

**5-HYDROXYTRYPTAMINE AND DEPRESSION: STUDIES
USING A NEUROENDOCRINE STRATEGY**

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of the degree of Doctor of Medicine
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ABSTRACT

5-Hydroxytryptamine and depression: studies using a neuroendocrine strategy

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The syndrome of depression is a common psychiatric disorder with considerable morbidity and mortality. Brain pathways involving 5-hydroxytryptamine (5-HT) are likely to form part of the biological substrate for mood and there is now considerable evidence that depressed patients have abnormalities in measures of 5-HT function. However the nature of the 5-HT abnormality and its relationship to clinical features and to potentially confounding factors such as weight loss remain unknown.

The present studies use a neuroendocrine challenge strategy to investigate aspects of 5-HT function related to depression. First, I study the effect of weight loss in normal volunteers and demonstrate that moderate weight loss through dieting lowers the plasma availability of the 5-HT precursor, L-tryptophan (TRP) in both sexes and that in women, but not men, brain 5-HT function, as measured by the prolactin (PRL) response to TRP, is altered. Second, I demonstrate that whereas the PRL response to infusion of the 5-HT uptake inhibitor, clomipramine, may provide an index of brain 5-HT function, its propensity to cause stressful side-effects warrants caution in interpretation of results. Using this challenge I show that depressed patients have blunted PRL responses compared to controls, particularly if they have features of melancholia, have attempted suicide or lost weight. This finding is not simply a reflection of impaired PRL secretion as I demonstrate that the PRL response to the dopamine antagonist, metoclopramide, is not altered in depression. Third, I investigate the hormone and temperature effects of a 5-HT_{1A} agonist, gepirone, in normal volunteers and show that it may prove a useful tool in assessing the function of this 5-HT receptor subtype in humans.

The implications of these studies are discussed. Weight loss is a potential confound for investigations of 5-HT function in depressed patients and may itself alter brain 5-HT function. This has implications for findings in depressed patients and for understanding the effects of dieting and the aetiology of eating disorders. The patient studies are consistent with, and add weight to, a substantial body of research showing decreased 5-HT function in depression although at present the site of the abnormality is not known. Use of specific 5-HT agonists, such as gepirone, will allow the investigation of 5-HT receptor subtype function in humans and will help to identify the nature of the 5-HT abnormality in depression.

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PUBLICATIONS

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ABBREVIATIONS

ACTH	adrenocorticotropic hormone
AHP	afterhyperpolarisation
ANOVA	analysis of variance
AUC	area under the curve with subtraction of the baseline value
BBB	blood brain barrier
BCAA	branch chain amino acids
BDI	Beck Depression Inventory
BFS	behavioural facilitation system
BIS	behavioural inhibition system
B _{max}	maximum specific binding (a measure of the number of binding sites)
BP	blood pressure
bpm	beats per minute
°C	degrees Celcius
cDNA	c-deoxyribonucleic acid
95% CI	95 percent confidence interval
CMI	clomipramine
CNS	central nervous system
CORT	cortisol
cpm	counts per minute
CRF	corticotrophin releasing factor
CSF	cerebrospinal fluid
5-CT	5-carbamidotryptamine
DA	dopamine

DCMI	desmethylclomipramine
DDrowsy	peak change in rating of drowsiness
DLightheaded	peak change in rating of lightheadedness
DNausea	peak change in rating of nausea
DOB	3H-4-bromo-2,5-dimethoxyamphetamine
DRN	dorsal raphe nucleus
DSM-III(-R)	third edition (revised) of the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders
DST	dexamethasone suppression test
ECG	electrocardiogram
ECT	electroconvulsive therapy
EEG	electroencephalography
F	statistic used in analysis of variance
FEN	fenfluramine
d-FEN	d-isomer of fenfluramine
G proteins	guanine nucleotides
GABA	gamma aminobutyric acid
GH	growth hormone
GnRH	gonadotrophin releasing hormone
GRF	growth hormone releasing factor
h	hour(s)
H	statistic used in Kruskal-Wallis one way analysis of variance by ranks
HAMA	Hamilton Rating Scale for Anxiety
HAMD	Hamilton Rating Scale for Depression
5-HIAA	5-hydroxyindoleacetic acid
HPA	hypothalamic-pituitary-adrenal (axis)

HPLC	high performance liquid chromatography
5-HT	5-hydroxytryptamine, serotonin
5-HTP	5-hydroxytryptophan
L-5-HTP	L-isomer of 5-hydroxytryptophan
HVA	homovanillic acid
IC ₅₀	concentration at which 50% inhibition occurs
IU	International Unit(s)
kcal	kilocalories
K _d	dissociation constant
kg	kilogramme(s)
K _M	kinetic parameter measuring uptake affinity
L	litre(s)
L-dopa	L-dihydroxyphenylalanine
LNAA	long chain neutral amino acids
LSD	lysergic acid diethylamide
M	molar
MAO	monoamine oxidase
MAOI	monoamine oxidase inhibitor(s)
MCP	metoclopramide
mCPP	m-chlorophenylpiperazine
ME	median eminence
mg	milligramme(s)
min	minute(s)
MK-212	6-chloro-2-(1-piperazinyl)-pyrazine
ml	millilitre(s)
mm	millimetre(s)
MRN	median raphe nucleus

NA	noradrenaline
ng	nanogramme(s)
8-OH-DPAT	8-hydroxy-2-(d-n-propylamino)-tetralin
pH	measure of acidity ($-\log_{10}[\text{H}^+]$)
PI	phosphatidylinositol
PIF	prolactin inhibitory factor
1-PP	1-(2-pyrimidinyl)-piperazine
PRF	prolactin releasing factor
PRL	prolactin
PVN	paraventricular nucleus
r	Pearson's product moment correlation
rho	Spearman's rank correlation
rpm	revolutions per minute
SCN	suprachiasmatic nucleus
SD	standard deviation
SEM	standard error of the mean
SWS	slow wave sleep
t	Student's t-test statistic
TAUC	total area under the curve
TCA	tricyclic antidepressant(s)
TRH	thyrotropin releasing hormone
TRP	L-tryptophan
TRP:LNAA	ratio of plasma tryptophan to long chain neutral amino acids
TSH	thyroid stimulating hormone, thyrotropin
U	Mann Whitney U test statistic
ug	microgramme(s)

uL	microlitre(s)
uM	micromolar
UV	ultraviolet
VIP	vasoactive intestinal polypeptide
V _{max}	maximum rate of uptake (5-HT into platelets)

PART 1

BACKGROUND

CHAPTER 1

5-Hydroxytryptamine and Depression

1.1 Introduction

The last decade has been a period of rapid progress in the neurosciences. Clinical research has inevitably lagged behind the 'cutting edge' of basic research; however for basic advances to make an impact on clinical disorders, particularly psychiatric illness, fundamental research must be applied to humans. This has limitations, is difficult, costly and time consuming, but necessary.

In this chapter I briefly consider the nature of depressive illness and the current status of basic research into 5-hydroxytryptamine (serotonin, 5-HT) systems in the mammalian brain before reviewing the evidence for abnormal 5-HT function in depressive illness. In Chapter 2 I discuss the neuroendocrine strategy in which 5-HT-mediated anterior pituitary hormone responses provide an index of brain 5-HT neurotransmission and I conclude by outlining the investigations undertaken in this thesis which address some of the questions raised by the foregoing review of 5-HT function in depression.

1.2 Depressive Illness

The symptom of low mood or depression occurs in many physical and psychiatric disorders and as part of normal life. It therefore needs to be distinguished from depressive illness in which depressed mood is one of a

cluster of symptoms which together form the syndrome of depression. In this thesis I will use the terms 'depressed' and 'depression' to refer to depressive illness.

Although the clinical picture presented by individual depressed patients may differ to some degree there is general agreement about which symptoms are central to the condition and about the range of symptoms that can be encountered (Hamilton, 1982, 1989).

The symptoms consist of:

- a) **Depressed mood** which is typically experienced as pervasive and not improved by events the patient would usually find pleasurable. Diurnal variation with mood lowest on awakening and some alleviation during the day is seen in more severe depressions, particularly in association with marked physical symptoms of depression (see below).
- b) **Anhedonia** or loss of pleasure in usual activities.
- c) **Anxiety** which is common in mild and moderately severe depression.
- d) **Lack of energy and tiredness.**
- e) Alteration in physical activity with **retardation** or **agitation.**
- f) **Disturbance of sleep** with generally broken sleep, difficulty getting to sleep (**initial insomnia**) and **early morning waking** which, in more severe depressions, is associated with being unable to get back to sleep

and gloomy brooding about the past or future.

- g) **Loss of appetite** with associated **weight loss**.
- h) Other physical symptoms occur such as **loss of libido**, **amenorrhoea** in women and **constipation**.
- i) **Pessimistic thoughts** (or depressive cognitions) are central features and concern the present, past and future. The patient considers himself worthless with nothing to live for. **Guilty thoughts** about past deeds or omissions preoccupy him and **suicidal thoughts or attempts** may occur.
- j) **Non-specific physical symptoms**, particularly aches and pains, and other **other psychiatric symptoms** such as hypochondriacal worries, obsessional, phobic, depersonalisation or hysterical symptoms, may occur or sometimes even predominate.
- k) **Psychotic symptoms** may occur in severe depressive illness, particularly **delusions** concerning the preoccupations outlined above - worthlessness, guilt, ill health. Persecutory delusions may occur but typically the patient accepts that he is to blame. **Hallucinations** may occur and are usually derogatory and accusing, only rarely having the characteristics of schizophrenic experiences.

There have been many attempts to classify depressive illness, none wholly successful. There is a strong clinical belief and historical tradition which distinguishes between two groups of depressed patients.

The first group consists of patients with symptoms best called melancholic (but often confusingly called endogenous or biological), namely loss of appetite with substantial weight loss, early morning waking, diurnal variation of mood, pervasive anhedonia, retardation and in severe cases, psychotic symptoms. Their depression often seems unrelated to stressors (hence **endogenous depression**). The second group have fewer melancholic symptoms, experience mood fluctuation with more anxiety and their depression appears related to adverse circumstances (**neurotic or reactive depression**). While there does appear to be a group of patients with 'endogenous' depression (Paykel, 1971), there is difficulty in establishing a clear boundary between this and 'neurotic' depression (Lewis, 1934) and recent studies have been unable to relate life events preceding illness to symptom profile or course of illness (Thomson and Hendrie, 1972; Paykel, 1974; Katschnig et al, 1986). Kendell (1969) has argued that depressive illness should be conceived in dimensional terms with symptoms occurring along a continuum between endogenous and neurotic.

A further classification has become generally accepted based on the work of Angst (1966) and Perris (1966), making a distinction between patients with recurrent episodes of depression alone (**unipolar affective illness**) from those with recurrent depression accompanied by one or more episodes of mania (**bipolar affective illness**). This distinction is supported by clinical features of the

illness, morbidity risk in relatives (Perris 1966) and by twin studies (Bertelsen et al, 1977).

The third edition of the American Psychiatric Association's Diagnostic and Statistical Manual of Mental Disorders (**DSM-III** and **DSM-III-R**) (American Psychiatric Association, 1980; 1987) abandons the endogenous/neurotic depression distinction and adopts operationalised criteria for a category called **Major Depression** in which a specified number of symptoms of the depressive syndrome have to occur in the absence of organic or other major psychotic illness. Criteria are provided for subclassification into melancholic or psychotic subtypes. This classification is widely used in research and I have adopted it for the studies in this thesis.

1.3 5-Hydroxytryptamine and Depression - Historical Background

In the nineteenth century scientists had observed that blood serum contained a substance that caused the contraction of smooth muscle organs. However, it was not until the mid-twentieth century that Page and colleagues in the USA, investigating hypertension, isolated this potent vasoconstrictor which they called serotonin (Rapport et al, 1948). This was subsequently identified as 5-HT and synthesised (Speeter et al, 1951). At about the same time in Italy, Erspamer characterised enteramine, a substance

found in enterochromaffin cells of the intestinal mucosa, as 5-HT (Erspamer and Asero, 1953). Following the demonstration of 5-HT in the mammalian brain (Twarog and Page, 1953) its structural similarity to lysergic acid diethylamide (LSD), a potent hallucinogen, led Woolley and Shaw (1954) to suggest that 5-HT might be involved in mental disorders.

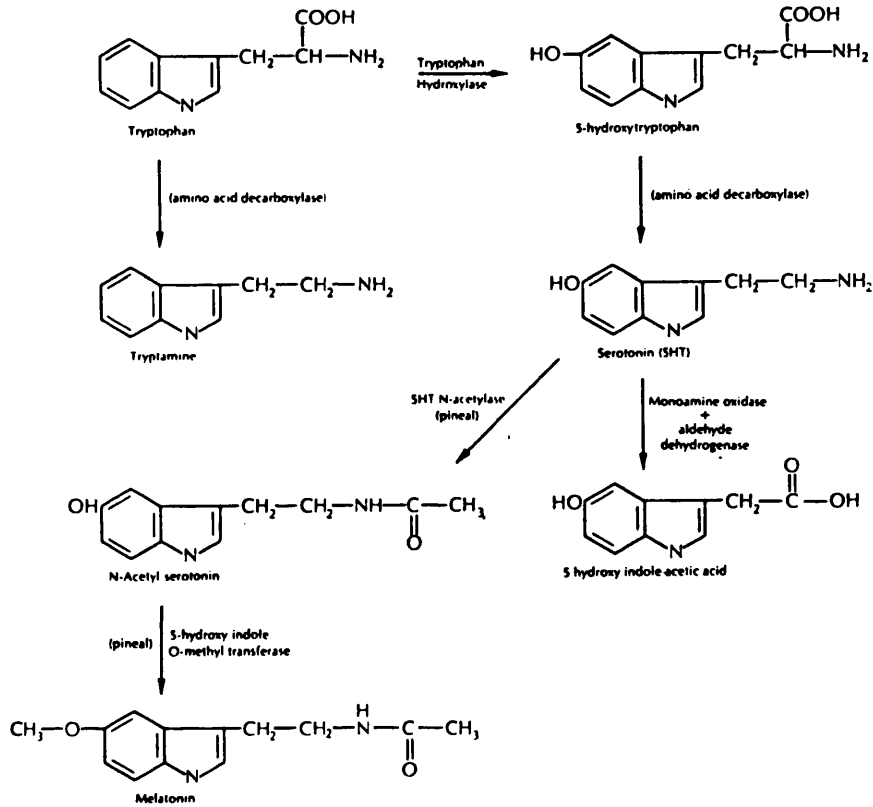
Over the following decade there developed the hypothesis that a deficiency in brain monoamine neurotransmission was of central importance in depression (Schildkraut, 1965; Coppen, 1967) based on observations about the effect of drugs on mood; first, that monoamine depletion by drugs such as reserpine could cause depression (see review by Goodwin and Bunney, 1971) and second, that drugs which enhance monoamine function have antidepressant properties. Coppen (1967) in particular argued for the central role of 5-HT in the aetiology of depression and after a further two decades of research he concluded that 'the serotonin hypothesis [of depression] has stood the test of time' (Coppen and Doogan, 1988).

1.4 Biochemistry of 5-HT

5-HT is synthesised within central neurones from L-tryptophan (TRP) (Figure 1.1). TRP is a rare essential amino acid (comprising about 1.5% of dietary protein) and is unique among amino acids in being highly bound to plasma

Figure 1.1

Metabolic pathways available for the synthesis and metabolism of 5-HT



(Reproduced from Cooper et al, 1982)

albumin (McMenamy and Oncley, 1958) so that less than 20% exists unbound (plasma free TRP). Over 90% of body TRP is metabolised through the kynurenine pathway in the liver (Young et al, 1978) and it is estimated that only 2% enters the brain to be available for 5-HT synthesis (Wood and Coppen, 1980). The rate of brain 5-HT synthesis depends on the availability of TRP because the hydroxylation step (TRP to 5-hydroxytryptophan, 5-HTP) is rate limiting and the enzyme responsible, TRP hydroxylase, is unsaturated with its substrate under normal conditions (Grahame-Smith, 1970). There has been a great deal of debate over the factors regulating TRP influx into the brain. Plasma concentrations of total TRP (Fernstrom and Wurtman, 1971), free TRP (Knott and Curzon, 1973) and the ratio of TRP to long chain neutral amino acids (LNAA) which compete for the same membrane carrier across the blood brain barrier (Fernstrom and Wurtman, 1972) can all be demonstrated to influence TRP influx. Under physiological conditions all three factors are likely to regulate TRP influx with the relative importance of each factor in practice depending on the circumstances of the study.

Aromatic L-amino acid decarboxylase converts 5-HTP to 5-HT. This enzyme may exist in two forms with different pH and temperature optima for reactions with 5-HTP and L-dopa (producing 5-HT and dopamine (DA) respectively) (Green and Grahame-Smith, 1975). However there appears to be little substrate specificity and it has been shown that

administration of 5-HTP to animals can result in the formation of 5-HT in non-5-HT neurones (Ng et al, 1972) where it can release catecholamines by displacement.

5-HT is stored in vesicles in complexes with a specific protein with high affinity for 5-HT, serotonin-binding protein. Following membrane depolarisation, 5-HT is released into the synapse by a calcium dependent process involving fusion of the vesicles with the presynaptic membrane (Tamir and Gershon, 1990).

5-HT is principally metabolised by monoamine oxidase (MAO) which exists in two forms, MAO-A with a higher affinity for noradrenaline (NA), 5-HT and tyramine than DA and phenylephrine, and MAO-B which has the reverse affinities (see review by Youdim and Finberg, 1983). The predominant form of MAO in the brain may be MAO-B (Youdim and Finberg, 1983). MAO metabolises 5-HT to 5-hydroxy-indoleacetaldehyde which is then dehydrogenated to 5-hydroxyindoleacetic acid (5-HIAA)(Figure 1.1) and excreted in the urine. Following synaptic release, 5-HT is taken back up into the presynaptic cell by an energy dependent process (see review by Cooper et al, 1982).

1.5 Distribution of 5-HT in the Brain

5-HT cell bodies are situated mainly in the raphe nuclei in the brainstem with a basic pattern common to all mammals studied (Takeuchi, 1988). Dahlstrom and Fuxe

(1964 classified indoleamine containing neurones into nine groups in the rat brain (designated B1-B9)(Figure 1.2).

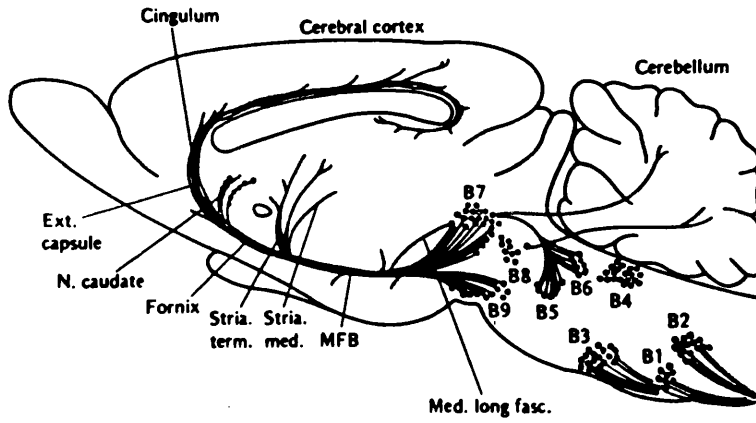
The following description of the localisation of 5-HT neurones is based on the review by Takeuchi (1988).

The caudal groups of 5-HT neurones (B1-B3) give rise to descending pathways projecting to the medulla and spinal cord and are less conspicuous in primates and humans than in rodents. The remaining groups of 5-HT neurones are situated within the pons and mesencephalon with the more rostral groups giving rise to ascending fibres innervating the striatum, limbic system and cerebral cortex. The B6 group lying beneath the rostral part of the fourth ventricle is continuous with the B7 group of neurones and these are the main components of the dorsal raphe nucleus (DRN). B8 neurones lying at the junction of the pons and midbrain form the median raphe nucleus (MRN).

The ascending 5-HT pathway provides the major 5-HT input to the forebrain. It is a large system of fibres originating from groups B6-B9, which ascends in two radiations, the periventricular path and the ventral tegmental radiation. These combine in the medial forebrain bundle and pass through the lateral hypothalamus. Distribution is widespread to the limbic system, striatum and neocortex (Tork, 1990). There is evidence of topographical organisation with neurones from the MRN projecting to hippocampus and in a relatively uniform manner to cerebral cortex while DRN neurones project mainly

Figure 1.2

Distribution of 5-HT pathways in the rat brain



(Reproduced from Cooper et al, 1982)

to striatum with minimal innervation of the hippocampus and a complex pattern of innervation of the cerebral cortex (Molliver, 1987).

1.6 5-HT Receptors

The first indication that 5-HT interacted with at least two distinct receptors came in the 1950s and culminated in the demonstration that 5-HT-induced contractions of guinea-pig ileum could be antagonised by dibenzylamine acting on 5-HT receptors on smooth muscle (D-receptors) and by morphine acting on 5-HT receptors on cholinergic neurones (M-receptors) (Gaddum and Picarelli, 1957). It is now clear that these antagonists are non-specific, but nevertheless the distinction between the two types of 5-HT receptor remains valid. The next major step was the classification, on the basis of ligand binding studies, of two 5-HT receptors in the CNS. 5-HT₁ receptors were characterised by nanomolar affinity for 5-HT; 5-HT₂ receptors by micromolar affinity for 5-HT but nanomolar affinity for spiperone (Peroutka and Snyder, 1979). Since then, significant advances have occurred and 5-HT receptors are now broadly classified into 5-HT₁, 5-HT₂, and 5-HT₃ receptor 'families' on the basis of ligand binding studies (Bradley et al, 1986). Changes in this method of classification are likely as knowledge of the molecular biology, chemistry and physiology of 5-HT receptors

progresses (see below). In the last two years, The 5-HT₂ receptor and two subtypes of the 5-HT₁ receptor (5-HT_{1A} and 5-HT_{1C}) have been cloned (Hartig, 1989). 5-HT receptors are now known to belong to either the superclass of guanine nucleotide (G protein)-coupled receptors (5-HT₁ and 5-HT₂ receptors) or that of ion channel-linked receptors (5-HT₃ receptors).

1.6.1 5-HT₁ Receptor Family

The 5-HT₁ receptor has been subdivided into 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} and 5-HT_{1D} receptor subtypes (Gozlan et al, 1983; Pazos et al, 1984; Hoyer et al, 1985; Heuring and Peroutka, 1987). 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors share similar characteristics but 5-HT_{1C} receptors may be more appropriately classified with 5-HT₂ receptors (see below) and will not be discussed as part of the 5-HT₁ receptor family. The 5-HT₁ receptor family share nanomolar affinity for 5-HT and 5-carbamiidotryptamine (5-CT) and micromolar affinity for 5-HT antagonists such as ketanserin and mesulergine. Recently specific radioligands have been developed for the 5-HT_{1A} receptor, the prototype being 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT) (Gozlan et al, 1983). The pyrimidinyl piperazines (buspirone, gepirone and ipsapirone) are also selective ligands at the 5-HT_{1A} receptor (Traber and Glaser, 1987) and are of experimental and clinical importance.

5-HT_{1A} receptors appear to be the oldest 5-HT₁ receptor

phylogenetically and have a heterogeneous distribution with high densities in the hippocampus and raphe nuclei (Pazos et al, 1988). 5-HT_{1B} receptors are species specific, present with high densities in the basal ganglia in rodents but absent from the brains of higher mammals including humans (Pazos et al, 1988). 5-HT_{1D} receptors, first identified in bovine brain membranes (Heuring and Peroutka, 1987) may serve the same function in higher mammals as the 5-HT_{1B} receptor in rodents and are present in the striatum, substantia nigra, neocortex and hypothalamus (Pazos et al, 1988).

The 5-HT₁ receptor family are G protein-linked and activation leads to the inhibition of adenylate cyclase (Peroutka, 1988). 5-HT_{1A} receptors are believed to act as cell body autoreceptors on raphe neurones and postsynaptic receptors on hippocampal pyramidal cells (Aghajanian et al, 1987). 5-HT_{1B} and 5-HT_{1D} receptors appear to act as presynaptic terminal autoreceptors (Hoyer and Middlemiss, 1989).

1.6.2 5-HT₂ Receptor Family

5-HT_{1C} and 5-HT₂ receptors share molecular, pharmacological and biochemical characteristics and will be discussed together.

5-HT_{1C} receptors are found in choroid plexus, basal ganglia and hypothalamus and differ from 5-HT₂ receptors in having high affinity for 5-HT, thus leading to

classification with 5-HT_{1C} receptors. However 5-HT_{1C} receptors bind with high affinity to classical 5-HT₂ receptor antagonists such as ketanserin and ritanserin (Hoyer, 1988), and share half of their overall amino acid sequence with 5-HT₂ receptors and 78% of their transmembrane domains (Hartig, 1989); indeed probes generated from 5-HT_{1C} receptor amino acid sequences were used to isolate 5-HT₂ receptor c-deoxyribonucleic acid (cDNA) (Pritchett et al, 1988). Both 5-HT_{1C} and 5-HT₂ receptors are linked to modulation of phosphatidylinositol (PI) turnover (Sanders-Bush and Conn, 1987). 5-HT₂ receptors correspond in general terms to the D-receptor of Gaddum and Picarelli (Bradley et al, 1986) and have nanomolar affinity for antagonists such as ketanserin, spiperone and mianserin (Hoyer, 1988). They are present in high densities in the cerebral cortex (Pazos et al, 1988). Recently studies have suggested that subtypes of the 5-HT₂ receptor exist in rat and human brain, the 5-HT_{2A} receptor labelled by ³H-4-bromo-2,5-dimethoxy-amphetamine (DOB) and with high affinity by ³H-ketanserin, and 5-HT_{2B} receptors labelled with lower affinity by ³H-ketanserin and corresponding to the classical 5-HT₂ receptor (Lyon et al, 1986; Pierce and Peroutka, 1989).

1.6.3 5-HT₃ Receptor Family

5-HT₃ receptors have been well characterised in the periphery where excitatory effects of 5-HT, corresponding to

the M-receptor of Gaddum and Picarelli, can be antagonised by selective antagonists such as ondansetron, granisetron and zacopride. It is only recently that 5-HT₃ binding sites have been demonstrated in the CNS (Kilpatrick et al, 1987), where the highest densities are in cortical areas and the dorsal vagal complex. At least three subtypes of the 5-HT₃ receptor have been proposed on the basis of potency differences of selective 5-HT₃ antagonists (Richardson et al, 1985). 5-HT₃ receptors, unlike 5-HT₁ and 5-HT₂ receptors, are ligand-gated cationic channels (Derkach et al, 1989).

1.6.4 Other 5-HT Receptors

Recently another subtype of the 5-HT₁ family has been proposed on the basis of radioligand binding studies, the 5-HT_{1E} receptor, which differs from the rest of the family in not having a high affinity for 5-CT (Leonhardt et al, 1989). A peripheral 5-HT receptor with a unique pharmacological profile that correlates with physiological effects of 5-HT in the gut has been reported, the 5-HT_{1p} receptor (Mawe et al, 1986). Finally, a brain 5-HT receptor which is positively coupled to adenylate cyclase has been reported, a possible 5-HT₄ receptor (Dumuis et al, 1988).

1.7 The Function of 5-HT Systems in the Brain

Soon after 5-HT was discovered in the central nervous system, Brodie and Shore (1957) suggested that behavioural motivation was under the control of two neurotransmitters, NA involved in a stimulatory system underlying exploration, motor excitability and aggression and 5-HT involved in an inhibitory system underlying sleep, rest and digestion. Since then 5-HT has been linked to a wide number of functions but this basic idea remains a useful framework. 5-HT function has been primarily investigated in animals by the use of manoeuvres which decrease or increase 5-HT function in a global or local way. A difficulty exists in interpreting the results of these manipulations, as, depending on the paradigm involved, any specific finding can be related to the model being studied. An example of this is the anticonflict effect of 5-HT lesions which can be interpreted as indicating decreased fear, increased motor activity, insensitivity to pain, or a failure to extinguish previously learnt behaviour (see below).

1.7.1 5-HT Lesions and Behaviour

5-HT lesions in rats using chemical or electrolytic means result in a number of characteristic behavioural alterations. Locomotor activity is often increased (Willner, 1985) and there is evidence that this is limited to novel environments with decreased activity in familiar

environments or conditions of low stimulation (Willner, 1985). Rats treated with drugs that interfere with 5-HT neurotransmission (Geller and Blum, 1970) or with chemical lesions of 5-HT pathways (Tye et al, 1977) show deficient passive avoidance learning in conflict tests. In these tests the rat has been taught that a behaviour is associated with reward (eg pressing a lever for food); subsequently, during the presentation of another stimulus, an aversive stimulus is also given (the lever gives an electric shock). In this conflict paradigm a normal animal will stop the rewarding behaviour (ie stop pressing the lever for food) but the 5-HT-lesioned animal will continue. This effect is also seen with the systemic administration of anxiolytics such as benzodiazepines and, interestingly, can also be produced with microinjections of benzodiazepines into the DRN (Thiebot et al, 1980). These observations have led to the suggestion that this experimental paradigm is demonstrating an anxiolytic action, but as described above, this is only one possible explanation. 5-HT-lesioned animals are less sensitive to pain, an effect which appears to have both a central (Watkins et al, 1981) and a spinal cord (Dickenson et al, 1979) component. Therefore could it be that the animals experience less aversion than normal animals? Another possible explanation of the apparent anti-conflict effect is the observation that 5-HT-lesioned animals are less able to extinguish learnt behaviour (ie they continue a learnt

behaviour even if it is no longer rewarded) (Deakin and Crow, 1986) although they do not appear to have deficient active avoidance learning (Willner, 1985).

Decreased 5-HT function is associated with animals being hypersensitive to startling stimuli (Davis and Sheard, 1974) and they display increased aggression such as muricidal behaviour (Albert and Walsh, 1982) and aggression following electric shock (Ellison and Bresler, 1974). In humans there is increasing evidence for a link between low 5-HT function and aggression and impulsiveness. Brown and colleagues (1979, 1982) found that low CSF 5-HIAA correlated with a life history of aggressive events. Violent offenders with impulsive personality disorders were found to have reduced CSF 5-HIAA (Linnoila et al, 1983; Virkkunen et al, 1987) and the PRL response to fenfluramine, a dynamic measure of brain 5-HT function (see 2.4) was found to be inversely correlated with measures of impulsive aggression in men with personality disorders (Coccaro et al, 1989).

1.7.2 5-HT and 'Basic' Functions

(i) Appetite and Feeding

Increasing 5-HT function in animals and humans decreases food intake (Blundell, 1984). Blundell (1984) proposes that this is due to an increase in satiety rather than a decrease in appetite (as occurs with amphetamine) as fenfluramine, a 5-HT releasing agent, decreases meal size

and rate of eating in rats with negligible effect on meal initiation. It also enhances the 'satiety sequence' which involves behaviours such as grooming and sleeping. In humans, however, it is difficult to disentangle effects on appetite and satiety as d-fenfluramine both lowered motivation to eat before meals (hunger or appetite) as well as decreasing meal size and tendency to snack between meals (satiety) (Blundell and Hill, 1987). The paraventricular nucleus of the hypothalamus (PVN) appears to be an important site mediating the effects of 5-HT on feeding (Leibowitz and Papadakos, 1978). Since the availability of more selective 5-HT agonists it has been possible to investigate the 5-HT receptor subtypes involved in the effects of 5-HT on feeding. In rats, 5-HT_{1A} agonists increase food intake (Gilbert and Dourish, 1987), an effect which appears to be mediated by the stimulation of somatodendritic autoreceptors in the raphe nuclei (Hutson et al, 1986); this results in decreased 5-HT neurotransmission. Postsynaptic 5-HT_{1B} and 5-HT_{1C} receptors may be involved in the hypophagic effects of 5-HT in rodents (Kennet and Curzon, 1988).

(ii) Sleep

Jouvet (1969) provided evidence that normal 5-HT function was necessary for the initiation and maintenance of sleep in cats. It is now clear that sleep is a complicated process neurochemically, involving an interplay

between a number of neurotransmitters and perhaps humoral factors (Gillin et al, 1978) and the role of 5-HT is less clear than Jouvett originally proposed but does seem to be involved in the suppression of vigilance. This is necessary for the induction of slow wave sleep (SWS) and prevention of waking (Wauquier and Dugovic, 1990). The roles of the different 5-HT receptor subtypes remain to be clarified but a recent consistent finding is that selective 5-HT₂ antagonists enhance SWS in humans (Idzikowski et al, 1986), suggesting that 5-HT₂ receptors play an inhibitory role in SWS.

(iii) Sexual Behaviour

The effect of manipulation 5-HT function on sexual behaviour has been primarily studied in the rat. 5-HT can inhibit or facilitate the expression of this behaviour depending on which receptor subtypes are activated and in which sex (Gorzalka et al, 1990). In females, activation of 5-HT_{1A} postsynaptic receptors inhibits lordosis while 5-HT_{1A} cell body autoreceptors and 5-HT_{1B} terminal autoreceptors facilitate this behaviour. Lordosis is primarily facilitated by stimulation of 5-HT_{1C/2} receptors. Male sexual behaviour is facilitated by 5-HT_{1A} receptors, probably at a postsynaptic site and inhibited by 5-HT_{1B} receptors. The role of 5-HT₂ receptors in the male is uncertain.

(iv) Temperature

5-HT appears to play a role in the control of body temperature. 5-HT_{1A} agonists cause hypothermia in rodents, an effect that may be due to decreased 5-HT function as a consequence of somatodendritic autoreceptor stimulation (Green and Goodwin, 1987) although there is some dispute about this (Hjorth, 1985). A recent study showing that microinjection of 5-HT_{1A} agonists into the DRN causes hypothermia does however support a presynaptic site of action (Hillegaart, 1991). Stimulation of postsynaptic 5-HT receptors causes hyperthermia in rats (Gudelsky et al, 1986) and humans (Mueller et al, 1986) and it has been suggested that this is mediated by 5-HT₂ receptors (Gudelsky et al, 1986), but 5-HT_{1C} receptor-mediation is also likely.

1.7.3 5-HT and Circadian Rhythms

The suprachiasmatic nucleus of the hypothalamus (SCN) is believed to play a central role in controlling circadian rhythms and lesions here have been shown to abolish neuroendocrine circadian rhythms (see review by Kordon et al, 1981). It seems likely that 5-HT is involved in the regulation of circadian rhythms as the SCN is heavily innervated by 5-HT neurones, at least in the rat (see 2.2), and 5-HT lesions have also been demonstrated to blunt or abolish circadian rhythms (see review by Kordon et al, 1981).

1.7.4 Overview of 5-HT Function

In a famous review Soubrie (1986) proposed that 5-HT systems were involved in impulsivity or the ability of an animal to tolerate delay before reward. Depue and Spoont (1986), developing a similar idea, have suggested that 5-HT neurones play a central role in a behavioural inhibition system (BIS) which interacts with a behavioural facilitation system (BFS) to determine behaviour (clearly also similar to the model of Brodie and Shore, 1957). Essentially the BIS compares actual environmental circumstances with an internal model of expected outcome. A mismatch, such as punishment, non-reward or uncertainty, results in the BIS inhibiting the BFS which is responsible for locomotion and incentive-reward motivation. The suggested neuroanatomical substrates of the BIS and BFS are the septohippocampal system and mesolimbic DA system respectively. 5-HT neurones from the raphe nuclei provide an input into the septohippocampal region; the net result of increasing 5-HT function is to reinforce the BIS while 5-HT lesions weaken its effect on the BFS. Depue and Spoont (1986) suggest that the effect of an underactive BIS is to decrease a dimension of 'constraint' in an animal's engagement with the environment and in human terms could be seen as underlying impulsivity and impulsive aggression. It is not exactly clear how mood fits into this formulation although they suggest that an increase in activity of the BIS may be associated with frustration (accompanying

signals of non-reward), fear (accompanying signals of punishment) and anxiety (accompanying signals of uncertainty). This model does not provide a simple association between depressed mood and alteration in 5-HT function, however it might be supposed that depression could accompany change in the function of the BFS (the reward system). Given that the BFS and BIS are integrated systems, an alteration in one will be associated with compensatory changes in the other so that it is possible to conceive decreased BFS function accompanying decreased BIS (and 5-HT) function. Support for such a relationship between 5-HT and DA systems comes from work by Agren and colleagues (1986) showing that in humans, levels of cerebrospinal fluid (CSF) 5-HIAA and the DA metabolite, homovanillic acid (HVA), correlate strongly.

1.8 5-HT Function in Depression

For more than two decades researchers have adopted four main approaches to the investigation of 5-HT function in depression, namely studies of post-mortem brain, CSF metabolites, blood and platelets, and neuroendocrine challenge tests. Less direct evidence has been derived from the effects of antidepressant treatments and from manipulation of 5-HT synthesis in patients and volunteers. The techniques of brain imaging (eg positron emission tomography) and molecular genetics (eg candidate gene

approaches for 5-HT receptor subtypes) have not yet yielded any information about 5-HT function in depression.

Recently it has been suggested that impaired 5-HT function is associated with suicide (Goodwin and Post, 1983; van Praag, 1984), particularly violent suicide (Traskman et al, 1981) and more generally with impulsivity and impulsive aggression (Brown et al, 1979; Coccaro et al, 1989; and see 1.7.1). This complicates the interpretation of data in depressed patients, where suicide attempts are common as are personality disorders which often include impulsive traits.

The evidence discussed below is principally in drug-free depressed patients but an important caveat is that in many studies, particularly earlier ones, the drug-free period may have been short and effects of previously administered psychotropic drugs still evident. In many investigations it is not clear how long the drug-free period should be to avoid this confound although three weeks is usually taken as a minimum period.

1.8.1 Post-Mortem Brain Studies

The problems associated with post-mortem brain studies are well recognised. These include accuracy of diagnosis, prior drug exposure, age, post-mortem delay and matching of controls (for discussion see van Praag et al, 1982; Cooper et al, 1986; Stanley et al, 1986a). Most studies have used the brains of suicide victims leading to difficulty in

distinguishing the relative contributions of depression and of suicide itself. In addition it must be recognised that knowledge of monoamine and metabolite levels, or even receptor numbers, does not necessarily provide information about physiological neurotransmission.

(i) Levels of 5-HT and 5-HIAA

That two reviews of essentially the same literature come to opposite conclusions exemplifies difficulties in interpreting the findings. Stanley and colleagues (1986a) conclude on balance that brainstem 5-HT and 5-HIAA are reduced in depression. Cooper and colleagues (1986) interpret the evidence as showing no consistent change compared to controls. The studies and their findings are summarised in Table 1.1. Four out of eight studies reported a reduction in brainstem 5-HT levels with no convincing evidence for an alteration elsewhere in the brain. Levels of 5-HIAA do not differ from controls in most studies. I interpret these data as suggestive, but by no means conclusive, of lowered brainstem levels of 5-HT (presumably in 5-HT cell-bodies) in suicide victims. Mann and colleagues (1989) in a recent review favour the relationship of this 'finding' with suicidal behaviour rather than depression per se, emphasising the difficulty in interpretation discussed above.

