 0.001). However there was no occasion by time interaction (F= 1.26; df 4, 19; p= 0.32).

Mean ± SEM AUC zolmitriptan levels following ketanserin were 6.2 ± 1.8 μgxHr/ml compared to 10.1 ± 3.2 μgxHR/ml following placebo, p= 0.13, (corresponding mean ± SEM peak zolmitriptan levels were 4.35 ± 1.1 μg/ml and 5.64 ± 1.3 μg/ml, p= 0.35).

To account for the effect of the variance of zolmitriptan levels on the GH levels, zolmitriptan level AUC values were regressed on the GH AUC values for each treatment arm and the residualised scores for GH AUC were entered into an ANOVA. A significant effect of occasion (placebo vs ketanserin) was observed (F=46.61; df 1, 5; p=0.001). Hence, despite zolmitriptan levels being lower in the ketanserin arm of the study, the effect of ketanserin on the GH response remained significant.

Fig. 4.5 Mean ± SEM plasma GH concentrations in 6 healthy men following administration of zolmitriptan (5mg orally) with placebo (open square) and zolmitriptan (5mg orally) with ketanserin (40mg) (solid square). Subjects were tested on two occasions in a double blind, cross over design. *p<0.05, **p<0.01 (Fisher's test of least significant difference).

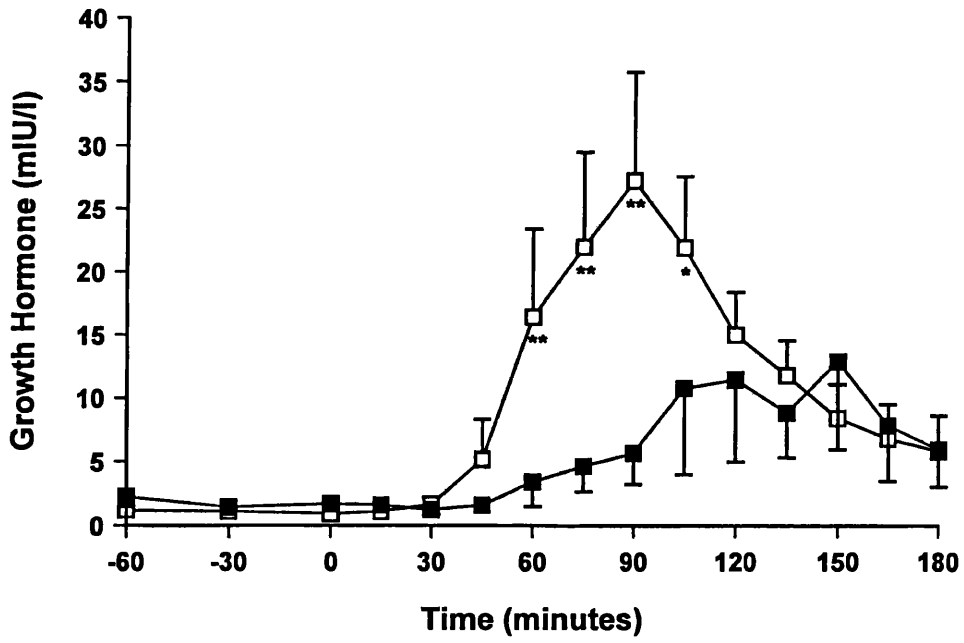
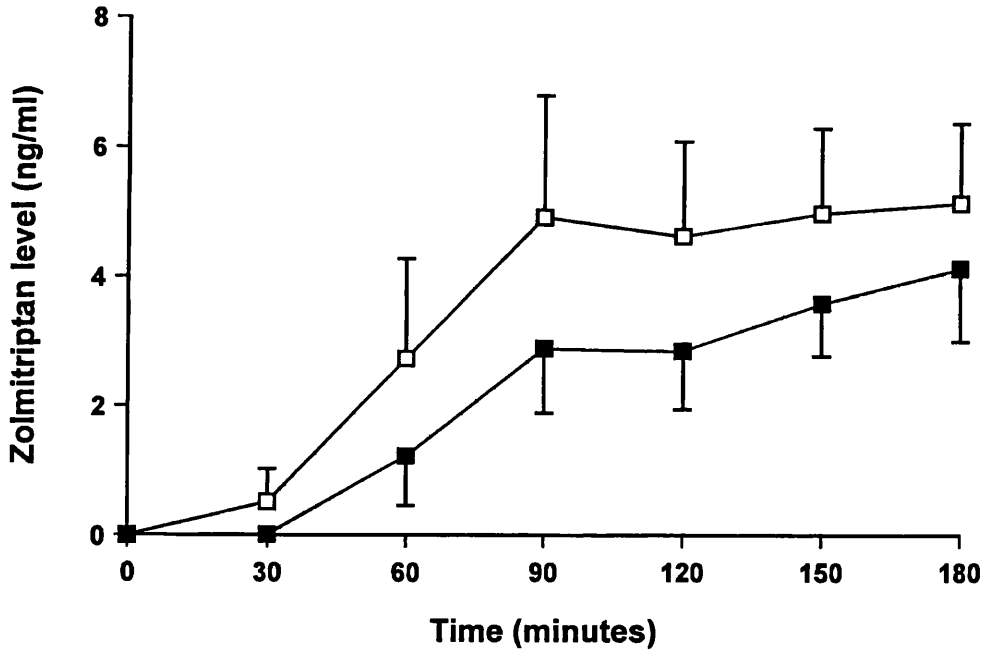


Fig. 4.6 Mean ± SEM plasma zolmitriptan levels in 6 healthy men follov ing administration of zolmitriptan (5mg orally) with placebo (open square) and zolmitriptan (5mg orally) with ketanserin (40mg) (solid square). Subjects were tested on two occasions in a double blind, cross over design. No significant difference was observed (ANOVA).



DISCUSSION OF ZOLMITRIPTAN STUDIES IN HEALTHY SUBJECTS

Zolmitriptan increased plasma GH in healthy men and women but had no effect on plasma PRL or body temperature. The GH response to zolmitriptan was attenuated by ketanserin suggesting that it may be mediated by 5-HT_{1D} receptors (Pauwels et al. 1996).

The ability of zolmitriptan to increase plasma GH is also shared by sumatriptan and rizatriptan (Herdman et al. 1994; Sciberras et al. 1997). This suggests that the induction of GH release may be a class property of 5-$HT_{1B/1D}$ receptor agonists. Zolmitriptan has a better blood brain barrier penetration than sumatriptan (Martin 1997; Sleight et al. 1990), which raises the possibility that it may produce more reliable increases in plasma GH. The neuroendocrine properties of sumatriptan and zolmitriptan are compared in table 4.1.

Comparison of zolmitriptan and sumatriptan in this respect is difficult because of the problems of comparing two drugs with somewhat differing receptor affinities as well as contrasting pharmacokinetic properties. With these reservations, the GH response to sumatriptan in healthy male and female volunteers from 6 earlier studies (where data was available) were examined (Herdman et al. 1994; Wing et al. 1996; Boeles et al. 1997; Yatham et al. 1997a; Cleare et al. 1998b, Whale and Cowen 1998a). When all these studies are taken together, 25 of 49 subjects showed a GH response to sumatriptan greater

than 5mIU/l. In contrast, in the present study, 12 of 14 subjects had GH responses to zolmitriptan above this threshold (p=0.02, chi squared test). This provides tentative evidence that zolmitriptan may induce more reliable GH release than sumatriptan, at tolerated doses, and may be a more reliable neuroendocrine probe.

Gender did not influence the GH responses to zolmitriptan in these studies, unlike other studies examining 5-HT function including the sumatriptan challenge (Whale and Cowen 1998), the fenfluramine challenge (Newman et al. 1998), L-TRP challenge following dieting (Anderson et al.1990b) and TRP depletion (Smith et al. 1997a).

The GH response to zolmitriptan was significantly antagonised by pretreatment with the 5-HT receptor antagonist, ketanserin. Ketanserin is primarily a 5-HT_{2A} receptor antagonist that is also able to block the human 5-HT_{1D} receptor with some preference over the 5-HT_{1B} receptor (Zgombick et al. 1995; Pauwels et al. 1996). Our findings therefore tentatively suggest that the GH response to zolmitriptan is mediated by 5-HT_{1D} receptors.

The possibility, however, that some other pharmacological property of ketanserin may be responsible for the attenuation of zolmitriptan induced GH release cannot be excluded. Against this is the fact the zolmitriptan has no significant affinity for the 5-HT_{2A} receptor (Martin 1996). In addition ketanserin did not antagonise the GH response to insulin hypoglycaemia suggesting that it does not produce a generalised reduction in stimulated GH release (Prescott et al. 1984). Ketanserin's lesser activity as an α and histamine

receptor antagonist would otherwise predict an inhibition of GH secretion (Muller et al.1999). Another relevant factor was that plasma levels of zolmitriptan tended to be lower in combination with ketanserin although allowing for this in a regression analysis did not remove the significant effect on GH responses. However, this possible pharmacokinetic effect of ketanserin as well as the small number of subjects studied means that conclusions concerning the role of 5-HT_{1D} receptors in zolmitriptan induced GH release must remain tentative. The study was sufficiently powered to show a difference in the GH AUC analysis. Hopefully future studies will be able to employ more selective 5-HT_{1D} receptor antagonists to examine this question.

Despite its effective stimulation of GH release, no effect of zolmitriptan on plasma PRL was found. Administration of sumatriptan to healthy men and women at midday lowers plasma PRL and this has been tentatively ascribed to the activation of 5-HT_{1B} autoreceptors on 5-HT nerve terminals (Herdman et al. 1994; Whale and Cowen 1998). The fact that zolmitriptan failed to produce this effect could suggest that an ability to lower plasma PRL is a idiosyncrasy of sumatriptan rather than a property of 5-$HT_{1B/1D}$ receptor agonists in general. Another possibility, however, is the differential binding affinity of sumatriptan and zolmitriptan for 5-HT_{1B} and 5-HT_{1D} receptors (Table 4.1). Whereas zolmitriptan has a moderate preference for 5-HT_{1D} over 5-HT_{1B} receptors, sumatriptan has a more equal binding affinity for the two receptor subtypes (Martin 1997). This raises the possibility that, at clinically used doses, zolmitriptan does not produce sufficient activation of presynaptic 5-HT_{1B} receptors to lower plasma PRL.

There have been few published reports on the effects of triptans on body temperature but there is evidence that activation of presynaptic 5-HT_{1B} receptors causes hypothermia in guinea pigs (Hagan et al. 1997). In one study in humans, a modest lowering of oral temperature following sumatriptan administration was found (Wing et al. 1996). In the present study, graphically mean temperature responses appeared lower following zolmitriptan but this was not a statistically significant reduction, perhaps supporting the proposal that at doses used to treat migraine, zolmitriptan does not activate 5-HT_{1B} receptors in the human brain.

In conclusion this study supports the ability of 5-$HT_{1B/1D}$ receptor agonists to increase plasma GH in humans and tentatively suggests that the pharmacological mechanism involves activation of 5-HT_{1D} receptors. 5-HT_{1D} receptors are located both postsynaptically (Bonaventure et al. 1997) and probably presynaptically in the raphe nucleus where they function as autoreceptors (Pineyro et al. 1995). Because increases in 5-HT neurotransmission are associated with increased GH release in humans it suggests that the GH response to zolmitriptan and other triptans is mediated via activation of postsynaptic 5-HT_{1D} receptors probably located in the hypothalamus. Zolmitriptan does not appear to be a probe for presynaptic receptors. Overall it appears to be a useful neuroendocrine probe, increasing GH secretion most likely via postsynaptic 5-HT_{1D} receptor stimulation, and a more reliable inducer of GH release than sumatriptan which is the 5-$HT_{1B/1D}$ receptor probe previously utilised in neuroendocrine challenge tests in depressed patients.

Table 4.1 Comparisons of neuroendocrine effects and 5-HT_1 receptor affinity (adapted from Martin 1997) for sumatriptan and zolmitriptan.

Neuroendocrine Response	Sumatriptan	Zolmitriptan
PRL	↓	0
GH	↑	↑↑
TEMP	↓	0

Receptor affinity *	Sumatriptan	Zolmitriptan
5-HT_{1A}	7.0	6.5
5-HT_{1B}	8.1	8.3
5-HT_{1D}	8.5	9.2
5-HT_{1E}	5.6	<5.0
5-HT_{1F}	7.6	7.1

* Ieceptor affinity figures are pKA at human receptors.

CHAPTER 5

THE ZOLMITRIPTAN CHALLENGE IN DEPRESSED PATIENTS AND THE EFFECT OF ANTIDEPRESSANT TREATMENT

INTRODUCTION

There is much evidence that impaired 5-HT neurotransmission contributes to the pathophysiology of major depression (see Chapter 1) but the precise nature of this abnormality, particularly in terms of the function of specific 5-HT receptor subtypes, is unclear. Current clinical data in this field are sparse because of the limited availability of selective probes for the various 5-HT receptors. Nevertheless, pharmacological challenge studies have suggested that the function of 5-HT_{1A} receptors is impaired in depressed patients (Cowen et al. 1994; Lesch 1992; Meltzer and Maes 1995; Sargent et al. 1997b), particularly in those whose symptoms meet criteria for melancholic depression.

As demonstrated in Chapter 4, the administration of zolmitriptan increases plasma GH, apparently mediated by activation of postsynaptic 5-HT_{1D} receptors. The aim of the present study was to use this GH response to zolmitriptan to assess the sensitivity of postsynaptic 5-HT_{1D} receptors in patients with major depression before and following treatment with SSRI antidepressants. On the basis of changes found in postsynaptic 5-HT_{1A} receptors in depression, I predicted that postsynaptic 5-HT_{1D} receptor function would be decreased in depressed patients, especially those with melancholic symptoms, and would be further diminished by SSRI treatment (Cowen and Charig 1987; Lesch 1992). Following the findings of chapter 4, it did not appear possible to examine presynaptic 5-$HT_{1B/1D}$ function with zolmitriptan as discussed in the aims in chapter 2.

METHODS

Subjects

Twenty six patients were recruited (10 men and 16 women) from clinics in primary care who on the basis of the SCID met criteria for Major Depression (as discussed in Chapter 3). Their mean age was 37.9 years (range 21 to 54 years) and mean weight 70.0 kg (range 51 to 96 kg). Fourteen subjects had never received psychotropic medication; the remainder had not received psychotropic medication for at least 2 weeks (mean 75 weeks). One woman was taking the oral contraceptive pill, two were postmenopausal, and one was taking hormone replacement therapy. Depressed subjects had a mean score on the HAM-D scale of 24.9 (range 16 to 39). Ten subjects met DSM-IV criteria for major Depression with Melancholia (see appendix).

A group of 25 healthy controls were selected from the volunteer register, and matched to the patient group as described in Chapter 3. This group consisted of 9 men and 16 women with mean age 39.9 years (range 23 to 54 years) and mean weight 71.5kg (range 56 to 102 kg). Controls had been free of psychotropic medication for at least 3 months.

Neuroendocrine tests

Methods are described in chapter 3. Subjects were brought to the laboratory in the morning having fasted for at least four hours. An indwelling venous cannula was inserted

and a 30 minute rest period allowed to elapse before, zolmitriptan (5mg orally), was administered. Blood samples were then removed at 15 minute intervals for a further 180 minutes. The placebo arm used in the studies described so far was omitted in this study due to the robust, reliable GH increase over placebo, as demonstrated above.

A sub group of 12 patients (3 men, 9 women) were rechallenged with zolmitriptan (5mg orally) following a trial of SSRI treatment of mean duration 33.9 days (range 27 to 76 days). Of this group, 7 patients received fluoxetine (20mg daily, except one subject who took 20mg on alternate days), 3 paroxetine (20mg), 1 citalopram (20mg) and 1 venlafaxine (150mg daily). At such a lower dose, venlafaxine appears to be predominately a 5-HT reuptake blocker (reviewed by Frazer 2001). Six healthy controls, similarly from the volunteer register, (4 men, 2 women) were also challenged twice with zolmitriptan (5mg orally), to examine the effect of repeated exposure to zolmitriptan on the GH response in untreated control subjects and ensure that rechallenge alone did not influence GH responses (as seen with the PRL response to rechallenge with clomipramine at 2 weeks; Gilmore et al. 1993). The mean interval between these two tests was 28.2 days (range 23 to 35 days). In both these studies female subjects received the second zolmitriptan challenge at the same stage of the menstrual cycle as the first. It is acknowledged that these two rechallenge groups were not matched for age and sex but a direct comparison was not the intention of this study. Sex was shown not to influence the GH response to zolmitriptan in the previous studies.

Biochemical measurements

Assay methods are described in Chapter 3.

Analysis of results

GH responses and plasma zolmitriptan levels were analysed as AUC calculated using the trapezoid method. AUC GH was highly correlated with peak GH change from baseline ($r=0.94$, $p<0.001$). Baseline CORT and GH were measured as mean concentration from the 3 baseline sample levels.

Patient / control differences were analysed using repeated measures ANOVA with 'time' as the within subject factor and 'depression' (presence or absence) as a between subjects factor and a one way ANOVA of AUC data was conducted with 'depression' as the between subjects factor. Post hoc differences between patients and controls were examined with Student's unpaired t test (two tailed). Within group comparisons were made using Student's paired t test (two tailed). Correlations were carried out using Pearson's product moment. Data from subjects rechallenged with zolmitriptan were analysed using a two way repeated measures ANOVA.

RESULTS

All subjects tolerated zolmitriptan well. VAS for subjective effects were not consistently used. High baseline GH levels led to 3 patients (one with melancholia) and 2 controls

being excluded. In the overall group of patients, mean ± SEM AUC GH did not significantly differ from those of controls, 5.9 ± 4.7 mIUxHr/l compared to 15.8 ± 4.0 mIUxHr/l , p= 0.12, (mean ± SEM peak GH responses 11.8 ± 3.4 compared to 16.3 ± 3.4 mIU/l, p= 0.36). The repeated measures ANOVA showed a main effect of time (F=11.69; df 14, 616; p<0.001) but no 'depression' by time interaction (F=0.62; df 14, 616; p=0.85). The one way ANOVA similarly showed no significant effect of 'depression' (F=2.58; df 1, 44; p= 0.12). Sex of subjects was not a significant covariate in the repeated measures ANOVA (p= 0.73). Three subjects described recent weight change: two weight gain (one of whom was melancholic) and one weight loss, with respective peak GH responses of 16.3, 0.5 and 20.2 mIU/l. The influence of duration of illness, and previous treatment could not be examined as it was not systematically recorded.

When the patients were divided into those with and those without the melancholic syndrome, however, the GH responses of patients with melancholic depression were significantly less than controls (Table 5.1 and Figs 5.1, 5.2, 5.3). The mean ± SEM AUC GH response in the 9 melancholic patients was significantly less when they were directly compared with 9 controls matched for gender, age and weight, -1.2 ± 3.5 mIUxHr/l compared to 22.0 ± 7.3 mIUxHr/l, p=0.015 (mean ± SEM peak GH 4.5±1.3 compared to 20.1± 5.9 mIU/l, p=0.03) (Fig 5.3). In figure 5.1, peak GH response is shown as opposed to AUC for visual clarity. The repeated measures ANOVA for this group showed a main effect of time (F=6.25; df 14, 224; p=0.002), a significant depression by time interaction (F=4.17; df 14, 224; p=0.014) and a significant effect of depression (F=6.63; df 1, 16; p=0.02). The one way ANOVA also showed a significant effect of depression (F=8.20; df

1, 16; p=0.011). As for the whole group, no influence of sex as a covariate in the repeated measures ANOVA of melancholic subjects was observed (p= 0.51).

Neither patient group differed from controls in baseline CORT concentration. Similarly, AUC of plasma zolmitriptan did not differ between patient groups and controls (Table 5.1). In patients there was no significant correlation of AUC GH with AUC plasma zolmitriptan levels, baseline CORT concentration or HAM-D score, (r=0.17, p=0.45; r=0.12, p=0.58; r=-0.20, p=0.36 respectively). Similarly, there was no significant correlation of AUC GH with AUC zolmitriptan levels or baseline CORT concentration in controls (r=-0.25, p=0.26; r=0.32, p=0.14 respectively).

In the 12 patients who were rechallenged with zolmitriptan after SSRI treatment the AUC GH response was significantly attenuated: 11.2 ± 5.9 compared to –6.1 ± 2.9 mIUxHr/l, p=0.005, (mean ± SEM peak GH 14.2 ± 4.8 compared to 1.9 ± 0.7 mIU/l, p=0.024; Fig 5.4). The repeated measures ANOVA showed no main effect of occasion (pre or post treatment) (F=4.37; df 1, 11; p=0.061), but a significant main effect of time (F=3.18; df 14, 154; p<0.001) and a significant occasion by time interaction (F=5.32; df 14, 154; p<0.001) (Fig 4.4). The mean ± SEM AUC of zolmitriptan levels was not significantly altered by SSRI treatment (9.8 ± 1.4 ngxHr/ml compared to 10.8 ± 1.4 ngxHr/ml, p= 0.45). The mean fall in HAM-D was 7.9. Five of the patients were responders as judged by a 50 per cent decline in HAM-D score. There was no correlation between fall in HAM-D and change in peak or AUC GH response to zolmitriptan (r=0.18, p=0.57 and r=-0.10, p=0.98 respectively).

Of this treated group, 5 subjects had major depression with melancholia, with responses similar to the whole group. Their mean ± SEM peak GH response fell from 6.6 ± 1.3 to 0.8 ± 0.7 mIU/l (p= 0.019), and HAM-D scores fell a mean of 5.2 points, although only one subject had at least 50% decline in HAM-D scores.

In the group of healthy controls who were tested on two occasions there was no significant difference between the AUC GH response to the first zolmitriptan challenge and the second: 15.7 ± 10.1 and 19.8 ± 14.2 mIUxHr/l, p=0.42 (mean ± SEM peak GH 16.9 ± 7.6 compared to 17.3 ± 8.9 mIU/l, p=0.81). The repeated measures ANOVA showed no main effect of occasion (F=3.49; df 1, 5; p=0.12), no effect of time (F=1.06; df 14, 70; p=0.40) and no significant occasion by time interaction (F=0.64; df 14, 70; p=0.82) (Fig 5.5).

Table 5.1 Mean ± SEM baseline CORT, peak and AUC GH, AUC plasma zolmitriptan level and HAM-D score in three groups of subjects undergoing zolmitriptan challenge.

	Controls n = 23	**Depressed Non melancholic n = 14**	**Depressed Melancholic n = 9**
Baseline CORT (μg/100ml)	23.8 ± 2.5	23.8 ± 2.0	20.8 ± 2.6
Baseline GH (mIU/l)	3.1 ± 0.9	4.7 ± 1.1	4.3 ± 1.1
AUC GH (mlU x Hr/l)	15.8 ± 4.0	10.5 ± 7.3	-1.2 ±3.5 *
Δ Peak GH (mlU/l)	16.3 ± 3.4	16.5 ± 5.2	4.5 ± 1.3 *
AUC Zolmitriptan level (ng x Hr/ml)	10.3 ± 1.5	9.8 ± 1.5	10.4 ± 1.9
HAMD score		24 (± 1.3)	26 (± 2.4)

* $p < 0.05$ (independent samples t test in comparison to individually matched control group).

Figure 5.1 Peak GH response over baseline following zolmitriptan (5mg orally) in depressed patients and controls. The responses of the patients with melancholic syndrome are significantly less than the controls ($p<0.05$, unpaired t test).

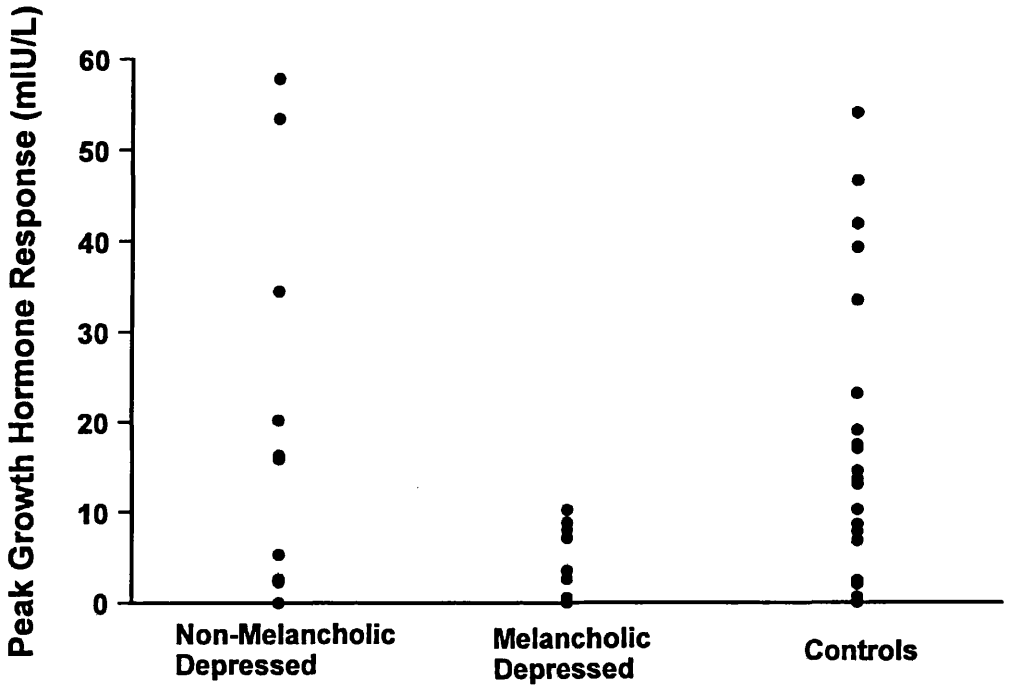


Figure 5.2 Mean ± SEM GH response following zolmitriptan 5mg at time '0' in 14 non melancholic depressed patients (open inverted triangles) compared with matched controls (open squares). No significant difference is observed between the two groups (ANOVA).

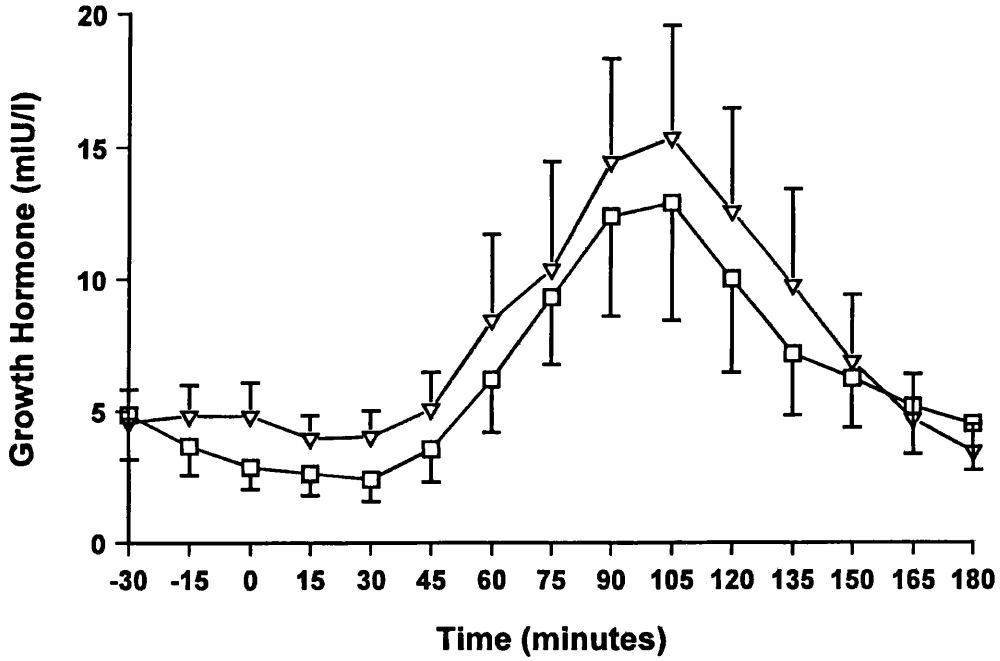


Figure 5.3 Mean ± SEM GH response following zolmitriptan (5mg) at time '0' in 9 melancholic depressed patients (closed inverted triangles) compared with matched controls (open squares). **p<0.01 (Fischer's test of least significant difference).

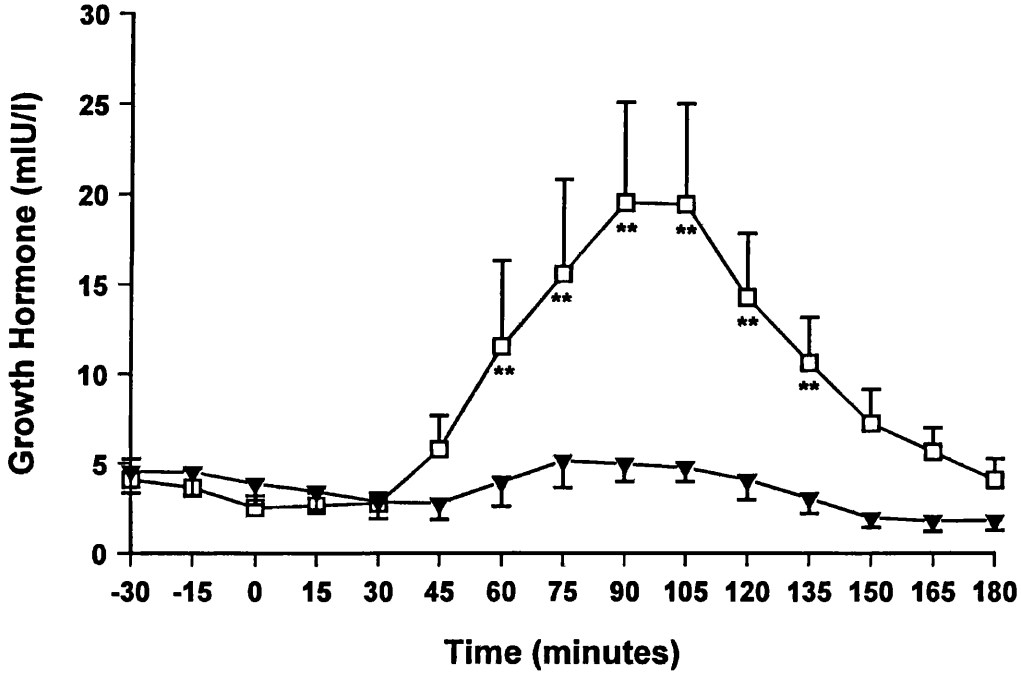


Figure 5.4 Mean ± SEM GH concentration in 12 depressed patients tested on two occasions, before (open inverted triangles) and after (closed inverted triangles) SSRI treatment (interval of mean 33.9 days). Patients received zolmitriptan (5mg orally) at time '0'. *p<0.05, **p<0.01 (Fischer's test of least significant difference).

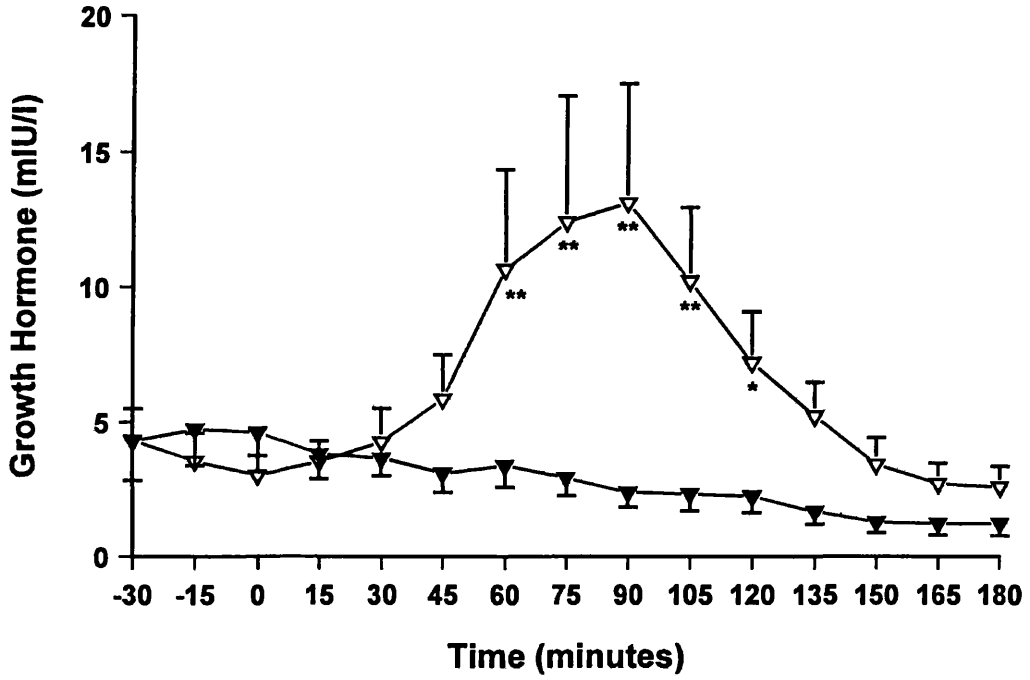
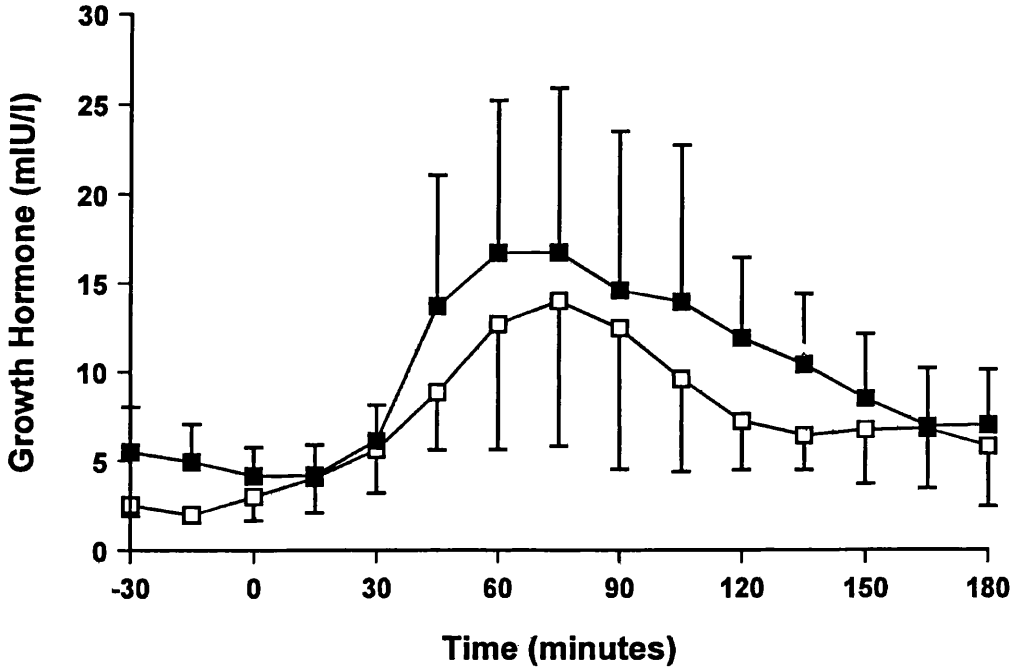


Figure 5.5 Mean ± SEM GH concentration in 6 healthy subjects tested on two occasions (mean 28.2 days interval), before (open squares) and after (closed squares). Subjects received zolmitriptan (5mg orally) at time '0'. No significant difference between the two groups is observed (ANOVA).