Table 1.1

5-HT and 5-HIAA levels in post-mortem brain from suicide victims versus controls

Study	Brain Stem		Cerebral Cortex		Other Areas	
	5-HT	5-HIAA	5-HT	5-HIAA	5-HT	5-HIAA
Shaw et al (1967)	↓					
Bourne et al (1968)	→	↓				
Pare et al (1969)	↓	→				
Lloyd et al (1974)	↓	→			→	→
Birkmayer and Riederer (1975) ^a	↓	↓			↓ ¹	↓ ¹
Beskow et al (1976)	→	±↓	→	±↓	→	±↓ ²
Cochran et al (1976)	→		→		→	
Owen et al (1983)				(↓)		
Crow et al (1984)				(↓)		
Korpi et al (1986)	→	→	→	→	→ ↓ ³	→ ↑ ⁴
Owen et al (1986)			→	→	→	↑ ⁵
Stanley et al (1986b)			→	→		
Arato et al (1987)			→	→		
McKeith et al (1987)				(↓)		

↑ Increased compared to controls

↓ Decreased compared to controls

→ Unchanged compared to controls

(↓) Decrease not statistically significant

±↓ Decrease disappears when post-mortem delay taken into account

a Only three subjects, non-suicide depressives, controls not defined

1 Basal ganglia

2 Putamen, thalamus, mesencephalon

3 Hypothalamus

4 Basal ganglia

5 Hippocampus

(ii) The 5-HT Transporter

The imipramine binding site is thought to be located on 5-HT nerve terminals associated with the 5-HT uptake site or transporter (Paul et al, 1984). Studies of [³H]-imipramine binding may therefore provide an index of 5-HT uptake site numbers and/or function. Table 1.2 summarises the findings. Overall there is no convincing evidence for a consistent change in imipramine binding sites in suicide victims.

(iii) 5-HT Receptor Binding

Table 1.3 summarises the studies to date which provide some evidence for an increase in 5-HT₂ receptor numbers in the frontal cortex of suicide victims and depressed non-suicide cases with little evidence for an alteration in 5-HT₁ receptor numbers. This finding has been interpreted as a consequence of decreased 5-HT neurotransmission (indicated by decreased brainstem 5-HT - see above) leading to an upregulation of postsynaptic 5-HT₂ receptors (Stanley et al, 1986a; Mann et al, 1989). While this appears initially plausible it is not made clear how brainstem decreases in 5-HT concentration alter cortical 5-HT₂ receptor numbers in the absence of any alteration in cortical 5-HT and 5-HIAA concentration (which presumably reflect 5-HT turnover at the presynaptic axon terminal). It also needs to be recognised that some investigators do not find that cortical 5-HT₂ receptors upregulate after

Table 1.2

Post-mortem studies of imipramine binding sites in suicide victims versus controls

Study	Frontal Cortex	Other Regions
Meyerson et al (1982)	↑	
Stanley et al (1982)	↓	
Perry et al (1983)		↓
Crow et al (1984)	↓ ¹	
Paul et al (1984)		↓
Owen et al (1986)	→	→
Meltzer et al (unpub) ²	→	
Arato et al (1987)	Φ	→
Gross-Isseroff et al (1989)	→	↑↓

- ↑ Increased numbers compared to controls
 ↓ Decreased numbers compared to controls
 → Unchanged numbers compared to controls
 Φ Altered left:right hemisphere ratio in suicide victims

- 1 Small subgroup of suicides with history of depression
 2 Cited in Mann et al (1989)

Table 1.3

Post-mortem 5-HT receptor numbers (B_{max}) in the frontal cortex of suicide victims and depressed non-suicide patients versus controls

Study	5-HT ₁	5-HT ₂
Owen et al (1983)	(↓)	(↓)
Stanley and Mann (1983)		↑
Crow et al (1984)		→ ¹
Mann et al (1986)	→	↑
Owen et al (1986)	→	→
McKeith et al (1987) ²	(↑)	(↑)
Cheetham et al (1988)		→ ³
Arora and Meltzer (1989a)		↑
Matsubara and Meltzer(unpub) ⁴	↑ ⁵	
Cheetham et al (1990)	↓ ⁵	
Yates and Ferrier (1990)	→ ⁵	
Yates et al (1990) ²		↑

- ↑ Increased compared to controls
 ↓ Decreased compared to controls
 → Unchanged compared to controls
 (↑) Increase not statistically significant
 (↓) Decrease not statistically significant

- 1 [³H]-LSD binding which does not distinguish between 5-HT₁ and 5-HT₂ receptors
 2 All or majority were depressed non-suicide patients
 3 Dissociation constant (K_d) increased indicating possible confounding effect of antidepressant medication
 4 Referred to in Arora and Meltzer (1989a)
 5 5-HT_{1A} subtype of 5-HT₁ receptor.

raphe lesioning which reduces 5-HT innervation to the cortex (eg Blackshear et al, 1981) so that this mechanism remains speculative.

1.8.2 CSF Studies

A large number of studies have compared lumbar CSF levels of the 5-HT metabolite, 5-HIAA. Most studies have measured baseline 5-HIAA levels and the results of these are summarised in Table 1.4. Pretreatment of subjects with probenecid inhibits active transport of 5-HIAA out of the CSF and may provide a more sensitive index of 5-HT turnover than baseline values. Studies utilising this technique are summarised in Table 1.5. Overall about half of the studies have found a decrease in CSF 5-HIAA in depressed patients (more using the probenecid accumulation technique) with only one study finding an increase in females alone. This provides reasonable evidence that lumbar CSF 5-HIAA is lower in depressed patients. However the interpretation of this finding is not straightforward. First, it is not clear how well lumbar CSF 5-HIAA reflects brain 5-HIAA levels. Most of originates from the lumbar spinal cord rather than the brain (Garelis et al, 1974) although Stanley and colleagues (1985) did find a good correlation between CSF and frontal cortex 5-HIAA levels. Second, it is usually presumed that lower 5-HIAA levels reflect lower 5-HT function but, as argued above (1.8.1), the relationship between metabolite levels and 5-HT function is

Table 1.4

Basal levels of 5-HIAA in the cerebrospinal fluid in depressed patients versus controls

Study	CSF 5-HIAA
Fotherby et al (1963)	→
Ashcroft et al (1966)	↓
Bowers et al (1969)	→
Papeschi and McClure (1971)	(↓)
Van Praag and Korf (1971)	↓
Coppen et al (1972)	↓
McLeod and McLeod (1972)	↓
Goodwin et al (1973)	→
Sjostrom (1973)	→
Ashcroft and Glen (1974)	↓ ¹
Takahashi et al (1974) ^a	↓
Jori et al (1975)	→
Subrahmanyam et al (1975)	↓
Asberg et al (1976)	↓ ^{2 3}
Banki et al (1977)	↓
Vestergaard et al (1978)	→
Berger et al (1980)	→
Curzon et al (1980)	↓
Oreland et al (1981)	→ ³
Traskman et al (1981)	↓ ⁴
Maas et al (1982)	→
Koslow et al (1983)	→ ↑ ⁵
Gerner et al (1984)	→
Asberg et al (1984)	↓
Gjerris et al (1987)	→
Jones et al (1990)	↓ ⁶

- ↑ Increased compared to controls
 ↓ Decreased compared to controls
 → Unchanged compared to controls
 (↓) Decrease not statistically significant

a Cited in Goodwin and Post (1983)

- 1 Unipolar patients only. Bipolar patients=controls
 2 Bimodal distribution with low 5-HIAA subgroup
 3 Suicidal lower than non-suicidal patients
 4 Suicide attempters especially if violent means
 5 Increased in female depressives only. Low suicide rate in patients
 6 Elderly depressives. Decreased in suicide attempters only

Table 1.5

Levels of 5-HIAA in the cerebrospinal fluid after probenecid pretreatment in depressed patients versus controls

Study	CSF 5-HIAA
Roos and Sjostrom (1969)	(+)
van Praag et al (1970)	↓
Korf and van Praag (1971)	↓
Sjostrom and Roos (1972)	↓
Sjostrom (1973)	↓
van Praag et al (1973)	↓
Jori et al (1975)	→
Banki et al (1977)	↓
Berger et al (1980)	→

↓ Decreased compared to controls
 (+) Decrease not statistically significant
 → Unchanged compared to controls

not clear. For example treatments thought to increase brain 5-HT function, fenfluramine (Chase and Shoulson, 1975) and tricyclic antidepressants (TCA) (Potter et al, 1985) result in lower CSF 5-HIAA levels.

The question as to whether low CSF 5-HIAA is a state or trait abnormality in depressed patients is not yet clear. Two studies of drug-free patients investigated when depressed and then in remission found no change in 5-HIAA levels (Coppen et al, 1972; Post et al, 1980) but two larger studies showed that 5-HIAA levels increased after recovery in patients with low initial metabolite levels (van Praag, 1977; Traskman-Bendz et al, 1984) suggesting that there may be a subgroup of patients who show normalisation of CSF 5-HIAA levels on recovery from depression.

One study has measured CSF 5-HT levels and found no difference between depressed patients and controls (Gjerris et al, 1987).

1.8.3 Peripheral Measures of 5-HT Function

While it is possible to measure a number of aspects of peripheral 5-HT function, there are major difficulties in relating most findings to neuronal function in the central nervous system (CNS).

(i) Body Fluid Measurements

As discussed in 1.4, brain 5-HT synthesis is dependent

on the availability of its precursor, the essential amino acid, TRP. Plasma total TRP, free TRP and the ratio of TRP to LNAA (TRP:LNAA) may all influence the synthesis of 5-HT within the brain. Studies in depression have yielded conflicting results with regard to plasma total TRP levels (Coppen et al, 1973; Riley and Shaw, 1976; Møller et al, 1979; DeMyer et al, 1981; Joseph et al, 1984; Maes et al, 1987a; Cowen et al, 1989; Anderson et al, 1990) and plasma free TRP levels (Coppen et al, 1973; Niskanen et al, 1976; Riley and Shaw, 1976; Møller et al, 1979). Reports have been more consistent with regard to the TRP:LNAA ratio with most (DeMyer et al, 1981; Joseph et al, 1984; Cowen et al, 1989; Anderson et al, 1990; Maes et al, 1990), but not all (Møller et al, 1980) studies showing a reduction.

The balance of evidence does appear to support a decrease in plasma TRP measures in depression, particularly the TRP:LNAA ratio, but it is not clear to what degree these changes are related to features of the depression itself and how much to nutritional status, in particular weight loss (Anderson et al, 1990).

(ii) Platelet Studies

The platelet has been used as a model of the brain 5-HT neurone because it possesses a 5-HT uptake system, 5-HT₂ binding sites and is easily accessible. There is a problem however in equating platelet and 5-HT neuronal function which is highlighted by a recent study in rats where

central 5-HT neurone lesioning, which caused profound changes in brain 5-HT levels and receptor binding, had no effect on platelet paroxetine binding (Moret and Briley, 1991). This suggests that state abnormalities in platelet function are likely to be due to peripheral mechanisms, although it remains possible that trait abnormalities in platelet measures could provide a reflection of similar trait disturbances in brain 5-HT neurones.

There is some consensus that 5-HT uptake into the platelet (V_{\max}) is decreased in depression (Tuomisto and Tukiainen, 1976; Coppen et al, 1978; Meltzer et al, 1981; Rausch et al, 1986; Faludi et al, 1988), with all but one of these (Faludi et al, 1988) finding no alteration in the uptake affinity for 5-HT (K_M) indicating that the decrease in V_{\max} is not due to modification of the uptake site. Coppen and colleagues (1978) found that this abnormality did not normalise after recovery and discontinuation of medication which is suggestive of a trait phenomenon; however Meltzer and colleagues (1981) found a correlation between treatment response and an increase in 5-HT uptake, suggesting a state marker, so this issue is not resolved. In addition it must be recognised that this finding is not specific for depression as it can occur in schizophrenia, migraine, cirrhosis and hypertension (Coppen and Doogan, 1988).

The imipramine binding site is related to the 5-HT transporter (Paul et al, 1984) and two recent reviews

(Langer and Schoemaker, 1988; Møllerup and Plenge, 1988), citing over 30 studies, show that about two thirds of studies report a decrease in the number of [³H]-imipramine binding sites (B_{max}) with only one study showing an increase (Møllerup et al, 1982). Langer and Schoemaker (1988) conclude that decreased imipramine binding is a relatively specific marker for depression. In contrast, the review by Møllerup and Plenge (1988) highlights both methodological problems and the lack of specificity of this finding for depression. They do finally conclude that a subgroup of depressed patients with decreased imipramine binding probably does exist but that evidence is conflicting as to whether this is a state or trait phenomenon.

It has been suggested that the 5-HT transporter has one site for imipramine binding and another for 5-HT and specific 5-HT uptake inhibitors (Møllerup et al, 1983; Sette et al, 1983; Habert et al, 1985; Møllerup et al, 1985). Paroxetine is a highly potent and specific 5-HT uptake inhibitor (Buus-Lassen et al, 1980) with a higher affinity than imipramine for platelet membranes although the number of binding sites is the same (Møllerup et al, 1983). This suggests that it might be a more reliable tool than imipramine for determining changes in the 5-HT transporter. One study in depression has reported no difference in paroxetine binding between controls and patients (D'haenen et al, 1988).

There have been only a few studies of platelet 5-HT₂ receptor binding in depression with two studies finding no difference from controls (Cowen et al, 1987; McBride et al, 1987) and three studies an increase in B_{max} (Biegon et al 1987; Arora and Meltzer, 1989b; Pandey et al, 1990). These findings essentially parallel the post-mortem findings in frontal cortex (see 1.8.1) and suggest that 5-HT₂ receptor numbers may be increased in depressed patients. However the small number of studies does not allow firm conclusions to be drawn.

1.8.4 5-HT Neuroendocrine Challenge Tests

The measurement of anterior pituitary hormone responses to 5-HT drug challenge offers a strategy for investigating the function of hypothalamic 5-HT systems (see Chapter 2 for discussion of principles and methodological considerations).

(i) 5-HT-Mediated Cortisol Responses

Before discussing these results it is important to recognise the overwhelming evidence for abnormal functioning of the hypothalamic-pituitary-adrenal (HPA) axis in depression with increased plasma cortisol levels, adrenal hyperplasia and abnormal adrenocorticotrophic hormone (ACTH) and cortisol responses to corticotrophin releasing hormone (CRF) and ACTH administration respectively (see reviews by Charlton and Ferrier, 1989;

Table 1.6

5-HT-mediated cortisol responses in depression

Study	Drug	Route	CORT Response
Meltzer et al (1984)	5-HTP	po	↑
Jacobsen et al (1987)	5-HTP	po	→ ¹
Maes et al (1987b)	5-HTP	po	↑ ²
Maes et al (1989)	5-HTP	po	↑ ²
Meltzer et al (1989)	L-5-HTP	po	(↓)
Weizman et al (1988)	FEN	po	↓
Asnis et al (1988)	FEN	po	→
Lopez-Ibor et al (1989)	FEN	po	↑ ³
Targum and Marshall (1989)	FEN	po	→ ⁴
Mitchell and Smythe (1990)	FEN	po	→
O'Keane and Dinan (1991)	d-FEN	po	↓
Golden et al (1990)	CMI	iv	(↓)
Kahn et al (1988)	mCPP	po	→
Heninger et al (1990)	mCPP	iv	→
Kahn et al (1990)	mCPP	po	→
Lesch et al (1990a)	IPS	po	↓
Meltzer (1990)	MK-212	po	→

- ↑ Increased compared to controls
 ↓ Decreased compared to controls
 → No different to controls
 (↓) Non-significant decrease compared to controls

- 1 Patients with seasonal affective disorder (but also meeting Research Diagnostic Criteria for major depressive disorder)
 2 Females only
 3 Major depressives compared to dysthymic disorder
 4 No cortisol response in patients or controls

CORT=cortisol

5-HTP=D,L-5-hydroxytryptophan; L-5-HTP=L-5-hydroxytryptophan,
 FEN=fenfluramine; d-FEN=d-isomer of fenfluramine; CMI=clomipramine;
 mCPP=m-chlorophenylpiperazine; MK-212=6-chloro-2-(1-piperazinyl)-
 pyrazine; IPS=ipsapirone

Kathol et al, 1989). This renders sensible interpretation of the effects of neurotransmitter manipulations difficult, if not impossible. Table 1.6 summarises the results of studies measuring cortisol responses to 5-HT challenge which overall provide no consistent evidence for an abnormality. The finding of increased cortisol responses in three out of five studies using 5-HTP without consistently similar findings using other 5-HT drug challenges suggests either that this response is not mediated by 5-HT pathways (5-HTP can displace catecholamines from nerve terminals, Ng et al, 1972) or else it is an effect specific to precursor challenge. One possibility, particularly if it is restricted to melancholic females as found by Maes and colleagues (1987), is that the enhanced cortisol response is due to weight loss, a finding seen with prolactin (PRL) and growth hormone (GH) responses to TRP challenge after weight loss in (predominantly female) depressed patients (Cowen and Charig, 1987; Deakin et al, 1990)

(ii) 5-HT-Mediated Growth Hormone Responses

Five studies have reported decreased GH responses to 5-HT precursor challenge (Table 1.7). In two studies the blunting was most evident in endogenous/melancholic patients (Deakin et al, 1990; Price et al, 1991) but no relationship with melancholic status was found in a third (Cowen and Charig, 1987). One study has looked at the

Table 1.7

5-HT-mediated growth hormone responses in depression

Study	Drug	Route	GH Response
Takahashi et al (1973)	5-HTP	po	↓
Koyama and Meltzer (1986)	TRP	iv	↓
Cowen and Charig (1987)	TRP	iv	↓
Deakin et al (1990)	TRP	iv	↓
Price et al (1991)	TRP	iv	↓ ¹
Lopez-Ibor et al (1989)	FEN	po	↓ ^{2 3}
Golden et al (1990)	CMI	iv	↑ ²
Heninger et al (1990)	mCPP	iv	↓

↑ Increased compared to controls

↓ Decreased compared to controls

1 In melancholic depressives

2 FEN and CMI do not consistently cause GH responses in normals

3 Major depressives compared to dysthymic disorder

5-HTP=5-hydroxytryptophan; TRP=L-tryptophan; FEN=d,1-fenfluramine;
CMI=clomipramine; mCPP=m-chlorophenypiperazine

effect of recovery (after withdrawal of antidepressant medication) and suggests that this abnormality normalises when the patient is euthymic (Upadhyaya et al, 1990) suggesting a state abnormality. In normal subjects there is no consistent stimulation of GH secretion by fenfluramine (Cowen and Anderson, 1986) or CMI (Golden et al, 1989) which make the findings of Lopez-Ibor and colleagues (1989) and Golden and colleagues (1990) (Table 1.7) difficult to interpret. Therefore further studies using challenge with alternative 5-HT drugs which do stimulate GH secretion in normal subjects are needed and it is of interest that one study using mCPP has also demonstrated a blunted GH response in depressed patients (Heninger et al, 1990). As yet, however, it cannot be concluded with certainty that 5-HT-mediated GH secretion is decreased in depression and, even if this is the case, there is still some uncertainty as to whether GH release following growth hormone releasing factor (GRF) stimulation is blunted in depressed patients (see Cowen, 1989). If this is the case, it would suggest that the abnormality in GH secretion occurs at the level of the pituitary somatotroph rather than the hypothalamic 5-HT neurone.

(iii) 5-HT-Mediated Prolactin Responses

There are now a reasonable number of studies looking at PRL responses to a variety of 5-HT drug challenges (Table 1.8). Eleven out of nineteen studies have found a definite

Table 1.8

5-HT-mediated prolactin responses in depression

Study	Drug	Route	PRL Response
Maes et al (1989)	5-HTP	po	↑ ¹
Heninger et al (1984)	TRP	iv	↓
Koyama and Meltzer (1986)	TRP	iv	(↓) ²
Cowen and Charig (1987)	TRP	iv	↓ ^{3 4}
Deakin et al (1990)	TRP	iv	↓ ³
Price et al (1991)	TRP	iv	↓ ⁵
Siever et al (1984)	FEN	po	↓
Siever et al (1986)	FEN	po	↓ ⁶
Asnis et al (1988)	FEN	po	→
Weizman et al (1988)	FEN	po	(↓) ⁷
Coccaro et al (1989)	FEN	po	↓ ⁸
Lopez-Ibor et al (1989)	FEN	po	↓ ^{4 9}
Targum and Marshall (1989)	FEN	po	(↓)
Mitchell and Smythe (1990) ^a	FEN	po	↓ ¹⁰
O'Keane and Dinan (1991)	d-FEN	po	↓ ¹¹
Golden et al (1990)	CMI	iv	↓
Heninger et al (1990)	mCPP	iv	→
Kahn et al (1990)	mCPP	po	→
Meltzer et al (1990)	MK-212	po	→

- ↑ Increased compared to controls
 ↓ Decreased compared to controls
 → No different to controls
 (↓) Non-significant/questionable decrease compared to controls

a Substantially the same group of patients as described in Mitchell et al, 1990

- 1 Females only (melancholic patients)
 2 Not statistically significant after covarying for plasma TRP levels which were lower in patients
 3 Excluding patients with severe weight loss
 4 Lowest in patients with major depression with melancholia
 5 Non-melancholic patients only
 6 Recovered depressives also had decreased PRL responses
 7 Response decreased as % of baseline, not in absolute terms
 8 Particularly in patients with suicidal behaviour
 9 Major depression compared to dysthymic disorder
 10 Endogenous/melancholic patients only
 11 Non-blunting associated with endogenous diagnosis and weight loss

TRP=L-tryptophan; FEN=fenfluramine; d-FEN=d-isomer of fenfluramine;
 CMI=clomipramine; mCPP=m-chlorophenypiperazine; MK-212=6-chloro-2-(1-piperazinyl)-pyrazine

decrease with a further three finding non-significant decreases. One study with 5-HTP found an increase (Maes et al, 1989), but only in melancholic females, with no PRL response in normal controls and a tendency for PRL levels to fall more in male depressives compared to controls. The authors suggest that there may be sex differences in 5-HT function in depression, but it is more likely that this is the confounding effect of weight loss which was apparent in the TRP studies by Cowen and Charig (1987) and Deakin and colleagues (1990) who both found that weight loss during the depressive episode resulted in enhanced PRL responses in females. Of the four studies that have found no difference between patients and controls, three have used post-synaptic agonists (mCPP and MK-212).

These studies therefore provide strong evidence that depressed patients have diminished PRL responses to 5-HT drugs which challenge the presynaptic neurone and further studies are clearly indicated using postsynaptic 5-HT agonists. Melancholic/endogenous subgroup has been associated with particularly low PRL responses in three studies (Cowen and Charig, 1987; Lopez-Ibor et al, 1989; Mitchell and Smythe, 1990) with the opposite association in two others (O'Keane and Dinan, 1991; Price et al, 1991). Suicidal behaviour/impulsive aggression (Coccaro et al, 1989) has also been associated with particularly low PRL responses. The study by Price and colleagues (1991), using intravenous TRP, deserves special mention because it

compared one hundred and twenty six patients to fifty eight controls; a very large study in this area of research. Given its size the results were somewhat equivocal although broadly in line with other studies. A note of caution in interpreting the results of this study is warranted however as the patients appear to have been a highly selected population (patients with suicidal behaviour were excluded and surprisingly few patients had significant weight loss) and a large proportion appear to have recently been on psychotropic drugs.

The question as to whether the blunted PRL response in depressives is a state or trait abnormality is uncertain as one study has reported that medication-free recovered depressives have decreased PRL responses to fenfluramine (Siever et al, 1986), while another suggests that the blunted PRL response to TRP reverts to normal in patients who are retested after recovery (Upadhyaya et al, 1990).

An abnormality in 5-HT-mediated PRL does not necessarily imply a change in 5-HT function as opposed to some other step in PRL release and/or clearance from plasma. Hormone release after drug challenge measures overall function through a series of steps from the 5-HT neurone to the pituitary lactotroph and an obvious possibility is that PRL secretion itself is abnormal in depression. The most widely used challenge of PRL secretion has been infusion of thyrotropin releasing hormone (TRH), a tripeptide, which acts directly on peptide

Table 1.9

TRH-induced prolactin responses in depression

Study	PRL Baseline	PRL Response
Maeda et al (1975)	→	↑
Brambilla et al (1978)	→	→
Naeije et al (1987)	→	→
Coppen et al (1980)	→	↑ ¹
Langer et al (1980)		→
Linkowski et al (1980)	↓ ²	↓ ³
Kirkegaard et al (1981)	→	→
Targum et al (1982)	→	↓
Winokur et al (1982)	→	→
Amsterdam et al (1983)	→	→
Winokur et al (1983)		→
Witschy et al (1984)	→	↓
Kjellman et al (1985)	→	→
Garbutt et al (1986)	↓ ⁴	(↓) ⁴
Zis et al (1986)	→	→ ⁵
Unden et al (1987)	→	→
Baumgartner et al (1988)	→	↑
Rubin et al (1989)	→	↑

↑ Increased compared to controls

↓ Decreased compared to controls

→ No different to controls

(+) Decreased absolute response but not when expressed as percentage of baseline

1 Subgroup without family history (sporadic depression)

2 In bipolar and postmenopausal unipolar patients but not in premenopausal unipolar patients

3 In pre- but not postmenopausal female depressed patients (males not studied)

4 Female but not male depressed patients

5 Compared to non-depressed psychiatric controls

receptors on the pituitary lactotroph to stimulate PRL secretion. This response is calcium dependent with PI as the second messenger (Gershengorn, 1986). Table 1.9 summarises the results from seventeen studies with adequate numbers which compared the PRL response to TRH in depressed patients and controls. Overall there is no consistent evidence for an abnormality in stimulated PRL secretion from these studies. Unfortunately they do not provide an adequate control for 5-HT-mediated PRL studies because the PRL responses in the two conditions are not comparable. 5-HT drugs produce a submaximal stimulation of PRL secretion while all the TRH studies used 100ug or more of TRH, a supramaximal stimulus producing maximal PRL responses (Jacobs et al, 1973). These TRH studies, therefore, would not have detected an alteration in pituitary lactotroph sensitivity (ie any shift in the dose-response curve), only in the total amount of PRL that could be released (often called pituitary PRL reserve). Therefore at present we do not have the information to exclude reduced lactotroph responsiveness in depression.

1.8.5 Evidence from 5-HT Depletion Studies and Antidepressant Treatment

While it is not possible to extrapolate directly from the mechanism of action of an effective treatment to pre-existing abnormality (eg diuretics which act on the kidney are effective in treating cardiac failure), it is

nevertheless of interest that enhancement of 5-HT function appears to be antidepressant and 5-HT depletion causes relapse.

(i) 5-HT Depletion

It has been long recognised that monoamine depletion by drugs such as reserpine may cause depression (Goodwin and Bunney, 1971), indeed this observation was one of the original planks of the monoamine hypothesis of depression. Further evidence, more specifically related to 5-HT, consists of two studies in which successfully treated depressed patients were temporarily caused to relapse by treatments designed to reduce 5-HT synthesis. In the first study Shopsin and colleagues (1976) induced depressive relapse in patients on tranylcypromine by the administration of the 5-HT synthesis inhibitor, para-chlorophenylalanine. In the second study, Delgado and colleagues (1990) induced a temporary relapse of depression by the oral administration of a TRP-free amino acid mixture. This manoeuvre causes an acute profound reduction in plasma TRP leading to reduced TRP entry into the brain and hence decreased 5-HT synthesis (see 1.4). Normal subjects also experience mild depressive symptoms if subjected to similar treatment (Young et al, 1986; Smith et al, 1987).

While these results are intriguing, it remains to be demonstrated that they are specific consequences of 5-HT

depletion and are not, for example, mediated by cognitive processes such as the misinterpretation of an unpleasant physical state.

(ii) Antidepressant Treatment

Many antidepressant treatments have acute effects on 5-HT function. TCA such as imipramine, clomipramine (CMI), specific 5-HT uptake inhibitors and monoamine oxidase inhibitors (MAOI) are all believed to increase intrasynaptic 5-HT levels acutely (Cooper et al, 1982; Fuller and Wong, 1990). This effect is not paralleled by clinical response which is typically delayed by two or more weeks. Interest has therefore turned to longer term effects of antidepressants on 5-HT measures (see Garattini and Samanin, 1988 for review and discussion).

Chronic administration of a variety of antidepressant drug treatments reduce 5-HT₂ receptor numbers in rat cortex without consistent effects on 5-HT₁ receptors (Anderson, 1983). However there are exceptions to this finding, namely that electroconvulsive shock increases 5-HT₂ receptor density and fluoxetine, a selective 5-HT uptake inhibitor and effective antidepressant, does not alter 5-HT₂ receptor density (Anderson, 1983). Of interest, in this regard, a recent post-mortem study in humans found that unmedicated depressed patients had increased numbers of cortical 5-HT₂ receptors while those who had received antidepressants had normal numbers, suggesting that

antidepressants may have 'normalised' the number of 5-HT₂ receptors (Yates et al, 1990).

Functional measures of 5-HT receptor sensitivity provide conflicting evidence about whether chronic antidepressant treatments enhance 5-HT neurotransmission. Electrophysiological studies by Blier and colleagues (1987) have demonstrated that a common action of a wide variety of antidepressant treatments is to increase hippocampal 5-HT₁ (probably 5-HT_{1A}) receptor responses by alteration of pre- or postsynaptic receptor sensitivity. In apparent contrast to this, however, chronic antidepressant treatment has been reported to reduce the behavioural syndrome following administration of the 5-HT_{1A} receptor agonist, 8-OH-DPAT (Goodwin et al, 1987a) while the corticosterone response appears unaltered (Aulakh et al, 1988a); both of these responses provide a measure of postsynaptic 5-HT_{1A} receptor function. Chronic antidepressants do however attenuate the hypothermia caused by 8-OH-DPAT (Goodwin et al, 1987a), a putative measure of presynaptic (autoreceptor) function (see 1.7.2). This is an effect likely to enhance 5-HT neurotransmission. However, attenuation of 8-OH-DPAT hypothermia is not found after all antidepressants and can be caused by diazepam (Martin et al, 1989) casting some doubt on this being a common and specific mechanism related to antidepressant action.

In humans, 5-HT precursors have little antidepressant efficacy when given alone (d'Elia et al, 1978), but do

enhance the antidepressant efficacy of MAOI and CMI (d'Elia et al, 1978). In contrast to animal work on cerebral cortex, chronic desipramine treatment in normal volunteers increased the number of platelet 5-HT₂ receptor binding sites (Cowen et al, 1986). 5-HT neuroendocrine challenge tests have been used to investigate the effects of antidepressants on 5-HT function. In normal volunteers, the PRL response to TRP infusion, a putative measure of 5-HT₁ function (see 2.4), was enhanced by desipramine (Cowen et al, 1986), lithium (Glue et al, 1986) and acute CMI administration (Anderson and Cowen, 1986). In depressed patients, desipramine (Charney et al, 1984; Price et al, 1989a), amitriptyline (Charney et al, 1984), tranylcypromine (Price et al, 1985), fluvoxamine (Price et al, 1989a) and lithium (Price et al, 1989b) have all been shown to enhance TRP-induced PRL responses, but mianserin (Cowen, 1988) and trazodone (Price et al, 1988) did not have this effect. Meltzer (1990) reports that fluoxetine treatment in depressed patients enhances the PRL and cortisol responses to L-5-HTP, and the cortisol response to MK-212, but that tricyclic antidepressants do not. Enhanced 5-HT-mediated neuroendocrine responses do not therefore appear to be a universal property of antidepressant drug treatment. Further, an association between the ability of a drug to enhance this response and antidepressant effect in individual patients, has not been found (Charney et al, 1984; Price et al, 1989a).

In conclusion, it is clear that a large number of antidepressant treatments enhance 5-HT₁ function in animal and human studies. The hypothesis that this effect is relevant to antidepressant efficacy is appealing and overall gains support when taken in conjunction with 5-HT depletion studies (see above). The conflicting evidence however suggests that enhancement of 5-HT₁ function is not necessary, and perhaps not sufficient, to effect an antidepressant response.

1.9 Summary

5-HT neurones from the brainstem are widely distributed in the forebrain (see 1.5) and evidence has been presented to show that they are involved in the regulation of a wide number of 'basic' functions such as appetite, sleep, sexual behaviour, temperature and circadian rhythms (see 1.7). They are also involved in a complex way in interactions with other animals and the environment. In an attempt to integrate the findings from different areas it has been suggested that 5-HT neurones may form the basis of a system for terminating or inhibiting behaviour and for disengaging from the environment particularly if there are negative consequences (1.7.4). Extrapolation from animals to humans is difficult and often of uncertain validity, however the evidence that exists is consistent with aspects of the above formulation. In particular the link between measures

suggestive of low 5-HT function and impulsive-aggression (see 1.7) suggests to me that adequately functioning 5-HT systems are necessary for self-control and inhibition of violent behaviour. It is notoriously difficult to use animal models to gain knowledge about human mood states and it is clear that in the end this must be done in human studies. However I would argue that the animal evidence is consistent with 5-HT being involved in affective states in humans where striking alterations occur in sleep and appetite with profound behavioural changes in the quality of interactions with other people and the environment.

I have reviewed the evidence for abnormal 5-HT function in depression in human studies (see 1.8). The major area of investigation has been the study of measures of 5-HT function in drug-free depressed patients. Assessment of these data is complicated by the bias toward reporting of positive results; this is particularly relevant in areas where the number of studies is small. I believe that there are only five findings where there are both a reasonable number of studies and sufficient consistency of result to be persuaded that an abnormality exists (in at least a subgroup of depressed patients). These are:

- 1 A reduction in CSF levels of 5-HIAA.
- 2 A reduction in platelet 5-HT uptake.
- 3 A reduction in platelet imipramine binding.
- 4 A reduction in plasma TRP:LNAA levels.
- 5 A reduction in 5-HT-mediated PRL release.

On the face of it this is an impressive body of evidence from which it is possible to hypothesise that depressed patients have a decrease in the function of central 5-HT neurones. Using the five lines of evidence above the formulation runs thus: decreased intraneuronal 5-HT due to diminished synthesis (4) and reuptake (2,3) would lead to lowered 5-HT turnover (1) and decreased 5-HT release (5). Much of this is also consistent with other less firm findings, eg lowered brainstem 5-HT levels and increased cortical 5-HT₂ receptor numbers in suicide victims.

As has been pointed out, however, taken individually each finding raises unanswered questions:

1 Does the reduction in lumbar CSF 5-HIAA reflect what occurs in the brain? Are there methodological explanations, eg prior antidepressant drug treatment? What does lower 5-HIAA tell us about 5-HT neurotransmission? Is this finding more related to a propensity to suicide rather than depression itself?

2 How does the peripheral finding of lower platelet 5-HT uptake relate to neuronal function? What does it tell us about 5-HT neurotransmission (5-HT uptake inhibitors supposedly enhance 5-HT function)? What do we make of its lack of specificity for depression?

3 The finding of decreased platelet imipramine binding raises the same questions as apply to platelet 5-HT uptake.

4 Although TRP:LNAA ratio is a peripheral finding there

is good reason from animal studies to believe this can influence brain 5-HT function. However, are the changes seen in depression large enough to have any effect? Is this change related to depression directly or is it simply a consequence of some other factor such as eating less or losing weight?

5 5-HT neuroendocrine tests potentially offer a method of assessing central 5-HT neurotransmission. There are however many methodological problems in the interpretation of the results; these are discussed fully in Chapter 2. I will just highlight here the lack of specificity of the drugs available, the question as to whether PRL secretion itself is altered in depressed patients, the potentially confounding effects of weight loss and side-effects (in particular nausea). In addition, if the abnormality is indeed due to decreased 5-HT function, it is not clear where the abnormality lies; is it in 5-HT synthesis or release, or in post-synaptic mechanisms such as receptor sensitivity or second messenger activity?

Addressing all of these issues is clearly beyond the scope of this thesis. Those which I do investigate are outlined at the end of Chapter 2 and are concerned with points 4 and 5 above.

CHAPTER 2

Neuroendocrine Challenge Tests of 5-HT Function

2.1 Introduction

The rationale behind neuroendocrine challenge tests has been described by Checkley (1980) and is based on the involvement of monoamine pathways in the control of anterior pituitary hormone secretion (see reviews by Muller et al, 1977; Tuomisto and Mannisto, 1985). Monoamine neurones innervate hypothalamic nuclei containing the cell bodies of anterior pituitary hormone releasing (and inhibitory) factor neurones. These send projections to the median eminence (ME), the terminals of which abut the capillaries of the hypothalamo-hypophyseal portal system. Stimulation of these neurones causes 'secretion' of releasing or inhibitory factors into the portal system whence they are transported to the anterior pituitary. Interaction of these factors with specialised anterior pituitary cells results in stimulation or inhibition of hormone secretion from those cells. Activation of a monoamine pathway by a drug with specific actions on that pathway leads to a characteristic pattern of hormone release detectable in venous blood. The magnitude of the hormone 'response' can be used as an index of neurotransmission in the monoamine system stimulated, provided that certain limitations of this approach are recognised and care is taken to control for factors known to influence the hormone response.

Checkley (1980) gives the following conditions that

need to be considered in the evaluation of neuroendocrine challenge tests:

- a) The same hormone response should result from the administration of all drugs which stimulate the specified receptor.
- b) The hormone response should be inhibited by appropriate antagonists.
- c) The hormone response should not be inhibited by antagonists of other receptors.
- d) The case is strengthened if antagonists of the specific receptor also inhibit hormone responses to physiological stimuli such as exercise.
- e) In the case of precursors or indirect agonists, any alterations in hormone response may occur as a consequence of changes in neurotransmitter release or uptake, as well as at the level of the receptor.

To establish the site of receptor stimulation further considerations are:

- f) If the hormone response is only produced by drugs that cross the blood brain barrier (BBB) then the receptor lies within the brain.
- g) If drugs that do not cross the BBB stimulate hormone secretion then the receptor may still lie within the brain provided it can be demonstrated in animal models that they lie within the ME, a structure lacking the usual BBB.

Criterion g) may be widened to consider the neuroanatomical

basis of a specific hormone response in animal models. For example, the existence of 5-HT neurones innervating releasing factor cells in the hypothalamus, in the absence of good evidence for pituitary innervation, lends support to a hypothalamic site of action for 5-HT-active drugs.

If an abnormality in hormone secretion is detected in a patient sample then the following methodological considerations are important:

- 1) Psychotropic drug exposure because of the effect on monoamine function (see Garattini and Samanin, 1988). It is usual to exclude current neuroleptic and antidepressant drug treatment but the usual three week washout period may not be enough (eg see Corn et al, 1984; Schittecatte et al, 1989). In addition benzodiazepines are often allowed but there is evidence that, at least acutely, they may alter hormone responses, for example to the 5-HT precursor, TRP (Nutt and Cowen, 1987).
- 2) Intake of recreational drugs such as alcohol, nicotine and caffeine should be considered although only excessive alcohol intake is likely to be important (Checkley, 1980).
- 3) Age, sex and ovarian activity must be controlled for as these may influence hormone secretion. For example oestrogens enhance PRL secretion (Tuomisto and Mannisto, 1985).
- 4) Psychological stress caused by the procedure (eg

venepuncture) may cause stress-related hormone secretion and it is therefore usual to have a 30- to 60-minute baseline period before administration of the challenge drug. In addition the drug itself may cause stressful side-effects and so influence hormone secretion.

- 5) Nutritional status is likely to be important. Preliminary evidence suggests that even moderate weight loss may alter hormone responses to TRP (Goodwin et al, 1987b).
- 6) Other influences such as physical activity and time of day can influence hormone secretion. The presence of certain physical illnesses and pregnancy will also alter hormone responses.

Three further points that are not made by Checkley (1980) need to be emphasised:

- 7) The hormone response does not only depend on the activity of the monoamine synapse with which the drug interacts, but on a series of steps leading from the monoamine neurone to the pituitary secreting cell together with the rate of clearance of the hormone from the plasma. In other words it tests the function of the overall system and an abnormality in any one of the steps could account for an abnormal finding in a patient population.
- 8) Kinetic factors must be considered with regard to any abnormality, in particular after oral administration of

a challenge drug. It is quite feasible that patients may have an altered rate of absorption of the drug leading to different plasma levels compared to controls.

- 9) It must be acknowledged that there is great inter-individual variation in hormone responses to challenge. This is true of most, if not all, biological measures in heterogeneous populations, and its consequence is that small changes are often obscured by the size of the variance. There is no easy solution to this and false negatives (Type II errors) are likely as are false positives (Type I errors) arising from all the other factors discussed above. This means that it is vital for studies to be replicated and for appropriate statistical analysis to be employed, particularly for negative studies, where large variance often means that substantial differences could be missed.

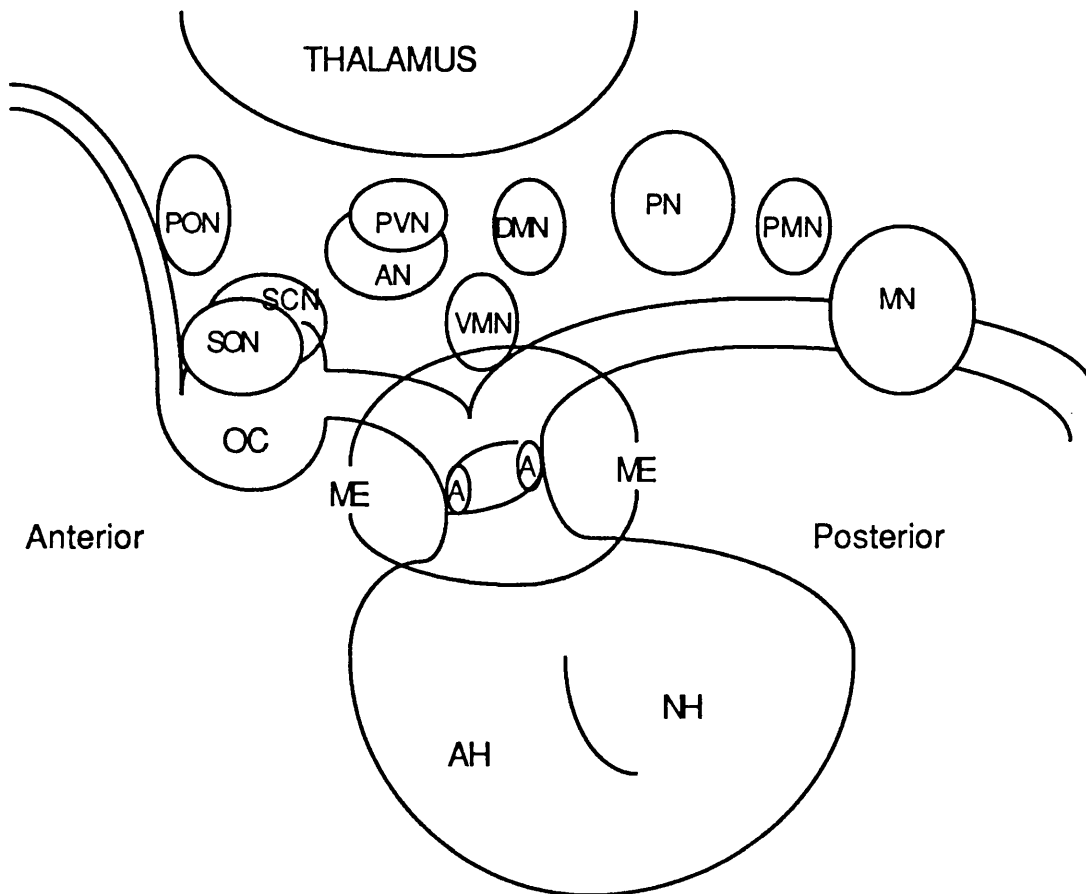
2.2 5-HT Innervation of the Hypothalamus and Pituitary: Relationship to Neuroendocrine Function

There are species differences in hypothalamic innervation by 5-HT fibres and this must be taken into account when attempting to extrapolate from animal studies to humans. A schematic diagram of hypothalamic nuclei in humans is given in Figure 2.1.

The hypothalamus as a whole is one of the highest 5-HT-

Figure 2.1

Schematic diagram of the major hypothalamic nuclei in the human brain



A	arcuate nucleus	POH	preoptic nucleus
AH	adenohypophysis	PMN	pre-mammillary nucleus
AN	anterior nucleus	PN	posterior nucleus
DMN	dorsomedial nucleus	PVN	paraventricular nucleus
ME	median eminence	SCN	suprachiasmatic nucleus
MN	mammillary nucleus	SON	supraoptic nucleus
NH	neurohypophysis	VMN	ventromedial nucleus
OC	optic chiasma		

innervated areas in the central nervous system with the highest concentration of 5-HT fibres found in the ventromedial and mamillary nuclei (Takeuchi, 1988). Both the DRN and MRN innervate the hypothalamus and there is evidence for a degree of topographical organisation, for example innervation of the anterior hypothalamus is particularly from the the MRN (Moore et al, 1978; Van de Kar and Lorens, 1979).

In rats, raphe lesions or interruption of caudal hypothalamic inputs abolish the PRL response to suckling (Barofsky et al, 1983) and 5-HT drug challenge (Fessler et al, 1984; Van de Kar et al, 1985) but not apparently the corticosterone response to p-chloroamphetamine (Van de Kar et al, 1985), raising the possibility that the 5-HT pathways mediating PRL and corticosterone secretion are different.

The SCN receives a dense 5-HT innervation in the rat and cat but this area is practically devoid of fibres in the monkey. A large proportion of 5-HT axon terminals in the SCN are opposed to, or form synaptic contacts with, cells containing vasoactive intestinal polypeptide (VIP)(Kiss et al, 1984; Bosler and Beaudet, 1985) which is a strong candidate to be a physiological prolactin releasing factor (PRF)(Muller and Nistico, 1989). Intraventricular injection of 5-HT has been shown to increase VIP concentrations in hypophyseal portal blood followed by an increased release of pituitary PRL (Shimatsu

et al, 1982). Injection of 5-HT into the anterior hypothalamus has been demonstrated to stimulate PRL secretion (Willoughby et al, 1988) although the failure of 5-HT antagonists to block this effect suggests that this could have been a non-specific action. In spite of the above, however, the lack of efferent projections from the SCN to the ME (Watts et al, 1987) indicates that the SCN is unlikely to be directly involved in 5-HT-mediated PRL release; the involvement of the SCN in hormonal circadian rhythms (see 1.7.3) appears to be mediated through one or more interneurons (Watts et al, 1987).

In the rat, the **PVN** is moderately innervated by 5-HT fibres in its parvocellular part which retrograde tracer studies indicate originate in cell groups B7-B9 (ie DRN and MRN) (Sawchenko et al, 1983). Using immunocytochemical detection, some 5-HT terminals in the PVN have been shown to synapse with corticotrophin releasing factor (CRF) cells (Soghomonian et al, 1988). Application of 5-HT onto isolated hypothalami causes increased CRF synthesis and release (Tuomisto and Mannisto, 1985) supporting a role for 5-HT in the regulation of CRF secretion. PVN lesions have been shown to abolish the PRL response to 5-HTP and stress (Minamitani et al, 1987) and Hokfelt and colleagues (1982) have demonstrated a parvocellular paraventricular system with nerve endings in the external layer of the ME which contain a potent PRL releasing agent, peptide histidine isoleucine amide (PHI) (Werner et al, 1983; Kaji et al,

1984). This has has 48% homology with VIP (Tatemoto et al, 1981). While electrical stimulation of the PVN was reported not to increase PRL levels it did enhance VIP-mediated PRL release (Michalkiewicz et al, 1987). These data strongly suggest that the PVN is involved in 5-HT-mediated ACTH and PRL release.

The **anterior hypothalamus** has thin varicose fibres with marked species differences in density. There are axosomatic contacts between 5-HT terminals and gonadotrophin releasing hormone (GnRH) neurones in the medial preoptic region (Kiss and Halasz, 1985) and this area is sexually dimorphic with regard to the distribution of 5-HT fibres, suggesting a role for 5-HT in the different reproductive functions in the two sexes (Takeuchi, 1988).

The **arcuate nucleus** has a low density of 5-HT axon terminals (Kiss and Halasz, 1986), but direct apposition of these terminals with DA cell groups, A12 and A13 has been demonstrated (Kiss and Halasz, 1986). These neurones comprise the tuberoinfundibular DA system involved in PRL secretion (Tuomisto and Mannisto, 1985) and it is likely that 5-HT is involved in regulating PRL secretion at this site. This is supported by recent evidence showing that PRL secretion is stimulated by microinjection of 5-HT into the basal hypothalamus (Willoughby et al, 1988). 5-HT axons have also been demonstrated in contact with pro-opiomelanocortin immunoreactive neurones in the arcuate nucleus (Kiss et al, 1984; Bosler and Beaudet, 1985) but the

functional significance of this is unclear.

5-HT innervation of the ME predominates in the outer layer (Soghomonian et al, 1988) and Nakai and colleagues (1983) demonstrated an intimate contact between 5-HT neurones and TRH axons in this area as well as providing evidence of co-localisation of 5-HT and TRH in the same axon. It is possible that this co-localisation subsumes a neuromodulatory role in addition to possible involvement of 5-HT in thyroid stimulating hormone (TSH) secretion, for which the evidence is conflicting (Tuomisto and Mannisto, 1985; Montagne and Calas, 1988). There is evidence that 5-HT is involved in gonadotrophin release through stimulating GnRH release from nerve terminals in the ME (Vitale et al, 1986).

In the rat, 5-HT-containing fibres have been described in the anterior pituitary but there remains uncertainty as to whether these are truly 5-HT neurones (Montagne and Calas, 1988). The weight of evidence is that 5-HT-stimulated PRL release does not occur at pituitary level; however GH and ACTH may be released by a direct action of 5-HT on the pituitary (Tuomisto and Mannisto, 1985; Montagne and Calas, 1988).

2.3 5-HT Receptor Subtypes and Hormone Secretion

The lack of selective compounds has made it difficult to characterise the 5-HT receptor subtypes mediating hormonal responses to 5-HT-active compounds.

2.3.1 Prolactin

In the rat, 5-HTP stimulated PRL secretion is likely to be mediated by 5-HT₂ receptors (Gartside and Cowen, 1990) although others have questioned this (Meltzer et al, 1983a). These receptors appear to be situated in the PVN as lesions here abolish the response (Minamitani et al, 1987). The 5-HT_{1C/2} receptor antagonists, ketanserin and ritanserin, have been reported to block the PRL response to fenfluramine, quipazine and 5-methoxy-N,N-dimethyl-tryptamine (Meltzer et al, 1983a; Di Renzo et al, 1989) supporting a role for 5-HT_{1C/2} receptors in PRL secretion. In humans the situation is less clear as ritanserin did not inhibit, indeed it enhanced, the PRL response to TRP infusion (Charig et al, 1986) although it was reported to lower PRL levels in functional and puerperal hyperprolactinaemic women (Falaschi et al, 1990). A putative 5-HT_{1C/2} agonist, 6-chloro-2-(1-piperazinyl)-pyrazine (MK-212) stimulated PRL secretion in humans (Lowy and Meltzer, 1988).

In rats, the role of 5-HT₁ receptors in PRL secretion is uncertain. Initial reports of PRL stimulation by the

5-HT_{1A} agonist, 8-OH-DPAT, in the rat (Simonovic et al, 1984; Aulakh et al, 1988b) have subsequently not been substantiated (Di Renzo et al, 1989; Van de Kar et al, 1989). Of interest, Willoughby and colleagues (1988) recently reported that microinjections of 5-HT and 8-OH-DPAT into the medial basal hypothalamus stimulated PRL secretion suggesting that 5-HT_{1A} receptors in this region may regulate PRL secretion. If this is the case then these receptors may lie on DA neurones in the arcuate nucleus (see 2.2). In humans there is preliminary evidence that 5-HT₁ receptors are involved in PRL secretion as pindolol, which has 5-HT_{1A/1B} antagonist properties, antagonised the PRL response to TRP (Smith et al, 1991). The 5-HT_{1A} agonist buspirone stimulates PRL secretion (Meltzer et al, 1983b) but it is likely that DA properties, at least partly, account for this effect (Meltzer et al, 1982a). m-Chlorophenylpiperazine (mCPP), initially thought to be a relatively selective 5-HT₁ receptor agonist, stimulates PRL secretion in humans (Mueller et al, 1985; 1986) but it is now apparent that this compound binds to other 5-HT receptor subtypes (Hoyer, 1988).

Evidence is lacking concerning 5-HT₃ receptor involvement in PRL secretion apart from one study in which granisetron, a 5-HT₃ antagonist, failed to antagonise the PRL response to TRP in volunteers (Anderson et al, 1988).

In summary, 5-HT₁ receptors appear to be involved in 5-HT-mediated PRL secretion. From animal studies there is

evidence that 5-HT₂ receptors may also play a role although this has yet to be established in humans.

2.3.2 Growth hormone

The rôle of 5-HT in GH secretion in animals is uncertain (Tuomisto and Mannisto, 1985; Cowen and Anderson, 1986). We have preliminary evidence that the GH responses to LTP and to buspirone in humans may be mediated by 5-HT₁ receptors as they are antagonised by pindolol (Smith et al, 1991; Anderson and Cowen, 1992). However the non-specific 5-HT antagonist metergoline failed to antagonise the GH response to both these challenges (McCance et al, 1987; Cowen et al, 1990). Not all 5-HT challenges stimulate GH secretion, notably the 5-HT releasing agent, fenfluramine, fails to (Anderson and Cowen, 1986) and therefore the role of 5-HT in human GH secretion remains unclear.

2.3.4 ACTH/Cortisol

There is good evidence that stimulation of 5-HT_{1A} receptors results in ACTH and corticosterone secretion in rats (Koenig et al, 1987; Gilbert et al, 1988; Koenig et al, 1988). Consistent with this, in humans, the 5-HT_{1A} agonists, ipsapirone (Lesch et al, 1989) and buspirone (Cowen et al, 1990) stimulate ACTH/cortisol secretion.

5-HTP-stimulated ACTH release in rats appears to be mediated by 5-HT₂ receptors (Gartside and Cowen, 1990) but there is disagreement as to whether ritanserin blocks this

response in humans (Facchinetti et al, 1987; Lee et al, 1991). MK-212-stimulated corticosterone secretion in rats appears to be mediated by 5-HT₂ receptors (Koenig et al, 1987) and this compound also stimulates cortisol secretion in humans (Lowy and Meltzer, 1988).

These results suggest that 5-HT₁ and probably 5-HT₂ receptors are involved in ACTH/cortisol secretion in humans.

2.4 5-HT Neuroendocrine Challenge Tests

Because species differences make direct comparison unreliable it is necessary to consider the less comprehensive data in humans in order to interpret neuroendocrine challenge studies. In a recent review of human neuroendocrine data (Cowen and Anderson, 1986) we concluded that there was strong evidence for 5-HT having a stimulatory role in the secretion of PRL with a likely but less certain role in stimulating GH and ACTH/cortisol. Evidence concerning the involvement of 5-HT in the secretion of other hormones is sketchy (Cowen and Anderson, 1986). Since that review more selective 5-HT agonists have been used in human subjects and been shown to stimulate GH and ACTH/cortisol secretion (see Table 2.1) but appropriate antagonist studies to characterise the responses have been few in number and hampered by the lack of selective compounds. Because cortisol is easier to measure than

Table 2.1

5-HT neuroendocrine challenges in humans

Drug	Action	Route	PRL	GH	CORT
5-HTP ^a	precursor	po	±	± ¹	± ²
TRP ^b	precursor	iv	+ ³	+ ⁴	±
FEN ^c	releaser and uptake inhibitor	po	+ ⁵	-	+ ⁶
CMI ^d	uptake inhibitor	iv	+ ⁷	-	+
mCPP ^{e,f}	5-HT agonist	po iv	+ ⁸ +	- +	+ ⁸ +
MK-212 ^g	5-HT _{1C/2} agonist	po	+	-	+
Buspirone ^h	5-HT _{1A} agonist	po	+ ⁹	+ ¹⁰	+
Ipsapirone ⁱ	5-HT _{1A} agonist	po	-	(+)	+ ¹¹

+ response; - no response; ± variable response; (+) probable response

a) Meltzer et al, 1982b; b) Charney et al, 1982; c) Quattrone et al, 1983; d) Golden et al, 1989; e) Mueller et al, 1986, f) Charney et al, 1987; g) Lowy and Meltzer, 1988; h) Meltzer et al, 1983b, Cowen et al, 1990a; i) Lesch et al, 1989

- 1 Antagonised by cyproheptadine (Nakai et al, 1974)
- 2 Antagonised (Lee et al, 1991)/not blocked (Facchinetti et al, 1987) by ritanserin
- 3 Enhanced by CMI (Anderson and Cowen, 1986) and ritanserin (Charig et al, 1986), antagonised by metergoline (McCance et al, 1987) and pindolol (Smith et al, 1991), not blocked by BRL 43694 (Anderson et al, 1988).
- 4 Enhanced by CMI (Anderson and Cowen, 1986), antagonised by pindolol (Smith et al, 1991), not blocked by ritanserin (Charig et al, 1986), metergoline (McCance et al, 1987) or BRL 43694 (Anderson et al, 1988)
- 5 Antagonised by metergoline (Quattrone et al, 1983)
- 6 Antagonised by cyproheptadine (Lewis and Sherman, 1984)
- 7 Antagonised by methysergide (Laakmann et al, 1983)
- 8 Antagonised by metergoline (Mueller et al, 1986)
- 9 Antagonised by metergoline (Gregory et al, 1990) but not blocked by pindolol (Anderson and Cowen, 1992)
- 10 Antagonised by pindolol (Anderson and Cowen, 1992)
- 11 Antagonised by pindolol (Lesch et al, 1990b)

5-HTP=5-hydroxytryptophan; TRP=L-tryptophan; FEN=fenfluramine; CMI=clomipramine; mCPP=m-chlorophenypiperazine; MK-212=6-chloro-2-(1-piperazinyl)-pyrazine
 cyproheptadine=5-HT_{2>1} antagonist; ritanserin= 5-HT_{1C/2} antagonist; metergoline=5-HT₁₊₂ antagonist; methysergide=5-HT₁₊₂ antagonist; BRL 43694=5-HT₃ antagonist; pindolol=β-blocker with 5-HT₁ antagonist properties

ACTH, most studies until recently have used plasma levels of this hormone rather than ACTH as an endocrine index of 5-HT function. This may not be justified as, first, it involves another (peripheral) step between the 5-HT synapse and plasma hormone response and, second, 5-HT may directly stimulate the adrenal secretion of cortisol (Van de Kar et al, 1985). The cortisol response to 5-HT drug stimulation may then not solely reflect the activity of the hypothalamic-pituitary axis.

Table 2.1 summarises the endocrine effects of some 5-HT drugs in humans with the limited antagonist data included. Consideration of these 5-HT challenges against the criteria outlined in 2.1 shows up the limitations of current 5-HT neuroendocrine probes. To a large extent the problem is one of specificity of both challenge drugs and antagonists. In particular metergoline and methysergide have DA agonist properties (Krulich et al, 1981) and metergoline has been shown to antagonise the PRL response to haloperidol in volunteers (Ellis et al, 1991) indicating that it cannot be used to characterise 5-HT-mediated PRL secretion. Cyproheptadine is an antagonist at muscarinic and histaminergic receptors (Leysen et al, 1981) and pindolol is a beta-adrenoceptor antagonist.

The hormone responses to TRP infusion have been the most thoroughly studied and provide evidence for 5-HT-mediation of PRL responses to this challenge, probably through 5-HT₁ receptors (Table 2.1). That the GH response

to TRP may also be mediated by 5-HT₁ receptors is suggested by preliminary results of blockade of the response by pindolol (Smith et al, 1990). The other 5-HT challenges have been less well studied and firm conclusions cannot be drawn. However it does seem that stimulation of central 5-HT receptors produces a broadly predictable pattern of hormone secretion, particularly stimulation of PRL and cortisol secretion, which is consistent with knowledge of 5-HT innervation of the hypothalamus in animal studies (see 2.2). The best approach currently to using 5-HT challenges in pathological conditions is therefore to test patients with more than one 5-HT drug. If similar abnormalities are seen across studies using different compounds which share the ability to stimulate 5-HT pathways, then this can provide strong evidence for an abnormality in 5-HT-mediated responses.

The story cannot end there however. As has been pointed out above (2.1) a neuroendocrine response is a reflection of the overall functioning of a series of steps, only one of which involves the 5-HT synapse. Further consideration has to be given to other explanations of any abnormality found, in particular whether there is a primary abnormality in hormonal secretion itself in the condition studied.

2.5 Questions Addressed in This Thesis

2.5.1 Weight Loss and Measures of 5-HT Function

There is preliminary evidence that weight loss increases the PRL response to TRP infusion in female dieters (Goodwin et al, 1987c) and depressed patients (Cowen and Charig, 1987; Deakin et al, 1990). As weight loss is common in depressive illness this is a serious confounding factor in the interpretation of results. Perhaps more importantly, weight loss could itself affect 5-HT function in which case the abnormal results in depression could be wholly or partly due to weight loss rather than to depression. In this thesis I address the following questions related to weight loss:

- a) Does weight loss indeed alter the PRL response to TRP challenge in normal volunteers and can the sex difference be replicated?
- b) If so, is this a specific effect related to 5-HT-drug challenge or does weight loss affect PRL responses to other (non-5-HT) stimuli?
- c) Does weight loss lower plasma TRP measures and how does this relate to PRL responses? One way in which dieting/weight loss could alter brain 5-HT function is through an effect on the availability of the 5-HT precursor, TRP (see 1.4).

2.5.2 5-HT-Mediated PRL Secretion in Depression

As I have discussed there are a number of methodological and conceptual problems concerning studies of PRL response to 5-HT-drug challenge in depression and I address the following questions:

- a) Do depressed patients have impaired PRL responses to an alternative 5-HT-active drug, intravenous CMI, and is this influenced by clinical features such as melancholia, suicide attempt and weight loss?
- b) How far are PRL responses to CMI related to the stressful side-effect of nausea?
- c) Are PRL responses to non-5-HT stimuli blunted in depression? If so, this would suggest that the finding of reduced 5-HT-mediated PRL release in depression is related to an abnormality in PRL secretion rather than to 5-HT function.

2.5.3 Measurement of 5-HT Receptor Function

Specific, direct 5-HT receptor agonists have only recently become available for use in humans. If these can be demonstrated to have hormonal and physiological effects they might offer a way to investigate 5-HT receptor function in vivo. I investigate whether a selective 5-HT_{1A} receptor agonist, gepirone, has effects on hormone secretion and temperature in normal volunteers.

PART 2

EXPERIMENTAL STUDIES

CHAPTER 3

General Methods

All the studies described in this thesis were approved by the Oxford Psychiatric Sector Ethics Committee.

3.1 Selection and Assessment of Subjects

3.1.1 Normal Volunteers

Normal volunteers were recruited from hospital staff and by advertisement. They received a semi-structured clinical interview, physical examination, electrocardiogram (ECG) and routine blood tests for haematology and biochemistry (full blood count, electrolytes, urea and liver function). Volunteers were excluded if they had any of the following: history of cardiac or endocrine abnormality; psychiatric disorder or substance abuse; alcohol intake above twenty one units per week for men, fourteen units per week for women; drug treatment in the preceding two months; significant weight change in the previous two months. For the dieting studies volunteers were required to be of average weight with a body mass index (weight in kg divided by height in metres², BMI) of between 20 and 27kg/m² and to have no evidence of an eating disorder as assessed by a score of less than twenty on the Eating Attitudes Test (Garner and Garfinkel, 1979).

3.1.2 Depressed Patients

Outpatients and inpatients from the Littlemore and Warneford Hospitals in Oxford and direct referrals from

General Practitioners were recruited. They met DSM-III criteria for unipolar major depressive episode (American Psychiatric Association, 1980) on the basis of a semi-structured clinical interview. They were free of antidepressant drug treatment for at least three weeks, in most cases much longer, and were on no current psychotropic medication apart from small doses of benzodiazepines. Patients received a physical examination, ECG and blood tests as described for the normal volunteers with the addition of thyroid function tests. They were excluded from the study if they had significant current physical illness or alcohol abuse or a history of endocrine abnormality.

Severity of depression was assessed using the twenty one item Hamilton Rating Scale for Depression (HAM-D) (Hamilton, 1967) administered by the author or a trained Research Nurse and the Beck Depression Inventory (BDI) (Beck et al, 1961), a self-rating scale. Patients were also rated on the Hamilton Anxiety Rating Scale (HAM-A) (Hamilton, 1969) and the Newcastle Scale for endogenous depression (Carney et al, 1965). Weight loss was assessed in two ways, first, using the weight loss rating item on the BDI which asks the patients to rate recent weight loss (explained to the patients as weight loss over the last month), and second, by administering an observer-rated questionnaire designed to detect amount of weight loss in the last month (see Appendix). Both of these measures were used to decide likely weight loss over the last month

and patients were then assigned to a 'no weight loss group' (5lb and less) and a 'weight loss group' (greater than 5lb).

3.2 Neuroendocrine Protocol

3.2.1 General Procedure

All neuroendocrine testing was performed with subjects fasting from midnight the night before. They attended a Research Unit where an intravenous cannula was inserted between 08.30h and 09.00h and maintained with heparinised saline. Subjects rested for 60 minutes before administration of the drug between 09.30h and 10.00h (time 0). Baseline blood samples were taken before, and at intervals after drug administration. During the period of testing subjects rested semi-supine and were not allowed to eat, drink or smoke.

3.2.2 L-Tryptophan Test

TRP (Optimax powder, Merck Ltd) was dissolved in a solution containing 0.72% sodium chloride and 0.05% sodium sulphite to a concentration of 10 grammes/litre and the solution sterilised by autoclaving. Subjects were given 100mg/kg, infused over 20 minutes from time 0 via a 0.8 micron Ivex-RF on-line filter (Millipore Corporation). Three baseline blood samples (10ml) were taken at 15-minute intervals starting 30 minutes before the infusion. A blood sample was taken at the end of the infusion (+20min) and

then every 15 minutes from 30 to 120 minutes after the start of the infusion (+30min to +120min).

3.2.3 Metoclopramide Test

Metoclopramide (MCP) (Maxolon, Beecham Research Laboratories) was diluted with sterile 0.9% sodium chloride to give 5ug/kg (Study 2) or 0.3mg (Study 6) in 1ml which was infused over 10 seconds (at time 0). Blood samples (5ml) were taken at 15-minute intervals from 30 minutes before to 90 minutes after the MCP infusion (+90min).

3.2.4 TRH Test

For the **low-dose TRH tests**, TRH (TRH-Roche, Roche Products Limited) was diluted with sterile 0.9% sodium chloride to give 0.1ug/kg (Study 2) or 6.25ug (Studies 4 and 5) in 1ml and infused over 10 seconds. Blood samples (5ml) were taken before, and at 10-minute intervals for 30 minutes after injection.

For the **high-dose TRH test**, 150ug TRH (1.5ml of a solution of 200ug in 2ml) was infused over 10 seconds and blood samples taken as for the low-dose TRH test.

3.2.5 Clomipramine Test

CMI for intravenous use (Anafranil injection, Geigy Pharmaceuticals) was made up to 5ml with 0.9% sodium chloride and administered by infusion pump (Sage Instruments) over 15 minutes starting at time 0. Blood

samples (10ml) were taken every 15 minutes from 30 minutes before the start of the CMI infusion to 75 minutes after the end of infusion (+90min).

3.2.6 Gepirone Test

Gepirone 10mg, 20mg (Bristol-Myers Company, Wallingford, Connecticut, USA) and placebo were administered in two capsules at time 0. Blood samples (15ml) were taken every 15 minutes from 30 minutes before to 30 minutes after drug administration and then every 30 minutes until +180min.

3.2.7 Measurement of Vital Signs and Side-Effects

Every 15 minutes during the CMI test subjects had pulse and blood pressure (BP) measured (Dynamap, Model 341A, Sage Instruments). 100mm visual analogue scales (eg for nausea: 0-not at all nauseated, to 100-extremely nauseated) appropriate to each test were administered to subjects at 15-minute intervals from -15min to the end of the test. The following ratings were made: TRP test - drowsiness; CMI test - nausea; gepirone test - nausea, drowsiness and light-headedness. In addition, during the CMI test, an observer assessment of the presence or absence of 'stressful nausea' during the test was made. Presence of stressful nausea was defined as observed retching or vomiting or a subjective complaint of nausea associated with evidence of autonomic stress - pallor, shivering, marked cardiovascular changes (bradycardia, tachycardia or hypotension).

Vital signs and side-effects were not formally rated during the TRH and MCP tests. For all tests subjects were asked to report any adverse experiences or alterations in their subjective state.

3.2.8 Plasma Samples

In all investigations except the gepirone study (Study 6, Chapter 10) blood samples were collected into lithium heparin tubes and plasma was separated at the end of the test period by centrifugation at 2000g for 10 minutes at 4°C. In the gepirone study blood samples were collected into EDTA tubes on ice and the plasma separated within 60 minutes. The plasma samples were immediately frozen and stored at -30°C until assay.

3.3 Plasma Assays

All plasma assays apart from branch chain amino acids (BCAA) were performed by technical staff in the Oxford University and MRC Clinical Pharmacology Research Unit, Littlemore Hospital. Plasma BCAA were kindly assayed by Mark Parry-Billings of the University of Oxford Department of Biochemistry.

3.3.1 Prolactin

Plasma PRL concentration was estimated using a double antibody ¹²⁵I radioimmunoassay.

Briefly, 50ul volumes of plasma sample or standard with 100ul each of sheep anti-prolactin (in 2% bovine serum albumin/sodium phosphate buffer, pH 7.5) and ^{125}I -PRL (3,500cpm in 100ul of 0.05M sodium phosphate buffer, pH 7.5) were pipetted into 75 x 12mm polystyrene tubes in duplicate. Tubes were vortex mixed and left to incubate at room temperature. After 24 hours, 100ul of anti-sheep Sac-cel (Immunodiagnosics) was added to the tubes which were vortex-mixed and left for 30 minutes at room temperature. At the end of this period 1ml of distilled water was added and the tubes were centrifuged at 2,500g for 20 minutes at 4°C. Following this the supernatants were aspirated to waste via a vacuum system and the residual antibody bound complex in the precipitate kept for radioactive measurement. The tubes were counted in a Canberra Pochard Crystal II gamma counter.

Results were interpolated from standard curves constructed of percentage of hormone bound versus PRL concentration. Plasma PRL concentrations are quoted as milli-international units per litre (mIU/L) of MRC 75/504 standard. Intra- and inter-assay variation were 7.4% and 11.8% respectively and the limit of detection was 20mIU/L.

3.3.2 Growth Hormone

Plasma GH concentration was estimated using a double antibody ^{125}I radioimmunoassay.

50ul volumes of plasma sample or standard with 200ul of

sheep anti-GH (in 2% bovine serum albumin/sodium phosphate buffer, pH 7.5) and 50ul ^{125}I -GH (20,000cpm in 50ul of 0.05M sodium phosphate buffer, pH 7.5) were pipetted into 75 x 12mm polystyrene tubes in duplicate. Tubes were vortex mixed and left to incubate at room temperature. After 24 hours, 100ul anti-sheep Sac-cel (Immunodiagnosics) was added to the tubes. These were vortexed and left for 30 minutes at room temperature. 1ml of distilled water was then added to the tubes which were centrifuged at 2,500g for 10 minutes at 4°C. Following this the supernatants were aspirated to waste via a vacuum system and the residual antibody bound complex in the precipitate kept for radioactive measurement. The tubes were counted in a Canberra Pochard Crystal II gamma counter.

Results were interpolated from standard curves constructed of percentage of hormone bound versus GH concentration. Plasma GH concentrations are quoted as milli-international units per litre (mIU/L) of IRP 80/505 standard. Intra- and inter-assay variation were 4.3% and 10.7% respectively and the limit of detection was 1mIU/L.

3.3.3 ACTH

Plasma ACTH concentration was estimated using a commercially available double antibody ^{125}I radioimmunoassay (Diagnostic Products Corporation, Los Angeles, USA).

100ul volumes of plasma sample or standard with 100ul of ACTH antiserum were pipetted into 75 x 12mm polystyrene

tubes in duplicate, vortex mixed and incubated at room temperature for 60 minutes. 100ul ^{125}I -ACTH was then added, the tubes vortex mixed and left to incubate at 4°C . After 24 hours, 1ml of cold precipitating solution (goat anti-rabbit gamma globulin and dilute polyethylene glycol in saline) was added to all tubes which were vortex mixed before being centrifuged for 15 minutes at 3000g at 4°C . Following this the supernatants were aspirated to waste via a vacuum system and the residual antibody bound complex in the precipitate kept for radioactive measurement. The tubes were counted in a Canberra Pochard Crystal II gamma counter.

Results were interpolated from standard curves constructed of percentage of hormone bound versus ACTH concentration. Plasma ACTH concentrations are quoted as picograms per millilitre (pg/ml). Intra- and inter-assay variation were 5.0% and 10.2% respectively and the limit of detection was 7pg/ml.

3.3.4 Cortisol

Plasma cortisol concentration was estimated using a single antibody ^{125}I radioimmunoassay using reagents from Bioanalysis Ltd, Cardiff.

50ul volumes of plasma or standard with 1ml of ^{125}I -cortisol and 200ul of solid-phase antiserum were pipetted into 75 x 12mm polystyrene tubes in duplicate. The tubes were then vortex-mixed and incubated for 90 minutes in a 37°C water bath. Following this the tubes were centrifuged

for 10 minutes at 1,200g at 4°C, the supernatant decanted and the tubes inverted on absorbent tissue to drain. The tubes were counted in a Canberra Pochard Crystal II gamma counter.

Results were interpolated from standard curves constructed of percentage of hormone bound versus cortisol concentration. Plasma cortisol concentrations are quoted as microgrammes per decilitre (ug/dl). Intra- and inter-assay variation were 6.9% and 12.6% respectively and the limit of detection was 0.5ug/dl.

3.3.5 Total Tryptophan

Plasma total TRP was assayed according to the method described by Bloxam and Warren (1974).

10ul volumes of plasma were added to 2ml of ice-cold 10% (w/v) trichloroacetic acid (TCA) contained in 4ml LP4 tubes. This was centrifuged at 2,500g for 15 minutes at 4°C to remove protein. Standards in the range 0-2 nanomoles TRP/tube were prepared similarly. For very high values of plasma TRP (following TRP infusion) the deproteinised plasma solution was further diluted with 10% TCA to give values within the range of the standard curve. To each sample/standard on ice was added 0.2ml of 2% (w/v) formaldehyde and 0.1ml of 6mM ferric chloride in 10% TCA, the tubes vortex-mixed and immediately transferred to a heated dry block (temperature 100°C) for 1 hour. The fluorescence in each tube was measured in a Hitachi model

2000 spectrofluorimeter (excitation wavelength 300nm, emission wavelength 440nm).

Results were interpolated from standard curves constructed of fluorescence versus TRP concentration. Plasma TRP concentrations are quoted as micromoles per litre ($\mu\text{mol/L}$). Intra- and inter-assay coefficients of variation were 4.4% and 9.4% respectively and the limit of detection was $0.5\mu\text{mol/L}$.

3.3.6 Free Tryptophan

Plasma free TRP was assayed according to the method described by Bloxam and colleagues (1977).

Using Millipore ultrafree 10,000 NMWL filters, 200ul volumes of plasma were pipetted into the filter reservoir. The sample was gassed with a 95% oxygen/5% carbon dioxide (CO_2) mixture by inserting a 19 gauge syringe needle into the reservoir above the sample for 30 seconds before quickly closing the top of the centrifuge tube to maintain the gas atmosphere. This prevents plasma pH increasing during centrifugation due to loss of CO_2 (which would result in a change in the proportion of TRP bound to albumin). The tubes were then inverted for 10 minutes before being spun for 30 minutes in an eppendorph benchtop centrifuge.

The filtrate was analysed for TRP as described above (3.3.5) using all of the filtrate (about 130ul) in the initial deproteinisation step.

3.3.7 Branch Chain Amino Acids

Plasma BCAA (leucine, isoleucine and valine) were assayed by a method modified from that of Livesey and Lund (1980).

Plasma extracts were prepared as follows: 250ul of plasma and 250ul of 10% perchloric acid were pipetted into plastic tubes and centrifuged for 2 minutes at 9,000g. 420ul of the supernatant was taken, to which was added 50uL 0.5M triethanolamine, 10ul universal indicator and the solution neutralised with 20% potassium hydroxide. The tubes were again centrifuged for 2 minutes at 9,000g and the supernatant stored frozen at -30°C until assay.

80uL of plasma extract or standard (0.4mM leucine) with 1ml assay mix (v/v 10:1:9 Tris/hydrochloric acid buffer: 20mg/ml nicotinamide adenosine dinucleotide solution: distilled water) were pipetted into 1ml plastic cuvettes in duplicate. The cuvettes were vortex-mixed and read in a Philips PU 8620 ultra violet (UV) spectrophotometer at 340nm. 10ul of buffered leucine dehydrogenase (Sigma, UK) (25 units enzyme: 0.5ml of 10mM dipotassium hydrogen phosphate) was added, the cuvettes vortex-mixed and left to incubate at room temperature for 1-1½ hours. At the completion of the reaction the cuvettes were again read at 340nm.

Results were calculated by taking the difference between the two spectrophotometer readings and calibrating

against the standard (0.4mM leucine). Average intra- and inter-assay coefficients of variation were about 5%.

3.3.7 Clomipramine

Plasma CMI concentration and that of the metabolite, desmethylclomipramine (DCMI), were estimated by high performance liquid chromatography (HPLC) with ultraviolet (UV) detection following solid phase extraction of CMI. An internal standard, maprotiline (Ciba Laboratories), was used to monitor CMI recovery.

Sorbent columns of the C2 type were attached to a Vac-Elut vacuum aspiration system. The columns were pre-treated with 1ml volumes of methanol, distilled/deionised water and finally with 0.5ml of 2.0M Tris. These reagents were loaded with a Pasteur pipette and aspirated by negative pressure to waste. The plasma samples and standards (0.5ml), internal standard (25uL of 100ug/ml maprotiline in 0.1M hydrochloric acid and 0.1% methanol) and 0.5ml of 2.0M Tris were carefully loaded with a Pasteur pipette followed by gentle aspiration to waste. The columns were then washed with single column loads of 0.2M Tris, mobile phase (to remove serum pigments), and distilled/deionised water. These were aspirated to dryness. The CMI and DCMI were finally eluted into eppendorf tubes with 2 x 0.25ml of the elution reagent (10.0mM potassium hydrogen orthophosphate:acetonitrile:methanol v/v 15:65:20; pH 3.0). Samples were then injected into the HPLC system.