DISCUSSION

These findings indicate that the GH response to zolmitriptan is impaired in patients with major depression and melancholia. In patients treated with SSRIs, the GH response to zolmitriptan was markedly attenuated suggesting an adaptive desensitisation of the 5-HT mechanisms involved in zolmitriptan induced GH release. In normal subjects rechallenged with zolmitriptan (without SSRI administration), no such desensitisation was observed.

This blunted GH response to zolmitriptan in depressed subjects could be explained by high CORT levels, as seen in some depressed subjects (Carrol et al. 1981), inhibiting the secretion of GH (Goodwin et al. 1992). There was however, no significant difference between baseline CORT in patients and controls (including melancholic patients), excluding the possibility of this effect. GH responses in depressed subjects do not appear to be blunted in general, as the GH response to GnRH (Amsterdam et al. 1982) and buspirone in melancholic subjects (Cowen et al. 1994) is not different from healthy controls.

The finding of blunted GH responses to zolmitriptan in patients with melancholic depression are similar to those of challenge studies using 5-HT_{1A} receptor ligands where blunted endocrine and thermic responses are particularly apparent in melancholic subjects (Cowen et al. 1994; Lesch 1992). Two other studies have reported blunted GH responses to sumatriptan in patients with major depression (Cleare et al. 1998b; Yatham et al.

1997a), giving greater confirmation that 5-$HT_{1B/D}$ receptor agonists have this effect, although results of sumatriptan challenge are difficult to interpret because of the number of healthy subjects who fail to produce useful GH responses (as discussed above). Yatham et al. (1997b) also found a blunted GH response to sumatriptan in patients with SAD in comparison to controls, implying that this disorder (and possible depressive disorder subtype) is also associated with impaired 5-$HT_{1B/D}$ receptor function.

These findings from challenge studies with directly acting 5-HT receptor agonists in depressed patients differ from those obtained with the 5-HT precursor, L-TRP, where endocrine responses are blunted in both melancholic and non melancholic subjects (Cowen and Charig 1987). This suggests that whereas depressed patients in general may have impaired presynaptic release of 5-HT, patients with melancholia may additionally exhibit impaired sensitivity of postsynaptic 5-HT receptors. This would be expected to result in a larger decrement in overall 5-HT neurotransmission. The findings of a reduced PRL response to clomipramine (Anderson et al. 1992b) and fenfluramine (Newman et al. 1998) in melancholia could also reflect such a further reduction in overall 5-HT neurotransmission. This could increase the severity of depressive symptoms or induce features particularly observed with the melancholic syndrome. Symptoms such as significant sleep disturbance, weight loss and psychomotor agitation (see appendix) are known to be particularly influenced by 5-HT (as discussed in chapter 1) which would fit with this hypothesis. The finding of a reduced placebo response in melancholic subjects (Peselow et al. 1992) and some reports of a greater response rate to antidepressant treatment (eg. Heiligenstein et al. 1994) also appears consistent with a greater overall

reduction in 5-HT neurotransmission. Reduced plasma TRP has also been associated with melancholia (Maes et al. 1987b), potentially reducing brain TRP availability for subsequent 5-HT production, but reduced plasma TRP is also observed with weight loss (Anderson et al. 1990b), confounding this finding.

The possible contribution of impaired postsynaptic 5-HT_{1D} receptor sensitivity to the depressive syndrome is unclear because there are few known functional correlates of 5-HT_{1D} receptor activation in animals or humans. High densities of postsynaptic 5-HT_{1D} receptors are found in basal ganglia, which might indicate involvement in depression associated psychomotor changes, again more likely with melancholic severity (Barnes and Sharp 1999) and consistent with the above hypothesis. In addition, 5-HT_{1D} receptors in cortex modulate glutamate release, which might suggest a role in the memory impairments experienced by depressed patients (Maura et al. 1998).

Recent weight loss is a feature of the melancholic syndrome and can influence neuroendocrine challenge results as described in chapter 2. Only one subject reported weight loss in this study (who was non melancholic), and their peak GH response was greater than the mean, perhaps consistent with previous findings with the L-TRP challenge (Goodwin et al. 1987). To clarify the influence of weight loss on this challenge, the study would need to be repeated in a group with such attributes. Weight loss does not appear to be a significant confounding factor on the results of this study.

In patients treated with SSRIs the GH response to zolmitriptan was markedly decreased.

This suggests that SSRIs produce a downregulation of postsynaptic 5-HT_{1D} receptors. There is also evidence that repeated SSRI treatment can downregulate postsynaptic 5-HT_{1A} and 5-HT_2 receptors, in both healthy subjects and depressed patients (Lesch 1992; Quested et al. 1997; Sargent et al. 1998a). The only other study to examine 5-$HT_{1B/1D}$ function following SSRI treatment was by Wing et al. (1996). This study examined the neuroendocrine response to acute sumatriptan in healthy subjects before and after 16 days of paroxetine treatment. Despite finding an initial reduction in PRL and temperature, there was no change in these responses following treatment. GH responses were not reported. If the PRL and temperature responses are mediated by presynaptic receptors, as discussed above, it implies that presynaptic 5-$HT_{1B/1D}$ function is not altered by SSRI treatment, whereas the blunted GH response to zolmitriptan following SSRIs possibly indicates reduced postsynaptic 5-HT function. Chronic SSRI treatment has been shown to enhance PRL levels in humans however (Sargent et al. 1998a) which may counteract any further blunting of the PRL response. The function of 5-$HT_{1B/1D}$ receptors may also vary between healthy and depressed groups, which would indicate a need to repeat this sumatriptan challenge post SSRI treatment in a depressed group to clearly examine any effect. Davidson and Stamford (2000) examined the sensitivity of 5-HT_{1B} and 5-HT_{1D} autoreceptor function in rats following 21 days of paroxetine treatment, and found no change in sensitivity of 5-HT_{1D} receptors, but densensitisation of 5-HT_{1B} receptors only when a 5-HT_{1A} antagonist was added. This is consistent with the findings of Wing et al. and is also consistent with the possible beneficial effect of adding pindolol to an SSRI. Yatham et al. (1997b) examined the effects of light therapy on the GH response to sumatriptan in a group of patients with SAD, finding a resolution of the

previously blunted GH response with treatment, implying that light therapy has a different mode of action (certainly as far as 5-$HT_{1B/1D}$ receptors are involved) than SSRIs.

Despite these effects of SSRIs on postsynaptic 5-HT receptors, it is likely that overall, SSRIs increase 5-HT neurotransmission because procedures such as TRP depletion, which lower synaptic 5-HT availability, reverse the antidepressant effects of SSRIs in recovered patients (Delgado et al. 1999). From this point of view the downregulation of postsynaptic receptors seen during SSRI treatment is presumably an adaptive response which limits, but does not remove, the increase in 5-HT neurotransmission produced by chronic 5-HT reuptake blockade and increased levels of synaptic 5-HT. This may explain the apparently paradoxical finding that some unmedicated patients with major depression have impaired postsynaptic 5-HT_{1D} receptor function, which is further diminished by effective treatment. This postsynaptic downregulation occurs at the time of onset of antidepressant effect so may be crucial to this (Stahl 1998). It could however not be relevant to the therapeutic effect and just be a marker of some other intracellular activity. It is also possible that the magnitude of postsynaptic downregulation is not equal between receptor groups and the relative neurotransmission at one receptor type may actually be increased due to a relatively greater downregulation of other receptors. Such increased specific receptor mediated neurotransmission despite downregulation, possibly most relevant at 5-HT_{1A} receptors (as implicated by an enhanced PRL response to TRP following SSRI treatment), could therefore equate with antidepressant activity. Consistent with this, impaired 5-HT_{1A} receptor function in depression appears to resolve following treatment as discussed later (Smith et al. 2000).

CHAPTER 6

THE CITALOPRAM CHALLENGE IN HEALTHY SUBJECTS

THE NEUROENDOCRINE EFFECTS OF CITALOPRAM IN HEALTHY SUBJECTS

INTRODUCTION

Previous chapters have discussed the use of pharmacological probes to measure brain 5-HT receptor function via changes in the plasma concentration of certain anterior pituitary hormones. Progress in this field was marred by the withdrawal of d-fenfluramine from the market in 1999, due to cardiotoxicity (Baumann et al. 2000). This further prompted the search for a neuroendocrine probe that can increase presynaptic serotonergic function in humans in a reliable and well tolerated manner, and has led to the examination of other available drugs that may act in this way, such as citalopram (Hyttel 1982; Joubert et al. 2000).

Citalopram's chemical structure is shown in figure 6.1. Citalopram is indicated for the treatment of depressive disorder in acute and maintenance phases and also for panic disorder. The recommended initiation dose for the treatment of depression is 20mg, increased to a maximum of 60mg. Adverse reactions are typically mild to moderate and usually occur within the first 2 weeks of treatment. The most common adverse reactions, above those seen at placebo rates, are nausea, somnolence, dry mouth, increased sweating and tremor (Electronic Medicines Compendium).

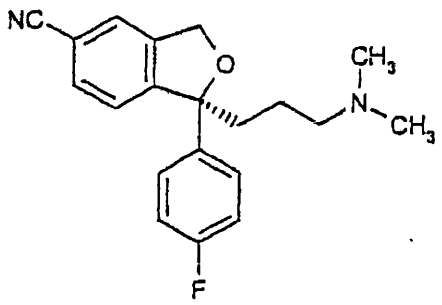


Fig 6.1 The chemical structure of citalopram.

Citalopram, as with other SSRIs, preferentially inhibits 5-HT reuptake at the presynaptic membrane. In vitro animal studies have shown a 3400 fold preference for blocking 5-HT over NA uptake (with even greater preference over DA uptake) (Hyttel et al. 1995). It is the most selective of currently available SSRIs, lacking inhibition at any other receptor sites. The highest affinity at other receptors, but at micromolar levels is at α_1 and histamine H_1 receptors (Hyttel et al. 1995).

Citalopram is a racemic mixture with pharmacological activity residing in the (+)-enantiomer with the 1-(S) absolute configuration (Hyttel et al 1995). This active enantiomer of citalopram has recently been released for clinical use (Owens et al. 2001).

Citalopram is highly lipophillic and is readily absorbed from the gastrointestinal tract when administered orally (time to peak levels is approximately 3 hours). The absolute

bioavailability of citalopram tablets is 80% of the intravenous dose (Baumann and Larsen 1995). Citalopram shows a biphasic elimination following a single dose, with a terminal half life of approximately 36 hours (Kragh-Sorensen et al. 1981).

Citalopram is metabolised to demethylcitalopram, didemethylcitalopram, citalopram-N-oxide and the deaminated propionic acid derivative. Citalopram is the predominant compound in plasma after a single dose, with the metabolites appearing at much lower levels (Baumann and Larsen 1995). It is 75% excreted in urine. The metabolites are less potent SSRIs and enter the brain less readily, so do not contribute to the overall pharmacological effect (Hyttel and Larsen 1985).

Intravenous citalopram is preferable in a neuroendocrine paradigm to ensure greater bioavailability and a theoretically more rapid hormonal response in comparison to the oral preparation.

A previous study by Seifritz et al. (1996) in healthy humans showed that a 20mg dose of intravenous citalopram robustly increased plasma PRL and CORT concentrations as also observed in rats (Meltzer et al. 1981). Seifritz et al. (1996) demonstrated that citalopram at this dose had no effect on body temperature or heart rate. There were draw backs in using citalopram as a neuroendocrine probe at this dose, however, as there were also significant increases in subjective ratings of distress, restlessness, sickness, nausea and dizziness. These subjective experiences could themselves influence the neuroendocrine response (Cowen 1998), and recruitment of subjects for such a drug challenge could be

problematic, diminishing the usefulness of this test.

The following experiment aimed to assess the neuroendocrine effects of two lower doses of intravenous citalopram (5mg and 10mg) to examine whether these doses would be sufficient to elevate PRL and CORT relative to placebo, in the absence of such adverse effects.

METHODS

Subjects

Eleven healthy volunteers (6 male, 5 female) mean age 37.2 years (range 21 to 62 years) and mean weight 73.6kg (range 56.9kg to 93.4kg) were recruited as described in Chapter 3. They had no significant physical illness and had been free of medication for at least three months.

Neuroendocrine tests

Subjects were tested on three occasions. They attended the laboratory at midday and received, over 30 minutes, an infusion of (a) citalopram 5mg, (b) citalopram 10mg, or (c) placebo, in a double blind, random order design. The infusion was prepared as discussed in Chapter 3. Further blood sampling was carried out at the end of the infusion and at 15 minute intervals thereafter for the next 150 minutes. The mean interval between the tests

was 11 days (range 5 to 44 days). Female subjects were tested in the early follicular stage of the menstrual cycle. Subjects were monitored by nursing staff throughout the testing sessions and questioned every 30 minutes about adverse effects.

Biochemical measurements

Assay methods are described in Chapter 3. GH responses were not examined as similar pharmacological challenges, such as clomipramine and fenfluramine, have no effect on GH (Laakmann 1990). Plasma citalopram level assays were not undertaken but would have been ideally, to control for pharmacokinetic variation between subjects.

Analysis of results

Hormone responses to citalopram and placebo were calculated as AUC using the trapezoid method with subtraction of baseline secretion extrapolated from time '0'. The AUC values were analysed with a one way ANOVA with 'treatment' (citalopram vs placebo) as the within subject factor. Post hoc comparisons between AUC values were compared using t tests for different treatment arms. Individual time point data were analysed using a two way ANOVA with 'occasion' (citalopram at each dose vs placebo) and 'time' as the main within subject factors.

RESULTS

Citalopram treatment elevated plasma PRL in a dose related manner (Table 6.1, Fig 6.2).

The one way ANOVA for PRL AUC showed a significant effect of treatment (F= 7.62; df 71, 17.09; p= 0.006). Post hoc testing showed significant differences in the AUC PRL response between placebo (mean AUC mIUxHr/l ± SEM was -64.1 ± 36.9) and citallopram 5mg (37.1 ± 23.6) (comparison p=0.010) and between placebo and citalopram 10mg (62.4 ± 25.8) (p=0.014), but not between the 5mg and 10mg doses of citalopram (p= 0.36).

The two way ANOVA for citalopram 10mg vs control showed a significant effect of occasion (F= 9.89; df 1, 10; p=0.010), a significant effect of time (F= 3.67; df 2.87, 28.74; p=0.025), and a significant occasion by time interaction (F= 4.35; df 3.75, 37.45; p=0.006). The ANOVA for citalopram 5mg vs control was not significant for occasion (F= 0.85; df 1, 10; p=0.38), but a significant effect of time (F= 3.17; df 2.94, 29.37; p=0.04) and of the occasion by time interaction was observed (F= 5.09; df 7.35, 73.45; p<0.001).

Citalopram treatment also elevated plasma CORT in a dose related manner (Table 6.1, Fig 6.3). The one way ANOVA for CORT AUC showed a significant effect of treatment (F= 5.81; df 20.00, 2.00; p= 0.010). Post hoc tests showed no significant difference in the AUC CORT response between placebo (with mean AUC mcgxHr/100ml ± SEM of -11.6 ±3.0) and citalopram 5mg (-6.3 ± 3.5) (comparison p=0.21) but a significant difference between citalopram 10mg (3.1 ±4.9) and placebo (p=0.005). No significant difference between the 5mg and 10mg doses of citalopram was observed (p= 0.090).

The two way ANOVA for citalopram 10mg vs control showed a significant effect of occasion ($F= 5.96$; df 1, 10; $p=0.035$), of time ($F=11.29$; df 3.35, 33.48; $p<0.001$), and of the occasion by time interaction ($F= 6.53$; df 4.05, 40.48; $p<0.001$). The ANOVA for citalopram 5mg vs control was not significant for occasion ($F= 0.2.19$; df 1, 10; $p=0.17$), but significant for time ($F= 9.26$; df 24.21, 2.42; $p=0.001$) and not significant for the occasion by time interaction ($F= 0.33$; df 22.86, 2.29; $p=0.75$).

Gender was not a significant covariate in the two way ANOVAs for citalopram 10mg vs control for either PRL ($p=0.46$) or CORT ($p=0.83$) data.

VAS scales for subjective ratings were unfortunately not completed for all subjects but it was noted when subjects felt significantly different during the challenge. Citalopram 10mg IV was well tolerated with no subjects vomiting throughout the study. No adverse effects were reported after placebo but after 5mg of citalopram 4 subjects reported drowsiness and 1 nausea. After 10mg citalopram 8 subjects reported drowsiness, 4 nausea and 1 light headedness. These effects occurred within the first 60 minutes of the start of the infusion and were of brief duration (1 hour at the most) and not severe.

These results are discussed at the end of this chapter.

Table 6.1

Mean ± SEM AUC responses for PRL and CORT following citalopram 10mg IV, citalopram 5mg IV and placebo infusion in 11 healthy subjects tested on 3 occasions.

	Citalopram 10mg	Citalopram 5mg	Placebo
PRL AUC Response mIUxHr/l	62.4 ± 25.8*	37.1 ± 23.6**	-64.1 ± 36.9
CORT AUC Response mcgxHr/l00ml	3.1 ± 4.9**	-6.3 ± 3.5	-11.6 ± 3.0

*p<0.05 ** p<0.01 for paired t tests in comparison with placebo values

Figure 6.2 Mean ± SEM plasma PRL (measured as change from baseline) in 11 healthy subjects who were tested on 3 separate occasions receiving (a) placebo (open squares), (b) citalopram 5mg (closed triangles) and (c) citalopram 10mg (closed circles), infused intravenously over 30 minutes starting at time '0'.

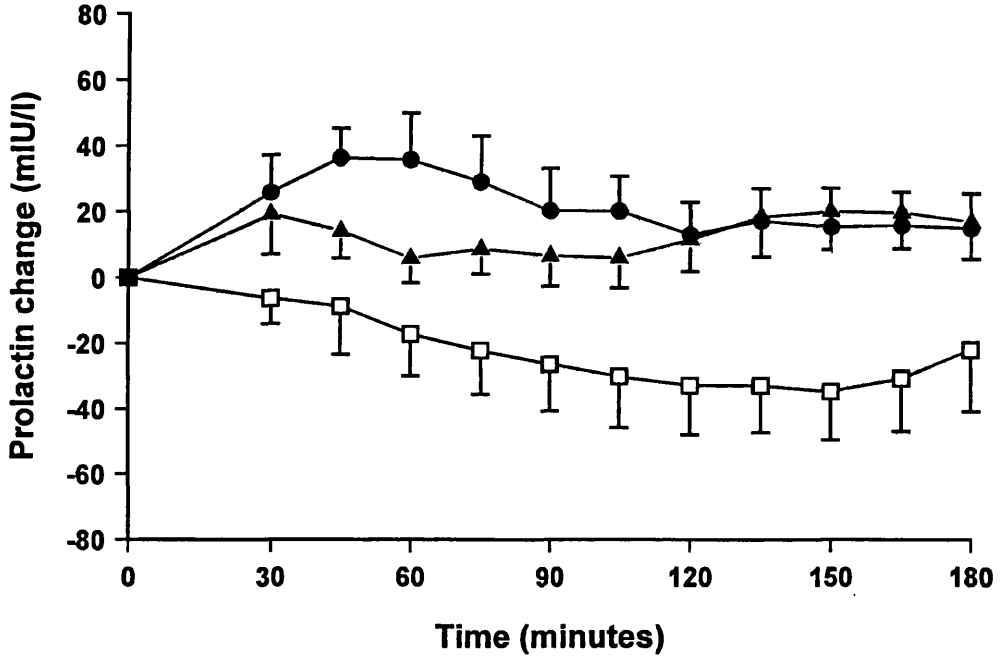
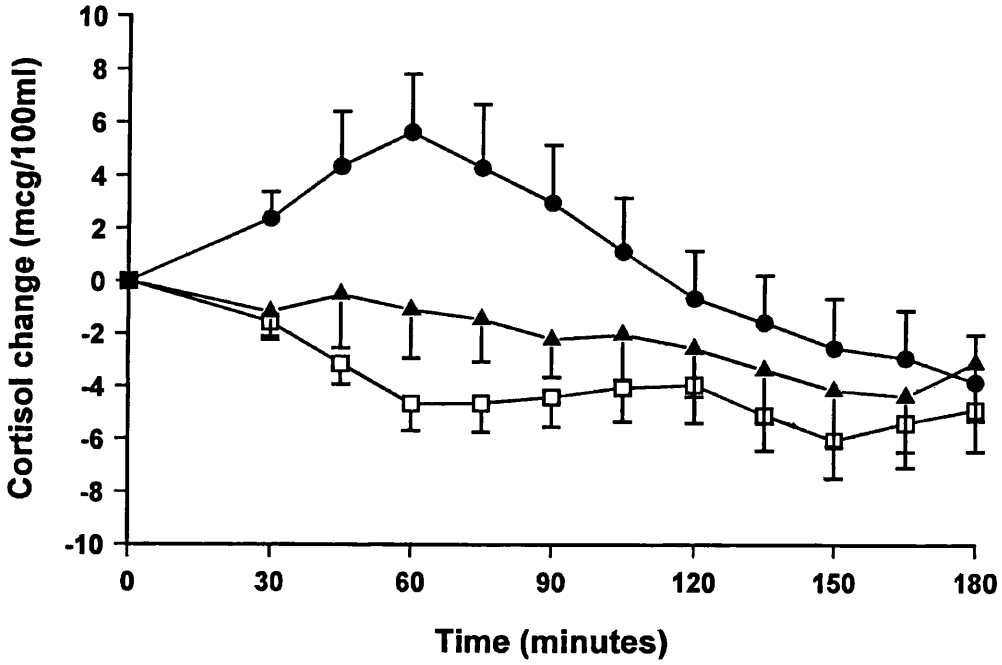


Figure 6.3 Mean ± SEM plasma CORT (measured as change from baseline) in 11 healthy subjects who were tested on 3 separate occasions receiving (a) placebo (open squares), (b) citalopram 5mg (closed triangles) and (c) citalopram 10mg (closed circles), infused intravenously over 30 minutes starting at time '0'.



DEFINING THE MODE OF ACTION OF THE NEUROENDOCRINE RESPONSE TO INTRAVENOUS CITALOPRAM

THE EFFECT OF CYPROHEPTADINE ON THE NEUROENDOCRINE RESPONSE TO CITALOPRAM

INTRODUCTION

Having established that citalopram enhances PRL and CORT release, the receptor mediated mode of action of this effect was investigated. As discussed in Chapter 1, d-fenfluramine reliably induces an increase in PRL, ACTH, and CORT which appears to be mediated mainly via activation of 5-$HT_{2A/C}$ receptors (Albinsson et al. 1994; Goodall et al. 1993; Park and Cowen 1995). D-fenfluramine has properties similar to citalopram in that it can block the presynaptic reuptake of 5-HT (although differs from SSRIs in causing 5-HT release). I therefore hypothesised that the PRL and CORT responses to citalopram were also mediated by 5-HT_2 receptors.

Cyproheptadine hydrochloride (Hoyer 1988) was the 5-HT_2 antagonist used for this study purely due to its availability. Cyproheptadine is licensed for use as an antiallergic-antipruritic and in the treatment of migraine and vascular type headache. The oral therapeutic dose range is from 4mg to 20mg a day for antipruritic effect and up to 8mg in

a 6 hour period for migraine. Its antiallergic therapeutic action is mediated through histamine receptors and antimigraine action via 5-HT receptors (Lehrer 2004). Cyproheptadine is contraindicated in asthma attacks, breast feeding, gut stenosis, urinary retention, glaucoma, with MAOIs, and in the elderly. It is well tolerated with the only common side effect being sedation. Peak plasma levels occur 6 hours following oral administration. Elimination is primarily in urine as a quaternary ammonium glucuronide conjugate, but it is also found in stools metabolised and unmetabolised (Electronic medicines compendium).

Cyproheptadine primarily acts as an antagonist at histamine and 5-HT_2 receptors (Stone et al.1961). It also has activity as an antagonist at ACh receptors, and much less so at DA and α receptors (Stone et al. 1961; Williams and Martin 1982). Lewis and Sherman (1985) used cyproheptadine pretreatment (0.06 mg/kg orally every 6 hours for 2.5 days) to successfully blunt the PRL response to fenfluramine. Cyproheptadine did not influence basal PRL or GH levels in this study. Sharpley et al. (1990) also used cyproheptadine to examine the influence of 5-HT_2 receptor antagonism on slow wave sleep. They used 4mg orally, a dose equivalent to that used by Lewis and Sherman, which successfully increased slow wave sleep and reduced REM sleep in healthy volunteers. The dose of 4mg was therefore chosen for this study as it appears to effectively block 5-HT_2 receptors, has no apparent influence on basal PRL levels (Lewis and Sherman 1985) and is likely to only have significant activity at 5-HT and histamine receptors (Stone et al.1961). Histamine antagonism alone does not appear to influence PRL secretion (Rivier and Vale 1977), although histamine agonism can enhance PRL secretion (Freeman et al.2000).

ACh is inhibitory to PRL release (Freeman et al. 2000) so cyproheptadine could theoretically enhance PRL secretion. Cyproheptadine can reduce ACTH secretion and plasma CORT levels (Electronic medicines compendium).

METHODS

Subjects

Six healthy subjects were recruited as described in Chapter 3, consisting 5 male and 1 female of mean age 40.7 years (range 21 to 52 years) and mean weight 75.2kg (range 59 to 88kg). This number of subjects is low although was adequate to show a clear effect of cyproheptadine on the PRL response to fenfluramine in the Lewis and Sherman (1985) study.

Neuroendocrine tests

Subjects were tested on two occasions, receiving intravenous citalopram (10mg over 30 minutes) on both occasions. Six hours prior to infusion subjects took either cyproheptadine, 4mg orally (as used by Sharpley et al. 1990), or placebo in a double blind, random, balanced order design. This ensured that on the cyproheptadine test day, plasma cyproheptadine was at peak level at the start of the citalopram infusion. Blood samples were taken as above. The mean interval between the tests was 7 days (range 5 to 10 days). The female subject was tested in the early follicular stage of her menstrual cycle. Subjects were monitored by nursing staff throughout the testing sessions and

questioned every 30 minutes about adverse effects.

Biochemical measurements

Assay methods are described in Chapter 3.

Analysis of results

Hormone responses were analysed using a two way ANOVA with 'occasion' (cyproheptadine vs placebo) and 'time' as the main within subject factors. The AUC values for PRL and CORT (calculated as above) were also analysed with a one way ANOVA with 'occasion' (cyproheptadine vs placebo) as the within subject factor.

RESULTS

Administration of cyproheptadine significantly lowered baseline plasma PRL and CORT concentration. For PRL, the mean ± SEM value at time '0' was 94 ± 9 mIU/L for the cyproheptadine challenge day and 109 ± 11 mIU/l for the placebo day (p=0.031). For CORT the corresponding values were 8.6 ± 2.1 mcg/100ml and 13.1 ± 2.3 mcg/100ml (p= 0.003). Despite this, cyproheptadine treatment failed to antagonise the PRL and CORT responses to citalopram, as shown in figures 6.4 and 6.5 respectively. To correct for the baseline influence on the cyproheptadine study arm, ANOVAs were undertaken on change from baseline data.

The ANOVA for PRL change from baseline, cyproheptadine vs placebo, showed no effect of occasion ($F=0.85$; df 1, 5; $p=0.40$), or time ($F=1.58$; df 1.7, 8.4; $p=0.26$), or occasion by time interaction ($F=0.62$; df 6.3, 31.7; $p=0.72$). The mean AUC PRL ± SEM response for citalopram / placebo was -2.2 ± 21.3 and for citalopram / cyproheptadine 21.5 ± 23.1 mIUxHr/l. The ANOVA for PRL AUC showed no significant effect of cyproheptadine vs placebo ($F=0.80$; df 1, 5; $p=0.41$) but with a power of 0.11 (α being set at 0.05). This demonstrates that the sample size was not large enough to show any clear difference between treatment days. PRL responses tended to be greater following cyproheptadine.

The two way ANOVA for CORT change from baseline, cyproheptadine vs placebo, showed no significant effect of occasion ($F=0.21$; df 1, 5; $p=0.67$), time ($F=2.55$; df 4.3, 21.7; $p=0.06$) or of occasion by time interaction ($F=1.92$; df 2.0, 9.9; $p=0.20$).
The mean AUC CORT ± SEM response for citalopram/placebo was 7.8 ± 5.7 mIU hr/l and for citalopram/cyproheptadine 8.5 ± 7.2 mIU hr/l (one way ANOVA comparison, $F=0.05$; df 1, 5; $p=0.84$). The power of this AUC ANOVA was very low at 0.05 (α being set at 0.05), again demonstrating that the sample size was not large enough to show any clear difference between treatment days

As with the previous study, VAS scales for subjective ratings were unfortunately not completed for all subjects. No adverse experiences were reported however.

The results of this study are discussed at the end of this chapter.

Fig 6.4 Mean ± SEM plasma PRL response in 6 healthy subjects challenged twice with citalopram 10mg IV in addition to placebo orally (closed circles) or cyproheptadine 4mg orally (closed stars). No significant difference is observed (ANOVA).

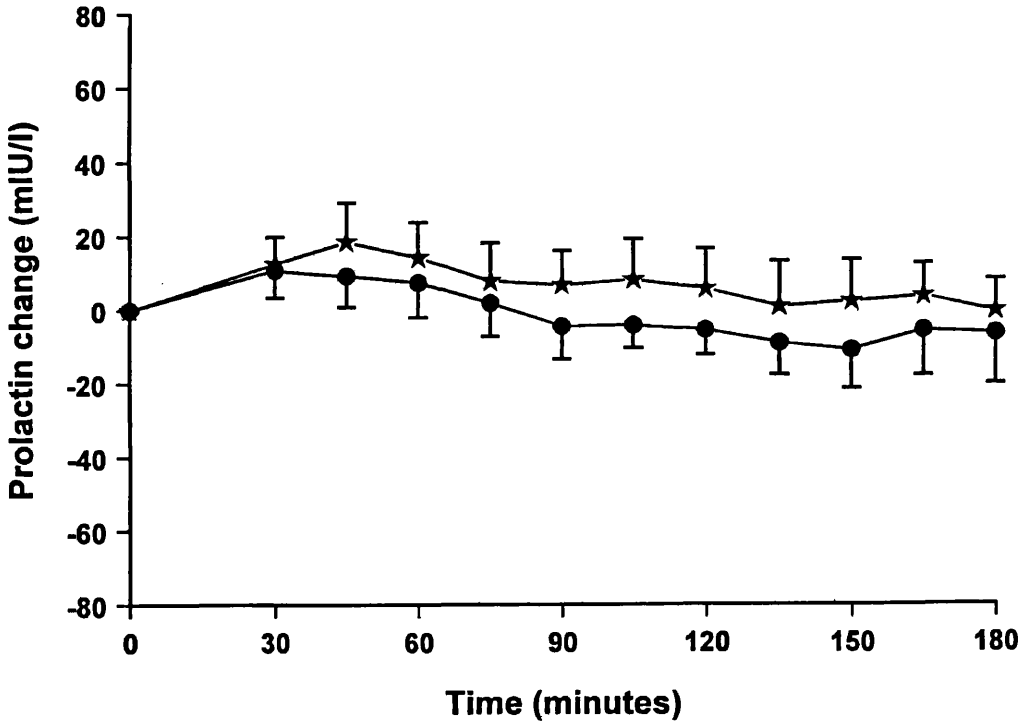
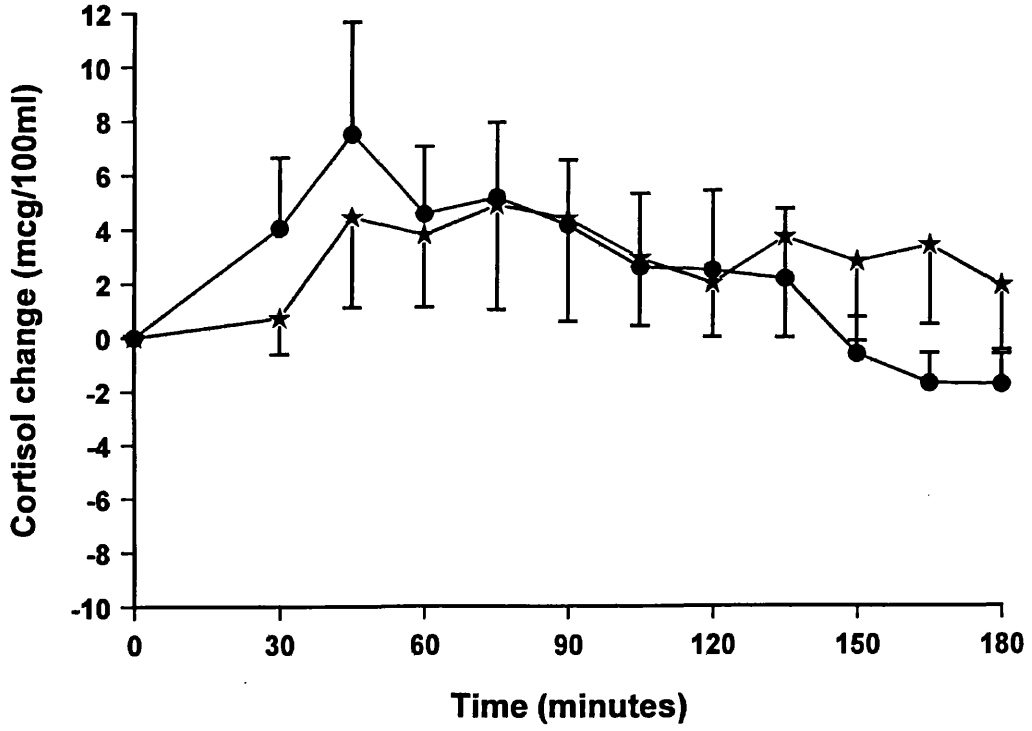


Fig 6.5 Mean ± SEM plasma CORT response in 6 healthy subjects challenged twice with citalopram 10mg IV in addition to placebo (closed circles) or cyproheptadine (closed stars). No significant difference is observed (ANOVA).