The HPLC system consisted of a Courtometric 3000 pump, a Milton Roy 3100 variable wavelength UV spectromonitor, a 10cm, 3µm cyanonitrile column with a 3cm, 5µm cyanonitrile guard column attached, a Rheodyne 4125 injection valve and a Milton Roy CI 4000 integrator for data reduction.

The mobile phase consisted of 5mM potassium hydrogen orthophosphate:acetonitrile:methanol (v/v 15:65:20; pH 7.0); this was run at a flow rate of 3.0ml/min. The detector wavelength was set at 215nm.

To calculate results a standard graph of peak height ratio of CMI/DCMI to internal standard versus plasma CMI/DCMI concentration was constructed. Plasma concentrations were estimated by interpolation of the standard curve by the system integrator. Plasma CMI concentrations are expressed as ng/ml. Intra- and inter-assay variation were 3.1% and 10.2% respectively and the limit of detection was 5ng/ml for CMI and desmethylclomipramine.

3.4 Analysis of Results

Plasma hormone responses and plasma TRP following TRP infusion are analysed as the area under the response curve with subtraction of the baseline (time 0 or +90min) value (AUC). The trapezoid method is used when discrete samples were taken (Studies 1, 3, 4, 5, 6) and summation with correction for time is used in Study 2 where a continuous

sampling method was used. In Studies 3 and 4 the presence of a PRL response is defined as a positive AUC and the absence of a response as a zero or negative AUC. In the gepirone study, changes in plasma hormone and temperature values are analysed over time as described below. Plasma CMI following CMI infusion is analysed as the total area under the curve (TAUC) using the trapezoid method. Visual analogue ratings are analysed as peak change in rating from baseline (DNausea, DDrowsy, DLight-headed). Pulse and BP values are analysed over time as described below.

Statistical analyses are carried out according to methods described by Cohen and Holliday (1982). Comparisons between two groups are made using unpaired t-tests (t) and Mann Whitney U tests (U) for parametric and non-parametric data respectively. Where the means of a number of different groups are compared, a one-way analysis of variance (ANOVA) is used to determine overall significance; with non-parametric data the Kruskal-Wallis one-way ANOVA by ranks (H) is used. Post-hoc range finding following one-way ANOVA testing is performed using unpaired t-tests for parametric data and Mann Whitney U tests for non-parametric data. Within subject comparisons are made with paired t-tests and Wilcoxon signed rank tests for parametric and non-parametric data respectively. Plasma hormone and temperature values in the gepirone study are analysed using a two-way repeated measures ANOVA with post-hoc range testing carried out using Tukey's test. Pulse and BP measurements are compared using

a two-way ANOVA with one between group factor (group) and one within group, repeated measures, factor (time) with unpaired t-tests for post-hoc testing. All tests are two-tailed.

Correlations between variables are assessed using Pearson's product moment correlation (r) or Spearman's rank correlation (ρ) for parametric and non-parametric data respectively.

Values quoted are mean \pm standard deviation (SD) for parametric data and median (range) for non-parametric data. In Studies 3 and 5, mean/median differences and 95% confidence intervals (95% CI) are used to interpret the data (Gardner and Altman, 1986; Campbell and Gardner, 1988).

CHAPTER 4

Study 1: The Effect of Weight Loss on Plasma Tryptophan
Measures and on the Prolactin Response to
L-Tryptophan in Normal Volunteers

4.1 Introduction

Weight loss is common in depressive illness and, as I have discussed in the introductory chapters, any investigation into biological correlates of depression must consider the effects of weight loss on the measures used. This has not been systematically studied in research into biological correlates of depression. With regard to the TRP challenge test of 5-HT function, one study (Goodwin et al, 1987b; 1987c) has demonstrated that normal female volunteers who lose a moderate amount of weight through dieting have an enhanced PRL response, while, in men this response does not appear to be altered by a similar degree of weight loss. The GH response to TRP was increased in both sexes. This has important implications for studies in depressed patients where two studies (Cowen and Charig, 1987; Deakin et al, 1990) did not find a significant blunting of the PRL response to TRP when all patients were grouped together. A blunting was demonstrable when patients with weight loss were excluded; the patients with severe weight loss showing an enhanced PRL response. The GH response to TRP was affected in the same direction by weight loss but in this case the difference between patients and controls was not obscured as it was with the PRL response.

The mechanism by which weight loss enhances the endocrine responses to TRP is unknown but a possible

mechanism is through a reduction in the availability of plasma TRP for 5-HT synthesis in the brain. This is suggested by animal studies in which lowering plasma TRP results in lowered brain 5-HT levels and enhanced PRL and cortisol responses to TRP (Gil-Ad et al, 1976) and to 5-HTP (Clemens et al, 1980). This is relevant to studies of depressed patients where a consistent finding of a lowered TRP:LNAAs ratio has been found (see 1.8.3).

The aims of this study were, first, to attempt to replicate the finding of Goodwin and colleagues (1987c) concerning the effects of weight loss on the PRL response to TRP infusion, and second, to test the hypothesis that this is related to a reduction in plasma TRP availability by measuring plasma TRP and competing amino acids levels before and after weight loss.

4.2 Methods

4.2.1 Subjects and Diet

Fifteen healthy volunteers (6 men, 9 women) were recruited as described under General Methods (Chapter 3). They were of average weight (mean body mass index (BMI) 25.2, range 22.4-27.6).

The subjects underwent neuroendocrine testing before and at the end of the third week of a calorie restricted diet (1000 kcal for women, 1200 kcal for men). The onset of dieting was delayed for one week after the first test in

the women so that the second test was at the same stage of the menstrual cycle. Six women were tested in the follicular phase of the menstrual cycle and 3 who were taking a low dose oestrogen oral contraceptive pill were tested during the pill-free week. To ensure comparability with the women, men also delayed the onset of their diet by one week.

The diet followed by the subjects was provided by the Oxford University Department of Nutrition and Dietetics. The subjects were instructed to follow a daily eating plan consisting of a fixed number of 'exchanges' of carbohydrate (CHO) and protein, a fixed amount of fat as low-fat spread and freely available food low in calories and protein (eg lettuce, cucumber). The women received 1000 kcal/day as 31% protein, 44% carbohydrate and 25% fat, and the men received 1200 kcal/day as 30% protein, 36% carbohydrate and 35% fat. Subjects kept a diary of food intake and were contacted twice-weekly when they received close guidance on their diet and had their compliance checked by discussion of their diary. They were weighed weekly.

4.2.2 Neuroendocrine Testing

TRP for infusion was prepared and administered as described in General Methods (Chapter 3).

Plasma PRL and total TRP were measured on all blood samples. The blood sample taken at -30min was used to measure fasting total and free TRP and BCAA (valine,

leucine and isoleucine) which compete with TRP for entry into the brain. Assay methods are described in General Methods (Chapter 3). Analysis of samples from each individual before and during dieting were always carried out in the same assay.

4.2.3 Subjective Ratings

Drowsiness was rated as described in General Methods (Chapter 3) as experience in our Unit and in the literature (Charney et al, 1982; Winokur et al, 1986) indicates that this is the only consistent subjective experience to TRP infusion. Nausea can occur at high doses (Winokur et al, 1986) but has not been a problem in our Unit at the doses employed in this study. Subjects were asked to report any nausea.

4.2.4 Statistical Analysis

The plasma PRL response and plasma total TRP profile after the TRP infusion were analysed as AUC as described in General Methods (Chapter 3). Because of evidence that dieting affects men and women differently in this test the sexes were analysed separately on TRP test measures. The effects of dieting within individuals were analysed using paired t-tests and men and women were compared using unpaired t-tests except in the case of peak change in drowsiness ratings (DDrowsy) which were compared using Wilcoxon's signed rank test (all two-tailed). Correlations

were performed using Pearson's product moment. Values are expressed as mean \pm SD and median (range) as appropriate.

4.3 Results

4.3.1 Weight Loss and Fasting Plasma Amino Acids

All subjects lost weight, and although the men lost more weight than women, the percentage weight loss was not significantly different (Table 4.1). Dieting did not affect the timing of the menstrual cycle and the results from women taking the contraceptive pill did not differ from the rest.

Dieting decreased plasma total TRP levels in both sexes (Figure 4.1, Table 4.2), but the reduction was considerably greater in women both in absolute (13.6 ± 7.0 vs 5.8 ± 2.2 $\mu\text{mol/L}$, $p=0.01$) and percentage terms (21.1 ± 10.0 vs 10.1 ± 3.8 %, $p=0.01$). There was also a significant decrease in the ratio of plasma total TRP to BCAA in the dieters as a whole although this fall did not reach statistical significance for the women ($p=0.08$) or men ($p=0.13$) considered separately (Table 4.2). Plasma levels of free TRP and BCAA were not altered by dieting (Table 4.2).

4.3.2 Tryptophan Test

(i) Prolactin Response

A TRP test of one subject had to be excluded from analysis because of technical problems with the infusion

Table 4.1

Subject characteristics and effects of weight reducing diet

	Women (N=9)	Men (N=6)
Age (years)	29.4 ± 4.9	33.7 ± 6.4
Body Mass Index (kg/m ²) ^a	24.6 ± 1.5	26.2 ± 1.3 [*]
Weight (kg) ^a	67.3 ± 4.7	81.1 ± 6.4 ^{**}
Weight Loss (kg)	-3.4 ± 1.0	-4.6 ± 0.8 [*]
Weight Loss (% of starting weight)	-5.0 ± 1.4	-5.7 ± 1.4

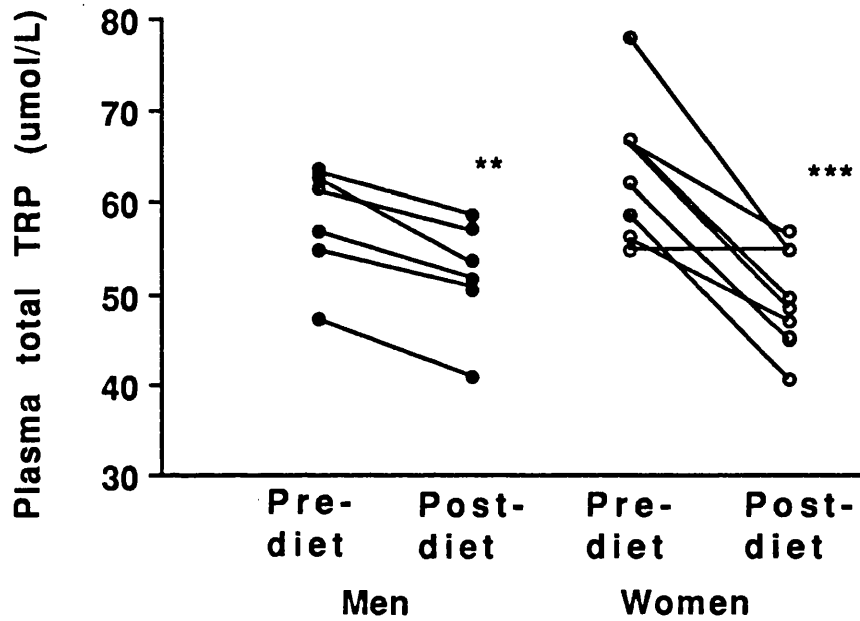
Data shown as mean ± SD

a Pre-diet values

* p<0.05 } men versus women (unpaired t-test)
 ** p<0.01 }

Figure 4.1

Effect of dieting on fasting plasma total tryptophan in normal volunteers



Individual values are shown before (pre-diet) and after (post-diet) dieting.

** $p < 0.01$ } post-diet compared to pre-diet (paired t-test)
 *** $p < 0.001$ }

Table 4.2

Effects of weight reducing diet on fasting plasma amino acids

	Total TRP ($\mu\text{mol/L}$)	Free TRP ($\mu\text{mol/L}$)	BCAA ($\mu\text{mol/L}$)	TRP:BCAA ratio
All (N=15)				
Pre-diet	60.8 \pm 7.3	5.8 \pm 2.5	369.1 \pm 83.4	0.174 \pm 0.048
Post-diet	50.8 \pm 5.8 ^{***}	6.8 \pm 3.0	357.5 \pm 87.3	0.150 \pm 0.047 [*]
Women (N=9)				
Pre-diet	62.8 \pm 7.6	4.8 \pm 2.2	341.9 \pm 90.9	0.194 \pm 0.050 ⁺
Post-diet	49.1 \pm 5.5 ^{***}	6.3 \pm 3.4	333.3 \pm 101.3	0.161 \pm 0.057
Men (N=6)				
Pre-diet	57.9 \pm 6.2	7.4 \pm 2.3	409.8 \pm 54.0	0.144 \pm 0.027
Post-diet	52.1 \pm 6.4 ^{**}	7.6 \pm 2.2	393.7 \pm 48.2	0.134 \pm 0.022

Data are mean \pm SD

+ p<0.05 versus pre-diet men (unpaired t-test)
 * p<0.05 } post- versus pre-diet (paired t-test)
 ** p<0.01 }
 *** p<0.001 }

and so the results of 6 men and 8 women are presented. Dieting resulted in a significantly enhanced PRL response to TRP infusion in women with no change in the response in men (Figure 4.2, Table 4.3). Baseline plasma PRL (time 0) was not altered by dieting (Table 4.3).

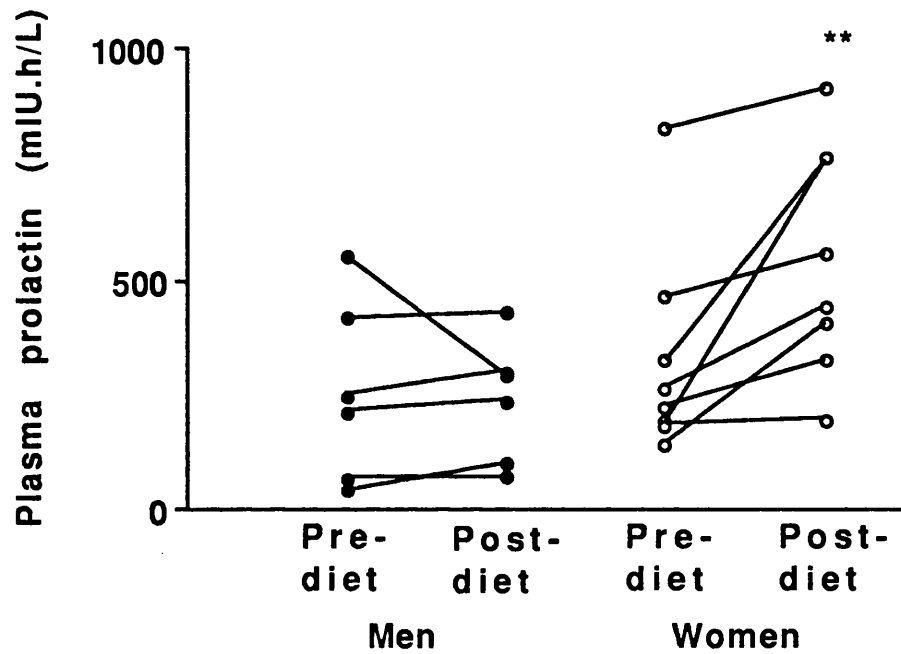
TRP infusion resulted in a large increase in plasma total TRP concentration (Figure 4.3). After dieting this increase was smaller in women whereas it was unchanged in men (Figure 4.3, Table 4.3).

(ii) Subjective Measures

An increase in drowsiness was experienced in all but two subjects before, and all subjects after dieting. Baseline (time 0) drowsiness did not differ between occasion in women (median (range): 2.5 (0-45) pre-diet versus 7.5 (0-60) post-diet, $p>0.1$) or men (20 (0-70) pre-diet versus 25 (0-45) post-diet, $p>0.1$). There was a trend to an increase in sedation after dieting in women (DDrowsy: 20 (0-95) pre-diet versus 45 (10-95) post-diet, $p<0.1$) but not men (25 (0-40) pre-diet versus 35 (20-55) post-diet, $p>0.1$). If all subjects were combined then sedation was significantly greater after dieting (22.5 (0-95) pre-diet versus 40 (10-95) post-diet, $p=0.04$). Only one female subject reported nausea which occurred during pre-diet TRP testing.

Figure 4.2

Effect of dieting on the prolactin response to tryptophan infusion in normal volunteers



Individual PRL responses (AUC) are shown before (pre-diet) and after (post-diet) dieting.

** $p < 0.01$ post-diet compared to pre-diet (paired t-test)

Table 4.3

Effects of weight reducing diet on tryptophan test measures

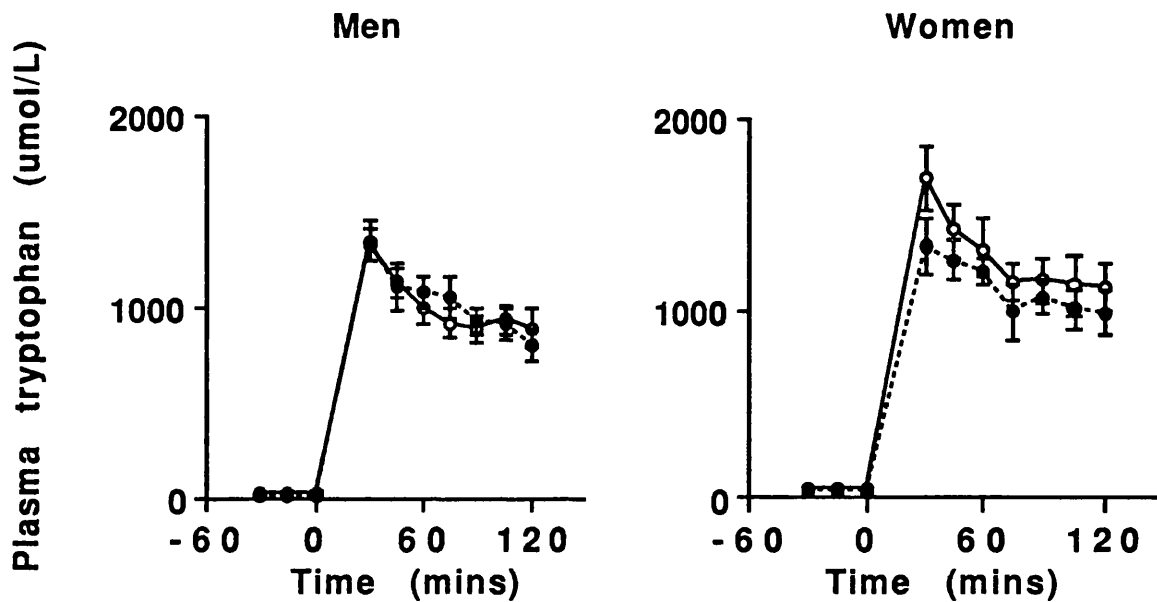
	Women (N=8)		Men (N=6)	
	Pre-Diet	Post-Diet	Pre-Diet	Post-Diet
PRL Baseline (mIU/L)	258 ± 58 ⁺⁺	217 ± 64	95 ± 15	96 ± 12
PRL AUC (mIU.h/L)	325 ± 225	518 ± 234 ^{**}	253 ± 198	236 ± 134
TRP AUC after TRP Infusion (µmol.h/L)	2250 ± 655	1959 ± 474 [*]	1785 ± 268	1848 ± 375

Data are mean ± SD

⁺⁺ p<0.01 versus pre-diet men (unpaired t-test)
^{*} p<0.05 } post- versus pre-diet (paired t-test)
^{**} p<0.01 }

Figure 4.3

Effect of dieting on plasma total tryptophan following tryptophan infusion in normal volunteers



Tryptophan (100mg/kg) was infused between time 0 and +25min

Data is shown as mean \pm SEM at each time point

○—○ - before dieting, ●---● - after dieting

Tryptophan levels were significantly lower in women after dieting ($p < 0.05$, paired t-test on AUC).

4.3.3 Correlations

Correlations were performed for all the subjects considered together and for men and women separately. There was a trend to a significant positive correlation between the change in plasma total TRP and the change in the PRL response in the subjects as a whole ($r=0.52$, $p<0.06$) but not in either sex considered separately (women: $r=0.30$, $p=0.47$; men: $r=0.22$, $p=0.68$). There was negative correlation in women between the change in TRP AUC following TRP infusion and the change in PRL response ($r=-0.79$, $p=0.02$) but no correlation in men ($r=0.45$, $p=0.38$) or the subjects as a whole ($r=0.05$, $p=0.88$).

There were no significant correlations between the following measures: weight loss vs change in plasma total TRP; weight loss vs change in TRP:BCAA ratio; weight loss vs change in plasma free TRP; weight loss vs change in PRL response; change in plasma total TRP vs change in TRP AUC; change in TRP:BCAA ratio vs change in PRL response; change in DDrowsy vs change in PRL response; change in DDrowsy vs change in TRP AUC (all $p>0.10$).

4.4 Discussion

The results of this study show that moderate weight loss through dieting reduces fasting plasma TRP in healthy volunteers and that in women, but not men, this reduction is associated with an enhanced PRL response to intravenous

TRP, a putative measure of brain 5-HT function. My interpretation of the data is that dieting alters brain 5-HT function in women, but not men, and that this is a consequence of a decrease in the availability of plasma TRP for brain 5-HT synthesis.

This study confirms an earlier report of an increase in the PRL response to TRP following dieting in women but not men (Goodwin et al, 1987c). While there is substantial evidence that this neuroendocrine response is mediated by brain 5-HT pathways (Cowen and Anderson, 1986; Cowen, 1987) it might be argued that the effect of dieting on TRP-induced PRL responses could reflect a more general facilitation of PRL release caused by weight loss. Goodwin and colleagues (1987b) reported no effect of dieting on the PRL response to 200ug of the direct pituitary lactotroph stimulant, TRH, but as discussed in 1.8, this dose produces a maximal PRL response (Jacobs et al, 1973) and is not directly comparable to the submaximal response following TRP. It is possible that pituitary sensitivity could be increased without affecting the maximal response (ie a shift of the dose response curve to the left). I investigate this in a further study (Chapter 5) by measuring the PRL responses to submaximal doses of the dopamine antagonist, metoclopramide (MCP) and TRH.

I believe that the present investigation reveals the mechanism by which dieting alters this neuroendocrine response, namely that plasma concentration of the 5-HT

precursor, TRP, is reduced. This finding has recently been reported by Goodwin and colleagues (1990) after reanalysing plasma samples from their dieting study (Goodwin et al, 1987b; 1987c) and is consistent with a report by Heraief and colleagues (1983) who found that a three day, very low calorie, protein-sparing, diet lowered plasma TRP and its ratio to competing amino acids. As discussed in 1.4, TRP availability determines brain 5-HT synthesis because TRP hydroxylase, the rate limiting enzyme in the synthetic pathway, is unsaturated with TRP under physiological conditions. In animal studies a diet with low TRP content reduces brain TRP levels and 5-HT turnover (Biggio et al, 1974). Further, when rats are fed a low TRP diet for several days, the PRL and corticosterone responses to subsequent challenge with the 5-HT precursors, TRP (Gil-Ad et al, 1976) and 5-HTP (Clemens et al, 1980) are enhanced, findings strikingly similar to our own. These enhanced neuroendocrine responses are believed to reflect a supersensitivity of brain 5-HT pathways, perhaps involving post-synaptic 5-HT receptors, as a result of 5-HT depletion (Clemens et al, 1980).

Accordingly I propose that moderate dieting in women lowers plasma TRP sufficiently to reduce brain 5-HT synthesis. Subsequent challenge with intravenous TRP causes an enhanced PRL response because, as in the animal studies, an adaptive supersensitivity of 5-HT pathways has developed.

In order to be available for brain 5-HT synthesis, TRP must be actively transported across the BBB. As discussed in 1.4, three factors are believed to be important in this process; first, the total concentration of TRP in the plasma (plasma total TRP), second, the amount of TRP in the plasma which is not bound to albumin (plasma free TRP) and third, the ratio of total TRP to neutral amino acids that compete for transport across the BBB. The relative importance of these mechanisms has been debated but probably depends on the experimental conditions. It appears that in the present subjects dieting reduced plasma total TRP and thereby the ratio of TRP to competing amino acids, but that free TRP was unaltered. Therefore, if my proposal is correct, the reduction in plasma total TRP is the crucial factor that leads to abnormal 5-HT-mediated neuroendocrine responses during dieting. While there was a trend towards a significant correlation between the fall in plasma total TRP and the increase in the PRL response in the whole subject group, the failure to find a more robust association is not surprising if the enhanced neuroendocrine response is the result of an adaptive change in the brain, rather than a direct consequence of reduced TRP availability.

Alterations in the regulation of TRP metabolism during dieting could alter the disposition of intravenous TRP, and this, rather than a change in brain 5-HT function, could explain the enhanced PRL responses to intravenous TRP in

females. However dieting did not result in increased plasma TRP levels following TRP infusion, indeed the data showed that in women, there was a more rapid clearance of intravenous TRP from plasma. This change cannot account for the increase in PRL response to intravenous TRP because it correlated inversely with the increased response. In other words, the increased plasma clearance of intravenous TRP caused by dieting in females tended to reduce the PRL response to intravenous TRP, but this was outweighed by more powerful changes acting in the opposite direction. It is of interest that two groups have reported that depressed patients have increased plasma TRP clearance following TRP infusion compared to controls (Koyama and Meltzer, 1986; Deakin et al, 1990) although two others found no difference (Cowen and Charig, 1987; Price et al, 1991). One possible explanation for this is enhanced metabolism of TRP by liver pyrrolase under the influence of raised plasma corticosteroid levels (Joseph et al, 1976). This study suggests that weight loss also increases the clearance of TRP from plasma perhaps by a similar mechanism, as plasma cortisol is known to increase in conditions of food restriction (Fichter and Pirke, 1984). Why this was only evident in female dieters is obscure.

It is important to consider why dieting might alter brain 5-HT function in women but not men, even though in both sexes, plasma TRP concentrations were reduced. There are two likely mechanisms which are not mutually exclusive

but indeed may be additive. First, plasma TRP levels fell more in women during dieting (perhaps related to increased plasma clearance as discussed above) and it is possible that this resulted in a significantly greater reduction of brain 5-HT synthesis. Second, in both human (Young et al, 1980) and non-human primates (Young and Ervin, 1984), rates of brain 5-HT turnover are greater in females than in males. A modest reduction in precursor availability may therefore be more likely to compromise brain 5-HT function in women than men.

My suggestion that a decrease in plasma TRP may alter 5-HT neuroendocrine responses in females is strongly supported by a recent study where a ten day low-TRP diet in healthy volunteers reduced plasma TRP levels and enhanced the PRL response to intravenous TRP (Delgado et al, 1989). Consistent with the present findings, the increase in PRL response was significant in women, but not men; in addition the women had a greater fall in plasma TRP. The reason why women appear more sensitive to manoeuvres which reduce plasma TRP is not clear.

4.5 Conclusions

This study suggests that dieting may alter brain 5-HT function in women but not men: what then are the potential implications? A major consequence is that investigations of 5-HT function in psychiatric disorders, particularly

depression, must assess weight loss and consider it as a potential confound in the interpretation of results. In addition weight loss must be considered when looking at possible aetiological factors involved in the development of psychiatric illness, especially eating disorders and depression. 5-HT pathways are involved in the control of food intake in animals, possibly by influencing satiety mechanisms (Blundell, 1984). Similarly in humans reduced brain 5-HT function has been linked to impaired appetite and satiety (Silverstone, 1985), a tendency to binge-eat (Kaye et al, 1988), poor impulse control (Depue and Spont, 1986) and of course depressive symptomatology (see Chapter 1). Since dieting commonly precedes the development of clinical eating disorders (Szmukler, 1985; Patton, 1988) it seems plausible that in predisposed individuals dieting-induced decreases in brain 5-HT function could contribute to the genesis of anorexia nervosa and bulimia nervosa. It has been argued that dieting is the key behaviour which puts individuals at risk of developing eating disorders (Szmukler, 1985), and it is well established that dieting is more common amongst women than men (Nylander, 1971). While this difference is likely to be a major factor in accounting for the preponderance of females with eating disorders, my data suggest that women may also be more biologically vulnerable to the effects of dieting than men.

CHAPTER 5

Study 2: The Effect of Weight Loss on Prolactin Secretion
in Normal Female Volunteers

5.1 Introduction

In the previous study I demonstrated that weight loss in dieting female, but not male, volunteers results in an increased PRL response to infusion of the 5-HT precursor tryptophan (Chapter 4). I suggested on the basis of these data that moderate weight loss alters brain 5-HT function in women but not men.

However PRL secretion is predominantly under inhibitory DA control (Tuomisto and Mannisto, 1985) and it was not possible in my previous study to exclude an effect of weight loss on DA function, or on PRL secretion itself, as the explanation for the altered PRL response to TRP. The present investigation addresses these questions by examining the effects of weight loss through dieting on the PRL responses to the DA antagonist, metoclopramide (MCP), and to TRH, which acts directly at pituitary level to stimulate PRL release. As only female dieters demonstrated an alteration in PRL responses to TRP this study was conducted in women only.

5.2 Subjects and Methods

5.2.1 Volunteers and Diet

Eleven female volunteers (aged 20 to 38 years, mean 26.9 years), non of whom had participated in the previous diet study, took part in this investigation. Recruitment

and selection criteria were the same as in the previous study (Chapter 4) and are described in General Methods (Chapter 3). Pre-diet weight and BMI are shown in Table 5.1. The subjects were tested before and at the end of the third week of a 1000 kcal diet. The onset of the diet was delayed for one week after the first neuroendocrine test so that as far as possible subjects were at the same stage of the menstrual cycle for both tests. One subject was taking a low-oestrogen contraceptive pill.

The subjects followed an identical diet protocol to the previous diet study (see 4.2.1).

Subjects completed a BDI (Beck et al, 1961) the day before each neuroendocrine test. This is a twenty-one item questionnaire covering symptoms related to depressive illness over the previous week. Item 19 (weight loss) was excluded from analysis.

5.2.2 Neuroendocrine Testing

This study was part of a larger study investigating the effects of dieting on overnight hormone secretion (Anderson et al, 1989a). Subjects had slept on the Research Unit the previous night for overnight hormone profiles to be measured. Blood sampling was performed via a specially adapted double lumen intravenous cannula, inserted the previous evening, which allowed continuous blood sampling using a peristaltic pump. The blood was heparinised as it was withdrawn and passed via fine bore tubing to a fraction

collector in an adjacent room (Matthews et al, 1985).

On the morning following the overnight sampling subjects were allowed out of bed but remained on the Research Unit and the continuous sampling cannula remained in situ. At 08.30 the subjects returned to their bed and had a second venous cannula inserted to allow administration of the challenge drugs. They were tested reclining without being allowed to sleep. After a 30-minute rest period, sampling was recommenced with blood being collected in 15-minute aliquots. After two samples had been collected, MCP at a dose of 5ug/kg was infused over 10 seconds (see General Methods, Chapter 3) at 09.30 (time 0). Continuous sampling continued over the next 90 minutes (to +90min) at which time TRH, 0.1ug/kg, was infused over 10 seconds (see General Methods, Chapter 3). Blood sampling continued in 10-minute aliquots for a further 30 minutes. These doses produce submaximal PRL responses which are more comparable to the PRL responses seen after TRP challenge than the maximal responses usually measured (see 1.8 and 5.4). Eight subjects had the combined MCP-TRH test and three had the MCP test alone.

Separation of plasma and measurement of plasma PRL were performed as described in General Methods (Chapter 3)

5.2.3 Analysis of Results

The PRL response to the drug challenges was expressed as AUC by taking the mean of the integrated hormone values

after drug challenge, subtracting the baseline and correcting for time.

Plasma hormone responses were compared using paired t-tests and BDI scores were compared using Wilcoxon's signed rank test (both two-tailed). Correlations were performed using Pearson's product moment (r). Group values are expressed as mean \pm SD; mean differences with 95% confidence intervals (95% CI) and significance levels (p) are used to interpret the data.

5.3 Results

All subjects lost weight as a consequence of the diet (Table 5.1). Nine women were tested at the same stage of their menstrual cycle on both occasions while two women had a menstrual cycle of three weeks and were tested at different stages of their cycle. Exclusion of these two subjects did not alter the results which are therefore presented for the subjects as a whole.

Dieting did not cause any obvious psychological upset in ten subjects who were happy about their weight loss. One subject did appear mildly depressed but this was in the context of a stressful house move which occurred unexpectedly during the diet. Some subjects spontaneously complained of feeling more irritable during the diet. There was no change in the overall BDI score (median (range): 1 (0-7) pre-diet versus 2 (0-10) post-diet,

Table 5.1

Subject characteristics and effects of weight reducing diet

	Subjects (N=11)
Age (years)	26.9 ± 5.6
Body Mass Index (kg/m ²) ^a	23.2 ± 1.3
Weight (kg) ^a	61.7 ± 5.2
Weight Loss (kg)	-3.1 ± 0.7
Weight Loss (% of starting weight)	-5.1 ± 1.2

Data shown as mean ± SD

a Pre-diet values

$p > 0.1$). The score decreased in four subjects, increased in three and stayed the same in four. The only item for which the score changed significantly was item 11 (irritability) with one person scoring before dieting and seven after dieting (0 (0-1) versus 1 (0-1), $p = 0.05$).

5.3.1 Prolactin Responses to Metoclopramide and TRH

Dieting did not significantly alter the PRL responses to MCP or TRH (Fig. 5.1, Fig. 5.2, Table 5.2). The PRL levels at +90min had not returned to baseline (time 0) values before the TRH infusion (Table 5.2) but there was no significant effect of dieting on either value (Table 5.2).

5.3.2 Correlations

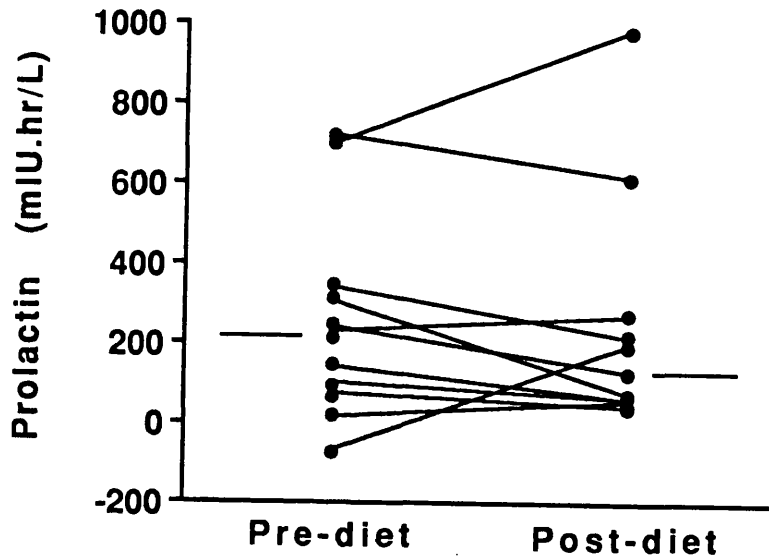
There were no significant correlations between PRL levels before drug infusion (time 0 and +90min) and the subsequent hormone responses (to MCP and TRH respectively) (all $p > 0.1$). The PRL responses to MCP and TRH did not correlate significantly ($p > 0.1$).

5.4 Discussion

The female subjects in this study were comparable to those in the previous study (Study 1, Chapter 4) and lost a similar amount of weight as a consequence of dieting. In contrast to the robust effects of weight loss on the PRL response to TRP infusion, however, dieting did not alter

Figure 5.1

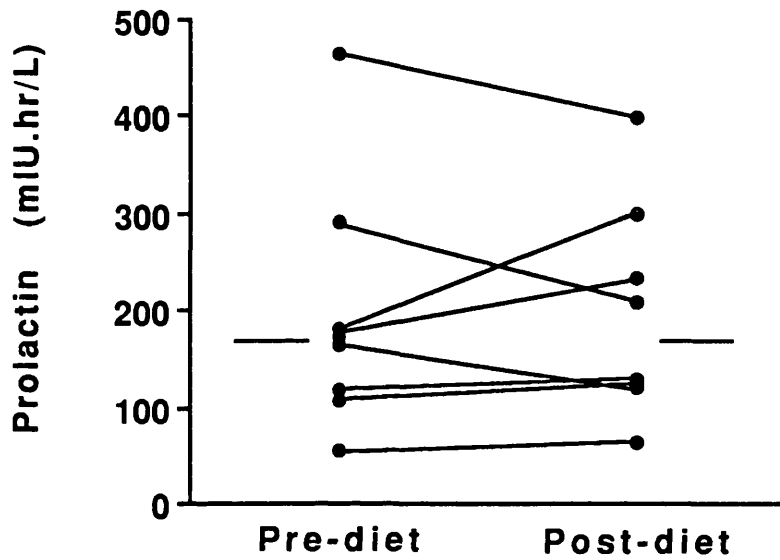
Effect of dieting on the prolactin response to metoclopramide infusion in female volunteers



Individual and mean PRL responses (AUC) are shown before (pre-diet) and after (post-diet) dieting.

Figure 5.2

Effect of dieting on the prolactin response to TRH infusion in female volunteers



Individual and mean PRL responses (AUC) are shown before (pre-diet) and after (post-diet) dieting.

Table 5.2

Plasma prolactin measures in female volunteers before and after dieting.

Measure	Pre-diet	Post-diet	Difference	95% CI
PRL baseline (mIU/L)	184 ± 70	169 ± 91		
PRL response to MCP (mIU.h/L)	224 ± 221	193 ± 228	-31 ± 127	-116 to 54
PRL +90min (mIU/L)	324 ± 141**	295 ± 164*		
PRL response to TRH (mIU.h/L)	172 ± 101	169 ± 89	-3 ± 71	-62 to 57

Values are Mean ± SD

* p<0.06 } versus PRL baseline (time 0)
 ** p<0.01 }

the PRL responses to submaximal stimulation by MCP or TRH. The 95% CI indicates that a 'true' effect of about 30% could have been missed at this level of significance, less than the 60% enhancement of the response to TRP.

MCP has been shown to be a DA antagonist (Peringer et al, 1976) and to raise PRL levels in humans (McCallum et al, 1976), an effect antagonised by DA agonists (McCallum et al, 1976; Quigley et al, 1980). The magnitude of the PRL responses achieved in our study are submaximal (McCallum et al, 1976) and comparable to those found with similarly low doses of the DA antagonist haloperidol (Rubin et al, 1976). It has been suggested that 5-HT agonism may also play a part in the PRL response to MCP (Fang and Shian, 1981; Jungmann et al, 1984), although others have found MCP to have no effect on brain 5-HT or 5-HIAA levels (Peringer et al, 1975). I believe that 5-HT agonism is unlikely to be important in causing the PRL responses in this study given the very low dose of MCP used and the comparability to results using haloperidol (Rubin et al, 1976).

These findings indicate that, at least in females, dieting does not alter DA-mediated PRL release. The striking alterations I showed in the PRL response to TRP (Chapter 4) are therefore unlikely to be caused by alterations in DA neurotransmission. Similarly, in the present study dieting did not alter the PRL response to a small dose (about 6ug) of TRH, a dose which causes a

submaximal release of PRL (Jacobs et al, 1973). This finding is consistent with a previous investigation where dieting did not alter the maximal PRL response to infusion of 200ug of TRH in either women or men (Goodwin et al, 1987b). Since TRH stimulates PRL by a direct effect on pituitary lactotrophs (Ishibashi and Yamaji, 1984; Gershengorn, 1986) these findings rule out a simple increase in pituitary lactotroph sensitivity or PRL reserve as an explanation for the altered PRL response to TRP infusion following dieting.