THE EFFECT OF PINDOLOL AND PENBUTOLOL ON THE NEUROENDOCRINE RESPONSE TO CITALOPRAM

INTRODUCTION

The previous study using cyproheptadine was unable to clarify whether the increase in PRL and CORT following citalopram infusion is likely to be mediated by 5-HT_2 receptors. Other neuroendocrine probes that influence presynaptic function (in common with citalopram) include L-TRP. The PRL increase following L-TRP infusion may be mediated via activation of postsynaptic 5-HT_{1A} receptors as it is antagonised by pindolol (Smith et al. 1991, as discussed in Chapter 2). It is therefore possible that the neuroendocrine effects of citalopram are also mediated by 5-HT_{1A} receptors.

The effects of pindolol and penbutolol on the hormone responses to citalopram were examined. Pindolol has classically been used to examine the involvement of 5-HT_{1A} receptors in neuroendocrine probe studies (eg. Smith et al. 1991), but this drug has partial agonist properties which therefore makes findings difficult to interpret (Hjorth and Carlsson 1986). Penbutolol was also used in this study as a potential antagonist of the neuroendocrine effects of citalopram, as it has clear 5-HT_{1A} receptor antagonist properties in animal models (Hjorth 1992), and functionally has acted more potently than pindolol at this receptor (Gartside 1999).

Penbutolol is a noradrenergic β_1 and β_2 receptor antagonist (with 4 times greater potency than the commonly used propranolol) and a 5-HT_{1A} receptor antagonist. It is indicated for the treatment of arterial hypertension, with a dose range of 20 to 80mg daily. This effect is achieved through central and peripheral β receptor blockade and related reduction in renin secretion. Penbutolol is well tolerated up to 80mg with most common side effects of headache, dizziness, fatigue and nausea reported infrequently (in less than 8% of subjects). Contraindications are cardiogenic shock, sinus bradycardia, atrioventricular conduction block, obstructive pulmonary disease and asthma. Metabolism of penbutolol is through conjugation, oxidation and subsequent urinary excretion. The oxidative metabolite has some but much less affinity for β receptors than the parent compound. Following oral use, penbutolol is rapidly and completely absorbed. Peak blood levels occur 2 to 3 hours following ingestion. Plasma half life is approximately 5 hours (Schwarz Pharma 1995).

Pindolol is also a noradrenergic β_1, β_2 and 5-HT_{1A} receptor antagonist, indicated for the treatment of arterial hypertension and the prophylactic treatment of angina pectoris. The dose range for both indications is between 10mg and 45mg per day in divided doses. Pindolol has intrinsic sympathomimetic activity which provides the heart with some basal stimulation, similar to normal resting sympathetic activity. Contraindications are as for penbutolol. Similarly, pindolol is well tolerated within therapeutic doses with most common but infrequent side effects as for penbutolol. Up to 40% of pindolol is excreted unchanged in the urine, while the rest is excreted by liver and kidney as inactive metabolites. Following oral use, pindolol is rapidly and more than 95% absorbed, with

bioavailability of 87%. Peak blood levels occur within 1 hour of ingestion. Plasma half life is approximately 5.5 hours (Electronic medicines compendium). Anderson and Cowen (1992a) used a predosing regime of pindolol 80 mg over 3 days to successfully blunt the GH and hypothermic responses to buspirone, and Smith et al. (1991) used 40mg over 2 days to successfully blunt the PRL and GH responses to L-TRP.

In a PET scanning study, concurrent to this study, Rabiner et al. (2000) examined the binding of pindolol and penbutolol in human brain in vivo. Both drugs exhibited occupancy effects at 5-HT_{1A} receptors, with higher binding at higher drug plasma levels. Pindolol displayed a preferential occupancy of the presynaptic 5-HT_{1A} receptor following a single dose of 10mg (which disappeared at 20mg), whereas penbutolol did not show any preferential pre or post synaptic occupancy at any dose tested (upto 80mg). Both pindolol 10mg and penbutolol 80mg were well tolerated, and therefore were used in the current neuroendocrine challenge. Using these findings, I hypothesised that pindolol 10mg would enhance the PRL and CORT responses to citalopram, due to preferential autoreceptor binding, and penbutolol 80mg would diminish these responses, if postsynaptic 5-HT_{1A} receptors were involved in mediating the endocrine response to citalopram.

METHODS

Subjects

Ten healthy subjects were recruited as described in Chapter 3, (5 male, 5 female) with mean age 36 years (range 22 to57 years), and mean weight 69.5kg (range 56.9 to 80.0kg). Two of the female subjects were postmenopausal, one of whom was taking constant dose hormone replacement therapy.

Neuroendocrine tests

Subjects were tested on three occasions, receiving intravenous citalopram (10mg over 30 minutes) on all occasions. On one of these occasions subjects received pindolol 10mg orally, on another penbutolol 80mg orally and on the other occasion, placebo in a double blind, randomised, balanced order design. Pindolol was given 2 hours before citalopram infusion and penbutolol was given 3 hours before infusion, to ensure peak plasma levels at time '0' (t_{max} of pindolol and penbutolol in plasma being 1 to 2 hours and 1 to 3 hours respectively, Sweetman 2002). Giving pindolol or penbutolol at these differing times does influence blinding, but this was improved by administering the placebo at either 2 or 3 hours before time '0' in a similarly random manner. Blood samples were taken as above. The mean interval between the tests was 11 days (range 3 to 22 days). Female subjects were tested in the early follicular stage of the menstrual cycle. Subjects were monitored by nursing staff throughout the testing sessions and questioned every 30 minutes about adverse effects.

Biochemical measurements

Assay methods are described in Chapter 3.

Analysis of results

PRL and CORT responses were initially analysed using a one way ANOVA on AUC data with 'treatment' (pindolol/penbutolol or placebo) as the within subject factor. Sub analyses of pindolol vs placebo and penbutolol vs placebo were undertaken using paired t tests. Two way ANOVAs with 'occasion' (pindolol vs placebo or penbutolol vs placebo) and 'time' as the main within subject factors were also examined.

RESULTS

A female subject was excluded from further analysis due to vomiting at 60 minutes following the start of citalopram infusion during one neuroendocrine challenge (later found to be the pindolol test day). No other adverse feelings were reported by subjects but ideally this should have been recorded with systematic VAS measures. A further female subject was excluded due to a large peak in PRL (>500mIU/l) during one session (placebo arm). For clarity, the results are summarised in Table 6.2 and shown in figures 6.6 and 6.7.

Mean baseline (time '0') PRL ± SEM on placebo, pindolol and penbutolol test days were 158 mIU/L ± 20, 123 mIU/L ± 14 and 121 mIU/L ± 17 respectively. Pindolol and

penbutolol PRL baselines were significantly lower than placebo (for t tests, p= 0.02, and p=0.01 respectively) but were not significantly different from each other (p=0.81). Mean baseline CORT ± SEM on placebo, pindolol and penbutolol test days were 9.7 mcg/100ml ± 1.7, 10.9 mcg/100ml ± 1.6 and 15.6 mcg/100ml ± 1.9 respectively. Penbutolol but not pindolol test day baseline CORT was significantly greater than placebo (for t tests, p=0.01 and p=0.46 respectively) and pindolol and penbutolol comparisons verged on significant difference (p=0.05). The following ANOVAs are undertaken using change from baseline data to take into account these baseline changes.

The one way ANOVA for PRL AUC clearly showed no effect of treatment in comparison to placebo (F=0.001; df 22, 1; p= 0.98). Mean PRL AUC in the pindolol and penbutolol treatment arms did not significantly differ from placebo, as shown in Table 6.2.

For pindolol vs placebo the two way ANOVA of PRL change from baseline response was not significant (for effect of occasion F=1.57; df 1,7; p=0.25; of time F=0.97; df 16.2, 2.3; p=0.41, and the occasion by time interaction F=1.61; df 16.1, 2.3; p=0.23).
For penbutolol vs placebo the two way ANOVA of PRL change from baseline response was similarly not significant (for effect of occasion F=1.55; df 1,7; p=0.25; of time F=1.36; df 2.7; 19.0; p=0.28, and the occasion by time interaction F=1.84; df 2.04, 2.14.27; p=0.20).

The one way ANOVA for CORT AUC also showed no effect of treatment in comparison to placebo (F=0.17; df 1, 22; p=0.69). Mean CORT AUC in the pindolol and penbutolol

treatment arms similarly did not significantly differ from placebo, as shown in Table 6.2.

For pindolol vs placebo the two way ANOVA of CORT change from baseline responses was not significant (for effect of occasion $F=0.46$; df 1,7; $p=0.52$; of time $F=1.70$; df 5.7, 39.8; $p=0.15$, and for the occasion by time interaction $F=2.21$; df 34.6, 4.9; $p=0.08$). For penbutolol vs placebo the ANOVA of CORT change from baseline responses was similarly not overall significant (for effect of occasion $F=1.17$; df 1,7; $p=0.32$; of time, significantly, $F=9.32$; df 7.4,51.8; $p<0.001$, and for the occasion by time interaction $F=1.31$; df 3.1, 21.5; $p=0.30$).

Comparisons of pindolol vs penbutolol test days were significantly different for PRL AUC: $F= 7.36$; df 1, 7; $p=0.03$, but not for CORT AUC: $F= 1.60$; df 1, 7; $p=0.25$.

These results are discussed below with the results from the other citalopram studies.

Table 6.2

Mean ± SEM baseline (time '0') and AUC responses for PRL and CORT following citalopram 10mg IV in 8 healthy subjects on 3 occasions pretreated with either, pindolol 10mg, penbutolol 80mg, or placebo.

	Pindolol (10mg)	**Penbutolol (80mg)**	**Placebo**
Baseline PRL ± SEM mIU/l	123 ± 14 *	121 ± 17 *	158 ± 20
Baseline CORT ± SEM mcg/100ml	10.9 ± 1.6	15.6 ± 1.9 *	9.7 ± 1.7
PRL AUC Response mIUxHr/l	-30.5 ± 12.5	24.8 ± 15.7	-3.6 ± 22.3
CORT AUC Response mcgxHr/100ml	1.8 ± 2.4	-4.4 ± 4.7	1.1 ± 2.3

*$p<0.05$ for paired t tests in comparison with placebo values

Fig 6.6 Mean ± SEM plasma PRL response to citalopram 10mg IV in 9 healthy subjects on 3 occasions pretreated with either placebo (closed circles), pindolol 10mg (closed triangles) or penbutolol 80mg (closed diamonds) in a randomised manner.

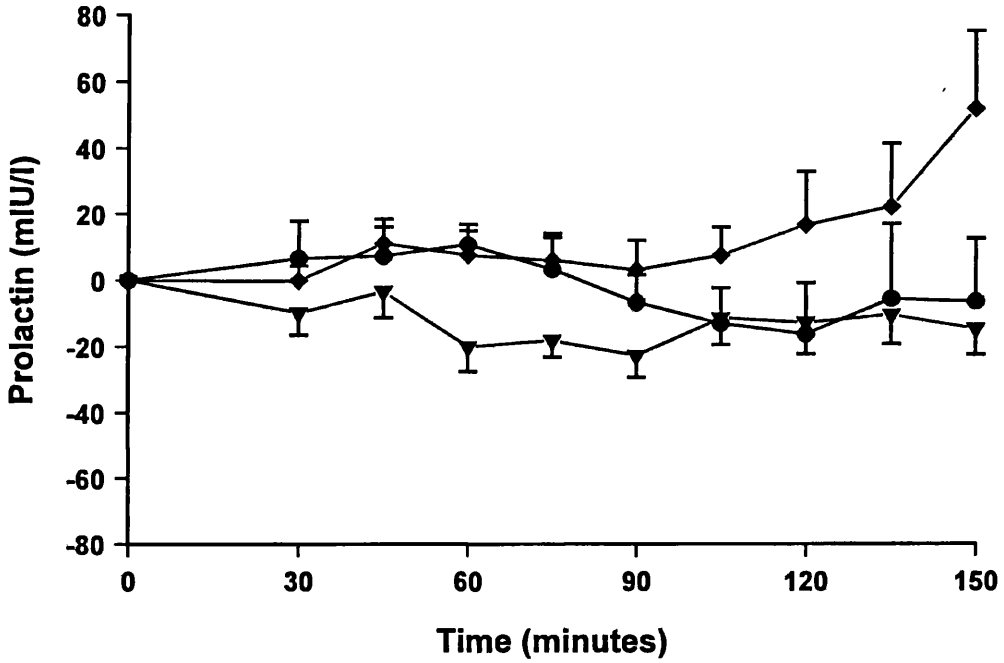
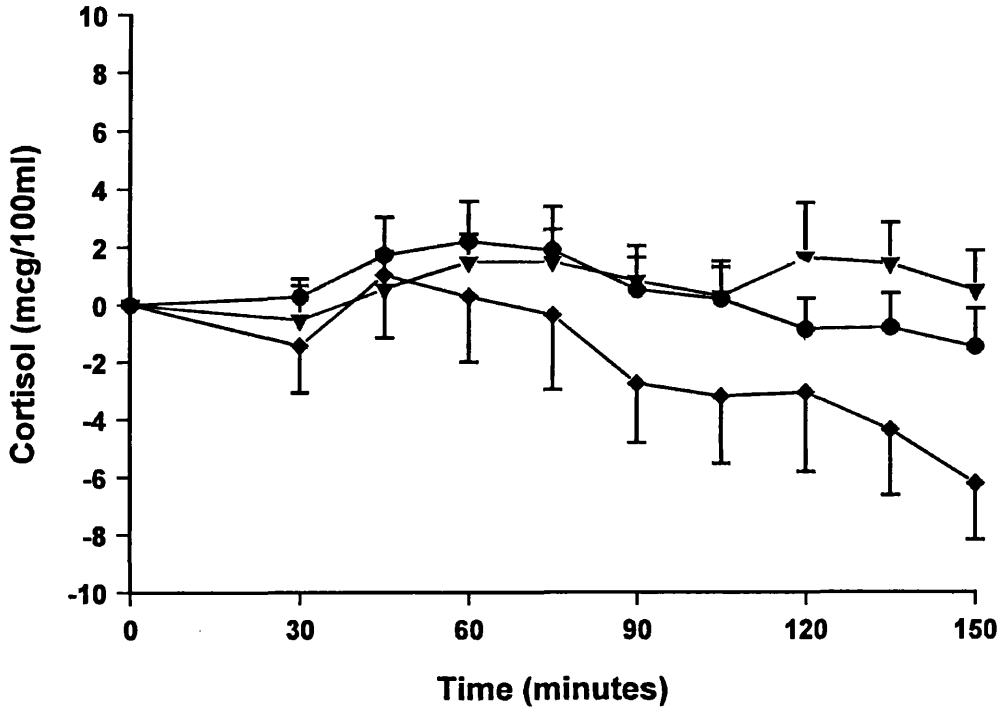


Fig 6.7 Mean ± SEM plasma CORT response to citalopram 10mg IV in 9 healthy subjects on 3 occasions pretreated with either placebo (closed circles), pindolol 10mg (closed triangles) or penbutolol 80mg (closed diamonds) in a randomised manner.