As expected there were no major mood changes during dieting but just over half of the subjects did experience an increase in irritability. Snaith and colleagues (1978) define irritability as a 'psychological state characterised by impatience, intolerance and poorly controlled anger'. In view of the link that has been proposed between low 5-HT function and impulsive aggression in humans (Linnoila and Virkkunen, 1991) and irritative aggression in animals (Soubrie, 1986) it is tempting to speculate that the increase in irritability seen in dieting could be related to a decrease in brain 5-HT function.

5.5 Conclusions

The results of this study show that dieting does not alter PRL secretion induced by MCP and TRH. This provides support for the enhanced PRL response to TRP being a

specific effect which I suggested in Chapter 4 is a consequence of an alteration in brain 5-HT function. Clearly it will be important to investigate this with different measures of 5-HT function, including more direct tests of 5-HT receptor function, to determine at what level this abnormality occurs. Possibilities include an alteration in neuronal handling of TRP as well as altered (pre- or postsynaptic) 5-HT receptor sensitivity.

The finding of increased irritability in these dieters is intriguing and requires replication in a further study with male and female dieters. If it does indeed hold true then its relationship to altered 5-HT function and the effect of manipulations with 5-HT-active drugs will be of great interest.

CHAPTER 6

Study 3: The Prolactin Response to Clomipramine Infusion in
Normal Volunteers

6.1 Introduction

CMI is a tricyclic antidepressant with a tertiary amine structure (see Figure 6.1). It potently inhibits monoamine uptake by presynaptic neurones with about a 5- to 10-fold selectivity for 5-HT over NA (Waldmeier et al, 1976; Hall and Ogren, 1981)(Table 6.1). CMI is however relatively non-specific in its actions and has a high affinity to α_1 -adrenoceptor and histamine₁ receptors with a lower affinity for muscarinic, 5-HT₂ and DA₂ receptors (Table 6.1). Behavioural studies indicate that CMI has little or no antagonist activity in measures of α_1 -adrenoceptor, DA or 5-HT₂ receptor function (Hall and Ogren, 1981). CMI is metabolised to the secondary amine, desmethyl-clomipramine (DCMI) which is a potent NA uptake inhibitor (Carlsson et al, 1969; Ross and Renyi, 1975)(Figure 6.1, Table 6.1). Therefore CMI has disadvantages, as far as specificity is concerned, compared to newer compounds such as fluoxetine, fluvoxamine, zimelidine and citalopram which are highly selective 5-HT uptake inhibitors (Green and Costain, 1981). However these compounds are not available in an intravenous preparation whereas there is substantial clinical experience with intravenous CMI (eg O'Flanagan, 1979). Acute oral administration of 5-HT uptake inhibitors, including CMI, has not been shown to consistently stimulate PRL secretion (Hughes, 1973; Francis et al, 1976; Jones et al, 1977; Syvalahti et al 1979)

Figure 6.1

Chemical structures of clomipramine and desmethylclomipramine

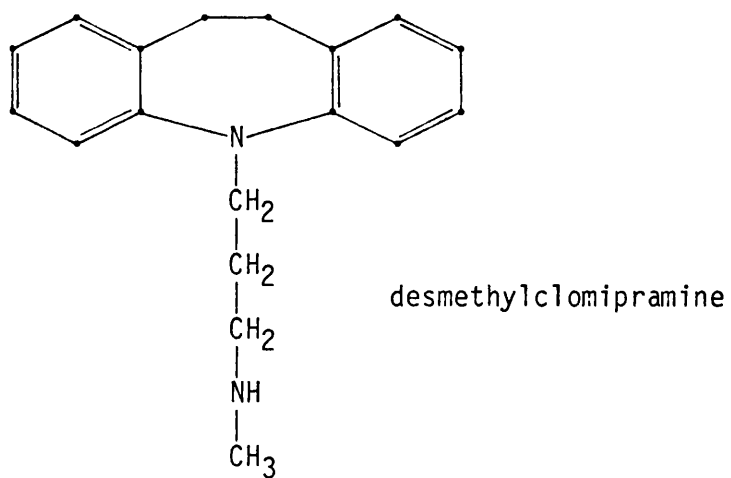
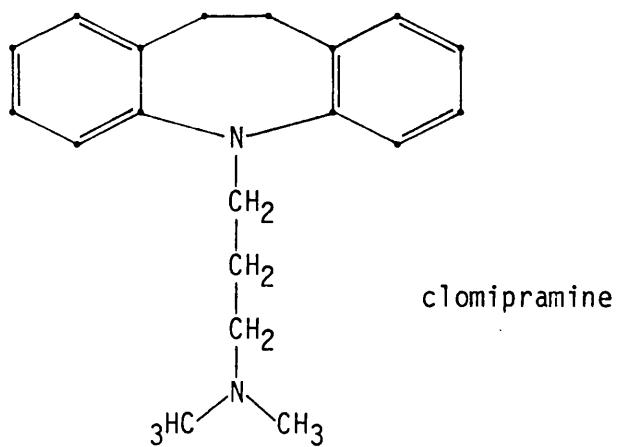


Table 6.1

Uptake inhibition and receptor binding properties of clomipramine and desmethylclomipramine ($IC_{50}, \mu M$)

Measure	Clomipramine	Desmethylclomipramine
Inhibition of 5-HT Uptake	0.018	0.24
Inhibition of NA Uptake	0.060	0.024
5-Hydroxytryptamine ($5-HT_1$)	21.2	
d-Lysergide ($5-HT_{1+2}$)	0.917	
Dihydroalprenolol (β -adrenoceptor)	38.9	
WB-4101 (α_1 -adrenoceptor)	0.035	
Clonidine (α_2 -adrenoceptor)	5.04	
Spiroperidol (dopamine ₂)	0.268	
Mepyramine (histamine ₁)	0.064	
Quinuclidinylbenzilate (muscarinic)	0.184	

Uptake inhibition studies were carried out on synaptosomal preparations from the rat midbrain-hypothalamus region (Ross and Renyi, 1975)

Receptor binding studies were performed on crude homogenates of rat brain (Hall and Ogren, 1981)

whereas there is good evidence that intravenous CMI does increase plasma PRL levels in humans (Lacey et al, 1977; Laakmann et al, 1984; Golden et al, 1989). This effect appears not to be due to NA-uptake inhibition as extensive animal and human data indicate that NA systems are of minor importance in PRL secretion (Tuomisto and Mannisto, 1985) and consistent with this, desmethylimipramine, a NA uptake inhibitor, is much less potent than CMI in stimulating PRL secretion (Laakmann et al, 1984). In addition, Golden and colleagues (1989) were unable to demonstrate detectable plasma DCMI levels after CMI infusion indicating that an appreciable effect on NA uptake is unlikely under these conditions. As has been discussed in 2.3, there is considerable evidence that 5-HT pathways are involved in PRL secretion. The proposed mechanism by which CMI stimulates PRL secretion is through an increase in synaptic 5-HT concentration (consequent on inhibition of 5-HT uptake into the presynaptic neurone) which then acts on postsynaptic 5-HT receptors (Golden et al, 1989). The only antagonist study to attempt to characterise this response is by Laakmann and colleagues (1983) who reported that the PRL response to CMI is blocked by methysergide, a 5-HT antagonist. Unfortunately, as has been discussed (2.4 , methysergide is non-selective and other properties (such as DA agonism) might explain this blockade.

A serious problem with CMI challenge is its propensity to cause stressful side-effects, particularly nausea and

vomiting. For example in the study by Laakmann and colleagues (1984), eleven out of twelve subjects experienced nausea and one vomited after administration of 25mg of CMI intravenously. Although there was no obvious relation between side-effects and PRL response it is well known that stress does stimulate PRL secretion (Anfilogoff et al, 1987; Meyerhoff et al, 1988) and it is therefore possible that the PRL response to CMI is a non-specific response to the presence of stressful side-effects.

This study investigated the utility of CMI challenge as a probe of 5-HT function in human subjects with particular emphasis on trying to distinguish the pharmacological effects of CMI from stress-related responses.

6.2 Methods

6.2.1 Subjects and Clomipramine Dose

Nineteen normal volunteers (thirteen males, six females, mean age 30 years, range 22-45) were recruited to receive the CMI infusion test as described under General Methods (Chapter 3). The study was conducted in an open manner although most subjects were not told the dose of CMI they would receive. Four males undertook more than one CMI test (three received two tests and one received three tests) with the rest of the subjects receiving only one CMI test, making a total of twenty four tests altogether. Doses of CMI administered were 0.075mg/kg (3 tests),

0.1mg/kg (thirteen tests), 0.125mg/kg (seven tests) and 0.15mg/kg (one test). The subjects who undertook more than one test received different doses on each occasion. A group of ten normal volunteers (six males, four females, mean age 28 years, range 22-37) who had received placebo capsules as part of another study running concurrently (Gregory et al, 1990) were used as placebo controls.

6.2.2 Neuroendocrine Testing

The neuroendocrine protocol, determination of side-effects, pulse and BP, and measurement of plasma PRL are described under General Methods (Chapter 3).

6.2.3 Analysis of Results

The PRL responses and DNausea were first analysed using the Kruskal-Wallis one-way ANOVA (H) with subsequent post-hoc Mann Whitney U tests (U) if significant. Baseline PRL levels were compared using a one-way ANOVA. Correlations were performed using Pearson's product moment correlation (r) and Spearman's rank correlation (rho).

6.3 Results

6.3.1 Side-Effects

Even at these relatively low doses (4.8-12.0mg), CMI infusion caused significant gastrointestinal side-effects. Five out of the twenty four infusions (21%) caused

stressful nausea (see 3.2.7 for definition) with vomiting in two subjects; eleven of the remaining nineteen infusions (58%) resulted in some nausea, although this was generally mild. There appeared to be a dose-related incidence of stressful nausea although individual susceptibility varied greatly. At CMI doses of 0.075, 0.1, 0.125 and 0.15mg/kg, stressful nausea occurred in 0/3 (0%), 2/13 (15%), 2/7 (29%) and 1/1 (100%) of subjects at respectively. Of the four subjects who received more than one dose, 3/4 experienced stressful nausea, in each case at the highest CMI dose they received (two at 0.125mg/kg and one at 0.15mg/kg). All of the subjects who experienced stressful nausea were male. None of the subjects who received placebo tablets experienced nausea.

Examination of visual analogue ratings of nausea (excluding CMI 0.15mg as there was only one test at this dose) revealed a significant difference between DNausea in the different groups ($H=17.66$, $df=3$, $p<0.01$). DNausea ratings were significantly higher than placebo in subjects receiving CMI 0.1mg (median (range): 30 (0-80) versus 0 (0), $U=10$, $p<0.01$) and CMI 0.125mg (median (range): 40 (0-100) versus 0 (0), $U=5$, $p<0.05$). Subjects who received CMI 0.1mg also had higher DNausea than those receiving CMI 0.075mg, none of whom experienced any nausea ($U=3$, $p<0.05$) but there was no difference in DNausea between subjects receiving CMI 0.1mg and 0.125mg ($U=37$, $p>0.05$). The five subjects who had stressful nausea had the highest DNausea

ratings (80 in three subjects, 100 in two subjects).

Exclusion of these subjects showed that nausea was generally mild but the dose relationship remained the same (Table 6.2).

There was no significant relationship between absolute CMI dose and visual analogue ratings of nausea (see 6.3.4 below).

6.3.2 Prolactin Responses

PRL responses in subjects with stressful nausea were analysed separately (N+) from those without severe side-effects. The results are shown in Figure 6.2 and Table 6.2. In the placebo group, PRL levels continued to decline after time 0 and this is reflected in a negative AUC. CMI infusion at a dose of 0.075mg/kg did not appear to alter this pattern but CMI 0.1mg/kg prevented the expected fall and CMI 0.125mg/kg produced a modest increase over baseline. The N+ group demonstrated a strikingly higher PRL response than other groups, an effect that could not be accounted for by CMI dose (Table 6.2) . The Kruskal-Wallis ANOVA showed a significant difference between groups including ($H=24.85$, $df=4$, $p<0.01$) or excluding the N+ group ($H=18.75$, $df=3$, $p<0.01$) and post-hoc testing showed the PRL responses to be dose related (Figure 6.2, Table 6.2).

There was a significant correlation between dose and PRL response (see 6.3.4) and it was clear from inspecting

Table 6.2

The prolactin response to clomipramine infusion in normal volunteers

Dose	Weight (kg) mean \pm SD	CMI dose (mg) mean \pm SD	DNausea median (range)	Basal PRL (mIU/L) mean \pm SD	CMI AUC ^a (mIU.h/L) median(range)
Placebo (6M:4F)	68 \pm 11	0	0 (0)	231 \pm 66	-52 (-161 to 2)
0.075mg/kg (3M)	80 \pm 20	6.3 \pm 1.9	0 (0)	153 \pm 67	-55 (-86 to 19)
0.1mg/kg (7M:4F)	73 \pm 12	7.3 \pm 1.2	20** ‡ (0 to 60)	193 \pm 70	7** (-40 to 291)
0.125mg/kg (2M:3F)	64 \pm 11	8.2 \pm 1.4	20* (0 to 60)	231 \pm 102	93** + (31 to 463)
N+ ^b (5M)	74 \pm 10	8.9 \pm 1.9	90** ++ ‡ Δ (80 to 100)	133 \pm 27	569** ++ (293 to 966)

DNausea and CMI AUC values compared using Mann Whitney U tests

* p<0.05 } versus placebo
 ** p<0.01 }

+ p=0.05 } versus 0.1mg/kg
 ++ p<0.01 }

‡ p=0.05 versus 0.075mg/kg

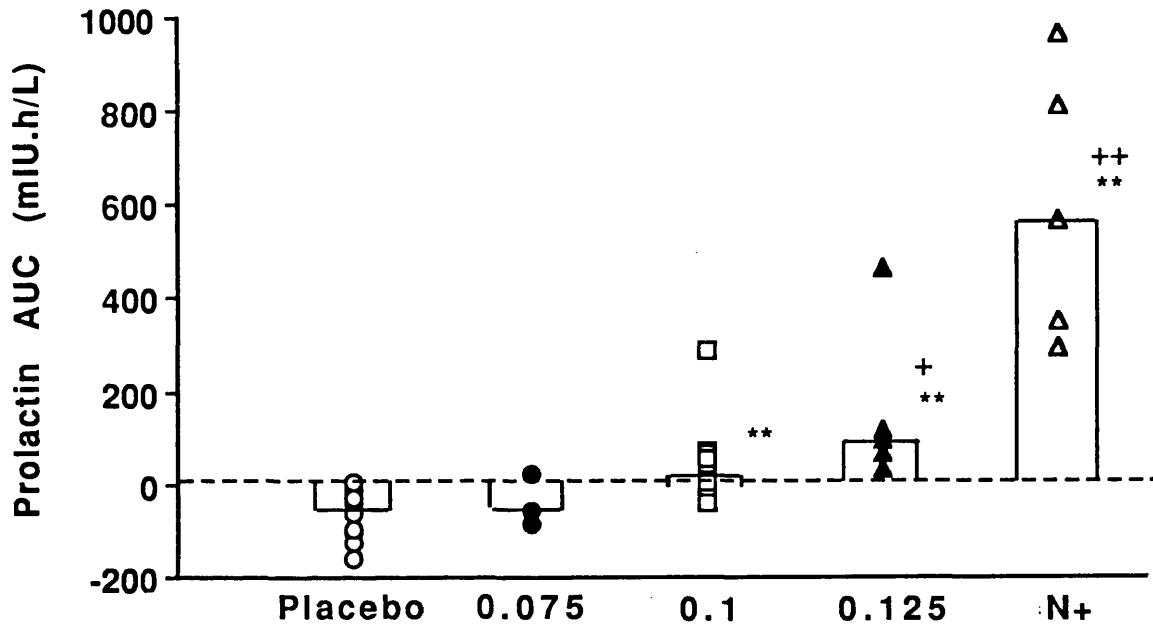
Δ p=0.01 versus 0.125mg/kg

a PRL response to CMI infusion (area under curve minus baseline)

b Subjects experiencing stressful nausea

Figure 6.2

Prolactin responses to placebo and clomipramine infusion in normal volunteers



Individual and median PRL responses are shown. 0.075, 0.01 and 0.125 denote doses of CMI in mg/kg. N+ denotes subjects experiencing stressful nausea

Responses compared using Mann Whitney U tests

- ** $p < 0.01$ versus placebo
- + $p = 0.05$ versus CMI 0.1mg/kg
- ++ $p < 0.01$ versus CMI 0.1mg/kg

the results that individuals who received less than 7mg of CMI, irrespective of body weight, were much less likely to have a PRL response than those who received 7mg or more (2/7 versus 11/12).

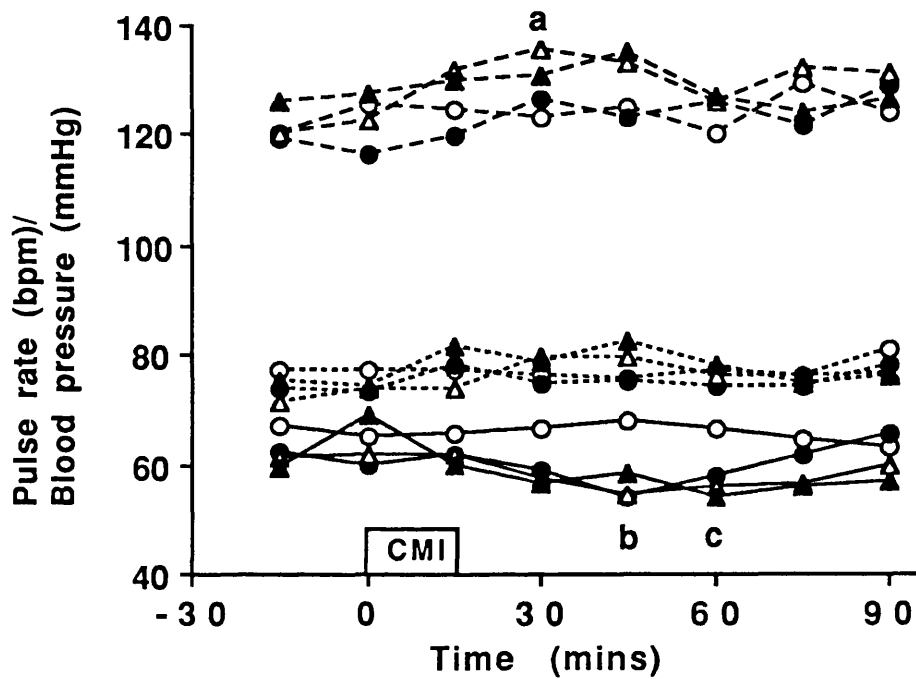
Baseline PRL levels (time 0) were significantly higher in female than male volunteers (mean \pm SD: 240 \pm 88 versus 177 \pm 60 mIU/L, $t=2.1810$, $df=32$, $p=0.04$). There were no significant differences between baseline PRL in the different groups (ANOVA: $F=2.2632$, $df=4,29$, $p>0.05$) (Table 6.2).

6.3.3 Cardiovascular Responses

Five subjects receiving placebo had pulse and blood pressure measurements performed and these were compared to the CMI 0.1mg/kg, 0.125mg/kg and N+ groups (Figure 6.3). One subject receiving CMI 0.075mg did not have cardiovascular measures recorded and therefore the group was too small to include in the analysis. ANOVA for pulse rate showed no significant overall group differences ($F=1.24$, $df=1$, $p=0.32$) but a significant change in pulse rate over time ($F=3.03$, $df=7$, $p=0.005$) with significant differences between groups at specific time points (group x time: $F=1.97$, $df=21$, $p=0.011$). Post-hoc range testing revealed a modest but significant difference between placebo and CMI infusion groups due to a decrease in pulse rate after CMI infusion with a nadir at +45 to +60min (Figure 6.3).

Figure 6.3

Cardiovascular responses to placebo and clomipramine infusion in normal volunteers



Mean values are shown. CMI was infused between time 0 and +15min

Solid lines represent pulse rate; fine dotted lines, diastolic BP; coarse dotted lines, systolic BP

Open circles represent placebo; closed circles, CMI 0.1mg/kg; open triangles, CMI 0.125mg/kg; closed triangles, subjects experiencing stressful nausea (N+)

Values compared using unpaired t-tests following significant ANOVA

- a $p=0.06$, CMI 0.125mg/kg versus placebo
- b $p<0.05$, CMI 0.1mg/kg and 0.125mg/kg versus placebo
- c $p<0.05$, N+ versus placebo

ANOVA for diastolic BP showed no significant overall group differences ($F=0.18$, $df=1$, $p=0.91$), a just significant change over time ($F=2.11$, $df=7$, $p=0.046$) but no significant differences between groups at specific time points (group x time: $F=0.99$, $df=21$, $p=0.48$)

ANOVA for systolic BP showed no significant overall group differences ($F=1.0$, $df=1$, $p=0.41$) but a significant change over time ($F=4.04$, $df=7$, $p=0.0004$) with significant differences between groups at specific time points (group x time: $F=1.82$, $df=21$, $p=0.020$). However, on post-hoc range testing, the differences between placebo and CMI infusion groups did not reach statistical significance although systolic BP tended to increase after CMI infusion with a peak at +30 to +45min (Figure 6.3)

6.3.4 Correlations

The PRL response was strongly negatively correlated with PRL baseline levels in the placebo group ($r=-0.84$, $p=0.002$) and 0.075mg/kg group ($r=-0.99$, $p=NS$), a relationship not present in any other group; indeed there was a trend to a significant positive correlation in the 0.25mg/kg group ($r=0.84$, $p=0.08$).

Exclusion of the N+ group from correlation analyses comparing PRL responses with absolute CMI dose and DNausea revealed that there was a significant correlation between the CMI dose and PRL response ($\rho=0.49$, $p=0.04$) but none between DNausea and PRL response ($\rho=0.22$, $p=0.36$).

In contrast, if the N+ group was included, there were significant correlations between both absolute CMI dose and PRL response ($\rho=0.53$, $p=0.009$), and DNausea and PRL response ($\rho=0.61$, $p=0.002$), the latter because those with severe nausea had very large PRL responses.

There was no significant correlation between absolute CMI dose and DNausea; this was true whether the N+ group was included ($\rho=0.23$, $p=0.29$) or excluded ($\rho=0.03$, $p=0.89$).

6.4 Discussion

This study confirms that CMI infusion stimulates PRL secretion in human volunteers and is consistent with other studies (Lacey et al, 1977; Laakmann et al, 1984; Golden et al, 1989). However even at the low doses employed here a substantial minority of volunteers had stressful side-effects which were associated with large PRL responses. In the absence of stressful nausea there was a modest dose-related PRL response to CMI infusion.

It is well recognised that 5-HT-active drugs can cause nausea; a good example clinically is the nausea and, more rarely, vomiting that can occur with selective 5-HT uptake inhibitors used in the treatment of depression (eg Benfield and Ward, 1986). The neural mechanisms controlling nausea and vomiting are still poorly understood and a wide range of stimuli can cause emesis. This 'emetic reflex' has been

viewed as a defence against ingested toxins with a hierarchical organisation consisting of taste and smell, sensory mechanisms in the proximal gut and the chemoreceptor trigger zone in the brainstem (Davis et al, 1986). Of great recent interest has been the discovery that 5-HT₃ receptor agonists are potent anti-emetics for some forms of emesis, in particular cytotoxic-induced vomiting (Miner and Sanger, 1986). 5-HT₃ receptors are present in the gut and 5-HT₃ antagonists can block 5-HT-induced depolarisation of vagal fibres (Richardson et al, 1985) and the 'vagally-dependent' phase of radiation-induced vomiting (Andrews and Hawthorne, 1987). 5-HT₃ receptors are also present in high density in the dorsal vagal complex (Reynolds et al, 1989), an area in the brainstem known to be important in triggering the vomiting response (chemoreceptor trigger zone) (Borison, 1974) and 5-HT₃ antagonists block the central component of cytotoxic-induced vomiting in the ferret (Higgins et al, 1989). Therefore it appears that 5-HT may be involved both peripherally and centrally in the emetic response through its action on 5-HT₃ receptors. The role of other 5-HT receptor subtypes is uncertain (Leslie and Reynolds, 1991).

It can be seen from the results that there does appear to be an association between stressful nausea and disproportionately large PRL responses, with a significant correlation between nausea ratings and PRL responses when the N+ group is included in the analysis. It is possible

to suggest two explanations for this association. First, the hormone response could be a result of stress (Anfilogoff et al, 1987; Meyerhoff et al, 1988) and therefore likely to be unrelated to the pharmacological effect of CMI on 5-HT function (although it is of course possible that 5-HT is involved in the stress response itself, eg De Meirleir et al, 1985; Prescott et al, 1984). Second, there could be an increased sensitivity of postsynaptic 5-HT receptors leading both to exaggerated neuroendocrine responses and to increased nausea in susceptible individuals. I believe that the first of these explanations is the more plausible and therefore that the presence of stressful nausea invalidates that individual's CMI test as a pharmacological test of 5-HT function.

I would contend that the situation differs when subjects experiencing stressful nausea are excluded as the PRL responses in this situation were dose related and did not correlate with the presence of mild nausea as measured by visual analogue ratings. In particular there were individuals who clearly had experienced some nausea but had no PRL response and others who had felt no side-effects who demonstrated a clear hormone response. This suggests that the PRL responses in the absence of stressful nausea may be related to the pharmacological actions of CMI.

The placebo condition in this study was not strictly comparable to the CMI test and is a weakness in the study design; it would have been preferable to study subjects

with a placebo infusion to control for experimental procedure. However I believe that this placebo group does provide a useful comparison with the CMI infusion groups. First, the PRL levels after placebo tablets in this study are comparable to those seen after saline infusion (Cowen et al, 1985; Golden et al, 1989) and, second, they appeared indistinguishable from the lowest dose of CMI, suggesting that the infusion procedure itself did not cause a PRL response. The negative correlation between PRL baseline and PRL AUC in the placebo and CMI 0.075mg/kg conditions reflects the morning fall in PRL levels from high levels during the latter part of sleep (Weitzman, 1976); those who start higher as a legacy of the nocturnal surge tend to fall further to basal daytime levels. The finding that females had higher baseline PRL levels than males is also consistent with the literature (Baumgartner et al, 1988).

The results of this study are therefore consistent with 5-HT mediation of PRL response to CMI infusion in the absence of stressful side-effects but unfortunately the appropriate antagonist studies (particularly of 5-HT₁ receptors) required to prove this in humans are difficult to perform. Metergoline and methysergide are the best non-selective (5-HT₁ and 5-HT₂) antagonists for human use but, as has been discussed in 2.4, both have DA agonist properties (Krush et al, 1981) and any inhibition of the PRL response is probably due to direct inhibition of PRL secretion at pituitary level through DA receptor

stimulation (Tuomisto and Mannisto, 1985; Ellis et al, 1991). More selective antagonists are now available in humans, for example the 5-HT_{1C/2} antagonist, ritanserin, and the 5-HT₃ antagonist, ondansetron, but neither of these were available at the time of the study. It is of interest that the 5-HT_{1C/2} antagonists ketanserin and ritanserin failed to inhibit the PRL response to TRP (Cowen and Anderson, 1986; Charig et al, 1987) and the evidence at present implicates 5-HT₁ receptors in PRL responses to TRP (see 2.3.1 and 2.4). Unfortunately there are no selective 5-HT₁ receptor antagonists currently available for human use. The beta-blocker pindolol is an antagonist at 5-HT_{1A+1B} receptors but potential cardiovascular complications precluded its use with CMI. If we turn to animal data there is good evidence for elevation of PRL levels following the intraperitoneal administration of selective 5-HT uptake inhibitors such as fluoxetine (Morgan and Herbert, 1978), fluvoxamine (Cella et al, 1983) and citalopram (Fessler et al, 1984). Support for this effect being mediated by 5-HT comes from the abolition of this response by destruction of 5-HT neurones innervating the hypothalamus (Fessler et al, 1984). As far as I know this response has not been investigated using selective 5-HT antagonists. The evidence taken as a whole does I believe point to 5-HT-mediation of the PRL response to intravenous CMI.

This study demonstrated a modest reduction in pulse

rate and increase in systolic BP after CMI infusion, an effect not seen after oral placebo. This finding is consistent with the ability of CMI to affect 5-HT and NA neurotransmission (Burgess, 1981). While the placebo group in this study was not strictly comparable to the CMI infusion groups, it is very unlikely that the infusion procedure itself is responsible as infusion of a small volume of normal saline does not lower pulse rate or increase BP (Golden et al, 1989; IM Anderson, unpublished observations). Studies of chronic CMI administration in depressed patients show that variable cardiovascular effects occur, the commonest being hypotension with sinus tachycardia (Symes, 1973; Burgess et al, 1978; Szarek and Goethe, 1984). Golden and colleagues (1989) reported that infusions of saline, CMI 10mg and CMI 20mg had no effect on cardiovascular measures in normal volunteers although in a later study, comparing normal controls and depressed patients, there was a decrease in pulse rate in the normal volunteers after CMI 10mg (Golden et al, 1990). In contrast, Laakmann and colleagues (1984) reported an increase in mean arterial BP without an effect on pulse rate after an infusion of 25mg of CMI. The reason for these discrepant findings is unclear.

6.5 Conclusions

In the absence of severe, stressful side-effects, elevation of plasma PRL following CMI infusion may be a consequence of increased synaptic 5-HT concentration, and hence 5-HT neurotransmission, in the hypothalamus (see 2.2). Severe nausea stimulates PRL secretion as part of a stress response and therefore the presence of severe side-effects during the CMI infusion is likely to swamp any selective pharmacological effect. The dose 'window' to achieve a PRL response without stressful nausea is extremely narrow, and not entirely predictable, indicating that close monitoring of side-effects, with exclusion of stressed individuals, is necessary if CMI infusion is to be used as a 5-HT neuroendocrine probe. Provided this precaution is taken, CMI-induced PRL secretion may provide an index of brain 5-HT function.

CHAPTER 7

Study 4: The Prolactin Response to Clomipramine and
Low-Dose TRH Infusion in Depressed Patients
and Controls.

7.1 Introduction

As discussed in Chapter 1, there are a number of lines of evidence that 5-HT function is impaired in depressed patients. Neuroendocrine challenge tests currently offer the only practical means of testing this hypothesis using a dynamic assessment of brain 5-HT function. Studies to date provide good evidence for blunted PRL responses to challenge by drugs believed to increase presynaptic 5-HT function, namely TRP and fenfluramine. The lack, until recently, of suitable specific 5-HT antagonists for human use (see 2.4) means that it has not been possible to conclusively characterise the PRL responses to these challenges, although there is evidence that the PRL response to TRP infusion is mediated by 5-HT₁ (probably 5-HT_{1A}) receptors (see 2.4). However if the same result can be demonstrated by the use of a number of compounds which differ structurally and in their mode of action, but which share the ability to stimulate 5-HT neurotransmission, then this provides strong circumstantial evidence that the abnormality involves 5-HT pathways. The 5-HT precursor, TRP, causes increased neuronal 5-HT synthesis (Moir and Eccleston, 1968) and therefore provides more 5-HT available for release, while fenfluramine releases 5-HT from pre-synaptic nerve terminals (Garattini et al, 1975) (see Cowen and Anderson, 1986 for discussion). CMI offers the chance to test depressed patients with a

compound that enhances 5-HT function in a different way - by inhibiting 5-HT uptake. In Chapter 6 I showed that the PRL response to CMI infusion may provide an index of brain 5-HT function provided great care was taken to exclude subjects who experienced stressful nausea.

As has been discussed in 2.1 the PRL response to 5-HT drug challenge is the result of a series of steps leading from the 5-HT synapse to the pituitary lactotroph which releases PRL. Any abnormality in the 5-HT-mediated PRL response in depressed patients could result from altered function occurring at a site other than the 5-HT synapse. In particular an abnormality in PRL secretion itself has to be excluded. The studies investigating TRH stimulated PRL secretion in depression are flawed through using large doses of TRH which provide a supramaximal stimulation to PRL secretion (see 1.8.4). In order to detect an alteration in pituitary lactotroph sensitivity rather than PRL reserve (measured by maximal PRL release) it is necessary to use a dose of TRH resulting in a submaximal stimulation of PRL secretion, comparable to that seen after 5-HT drug stimulation.

This study investigated the PRL response to CMI and to low-dose TRH in depressed patients compared to controls. The relationship between PRL response and melancholia, suicide attempt and weight loss were of particular interest given the evidence that these may be associated with altered 5-HT function (see 1.8 and Chapter 4).

7.2 Methods

7.2.1 Subjects

Fifteen depressed patients were recruited and assessed as described under General Methods (Chapter 3) and received CMI and TRH infusion tests. Two female patients were excluded from analysis as they experienced stressful nausea as defined in General Methods (Chapter 3). Another female patient was discovered to have no CMI in her plasma and her CMI test results were therefore discarded but her TRH test results were used. A further female patient did not receive a TRH test because venous access was lost. The results from twelve CMI tests and twelve TRH tests were therefore available for analysis and patient details are shown in Table 7.1. There were seven inpatients, three patients (two males, one female) reached criteria for melancholia, one female patient had endogenous depression (as defined by the Newcastle Scale) and five (three males, two females) had lost more than 5lb in weight in the preceding month. Four patients (two males, two females) had made suicide attempts (paracetamol overdose, attempted drowning, attempted gassing, wrist cutting) and a fifth male patient was included in the 'suicide' group as he had planned to crash his car following the writing of a suicide note but was intercepted in time. Of particular note, 10/12 patients had never previously received antidepressant medication; one inpatient had received a single dose of

Table 7.1

Details of depressed patients and controls in the clomipramine and low-dose TRH study

	Clomipramine Test		Low-Dose TRH Test	
	Controls	Patients	Controls	Patients
Sex (M:F)	6:6	6:6	6:6	6:6
Age (years)	31.3 ± 8.6	31.2 ± 9.2	31.2 ± 8.7	31.2 ± 9.2
Weight (kg)	70.3 ± 13.6	70.3 ± 13.6	69.8 ± 13.8	70.0 ± 13.7
HAMD (21 item)		22.3 ± 4.9		22.0 ± 5.2
BDI		24.2 ± 10.3		23.3 ± 10.5
Newcastle Score		4.4 ± 0.8		4.3 ± 0.9
HAMA		17.2 ± 7.7		17.9 ± 8.0
Drug Free Period				
Never		10		10
3+ Months		1		1
3 Weeks-3 Months		1		1
Menstrual Stage				
Follicular	-	3	-	3
Mid Cycle	1	1	-	-
Luteal	2	1	2	1
Irregular/Unknown	2	1	3	2
Contraceptive Pill	1	-	1	-

Values are mean ± SD

temazepam. The patients were compared to age, sex and weight matched controls (see Table 7.1).

7.2.2 Neuroendocrine tests

The CMI infusion tests were prepared and carried out as described in General Methods (Chapter 3). On the basis of the results obtained in Study 3 (Chapter 6) the CMI dose was banded according to weight as follows:

Subjects weighing below 50kg received 6.5mg;

50-60kg received 7.0mg;

60-70kg received 7.5mg;

70-80kg received 8.0mg;

80-90kg received 8.5mg.

All controls received the same CMI dose as their matched patient except for one female control who received a lower dose (the patient received 7mg and the control 6.5mg).

The low-dose TRH tests were prepared and administered as described in General Methods (Chapter 3) and given at the end of the CMI test, immediately after the +90min blood sample.

Nausea ratings and pulse and blood pressure were measured during the CMI infusion test and plasma PRL and CMI were estimated as described in General Methods (Chapter 3).

7.2.3 Analysis of Results

The PRL responses to CMI infusion were analysed as the

AUC from time 0 to +90min (CMI AUC) as described in General Methods (Chapter 3). The PRL responses to low-dose TRH were similarly calculated from time +90 to +120 with subtraction of the +90 value (TRH AUC). Plasma CMI following CMI infusion (TAUC) was calculated as described in General Methods (Chapter 3). CMI AUC values and nausea ratings (DNausea) were compared using Mann Whitney U tests (U); TRH AUC, TAUC and baseline PRL values were compared using unpaired t-tests (t). The effects of CMI infusion on cardiovascular measures were analysed by two-way ANOVA as described in General Methods (Chapter 3).

7.3 Results

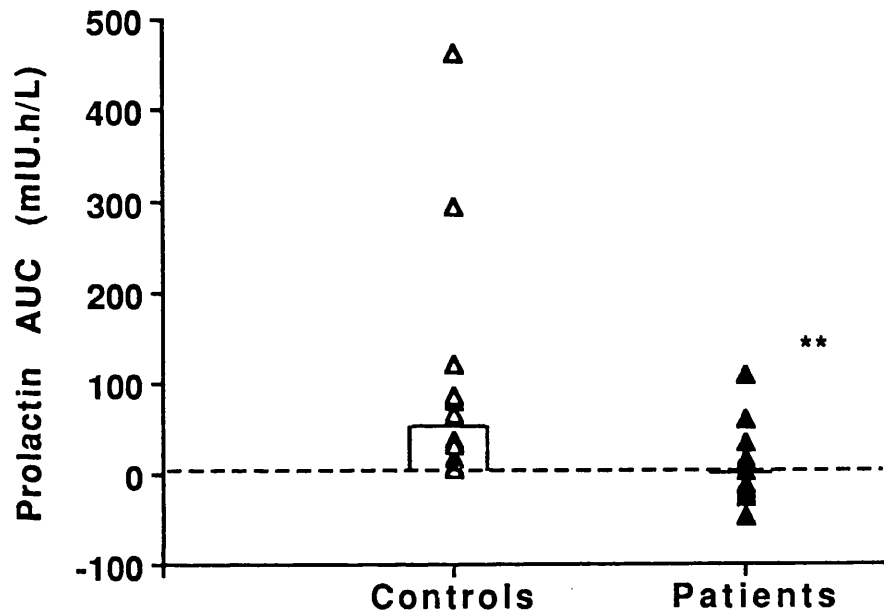
7.3.1 Clomipramine Test

(i) Prolactin Response

Patients had a significantly smaller PRL responses to CMI infusion than controls (Figure 7.1, Table 7.2). If men and women were considered separately, the male depressives had significantly lower responses compared to male controls (median (range): -4.8 (-49 to 33.5) versus 50.6 (17.3 to 87.3) mIU.h/L, $U=2$, $p=0.01$) whereas the response was not significantly lower in female depressives compared to female controls (median (range): -0.8 (-24.5 to 109) versus 75.6 (5 to 463) mIU.h/L, $U=7$, $p>0.05$). The three melancholic patients had the lowest PRL responses, statistically different to controls and non-melancholic

Figure 7.1

Prolactin responses to clomipramine infusion in depressed patients and controls



Individual and median PRL responses are shown

Groups compared using Mann Whitney U test

** $p < 0.01$ versus controls

Table 7.2

The prolactin response to clomipramine infusion in depressed patients and controls

Subjects	N	CMI AUC (mIU.h/L) ^a Median (Range)
Controls	12	50.6 (5.0 to 463.0)
Patients	12	-4.8 (-49.0 to 109.0)**
Melancholia-	9	11.3 (-19.5 to 109.0)*
Melancholia+	3	-26.5 (-49.0 to -24.5)** +
Suicide-	8	13.8 (-24.5 to 109.0)
Suicide+	4	-23.0 (-49.0 to -11.3)** ‡
Weight Loss-	7	16.3 (-19.5 to 109.0)
Weight Loss+	5	-24.5 (-49.0 to -11.3)** Δ

- and + indicate absence and presence respectively of feature
Responses compared using Mann Whitney U tests

* p<0.05 } versus Controls
 ** p<0.01 }
 + p=0.01 versus Melancholia-
 ‡ p<0.05 versus Suicide-
 Δ p<0.05 versus Weight Loss-

a PRL response to CMI infusion (area under curve minus baseline)

depressives (Table 7.2). Suicidal patients also had significantly lower PRL responses than non-suicidal patients (Table 7.2) as did patients with weight loss compared to those without weight loss (Table 7.2). Baseline PRL concentrations did not differ between patients and controls (219 ± 120 mIU/L versus 187 ± 99 mIU/L, $t=0.7204$, $df=22$, $p=0.48$) or between female and male patients (222 ± 105 versus 216 ± 144 , $t=0.0710$, $df=10$, $p=0.94$) although in controls there was a trend for females to have a higher baseline than males (234 ± 98 mIU/L versus 139 ± 80 mIU/L, $t=1.8462$, $df=10$, $p=0.095$).

(ii) Plasma Clomipramine levels

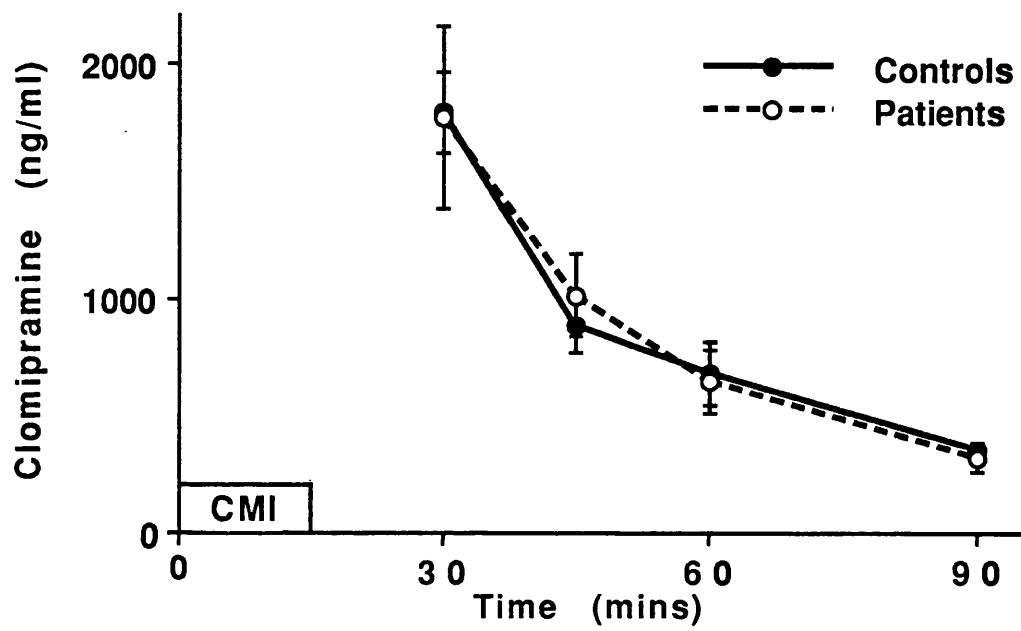
Results were available from ten matched pairs of patients and controls. There was no difference between plasma CMI levels in the two groups (TAUC: 799 ± 438 ng.h/ml in patients versus 789 ± 280 ng.h/ml in controls, $t=0.0593$, $df=18$, $p=0.95$) (Figure 7.2). DCMI was not detected.

(iii) Side-Effects

The CMI infusion was well tolerated apart from the two female patients who were excluded because of stressful nausea. More controls than patients reported nausea (10/12 versus 6/12) although this was generally mild and there was no significant difference between DNausea ratings (median

Figure 7.2

Plasma clomipramine levels following clomipramine infusion in depressed patients and controls



Values are mean \pm SEM. CMI was infused between time 0 and +15min

(range): 40 (0-60) in controls versus 5 (0-70) in patients; U=39, $p>0.05$).

(iv) Cardiovascular Responses

ANOVA revealed significant changes in pulse, diastolic and systolic BP over time (Time - **pulse**: $F=9.52$, $df=7,147$, $p=0.0001$; **diastolic BP**: $F=4.68$, $df=7,147$, $p=0.0001$; **systolic BP**: $F=4.63$, $df=7,147$, $p=0.0001$). Inspection of the results showed that CMI infusion decreased pulse rate and increased both diastolic and systolic BP (data not shown). There were no overall differences between controls and patients in the 3 measures (Group - **pulse**: $F=0.89$, $df=1,21$, $p=0.36$; **diastolic BP**: $F=1.21$, $df=1,21$, $p=0.28$; **systolic BP**: $F=0.65$, $df=1,21$, $p=0.43$) and no significant differences at specific time points (Group x Time - **pulse**: $F=1.35$, $df=7,147$, $p=0.23$; **diastolic BP**: $F=1.66$, $df=7,147$, $p=0.12$; **systolic BP**: $F=1.48$, $df=7,147$, $p=0.18$).

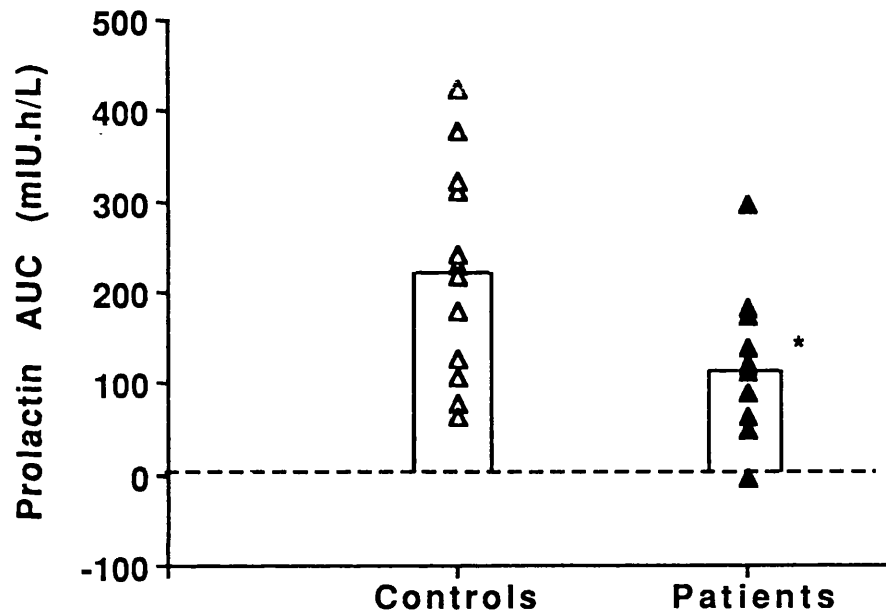
7.3.2 Low-Dose TRH Test

(i) Prolactin Response

The pattern of PRL responses to TRH was essentially the same as seen with CMI infusion. Patients had significantly smaller PRL responses to TRH infusion than controls (Figure 7.3, Table 7.3). If men and women were considered separately, the male depressives did not have significantly lower responses compared to male controls (mean \pm SD: 102.8 ± 69.4 versus 160.0 ± 67.3 mIU.h/L, $t=1.4477$, $df=10$,

Figure 7.3

Prolactin responses to low-dose TRH infusion in depressed patients and controls



Individual and mean PRL responses are shown

Groups compared using unpaired t-test

* $p < 0.02$ versus controls

Table 7.3

The prolactin response to low-dose TRH in depressed patients and controls

Subjects	N	TRH AUC (mIU.h/L) ^a Mean ± SD
Controls	12	222.8 ± 118.2
Patients	12	119.3 ± 76.2*
Melancholia-	9	139.1 ± 72.7 ⁺
Melancholia+	3	59.9 ± 61.3**
Suicide-	7	150.3 ± 69.8
Suicide+	5	76.0 ± 68.0**
Weight Loss-	7	133.0 ± 81.8 ⁺
Weight Loss+	5	100.2 ± 71.6*

- and + indicate absence and presence respectively of feature
Responses compared using unpaired t-tests.

+ p<0.07
* p<0.025
** p<0.01 } versus Controls

a PRL response to low-dose TRH infusion (area under curve minus baseline (+90min))

p=0.17) whereas the response was significantly lower in female depressives compared to female controls (mean \pm SD: 135.8 \pm 85.4 versus 285.7 \pm 129.2 mIU.h/L, t=2.310, df=10, p=0.04) . When patients were divided into presence and absence of melancholia, suicide attempt and weight loss, the group with each of these factors had a lower mean PRL response than the group without (Table 7.3). This difference was not statistically significant though a trend was present for both melancholia and suicide attempt (both p<0.1). Baseline PRL concentrations (+90min) did not differ between patients and controls (199 \pm 117 mIU/L versus 201 \pm 115 mIU/L, t=0.0459, df=22, p=0.96)

(ii) Side Effects

The low-dose TRH infusion was extremely well tolerated. Very mild, fleeting sensations of flushing, tight chest or nausea were experienced by some subjects.

7.3.3 Correlations

Correlations are summarised in Table 7.4. In the subjects as a whole there was a significant correlation between the PRL responses to CMI and low-dose TRH infusion which was also apparent in the patients alone but not the controls. The PRL response to CMI infusion did not correlate with measures of severity of depression but there was a trend to a negative correlation with weight loss in the past month. In contrast the PRL response to TRH did

Table 7.4

Correlations between the prolactin responses to clomipramine, low-dose TRH and other variables

	CMI AUC ^a	Baseline PRL	TAUC ^b	DNausea	HAMD	BDI	Weight Loss ^c
<u>CMI AUC^a</u>		<u>(Time 0)</u>					
All		0.05	0.07	0.26			
Controls		0.34	0.01	-0.41			
Patients		0.10	0.24	0.41	-0.38	-0.05	-0.53 ⁺
<u>TRH AUC^d</u>		<u>(Time +90)</u>					
All	0.56**	0.23					
Controls	0.19	0.35					
Patients	0.58 ^Δ	0.09			-0.58*	-0.30	0.05

Correlations performed using Spearman's rho

- + p<0.08
- Δ p<0.06
- * p<0.05
- ** p<0.01

- a PRL response to CMI infusion (area under curve minus baseline)
- b Plasma CMI after CMI infusion (area under curve)
- c Estimated absolute weight loss in last month
- d PRL response to TRH infusion (area under curve minus baseline)

not correlate with weight loss but did show a modest negative correlation with severity of depression as measured by the HAMD. There were no significant correlations with anxiety as measured by the HAMA or with Newcastle Scale scores ($p > 0.1$).

7.4 Discussion

The main findings in this study were that depressed patients showed blunted PRL responses to both CMI and TRH infusion. In each case there was a similar pattern of lower responses in depressed patients with a diagnosis of melancholia, suicide attempt or weight loss compared to those without these features.

The finding of a reduced PRL response to CMI infusion in depressed patients adds further weight to the now considerable evidence for reduced 5-HT-mediated PRL secretion in depression (see 1.8.4). Although the melancholic subgroup was small, I found that it had the lowest PRL responses but I was not able to demonstrate a correlation between Newcastle Scale scores and PRL responses. Six other studies have looked at the relationship between melancholia/endogeneity and PRL response; three find an association with blunting (Cowen and Charig, 1987; Lopez-Ibor et al, 1989; Mitchell and Smythe, 1990), one no association (but patients with weight loss were excluded (Deakin et al, 1990) and two an association

with non-blunting (O'Keane and Dinan, 1991; Price et al, 1991). As discussed in 11.3.3, DSM-III melancholia and Newcastle Scale endogeneity differ in important ways and identify different patients (although there is overlap) and this may partly explain the apparent discrepancies in results. The association of melancholia with blunting in this study is unlikely to be simply a reflection of severity of depression as there was no correlation between HAMD score and PRL response to CMI. It is of interest in this context that there is also evidence for decreased plasma availability of the 5-HT precursor, TRP, in melancholic compared to non-melancholic patients (Anderson et al, 1990).

In this group of depressed patients my findings are consistent with those of Coccaro and colleagues (1989) who found reduced fenfluramine-stimulated PRL responses in patients who had attempted suicide. In contrast two further studies using fenfluramine have found either no association with suicidal thoughts or acts (O'Keane and Dinan, 1991) or a weak positive correlation between the PRL response and suicidal ideation (Mitchell et al, 1990). The present study therefore adds support to there being an association between reduced 5-HT function and suicide but overall the evidence from 5-HT neuroendocrine studies in depression is inconsistent at present.

The finding that depressed patients with weight loss have reduced CMI-stimulated PRL responses compared with

those without weight loss needs discussion in view of the evidence I have presented that weight loss alters the PRL responses to TRP in normal female dieters (Chapter 4) and female depressed patients (Cowen and Charig, 1987; Deakin et al, 1990) without, however, any apparent effect in men. This raises the question as to whether the blunted PRL response to CMI infusion in this study is a consequence of weight loss, and not directly related to depression. Although this interpretation cannot be conclusively excluded at present I think it is unlikely to be the whole explanation of the results, first because depressed patients without significant weight loss did have lower PRL responses than controls although this failed to reach statistical significance (perhaps because of the small numbers), second, because there is a significant blunting of the PRL response in male depressives whereas in neither my study (Chapter 4) nor that of Goodwin and colleagues (1987b) did weight loss alter TRP-stimulated PRL secretion in men. Consequently my favoured explanation for the association between weight loss and blunted PRL responses in this study is that weight loss is linked to melancholia and biological symptoms of depression and it is these which are related to lowered 5-HT function (also a similar situation to that found with plasma TRP in depressed patients, Anderson et al, 1990). Unfortunately the numbers are too small to be able to address this question by statistical methods.

The finding that the PRL responses to both CMI and low-dose TRH are blunted raises the possibility that the blunted PRL responses to 5-HT challenge widely reported in depression (see 1.8.4 and Table 1.8) are a consequence of abnormal PRL secretion rather than an abnormality in 5-HT function. Indeed I found that the PRL responses to CMI and TRH correlated, particularly in depressed patients, suggesting a common mechanism underlying the abnormality. However before accepting this conclusion a methodological difficulty presents itself - the test protocol, involving the low-dose TRH test following the CMI infusion test, means that it is not possible to exclude an interaction between CMI and TRH, especially as there is evidence from animal work for an interaction between 5-HT and TRH (see Chapter 8 for discussion). For this reason a normal volunteer study was undertaken in order to investigate a possible interaction and this is described in Chapter 8).

As has been discussed in 6.4, stressful side-effects may cause PRL secretion through a non-specific mechanism. More controls than patients did experience some degree of nausea and this explanation for the difference in PRL response needs to be considered. Against this interpretation, however, the degree of nausea was generally mild, was not experienced as stressful, and there was no correlation between DNausea rating and PRL response to CMI and no significant difference in DNausea ratings between depressed patients and controls. In addition the blunting

of the PRL response to low-dose TRH in depressed patients cannot be simply explained by the presence of stressful side-effects during the CMI test and indicates that a different explanation must be sought.

Plasma CMI concentrations following CMI infusion were nearly identical in patients and controls making it unlikely that a difference in CMI availability is the explanation for the difference in PRL responses.

The stage of menstrual cycle at which female patients and controls were tested was not ideally controlled for in this study (Table 7.1) and there is evidence that PRL responses to drug challenge may differ through the menstrual cycle (Buckman et al, 1976), an effect believed to be due to the modulatory effect of oestrogen on PRL secretion (Buckman et al, 1976; Ojeda et al, 1977). However the fact that male patients demonstrated blunted PRL responses indicates that this cannot account for the findings.

The present study confirmed the findings of the normal volunteer study with regard to the cardiovascular effects of CMI infusion (see Chapter 6). This may be a peripheral effect of CMI and could in part be attributable to its 5-HT uptake inhibitor properties (see Chapter 6). If this is the case then it suggests that controls and depressed patients do not differ in peripheral 5-HT function as the two groups did not differ in their cardiovascular responses.

7.5 Conclusions

This study adds to the growing evidence for an abnormality in 5-HT-mediated PRL secretion in depressed patients. In spite of small numbers it also provides support for previous findings suggesting that patients with melancholic depression and suicide attempts have particularly low PRL responses. Weight loss remains a potential confound in the interpretation of these results, although, for the reasons discussed above, I believe it is unlikely to wholly account for the abnormality.

In addition, the blunting of the PRL response to low-dose TRH challenge raises an important question about the cause of the abnormal PRL response - is it due to an alteration in PRL secretion rather than in 5-HT function? As has been discussed the methodology of this study prevents a firm conclusion being drawn but the question is addressed in Studies 5 and 6 (Chapters 8 and 9).

CHAPTER 8

Study 5: The Effect of Clomipramine Pretreatment on the
Prolactin Response to TRH.

8.1 Introduction

In Chapter 7 I demonstrated that the submaximal PRL response to a low dose of TRH was blunted in depressed patients and, furthermore, that it correlated with the PRL response to CMI in the same patients. One interpretation of these data is that PRL release is itself impaired in depression, but the methodology of the study did not exclude an alternative explanation, that of an interaction between the previously administered CMI and subsequent TRH infusion. Another possible explanation for the result is that 5-HT is involved in the PRL response to low-dose TRH (see discussion below).

Because of the importance of this result, I investigated the effect of CMI pretreatment on the submaximal and maximal PRL responses to TRH in normal volunteers.

8.2 Methods

8.2.1 Subjects

Six normal male volunteers were recruited as described in General Methods (Chapter 3). Mean age (range) was 33.5 (30-38) years and mean weight (range) was 70.0 (54-80) kg.

8.2.2 Neuroendocrine Testing

Neuroendocrine testing was carried out following the

general protocol described in General Methods (Chapter 3).

Volunteers received two TRH tests preceded by either oral CMI 20mg or matching placebo capsules. The tests were separated by one week and administered in a balanced order with the subject blind to the condition. Maximum plasma CMI concentration following oral administration occurs at between 2-3 hours with minimal levels of the the metabolite, DCMI, occurring at this time (Luscombe, 1979). The dose and timing of CMI administration in this study was designed to result in plasma CMI concentrations comparable to those resulting from the intravenous dose used in the depressed patient study (Luscombe, 1979) and we have previously used a similar protocol to investigate the effect of CMI pretreatment on the PRL response to TRP infusion (Anderson and Cowen, 1986). This protocol does not alter basal PRL levels (Anderson and Cowen, 1986). Subjects took CMI or placebo at 08.00h and the intravenous cannula was inserted at 09.00h. Following a 50-minute rest period, two baseline blood samples were taken at 10-minute intervals and 6.25ug (low-dose) TRH was administered intravenously over 10 seconds at 10.00h. Three blood samples were taken at 10-minute interval until +30min, following which, 150ug (high-dose) TRH was infused over 10 seconds and 10-minute blood sampling continued until +60min.

The TRH infusions were prepared as described in General Methods (Chapter 3). These two doses (6.25ug and 150ug) of

TRH produce half-maximal and maximal PRL responses respectively (Jacobs et al, 1973).

Side-effects are generally mild and fleeting after TRH and visual analogue ratings were not administered but subjects were asked about any adverse reactions at the end of the test.

Plasma PRL levels were determined as outlined in General Methods (Chapter 3).

8.2.3 Analysis of Results

The PRL response to 6.25ug TRH was analysed as AUC from time 0 to +30min with subtraction of the time 0 value (AUC_{0-30}); the response to 150ug TRH was analysed as AUC from +30min to +60min with subtraction of the +30min value (AUC_{30-60}). Values on the two occasions were compared using two-tailed paired t-tests.

8.3 Results

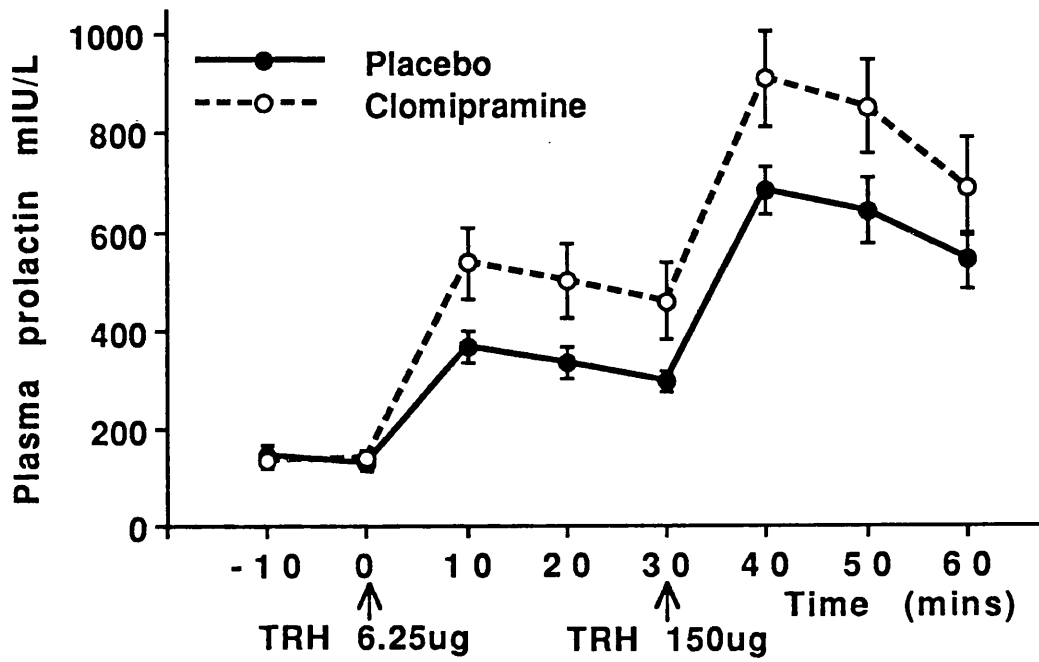
All subjects tolerated the tests well with 5 out of 6 reporting the usual effects of mild, fleeting flushing and nausea. No obvious difference in these effects between the two tests were reported after TRH 6.25ug but three subjects reported an increased intensity of nausea with the combination of CMI and TRH 150ug.

The results are shown in Figures 8.1, 8.2 and Table 8.1. CMI pretreatment enhanced the PRL response to TRH

Figure 8.1

The effect of clomipramine pretreatment on the prolactin responses to low- and high-dose TRH infusion in six male volunteers

a) Plasma prolactin levels following TRH

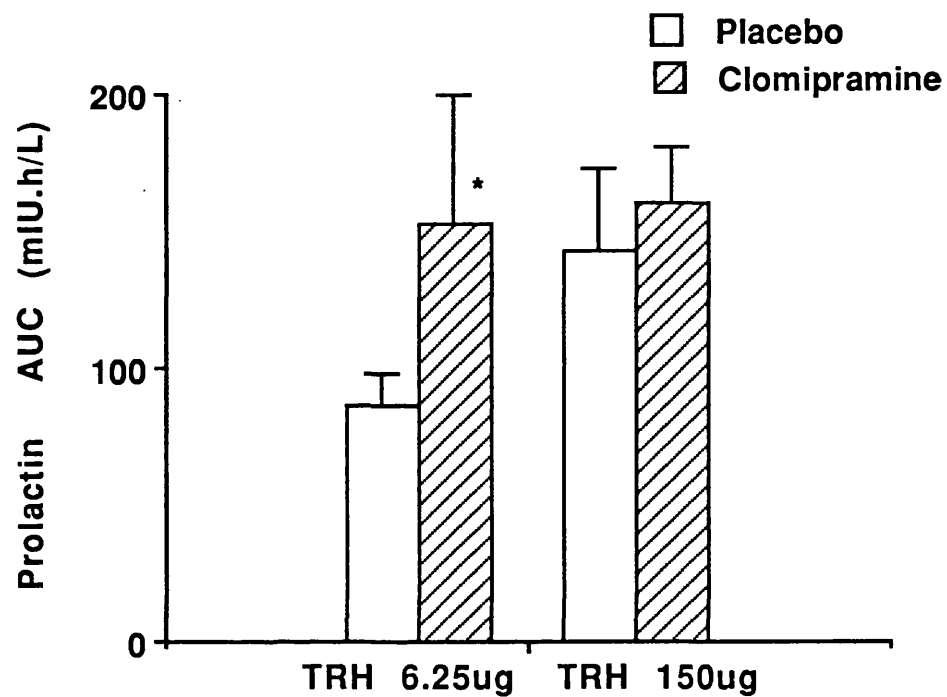


Values are mean \pm SEM

Figure 8.2

The effect of clomipramine pretreatment on the prolactin responses to low- and high-dose TRH infusion in six male volunteers

b) Prolactin responses (AUC) to low- and high-dose TRH



Values are mean \pm SEM

Occasions compared using paired t-tests

* $p < 0.025$ versus placebo pretreatment

Table 8.1

The effect of clomipramine pretreatment on the prolactin response to low- and high-dose TRH in six male volunteers

Pretreatment	TRH 6.25 μ g		TRH 150 μ g	
	PRL time 0 (mIU/L)	PRL AUC ₀₋₃₀ ^a (mIU.h/L)	PRL +30min (mIU/L)	PRL AUC ₃₀₋₆₀ ^b (mIU.h/L)
Placebo	131 \pm 41	87 \pm 30	296 \pm 49	143 \pm 43
Clomipramine	141 \pm 33	153 \pm 72**	469 \pm 189*	160 \pm 33

Values are mean \pm SD

Responses compared using paired t-tests.

* p<0.05 } versus placebo
 ** p<0.025 }

- a PRL response to TRH infusion from time 0 to +30min (area under curve minus baseline)
 b PRL response to TRH infusion from +30min to +60min (area under curve minus baseline)

6.25ug but not to TRH 150ug. CMI pretreatment did not alter basal PRL levels (time 0).

8.4 Discussion

The main finding of this study was that CMI pretreatment enhanced the PRL response to TRH 6.25ug, a dose producing a submaximal stimulation of PRL secretion. No such enhancement was seen with TRH 150ug.

These data are consistent with CMI pretreatment increasing the sensitivity of pituitary lactotrophs to TRH stimulation without increasing the maximum response, ie a shift of the dose response curve to the left. Caution in adopting this interpretation is however warranted because the PRL levels at +30min were significantly higher in the CMI-pretreatment condition and this could have influenced the PRL response to high-dose TRH. Evidence is conflicting as to the expected effect of a raised PRL baseline on the PRL response to stimulation. PRL may inhibit its own secretion through a short-loop positive feedback which induces hypothalamic DA activity (McCann et al, 1984; Tuomisto and Mannisto, 1985); this would be expected to suppress the PRL response. In contrast, elevated PRL levels due to DA blockade have been shown to lead to enhanced PRL responses to high-dose TRH (Loosen et al, 1986) and I found a trend towards a positive correlation between PRL baseline and the PRL response to CMI 0.125mg/kg

(Chapter 6).

In apparent contradiction to my finding with the higher dose of TRH, chronic CMI treatment in depressed patients has been reported to enhance the PRL response to 400ug TRH (Langer et al, 1981). However two factors mean that the study by Langer and colleagues (1981) is not directly comparable to the present one: first, chronic administration of CMI is likely to have very different overall effects to acute, single dose administration, including effects on DA function (Garattini and Samanin, 1988); second, recovery from depression might have influenced the PRL response to TRH. To resolve this issue a separate study using only high-dose TRH stimulation of PRL is required.

The present study indicates that it is indeed very likely that an interaction occurred between the CMI and low-dose TRH infusions in Study 4 (Chapter 7). The six male control subjects in Study 4 (who had TRH 6.25ug following intravenous CMI) had PRL responses comparable to the those following CMI pretreatment in the current study (mean \pm SD: 160 \pm 67 vs 153 \pm 72 mIU.h/L); these were significantly higher than the responses following placebo pretreatment in this study (87 \pm 30 mIU.h/L, $t=2.4176$, $df=7$, $p<0.05$, unpaired t -test). Depressed men had intermediate PRL responses (103 \pm 69), rather closer to the placebo pretreatment condition.

An interesting question is raised about the nature of

the interaction between CMI and TRH and its implications for the use of TRH as a 'non-5-HT' stimulus of PRL secretion. There is now much evidence for an interaction between TRH and 5-HT in the CNS. Co-localisation occurs in the same neurone (see 1.5), 5-HT may bind to high affinity TRH receptors (Teshima et al, 1986) and TRH administration increases the number of 5-HT₁ receptors in the limbic forebrain and hippocampus of rats (Funatsu et al, 1985). TRH potentiates the 5-HT behavioural syndrome caused by tranylcypromine and TRP (Green and Grahame-Smith, 1974) and high dose TRH and the TRH analogue, MK-771 have been reported to increase 5-HT and 5-HIAA levels in rat brain areas including the hypothalamus (Rastogi et al, 1981) although very low-dose TRH appears to have the opposite effect (Grosman and Rubio, 1987). TRH and its analogues produce a behavioural syndrome similar (but not identical) to the 5-HT behavioural syndrome with some of the same pharmacological characteristics, suggesting 5-HT involvement (Bennett et al, 1987; Pranzatelli, 1988; Fone et al, 1989). Clearly any interaction between CMI and TRH to enhance the PRL response to the latter must occur at the level of the anterior pituitary and it is of interest that in rats 5-HT and TRH may interact in stimulating PRL secretion in isolated pituitary preparations (Apfelbaum, 1987); however the action of 5-HT antagonists on high-dose TRH-induced PRL secretion in humans has been inconsistent (Egge et al, 1977; Sartani et al, 1984).

The mechanism by which CMI enhances the PRL response to TRH is therefore not clear but may be through an enhancement of 5-HT function. This could occur at the level of the pituitary (Apfelbaum, 1987) or alternatively it is possible that CMI increases the 'tone' of another PRF such as VIP or PHI (McCann et al, 1984) which then interacts to augment the TRH stimulus to PRL (cf the synergism between CRF and vasopressin in ACTH secretion, Salata et al, 1988). Whatever the mechanism, there is the possibility of a '5-HT component' in the submaximal PRL response to TRH (which is not evident in the maximal response).

8.5 Conclusions

This normal volunteer study demonstrates that CMI pretreatment enhances the PRL response to low-dose TRH administration. This interaction is likely to have occurred in Study 4 (Chapter 7) making it impossible to draw conclusions about PRL secretion itself from that study.

One interpretation of the data in this study is that CMI pretreatment shifts the TRH-PRL dose response curve to the left, probably as a result of an interaction between TRH and increased 5-HT function at hypothalamic or pituitary level. It is also possible that there is a '5-HT component' to the PRL response to low-dose TRH although it

is not clear whether this is significant when TRH is used alone. It would be of interest to examine the effect of specific 5-HT antagonists on the PRL response to low-dose TRH to clarify this matter. Until this has been done the use of TRH to stimulate submaximal PRL secretion needs to be tempered with caution when investigating conditions, such as depression, in which 5-HT function is believed to be abnormal.

CHAPTER 9

Study 6: The Prolactin Response to Low-Dose Metoclopramide
Infusion in Depressed Patients and Controls

9.1 Introduction

Following on from Studies 4 and 5 (Chapters 7 and 8) there remains the question as to whether PRL secretion itself is abnormal in depressed patients. As discussed in Chapter 5 (5.4), the major inhibitory influence on PRL secretion is DA, which is believed to be prolactin inhibitory factor (PIF) (Tuomisto and Mannisto, 1985). PRL secretion can be stimulated by the administration of DA antagonists (Tuomisto and Mannisto, 1985; McCallum et al, 1976) and therefore DA antagonist challenge provides an alternative strategy to TRH administration for the assessment of PRL secretion. One study found an enhanced PRL response to the DA antagonist, MCP, in patients during a depressive episode compared to when euthymic (Joyce et al, 1987), but the dose used (10mg) provided a supramaximal stimulus to PRL secretion which does not allow comparison with the submaximal PRL response to CMI infusion (see 1.8.4).

In order to assess PRL secretion in depressed patients using a DA antagonist challenge I therefore adopted a low-dose strategy and tested depressed patients and controls using 0.3mg MCP which stimulates a submaximal hormonal response (see Chapter 5).

9.2 Methods

9.2.1 Subjects

Twelve depressed patients were recruited and assessed as described in General Methods (Chapter 3). Patient details are given in Table 9.1. There were seven inpatients, five patients (three males, two females) reached criteria for DSM III melancholia, three (one female, two males) had endogenous depression as measured by the Newcastle Scale (Carney et al, 1965) and six (two males, four females) had lost more than 5lb in weight in the preceding month. Three patients (two males, one female) had made suicide attempts. Three patients had also participated in Study 2 (Chapter 5) and had had combined CMI and TRH infusion tests on the previous day. Antidepressant drug status is given in Table 9.1; in addition one patient had stopped lithium and temazepam two weeks before testing and one patient had had a single dose of temazepam the day before testing.

The patients were compared to age, sex and weight matched controls (see Table 9.1).

9.2.2 Neuroendocrine Testing

Neuroendocrine testing followed the general protocol as described in General Methods (Chapter 3). The low dose of MCP used (0.3mg) produces submaximal PRL responses in normal volunteers (see Chapter 5).

Table 9.1

Details of depressed patients and controls in the low-dose metoclopramide study

	Controls	Patients
Sex (M:F)	7:5	7:5
Age (years)	40.8 ± 12.1	40.9 ± 11.5
Weight (kg)	70.5 ± 10.3	69.8 ± 11.9
HAMD (21 item)		26.9 ± 5.6
BDI		33.3 ± 12.1
Newcastle Score		4.8 ± 1.2
HAMA		21.1 ± 7.6
Drug Free Period		
Never		5
3+ Months		5
3 Weeks-3 Months		2
Menstrual Stage		
Follicular	1	3
Mid Cycle	-	-
Luteal	-	-
Irregular/Unknown	3	-
Contraceptive Pill	-	-
Post Menopausal	1	2

Values are mean ± SD

Plasma PRL was estimated as described in General Methods (Chapter 3).

9.2.3 Analysis of Results

The PRL response to MCP infusion was analysed as the AUC (MCP AUC) as described in General Methods (Chapter 3). The results were not normally distributed and were compared using Mann Whitney U tests (U). The median difference between the two groups and 95% confidence interval (95% CI) were calculated as described by Campbell and Gardner (1988). Baseline PRL concentrations were compared using unpaired t-tests (t). Correlations were performed using Spearman's rank correlation test (ρ).

9.3 Results

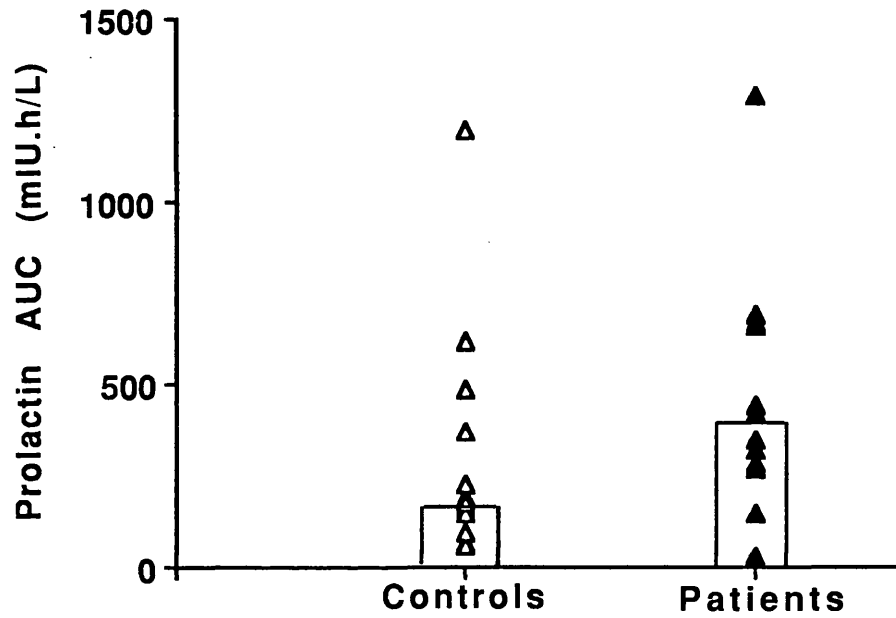
The MCP test was extremely well tolerated with no subject experiencing any side-effects.

9.3.1 Prolactin Response

There were no significant differences between the PRL responses in depressed patients compared to controls although the patient group had a higher median response (Figure 9.1, Table 9.2). This was also the case if men and women were considered separately (men: 287 (32 to 695) in patients versus 186 (147 to 618) in controls, $U=20$, $p>0.05$; women: 423 (318 to 1292) in patients versus 95 (57 to 1196)

* Figure 9.1

Prolactin responses to low-dose metoclopramide infusion in depressed patients and controls



Individual and median PRL responses are shown

Table 9.2

The prolactin response to low-dose metoclopramide infusion in depressed patients and controls

Subjects	N	MCP AUC (mIU.h/L) ^a Median (Range)	Difference Median (95% CI)
Controls	12	180.5 (57 to 1196)	172.5 (-29 to 323)
Patients	12	386.5 (32 to 1292)	
Melancholia-	9	443 (146 to 1292)	
Melancholia+	3	318 (32 to 695)	
Suicide-	8	334 (146 to 667)	
Suicide+	4	569 (32 to 1292)	
Weight Loss-	7	665 (273 to 1292)	
Weight Loss+	5	334 (32 to 443)	

- and + indicate absence and presence respectively of feature

Responses compared using Mann Whitney U tests.

a PRL response to MCP infusion (area under curve minus baseline)

in controls, $U=6$, $p>0.05$). Analysis of patients' results according to presence or absence of melancholia, suicide attempt or weight loss did not reveal any significant differences between subgroups or compared to controls (Table 7.2). Baseline PRL concentration (time 0) did not differ between patients and controls (186 ± 111 versus 190 ± 106 mIU/L, $t=0.083$, $df=22$, $p=0.93$).

9.3.2 Correlations

There were no significant correlations between MCP AUC and severity of depression (as measured by HAMD or BDI), anxiety (HAMA), Newcastle Scale scores or weight loss (all $p>0.2$).

9.4 Discussion

This study found that depressed patients and controls did not differ significantly in their PRL responses to a low-dose challenge by the DA antagonist, MCP.

As has been discussed in Chapter 5, MCP is a DA antagonist (Peringer et al, 1976) and raises plasma PRL levels in humans (McCallum et al, 1976) through DA mechanisms (McCallum et al, 1976; Quigley et al, 1980). Submaximal PRL responses were achieved through the use of a low dose of MCP (about 5ug/kg) in order to be comparable to responses to 5-HT-drug challenge.

This study was carried out to investigate the

possibility that depressed patients have a general blunting of PRL release. Although no blunting of DA-mediated PRL release was demonstrated it has to be acknowledged that the number of subjects was small and a true difference between patients and controls could have been missed (ie a type II error). However the 95% CI for the median difference between the two groups indicates that it is unlikely that this study has missed a 'true' blunting of the PRL response in depressed patients of the degree seen in the CMI and low-dose TRH studies (a possible 17% compared to about 50%). In contrast, it is possible that a substantial 'true' increase in the PRL response of about 180% was not detected at this level of confidence. In light of this it is therefore interesting that a study using a high-dose MCP strategy found enhanced PRL responses when patients were depressed compared to when they were euthymic (Joyce et al, 1987).