DISCUSSION OF CITALOPRAM STUDIES IN HEALTHY SUBJECTS

There is now much evidence that intravenous administration of SSRIs and other less specific drugs (such as clomipramine), in humans, increases plasma PRL and CORT (Laakmann 1990; Raap and Van de Kar 1999). Consistent with this, the citalopram dose response study shows that relatively modest doses of intravenous citalopram increase plasma PRL and CORT in healthy volunteers. These hormones were most robustly increased in comparison to placebo following 10mg intravenous citalopram, although direct comparisons between 10mg and 5mg showed numerical but not statistical difference. Blinded clinical observations indicated that both doses of citalopram were well tolerated but further studies with standardised subjective rating scales would be needed to confirm this impression, and exclude any potential bias of subjective effects. Optimal PRL responses to citalopram in larger studies are therefore likely to be attained at the dose of 10mg, as opposed to the apparently less well tolerated dose of 20mg (Seifritz et al. 1996). To confirm this finding, a within subject comparison between citalopram at 10mg and 20mg would need to be undertaken. Gender did not influence the hormonal responses to citalopram (as with zolmitriptan).

The release of PRL and CORT following SSRI administration is likely to reflect increased synaptic concentrations of 5-HT at the hypothalamic level, but the receptor mechanisms involved to influence these hormones have been difficult to establish clearly. Studies with selective 5-HT receptor antagonists in both humans and animals have

suggested that a variety of postsynaptic 5-HT receptors (5-HT_{1A}, 5-HT_{2A}, 5-HT_{2C}) are involved in 5-HT mediated PRL and ACTH release (Raap and Van de Kar 1999; Van de Kar 1991). For example, the PRL response to the 5-HT releasing agent, d-fenfluramine, is abolished by the 5-$HT_{2A/2C}$ receptor antagonist, ritanserin (Goodall et al. 1993), but the ability of pindolol, a 5-HT_{1A} receptor antagonist, to block this response is equivocal (Park and Cowen 1995).

The initial hypothesis was that the PRL and CORT responses to citalopram are mediated by 5-$HT_{2A/2C}$ receptors. Ritanserin was not available for use, and therefore the less selective 5-$HT_{2A/2C}$ receptor antagonist, cyproheptadine (Hoyer 1988) was used. In this study, pretreatment with cyproheptadine at a dose sufficient to lower baseline plasma PRL and CORT, did not attenuate the endocrine responses to citalopram, but the study was underpowered to detect such a difference. Lewis and Sherman (1985) did not demonstrate this baseline change in PRL with cyproheptadine at an equivalent dose, but the baseline CORT reduction was predicted (Electronic medicines compendium). They did demonstrate that cyprophеptadine blocked the PRL response to fenfluramine, however. These preliminary data therefore can not clarify whether citalopram induced endocrine responses are mediated predominantly by 5-$HT_{2A/2C}$ receptors, and the study would need to be repeated with cyproheptadine and a larger sample or another 5-$HT_{2A/2C}$ receptor antagonist with less activity at other receptors. Other receptor subtypes may also be sufficiently involved in the endocrine responses to citalopram to be able to overcome the effects of 5-$HT_{2A/2C}$ receptor blockade.

The second hypothesis was that the PRL and CORT responses to citalopram are mediated by 5-HT_{1A} receptors and that pindolol 10mg would enhance and penbutolol 80mg diminish the endocrine effects. Neither pindolol nor penbutolol demonstrated a significant effect on PRL and CORT responses to citalopram in this study (table 6.2). This would initially suggest these responses are not mediated by 5-HT_{1A} receptors. If in this sample, pindolol bound preferentially to presynaptic receptors (as predicted, Rabiner et al. 2000) then its partial antagonistic activity (Hjorth and Carlsson 1986) at this site may have prevented any significant change in hormone secretion following citalopram in comparison to placebo. Some postsynaptic binding still occurs with pindolol 10mg (Rabiner et al. 2000) and this may have been enough to negate the postsynaptic effect of a rise in 5-HT release occurring with the influence of citalopram and presynaptic blockade, again with no net effect on hormone secretion. Similarly, if penbutolol has no preferential pre or postsynaptic binding (Rabiner et al. 2000) the net hormonal effects of citalopram could be negligible. In animal models, both pindolol and penbutolol have been shown to enhance serotonergic activity following acute SSRI infusion (Gartside et al. 1999; Gundlah et al. 1997) which may enhance the validity of the trend to an increase in PRL above placebo in the penbutolol arm at the end of the blood sampling period, as shown in Fig 6.5. This study would need to be repeated with a longer sampling period to examine whether penbutolol indeed had such an effect. This could indicate a potentially useful property of penbutolol, in the augmentation of the antidepressant effect of SSRIs. Overall, it is unlikely that postsynaptic 5-HT_{1A} receptors mediate the PRL response to citalopram.

Baseline PRL was significantly less for the pindolol and penbutolol test days in

comparison to placebo. This raises the possibility that both drugs alone can reduce tonic brain 5-HT neurotransmission and highlights the importance of including a placebo arm in such a trial to gain a true impression of the impact of these drugs on the neuroendocrine responses to citalopram. The mechanism of this PRL reduction could be antagonism of postsynaptic 5-HT_{1A} receptors or partial agonist activity at presynaptic 5-HT_{1A} receptors. The β adrenoceptor antagonist effect of pindolol and penbutolol may also influence PRL secretion via changes in NA neurotransmission. Propranolol, similarly a β adrenoceptor antagonist, appears to enhance PRL secretion in some paradigms (the desimipramine challenge, Laakmann et al. 1986) but has no effect in others (the L-TRP challenge, Upadhyaya et al. 1990). In sheep, NA inhibits PRL secretion which is reversed in vitro by propranolol (Colthorpe et al. 2000). β adrenoceptor antagonist effects are therefore unlikely to inhibit PRL secretion. This reduction in baseline PRL could potentially influence the subsequent PRL response when citalopram is administered. To identify such an effect, again a full cross over design would need to be used so that the effect on PRL secretion of penbutolol alone without citalopram could be accurately defined.

At this stage it is therefore unclear which receptors mediate the neuroendocrine responses to citalopram but using currently available ligands, clarification of the involvement of postsynaptic 5-HT_{1A} or 5-$HT_{2A/2C}$ receptors has not been possible. More selective antagonists, acting only at postsynaptic areas would be required and this should be investigated as they become available. Recent studies from the laboratory of Dr Trevor Sharp have shown that PRL and CORT responses to citalopram in rats are not

antagonised by either selective 5-HT_{1A} or selective 5-HT_2 receptor antagonists or by both antagonists given together (Sharp T, personal communication). Although this leaves open the question of which receptors mediate the endocrine responses to citalopram, it does suggest that the findings here are valid.

CHAPTER 7

THE CITALOPRAM CHALLENGE IN DEPRESSED SUBJECTS

INTRODUCTION

Having shown the effects and tolerability of citalopram 10mg administered intravenously in healthy subjects, and attempted to define the mode of action of the neuroendocrine responses, the next stage was to apply this test to a sample of depressed patients.

I hypothesised that the PRL and CORT responses to citalopram would be blunted in a group of depressed subjects, in comparison to a healthy control sample, as observed with other presynaptic acting neuroendocrine probes such as fenfluramine (Stahl 1993). The subsequent study examines this hypothesis.

METHODS

Subjects

Fourteen depressed subjects were recruited as described in Chapter 3. All had DSM-IV Major Depressive disorder (see appendix I) identified through the SCID, and had been drug free for at least three months. Equal numbers of each sex were recruited, 7 men and 7 women, of mean age 45 years (range 25 to 62) and mean weight 74.7 kg (range 55.0 to 104.8). Mean HAM-D and BDI scores were 20 (range 9 to 33) and 25 (range 8 to 43) respectively. Only two subjects were identified as melancholic type depressive disorder, negating the usefulness of such a subanalysis. Fourteen controls were selected from a volunteer list, individually matched to the depressed group as described in the methods section. This group had a mean age of 41 years (range 21 to 57) and mean weight 71.1kg

(range 56.0 to 94.8). One patient with a weight of 104.8 did not have a close match for this variable and hence a control with the nearest weight of 94.8kg was selected.

Neuroendocrine tests

All subjects were tested on two separate days in a double blind, balanced order, placebo controlled design. The mean ± SEM interval between the two tests was 10 ± 2 days with no significant difference between the groups. Subjects fasted after a light breakfast and came to the research unit at 12.00 hours. After a 30 minute rest period for removal of baseline venous samples for PRL and CORT estimation, citalopram 10mg (diluted in 5ml saline) or 5ml saline (placebo) were administered over 30 minutes. Blood sampling continued at 15 minute intervals for a further 150 minutes. All female subjects were tested in the first half of the menstrual cycle.

Biochemical measurements

Assay methods are described in Chapter 3. The inter and intra assay coefficients of variation of the PRL assays over the range encompassed by the standard curve were 5% and 1% respectively (as opposed to those stated in Chapter 3). CORT was analysed using RIA with inter and intra assay coefficients of variation over the range encompassed by the standard curve of 10% and 1% respectively.

Analysis of results

Baseline plasma PRL and CORT levels between patient and control groups were analysed by unpaired t tests between means of the 3 baseline measures taken on the placebo test day (at time points -30, -15 and 0).

AUC PRL and CORT responses were examined in ANOVAs with citalopram and placebo test days as 'occasion' within subject factors and 'depression' presence or absence as a between subjects factor.

To more simply and accurately analyse this dataset, patient and control PRL and CORT responses are expressed as ΔAUC values, calculated by subtraction of the individual placebo challenge AUC values from their respective citalopram response AUC values. The difference between these values was investigated using univariate ANOVA of ΔAUC values with depression (presence or absence) as the between (fixed) subjects factor. The ANOVAs on raw values for PRL and CORT were not undertaken due to the large number of levels in this analysis, which would make interpretation of the results more difficult.

RESULTS

Due to a high baseline PRL, one depressed subject (and therefore their matched control) was excluded from the study. Mean ± SEM placebo test day baseline scores for PRL for patient and control groups were 164 ± 20 and 157 ±18 mIU/l respectively, with no

significant difference (unpaired t test t= -0.26; df 23.6; p= 0.80). Mean ± SEM placebo test day baseline scores for CORT for patient and control groups were also non significantly different at 18.4 ± 2.2 and 16.4 ± 2.7 mcg/100ml respectively (unpaired t test t= 0.58, df 24.7, p= 0.57).

For the ANOVA of PRL AUC values with the within subject factor of occasion (citalopram or placebo) and between subject factor of depression (patient or control), a highly significant effect of occasion (F=14.29; df 1, 24; p=0.001) and a significant occasion by depression interaction (F=7.30, df 1, 24; p=0.012) was observed. Mean PRL ΔAUC ± SEM scores for depressed subjects and controls were 14.8 ± 19.9 and 89.1 ± 19.0 mIU hr/l respectively. The univariate ANOVA of PRL ΔAUC scores, with depression as fixed factor and ΔAUC as dependant variable showed a significant effect of depression (F=7.30, df 24, 1; p=0.012). The post hoc independent samples 2 tailed t test for PRL ΔAUC between depressed subjects and controls was similarly significant (t=2.70; df 24; p=0.012). Figures 7.1 and 7.2 demonstrate graphically the effect of depression on PRL responses to citalopram.

The ANOVA of CORT AUC values comparing citalopram and placebo test days with depression as a between subjects factor showed a significant effect of occasion (F=11.21; df 1,26; p=0.002) but no significant occasion by depression interaction (F=2.45; df 1, 26; p=0.13). Mean CORT ΔAUC ± SEM scores for depressed subjects and controls were 5.7 ± 4.9 and 15.7 ± 4.0 mcgxHr/100ml respectively. The univariate ANOVA of CORT ΔAUC values showed no effect of illness (F=2.45; df 26, 1; p=0.13). Figures 7.3 and 7.4

show these effects, suggesting a trend to blunting of the CORT response in the depressed group in comparison to healthy controls.

Gender was not a significant covariate in the ANOVAs of either PRL AUC ($p=0.38$) or CORT AUC ($p=0.27$) data. No correlations were observed between PRL ΔAUC and Beck ($r=-0.58$; $p=0.85$) or Hamilton ($r=0.048$, $p=0.88$) depression rating scores or CORT ΔAUC and Beck ($r=-0.16$; $p=0.60$) or Hamilton scores ($r=0.22$; $p=0.48$) in the patient group. In individual subjects, PRL ΔAUC did not correlate with CORT ΔAUC ($r=0.054$; $p=0.86$, for the depressed group).

Although accurate comparative analysis of data between melancholic and non melancholic subjects is not possible, due to there only being two melancholic subjects, their individual PRL ΔAUC scores were 58.0 and 14.8 mIU hr/l, and their CORT ΔAUC scores were 6.4 and -10.7 mcgxHr/100ml respectively. These do not appear to be markedly different from the spread of data from non melancholic subjects although obviously a larger number of melancholic subjects would be needed to test this hypothesis.

No depressed subjects described recent weight change, although excluding this as a confounding factor is not fully possible as these were subjective reports. The influence of duration of illness, and previous treatment could not be examined as it was not systematically recorded.

As before, citalopram was well tolerated with no adverse effects reported by subjects. Ideally this should have been recorded systematically with VAS to more clearly exclude any bias due to subjective effects.

DISCUSSION

These findings indicate that the PRL response to citalopram is blunted in patients with Major Depressive disorder. The CORT response to citalopram in this group shows a trend to blunting. This is consistent with the reliable body of data that shows such PRL secretion reduction in depression with similar presynaptic acting ligands (Cowen 1998), specifically clomipramine (Anderson et al. 1992b; Golden et al. 1992). Another group has recently examined the influence of neuroendocrine responses to citalopram 20mg IV in depression, with very similar findings for PRL and CORT (Kapitany et al. 1999). Similar rates of side effects were observed in the Kapitany et al. study as with the Seifritz et al. (1996) study with 20mg citalopram in healthy subjects. The lack of significant reduction in CORT, despite an observed trend in both studies, may indicate a need to test a larger number of subjects to clarify this finding.

Citalopram levels were not examined in this study but this would have been ideal to examine any difference in pharmacokinetic properties between these two subject groups. Such a difference would be unlikely but cannot be excluded.

It is notable that baseline PRL and CORT did not vary between patient and control groups, which may otherwise have influenced the subsequent endocrine response to citalopram. Absolute CORT values have been shown to differ in some groups of depressed patients in comparison to healthy subjects (Dinan 1994), but this is not replicated in this sample and is discussed further in chapter 8.

As discussed in the previous chapter, the receptor mediated mechanism of the PRL response to citalopram has not been defined. These findings are likely however to represent disturbed presynaptic serotonergic functioning in depressed subjects. Brain imaging studies in patients with depressive disorders have identified decreased availability of 5-HT reuptake sites in midbrain and brain stem (Malison et al. 1998; Willeit et al. 2000) which are consistent with these neuroendocrine findings.

Figure 7.1 Mean ± SEM PRL concentration change in 13 depressed patients (closed symbols) and matched controls (open symbols) tested on two occasions with citalopram 10mg IV (circles) and placebo (squares).

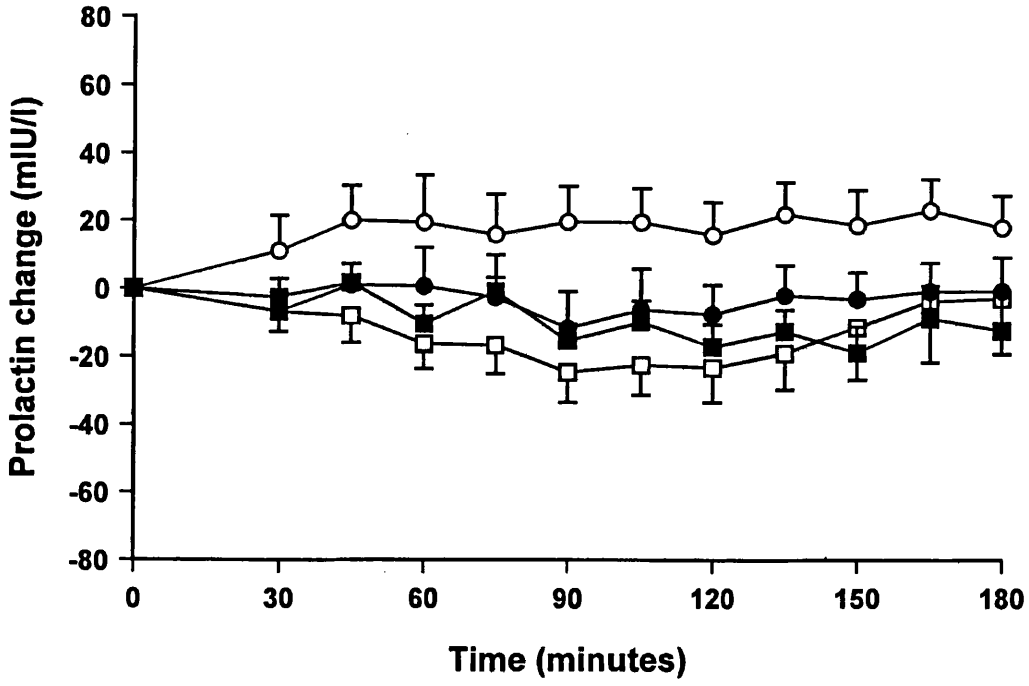


Figure 7.2 Individual ΔAUC PRL responses of acutely depressed subjects (n= 13) and matched healthy controls. ΔAUC PRL was calculated by subtraction of the individual placebo challenge AUC PRL values from their respective citalopram 10mg AUC PRL response values.