9.5 Conclusions

From the results in this study, blunted PRL responses to 5-HT-drug challenge found in depressed patients cannot simply be explained by a general reduction in PRL release by the pituitary or by an abnormality in DA inhibition of PRL release.

It must be acknowledged however that the mechanisms by which a PRF such as TRH, and a DA antagonist such as MCP

cause PRL release from the pituitary are different, with separate receptors and second messengers in the pituitary lactotroph (Tuomisto and Mannisto, 1985; Gershengorn 1986). Even if DA-mediated PRL release is normal in depression it is still possible that PRL release produced by a PRF could be impaired. In a study carried out in our Unit subsequent to those described in this thesis, we have shown that the PRL response to TRH is not blunted in depressed patients (Anderson et al, 1992). This supports my suggestion that the results in the depressed patient study in Chapter 7 are confounded by an interaction between CMI and TRH. However these results do not exclude an abnormality in the PRL release to other PRFs (eg VIP or PHI).

CHAPTER 10

Study 7: The Effects of Gepirone on Neuroendocrine Function
and Temperature in Male Volunteers

10.1 Introduction

The azapirone gepirone is an analogue of the non-benzodiazepine anxiolytic, buspirone, and like buspirone demonstrates a high affinity for brain 5-HT_{1A} receptors in ligand binding studies (Peroutka, 1985; Hamon et al, 1986). However, gepirone differs from buspirone in having minimal activity at dopamine receptors (McMillen and Mattiace, 1983; Eison et al, 1985) and appears to be a selective 5-HT_{1A} receptor agonist.

Electrophysiological studies indicate that gepirone mimics the inhibitory effects of 5-HT on postsynaptic 5-HT_{1A} receptors in the rat hippocampus and on presynaptic 5-HT_{1A} receptors on raphe cell bodies (Traber and Glaser, 1987; Sprouse and Aghajanian, 1987; Blier and de Montigny, 1987). In behavioural studies gepirone reduces body temperature in rodents (Green and Goodwin, 1987; Koenig et al, 1988); in the mouse this effect is probably mediated by stimulation of presynaptic 5-HT receptors (Green and Goodwin, 1987). High doses of gepirone to rats induce a 5-HT-mediated behavioural syndrome probably through activation of post-synaptic 5-HT_{1A} receptors (Eison et al, 1986).

As discussed in Chapter 2 there is considerable evidence in animals and humans that 5-HT is involved in the secretion of PRL and ACTH. In the present study I used gepirone to assess the role of 5-HT_{1A} receptors in the

control of neuroendocrine function and temperature in humans.

10.2 Methods

10.2.1 Subjects and Drug Administration

Twelve healthy male volunteers (mean age 26.8 years, range 22-32 years; mean weight 72.9kg, range 60.5-81.8kg) were recruited as described in General Methods (Chapter 3). They each received the following three oral drug treatments in a single blind balanced order design: a) placebo; b) gepirone 10mg; c) gepirone 20mg. Medication consisted of two identical capsules on each occasion. The gap between any two treatments ranged from one to three weeks.

10.2.2 Neuroendocrine Testing

Neuroendocrine testing was carried out following the general protocol described in General Methods (Chapter 3). An intravenous cannula was inserted at 08.30h and gepirone or placebo capsules were administered at 09.30h (time 0). Blood samples were taken every 15 minutes from 30 minutes before (-30min) until 30 minutes (+30min) after drug administration, and then every 30 minutes until +180min.

Blood samples were taken into EDTA coated tubes on ice, centrifuged within 60 minutes and the plasma frozen immediately. Samples were stored at -30°C until assayed for PRL, GH, ACTH and cortisol as described in General

Methods (Chapter 3).

During each neuroendocrine test oral temperature was taken at 30-minute intervals from -30min by a glass mercury thermometer which remained in situ for ten minutes during each recording. Also at 30-minute intervals subjects completed 100mm visual analogue ratings for drowsiness, light-headedness and nausea (see General Methods, Chapter 3).

10.2.3 Statistical Analysis

Distributions of the hormone data and temperature were checked and those not approximating satisfactorily to normality were \log_{10} transformed. The data were analysed by a two way repeated measures ANOVA with Tukey's test as a post-hoc range test. For the purposes of subsequent correlations the hormone responses were also measured as the AUC. Maximum changes in visual analogue ratings from baseline (time 0) (DDrowsiness, DLight-headedness and DNausea) were compared by the Wilcoxon signed rank test and correlations carried out using Spearman's rank correlation (ρ).

10.3 Results

10.3.1 Hormone Responses

(i) Prolactin

Administration of gepirone significantly altered plasma

PRL concentration (Figure 10.1). The ANOVA on \log_{10} transformed data showed a significant effect of drug treatment ($F=5.12$; $df=2,22$; $p=0.0149$), a significant effect of time ($F=15.79$; $df=9,99$; $p=0.0001$) and a significant interaction between drug treatment and time ($F=2.69$; $df=2,22$; $p=0.0004$). Post-hoc testing showed that following gepirone 20mg, plasma PRL was significantly increased. In contrast after gepirone 10mg, PRL concentrations tended to decline more than placebo and at 180 mins were significantly lower (Figure 10.1).

(ii) Growth Hormone

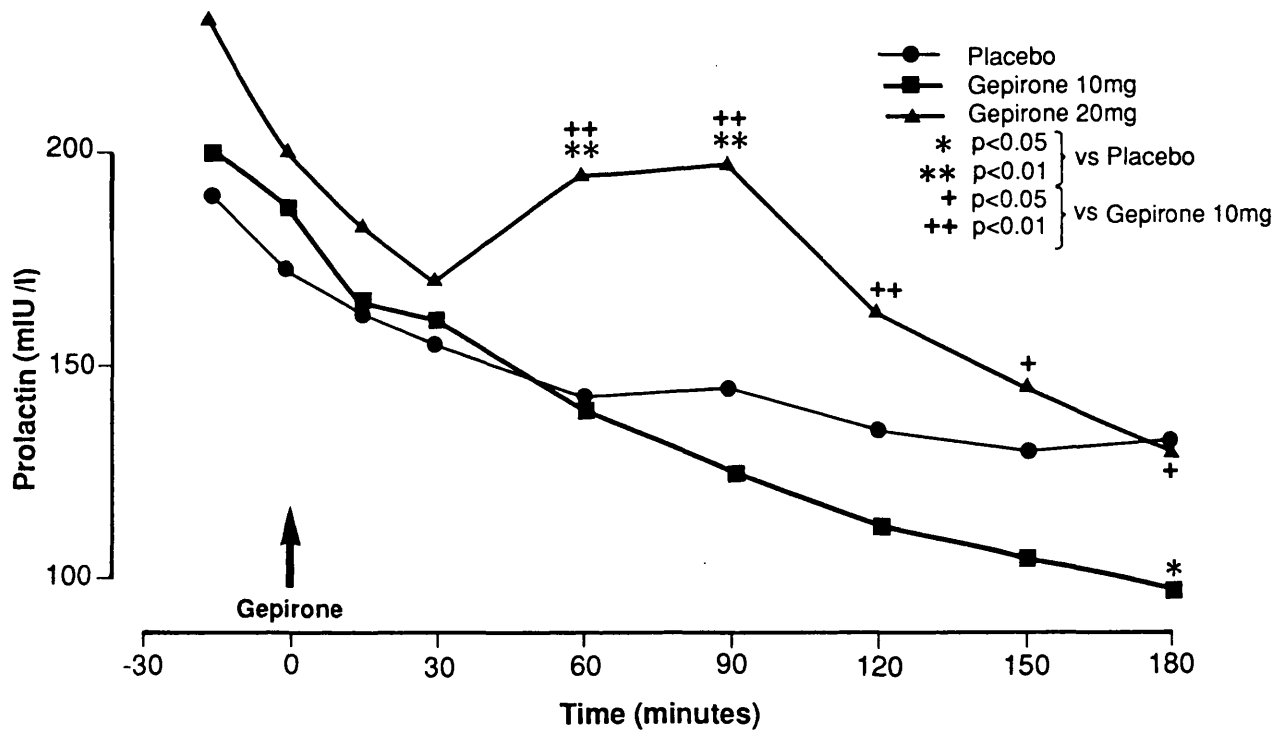
Three subjects had elevated baseline GH levels on at least one occasion and therefore results for nine subjects are presented. ANOVA on \log_{10} transformed values showed significant main effects of drug treatment ($F=7.8$; $df=2,16$; $p=0.0043$) and time ($F=8.66$; $df=10,80$; $p=0.0001$). There was also a significant interaction between drug treatment and time ($F=4.06$; $df=20,160$; $p=0.0001$) (Fig 2). Post-hoc testing showed that both doses of gepirone significantly elevated plasma GH levels with a larger and more sustained response from 20mg (Figure 10.2).

(iii) ACTH and Cortisol

Gepirone also increased plasma ACTH. The ANOVA showed significant effects of drug treatment ($F=4.58$; $df=2,22$; $p=0.027$) and time ($F=2.61$; $df=9,99$; $p=0.0094$) and a

Figure 10.1

Effects of gepirone and placebo on plasma prolactin in twelve male volunteers

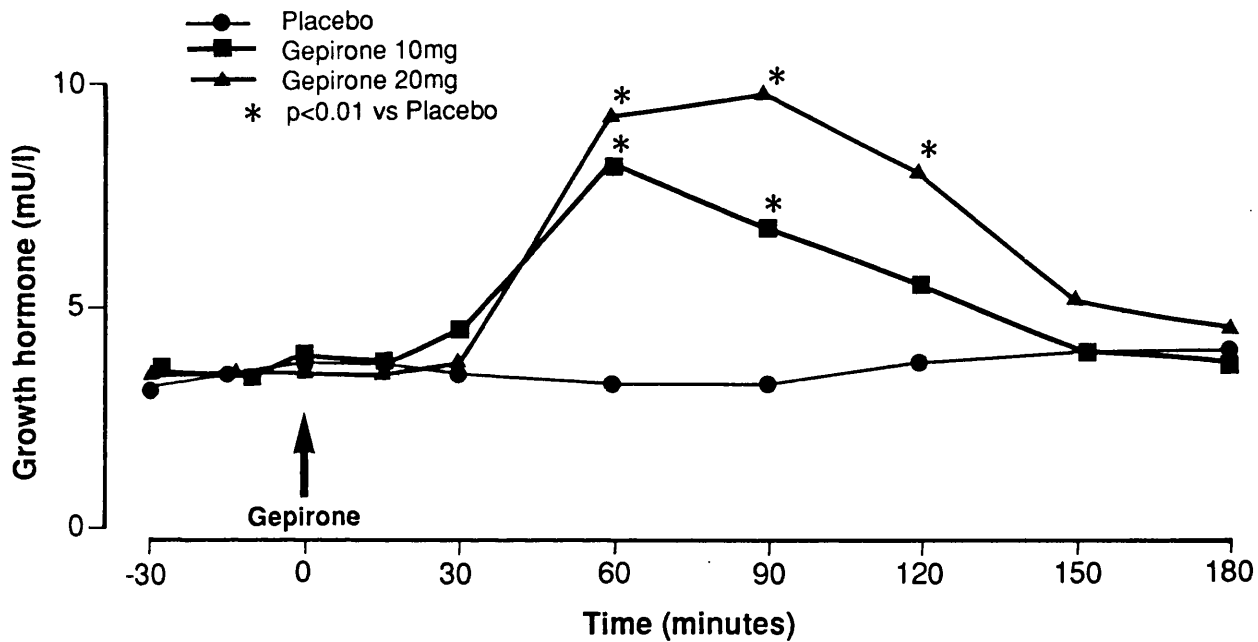


Values are geometric means

Subjects were tested on three occasions with each of the three treatments shown. There were significant differences between the treatments at the time points indicated (Tukey's test)

Figure 10.2

Effects of gepirone and placebo on plasma growth hormone in nine male volunteers



Values are geometric means

Subjects were tested on three occasions with each of the three treatments shown. There were significant differences between the treatments at the time points indicated (Tukey's test)

significant interaction between drug treatment and time ($F=2.7$; $df=18,198$; $p=0.0004$). Examination of the data revealed that gepirone 20mg significantly increased plasma ACTH compared to placebo but gepirone 10mg did not (Figure 10.3). Essentially similar findings were obtained for the effect of gepirone on plasma cortisol (\log_{10} transformed data)(drug effect: $F=7.17$; $df=14,140$; $p=0.0001$; time $F=4.46$; $df=9,99$; $p=0.0001$; drug x time $F= 2.75$, $df=18,198$; $p=0.0003$)(Figure 10.4).

10.3.2 Oral temperature

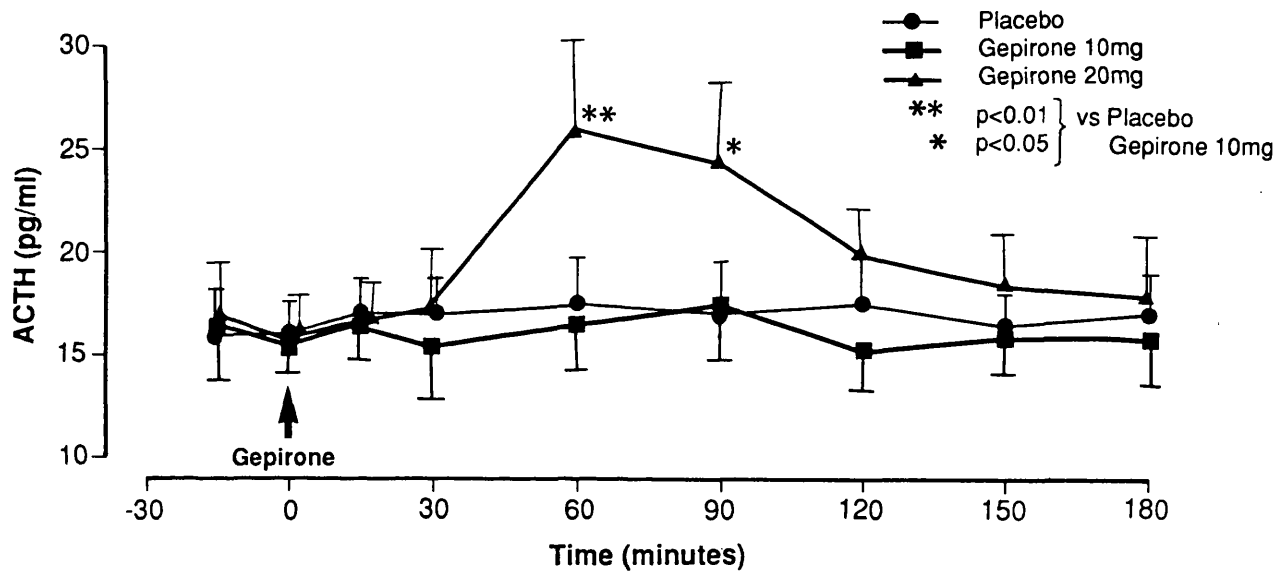
Gepirone significantly lowered oral temperature in the eleven subjects from whom data were available. There was a significant effect of drug treatment ($F=7.56$; $df=2,20$; $p=0.0036$) and time ($F=4.46$, $df=7,70$; $p=0.0004$). There was also a significant interaction between drug effect and time ($F= 4.52$; $df=14,140$; $p=0.0001$). Post-hoc analysis revealed that gepirone 20mg, produced a small but highly significant decrease in oral temperature (Figure 10.5).

10.3.3 Visual analogue ratings

Following gepirone 10mg there was a significant increase in ratings of light-headedness. Gepirone 20mg, was associated with significant but modest increases in feelings of nausea, drowsiness and light-headedness (Table 10.1).

Figure 10.3

Effects of gepirone and placebo on plasma ACTH in twelve male volunteers

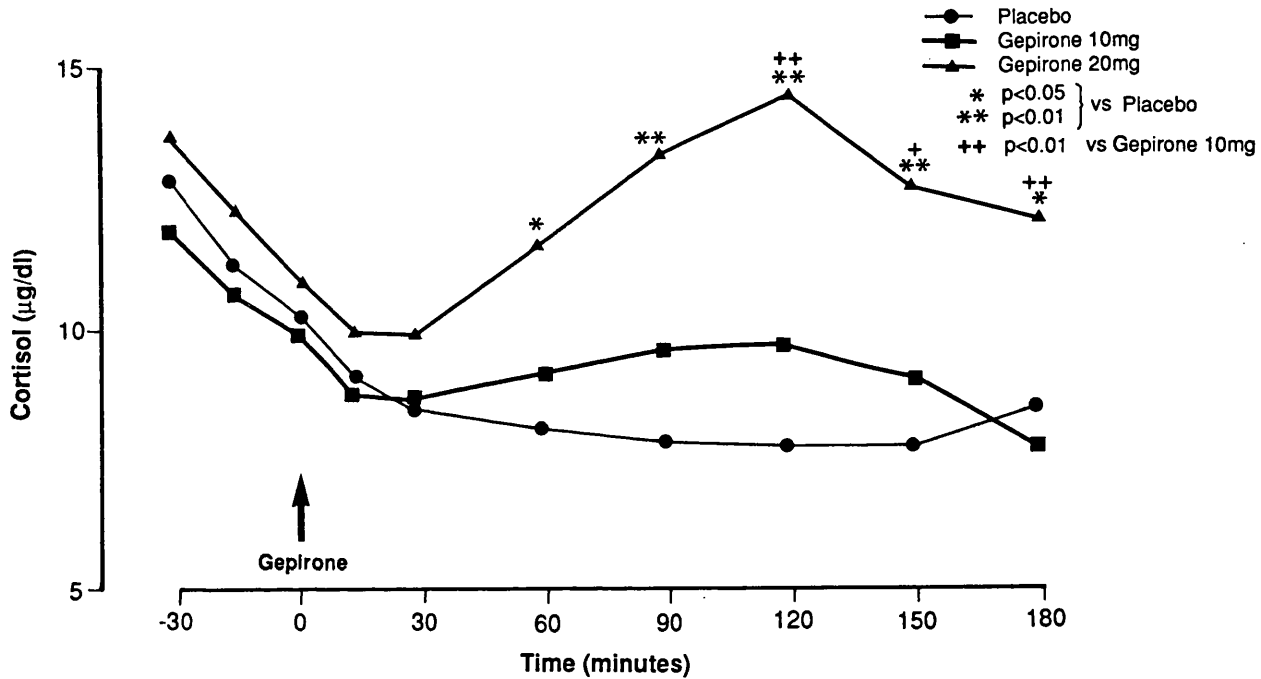


Values are mean \pm SEM

Subjects were tested on three occasions with each of the three treatments shown. There were significant differences between the treatments at the time points indicated (Tukey's test)

Figure 10.4

Effects of gepirone and placebo on plasma cortisol in twelve male volunteers

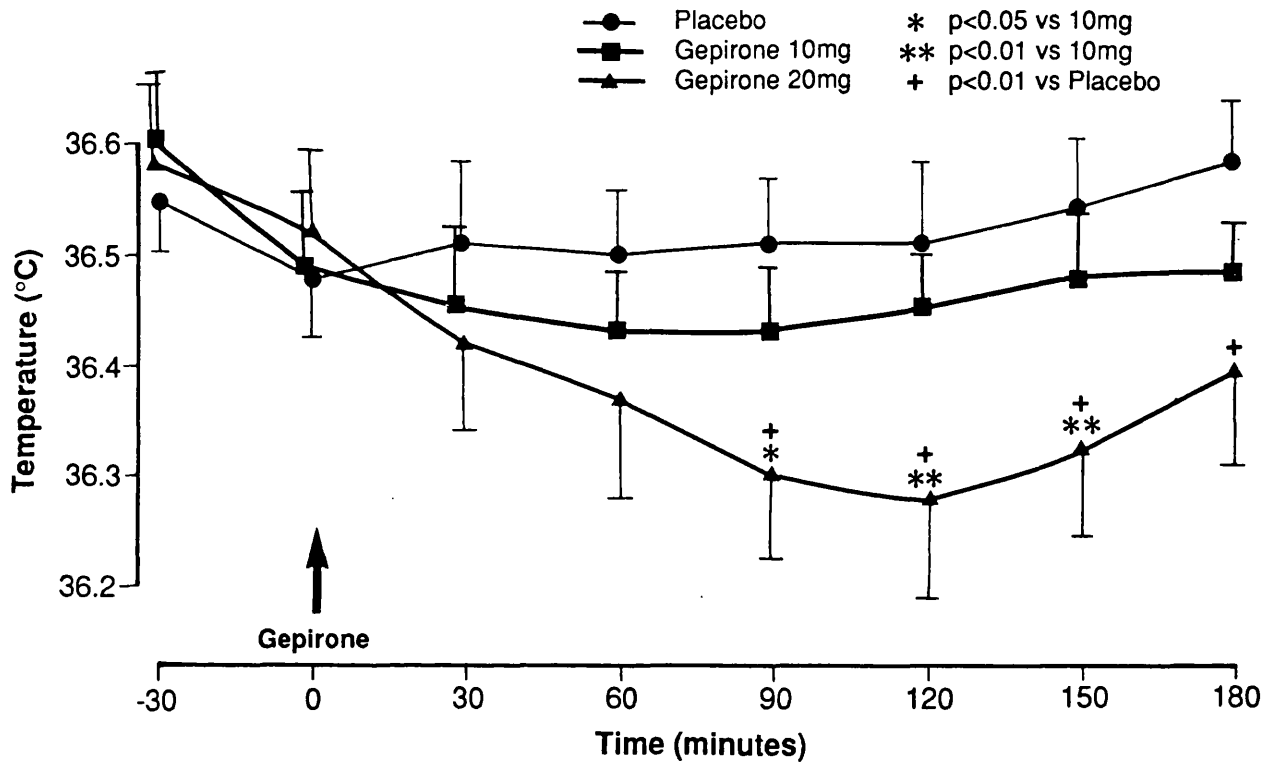


Values are geometric means

Subjects were tested on three occasions with each of the three treatments shown. There were significant differences between the treatments at the time points indicated (Tukey's test)

Figure 10.5

Effects of gepirone and placebo on oral temperature in eleven male volunteers



Values are mean \pm SEM

Subjects were tested on three occasions with each of the three treatments shown. There were significant differences between the treatments at the time points indicated (Tukey's test)

Table 10.1

Effect of gepirone on visual analogue ratings

	Placebo	Gepirone 10mg	Gepirone 20mg
DDrowsy	5 (0-20)	17.5 (0-50)	30 (10-70)*
DNausea	0 (0-0)	0 (0-5)	10 (0-40)* +
DLight-headed	0 (0-10)	10 (0-60)*	27.5 (0-70)*

Values are median (range)

* p<0.01 versus placebo

+ p<0.01 versus gepirone 10mg

10.3.4 Correlations

Following gepirone 10mg there was a single significant correlation between feelings of drowsiness and increase in plasma growth hormone ($\rho=0.73$; $p<0.02$). Correlations following gepirone 20mg are summarised in Table 10.2. There was a significant correlation between cortisol response and DNausea and the increase in plasma PRL correlated positively with the increases in ACTH and cortisol. There was also a significant correlation between DLight-headedness and DNausea. No other significant correlations were obtained.

10.4 Discussion

These results demonstrate significant effects of gepirone on neuroendocrine function and temperature in human subjects. It is likely that these effects are mediated by 5-HT_{1A} receptors because gepirone is a relatively selective ligand for this receptor subtype (Peroutka, 1985; Hamon et al 1986). Gepirone is metabolised to 1-(2-pyrimidinyl)-piperazine (1-PP) which has alpha₂-adrenoceptor properties (Bianchi and Garratini, 1988). However, an alpha₂-adrenoceptor antagonist would not be expected to increase plasma cortisol in normal human subjects (Tartar and Vigas, 1984), and rodent data suggest that the ability of 1-PP to increase plasma corticosterone is substantially less than that of gepirone (Matheson et

Table 10.2

Correlations between visual analogue ratings and hormone responses to gepirone 20mg

	DDrowsy	DNausea	DLight-headed	GH	PRL	ACTH	Cortisol
DDrowsy	-	-0.13	-0.01	0.14	-0.23	-0.28	-0.15
DNausea		-	0.61*	-0.48	0.48	0.33	0.63*
DLight-headed			-	-0.47	0.04	-0.08	0.30
GH				-	-0.28	-0.42	-0.05
PRL					-	0.73**	0.58*
ACTH						-	0.41

* p<0.05

** p<0.01

al, 1988). In addition, α_2 -adrenoceptor agonists rather than antagonists are associated with increases in plasma growth hormone (Checkley, 1980) and decreases in body temperature (Zacny, 1982; Glue and Nutt, 1988).

In the rat a number of 5-HT_{1A} agonists, including gepirone, elevate plasma ACTH and corticosterone, an effect antagonised by the 5-HT₁ receptor antagonist, pindolol (Gilbert et al 1988; Koenig et al 1987; 1988). The recent report that ipsapirone elevates plasma ACTH and cortisol in humans, and that this response is antagonised by pindolol (Lesch et al, 1989, 1990) taken together with this study indicates that 5-HT_{1A} receptors are likely to be involved in ACTH release in humans. However, the present data do not exclude a direct effect of gepirone at anterior pituitary level.

Administration of the 5-HT_{1A} agonist, 8-OH-DPAT, to rats increases plasma PRL, although this effect is somewhat inconsistent (Simonovic et al, 1984; Aulakh et al, 1988b; Van de Kar et al, 1989), at least in male rats (Carlsson and Eriksson, 1986). Similarly in humans, the picture is uncertain for while it is well recognised that buspirone elevates plasma PRL (Meltzer et al, 1983b; Seppala et al, 1986), ipsapirone does not (Lesch et al, 1989), even though they are both 5-HT_{1A} agonists. It has been suggested that buspirone elevates PRL by antagonism of pituitary DA₂ receptors (Meltzer et al, 1982a) and consistent with this I have recently shown that pindolol does not block this

response (Anderson and Cowen, 1992). This is unlikely to explain the ability of gepirone 20mg to stimulate PRL secretion in this study as gepirone does not appear to have significant postsynaptic DA effects (McMillen and Mattiace, 1983; Eison et al, 1985), indeed Nash and Meltzer (1989) have recently provided evidence for a DA₂ agonist-like effect of gepirone in lowering PRL secretion from rat anterior pituitary tissue. It is possible that the ability of gepirone, but not ipsapirone, to increase plasma PRL in humans is attributable to the fuller agonist action of gepirone at postsynaptic 5-HT_{1A} receptors (Traber and Glaser, 1987).

As a further complication there were indications that that plasma PRL concentrations may in fact have been lowered after gepirone 10mg. If this finding is confirmed it may reflect a DA₂ agonist action of gepirone as suggested by Nash and Meltzer (1989), although it is difficult to explain simply why the 20mg dose did not have this effect. An alternative explanation is that the predominant effect of low dose gepirone is to inhibit 5-HT neurotransmission through activation of 5-HT_{1A} autoreceptors, an effect which at higher doses is outweighed by direct stimulation of postsynaptic 5-HT_{1A} receptors mediating PRL release. Clearly further studies are needed to resolve this issue.

However, with the other hormones studied there was no evidence that gepirone produced a biphasic dose response

effect. Indeed both 10mg and 20mg doses of gepirone produced an elevation of plasma GH. This effect is opposite to that reported in rodents where administration of 8-OH-DPAT lowered plasma GH levels (Aulakh et al, 1988b). The role of 5-HT pathways in GH release is complex and has been difficult to establish (Tuomisto and Mannisto, 1985). However, Meltzer et al (1983b) reported that buspirone elevated plasma GH levels in human subjects, a finding which we have recently confirmed and shown to be blocked by pindolol which has 5-HT_{1A} antagonist properties (Anderson and Cowen, 1992). Ipsapirone has been shown to increase mean GH levels in male volunteers, although this was not statistically significant (Lesch et al, 1989). The small numbers of subjects in that study preclude any final conclusion about the ability of ipsapirone to stimulate GH release. My results showing a dose related stimulation of GH by gepirone taken together with the ability of pindolol to block the GH response to buspirone suggests that 5-HT_{1A} receptors are involved in this response although other explanations such as DA receptor mediation cannot be excluded until further antagonist studies are carried out.

Administration of 8-OH-DPAT to rodents lowers core temperature (Goodwin and Green, 1985). While there is strong evidence in the mouse that this effect is mediated by presynaptic 5-HT_{1A} receptors, probably located on raphe cell bodies, (Green and Goodwin, 1987) there is some disagreement as to whether this mechanism obtains in the

rat (Hjorth 1985; Green and Goodwin, 1987; Hutson et al 1987). It is however noteworthy that a recent investigation in rats found that direct injection of 8-OH-DPAT into the DRN elicited a hypothermic response which could be antagonised by pindolol (Hillegaart, 1991), supporting a presynaptic site of action. Despite the limitations of our temperature measurement the findings suggest that stimulation of 5-HT_{1A} receptors in humans may decrease body temperature. This raises the interesting possibility that reduction in temperature following gepirone could provide an index of presynaptic 5-HT_{1A} receptor function in humans.

Drugs that increase brain 5-HT function commonly produce nausea and light-headedness. As has previously been discussed (Chapters 2 and 6), these symptoms, if sufficiently pronounced, may lead to secretion of anterior pituitary hormones through 'stress' mechanisms, not necessarily involving 5-HT pathways. In the present study there were significant correlations between DLight-headedness and GH responses following gepirone 10mg and between DNausea and increases in plasma cortisol following gepirone 20mg with no significant positive correlations with PRL and ACTH responses. The ratings recorded were modest and in my experience unlikely on their own to account for significant endocrine responses. The GH response to gepirone 10mg, in particular, is extremely unlikely to be stress-related as there was no suggestion of

any stimulation of PRL, ACTH or cortisol secretion. This suggests that gepirone produced a correlated effect on GH secretion and light-headedness. In the case of the cortisol response to gepirone 20mg, however, the possibility exists that it is at least partly attributable to stress factors. Clearly antagonist studies are required to settle this issue.

10.5 Conclusions

In summary these findings suggest that 5-HT_{1A} receptors may be involved in the secretion of anterior pituitary hormones and in the control of temperature in humans. As discussed in 1.7 and 2.3 other 5-HT receptors are also believed to be involved in neuroendocrine and temperature changes. For example, in humans, administration of mCPP and MK-212, probable 5-HT_{1C/2} receptor agonists, increase plasma PRL and cortisol (Mueller et al, 1985; Lowy and Meltzer, 1988) and mCPP elevates body temperature (Mueller et al, 1985). The effects of mCPP are antagonised by metergoline which is consistent with 5-HT-mediation (Mueller et al, 1986), however it is difficult to reach firm conclusions about the role of different 5-HT receptor subtypes in neuroendocrine and temperature regulation in humans because of the lack of specificity of the agonists employed. Both mCPP and MK-212 have a significant affinity for other 5-HT receptor subtypes (Hoyer, 1988). Therefore

studies are needed to determine the effect of specific 5-HT receptor antagonists on the endocrine and temperature responses to gepirone; it will be of interest to determine whether activation of 5-HT_{1A} receptors is indeed the mechanism underlying these effects.

PART 3

CONCLUSIONS

CHAPTER 11

Summary and Discussion

11.1 Introduction

In the introductory chapter to this thesis I undertook a broad review of the current information and thinking about 5-HT systems in the brain before focussing down on the evidence for abnormal 5-HT function in depressed patients. I would like to raise two general points that can be drawn from that review before going on to discuss specific issues in the course of this chapter. First, we are only beginning to develop a concept of the role that 5-HT systems play in the normal brain and hence the nature of the link between 5-HT and a syndrome such as depression remains obscure; for example we do not know whether an alteration in 5-HT function is directly related to mood change, is a secondary phenomenon or even a reflection of a vulnerability factor. Second, the progress in understanding the complexity of central 5-HT systems, particularly the type and distribution of 5-HT receptor subtypes, means that we can no longer approach 5-HT function in the brain as a simple unitary entity. The complexity must be acknowledged and we need to devise ways of looking at the functioning of specific types of 5-HT receptors and particular parts of the brain.

The evidence that I have reviewed provides support in the most general way for an association between abnormal 5-HT function and depressive illness, but casts limited light on the nature of the relationship. 5-HT

neuroendocrine challenge tests, for all their limitations, uniquely (to date) provide knowledge about a specific brain region, the hypothalamic-pituitary axis, and are able to give an index of the functioning of a 5-HT neurone-synapse-effector unit. In the rest of this chapter I will draw together the findings from the investigations in this thesis. I have employed a neuroendocrine strategy to investigate brain 5-HT function in weight loss and depression, and explored the potential for measuring human 5-HT_{1A} receptor responsivity.

11.2 Weight Loss: Relationship to 5-HT Function and Depression

On the basis both of the frequent occurrence of weight loss in depressive illness, and the evidence for weight loss itself affecting 5-HT neuroendocrine measures, I investigated the effect of weight loss on the PRL response to TRP infusion in normal volunteers. This is one of the most investigated neuroendocrine measures of 5-HT function and has provided one of the most consistent findings in depressed patients, a blunting of the response. It is also a response which appears to be affected by weight loss in both normal and depressed subjects.

To summarise the findings of the dieting studies (Chapters 4 and 5), I demonstrated that moderate weight loss through calorie restriction enhanced the PRL response

to TRP in women but not men. There was also a fall in plasma total TRP and the TRP:BCAA ratio in both sexes although it was greater in women than men. A control study investigating PRL release to the DA antagonist, MCP, and to the direct, lactotroph stimulant, TRH, indicated that the alteration in 5-HT-mediated PRL release could not be explained by an alteration either in DA function or in the sensitivity of the pituitary to stimulation.

These results raise a number of questions, (i) where is the abnormality and why is it confined to women dieters, (ii) what implications do these findings have for dieting and for psychiatric disorders and (iii) how do they relate to studies of 5-HT function in depression?

11.2.1 Weight Loss and 5-HT Function

The finding of an enhanced PRL response to TRP infusion immediately leads one to ask if this does indeed indicate an abnormality in 5-HT function. As has been discussed in 2.1, an issue for neuroendocrine studies in general is that they are assessing the function of a system in which the monoamine neurone and synapse play but one part. The picture is further complicated by the use of a precursor challenge such as TRP which has to be actively transported across the BBB, taken up into 5-HT neurones and synthesised into 5-HT (see 1.4) because an alteration in the handling of TRP could also explain the result.

In trying to identify the site of the abnormality, the

control study I carried out in Chapter 6 rules out one major possibility, an alteration in PRL secretion itself. Beyond that the current information about dieting in normal volunteers does not allow any definite conclusions, however it is possible to look at the literature on anorexia nervosa where investigations of 5-HT have been carried out in patients who have severe weight loss through calorie restriction. Caution is warranted because the patients are clearly in a more extreme and chronic state of malnutrition with consequent metabolic and endocrine disturbance, usually have altered mood states and often the nutritional status is not clear - for example they may be gaining weight through refeeding. In low-weight anorexics neuroendocrine investigations, in contrast to the studies in dieters, report normal (Goodwin et al, 1989) or blunted (Brewerton et al, 1990) PRL responses to TRP infusion and blunted PRL responses to mCPP (Brewerton et al, 1987; 1990). CSF studies have tended to indicate reduced 5-HIAA at low weight which increases with weight gain (see review by Jimerson et al, 1990), suggesting reduced 5-HT turnover associated with low weight. Although the CSF findings would fit in with my suggestion of lowered 5-HT function in female dieters, the increased PRL responses in dieting and decreased/normal PRL responses in low-weight anorexia appear to conflict. Possible hormonal explanations for this, that low oestrogen or high cortisol levels found in anorexic patients might lead to reduced PRL secretion do

not seem to be supported in practice as PRL responses to TRH and sulpiride are in normal anorexia nervosa (Giusti et al, 1981). Further neuroendocrine investigations are clearly needed to test the hypothesis that an abnormality in brain 5-HT neurotransmission is involved in the increased PRL to TRP in female dieters. The measurement of hormone responses to indirect 5-HT agonists such as fenfluramine and CMI, which act on the presynaptic nerve terminal, would furnish information about overall 5-HT synaptic function and the use of directly acting postsynaptic agonists such as gepirone or mCPP would test the sensitivity of postsynaptic receptors. If it were possible to measure CSF 5-HT and 5-HIAA then I would predict lower levels after dieting (as found in anorexia nervosa) if 5-HT synthesis and turnover are indeed reduced.

If we approach the issue from the direction of the reduction in plasma TRP, the animal evidence clearly shows that reduced availability of TRP does lead to decreased brain 5-HT turnover and enhanced endocrine responses to precursor challenge (Gil-Ad et al, 1976; Clemens et al, 1980). Most importantly, modest reduction in plasma TRP in non-dieting humans has been shown to cause enhanced PRL responses to TRP challenge (Delgado et al, 1989), strongly supporting my proposal that this is the mechanism that occurred in the female dieters. I believe that the explanation most consistent with the evidence is that the enhanced neuroendocrine response in female dieters is due

to an adaptation in the brain to compensate for reduced TRP availability. This hypothesis should be testable by restoring plasma TRP levels to normal during dieting using TRP supplements; my prediction would be that this would prevent the enhancement of the PRL response to TRP challenge. The withdrawal of TRP from the market because of association with the eosinophilia-myalgia syndrome (Committee on Safety of Medicines, 1990), thought to be due to contamination during production (Cowen, 1990), means the study cannot be undertaken at present. Whether the brain's adaptation to decreased TRP availability occurs at the level of TRP transport or metabolism, or at the level of 5-HT neurotransmission remains to be discovered.

Why do women seem more vulnerable than men to the effects of dieting? The men in my study (Chapter 4) did not show an alteration in TRP-mediated PRL release even though dieting lowered their plasma TRP. I suggested (4.4) that this could be because of the higher 5-HT turnover in females of many species, including humans, which could make them more vulnerable to a precursor deficit. In addition, female dieters showed a greater decrease in plasma TRP than men suggesting that, in them, precursor availability for 5-HT synthesis was more compromised. The reason for these sex differences is not clear (4.4).

11.2.2 Dieting and Psychiatric Disorders

Do these findings in volunteers have implications

either for dieting or psychiatric disorders? Considering dieting first, it is well recognised that 5-HT plays a role in appetite, satiety and perhaps nutrient selection in animals and humans (see 1.7.2). There is considerable evidence that the PVN of the hypothalamus mediates the effects of 5-HT on appetite; local injection of 5-HT causes anorexia in animals and PVN lesions abolish the effect of systemic 5-HT drugs on appetite (1.7.2). I have given evidence that the PVN also appears to be involved in 5-HT-mediated PRL secretion (see 2.2). Therefore this neuroendocrine test is providing an index of 5-HT function in the very part of the brain that is involved in 5-HT effects on appetite. Drugs that increase 5-HT function suppress appetite and aid weight loss in humans (Silverstone, 1983; Blundell and Hill, 1987; Ceci et al, 1989) and so it is possible to speculate that reduced 5-HT neurotransmission in the PVN plays a role in the hunger experienced during dieting. My data suggest that it is a peripheral effect of dieting (the reduction in plasma TRP) that leads to this reduction in brain 5-HT function, ie the periphery is providing a 'signal' to the brain to stimulate food intake. This is very similar to the suggestion made by Wurtman and Wurtman (1984) that reduced peripheral TRP availability (low TRP:LNAA ratio) results in an increased intake of carbohydrate relative to protein, an effect mediated through reduced brain 5-HT synthesis. An important implication of this is that it might be possible

to aid weight loss in dieters by abolishing the peripheral signal, rather than giving a centrally acting 5-HT drug. An obvious possibility, as discussed above, would be to give TRP supplements during dieting.