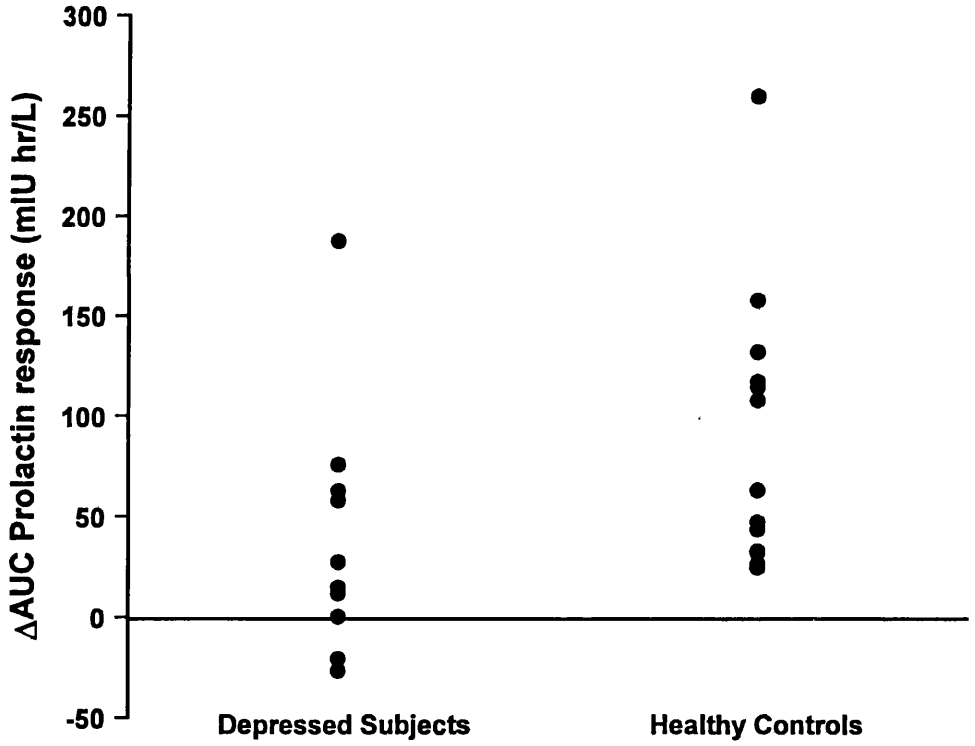


Figure 7.3 Mean ± SEM CORT concentration change in 13 depressed patients (closed symbols) and matched controls (open symbols) tested on two occasions with citalopram 10mg (circles) and placebo (squares).

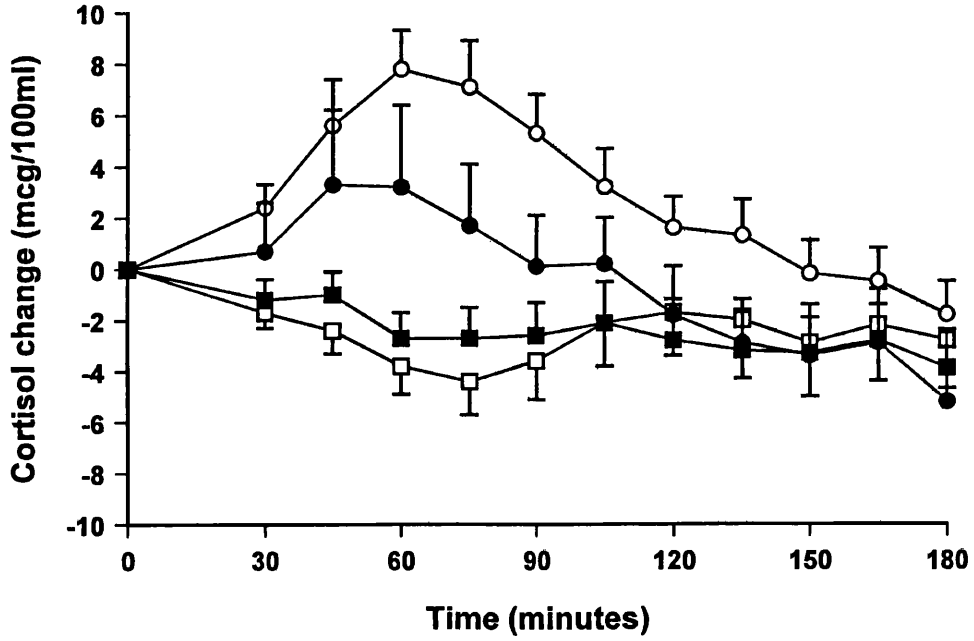
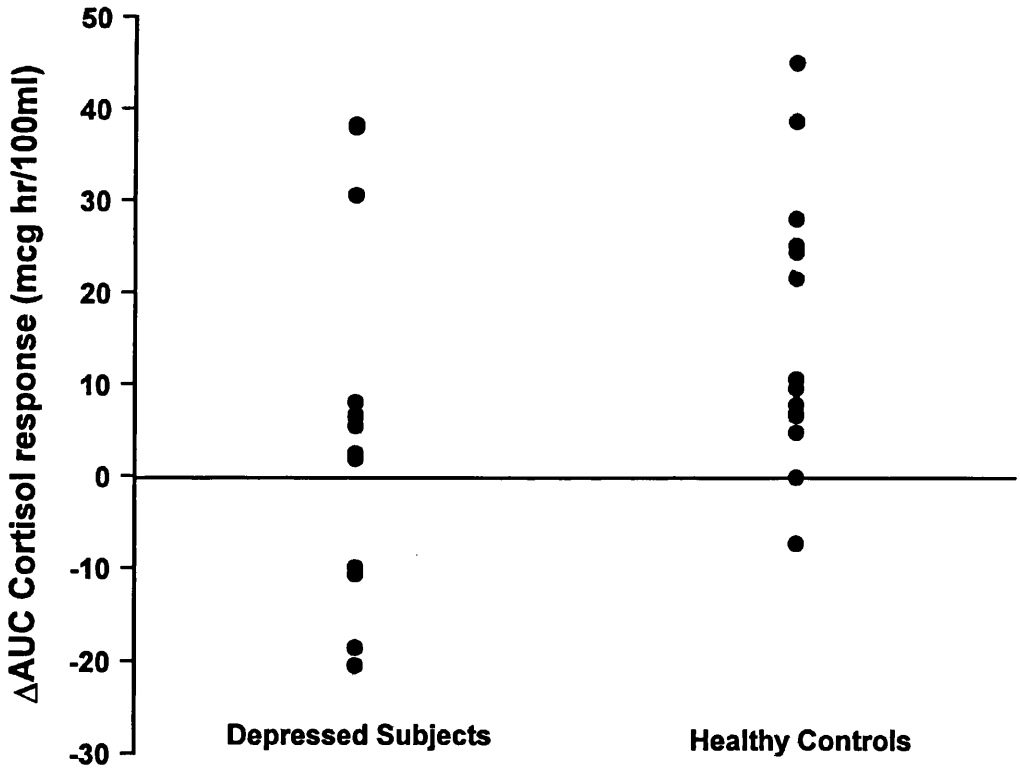


Figure 7.4 Individual ΔAUC CORT responses of acutely depressed subjects (n= 13) and matched healthy controls. ΔAUC CORT was calculated by subtraction of the individual placebo challenge AUC CORT values from their respective citalopram 10mg AUC CORT response values.



PART 3

CONCLUSIONS

CHAPTER 8

CONCLUSIONS

5-HT FUNCTION IN DEPRESSION

The aetiology of depressive disorders remains unclear. The heterogeneity of clinical symptoms, apparent causative factors, levels of vulnerability and response to existing treatments is not fully explained by current knowledge of this disorder. The level of mortality and morbidity for the individual and society (Scott and Dickey 2003) drives active investigation into the biological substrates of depressive disorders and the development of more effective treatments.

Chapter 1 reviewed the complexity of depressive disorders and 5-HT function in the human brain. At this stage, we are still identifying the role of 5-HT in the normal brain, so it could be argued that examining 5-HT function in the disordered brain is premature. Overall the evidence suggests that changes in brain 5-HT function are associated both with the state of depression and its pharmacological treatment. The evidence for this view comes from numerous lines of enquiry, such as CSF studies, postmortem studies, TRP depletion, neuroendocrine tests, neuroimaging and the effective antidepressant action of 5-HT promoting agents. The exact nature of the link with low brain 5-HT function and depression remains unclear however. Equally, how pharmacological potentiation of a single neurotransmitter leads to clinical antidepressant effect is unknown, although the complex interplay between monoamines and their resultant effect on intracellular events is starting to be elucidated.

NEUROENDOCRINE STUDIES

Neuroendocrine studies primarily give the investigator information about hypothalamic and pituitary function, in which 5-HT systems play an important regulatory role. The development of these studies has been valuable in the further investigation of functional changes in depressive disorders, and investigating the effects of antidepressant treatments at the receptor level. While PET imaging provides information about receptor density it has not provided information about receptor function. The use of pharmacological functional MRI offers possibilities in this direction (Anderson et al. 2002) but at present neuroendocrine studies are the simplest and most accessible means of measuring brain neurotransmitter function in humans.

Neuroendocrine studies however have a number of methodological difficulties (see Chapter 2). For example, they rely on knowing the mechanism of action of the drug probe used, and controlling for parameters that may influence hormone secretion such as age, gender, weight, hormonal status and previous psychotropic drug treatment.

To date this field of investigation has been marred by the poor availability of selective receptor ligands, which are required to clearly define whether a specific receptor type mediates the changes in hormone secretion observed. The large number of partially selective ligands (such as ketanserin for the 5-HT_{1D} receptor or pindolol for the 5-HT_{1A} receptor) has helped to give a possible idea of which receptors are involved (eg. the

5-HT_{1A} receptor possibly mediates the PRL response to L-TRP). Replication of some of the neuroendocrine findings using different probes with the same pharmacodynamic properties has also strengthened the evidence.

The main limitation of neuroendocrine studies is extrapolating changes in serotonergic activity influencing hormone secretion at the level of the pituitary to serotonergic activity in other parts of the brain. The results of neuroendocrine studies are therefore valuably combined with findings from other experimental paradigms such as functional imaging to give a wider view of what biological changes occur in depressive disorders.

This thesis set out to investigate two new potential neuroendocrine probes, zolmitriptan and low dose citalopram, which had hypothesised advantages over existing probes. In all of these studies, common confounding factors of neuroendocrine challenges of weight loss (despite including melancholic subjects), physical illness, recent psychoactive drug exposure, stage of menstrual cycle and external environmental stress were adequately excluded or controlled for.

THE ZOLMITRIPTAN CHALLENGE

Zolmitriptan, licensed for the treatment of migraine, markedly increased plasma GH in healthy subjects but had no effect on plasma PRL or body temperature. The GH response was blocked by ketanserin, in an adequately powered study, suggesting that it may be

mediated by 5-HT_{1D} receptors (Pauwels et al. 1996). In patients with major depressive disorder with melancholia, this GH response to zolmitriptan is impaired. In patients treated with SSRIs, the GH response to zolmitriptan was markedly attenuated. Healthy subjects rechallenged with zolmitriptan (without SSRI administration) showed no such desensitisation. These findings suggest an impaired 5-HT_{1D} receptor function in melancholic subjects with antidepressant treatment causing a further adaptive desensitisation of the 5-HT_{1D} receptor function. The hypothesis concerning 5-HT_{1B} autoreceptor dysfunction in depression could not be tested with this probe. It is not clear whether the blunted GH response to zolmitriptan is a depressive state dependent phenomenon or whether it may represent a vulnerability factor to depression (as discussed below). It would be useful to investigate this by challenging a group of recovered depressed subjects with zolmitriptan.

Zolmitriptan therefore appears to be a reliable, well tolerated neuroendocrine probe, possibly of postsynaptic 5-HT_{1D} receptor function. This mode of action will need to be clarified with the introduction of more selective ligands. It appears to enhance GH secretion more reliably than the existing 5-$HT_{1B/1D}$ receptor probe sumatriptan. Concerning the criteria for a valid probe (Yatham and Steiner 1993), zolmitriptan has a large, robust and consistent hormone response, which appears to be due to a direct effect of the drug on the 5-HT system with the influence of other factors excluded, and is free from stressful side effects. A dose dependent relationship between the drug and the hormone response was not demonstrated in this study but is suggested when these GH results with 5mg are compared with the magnitude of GH responses from the more recent

study by Moeller et al. (2000) who used 2.5mg (although a within subjects study would be necessary to prove this).

The specific functional relevance of 5-HT_{1D} receptors and their role in depressive disorders is as yet unknown. They appear in small numbers in the human basal ganglia, and could therefore have a role in movement and cognitive disturbances in depression. Despite low numbers, such receptors could have a dramatic regulatory influence over brain function. Disturbance of 5-HT_{1D} receptor function could equally be incidental to the aetiology of depressive disorders. Several studies have examined the role of 5-$HT_{1B/D}$ receptors in obsessive compulsive disorder, with reports of sumatriptan, but not zolmitriptan, significantly enhancing the GH response in these subjects in comparison to controls (Boshuisen and den Boer 2000). Acute challenge with sumatriptan has been shown to both enhance and diminish obsessive symptoms (Stein et al. 1999) and longer treatment with sumatriptan does not appear to improve obsessive symptoms (Koran et al. 2001). Preliminary studies have also linked obsessive compulsive disorder with genetic variation of the 5-HT_{1B} receptor gene (Camarena et al. 2004), which may explain the difference between the GH response to sumatriptan and zolmitriptan in this group, due to zolmitriptan having a greater affinity for 5-HT_{1D} than 5-HT_{1B} receptors. There are no specific 5-HT_{1D} receptor ligands available in humans however, to clearly test potential therapeutic effects with either obsessive or depressive symptoms.

THE CITALOPRAM CHALLENGE

Data from this thesis indicates that low dose intravenous administration of citalopram (10mg) has potential value as a 5-HT neuroendocrine probe, with associated increased PRL and CORT secretion. As with zolmitriptan, the utility of the citalopram neuroendocrine test will be increased if the 5-HT receptor mechanisms underlying its effects are more clearly established. It could not be clearly concluded whether 5-HT_{1A} or 5-HT_2 receptors are involved in mediating these hormonal changes from these studies. Using the criteria for a valid neuroendocrine probe (Yatham and Steiner 1993), citalopram 10mg IV has a reasonably large, robust and consistent hormone response (confirmed recently by Lotrich et al. 2004), which appears to be due to a direct effect of the drug on the 5-HT system with the influence of other factors excluded, and few subjects report stressful side effects. A dose dependent relationship between the drug and the hormone response is demonstrated. Findings from these studies confirm that acute major depression is associated with blunted 5-HT mediated PRL release, most likely representing impaired presynaptic functioning. It was not possible to clearly examine the influence of melancholia on the responses to citalopram challenge in this study, but one could hypothesise that the neuroendocrine response may be more robust, as observed with fenfluramine (Newman et al. 1998).

Hennig and Netter (2002) recently showed that an acute challenge of citalopram 20mg orally increased CORT but not PRL in a sample of 48 male subjects and was apparently well tolerated (replicated by Nadeem et al. 2004). It is unclear why IV citalopram

increases PRL and oral citalopram does not, but similarly, IV clomipramine increases PRL and oral clomipramine does not (Laakman et al. 1984). The PRL response may require higher plasma levels of citalopram or clomipramine than are produced by oral administration. Oral SSRIs can, however, elevate PRL after long term treatment (Cowen and Sargent 1997).

The recently developed active S- isomer of citalopram (Owens et al. 2001) may be even more effective at enhancing the reliability of such hormonal responses. However, Nadeem et al. (2004) examined the salivary CORT response to this isomer and the racemic mixture of isomers, finding no significant difference in change of salivary CORT between the two.

To examine whether 5-HT disturbance may be a vulnerability factor to depressive disorders, an extension to the citalopram study in Chapter 7 was undertaken (Bhagwager et al. 2002). A group of recovered depressed patients were recruited who had been euthymic for at least 6 months and had been off medication for at least 3 months. They underwent citalopram challenge as in Chapter 7. The control group was not individually matched to these patients however, as undertaken in the preceding studies, but mean age, weight and sex ratio was similar between groups. The PRL response to citalopram was also blunted in these subjects in comparison to controls, but not significantly different to the acute depressed group described above. Weight loss may complicate interpretation of these findings (as described above, Cowen 1998), but such changes were also not apparent in this recovered sample. Along with the finding that subjects who have had a

previous episode of depression are at a high risk of future episodes (Kendler et al. 1993), these results suggest that a blunted PRL response to citalopram in comparison to healthy controls may be a trait marker indicating vulnerability to major depression, as discussed further below.

The studies in this thesis therefore support a dysfunction of 5-HT in depressive disorders, and offer the investigator two further tools for neuroendocrine investigations. At a time when neuroimaging is being developed to measure the activity of 5-HT systems, these probes are also a useful addition for associated activation of presynaptic 5-HT function (citalopram) and postsynaptic 5-HT_{1D} receptors (zolmitriptan).

INVESTIGATIONS OF 5-HT FUNCTION IN DEPRESSION

To bring together the findings of the preceding chapters concerning 5-HT function and depression in humans, firstly it is clear from TRP depletion studies that a significant proportion of subjects are vulnerable to depressive symptom relapse in response to a lowering of brain 5-HT synthesis (Smith et al. 1997a). This is particularly, but perhaps not surprisingly, evident in subjects who are treated with SSRIs. Importantly, in healthy subjects without this vulnerability, diminishing brain activity through TRP depletion is insufficient to cause depression. Post mortem studies in depressed subjects on the whole have provided inconsistent results although the increase of globus pallidus 5-$HT_{1B/1D}$ receptors may be an important finding. Platelet 5-HT studies have similarly shown

inconsistent results but again the possible increase in 5-HT_{2A} receptor density may be important. The clearest neuroimaging findings in depressive disorder are a replicated reduction in 5-HT_{1A} and 5-HT_{2A} binding (the latter being inconsistent with the platelet results). Preliminary imaging studies also indicate a reduction in 5-HTT binding, consistent with downregulation following reduced stimulation due to reduced synaptic 5-HT. Neuroendocrine studies have provided a greater body of replicated findings which may represent a more realistic measure of overall 5-HT function and 5-HT receptor function (as opposed to receptor density). In depression, these studies have shown a likely reduction in presynaptic 5-HT_{1A} and postsynaptic 5-HT_{1A} function. The zolmitriptan studies in this thesis add to the existing data to indicate a reduction in postsynaptic 5-HT_{1D} function in melancholic subjects. These studies do not appear to give information on presynaptic 5-$HT_{1B/1D}$ function. The studies of postsynaptic 5-HT_2 receptors are difficult to interpret but possibly indicate an increased function (consistent with platelet findings). If L-TRP, fenfluramine and clomipramine are probes of overall presynaptic function (or possibly net 5-HT function) then this is diminished in depression, which is further confirmed by the results from this thesis with citalopram. It is unclear whether these findings are a primary dysfunction, causing depressive symptoms or a result of another primary abnormality. Reduced presynaptic function and downregulated postsynaptic receptors however, are consistent with reduced overall 5-HT neurotransmission and the associated emergence of depressive symptoms. Reduced presynaptic 5-HT_{1A} autoreceptor function is an understandable adaptation to reduced overall presynaptic 5-HT output, and appears inconsistent with theories of autoreceptor hypersensitivity in depression. 5-HT receptor dysfunction and overall reduced 5-HT

neurotransmission appears particularly likely in subjects with major depression with melancholic features.

Several investigators have examined whether these 5-HT system abnormalities found in depressive disorders change following illness resolution. Such a differentiation of trait from state markers could indicate persistent biological vulnerability factors to depressive disorder which may have been present before illness onset, but equally may represent a biological scar following a depressive episode that makes the subject more vulnerable to further episodes, as observed clinically. The PRL response to L-TRP resolves (Upadhyaya et al. 1991; Smith et al. 2000), but the PRL responses to fenfluramine (Coccaro et al 1987; Shapira et al.1993; Flory et al 1998), clomipramine (Golden et al. 2002) and citalopram (Bhagwager et al. 2002, as above) remain blunted in recovered depressed subjects. The difference between L-TRP and the other probes is that the L-TRP response is blocked by pindolol and therefore possibly mediated by postsynaptic 5-HT_{1A} receptors. 5-HT_{1A} receptor function therefore appears impaired in depression and resolves following treatment. If fenfluramine, clomipramine and citalopram measure presynaptic 5-HT function, then presynaptic dysfunction appears to be a trait abnormality in depression. On its own, this dysfunction is not enough to cause depression. This finding is consistent with TRP depletion causing a reduction of presynaptic 5-HT and subsequent recurrence of depressive features. An attractive hypothesis is that hypercortisolaemia following stressful events reduces brain TRP availability, through induction of peripheral TRP pyrrolase (causing increased metabolism of peripheral TRP) and therefore precipitates a depressive episode in vulnerable subjects (Cowen 2002). Such a vulnerability in 5-HT

function could be caused by genetic abnormalities, early environmental influence or as a consequence of a previous episode of major depression.

INVESTIGATIONS OF THE EFFECT OF SSRIS

The clearest findings of the effects of SSRIs on the 5-HT system are reduced neuroendocrine function mediated via postsynaptic 5-HT_{1A}, 5-HT_{1D} (shown in this thesis) and 5-HT_{2C} receptors. 5-HT_{1A} receptors in one imaging study also remained reduced following treatment but were not reduced further than untreated subjects (Sargent et al. 2000). Both postmortem and imaging studies have also indicated a reduction in postsynaptic 5-HT_{2A} receptor numbers with treatment. As discussed, this is consistent with an adaptive downregulation of postsynaptic receptors following enhanced presynaptic 5-HT output and appears at first sight to contradict the idea of an overall increase in 5-HT neurotransmission; however, if the increase in presynaptic 5-HT is sufficiently large, net 5-HT neurotransmission may still be enhanced. It is unclear whether this postsynaptic downregulation is necessary for antidepressant action. Presynaptic 5-HT_{1A} receptor function appears reduced following SSRI treatment (using temperature response as an index of function) which is consistent with theories of antidepressants having their action via autoreceptor desensitisation, as observed in rats, thereby enhancing levels of synaptic 5-HT. The function of presynaptic 5-HT_{1B} receptors (probed with sumatriptan) appeared unchanged following SSRI treatment in healthy subjects, again consistent with rat studies. The enhanced neuroendocrine response to L-TRP following SSRI treatment may indicate overall increased presynaptic 5-HT

function which would be expected following these treatments. SSRIs alone can increase basal PRL secretion which is also consistent with overall increased 5-HT function. These changes associated with SSRI treatment assist in understanding the process by which they have therapeutic effect and may give investigators clues as how to develop more effective or better tolerated antidepressants.

FURTHER INVESTIGATIONS INTO DEPRESSIVE DISORDERS

The development and growth of brain imaging techniques has offered much hope for the future investigation of depressive disorders. For example, the reduced presynaptic function of 5-HT neurones in depression implied from the citalopram challenge could be further investigated by examining presynaptic 5-HT release with PET, as has been examined with DA release in schizophrenia (Laruelle 2000, Abi-Dargham et al. 2000). To date, however, such PET studies have not been successful, possibly relating to the nature of the 5-HT system rather than the ligands used (Rabiner et al. 2002). The ligand [^{11}C] DASB has recently been developed for in vivo imaging of the 5-HTT and studies in depressed subjects are awaited (Ginovart et al. 2003). If such paradigms are worked out, it would be valuable to correlate PET findings with neuroendocrine findings within studies and examine whether these have any prognostic value (as with the DA studies, eg. Abi-Dargham et al. 2000).

It is clear that receptor function changes are just one of many events that occur in depressive disorders. Several authors have now examined the phenomenon of brain

atrophy in affective disorders. Sheline et al. (1996) found smaller hippocampal volumes in recurrent depressed subjects compared to controls using MRI. Shah et al. (1998) found reduced left sided grey matter density (including hippocampus) in chronic depressed subjects using MRI, and Drevets et al. (1997) demonstrated decreased function of prefrontal cortical areas in familiar unipolar depressed subjects using PET. Mathew et al. (2003) recently demonstrated that macaques exposed to early adverse rearing 10 years previously demonstrated changes in magnetic resonance spectroscopic images of anterior cingulate brain regions in comparison to controls, indicating reduced neuronal integrity and metabolism.

One theory to explain such findings in depressive disorders is of raised CORT (as discussed in Chapter 1) having a toxic effect on susceptible neurone groups, such as the hippocampus, as demonstrated in controlled animal experiments (Hellsten et al. 2002). Studies have demonstrated this hypercortisolaemia in depressive disorders (Dinan 1994) but it is not a consistent finding in all subjects. The depressed subjects examined in this thesis (in Chapters 5 and 7) did not have baseline plasma CORT levels that differed from healthy controls, despite abnormalities in 5-HT function as demonstrated by zolmitriptan and citalopram challenges. This absence of a difference in plasma CORT persisted even when comparing melancholic subjects and controls. Strickland et al. (2002) examined salivary CORT in a large community sample of ICD-10 depressed subjects, which again did not differ from healthy controls, even in the sub sample of severely depressed subjects. Hypercortisolaemia therefore cannot be considered a ubiquitous phenomenon in depressive disorders and therefore is not likely to be a common route to the development

of depression and associated findings such as focal brain atrophy. The theory that severe or melancholic depression is associated with hypercortisolaemia is also inconsistent with the above findings. Recent severe life events have been associated with hypercortisolaemia however (Strickland 2002), which appears to be the confounding factor in the association with depressive disorders.

Several studies have now shown the effect of antidepressant treatments on neuronal integrity and plasticity in animal models. Both electroconvulsive shock (ECS) treated rats (Scott 2000) and chronic lithium treated mice (Chen 2000) showed enhanced hippocampal cell generation in comparison to controls. Hellsten (2002) eloquently demonstrated how the neurotoxic effects of corticosterone can be reversed with ECS. In corticosterone treated rats, neurogenesis was decreased by 75%, which was counteracted by a single ECS. Multiple ECS further enhanced neurogenesis, with no difference from non corticosterone treated rats after 5 ECS. Czeh (2001) also demonstrated the effects of tianeptine, a modified TCA, in tree shrews subjected to chronic psychosocial stress. The untreated animals showed reduced hippocampal neuronal proliferation and cell loss which was prevented in the tianeptine treated group.

A further hypothesised mechanism for the enhancement of cellular growth following antidepressants involves nerve growth factors. BDNF is one of these nerve growth factors which collectively play a critical role in the development of neuronal systems and the survival and function of neurones throughout life (Thoenen 1995). BDNF is expressed in high levels in hippocampus and cortex of mature brain. The action of BDNF is mediated

by binding to TrkB receptors which triggers a cascade of intracellular events which subsequently have a diverse effect on cellular function (Duman 1998). Chronic administration of antidepressants, including SSRIs, upregulates the cAMP pathway at several levels, including increased expression of the cAMP response element binding protein (CREB). CREB can subsequently increase the gene expression of BDNF (Nibuya et al. 1995). The time of chronic antidepressant administration in these studies is consistent with the time for therapeutic effect of antidepressants (Duman 1998). Other psychotropics do not appear to upregulate BDNF (Nibuya et al. 1995). Acute manipulation of 5-HT levels appears to have a paradoxical effect on BDNF expression in comparison to chronic manipulation. Acute elevation of rat brain 5-HT using p-chloroamphetamine (PCA) or paroxetine caused reduced hippocampal BDNF mRNA expression, whereas acute depletion using multiple injections of PCA caused increased hippocampal BDNF mRNA expression (Zetterstrom et al. 1999). Other research paradigms have supported the involvement of BDNF in the mode of action of antidepressants, including animal behavioural models: infused BDNF has antidepressant effects in the forced swim test and learned helplessness models (Siuciak et al. 1996). Stress also reduces expression of BDNF (Smith et al. 1995), which could influence cell death in vulnerable brain areas and could theoretically be reversed by antidepressant treatment.

This neurogenesis model for the action of antidepressants suggests new targets for potential antidepressant agents at many different levels including increasing expression of the BDNF-TrkB system and increasing cAMP pathway function. Several 5-HT receptors

are coupled directly to the cAMP system (see Chapter 1) but only 5-HT_4 and 5-HT_7 are significantly expressed in hippocampus and limbic areas. Both 5-HT_4 (Bijak et al. 1997) and 5-HT_7 (Sleight et al. 1995) have altered function following chronic antidepressant treatment in animal models. Selective ligands for these receptors may have valuable antidepressant properties. Stimulating 5-HT_{1A} receptors causes inhibition of cAMP pathways, which may explain the antidepressant benefits of pindolol, a 5-HT_{1A} receptor antagonist (Whale et al. in preparation). 5-HT_{2A} receptors have influence on the phostphatidylinositol system and are present on GABA neurones in the hippocampus. Duman's group have demonstrated that 5-HT_{2A} receptors regulate BDNF expression in this area (Duman 1998), increasing interest in 5-HT_{2A} ligands.

Combining these investigations and theoretical models of antidepressant activity will hopefully lead to the development of tolerable, more effective antidepressant agents and help lift the psychosocial burden that depressive disorders currently impose.

Appendix

The Diagnostic and Statistical Manual of Mental Disorders (DSM-IV) guidelines for diagnosis of Major Depressive Disorder

Criteria for Major Depressive Episode

A. Five (or more) of the following symptoms have been present during the same 2 week period and represent a change from previous functioning; at least one of the symptoms is either (1) depressed mood or (2) loss of interest or pleasure.

Note: Do not include symptoms that are clearly due to a general medical condition, or mood incongruent delusions or hallucinations.

(1) depressed mood most of the day, nearly every day, as indicated by either subjective report (eg. feels sad or empty) or observation made by others (e.g. appears tearful). Note: In children and adolescents, can be irritable mood.

(2) markedly diminished interest or pleasure in all, or almost all activities most of the day, nearly every day (as indicated by either subjective account or observation made by others)

(3) significant weight loss when not dieting or weight gain (eg. a change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day. Note: In children, consider failure to make expected weight gains.

(4) insomnia or hypersomnia nearly every day

(5) psychomotor agitation or retardation nearly every day (observable by others, not merely subjective feelings of restlessness or being slowed down)

(6) fatigue or loss of energy nearly every day

(7) feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self reproach or guilt about being sick)

(8) diminished ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others)

(9) recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide

B. The symptoms do not meet criteria for a Mixed Episode

C. The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.

D. The symptoms are not due to the direct physiological effects of a substance (eg. a drug of abuse, a medication) or a general medical condition (eg. hypothyroidism).

E. The symptoms are not better accounted for by Bereavement, ie. after the loss of a loved one, the symptoms persist for longer than 2 months or are characterized by marked functional impairment, morbid preoccupation with worthlessness, suicidal ideation, psychotic symptoms, or psychomotor retardation.

Criteria for Melancholic Features

A. Either of the following, occurring during the most severe period of the current episode:

   1) loss of pleasure in all, or almost all, activities

   (2) lack of reactivity to usually pleasurable stimuli (does not feel much better, even temporarily, when something good happens)

B. Three (or more) of the following:

   1) distinct quality of depressed mood (ie. the depressed mood is experienced as distinctly different from the kind of feeling experienced after the death of a loved one)

   (2) depression regularly worse in the morning

   (3) early morning wakening (at least 2 hours before usual time of awakening)

   (4) marked psychomotor retardation or agitation

   (5) significant anorexia or weight loss

   (6) excessive or inappropriate guilt

Abbreviations

ACTH	adrenocorticotrophic hormone
cAMP	cyclic adenosine monophosphate
AMPT	α-methyl-p-tyrosine
ANOVA	analysis of variance
AUC	area the under the curve method
BDI	Beck depression inventory
BDNF	brain derived neurotrophic factor
Ca^{2+}	calcium ion
CBT	cognitive behavioural therapy
CNS	central nervous system
COMT	catechol O-methyltransferase
CORT	cortisol
CRH	corticotrophin releasing hormone
CSF	cerebrospinal fluid
DA	dopamine
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders Edition 4
DST	dexamethasone suppression test
ECS	electroconvulsive shock
ECT	electroconvulsive therapy
EEG	electoencephalogram

GABA	gamma aminobutyric acid
GH	growth hormone
GHRH	growth hormone releasing hormone
GnRH	gonadotrophin releasing hormone
HAM-D	Hamilton depression rating scale
HPA axis	hypothalamic pituitary adrenal axis
HPLC	high performance liquid chromatography
HVA	homovanillic acid
IPT	interpersonal therapy
IV	intravenous
K^+	potassium ion
L-Dopa	L-Dihydroxyphenylalanine
LSD	lysergic acid diethylamide
MAO	monoamine oxidase
MAOI	monoamine oxidase inhibitor
mCPP	m-chlorophenyl piperazine
MHPG	3-methoxy-4-hydroxyphenylglycol
MRI	magnetic resonance imaging
mRNA	messenger ribonucleic acid
NA	noradrenaline
NNT	number needed to treat
PCA	p-chloroamphetamine
PCR	polymerase chain reaction

PET	positron emission tomography
PRL	prolactin
RCT	randomised controlled trials
REM	rapid eye movement (sleep)
RIA	radioimmunoassay
rTMS	repetitive transcranial magnetic stimulation
SAD	Seasonal affective disorder
SCID	Structured Clinical Interview for DSM-IV
SEM	standard error of the mean
SNRI	selective noradrenaline reuptake inhibitor
SSRI	selective serotonin reuptake inhibitor
SPECT	Single photon emission computerised tomography
TCA	tricyclic antidepressant
TRH	thyrotrophin releasing hormone
TRP	tryptophan
TSH	thyroid stimulating hormone
VAS	visual analogue scale
WAY-100635	[N-(2-(4-(2-methoxyphenyl)-1-piperazinyl)ethyl)-N-(2 -pyridyl)cyclohexanecarboxamide]
5-HIAA	5-hydroxyindoleacetic acid
5-HT	5-hydroxytryptamine
5-HTT	serotonin transporter protein
8-OH-DPAT	8-Hydroxy-2-(di-n-propylamino) tetralin

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