The involvement of 5-HT in mood and impulse control also raises the question as to whether there are adverse effects caused by dieting that could be linked to 5-HT function. The effect of weight reduction on mood is variable depending on the population studied and the method and rate of weight loss (see reviews by Stunkard and Rush, 1974; Wing et al, 1984). Early studies claimed a high incidence of adverse emotional reactions, perhaps related to the inclusion of vulnerable individuals and severe weight loss regimens (Stunkard and Rush, 1974), whereas more moderate weight loss appears to be less problematic in more recent reports (Wing et al, 1984) and this was borne out in my investigations. However the preliminary evidence I obtained for an increase in irritability during dieting (Chapter 6) is consistent with informal questioning of dieters. Irritability is an unpleasant psychological state which, if attributed to dieting, would be expected to make it more difficult to persist with the diet. Irritability is probably related to impulsive aggression (Galbraith, 1985) which has been associated with low 5-HT function in animals and humans (see 1.7). This raises the question as to whether the irritability caused by dieting might be related to decreased 5-HT function. Developing this line of

reasoning, the model of Depue and Spoont (1986) proposes that 5-HT systems in the brain are involved in constraining behaviour and reducing impulsivity (see 1.7.1); it is therefore possible to speculate that low 5-HT function both reduces constraint (increases impulsivity) and increases the desire to eat. The unfortunate dieter then finds it impossible to resist the increased temptation to overeat.

The control of appetite and eating is complex and dieting is subject to many influences, social and psychological as well as physiological. It is important therefore not to overemphasise the part of one neurotransmitter, but it seems plausible to me that decreased 5-HT function could contribute to difficulties experienced during dieting. This is an area that needs further exploration with both male and female dieters, using better measures designed to detect psychological changes. The finding of increased irritability in female dieters needs to be confirmed and looked for in male dieters. The relationship between dieting-induced changes in psychological measures and 5-HT function could then be investigated and the effect of pharmacological intervention assessed. It is possible to envisage important consequences for the management of obesity where measures to help successful weight loss are important.

Turning now to possible implications for psychiatric disorders, it has been argued that dieting is the key behaviour which puts an individual at risk of developing an

eating disorder (Szmukler, 1985) and certainly dieting commonly precedes the development of clinical eating disorders (Szmukler, 1985; Patton, 1988). As discussed above, an effect of dieting to lower 5-HT function might be expected to reinforce the tendency to binge, both by stimulating appetite and by reducing restraint over behaviour, hence playing a part in the maintenance of bulimia nervosa by encouraging the 'diet-binge cycle'. The effect of treatment of bulimia nervosa with drugs that increase 5-HT function provides support for this; tricyclic antidepressants (Walsh, 1991a), 5-HT uptake inhibitors (Walsh, 1991b) and fenfluramine (Russell et al, 1988) have all been shown to reduce binge frequency. Treatment with TRP however has not shown consistent effects (Krahn and Mitchell, 1985; Mira and Abraham, 1998) but it is possible that this is due to an increased clearance of TRP from plasma (see Chapter 4) reducing the therapeutic effects. A further consequence of lowering 5-HT function may be to contribute to the development of depressive symptoms of which there are a high incidence in eating disorders (Levy et al, 1989). The predominance of females with eating disorders is commonly believed to be due to social and cultural factors leading to pressures to be slim and hence to dieting which is indeed more common in women than men (Nylander, 1971). However if my hypothesis is correct then women may also be more biologically vulnerable than men to develop eating disorders through a greater susceptibility

to reduced brain 5-HT function as a consequence of dieting.

In the dieting studies in this thesis the normal volunteers were selected to be of average weight and without evidence of eating or other psychiatric disorder. It could therefore be argued that they are likely to be relatively invulnerable to psychiatric consequences as a result of dieting. This raises the question as to whether subjects with a vulnerability to develop eating disorders might respond differently to normal volunteers. The increased PRL response to TRP infusion in normal female dieters could be seen as a homeostatic mechanism to maintain 5-HT function (for example through an increase in postsynaptic receptor sensitivity). Jimerson and colleagues (1990) argue, on the basis of the absence of this effect in eating disorder patients, that it is the failure of this compensatory up-regulation (see 11.2.1) that distinguishes patients from normals, with resultant clinical consequences.

If we now consider weight loss in the context of depressive illness, from the arguments above it seems plausible that weight loss might further lower 5-HT function in depressed patients. Evidence in normal subjects (Young et al, 1986; Smith et al, 1987) and remitted depressed patients (Delgado et al, 1990) indicates that acute lowering of brain 5-HT synthesis leads to the occurrence/reoccurrence of depressive symptoms. It is therefore of interest that, in animal models of depression,

Soubrie and colleagues (1988) showed that food restriction attenuated the response to antidepressants and, in a human study, depressed patients who had lost weight failed to show the expected increase in the PRL response to TRP following treatment with amitriptyline (Cowen et al, 1990b). These results suggest that weight loss might interfere with the augmentation of 5-HT function usually seen with antidepressants. It is conceivable therefore that weight loss might worsen a depressive state through its effect on brain 5-HT function and prevent or reduce the therapeutic response to antidepressants, possibly even precipitating relapse in remitted patients.

11.2.3 Weight Loss and Investigation of 5-HT Function in Depression

If weight loss alters measures of 5-HT function what should we make of findings in depression where weight loss is common? In Chapter 1 I concluded that there was good evidence for the following abnormalities in 5-HT function in depressed patients; reductions in CSF levels of 5-HIAA, platelet 5-HT uptake, platelet imipramine binding, plasma TRP:LNAA levels and 5-HT-mediated PRL release. I am not aware of direct evidence for the effects of moderate weight loss on CSF 5-HIAA, or platelet 5-HT uptake but the possibility that some of the findings in depression may be at least partly accounted for by calorie restriction/weight loss is suggested by the findings of reduced CSF

5-HIAA in anorexia nervosa (reversible by weight gain) (see Jimerson et al, 1990), however platelet 5-HT uptake does not appear to be reduced in eating disorders (see Jimerson et al, 1990). Although platelet imipramine binding is reported to be decreased in anorexia (Weizman et al, 1986) and bulimia nervosa (Marazziti et al, 1988) (patients with major depression were excluded) Goodwin and colleagues (1987d) failed to demonstrate a reduction in imipramine binding following dieting in normal volunteers. Clearly further consideration must be given to the effects of weight loss on these measures, and until this is done, a question mark must remain over their interpretation in depression.

Dieting does reduce plasma TRP and its ratio to competing amino acids and this has an important bearing on the finding of a reduced plasma TRP:LNAA ratio in depression. Does weight loss account for the finding? We have attempted to investigate this by carefully looking at the relationship between weight loss and plasma TRP measures in depressed patients (Anderson et al, 1990). Our findings showed a complex interaction between melancholia, weight loss and sex in predicting low plasma TRP and TRP:BCAA levels. Melancholia was the strongest predictor while weight loss was relevant in females but not males. However the high concordance between melancholia and weight loss in women made distinguishing their individual effects difficult. Weight loss certainly appears to contribute

significantly to reduced plasma TRP measures in depression, particularly in women, but probably is not the whole story, and other features related to melancholia, such as raised cortisol levels (Badawy 1977; Maes et al, 1990) may be important.

Turning now to the last consistent finding in depressed patients, that of blunted PRL responses to 5-HT challenge, the importance of weight loss has been recognised when using the TRP infusion test (Cowen and Charig, 1987; Deakin et al, 1990). In depressed female patients, as with normal volunteers, weight loss is associated with enhanced PRL responses and it is only after exclusion of these subjects that a blunted response is apparent. I have suggested that the likely explanation for the enhanced PRL response to another 5-HT precursor, 5-HTP, which was confined to melancholic females (Maes et al, 1989), was that weight loss confounded the results. The problem of inconsistent hormone responses with the 5-HTP test (Cowen and Anderson, 1986) is also illustrated in that study; male subjects did not have PRL responses. Therefore, although weight loss does affect the TRP test, it clearly cannot account for blunted PRL responses. If both the blunted PRL response due to depression and the enhanced response due to weight loss reflect changes in 5-HT function, then the alterations cannot be the same in the two cases. I have argued for diminished brain 5-HT function in both depression and weight loss, which, if true, means that different

mechanisms must underlie the reduction in the two conditions.

The effect of weight loss on the studies using fenfluramine has only been reported in two studies, one finding a non-significant association with a blunted response (Mitchell and Smythe, 1990/Mitchell et al, 1990) and the other with a non-blunted response (O'Keane and Dinan, 1991). The two studies differed methodologically with the latter using d-fenfluramine which is believed to be more specific in its effect on 5-HT function than the d,l-isomer which may also interact with DA neurones (Invernizzi et al, 1986). However it is difficult to believe that this explains the difference as weight loss does not appear to alter DA-mediated PRL secretion (see Chapter 6). The problem of disentangling the effects of weight loss from those of melancholia is also apparent in these studies and O'Keane and Dinan (1991) do not explain how they assessed weight loss. At present therefore it is not known what effect, if any, weight loss has on 5-HT function as assessed by the fenfluramine test and a normal volunteer dieting study would be helpful to clarify this. In my depressed patient study using CMI infusion (Chapter 8) I did find that weight loss was associated with a blunted PRL response while Golden and colleagues (1990) in their small CMI study did not report the effects of weight loss. We attempted to examine the effect of weight loss on this test in a pilot study using normal volunteers. The

dose of CMI we used produced only minimal PRL responses and while we did not observe enhanced PRL responses as a consequence of dieting we were unable to exclude the possibility that they were blunted (Cowen and Anderson, 1990). Therefore at present it is not possible to say for certain whether weight loss itself may cause blunted PRL responses to some 5-HT challenges. Looking at 5-HT neuroendocrine tests in depression, taken as a whole, I do not believe that the consistent finding of blunted PRL responses can simply be attributed to weight loss, however the question remains open as to its contribution to the results when fenfluramine and CMI are used.

In conclusion, any future investigations of 5-HT function in depressed patients must take weight loss into account and in particular an attempt has to be made to disentangle the effects of weight loss and melancholia and the interaction with sex. Because of the substantial overlap a large enough patient group is required to yield subgroups big enough to analyse; from our study looking at amino acid levels (Anderson et al, 1990) this means at least thirty depressed patients and ideally double that number.

I have not so far discussed an important methodological issue; that of assessment of weight loss in depressed patients. In practice it is only possible retrospectively with the inevitable inaccuracy that this entails. My experience is that in the majority of cases a simple

questionnaire such as the weight question on the BDI gives similar results to an observer administered questionnaire (Appendix 1) but in a minority there are serious discrepancies. Third party information can be useful but is rarely quantifiable and in the end it is often necessary to gather all possible information and make a best guess. In addition it is possible to analyse the data with, and without, the individuals whose status is uncertain. In practice, the apparent similarity in the results when weight loss was assessed retrospectively in depressed patients (Cowen and Charig, 1987; Deakin et al, 1990) and prospectively in volunteers (Chapter 4), suggests to me that it is possible to make a reasonable retrospective assessment of weight loss.

11.3 5-HT Function in Depression

In Chapter 1 I discussed the evidence for abnormal 5-HT in depression and, as mentioned above, concluded that there were five sets of consistent findings which warranted further exploration (see 1.9). In Chapter 2 I argued that neuroendocrine challenge tests currently offer the only practical way of assessing 5-HT neurotransmission in vivo, as long as certain methodological issues are recognised and addressed. The two that I will return to here with regard to the PRL response to 5-HT-drug challenge are, first, the specificity of the challenge, and second, the site of the

abnormality. To conclude this section I will then discuss the effect of clinical variables on the results and finally the clinical implications.

11.3.1 Specificity of 5-HT Neuroendocrine Tests

It is possible to criticise TRP (given intravenously), d,l-fenfluramine and CMI on the grounds that they affect catecholamine as well as 5-HT systems and so the need to characterise the hormone responses elicited becomes crucial. As has been discussed in 2.4, the major difficulty preventing this has been the lack of adequate 5-HT antagonists. In particular, the commonly used non-specific 5-HT antagonists, methysergide and metergoline are not helpful in characterising PRL responses because of their DA agonist properties (Krulich et al, 1981; Ellis et al, 1991). This unfortunately leaves a dearth of direct pharmacological evidence for the 5-HT-mediation of the PRL responses to fenfluramine and CMI although the evidence for 5-HT₁ receptor mediation of the PRL (and GH) responses to TRP is accumulating. However, I have argued (Chapter 8) that the demonstration of the same result, using different drugs which share a common property, does provide evidence for it being that property which is responsible for the hormone response.

My normal volunteer study indicated that an overlooked, or at least underacknowledged, factor in hormone responses to 5-HT-drug challenge is the presence of stressful side-

effects especially nausea. The TRP test is generally agreed to be well tolerated (Charney et al, 1982; Winokur et al, 1986) and that has been the experience in our unit with robust hormone responses occurring in the absence of side-effects apart from sedation. The situation with d,l-fenfluramine is less clear, with some authors claiming no side-effects (eg Lewis and Sherman, 1984) but others reporting them to be intolerable (O'Keane and Dinan, 1991). The latter authors found d-fenfluramine to be nearly side-effect free apart from sedation. The situation is somewhat similar with CMI infusion. Golden and colleagues (1990) reported no spontaneous complaints of nausea with CMI 10mg but 3/10 subjects complained of nausea at 20mg, one vomiting. Visual analogue scales did detect an increase in nausea at both doses (the severity was not reported) but the authors conclude that this was not responsible for the hormone responses to CMI 10mg. In contrast a sizeable number of subjects in my normal volunteer study (Chapter 6) had nausea following a dose of about 10mg which, in some, was experienced as stressful and associated with large PRL responses. This resulted in my having to exclude subjects who experienced stressful nausea in my depressed patient study (Chapter 7) and carefully compare nausea ratings in relation to results. I concluded that nausea was unlikely to account for the results, first, because the nausea was generally mild in the subjects included in the study with no correlation between nausea ratings and PRL response and,

second, because of the parallel finding with the sequential low-dose TRH test which was unrelated to nausea.

In conclusion it has to be acknowledged that the issue of specificity remains a difficulty with 5-HT challenge tests taken individually but, if considered together, the similarity of results in spite of different pharmacology and side-effects argues in favour of a common mode of action - 5-HT mediation of the PRL response.

11.3.2 Site and Mechanism of Abnormality

My finding of a blunted PRL response to CMI challenge presented in Chapter 7 is consistent with previous studies using TRP and fenfluramine; that three distinct 5-HT challenges have found the same result adds further weight to there being reduced 5-HT-mediated PRL release in depression. However, as I discussed in Chapter 2, the question as to the site of abnormality has not yet been answered. It is tempting simply to align the result with other evidence for diminished 5-HT function in depression and conclude that the abnormality must be in the 5-HT link. The importance of the finding - the only dynamic measure reflecting brain 5-HT function - and the knowledge that other aspects of neuroendocrine function are abnormal in depression, do not allow this. I therefore attempted to begin tackling this issue by asking the obvious question - is PRL secretion itself abnormal in depressed patients? As I argued in 1.8.4 the extensive literature on TRH-

stimulated PRL secretion in depressed patients misses the vital point as to whether the sensitivity of the pituitary release of PRL is altered because of the use of supra-maximal doses of TRH. The results presented in this thesis unfortunately do not give the final answer because of an unforeseen interaction between CMI and TRH in the depressed patient study which means that the blunted response to low-dose TRH cannot be taken as evidence of impaired PRL release. A subsequent investigation in our research unit has used the TRH test alone in depressed patients and found no blunting of the PRL response (Anderson et al, 1992); this is consistent with the investigation into DA-mediated PRL release I have presented (Chapter 9), and I believe the evidence is now strongly in favour of normal PRL release at the pituitary level in depression.

Fenfluramine and CMI act at the level of the 5-HT synapse indicating that the abnormality in 5-HT-mediated PRL secretion in depression occurs somewhere between 5-HT release from the nerve terminal and PRF release in the median eminence. The use of postsynaptic 5-HT agonists will be of great help in further clarifying the situation; preliminary results with mCPP and MK-212 which probably act on 5-HT_{1C/2} receptors have shown normal PRL responses in depression (see 1.8.4) suggesting either that the postsynaptic receptor/releasing factor 'link' is normal or that a different 5-HT receptor is involved. It is of interest that a recent study using the 5-HT_{1A} agonist

ipsapirone has shown diminished hypothermic responses in depressed patients. As I have discussed in Chapter 10, 5-HT_{1A} agonists probably cause hypothermia by stimulating presynaptic 5-HT_{1A} somatodendritic autoreceptors. If this preliminary result is replicated it will tend to support a presynaptic site for the 5-HT abnormality in depression, although diminished 5-HT_{1A} autoreceptor sensitivity cannot directly explain decreased terminal 5-HT release, rather it would be expected to increase it.

The evidence presented so far is therefore consistent with a reduction in 5-HT neuronal (presynaptic) function in depression. However Deakin and colleagues (1990) suggest an alternative mechanism mediating the reduction in 5-HT-mediated PRL secretion. They point out that in animal studies corticosteroid treatment results in reduced 5-HT (particularly 5-HT₁) behavioural responses (Nausieda et al, 1982; Dickinson et al, 1985) and reverses adrenalectomy-induced increases in 5-HT₁ receptor binding in the forebrain (Biegon et al, 1985; De Kloet et al, 1986). One function of 5-HT₁ receptor systems in animals appears to be adaptation to adverse events (Kennet et al, 1985; 1987). In agreement with others (Lopez-Ibor et al, 1988; Mitchell and Smythe 1990), Deakin and colleagues (1990) found that basal cortisol levels were inversely correlated with the magnitude of the PRL response. They suggest that chronic stress leads to raised cortisol levels (they found such a correlation) which results in reduced 5-HT₁ function and

impaired resistance to aversive events, hence depression supervenes. Their hypothesis therefore implicates the postsynaptic 5-HT₁ receptor system in the blunted PRL responses in depressed patients.

From the above discussion it is apparent that further studies using more selective 5-HT agonists to probe pre- and postsynaptic function are needed in order to clarify the nature of the abnormality indicated by the investigations to date.

11.3.3 5-HT Function in Relation to Clinical Features

A number of clinical features have been investigated in relation to 5-HT-mediated PRL responses in depressed patients.

(i) Severity of Depression and Melancholia

Most of the studies summarised in Table 1.8 measured the severity of depression using the HAMD scale; in agreement with my findings using the CMI test none reported an significant correlation with PRL response in depressed patients as a whole, although Price and colleagues (1991) found a modest positive correlation in the melancholic subgroup alone.

The measurement of endogenicity/melancholia has generally used either the DSM-III melancholia category or the Newcastle scale (Carney et al, 1965). These classifications do not overlap completely with the Newcastle

Scale providing more restrictive criteria (eg see Mitchell and Smythe, 1990). Although my numbers were small, I found that melancholic patients had the most blunted responses while there was no correlation with Newcastle Scale score. Of the three other studies that have reported comparison of DSM-III melancholic status with PRL responses, two have found an association with blunting (Cowen and Charig, 1987; Mitchell and Smythe, 1990) whereas the third, the largest 5-HT neuroendocrine study so far reported (Price et al, 1991), found only non-melancholic patients to have blunted responses. Four studies have reported the comparison between Newcastle Scale scores and PRL responses. Two found an association with blunted responses (Lopez-Ibor et al, 1989; Mitchell and Smythe, 1990), one no association (but patients with weight loss were excluded) (Deakin et al, 1990) and one an association with non-blunted responses (O'Keane and Dinan, 1991). The evidence to date therefore is somewhat conflicting with regard to there being a relationship between melancholia/endogeneity and blunted 5-HT-mediated PRL responses although, arguably, the evidence is more suggestive with regard to melancholia than endogeneity. It is worth pointing out that a major difference between DSM-III melancholia and Newcastle Scale endogeneity is that the latter rates down (and hence tends to exclude) patients with personality difficulties, psychosocial stresses and anxiety, factors that have no weighting in the diagnosis of melancholia. Deakin and

colleagues (1990) hypothesise that chronic psychosocial difficulties and stress may be related to reduced 5-HT¹ function and reduced PRL responses to TRP (see 11.3.2 above) while Coccaro and colleagues (1989) found a relationship between personality disorder and blunted PRL responses to d,l-fenfluramine. If these factors are related to reduced 5-HT function then the lack of a correlation between Newcastle Scale endogeneity and reduced 5-HT-mediated PRL responses is not surprising.

Melancholia, on the other hand, is associated with physical symptoms of depression and I would not find it surprising if this group of patients show the most marked abnormality in a physical measure. It is of interest that we have also found melancholia to be particularly associated with another measure related to 5-HT function, reduced plasma TRP availability (Anderson et al, 1990).

(ii) Suicide Attempts

As has been reviewed in 1.8 there is evidence for an association between low 5-HT function and suicide attempts, particularly with violent methods. In my study (Chapter 7) I found that those patients who had attempted suicide had lower PRL responses than those who had not. Only two other studies have compared suicide attempts and 5-HT-mediated PRL responses. Coccaro and colleagues (1989) found an association with reduced PRL response to d,l-fenfluramine which cut across diagnostic categories of current and

remitted major depressive disorder and personality disorder. O'Keane and Dinan (1991) reported no association between suicide attempts and PRL responses to d-fenfluramine. It is of great interest that patients who had current suicidal behaviour were excluded from the large study described by Price and colleagues (1991). This study found only modest evidence for blunted PRL responses in depressed patients which raises the question as to whether they had excluded the very patients most likely to have reduced brain 5-HT function (see 1.8). Four studies have looked at suicidal ideation as measured on the HAMD. Two found a weak positive correlation with PRL response (Cowen and Charig, 1987; Mitchell et al, 1990), however in the former this disappeared when weight loss was taken into consideration. Two studies (O'Keane and Dinan, 1991; Price et al, 1991) found no correlation between suicidal ideation and PRL response, as was the case in my study (data not presented). There is therefore no good evidence to support a link between suicidal ideation and blunted PRL response. If there is indeed an association between suicide attempt and low 5-HT function then it may be mediated through the tendency to act on suicidal impulses rather than directly related to suicidal ideation itself (see 1.7.4).

(iii) Weight Loss

This has been discussed at some length above (11.2.3). Weight loss appears to have different effects on individual

5-HT challenges and cannot account for the general blunting of PRL responses. As there is reason to believe that weight loss may alter 5-HT function it clearly needs to be carefully assessed when investigations are undertaken.

(iv) Anxiety, Personality, Psychosocial Factors and Treatment Response

These have not been investigated consistently across studies. I found no correlation between anxiety scores on the HAMA and PRL responses to CMI although O'Keane and Dinan (1991) report an association between blunted PRL responses to d-fenfluramine and anxiety items on the HAMD. Studies investigating 5-HT-mediated PRL responses in anxiety disorders have found normal (Charney and Heninger, 1986; Charney et al, 1987) or increased (Targum and Marshall, 1989) responses suggesting that anxiety per se is not associated with blunting. Personality as assessed by the Eysenck Personality Inventory was not found to be related to blunted PRL responses in one study (O'Keane and Dinan, 1991) but Coccaro and colleagues (1989) found that patients with borderline personality disorder had blunted PRL responses although this association disappeared when the effect of impulsive aggression was taken into account. Deakin and colleagues (1990) looked at a number of factors including duration of illness, chronic psychosocial difficulties, acute precipitant, premorbid neurotic traits/impaired personality and response to treatment as

assessed six months later. None of these showed a relationship to the PRL response to TRP.

11.3.4 Implications

What then are the implications of these findings for understanding the biology of depression and its treatment? It is important to recognise that just because there is an alteration in the functioning of a certain neurotransmitter system in depressed patients this does not imply that it is the only, or even the most important, abnormality. It seems almost certain that there will be a pattern of abnormalities in interconnected systems, reflecting the functional organisation of the brain rather than just individual neurotransmitter systems. For example the well established abnormalities in the HPA axis (Charlton and Ferrier, 1989; Kathol et al, 1989) must also be an expression of the disrupted functioning of the depressed brain, but what relationship this has to altered 5-HT function is obscure. It is likely that some changes will be secondary or even homeostatic. I have suggested for example that if a catecholaminergic reward system such as the BFS (see 1.7.4) is underfunctioning in depression that it would be expected that a functionally opposed or balancing system such as the BIS (related to 5-HT function) would have reduced function in an attempt to maintain behavioural homeostasis. There is some evidence for this in the high degree of positive correlation that occurs in

the CSF between 5-HIAA and HVA (Agren et al, 1986; Roy et al, 1988) and it has been suggested that 5-HIAA 'drives' HVA (Agren et al, 1986). Perhaps depressive illness only occurs when systems become uncoupled and homeostasis fails. Some features of depressive illness such as disrupted sleep are congruent with what would be expected from a knowledge of the effects of decreased 5-HT function, others such as reduced appetite and libido are difficult to explain directly; could it be that a decrease in 5-HT function is in some cases a failed attempt to compensate for changes in another system?

The above discussion presumes that the alteration in 5-HT function is a state abnormality but this has not been proven. The evidence as far as 5-HT-mediated PRL responses is concerned is mixed. Upadhyaya and colleagues (1990) report that the PRL response to TRP infusion returns to normal after recovery but two other studies using fenfluramine in cross-sectional studies suggest that recovered depressives have blunted PRL responses (Siever et al, 1986; Coccaro et al, 1989). Low 5-HT function in relation to impulsive aggression is seen as a trait phenomenon (Linnoila and Virkkunen, 1991) suggesting an alternative hypothesis, that of low 5-HT as a vulnerability factor for depression. There is some support for this hypothesis; healthy volunteers with a family history of depression are more likely to have low levels of CSF 5-HIAA than those without such a family history (Sedvall et al,

1980). CSF 5-HIAA levels appear to have a heritable component although social and environmental factors appear equally, if not more, important (Oxenstierna et al, 1986), opening the door for a biological correlate of the interaction between heredity and early experience in the aetiology of depressive illness. In addition the chronic stress mechanism suggested by Deakin and colleagues (1990 - see 11.3.2 above) suggests a way in which life events and psychosocial difficulties may contribute to vulnerability as well as precipitate and maintain a depressive illness. The finding of particularly low 5-HT function in patients with suicide attempts suggests that this could 'release' suicidal behaviour in the context of depression through increased impulsivity/impulsive aggression (Coccaro, 1989; Linnoila and Virkkunen, 1991). The above formulation does not exclude the combination of state and trait components of reduced 5-HT function in depressive illness, ie trait low 5-HT function could be a vulnerability factor for depression, which, when it occurs leads to a further lowering of 5-HT function and to an increased risk of suicide. This is consistent with investigations of depressed patients studied during illness and remission which suggest that there might be a subgroup whose CSF 5-HIAA levels are in the low-normal range when euthymic but who develop very low levels when depressed (van Praag, 1977; Traskman-Bendz et al, 1984).

A number of testable hypotheses flow from this. First,

it suggests that depressed patients in remission may fall into a number of subtypes. Recurrent depressives and perhaps those with a younger onset of first illness would be likely to have reduced measures of 5-HT function when euthymic, while 'sporadic' and late onset remitted depressives might be expected to have normal or near normal 5-HT function. If there are both state and trait elements to reduced 5-HT function then cross sectional studies might show that recovered depressives have measures of 5-HT function which are intermediate between normals and depressed patients, while longitudinal studies would show that depressed patients have lower 5-HT function when depressed compared to when euthymic. The three studies utilising 5-HT-mediated PRL secretion are essentially consistent with this (Siever et al, 1986; Coccaro et al, 1989; Upadhyaya et al, 1990).

Second, groups of people who have been demonstrated to have low 5-HT function should have an increased predisposition to depressive illness. There is evidence consistent with this. Coccaro and colleagues (1989) found that patients with borderline personality disorder had particularly low PRL responses to d,l-fenfluramine; these patients are prone to episodes of depression (Barrash et al, 1985). Other psychiatric diagnoses have been associated with reduced 5-HT function, for example alcoholism (Linnoila and Virkkunen, 1990) and eating disorders (Jimerson et al, 1990, see also 11.2) both of

which are associated with secondary depression (Pottenger et al, 1978; Levy et al, 1989). Obsessive-compulsive disorder (OCD) is however difficult to fit simply into this picture. It is associated with a significant incidence of secondary depression but a lower rate of suicide than primary depression (Freeman, 1983). Investigation into 5-HT function has suggested a different pattern to that in depression with possibly raised CSF 5-HIAA but blunted PRL and cortisol responses to mCPP and fenfluramine (Insel, 1991; Hollander et al, 1991). There is therefore evidence consistent with both increased 5-HT turnover and decreased function which might reflect separate pathology to that seen in primary depression. Possibly we are seeing here a dissociation between the 5-HT systems associated with mood and with impulse. Anatomically this is quite possible as depressed mood has been proposed to reflect changes in 5-HT function in limbic areas (Jimerson et al, 1990) and OCD and impulse control with frontal lobe function (Barratt and Patton, 1983; Machlin et al, 1991).

A third consequence of my formulation is that treatments which increase 5-HT function should be beneficial in depressive illness, both in acutely relieving depression and in reducing vulnerability to relapse. Selective 5-HT uptake inhibitors clearly are effective on both counts (Montgomery et al, 1988; Aberg-Wistedt A, 1989). There is evidence that 5-HT_{1A} agonists have antidepressant properties (Amsterdam et al, 1987) and as I

discussed in 1.8.5, many effective antidepressants appear to increase 5-HT neurotransmission. My formulation does not require that a primary action on 5-HT systems is necessary for antidepressant efficacy but does raise the question as to why fenfluramine, which, at least acutely, increases 5-HT function, is not an antidepressant, indeed it may cause depression (Silverstone, 1983). A possibility is that as a 5-HT-releasing agent (Garattini et al, 1975), chronic fenfluramine could lead to 5-HT depletion (ie an unchanged or even decreased level of 5-HT function). In addition it has been shown to have a neurotoxic effect on 5-HT neurones (Molliver and Molliver, 1990).

Fourth, drugs which decrease 5-HT function chronically should lead to an increased chance of depression. As discussed in 1.8.5 drugs which deplete monoamines, including 5-HT, can give rise to depression and the efficacy of antidepressants can be reversed by reducing brain 5-HT function. There does not appear to be any consistent evidence to show that chronic use of 5-HT antagonists causes depression but this of course raises the issue as to which 5-HT receptor systems are important in the aetiology of depression as well as the lack of knowledge of the chronic, as opposed to acute, effects of many drugs.

A fifth implication arising from my formulation is that depressed patients should be more impulsive and/or aggressive than non-depressed subjects and their

impulsivity/aggression should be greater in the depressed state compared to when euthymic. I know of no direct evidence on this subject probably because impulsivity/impulsive aggression is usually assessed as a trait characteristic, is probably not directly amenable to self-report and does not correlate well with performance measures which might allow a state assessment (Barrett and Patton, 1983). Having said that it is clear clinically that irritability and hostility are common in depressive illness and suicide attempts are not infrequently impulsive and associated with aggressive feelings towards others as well as the self. Of interest, one performance measure which has been shown to correlate with impulsivity, underproduction of time intervals (judging less time to have passed than in fact has), is also found in depressive illness (Barrett and Patton, 1983). It would be of interest to pursue this issue further and relate the findings to 5-HT function.

The clinical implications of the findings of low 5-HT function in depression are well known and include the development of drugs with specificity for the 5-HT system (it must be said that this is more in the hope of fewer side-effects and safer drugs than of increased efficacy) and prediction of antidepressant response to 5-HT-active drugs (eg Møller et al, 1986). New compounds are likely to include drugs with 5-HT agonist properties (5-HT_{1A} agonists have already been tested) and perhaps 5-HT receptor

antagonists, particularly those acting on autoreceptors, may be useful. The problem of the recurrence of depression is becoming recognised and long-term drug prophylaxis is increasingly an issue (Montgomery et al, 1988). This will require careful risk-benefit assessment in a society where the long-term detrimental effects of psychotropic drugs are increasingly debated; it will also require the availability of compounds which patients will be prepared to continue when they feel well (ie few side-effects as well as high safety). It would be useful to have a biological marker of vulnerability to depression which would allow the design of specific interventions and rational prophylaxis; perhaps some measure of 5-HT function may provide this.

The prevention of suicide is major clinical consideration in relation to pharmacological treatment. First, prescribing drugs which are relatively safe in overdose has been increasingly advocated because suicide attempts are frequently impulsive and use the nearest available means (often the antidepressants prescribed for the depression). Second, drugs might have a role in promoting or inhibiting suicidal impulses/actions. This has been highlighted by case reports suggesting the induction of suicidal feelings by fluoxetine (Teicher, 1990). Naturally this has stimulated interest and concern, but there is at present no evidence for an increased incidence of suicide in patients treated with 5-HT uptake inhibitors and even some suggestion that the emergence of

suicidal ideation is lower on 5-HT uptake inhibitors compared to tricyclic antidepressants (Beasley et al, 1991). The prediction and hope, of course, is that 5-HT-active drugs might protect against suicide attempts but the preliminary evidence so far is conflicting with one open study suggesting that fluvoxamine prevented future suicide attempts (Banki, 1991), another that fluoxetine had no effect in frequent repeaters (Montgomery SA, Abstract presented at British Association for Psychopharmacology Summer Meeting, 1991).

In summary, the demonstration of low 5-HT function in depressive illness continues to generate further questions and research needs to be directed towards further characterising this abnormality in terms of 5-HT receptor systems and neuroanatomical basis. The relationship between findings in depressive illness, impulsive aggression and other psychiatric diagnoses (such as the eating disorders and OCD) may give insights into neurochemistry of depression. Clinically, issues of treatment, prophylaxis and suicide prevention are of great importance and are beginning to be influenced by research findings.

11.4 Investigation of 5-HT Receptor Function

In Chapter 10 I demonstrated that the 5-HT_{1A} agonist gepirone has neuroendocrine and temperature effects in

humans. As will have become clear in this chapter the bulk of evidence concerning abnormal 5-HT in psychiatric conditions is general in nature; measures of 5-HT turnover or precursor availability, 5-HT uptake or overall neurotransmission. This contrasts with the increasing knowledge of the intricacies of 5-HT neuronal organisation, the 5-HT receptor subtypes that form a subtle orchestra upon which can be played different melodies with varying textures. Future advances are going to require an ability to investigate 5-HT receptor systems in specific neuro-anatomical locations. Hormone and temperature control are anatomically localised to the hypothalamus and provide a unique opportunity for investigating 5-HT function in a known part of the brain. The constraint, of course, is that we cannot be sure that this is an important area for the subject we are investigating. However in the case of depression, and perhaps more so of weight loss, I would argue that it is an excellent place to start given that the most consistent biochemical abnormality in depression is that of overactivity of the HPA axis (Charlton and Ferrier, 1989; Kathol et al, 1989) and that the hypothalamic PVN mediates the effects of 5-HT on appetite (see 1.7.2 and 11.2.1).

As discussed in Chapter 10, gepirone provides the potential for assessing both pre- and postsynaptic 5-HT_{1A} receptor function. Evidence accumulating from antagonist studies involving the related compounds buspirone and

ipsapirone supports 5-HT_{1A} receptor-mediation of the GH, ACTH and temperature responses. As yet the mediation of the PRL response is not certain. In depression there is preliminary evidence using ipsapirone that there is a blunting of the hypothermic response suggesting a decrease in sensitivity of the 5-HT autoreceptor or post-receptor pathways (Lesch et al, 1990c). Lesch and colleagues (1990d) also found that amitriptyline further blunted this response, a result that we have been able to replicate with buspirone (da Roza Davis et al, 1991) suggesting that tricyclic antidepressants alter presynaptic 5-HT_{1A} receptor function in humans. Clearly studies involving postsynaptic 5-HT_{1A} receptor-mediated responses in depression will be of great interest. Lesch and colleagues (1990a) have reported blunted ACTH and cortisol responses to ipsapirone in depressed patients, but this may simply reflect alteration in HPA axis function rather than 5-HT function (see 1.8.4). Further studies investigating the PRL and GH responses to 5-HT_{1A} agonists are clearly warranted.

I hope to also apply this approach to studying the effect of dieting in normal volunteers as there are many unanswered questions about the effect of this 'normal' activity on 5-HT function (see 11.2). The neuroendocrine challenge strategy has been applied to many psychiatric disorders and 5-HT_{1A} receptor agonist challenge with compounds such as gepirone will allow further progress

along the road of identifying (or rejecting) specific abnormalities of 5-HT receptor function.

11.5 Conclusions

In this thesis I have used a neuroendocrine challenge strategy in three different areas; to investigate 5-HT function in dieting normal subjects and in depressed patients, and to investigate the effect of 5-HT_{1A} receptor activation in humans. My conclusions can be briefly stated:

(i) Weight loss may cause a change in brain 5-HT function in women as a consequence of diminished plasma TRP availability. This has implications for problems encountered during dieting, the aetiology of eating disorders and the investigation of depressive illness where weight loss is common.

(ii) 5-HT neuroendocrine challenge tests are problematic because of lack of specificity and the propensity to cause stressful side-effects such as nausea. Investigations using this strategy must therefore continue in attempts to characterise hormonal responses by the use of specific antagonists and report side-effects which may compromise the interpretation of results.

(iii) There is good evidence for reduced 5-HT function in depressed patients apart from any confounding effects of weight loss and side-effects. A diagnosis of melancholia

and a previous suicide attempt are associated with particularly low 5-HT function. I suggest that the reduction in 5-HT function seen in depressed patients may have both state and trait components.

(iv) Future investigations using the neuroendocrine strategy will require more specific challenges of 5-HT receptor function. I have shown that 5-HT_{1A} receptor function may be amenable to investigation using gepirone and related compounds.

What of alternative approaches to attempting to answer the intriguing questions raised in the course of this thesis? Brain imaging techniques and molecular genetics are the great hopes for the immediate future but as yet imaging the 5-HT system by positron emission tomography awaits suitable ligands (Frost, 1990) and molecular genetic techniques have achieved limited success in psychiatry for a variety of reasons (Baron et al, 1990). However investigated, the mysteries and complexities of 5-HT functioning in the human brain are likely to continue to stimulate research for the foreseeable future.

APPENDIX

WEIGHT QUESTIONNAIRE

NAME: DATE:

AGE:

1) WHAT IS YOUR NORMAL WEIGHT/WEIGHT BEFORE YOU WERE ILL?

2) HOW MUCH DO YOU WEIGH NOW? (ASK BEFORE WEIGHING)

3) HAVE YOU LOST OR GAINED WEIGHT DURING THE LAST MONTH?

IF SO HOW MUCH?

HAVE YOU BEEN TRYING TO LOSE OR GAIN WEIGHT?

4) HOW LONG HAVE YOU BEEN UNWELL?WEEKS

5) HOW MUCH WEIGHT HAVE YOU LOST/GAINED SINCE YOU HAVE
BEEN UNWELL?

6) MEASURED WEIGHT

7) MEASURED HEIGHT

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